THE SAPROLEGNIACEAE

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WITH

NOTES ON OTHER WATER MOLDS

BY

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WITH SIXTY-THREE PLATES

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INTRODUCTION

SINCE the appearance about thirty years ago of Humphrey's fine monograph on the *Saprolegniaceae* and the almost simultaneous treatment of the European species by Fischer in Rabenhorst's Flora, there has been much work done on the water molds, both in this country and Europe. A number of new species have been described, fertilization and other cytological phenomena investigated, and a considerable amount of experimenting done on the effect of various media on growth and reproduction. It has been my purpose to get together a treatment of all known species and to describe and illustrate all species I have seen in the living state. Most cytological and physiological details of importance appearing in the literature since Humphrey's work have been included or referred to under the species involved, and I have not thought it worth while to write a long introduction covering these points. Researches on chondriosomes and our own work on the structure and behavior of the emerging spores will, however, be referred to in this introduction.

It will be well to call attention first to several conclusions arrived at by Humphrey that have since been shown to be erroneous. In regard to sexual reproduction Humphrey decides from observations by himself and others that fertilization no longer occurs in the *Saprolegniaceae.* This we now know to be incorrect. A true sexual fusion occurs in *Achlya americana* var. *cambrica*, *A. polyandra*, *A. deBaryana*, *Saprolegnia monoica*, *S. mixta*, *S. diclina* and *Aphanomyces laevis* (see these species for details), and it is highly probable that it occurs in a number of others where antheridia are well developed. Credit is due to Trow who in the face of much criticism carried forward to a convincing conclusion his assertion of sexuality in this family.

Humphrey was also wrong in his conclusion ('92, p. 77), contrary to the opinion of deBary, Strasburger and Büsgen, that "In general, there can be little doubt that the spores of the genera now under discussion [*Saprolegnia*, *Achlya*, *Aphanomyces*] leave the sporangia automatically." In my opinion the evidence against this view is overwhelming. In the first place, in genera like *Achlya* and *Aphanomyces*, where the first swimming stage is suppressed, the spores certainly have no ciliary action (even where cilia are present) sufficient to drive the spores at a high rate of speed through an opening so small as to constrict them. Where cilia have been demonstrated within the sporangium their action is very feeble and spasmodic and leads only to a slight rocking or jerking motion. Again, the spores move as a mass and not individually. As the first spores escape the others draw away evenly from the cell wall until as the emptying proceeds the spores form an axial column which remains solid and moves toward the opening, the spores having their long axes all parallel to the long axis of the sporangium. The spores on the bottom of the column can be plainly seen in places where they are slightly separated and it is perfectly obvious that if any ciliary action is going on at all it is negligible as a motive force. Absolutely conclusive are the cases where large masses of protoplasm are ejected from a sporangium in undifferentiated form, the entire contents at times plastically pouring out of the small mouth (see page 9). A similar case is the ejection of the bladders from the sporangia of *Pythium*, *Rhipidium* and *Araiospora*. In *Aphanomyces* the main bodies of the elongated spores becomes separated by an obvious interval soon after the sporangium opens (they are actually connected by a slender strand of protoplasm), and if one gets caught in the opening all the others in the row stop suddenly and start forward again only when the obstruction is removed, showing that the spores are being passively carried forward by the surrounding medium. In *Leptolegnia* the spores emerge with force but do not swim actively away as would be expected if this force were continued. They slowly move about at the sporangium mouth reshaping themselves along another axis preparatory to active swimming. It often happens in *Achlya* that one or more groups of spores on emerging become separated from the main mass by some distance (as several times the diameter of the spores). If one of these masses is touched all the groups are moved or jerked by the impulse. This shows a connecting medium less fluid than water, and this medium we now know to be delicate strands of protoplasm (see page 4).

Just what causes the internal pressure that drives out the spores has not been known with certainty. There is a shrinkage in the size of the sporangium during the discharge, but this could account for only a small part of the result. A sporangium of *Achlya racemosa* measured by us was 21μ thick before and 18.7μ thick after the discharge. Aside from this there would seem to be only two possible alternatives, the simple absorption of water due to higher osmotic tension within, or the swelling of a gelatinous medium by absorption of water. All the phenomena could be explained by the assumption that the expulsion is due to the swelling of a peripheral substance inside the sporangium, and that this is the cause was the opinion of deBary, as clearly expressed in his Comparative Morphology of the Fungi, etc., p. 82. The only difficulty in the way of the acceptance of the assumption that a swelling mucus is the cause of expulsion is the entire failure so far of all efforts to demonstrate the presence of such a mucus by selective stains. Mr. H. R. Totten, Instructor in this laboratory, has carried out a series of experiments on the emerging spores of *Achlya* and has used various stains in an attempt to determine the presence of a mucus. He reports as follows: "I have been unable to demonstrate the presence of a mucus in the sporangium while the spores are moving towards the central axis, or about the escaping spores, or about the spherical mass of spores at the tip of the sporangium after their escape. The following stains have been used: Delafield's Haematoxylin, Methylene Blue following Lead Acetate and without Lead Acetate, Chloroiodide of Zinc, India Ink after growing plants in 1% Congo red." On account of the even distribution of pressure throughout the sporangium, which would follow if the expellent material were simply a watery sap, the grouping of the spores into an axillary row while emerging, and their evident attraction to each other would have to be explained on the assumption that they actively cling and press together. Heretofore no explanation has been offered for these striking facts except the very unsatisfactory suggestion of a kind of liking for each other, the adelphotaxy of Hartog. Humphrey (p. 76) favors this explanation and also asserts the opinion that there is no material connection between the spores. Realizing the unsatisfactory state of this whole matter, we have been carrying on recently with the help of several of our students an examination into the spore discharge in *Achlya*, *Aphanomyces* and *Leptolegnia*, and we are now prepared to add some new data on this puzzling subject.

Rothert ('88) has shown that the spores of *Achlya polyandra* are connected by threads on emerging, and later ('03) found that the same is true for *Aphanomyces*. Mr. J. N. Couch, Instructor in this laboratory, has confirmed these observations and has proved that there is a material connection between the spores in *Achlya*, *Aphanomyces* and *Leptolegnia* while in the sporangium, and that in the first two this connection is maintained until the spores have taken their position in the apical ball. It is our opinion that these connecting strands play the most important part in drawing the spores into a ball in *Achlya* and *Aphanomyces* and in holding them there. This remarkable phenomenon has heretofore received no adequate explanation. Rothert thinks the spores are held together in a ball by a gum but as said above we find no such substance. In his first treatment of the genus *Aphanomyces*, deBary demonstrated the presence of a slender filament of protoplasm connecting the emerging spores in the sporangium ('60, pl. 19, fig. 3), but he said that at times they were without this connection. In our experience, when this connection is absent it is evidence that the sporangium will not empty and that the spores have reorganized themselves for a rest.

In *Leptolegnia*, Petersen (Ann. Myc. 8: 522. 1910) found that the spores were linked together by their cilia on passing through the sporangium. Mr. Couch finds that the cilia are formed from the original protoplasmic layers connecting the spore origins while they are against the cell wall, that these threads unless jerked apart by a too vigorous rocking of the spores persist and thus the spores as they emerge are connected with four protoplasmic threads, two in front and two behind. After emerging from the sporangium two of these four connections persist as cilia, but which two has not yet been clearly demonstrated.

In the case of *Achlya*, Mr. Couch finds a similar condition. A sporangium of *Achlya dubia* in the act of discharging its spores was killed with 1% osmic acid, and the discharged spores instead of forming a close mass at the tip of the sporangium separated a short distance from each other but remained attached in a loose cluster to the tip of the sporangium as if held together by a mucus or by delicate threads of protoplasm. At this moment the sporangium was dragged gently by the observer for a distance of several millimeters. At first the entire mass moved, and then the spores began stringing out, the ones behind being pulled apparently by those in front. A cover glass was then applied and the spores stained with a 1% solution of Fuchsin and methyl violet in 95% alcohol, to which was added three times its volume of water. This stain brought out unmistakable connections of delicate protoplasmic threads between the spores. Many spores had become separated from the general mass and it was observed that these connecting protoplasmic threads were on both ends of some spores, while on other spores the threads were in the normal position for cilia. Furthermore, spores on which these threads occupied all intermediate positions between the above two were observed. Similar results were obtained with *A. conspicua*, *A. apiculata* and its variety *prolifica*, *A. Orion*, and *A. flagellata*. Mr. Couch's work on this problem is still in progress and will be published with illustrations in a future number of the Journal of the Elisha Mitchell Scientific Society.

In discussing the peculiar behavior of the spores in *Achlya paradoxa* (now *Protoachlya*) I gave some years ago ('14, p. 294) a short review of the variations from the supposed normal behavior of the sporangia and spores in the *Saprolegniaceae*, as recorded in the literature. As rather numerous records have appeared since 1914, I think it worth while to reprint this review with such additions and changes as are needed to bring it up to date.

In the case of *Achlya* a departure from or modification of the usual grouping of the spores at the sporangium tip has been recorded in a few instances. In the first place it must be remembered that the spores in this genus are not perfectly quiescent during and immediately after emergence. A slight amoeboid motion is observable at all times from their initial formation to the appearance of the encysting membrane. Added to this is a certain feeble jerking and rotation due to the presence of cilia that has been recorded by several observers since Cornu first described it in 1872. On page 11 of his monograph Cornu says that these cilia have just enough agility to cause the escape of the spores from the sporangium, thus implying, in error, that they are the cause of the escape. The presence of cilia on the emerging spores of *Achlya* is strongly asserted by Hartog ('87) who also predicts that they will be found in all species of *Achlya* and *Aphanomyces* ('88).

Hartog also says in the first of these papers that the spores of *Achlya* after forming a ball revolve on their long axes for a short time before the cyst is formed, and that sometimes a few spores will detach themselves and swim away a short distance. In the second paper he says: "When the sporange is discharged near the margin of the hanging drop or in a thin layer of water on a slide, we constantly see single spores escape from the mass, swim away, and encyst apart." This important observation has been frequently overlooked by subsequent workers, but I can confirm it positively for *Achlya caroliniana*. In this case if the sporangium is put on the slide in a very thin layer of water the spores will swim slowly apart on emerging and scatter themselves over a limited area near the mouth of the sporangium. By addition of iodine solution the cilia were clearly seen. In the case of *Achlya deBaryana* I have ('11) recorded the occasional breaking up of the spore mass into scattered groups and the same habit I have often observed in *A. racemosa*, *A. colorata*, and *A. hypogyna*, and in the last the spores are ciliated on emerging. Humphrey in his monograph also demonstrated the presence of cilia on the escaping spores of *Achlya americana*, and says, "In *A. polyandra* one can hardly fail to notice the very marked ciliary motion within the sporangium during the escape of the spores." It will be noted, however, that in none of these cases do any of the spores swim away regularly and under ordinary circumstances when first discharged.

In case of bacterial contamination, or foulness from any cause, or where the parts are put in liquid nutrient media, there is a strong tendency for the spores to be retained in the sporangium, or if discharged for them to sprout at once without a second swimming stage. There has arisen a loose way of speaking of all sporangia when the spores are retained, or even in part retained, as dictiosporangia, a term that should be used only when spores emerge singly through the wall of the sporangium and escape for (what is homologous with) the second swimming stage. As one might expect, there is variation in *Dictyuchus* itself in this respect, the spores frequently sprouting by the *Aplanes* method (see below). Variations in the discharge and behavior of the spores are recorded in the following cases (many others are recorded under the species in this book):

Achlya aplanes Maurizio ('94). The behavior of the spores as described in this case is very peculiar. There is no swimming stage, the spores on emerging sprouting into tubes. Frequently they do not emerge at all, but remain in the sporangium and sprout there. (That these statements hold true regularly under normal conditions must, I think, remain doubtful until the plant is studied by some one else).

Achlya caroliniana Coker ('10). The spores may be retained and sprout as in *Aplanes*, or under certain circumstances may emerge in a motile condition. They may also emerge from several mouths. Later observation by me shows that under certain conditions as on egg yolk in 1 per cent KN_2PO_4 the spores may not stick to the sporangium mouth, but fall to the bottom in open order. This is true also of *A. flagellata* (see our treatment).

Achlya deBaryana Humphrey (*Achlya polyandra* deBary): Coker ('12). Figs. 7 and 8, of plate 78, show reduced sporangia with spores in a single row, the spores emerging exactly as in *Dictyuchus*. They also frequently sprout as in *Aplanes*.

Achlya glomerata Coker ('12a). In fig. 7, plate 79, is shown a sporangium with the spores sprouting as in *Aplanes*.

Achlya polyandra Hildebrand: Ward ('83). In plate 22, fig. 8, is shown a sporangium with the spores emerging just as in *Dictyuchus*. The retention of the spores in this case he was able to bring about by poor aeration, i.e., placing the culture in an air-tight chamber.

Achlya prolifera (Nees) deBary ('52). In plate 7, fig. 28, is shown the sprouting of the spores at the mouth of the sporangium, the second swimming stage omitted. In all the species of *Achlya* that I have studied the second swimming stage may be easily suppressed.

Achlya racemosa Hildebrand: Pringsheim ('73). In plate 22, figs. 1, 2, and 3, are shown sporangia emptying exactly as in *Dictyuchus*. Under the name of *Achlya lignicola*, which I am treating as a variety of *A. racemosa*, Hildebrand figures a sporangium with many of the spores remaining undischarged ('67, pl. 16, fig. 2). In both *A. racemosa* and *A. colorata* the emptying spores hang together imperfectly and often separate into several groups.

Achlya dubia Coker (see p. 135). In this species the sporangia discharge their spores in part as in *Achlya* and in part as in *Thraustotheca*; also not rarely as in *Dictyuchus*.

We have found in *Achlya caroliniana, A. proliferoides, A flagellata* and *A. Klebsiana* that sporangia may open and discharge spores through more than one papilla. This tendency is most distinctly shown in the last species, which see for other peculiarities in above behavior.

Achlya hypogyna. Spores ciliated on emergence and may show a sluggish motion. See p. 102.

Protoachlya and *Isoachlya.* For the behavior of the spores and sporangia in these genera, see our treatment.

Thraustotheca clavata (deBary) Humphrey: Weston ('18, pl. 4, fig. 31) shows the spores sprouting as in *Aplanes.*

Dictyuchus monosporus Leitgeb ('69). In plate 23, fig. 8, Leitgeb shows a sporangium with spores sprouting after the manner of *Aplanes.* This variation I have many times seen in *Dictyuchus sterile,* which is common at Chapel Hill (pl. 52, fig. 4). Weston ('19, pl. 23, fig. 18) shows a similar case in a sterile *Dictyuchus.* He also finds that a spore may in some cases emerge from its cyst in the same form and swim again ("repeated emergence").

Aplanes androgynus (Archer) Humphrey. See our discussion of this species.

Aphanomyces stellatus deBary: Sorokine ('76). In plate 7, figs. 10 and 18, are shown sporangia discharging their spores in the exact manner of *Dictyuchus.* Sorokine also shows sprouting at the mouth of the sporangium, and sporangia with spores in more than one row. See also Humphrey ('92, p. 79) for omission of second swimming stage. We find that spores retained in the sporangium may sprout as in *Aplanes.*

Leptomitus lacteus (Roth) Agardh: Humphrey ('92) says on p. 136: "While the zoospores ordinarily escape from the sporangia, they sometimes become encysted within them (fig. 117). It is this fact, probably, which led Braun to state ('51) that the spores of *Leptomitus* are arranged in a row in the spore cases, and that no active gonidia seem to occur." We have often seen some of the spores retained.

Apodachlya pyrifera Zopf ('88). The spores normally encyst at the mouth of the sporangium and then emerge for a swimming stage as in *Achlya.* However, they may, on occasion, swim away in emerging, or they may encyst in part in the sporangium. In our form of the species it is much more usual for the spores to swim at once on emergence.

Monoblepharis macrandra Woronin ('04). In this species some or all of the spores may be retained in the sporangium and sprout there. Normally the spores on emerging show amoeboid movements.

Saprolegnia asterophora deBary ('60). In plate 20, fig. 25, deBary shows a partly emptied sporangium. the remaining spores sprouting into tubes.

Saprolegnia ferax (Gruith.) Thuret ('50). In plate 22, fig. 8, Thuret shows an unopened sporangium with the spores sprouting in position. This is a good example of the *Aplanes* method. In this species Pringsheim ('73) gives an interesting case (fig. 12, plate 21) of the contents of an egg turning immediately into a sporangium the spores being retained and sprouting in position. In figs. 1a, b, c, plate 20, he shows spores that had been retained in a partly discharged sporangium. These had sprouted in position to short tubes which became sporangia and discharged small spores.

In the case of a parasite on fish that he considers *Saprolegnia ferax*, Smith ('78) gives a figure showing spores sprouting inside the sporangium at one end while others are swimming out at the other. Such a combination is probably fanciful.

Saprolegnia monoica Pringsheim: Huxley ('82) describes the regular occurrence towards the end of active growth of sporangia of the *Aplanes* type. He calls them, improperly, "dictiosporangia." In this plant, which was a parasite on salmon, it is noteworthy that Huxley found no motion in the spores but only a passive drifting about when discharged. In a similar (probably the same) plant, found as a parasite on fish, Unger ('44) gives a figure (fig. 11, plate 1) showing a few spores left in the sporangium and sprouting there into long tubes. In this parasite he records the spores as swimming on leaving the sporangium, not floating away as in Huxley's plant.

Saprolegnia torulosa deBary: Lechmere ('10) illustrates in fig. 33, plate 2, a sporangium with spores sprouting after the manner of *Aplanes*. He later gives ('11, fig. 2) another example. In his first paper he shows that the second swimming stage may be suppressed. DeBary ('84, p. 117) says that the second swimming stage may be omitted in any species of *Saprolegnia*, and this we can confirm for the species studied.

Saprolegnia furcata Maurizio ('99). The spores are frequently retained and sprout as in *Aplanes*.

Saprolegnia sp.?: Pringsheim ('60). In plate 22, fig. 9, is shown a sporangium emptying as in *Dictyuchus*. It is attached to a hypha which also bears a sporangium of the normal *Saprolegnia* type. Miss Collins ('20, figs. 3–11) also illustrates sporangia of both the *Dictyuchus* and *Aplanes* types, or the two combined, in a sterile species of *Saprolegnia*.

In both *Saprolegnia* and *Achlya* it frequently happens that the discharge of the spores is only partial, a few, or even a good many spores being left in the sporangium. These retained spores may emerge from their cysts, as normally, for a second swimming stage, moving about within the sporangium until they find their way out by its mouth, if they ever do. This is shown by Hildebrand ('67) for his *Achlya polyandra*, by Lechmere ('10) for *Saprolegnia torulosa* (?), plates 1 and 2, figs. 22, 23, 30, 31, also in fig. 2 of his 1911 paper, and very strikingly in our form of *A. Klebsiana* (which see). Lechmere erroneously calls this the *Dictyuchus* type of asexual reproduction. It is doubtful if the sterile species of *Saprolegnia* (a parasite on fish) studied by him in his first paper is *Saprolegnia torulosa*. It is more apt to be our *S. parasitica*.

Another peculiar and rare variation in the behavior of the sporangial contents is described and figured by Horn ('04) in a plant he calls *Achlya polyandra* deBary (which may be our *A. imperfecta*). At a temperature of 31° to 32° Celsius, sporangia were formed which emptied large masses of protoplasm through several openings. These masses, then, by direct division formed spores, some of usual size (10µ), some larger (up to 40µ in diameter). If now brought to room temperature these small spores escaped from their cysts and swam. The larger ones germinated directly. Horn also mentions the occasional appearance of double spores from normal sporangia. The discharge of large and irregular masses of protoplasm from the sporangia had been figured long ago by Leitgeb ('69), for *Saprolegnia monoica* as *Diplanes*. In plate 24, fig. 5 he shows several such masses, some with cilia at different points, also several double zoospores. In *Achlya imperfecta* from Chapel Hill, I have observed several times the emptying of the entire protoplasm from a sporangium at the tip, the mass falling at once to the bottom as a long contorted rope. This is conclusive evidence that the spores are discharged by internal pressure and not through their own motion. In numerous other species I have seen masses of protoplasm much larger than spores discharged from the sporangium and these may have several sets of cilia (pl. 1, fig. 12; pl. 7, fig. 1; pl. 39, fig. 4; pl. 60, figs. 12 and 14).

It will, of course, be understood that the variations reviewed above are in no sense fortuitous or accidental. They are the results of environmental conditions and many of them may now be induced at will by the investigator.

In this connection I feel it necessary to give a word of caution against the attitude adopted by Lechmere in his two papers in the New Phytologist, both of which are referred to above. In the summary of his first paper he says: "As the result of keeping a species of *Saprolegnia* under observation for a period of five months it has been found possible to obtain on the same mycelium the methods of asexual reproduction which are characteristic of six different genera." If this claim is examined it will be seen that outside of its own genus (*Saprolegnia*) the species he describes cannot with accuracy be said to show the methods of asexual reproduction of any other genera except *Aplanes* and *Leptolegnia*, and even in these cases only in certain details, not in all. The sporangial variations cited do not look like the sporangia of the genera in question and neither do the spores within them, and no one familiar with these genera would be misled into placing them there unless one's attention be focused on the wording of keys rather than on the plants themselves. Such variations as these do not, as Lechmere implies, create doubt of the validity of the presently accepted classification of the *Saprolegniaceae*.

The egg structure falls into two main types. In all cases the fatty reserve is on or near the periphery, but in one type it is in the form of small droplets entirely surrounding the protoplasm, while in the other it is collected into one or a few larger drops on one side. The first of these types is called centric, the second eccentric, but intergrading types occur which connect the two extremes, and for certain of these I have found it useful to introduce the word subcentric. The three terms may be defined as follows:

A *centric* egg has one or two layers of small fat droplets entirely surrounding the central protoplasm.

A *subcentric* egg has the protoplasm surrounded by one layer of droplets on one side and two or three layers on the other, or rarely with the droplets entirely lacking on part of one side as in *Achlya oblongata*; this last condition connecting directly with such eccentric structure as is shown by *Pythiopsis cymosa*.

An *eccentric* egg has one large drop on one side either outside the protoplasmic surface or barely enclosed by a thin layer of protoplasm, or several large drops enclosed in the protoplasm on one side, or a lunate row of small drops (in optical sections) on one side, as in *Pythiopsis cymosa*.

The egg structure is of much systematic significance. It is always the same in a given species and is often the same throughout an accepted genus, or else in groups of obviously related species within a genus.

Since Humphrey's review of the cytology of spore formation there is nothing of importance to add, except the work by Rothert referred to above and the careful study by Weston of the exact shape of the spores in *Achlya* ('17), *Thraustotheca* ('18) and *Dictyuchus* ('19). The cytology of the vegetative threads of *Saprolegnia* and *Achlya* has, on the other hand, received considerable attention from Dangeard ('16), Meyer ('04), Randolph ('12) and Guilliermond ('20, '20b). These authors find this material excellent for the study of the minute bodies known as chondriosomes (chondriomes, chondriokonts, mitochondria). The last named author has also studied carefully the origin of the vacuoles in *Saprolegnia* and as he has given much attention to these subjects in a number of well balanced studies it will be of interest to give some of his conclusions in a recent summary of his results ('20), as follows (translated and adapted):

"With the help of vital stains one may see that in very young filaments the system of vacuoles is composed of long little canals that anastomose in a network which by swelling and fusion finally form a large vacuole, a sort of large canal occupying the whole axis of the filament. The little canals and the network are filled with a substance in solution that stains quite strongly. When the central canal is formed this substance, being more diluted, stains in a much paler manner. The system of vacuoles also contains, except in the initial stages, bodies that stain in a more marked manner than the substance dissolved in the vacuolary sap. Some show only after vital staining; they are strongly stained and in a very metachromatic manner. The others appear in the form of corpuscles, often quite large, with a refraction quite marked and visible without staining. Osmic acid gives them a slightly gray tint. The vital stains color them only feebly. After fixing in alcohol or formalin and staining with methyl blue the system of vacuoles does not show the characters shown in the living state. The vacuoles are uncolored except for *only* little grains, some strongly colored with deep violet and others tinted with pale blue. Haematoxylin does not color the first and gives to the second a diffuse tint. These two groups of corpuscles, which are perhaps only different states of the same substance, do not show the characters of the granules drawn in the fungi under the name of metachromatic bodies.

"The employment of appropriate methods allows one to obtain excellent differentiation of the chondriosome which appears in the same manner that it showed when living. These methods do not stain the contents of the system of vacuoles except in exceptional cases, when the preparation has turned out badly. The fixations of commercial formalin followed by staining with iron haematoxylin put in evidence with the greatest clearness the nuclei and the chondriosomes. The methods of Benda and of Kull differentiate also the fatty granules which appear brown with osmic acid. With all these methods the chondriosome appears to be made up of numerous particles (chondriokonts) of variable length, which are differentiated very clearly by their intense coloration from the cytoplasm of very little color. These elements have a form so clear that it is sometimes difficult to distinguish them from bacteria that are sometimes found collected on the wall of the fungus. In certain filaments these chondriokonts undergo certain modifications of form which also show in the living material; they can take the form of short, thick-set clubs, of spindles, and even be transformed into vesicles. It is difficult to decide whether these forms are in relation to the cellular metabolism or represent alterations of the chondriosomes. The chondriosomes show a greater resistance to the fixatives containing acetic acid than similar bodies in general and than those of the other fungi in particular." To summarize, Guilliermond finds in the *Saprolegnia* studied: 1st, chondriosomes quite characteristic and comparable to those of the other plants and animals; 2nd, little fatty globules; 3rd, a system of vacuoles filled with a substance endowed with selective power against the vital stains, but which has not the characters of metachromatine.

Aside from the dates and places of collection given incidentally and usually in meager numbers by systematic writers, little is known of the occurrence, periodicity and environmental factors involved in the growth of the *Saprolegniaceae*. The one paper of importance dealing with these subjects is by Petersen in his treatment of the Danish water molds ('10, p. 504). His valuable notes are in most cases in agreement with our experience. Certain differences are found and to be expected, due to the cold climate of Denmark, as that the period of the *Saprolegniaceae* begins in the spring and generally closes in November. With us there is no closed season and we find water molds wherever the water is open any day in winter. In Denmark *Apodachlya* is common, while *Leptomitus* is not recorded by Petersen. In Chapel Hill *Leptomitus* is very common, while *Apodachlya* has been found but once.

Our experience clearly shows that vegetative threads and gemmae are in all species killed by drying up, and that mature eggs may resist drying. This is also the conclusion of Petersen. He makes the interesting statement that while efforts to secure cultures from dry material in many cases failed, yet growth had been obtained from material which had been in a dried state for several years. As regards cold, Petersen thinks that freezing for a short time need not have a deadly effect on the mycelium, but that freezing for a long time is absolutely destructive. We find that in all but one of the species (*Pythiopsis cymosa*) we have tested, a culture allowed to freeze solidly over night is all killed except the eggs. We also find, with Petersen, that darkness has no bad effect on laboratory cultures. The greater abundance of individuals or species in open sunny marshes and ditches is probably due to the large amount of other organic life in such places.

As to parasitism, of which much remains unknown, Petersen finds that fish are little attacked in natural conditions in Denmark and then only as a result of previous injury. He seems to think that frog eggs may be attacked in the living condition. We have often seen eggs of frogs and salamanders overgrown with water molds, but we have no proof that these plants were the actual cause of the death of the eggs. Petersen also announces that *Leptolegnia caudata* is the fungus which attacks and kills great numbers of the crustacean *Leptodora* in his country. We have not found it a parasite here.

In order to throw light if possible on the seasonal distribution of the different species we have made up the table that follows this page.

Between February 15, 1912, and December 15, 1913, the number of collections made from all sources around and in Chapel Hill was 593. A record of the species found in each collection was kept and from these records and the number and date of the collections the table was drawn up. It shows the per cent of all collections in which each species occurred for each month, for spring, summer, fall and winter; for the six warmest months (April–September inclusive) and the six coolest months, and finally the per cent of the whole number of collections in which the species was found compared to the total number of collections made in the entire period. For example, *Achlya apiculata* was found 89 times in 593 collections or in 15% of all collections. It was found 33 times in 138 collections made in March, or in 23.9% of the March collections. The number of collections made in each month varied considerably, for example, 24 collections were made in July and 138 in March. While this does not interfere with accuracy of the percentages, it does give opportunity for a larger variation of percentages in months with fewest collections, because of the various accidental factors involved in collecting. That is, the larger the number of collections the less the percentage of error in the figure showing the occurrence of a certain species at a certain place for a certain time. As would be expected, the number of collections in warm months was less than in the cool months, due to the absence of members of the staff on holidays. There were 237 collections made in the six warm months and 356 in the six cool months. Since 1913 we have continued steadily at work on this group, but have kept no exact data as to the number of collections, which will easily run into several thousands.

It will be noted that *Achlya flagellata* and *A. proliferoides* have been run together in the table. This is due to the fact that the two species were not distinguished for some time after the records began and went in

PERIODICITY TABLE

Showing Percent of Occurrence of the Species Noted in 593 Collections made from Feb. 15, 1912, to Dec. 12, 1913. For full Explanation see p. 13

Species	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Winter	Spring	Summer	Fall	Warm	Cool	% of all
Pythiopsis cymosa	5.2	0	0	0	0	0	0	0	0	0	0	4.0	2.6	0	0	0	0	1.2	.7
Pythiopsis Humphreyana	0	7.0	1.4	0	1.6	0	0	0	0	0	0	0	3.2	1.0	19.2	0	0.4	2.0	1.3
Saprolegnia diclina	1.7	1.4	3.6	19.8	15.9	40.0	12.5	21.7	10.0	19.0	29.4	0	1.3	11.3	19.2	18.9	17.0	6.1	10.6
Saprolegnia ferax	0	5.6	25.4	19.8	22.2	0.	0	0	0	0	29.4	4.0	2.6	23.1	0	1.1	13.0	13.5	12.3
Saprolegnia monoica var.	32.8	38.0	21.7	12.1	15.9	0	8.3	8.7	0	19.0	8.8	68.0	40.9	17.5	7.7	7.4	10.2	28.8	21.1
Isoachlya monilifera	1.7	8.5	13.0	0	0	0	0	0	0	0	0	0	4.6	6.1	0	0	0	7.2	4.2
Protoachlya paradoxa	1.7	4.2	4.4	4.4	20.6	0	0	0	0	0	0	0	2.6	7.9	0.	0	6.9	2.9	4.4
Achlya hypogyna	0	0	0	0	9.5	40.0	4.2	4.4	10.0	4.8	29.4	0	0	2.1	7.7	15.8	5.7	3.2	4.2
Achlya racemosa	5.2	1.4	1.3	0	0	0	0	0	0	0	0	0	2.6	6.2	0	0	0	6.4	3.7
Achlya colorata	0	7.0	8.7	5.5	1.6	0	0	0	0	0	2.9	0	3.2	6.2	0	1.1	2.4	5.2	4.0
Achlya flagellata and A. proliferoides	12.1	28.2	26.8	46.2	31.7	0	29.1	43.5	50.0	28.6	11.8	0	17.5	33.9	32.7	31.6	40.2	21.3	29.2
Achlya caroliniana	0	1.4	1.4	1.1	0	0	0	0	0	0	0	0	0.6	1.0	0	0	0.4	0.9	0.7
Achlya apiculata	24.1	9.9	23.9	25.3	3.2	0	0	0	0	4.8	2.9	3.2	18.8	19.9	0	2.1	10.2	18.4	15.0
Achlya glomerata	0	1.4	0.7	1.1	3.2	0	0	0	0	0	0	0	0.6	1.4	0	0	1.2	0.6	0.8
Thraustotheca clavata	1.7	0	0.7	1.1	4.9	0	0	0	0	0	0	0	0.6	1.7	0	0	1.6	0.6	1.0
Dictyuchus sterile	5.2	8.5	3.6	12.1	15.9	20.0	12.5	26.1	5.0	33.3	8.8	1.2	7.8	8.9	19.2	12.6	13.1	7.8	10.1
Leptolegnia caudata	1.7	0	0.7	0	0	0	4.2	0	2.5	0	0	0	0.6	0.3	1.9	1.0	0.8	0.6	0.7
Aphanomyces (three species)	20.7	14.1	10.9	25.3	31.7	20.0	37.5	39.1	57.5	61.9	38.2	20.0	17.5	19.8	36.5	51.6	34.6	19.6	25.8
Leptomitus lacteus	1.7	0	7.2	0	0	0	0	0	0	0	0	0	0.6	3.1	0	0	0	3.2	1.9

as one. Their records were, therefore, continued as one. In *Aphanomyces* the species occurring in Chapel Hill, except *A. parasitica*, have all been combined, as the genus may be quickly determined by the sporangia, while the species in two cases are often to be settled only after a series of cultures to secure the reluctant production of oogonia.

A table like this can be only suggestive and proves nothing rigidly except the relative abundance of the species at different seasons in any one place. This may be shown by taking the case of *Leptomitus*. It would seem from this table to be a rare species in Chapel Hill, whereas it may be had any day by collecting in streams containing sewage. This point makes a word necessary as to where the collections were made. In order to make the record mean as much as possible, the collections were regularly made in a series of stations of considerable variety and the great majority of collections were made in the same series of stations. The usual series were several collections in the Arboretum spring and branch, continuing into Battle's branch; several in Terra Cotta spring and the pool below on Glen Burnie farm; several in the seepy marsh at the foot of Lone Pine hill on the same farm, one collection occasionally in Lone Pine spring, and several collections in the sedgy marsh in front of the cemetery. Not rarely collections were made in Howell's spring and branch and its branches, and scattering collections were made on occasion in many other places, as Bowlin's Creek and Morgan's Creek, New Hope Creek, various springs, marshes, troughs, etc.

When we analyze the table we see that for the great majority of species spring is the most favorable season for growth. There are eleven species which were found in a greater per cent of the collections in spring, three in winter, three in summer and two in fall. But if we compare the six cold and the six warm months we find little or no difference, ten appearing more frequently in the warm and nine in the cold months.

The present volume is concerned primarily with the *Saprolegniaceae*, but we have noted the genera and species of the other families of the order *Saprolegniales* and have treated in some detail all species in this order that we have found in North Carolina. All are small saprophytic or parasitic fungi with an obvious mycelium growing in water. We have also added such fungus parasites of the water molds as have appeared in our collections. In regard to the culture media, when the word "ant" is used it refers to the adult worker of the small white termite that is common in old logs and stumps. The "spring water" mentioned in many notes was taken from the spring in the Arboretum of the University.

All the drawings have been made by camera lucida and nearly all from living material. The author is responsible for the photographs and for the majority of the drawings, but many of the more recent ones have been made by Mr. Couch. Plates 24 and 59 were prepared by Mr. F. A. Grant, former Assistant in Botany. Miss Alma Holland, Assistant in Botany, has inked in nearly all of the plates.

Key to the Families of the Saprolegniales

Threads of the plant (mycelium) not constricted into joints at intervals; oogonia containing one or, more often, several to numerous eggs in the formation of which all the protoplasm of the oogonium is used; eggs always smooth, not completely filling the oogonium except in *Leptolegnia;* antheridia present in most species, but even when present fertilization is not always effected; asexual spores biciliate, diplanetic or monoplanetic Fam. *Saprolegniaceae* (p. 17)

Plant often with a distinct stalk, the threads constricted at intervals into joints, which in the vegetative region are usually connected by small channels through the nodes; oogonia and antheridia present in most species, the egg always single and with periplasm, often with a sculptured surface; spores monoplanetic.... Fam. *Leptomitaceae* (p. 169)

Plant with a distinct enlarged stalk or, if the base is not distinctly differentiated, then branched in a dichotomous or verticillate way; peculiar resting cells present which are probably parthenogenetic eggs. These have thick, brown, distinctly pitted walls and completely or almost completely fill the thin-walled oogonium, out of which they often slip at maturity. Antheridia absent. Spores with one (or two?) cilia, monoplanetic. In one genus (*Allomyces*) the plant body is divided into cells by cross walls, a condition unknown in any other member of the order.................. Fam. *Blastocladiaceae* (p. 180)

Plant slender, branched, without a distinct stalk; not constricted into joints; egg single, usually sculptured, in some species ripening outside the oogonium, fertilized by an *active sperm* with one cilium. Spores uniciliate, monoplanetic. The family is by some authors placed in an order of its own. It is distinguished from all other fungi by the retention of an active sperm.............................. Fam. *Monoblepharidaceae* p. 178)

SAPROLEGNIACEAE

Key to the Genera

1. Sporangia rare or absent; oogonia with very thick pitted walls, the antheridia arising from immediately below them and running up their sides.............*Aplanes* (p. 76)
1. Not as above..2
2. Spores normally leaving the sporangium by a common mouth.......................3
2. Spores not leaving the sporangium by a common mouth (see also *Achlya dubia*).....8
3. Spores all (normally) swarming separately on escaping from the sporangium.........4
3. Spores all collecting in a hollow sphere or an irregular group at the mouth of the sporangium on escaping...7
3. Part of the spores on emerging swimming away or sluggishly jerking away and encysting separately from the remainder, which stop at the sporangium mouth. Sporangia rounded at the tip and not tapering, in great part proliferating cymosely as in *Achlya*, but at times threads may grow through empty sporangia as in *Saprolegnia*. Eggs centric..*Protoachlya* (p. 90)
4. Sporangia not thicker than the vegetative hyphae; zoospores in a single row *Leptolegnia* (p. 157)
4. Sporangia usually thicker than the hyphae; zoospores not in a single row..........5
5. New sporangia formed within the empty ones....................*Saprolegnia* (p. 22)
5. New sporangia formed in greater part by cymose branching........................6
6. Antheridia on every oogonium, androgynous.......................*Pythiopsis* (p. 17)
6. Antheridia absent, or on less than half the oogonia, diclinous........ *Isoachlya* (p. 81)
7. Sporangia usually thicker than the vegetative hyphae; zoospores not in a single row *Achlya* (p. 95)
7. Sporangia not thicker than the vegetative hyphae; zoospores in a single row *Aphanomyces* (p. 160)
8. Spores encysting within the sporangium, then emerging separately through the sporangium wall and swarming.................................*Dictyuchus* (p. 150)
8. Spores set free by the breaking up of the sporangial wall..........*Thraustotheca* (p. 148)

PYTHIOPSIS de Bary, 1888, p. 609.

Hyphae slender, much or little branched. Sporangia typically short and plump, spherical, oval, pyriform with a distinct apical papilla, or varying to elongated and irregular, primarily borne at the tips of the hyphae and multiplied from lateral stalks below the old ones to form more or less dense clusters. Spores emerging and swimming as in *Saprolegnia*, pip-shaped with two apical cilia, sprouting after the first encystment (monoplanetic). Gemmae resembling the sporangia or oogonia, formed plentifully, often in chains, producing zoospores after a rest. Oogonia borne like the sporangia and gemmae and resembling them in youth, typically spherical, oval or pyriform with unpitted walls, smooth or with a few blunt papillae. Antheridia short and thick, typically androgynous from the close neighborhood of the oogonia. Eggs one or few, eccentric, with a lunate cap of droplets on one side in *P. cymosa;* structure doubtful in *P. Humphreyana.*

Key to the Species

Sporangia globular or clavate; oogonia sometimes with a few blunt outgrowths; egg single, 14.8–18.5μ thick.. *P. cymosa* (1)

Sporangia occasionally elongated; oogonia always smooth; eggs generally one, occasionally 2, and very rarely 4, about 30μ thick........................ *P. Humphreyana* (2)

1. **Pythiopsis cymosa** deBary. Bot. Zeit. **46**: 631, pl. 9, fig. 1. 1888.

Plate 1

Hyphae slender, 14.8–22.5μ in diameter at base, short or moderately long. Sporangia globular or clavate. Spores 8.6–10.8μ, most about 9μ; monoplanetic. Oogonia plentifully formed in old cultures, spherical to oblong or pear-shaped, unpitted, smooth, or sometimes with a few blunt outgrowths, terminal or rarely intercalary, 18–30μ in diameter, a few smaller. Eggs mostly 14.8–18.5μ in diameter, but sometimes up to 24μ, single (Humphrey says rarely two to an oogonium), eccentric, as described above. Antheridial branches short or none, usually arising from just below the basal walls of the oogonia, rarely diclinous. Antheridia one or two to each oogonium, clavate; antheridial tubes present, at times growing up through the basal wall of the oogonium. Gemmae of more or less globular or ovoid shape are formed in quantity and are often arranged in chains. After a rest these also form zoospores.

Rather rare in springs and branches, as Terra Cotta spring, branch in Pritchard's pasture, Glen Burnie meadow, etc. Collected seven times, and all in winter. Heretofore reported from America only by Humphrey, from Amherst, Mass. See his plate 17, figs. 60–68; also Minden ('12), figs. 3a, 3b on p. 556.

The species may be easily distinguished from *P. Humphreyana* by smaller oogonia with sometimes a few blunt outgrowths, smaller eggs, smaller sporangia, presence of basal antheridia, and absence of elongated forms of sporangia. See Mycologia **6**: 285, pl. 147, 1914, from which the figures and most of the following notes are taken:

The sporangia, oogonia and antheridia are well shown by deBary and Humphrey, but variations occur that were not observed by them. The antheridial cells, as formed in about one-half the oogonia, are unique in position. They arise by the enlargement of the hypha immediately below the oogonium and the growth of this segment along the base of the oogonium for a short distance. A tube is formed near the septum and enters to the egg. As the antheridial cell is in close contact with the oogonial wall from the septum out, the position of the septum be-

PLATE 1

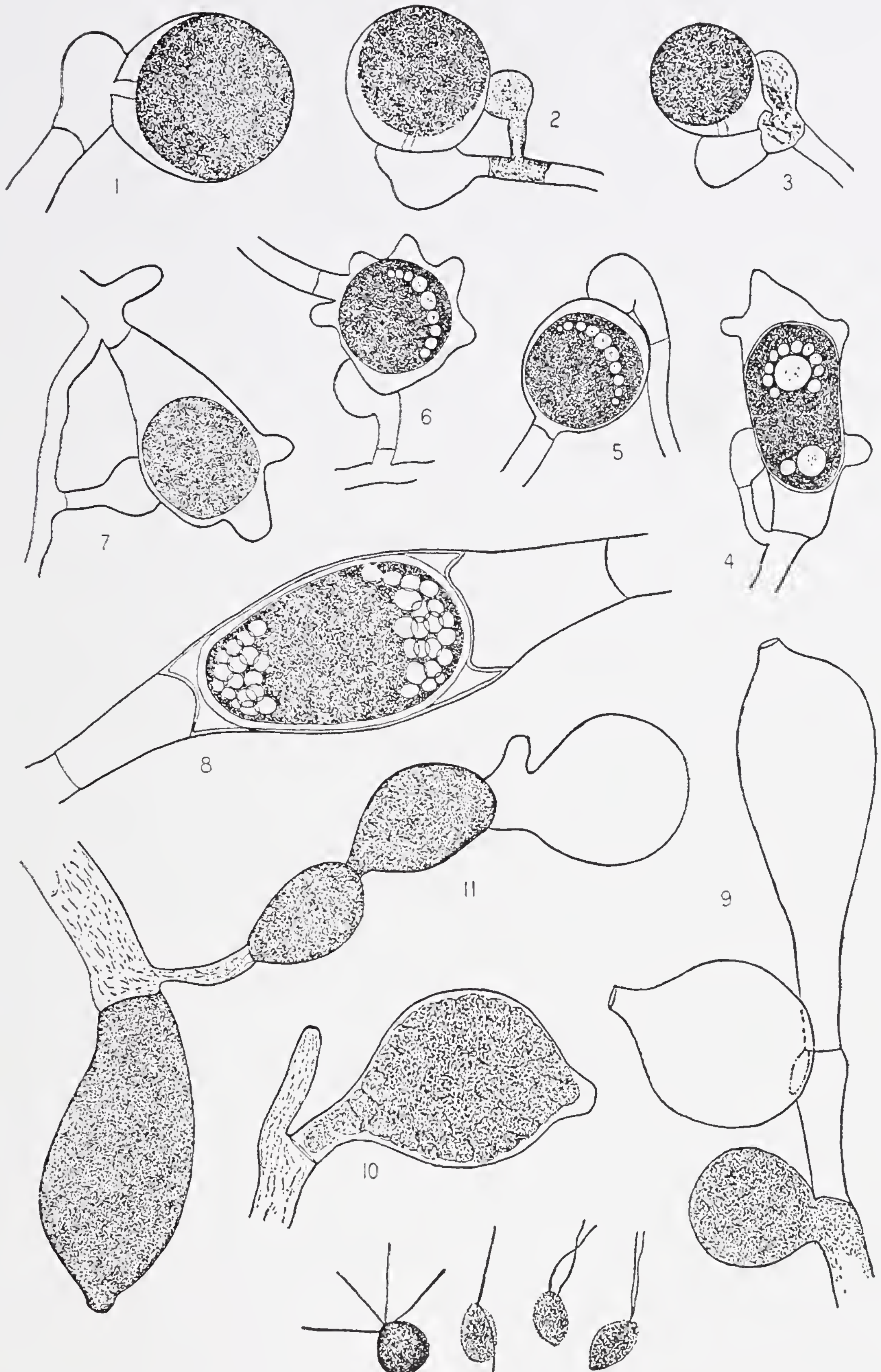
PLATE 1

(From Mycologia **6**: Pl. 147. 1914)

Pythiopsis cymosa

Fig. 1. Oogonium with a typical sub-basal antheridium. × 720.
Fig. 2. Ditto, with an additional lateral antheridium. × 720.
Fig. 3. Ditto, with two sub-basal antheridia. × 720.
Figs. 4, 5, 6 and 7. Oogonia of various forms with antheridia of various origin. × 720.
Fig. 8. An abnormal double oogonium, apparently without an antheridium. × 720.
Fig. 9. Sporangia of typical shapes. × 447.
Fig. 10. Sporangia about one minute before discharge showing the spores in the compression stage. × 447.
Fig. 11. Gemmae; one having discharged spores by a basal papilla. × 447.
Fig. 12. Spores, killed while swimming: one with four cilia and double size. × 720.

PLATE 1



PYTHIOPSIS CYMOSA.

comes obscured and the oogonium seems to be seated at maturity on a large, swollen, basal cell. Under high power, however, the original septum may be seen as a somewhat thicker disc. This form of antheridium, as shown in fig. 1 and in one of the two in fig. 2, is not exactly illustrated in either deBary's or Humphrey's figures. From this strictly basal and closely pressed antheridium we have in the remaining half of the oogonia all sorts of variations. The antheridium may be elevated on a stalk that varies from nothing to half the length of the oogonium and in very rare cases the antheridium may be even of diclinous origin (figs. 5 and 6). The appearance of several antheridia on one oogonium is of rather frequent occurrence in our cultures. This is not recorded by deBary or Humphrey. From figures 3 to 7 an idea may be gained of the variations observable in both antheridia and oogonia. DeBary does not give the size of the eggs. We find them to vary from 14.8 to 24μ, with an average of about 19.5μ. This is a little larger than the figures given by Humphrey.

The remarkable intercalary oogonium shown in fig. 8 is unique. Its single egg was 27.8 by 50μ in size and a large number of oil drops were grouped at each end. An antheridial cell was also cut off at each end, but no antheridium could be made out.

The peculiar jelly-like outer layer that deBary noticed on the oogonia in October cultures was also seen by Humphrey in a few cases. By careful observation we have been able to make out this layer in the majority of young oogonia. It is probably present on all at a certain stage, but in clean cultures free from bacteria is very hard to trace. Its presence and outline is hardly discernible, except for the bacteria and other minute particles that stick to it. As remarked by Humphrey, it is hardly possible that this hyaline gelatinous outer sheath can be a "periplasm" secreted from the oogonium contents, as deBary suggests. It is more apt to be due to the gelatinization of a thin outer layer of the wall of the oogonium.

In a typical clean culture in spring water on a mushroom grub the sporangia varied from 37 to 56μ in diameter, the majority being from 44 to 48μ broad.

In figures 9 and 10 are shown sporangia of usual appearance. When the sporangia proceed at once to the formation of spores the discharge is usually at the tip (fig. 9). If a rest occurs, the immergence tube is as apt to appear at the base, as shown in fig. 11. After the first sudden release of pressure the spores do not rush out as in *Achlya* and *Saprolegnia*, but emerge much more quietly as they find the opening. The last ones often swim around a long time in the sporangium before finding an exit. The spores are pear-shaped, with two cilia at the small end.

On coming to rest they round up. In fig. 12 are shown three normal spores and an anomalous double one with four cilia. This is not a case of fusion after emergence, but of imperfect segmentation of the protoplasm. (See remarks on page 9).

Cultures on various media gave the following results:

On corn meal agar. Formed plentiful gemmae, mostly in groups, but no oogonia.

On boiled butter bean, on white of egg, and on termites. Showed strong growth, a great many gemmae, and after some time numerous oogonia.

On boiled willow twig. Showed moderate growth and a few oogonia.

A culture in pea agar was left outside on the night of January 14, 1917. It was frozen solid, but was not killed.

The following experiments were made to determine the best method of preserving live cultures:

A culture on a grub was placed in distilled water in the spring of 1917, but when tested the following September was found to be dead.

A culture on corn meal agar was placed in a vial March 18, 1913, and was found to be dead when tested December 1, 1913.

2. **Pythiopsis Humphreyana** Coker.* Mycologia **6**: 292, pl. 148. 1914.

PLATE 2

Vegetative growth of long, slender, sparingly branched hyphae about 11–14μ thick which are stoutest in the neighborhood of the reproductive bodies, and which after maturity disorganize rather quickly. Sporangia varying in shape from spherical, oval or pyriform to elongated, tapering and irregular forms, discharging by a short or rather long papilla and usually proliferating from below in a cymose manner; spores monoplanetic, pear-shaped and with two cilia, about 8.9μ in diameter on coming to rest. Oogonia generally borne exactly like the sporangia and not to be distinguished from these when young, apical and often in groups by cymose branching, usually spherical with a basal neck, sometimes pear-shaped and rarely longer and more irregular, varying greatly in size, diameter from 33 to 89μ, averaging about 43μ; wall always smooth and unpitted, about 1.4μ thick. Eggs generally one, occasionally two and very rarely four, centric,† diameter from 24 to 40μ, averaging about 30μ, the wall about 2μ thick, not so nearly filling the oogonium as in *P. cymosa;* antheridia short-clavate, terminating a stalk that usually arises from immediately below the oogonium, but sometimes of more distant origin, or rarely diclinous, one, two or occasionally more on every oogonium and generally applied to its top or distal half, with an antheridial tube which reaches and apparently fer-

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*Most of the data given herewith is taken from the original description, and all the drawings except two were published there also.

†They were reported as centric in the original description, but we are not now sure of this. Slides fail to show this character clearly.

PLATE 2

PLATE 2

(Figs. 3–16 taken from Mycologia **6**: Pl. 148. 1914)

PYTHIOPSIS HUMPHREYANA

Fig. 1. A cluster of oogonia some with diclinous, others with androgynous antheridia. × 370.

Fig. 2. Oogonia borne racemosely with androgynous antheridia. × 370.

Fig. 3. Sporangia of the globular form. × 335.

Fig. 4. Two sporangia with an oogonium and antheridium. × 335.

Figs. 5–10. Sporangia of various forms. Nos. 3 and 4 × 185; others × 125.

Fig. 11. An oogonium with two eggs and two antheridia, one of which is diclinous. × 335.

Fig. 12. A young oogonium with antheridium. × 335.

Fig. 13. Two sporangia with oogonia in close proximity. × 185.

Fig. 14. A sporangium surrounded by several oogonia. × 185.

Fig. 15. An oogonium with a diclinous antheridium, showing plainly the antheridial tube. × 335.

Fig. 16. An abnormal oogonium with four eggs. × 335.

PYTHIOPSIS HUMPHREYANA.

tilizes the egg. Gemmae resembling sporangia or oogonia are present in quantity, and after a rest form spores or germinate with tubes.

Rather rare: occurring only eight times in our numerous collections, as in brook behind Dr. Henderson's residence, February 29, 1912; on south side Glen Burnie meadow, March 18, 1912; in brook in Battle's Park, March 25, 1912; in branch south of campus, May 13, 1912, etc.

The species is sharply separated from *P. cymosa* by the much larger and always smooth oogonia, larger eggs, larger sporangia, absence of strictly basal antheridia and presence of elongated forms of sporangia. Illustrations of the globular type of sporangia, which are the first to appear in clean and vigorous cultures, are given in figures 3, 4, and 6. They are of the same appearance as those of *P. cymosa*. The papilla is usually formed at the tip when growth is active, but if there is a rest it is as apt to be formed at any other point (figs. 4 and 6). Intermediate and elongated forms are shown in figs. 5–10 and 13. As in *P. cymosa* the internal pressure is dissipated before the last spores emerge and it is often many minutes before all find the exit. As shown in the figures, the papilla may be quite abrupt or may gradually taper into the body of the sporangium.

The oogonia are often closely associated with the sporangia (figs. 13 and 14), but the more common arrangement is a terminal oogonium on a rather short lateral branch, as shown in fig. 2, with a single stalked antheridium arising from immediately below it. The antheridial branch almost invariably carries but a single antheridium, which is short, thick and densely filled with protoplasm. The antheridial tube is quite conspicuous and its behavior is such that there is scarcely any doubt that fertilization takes place. The protoplasm of the antheridium passes into the tube and soon afterward no protoplasm or tube can be seen, indicating the discharge of the former and collapse of the very thin-walled tube. The tubes are distinctly shown in figs. 13–15.

Oogonia with two eggs are not very rare. One of these with two antheridia is shown in fig. 11. Oogonia with four eggs were seen twice. One of these, of anomalous shape, is shown in fig. 16. The occurrence of more than one egg in the oogonium of *P. cymosa* is quite rare. Humphrey saw two eggs only once and our cultures of that species have not produced any such oogonia. DeBary says that as many as three eggs may occur in *P. cymosa* but their appearance is evidently of great rarity.

SAPROLEGNIA Nees v. Esenbeck, 1823, p. 513.

Saprophytic on animal or plant remains, or parasitic in some species on aquatic animals as fish, frog eggs, etc. Exposed hyphae branched or more or less simple, straight or crooked, usually tapering gradually outward, more or less pointed, springing from an intricately branched, in part rhizoid-like mycelium within the substratum; all vegetative parts colorless in transmitted light, white in reflected light, the threads not septate or constricted until the approach of reproductive stages. Sporangia at first terminal on main threads, typically long-clavate and thicker toward the distal end, or at times slender-fusiform, often irregular and polymorphic in older cultures; at maturity opening typically by an apical mouth, the spores emerging rapidly one by one through pressure from within; typically proliferating within the older ones in a "nested" fashion, but often also as in *Achlya* (see, for example, p. 26). Spores pip-shaped, with two apical cilia, swimming away as soon as discharged, soon coming to rest and encysting in spherical form; after a few hours emerging again through a minute opening in the cyst and swimming again more actively in a somewhat kidney-shaped form with two lateral cilia, finally coming to rest on a nutrient substratum (if such is available) and sending into it a slender tube which grows and branches into the extensive mycelium within. Resting bodies, called gemmae or chlamydospores, of very variable shape and size formed in greater or less number; often in chains like beads; after resting for a few days the contents producing spores of the usual type which emerge by a variously formed mouth. Oogonia terminal on main threads or on long or short lateral branches, or in some species intercalary singly or in chains; shape spherical or oval or pyriform or when intercalary sometimes fusiform; wall smooth or papillate, often pitted. Eggs one or many in an oogonium, formed of all its contents, but never completely filling it, smooth, the protoplasm entirely surrounded by one or two layers of fatty food material (centric or subcentric); undergoing a resting period before sprouting. Antheridia present or absent, of various origin and appearance, usually terminating slender antheridial branches which are short or long, simple or branched, and originating from the same threads on which the oogonia they reach are borne (androgynous), or from other threads (diclinous); antheridia when present often forming one or more slender tubes which enter the oogonia through thin places and reach the eggs. Fertilization has been shown to take place in *S. diclina* and *S. mixta* by Trow and in *S. monoica* by Claussen (see these species for details). For a good account of spore development in *S. ferax* see Rothert (1888).

Natural Key to the Species*

Sub-genus Eusaprolegnia: Eggs averaging more than two in an oogonium, truly centric, with a single layer of oil drops completely surrounding the protoplasm, or subcentric, with two rows on one side and one on the other; oogonia with or without pits, smooth or (in one species) with a few warts on some of the oogonia

Diclina Group: Oogonia not in chains or if so not tending to separate at maturity; their walls not thick and the pits, if present, not very conspicuous; antheridia present, their supporting branches at least in part long and branched

1. Antheridia present on about a third of the oogonia, diclinous.. *S. crustosa* var. III (p. 70)
1. Antheridia present on all or nearly all the oogonia.......................... 2
2. Zoospores less than 20μ thick.. 3
2. Zoospores more than 20μ thick.. 6
3. Antheridia all or mostly diclinous.. 4
3. Antheridia all or mostly androgynous.. 5
4. Spores not of two kinds
   Oogonial wall with few pits; eggs 12–22μ thick......*S. crustosa* var. I (p. 69)
   Oogonial wall with few pits; eggs 17–27μ thick......*S. crustosa* var. II (p. 69)
   Oogonial walls without pits (except where the antheridia touch); eggs 20–26μ thick...*S. diclina* (1)
   As above, but eggs averaging 30μ thick...................*S. Kauffmaniana* (2)
   Oogonial wall with numerous pits; eggs 12–22.5μ thick.......*S. stagnalis* (p. 70)
   Oogonial wall with less numerous pits; most of the eggs 25–27μ thick..*S. delica* (3)
4. Spores of two kinds, large and small..........................*S. anisospora* (4)
5. Oogonial stalks bent and often coiled; oogonial wall unpitted or the pits few and inconspicuous.......................................{ *S. spiralis* (p. 71) / *S. furcata* (p. 72) }
6. Oogonia borne inside the host..............................*S. curvata* (p. 72)

Ferax Group: Oogonia not in chains, or if so not separating at maturity; their walls thick and usually with conspicuous pits; antheridia at times absent, but usually present on a varying number of the oogonia; their supporting branches short, but not arising from immediately below the oogonia. In *S. parasitica* which probably belongs in this group the oogonia are absent.

Antheridia not on all the oogonia (rarely over 50%), at times absent; androgynous and diclinous
   Antheridia usually on not more than 15% of the oogonia, often very few or none; oogonia often found in old sporangia and then cylindrical; eggs commonly about 26μ thick....................................*S. ferax* (5)
   Antheridia absent; oogonia never or rarely cylindrical, eggs usually 21–24μ thick.. *S. lapponica* (p. 73)
   (See also *S. esocina* (p. 41)
   Antheridia usually on about half the oogonia which are never cylindrical; eggs usually 24-25μ thick....................................*S. mixta* (6)
   As above but eggs usually 16–19μ thick...... *S. mixta* var. *Asplundii* (p. 74)
   Antheridia on about 75% of the oogonia..................*S. floccosa* (p. 74)

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* The absence of oogonia in *S. parasitica* makes its position doubtful. For an artificial key see below. American species are followed by a number, others by page reference.

Antheridia on all or nearly all the oogonia; androgynous or rarely diclinous

Oogonia commonly on short, lateral branches, spherical with a neck, eggs usually five or more in an oogonium, 16–22μ thick .... *S. monoica* (7)

As in *S. monoica*, except that the eggs are usually less than five in an oogonium and mostly 25–27μ thick.................... *S. monoica* var. *glomerata* (8)

As in *S. monoica*, but no oogonia produced except in a solution of lucin and levulose.. *S. monoica* var. *vexans* (9)

As in *S. monoica* except that the oogonia have longer stalks and few or no pits, and that the vegetative threads are longer and more slender *S. monoica* var. *montana* (p. 75)

Oogonia commonly on rather short lateral branches or also intercalary, spherical to barrel-shaped or even thread-like with a row of eggs; eggs 25–27μ thick in larger oogonia, 17–22μ thick in small ones; antheridial branches at times containing eggs............................ *S. paradoxa* (p. 75)

Eggs larger than above, most about 30–33μ thick, not often single *S. litoralis* (10)

Eggs still larger, most about 38μ thick, very often single.... *S. megasperma* (11)

Oogonia absent; parasitic on fish, etc. (position doubtful).... *S. parasitica* (12)

Hypogyna Group: Oogonia not in chains, their walls with conspicuous pits. Antheridia on all or nearly all the oogonia, androgynous, normally formed from a cell cut off from the oogonial stalk immediately beneath the oogonium.. *S. hypogyna* (13)

Torulosa Group: (Position doubtful; may go properly in *Isoachlya*); oogonia in large part in chains; antheridia absent or very few................ { *S. torulosa* (14); *S. rhaetica* (p. 67); *S. variabilis* (p. 67) }

Sub-genus Pseudosaprolegnia: Eggs as a rule only one or two in an oogonium, subcentric, i. e., with the protoplasm completely surrounded by small oil drops which are in a double layer on one side and a single layer on the other. Oogonia covered with blunt papillae.

Asterophora Group: There is but one species in the subgenus........... *S. asterophora* (15)

Artificial key to the Species*

1. Oogonia absent; parasitic on fish................................ *S. parasitica* (12)
1. Oogonia present.. 2
2. Spores very large (25μ thick); oogonia formed within the substratum *S. curvata* (p. 72)
2. Not as above; oogonium covered with blunt papillae; eggs only 1-3 in an oogonium *S. asterophora* (15)
2. Not as above; eggs 9–30 in an oogonium; 25–27μ thick; antheridial branches at times containing eggs.. *S. paradoxa* (p. 75)
2. Not as above in all respects.. 3
3. Oogonia in chains, androgynous antheridia on each oogonium See *Aplanes androgynus* (p. 77)
3. Oogonia in chains, easily separating, antheridia absent See *Isoachlya monilifera* (p. 88)

*American species are followed by a number, others by a page reference.

3. Oogonia often in chains but mostly single; not tending to separate; antheridia present on some (usually few) of the oogonia, diclinous.......See *Isoachlya toruloides* (p. 82)
3. Oogonia mostly in chains; not separating; antheridia none or very few, androgynous or diclinous .... *S. torulosa* (14); *S. rhaetica* (p. 67); *S. variabilis* (p. 67); see also *Isoachlya* (p. 81)
3. Oogonia not normally in chains; antheridia present on all or nearly all the oogonia 4
3. Oogonia not normally in chains; antheridia absent or present on only a part (rarely as many as 75%) of the oogonia, mostly androgynous..................... 10
3. Oogonia not in chains; antheridia present on only about a third of the oogonia, diclinous .... *S crustosa* var III (p. 70)
4. Antheridia all or nearly all diclinous.................................... 6
4. Antheridia all or mostly androgynous...................................... 5
5. Oogonial stalks bent and often coiled; oogonial wall unpitted or the pits few and inconspicuous .... *S. spiralis* (p. 71); *S. furcata* (p. 72)
5. Oogonial stalks not coiled or much contorted, oogonial wall usually with pits which are often conspicuous.. 7
6. Spores of two kinds, large and small............................*S anisospora* (4)
6. Spores not of two kinds.. 9
7. Antheridial branches present and not rising from immediately below the oogonia; antheridia on all or nearly all the oogonia.............................. 8
7. Antheridial branches normally absent; antheridia cut off from the top of the oogonial stalk ....*S. hypogyna* (13)
7. Antheridial branches at times absent; if present arising from immediately below the oogonium (antheridia always present)............see *Aplanes Treleaseanus* (p. 79)
8. First oogonia mostly terminal on main hyphae, often oval; eggs large, mostly 30–33μ thick ....*S. litoralis* (10)
8. Oogonia commonly on short, lateral branches; eggs very large, 30–52μ thick *S. megasperma* (11)
8. Oogonia commonly on short, lateral branches, spherical with a neck, eggs usually five or more in an oogonium, 16–22μ thick...........................*S. monoica* (7)
8. As in *S. monoica*, except that the eggs are usually less than five in an oogonium and mostly 25–27μ thick.............................*S. monoica* var. *glomerata* (8)
8. As in *S. monoica* but no oogonia produced except in a solution of lucin and levulose *S. monoica* var. *vexans* (9)
8. As in *S. monoica* except that the oogonia have longer stalks and few or no pits, and that the vegetative threads are longer and more slender...*S. monoica* var. *montana* (p. 75)
9. Oogonial wall without pits (except where the antheridia touch); eggs 20–26μ thick *S. diclina* (1)
9. Oogonial wall without pits (except where the antheridia touch); eggs averaging 30μ thick ....*S. Kauffmaniana* (2)
9. Oogonial wall with numerous pits; eggs 12–22.5μ thick.............*S. stagnalis* (p. 70)
9. Oogonial wall with less numerous pits; eggs mostly 25–27μ thick..........*S. delica* (3)
9. Oogonial wall with few pits; eggs 17–27μ thick..............*S. crustosa* var. II (p. 69)
9. Oogonial wall with few pits; eggs 12–22μ thick...........*S. crustosa* var. I (p. 69)
10. Antheridia usually on not more than 15% of the oogonia, often very few or none; oogonia often found in old sporangia and then cylindrical; eggs mostly about 26μ thick ....*S ferax* (5)

10. Antheridia absent; oogonia never or rarely cylindrical, eggs usually 21–24μ thick *S. lapponica* (p. 73)
See also *S. esocina* (p. 41)
10. Antheridia usually on about half the oogonia which are never cylindrical; eggs mostly 24–25μ thick .... *S. mixta* (6)
10. As above, but eggs mostly only 16–19μ thick....... *S. mixta* var. *Asplundii* (p. 74)
10. Antheridia on about 75% of the oogonia.......................... *S. floccosa* (p. 74)

1. **Saprolegnia diclina** Humphrey. Trans. Am. Phil. Soc. **17**: 109, pl. 17, figs. 50–53. 1892 [1893].
Saprolegnia dioica deBary. Bot. Zeit. **46**: 619, pl. 10, figs. 12 and 13. 1888.
Not *S. dioica* Pringsheim or *S. dioica* Schroeter (which see under *S. ferax* and *S. mixta*).

Plates 3, 4 and 14.

Main hyphae of moderate size and length, little branched. Sporangia only slightly enlarged and broadest near the end, repeatedly proliferating inwardly, but also not rarely arising laterally from beneath the discharged ones, as in *Achlya*. Spores 11–11.5μ in diameter. Gemmae very abundant and variable in shape, long and pointed or stocky and knotted, the longer ones rather characteristic for this species, the other forms much as in *S. delica*, etc. By single spore cultures made several times the species has proved to be not dioecious (heterothallic) although highly diclinous, the mycelium from a single spore producing both oogonia and antheridia. Oogonia spherical or oval or pear-shaped, usually with a short neck, mostly terminating the main branches, but not rarely intercalary, occasionally two or three to five in a chain, rarely on short lateral branches or cylindrical in empty sporangia, very variable in size, even in the same cultures, 35–100μ in diameter; walls rather thin, without pits except where the antheridia touch. Antheridial branches arising diclinously from near or distant hyphae, branching, delicate, slender, and soon disappearing after the antheridia have been cut off. Antheridia on every oogonium, numerous, often completely covering the oogonium, usually slender and not much larger than the branches, occasionally somewhat swollen and tuberous, only moderately dense, but remaining visible for a long time after the antheridial branches have disappeared. Antheridial tubes nearly always invisible (if present). In only two cases were they seen. (But see below for Trow's statement as to fertilization.) Eggs 20–26μ in diameter, most about 23–24μ, varying little in size in any one oogonium, one to twenty or more in an oogonium, usually six to twelve, centric.

Common in branches and small streams, such as Arboretum brook, Battle's brook, branch back of athletic field, etc. Collected in Chapel Hill 76 times between February 29, 1912, and December 16, 1913 (see table), and found many times since.

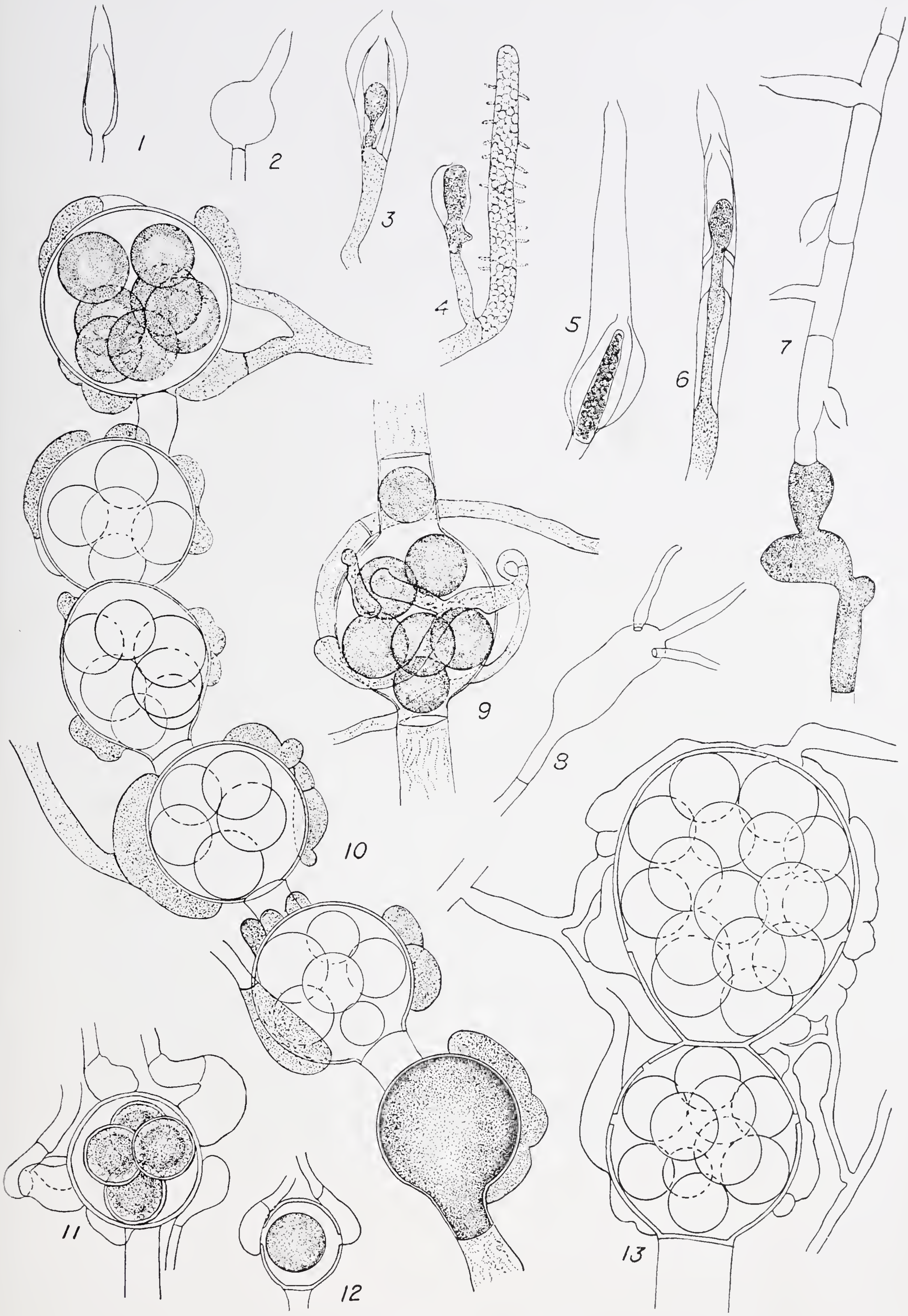
PLATE 3

PLATE 3

Saprolegnia diclina

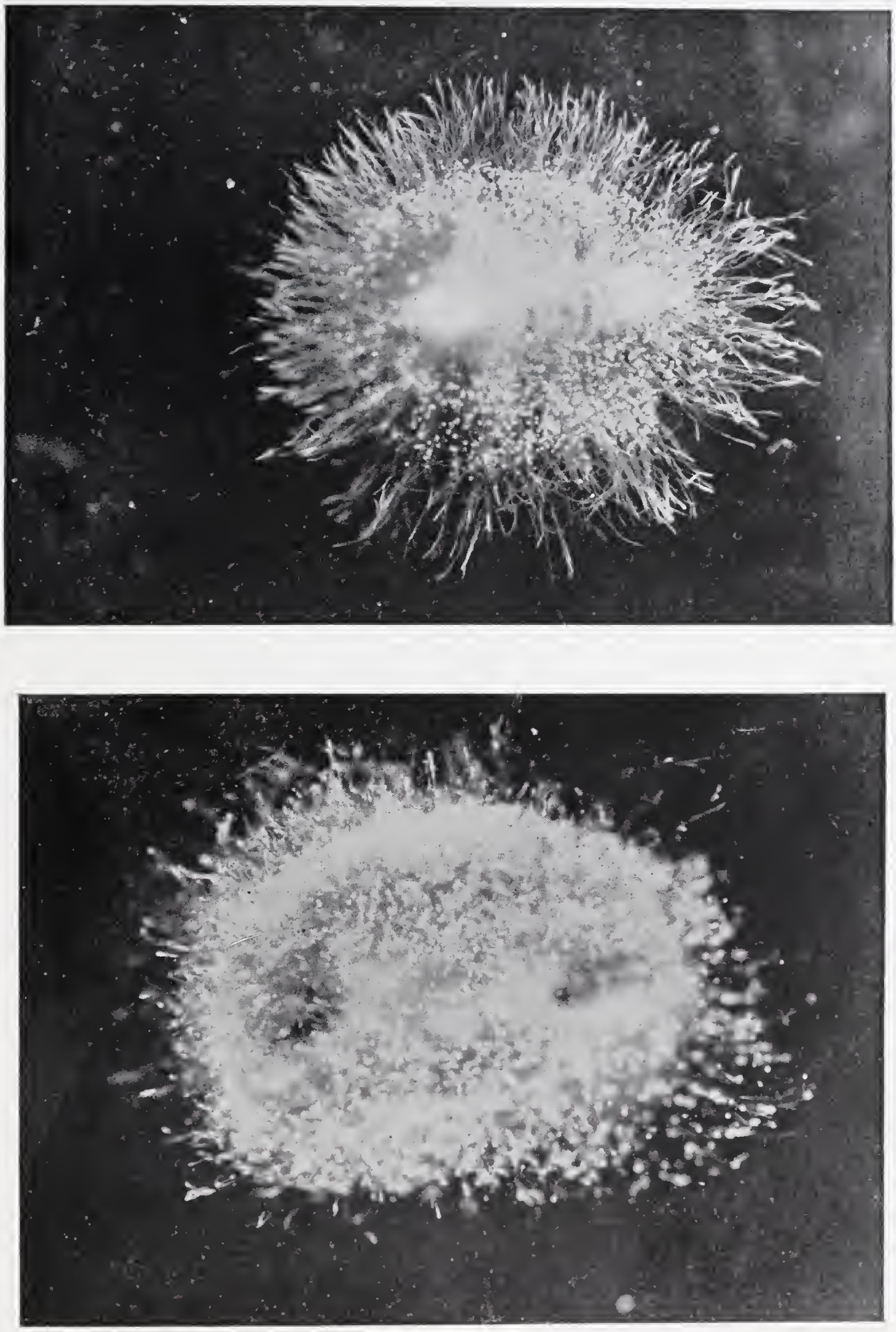
Fig. 1. Small sporangia. × 167.
Fig. 2. Sporangium with long papilla. × 167.
Fig. 3. Nested sporangia. × 167.
Fig. 4. Sporangia, one of which contains sprouting spores. × 167.
Figs. 5 and 6. Various forms of sporangia. × 167.
Figs. 7 and 8. Gemmae that have become sporangia. × 167.
Fig. 9. Intercalary oogonium with characteristic antheridia. × 447.
Fig. 10. Chain of oogonia. × 447.
Figs. 11 and 12. Oogonia. × 447.
Fig. 13. Oogonia containing large number of eggs. × 447.

PLATE 3



SAPROLEGNIA DICLINA.

PLATE 4



SAPROLEGNIA MONOICA VAR. GLOMERATA [ABOVE]. × 6.
SAPROLEGNIA DICLINA [BELOW]. × ABOUT 4.

Distribution: Chapel Hill, Massachusetts, Pennsylvania, Alabama, Louisiana. Also listed without notes by Kauffman as found by Pieters in Michigan ('15, p. 195).

For points of difference between this and *S. anisospora*, which is nearest, see that species. From *S. delica* it is distinguished by the far less numerous and larger oogonia, smaller and more numerous eggs, diclinous antheridia only, and the quickly disappearing antheridial branches; and from *S. ferax* by the numerous antheridia and the slightly pitted oogonia. For other illustrations see Minden ('12), fig. 1e on p. 520; Tiesenhausen ('12), figs. 4 and 5.

This is one of our commonest species, but is often difficult to determine with certainty because of the infrequency of sexual reproduction at room temperature. While the oogonia are not rare under ordinary conditions they are not nearly so abundantly formed as in other species, and sometimes many cultures will be made before they are found. However, sexual reproduction can be greatly increased by lower temperature, and can nearly always be obtained in an ice box.

Trow states ('95) that fertilization always occurs in this species (as *S. dioica* deB.) and in practically the same way as in *S. mixta*, which see for some detail.

To find if the plant is really dioecious cultures were made from a single spore from No. 10 of October 25, 1912, and from this the following five cultures were made:

Two cultures on ants were made about January 26th. One was put on the window sill outside, the other kept in the laboratory. By February 10th the one outside had formed 23 typical oogonia that were crowded with numerous antheridia of the usual distant origin. The oogonia were formed close in to the ant, as commonly, and the antheridial branches were very delicate, but in the unusual cold they did not disappear so soon. All the 23 oogonia were measured and they were found to average 62.98μ in diameter. The largest was 86.95μ, the smallest 40.7μ. The largest had about 20 eggs, the smallest 2 eggs. The eggs in this culture ran very regular, about 24μ in diameter. After staying out for about three weeks without a freeze this culture was frozen for about 36 hours. Taken in and tried it was found to be totally dead (unless vitality remained in the eggs: they would not sprout at the time). The numerous gemmae were all collapsed and dead. The culture inside also formed oogonia of usual character; a few were cylindrical. Antheridia very numerous and thickly enveloping the oogonia. Antheridial branches very ephemeral. As usual the oogonia were sparse, only about 50 in the culture. Some were very small and with only one egg, others quite large.

On an ant in watch glass with water from Arboretum spring. Many sporangia, about 6 oogonia; no gemmae.

On an ant in watch glass with rain water. Exactly like the above in appearance and result, except that there were no oogonia. New ant put in and again only sporangia.

On an ant in watch glass with distilled water. No oogonia.

Other cultures from No. 10 of August 25, 1912.

In egg yolk broth only, in test tube. Abundant growth, dense at surface, delicate below. Surface mat with a vast number of good sized gemmae of all shapes. A good many oogonia (several hundred) with fine healthy eggs of usual size. Antheridia only one or two to an oogonium, or seemingly absent from many. (Scarcity or absence of antheridia made these very favorable oogonia for study. The wall is rather thin, and no pits are to be seen.)

On corn meal agar. Growth very strong and healthy. Many large gemmae of usual varied shapes. Nothing else.

In pea broth. Culture first made in test tube. After a month or more a transfer was made to watch glass with new pea broth to see if the plant was alive. It was and grew well. This last showed a great many rather small gemmae and no sexual reproduction or sporangia.

In egg yolk broth on ant. Infected by a bit of corn meal agar culture. Rapid and extensive growth throughout dish. Many gemmae and sporangia and a good many scattering oogonia of normal appearance with antheridia on each, but much fewer than usual. Eggs of normal size and number, but in some cases not maturing.

In ½ maltose peptone + ½ egg yolk broth. Extensive growth of fine hyphae. No reproduction of any sort.

On corn meal egg yolk agar. Growth luxuriant, covering dish. Immense number of gemmae. No oogonia.

On ant in pea broth (50 peas boiled an hour or more in 500 c.c. of water) in sterile bottle. It grew well and when examined a month later showed an extensive mass of vegetative strands and a very few empty sporangia.

Cultures from No. 9 of August 26, 1912.

In pea broth on yolk of egg, single spore culture. Growth strong and heavy. Numerous sporangia and large gemmae. No oogonia.

On corn meal agar, single spore culture. Growth very strong and healthy. Many large gemmae of usual varied shapes. Nothing else.

On yolk of egg in distilled water. Abundant sporangia, very abundant gemmae and a good many oogonia with many antheridia to each.

In maltose 5% + peptone .01%, single spore culture. Luxurious growth filling dish. A good many sporangia, some liberating spores, some not—in last case the contained spores often sprouted in position. A few small gemmae were formed.

In ½ haemoglobin .05% + ½ KN_2PO_4 .1 % on ant (the culture was first grown to a good size in pure water, where many sporangia were formed, and then transferred to above solution). The sporangia did not empty and many gemmae formed.

The following cultures were all made from a pure culture on corn meal agar; the first six contained a .2% solution of the salt ½ and a .05% solution of haemoglobin ½:

In KNO_3 + haemoglobin. Delicate growth of rather limited extent. Many cylindrical sporangia, most of which retained the spores which sprouted by hyphae in position. Some of the sporangia discharged a few spores, but most remained behind. In such cases the remaing spores sent sprouts through the open tip as well as through the sides. Gemmae very few and small. No sexual reproduction.

In KH_2PO_4 + haemoglobin. Delicate growth of rather limited extent. A good number of gemmae of usual normal shapes. No sporangia and no sexual reproduction.

In NAH_2O_4 + haemoglobin. Practically no growth.

In $Ca_3(PO_4)_2$ + haemoglobin. No growth.

In K_2SO_4 + haemoglobin. Delicate limited growth. Many normal sporangia, often proliferating, a good many retained all or part of the spores, which did not sprout in position. Gemmae rather few and simple. No sexual reproduction.

In $Ca(NO_3)_2$ + haemoglobin. Delicate, limited growth. A few sporangia, all retaining the spores—no spores sprouting. A few scattered gemmae. No sexual reproduction.

In lactic acid, 1 drop to 50 c.c. H_2O. No growth.

In tannic acid, 1 drop to 10 c.c. H_2O. No growth.

The following experiments were made to test the best method of preserving live cultures:

Single spore culture (No. 10 of October 25, 1912) was put in vial of water in May, 1913, by cutting out piece of corn meal agar on which it was growing. The vial was closed with a plug of cotton and put in a dark place over summer. In December, 1913, tests showed the culture dead, except for about ½ the eggs, and they could not be made to sprout.

The same test was made for culture No. 5 of April 19, 1913, and also a similar test using a termite ant instead of a bit of agar. The first was found to be dead in December, the latter alive.

The same two tests were made for culture No. 2 of May 15, 1913, with exactly the same results.

Another test was made by putting a vigorous growth (single spore culture of No. 10 of October 25, 1912) in an aquarium jar with algae on the laboratory table on March 7, 1913. No growth appeared on insects dropped in the jar September 18, 1917.

2. **Saprolegnia Kauffmaniana** Pieters.* Bot. Gaz. **60**:488, pl. 21. 1915.

We have not found this, and the description is from Pieters, omitting references to figures. The species is very near our *S. diclina*, of which it may be only a variety with larger eggs. For comparison between this and *S. anisospora* see under the latter.

"This species was collected from algal material in the botanical laboratory of the University of Michigan, of unknown source, but presumably from around Ann Arbor.

"Vegetative growth like that of *S. ferax*, with firm stiff hyphae; sporangia freely produced and of the same size and appearance as in *S. ferax;* gemmae round, oval, or irregular in shape, mostly single, sometimes in chains and freely produced; oogonia very large, on long or short stalks, or intercalary; scattered; oval- or club-shaped, very rarely almost round, the usual size being about 70–80×100–250µ. The smallest oogonium noted was 30×70µ; oögonium wall thin and smooth, without pits; oospores from 3 or 4 in small oogonia to very many in large ones, averaging about 20–30 oospores per oogonium; oospores average about 30µ in diameter, contents granular without any conspicuous oil drop; antheridia nearly always present, only occasionally absent on inter-

* Dr. Pieters, who published this as *S. Kaufmanniana*, an incorrect spelling of Dr. Kauffman's name, in reply to a letter, consents to a change to *S. Kauffmaniana*.

calary oogonia, diclinous, of various shapes from clavate to clasping or irregular, often curving part way round the oogonium, and borne on slender antheridial branches; usually more than one on an oogonium.

"This species seems to differ decidedly from all others described, especially in the large, thin-walled oogonia without pits. Rarely two oogonia were observed in series. This species may be related to *S. anisospora*, of which species little is known, though no evidence of two kinds of zoospores was found in the present species. Besides its marked morphological characters, *S. Kauffmaniana* is interesting from the fact that it is especially sensitive to the concentration of haemoglobin. Oogonia were but sparingly produced on flies, many cultures having none, and no culture having more than a few. Tests were made by transferring vigorous mycelium to haemoglobin solution, and it was found that only where the haemoglobin had a concentration of 0.025 per cent were oogonia formed."

3. **Saprolegnia delica** n. sp.

Plates 5 and 6.

Growth delicate and lax, but uniform and symmetrical, the hyphae straight and simple at first, then much branched. Sporangia long, nearly cylindrical or later irregular, abundant and symmetrical in most young cultures, the later ones often irregularly inflated or bent, repeatedly proliferating from within, and not rarely laterally from below; spores about 10.5–11.5μ in diameter; gemmae plentiful or few (not nearly so abundant as in *S. diclina*), spherical or pyriform to fusiform or clavate, often in moniliform chains; oogonia typically spherical, abundant on most media, terminating the main branches and also racemosely borne throughout on rather long or rarely short lateral branches that are usually two or more times as long as the diameter of the oogonia; wall smooth, colorless, thin, about 1.8μ thick, furnished with rather few pits about 3.7–8.5μ in diameter which are not nearly so conspicuous as in *S. monoica*, *S. mixta* or *S. ferax;* diameter of oogonia on termites about 40–63μ, averaging about 55μ, on fly 42–70μ, averaging about 60μ; eggs mostly 1–6, often 8, and very rarely up to 16 (in abnormal cases when large oogonia are filled with very small eggs there may be up to 40), centric, quite dark when young (in transmitted light), lighter at full maturity, averaging about 25–27μ, with extremes of 14.8–33μ, the smallest often in oogonia of normal size and not rarely mixed with the larger. Antheridial branches abundant, often long and rambling, the larger part diclinous, rather stout and persistent; antheridia present and usually numerous on nearly all or all oogonia (95–100%), each oogonium typically furnished with at least one diclinous antheridium, and at times with androgynous ones also, occasionally absent from oogonia that terminate long branches and are therefore removed some distance from the main mass; pear-shaped or irregularly tuber-shaped, well filled with protoplasm; antheridial tubes present and not inconspicuous.

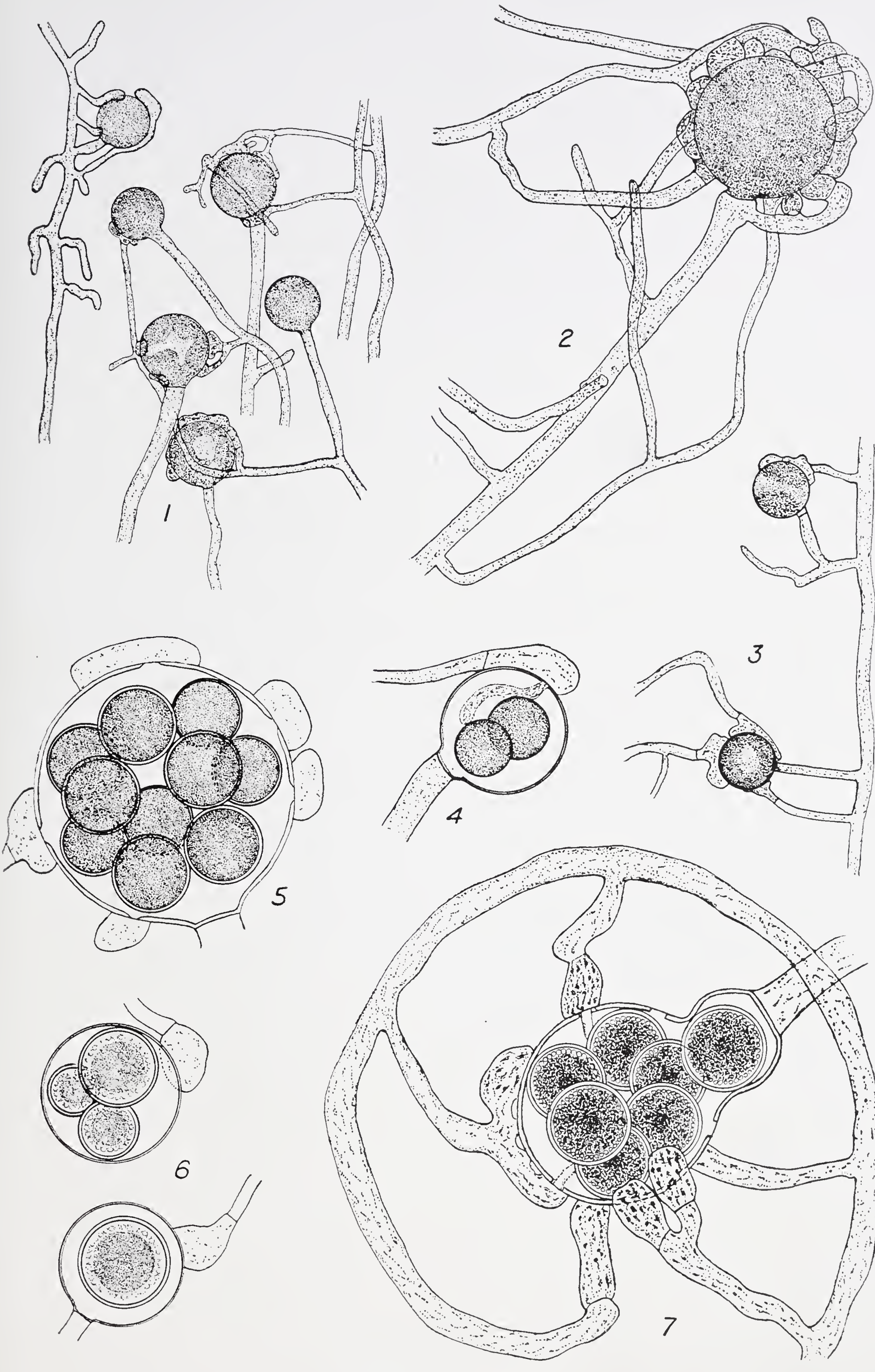
A very common species, appearing in marsh at foot of Lone Pine hill, edge of pond on Glen Burnie farm, brook behind athletic field,

PLATE 5

PLATE 5

Saprolegnia delica

Fig. 1. Habit of fruiting. × 188.
Fig. 2. Oogonium with androgynous and diclinous antheridia. × 503.
Fig. 3. Oogonia with androgynous and diclinous antheridia. × 188.
Fig. 4. Oogonium showing fertilizing tube. × 503.
Fig. 5. Oogonium. × 503.
Fig. 6. Oogonia with ripe eggs. × 503.
Fig. 7. Oogonium with curiously developed antheridia. × 503.



SAPROLEGNIA DELICA.

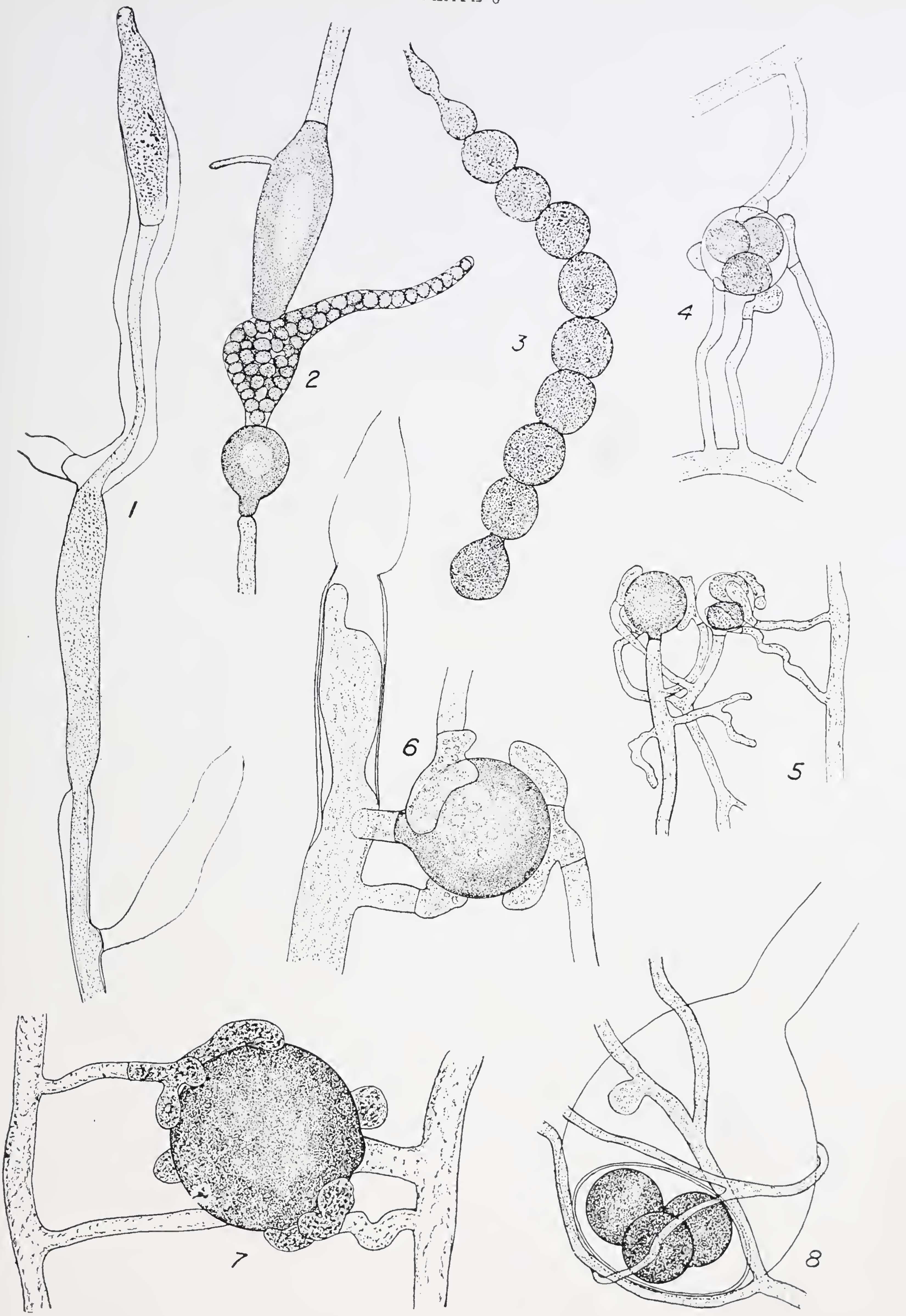
PLATE 6

PLATE 6

Saprolegnia delica

Fig. 1. Sporangia. × 278.
Fig. 2. Gemmae, the one in the center having metamorphosed into a sporangium. × 278.
Fig. 3. Gemmae. × 188.
Fig. 4. Oogonium with androgynous and diclinous antheridia. × 278.
Fig. 5. Oogonia. × 188.
Fig. 6. Oogonium and sporangia. × 503.
Fig. 7. Oogonium. × 503.
Fig. 8. Abnormal condition, showing a small oogonium formed within the larger aborted original (in maltose-peptone solution). × 503.

PLATE 6



SAPROLEGNIA DELICA.

etc., also in creek three miles north of Durham, N. C. Cultures from a single spore produce typical growth with oogonia and antheridia.

It is strange that this has not been described before, but there seems no doubt that it is new. The thin oogonial wall with few rather obscure pits, together with the preponderance of diclinous antheridia, would indicate a relationship to the *Diclina* group. It seems nearest *S. anisospora*, which see for resemblances and differences. From *S. diclina* it is distinguished by abundant oogonia, with fewer eggs (on average) and pitted walls, at least a few of which have androgynous antheridia at some stage of the culture; by the larger and more distinct antheridial branches which last longer; by the more definite and thicker antheridia, which are not so intricately wound over the oogonia (though sometimes so numerous as to cover it); by much fewer gemmae, and by the appearance of a part of the oogonia on lateral branches.

It does not seem possible to refer this to *S. semidioica* Petersen, as the antheridia in that species, so far as can be made out from the description, are essentially androgynous, arising from beneath oogonia and passing to the oogonia above as well as to others near. Moreover the eggs of Petersen's plant are too numerous (6–15) and small (22μ). We agree with Minden in referring that species to *S. monoica* (which see).

In regard to the origin of its antheridial branches the species is peculiar and apparently whimsical. As said above, the majority are always diclinous (in the media studied) and in most vigorous cultures on insects the proportion of oogonia with diclinous antheridia only is very high, usually over 95% and often 99%. However, as such cultures get older the per cent of such oogonia may drop to around 80% and on a bit of boiled corn grain the oogonia with androgynous antheridia may reach 25% (oogonia with androgynous antheridia nearly always have diclinous ones also), and on whole egg agar in distilled water nearly 50%. Most androgynous antheridia are found away from the periphery of the culture where racemosely borne oogonia are rather plentiful, but on a corn grain even the terminal oogonia on main hyphae may often have androgynous antheridia arising beneath (with diclinous ones also, as a rule). The diclinous antheridia very often arise from hyphae which also bear oogonia, as shown in the figures.

There is a decided preponderance of apical oogonia. Where sporangia are not formed abundantly nearly all the oogonia are apical. In the contrary case many are borne also on short lateral branches.

The first four cultures below were made from a single spore culture of No. 9 of November 25, 1912:

On bit of egg yolk in equal parts maltose 5% and peptone .01%. Extensive growth and very abundant gemmae. No sporangia or sexual reproduction.

In maltose + peptone alone. Growth good, filling dish. No sporangia or gemmae. Many inflated oogonia, with normal antheridial threads of diclinous origin wrapped about them, but no antheridia cut off; a good many of the oogonia with eggs in a spherical or elliptic secondary oogonium inside and at the tip of an inflated and now empty primary one, many without eggs (pl. 6, fig. 8). The wall of the inside oogonium may be pitted and that of the outside bladder also if it is thick enough. (This experiment repeated twice with single spore culture from No. 2 of January 6, 1913, again twice with No. 4 of January 15, 1913; again with No. 6 of January 15, 1913, and again with No. 6 of February 20, 1913—in all cases with the same results).

On white of egg in distilled water. Growth delicate and short, but dense and healthy. Very few if any sporangia. Abundant oogonia, with healthy eggs, averaging very small. About ½ with only one egg. Antheridia on every oogonium. Where there is only one egg there is usually only one antheridium, otherwise usually two or several. Antheridial branches not quickly disappearing.

On corn meal egg yolk agar. Luxuriant growth, filling dish. Immense number of oogonia with one or more antheridia on each, all apparently of diclinous origin. The oogonia were nearly all in a layer just below the surface of the agar; a few were borne above the surface.

On whole egg agar in distilled water. Oogonia terminal on main branches (generally), or on long lateral branches which are two or more times (rarely only as long as) the diameter of the oogonium. Antheridia on each oogonium, often there are both androgynous and diclinous antheridia, nearly always some diclinous; androgynous also in about ½ cases. The number of androgynous antheridia varies a good deal in different parts of the culture; they seem to be present where the growth is thickest (No. 2 of January 6, 1913).

In equal parts of maltose and peptone and egg yolk broth. Growth extensive and delicate. Many oogonia, nearly all inflated, many maturing good eggs, many not. Antheridial threads very numerous, and apparently all diclinous. Antheridia not cut off, except in a very few cases. No sporangia or gemmae. When eggs are formed they are cut off in a special wall in the tip of a bladder, just as in maltose + peptone alone (single spore culture from No. 2 of January 6, 1913).

In corn meal egg yolk gelatin. Grew well and covered the dish, but after a few days liquified the medium. No reproduction of any kind (No. 4 of January 15, 1913).

On whole egg agar in distilled water. Oogonia usually terminal on main threads; often lateral on long threads, i. e., on stalks two or more times their diameter. Antheridia on every oogonium, nearly always (only two exceptions seen) diclinous where the oogonium terminates a main branch. In a great majority of cases (about 90%) also diclinous where oogonia are on lateral branches. Short branches and bumps almost entirely absent beneath the oogonia (No. 6 of January 15, 1913).

The next three cultures were made with collection No. 1 of November 12, 1917:

On corn meal agar. Growth fairly vigorous, covering plate. Sporangia, containing spores which remained in sporangia, not sprouting, fairly abundant. Many oogonia with eggs. Antheridia present on about 90% of oogonia, mostly diclinous, but a fair number of androgynous ones also.

On corn meal agar cut from agar of preceding experiment in pure water without grub. Few small sporangia formed and emptied on and around the edges of the agar. Good many oogonia with eggs and androgynous and diclinous antheridia. Oogonia initials were present when agar was cut and put in water.

On boiled corn grain. Growth vigorous, somewhat more curled and branched than on grubs and flies. Sporangia produced as normally. Many oogonia with eggs; mostly terminal on main hyphae. Fully 50% of oogonia had no antheridia. This culture was repeated with the same results except that about 75% of oogonia produced were furnished with antheridia.

The following experiments were to test the best method of preserving live cultures:

Single spore culture from No. 9 of November 25, 1912, put in aquarium jar with algae on March 3, 1913. Was found to be dead when tested for life on September 18, 1917.

Single spore culture from No. 2 of January 6, 1913, put in an aquarium jar with algae on the laboratory table on March 7, 1913. Was found to be dead when tested for life on September 16, 1917.

Single spore culture of No. 6 of January 15, 1913, put in an aquarium jar with algae on the laboratory table on March 3, 1913. Was found to be dead when tested for life on September 16. 1917.

Single spore culture from No. 9 of January 25, 1912, put in vial on corn meal agar April 10, 1913, and was found to be alive December 1, 1913.

Single spore culture from No. 4 of January 15, 1913, put in vial on ant larvae April 10, 1913, and was found to be alive December 1, 1913, and furnished growth from gemmae. All eggs had disorganized.

4. **Saprolegnia anisospora** deBary. Bot. Zeit. **46**: 619, pl. 9, fig. 4. 1888.

PLATES 7, 8, 9, AND 10.

Main hyphae about 5–8 mm. long on a mushroom grub, of moderate size at base, but quickly becoming smaller, the culture appearing quite delicate in comparison with many other species; main hyphae from 40μ in diameter below to 11μ or even less near the tip. Sporangia usually borne on larger branches than the oogonia (but a good many oogonia also borne on the larger branches), usually rather stocky and irregular and largest in the middle or near the base, sometimes regularly tapering towards the end, very variable in size in the same culture, about 8.6–15.2μ, rarely up to 16.6μ thick, usually thicker than the strand that bears them, often short and broad; proliferating as usual in *Saprolegnia*, or when in distilled water the greater part as in *Achlya*. Dictiosporangia have been several times observed. Spores remarkable in being of two kinds, large and small and often intermediate sizes, usually in separate sporangia without constant regard to the size of the latter, a single sporangium usually with spores of only one size, but occasionally they are mixed; the smallest spores about 8–9μ in diameter, others from 10.5–11.5μ, the large ones from 13.7–14.8μ; small and large spores similar in structure, but the small are greatly in excess of the large ones; in nearly all cultures there are formed in addition a few very large spores at least twice the bulk of the ordinary large spores, these appearing usually mixed with the latter.

Oogonia numerous and formed in all ordinary culture media, borne usually on the tips of long slender branches which arise from near the substratum, often intercalary (very rarely two or three in a row), varying to laterally sessile or on short or rather long lateral branches; typically spherical with a short neck when apical, but at times oval to pear-shaped, and when intercalary oblong to flask or spindle-shaped with long necks; 33–92μ in diameter, most about 55–65μ; at maturity with moderately thick walls that appear unpitted except beneath each antheridium, where there is always a distinct circular pit (when young, just before the formation of the egg initials, the oogonia present a spotted appearance from the arrangement of droplets of oil in the protoplasm, as is usual in the family). Eggs 1–20, mostly 4–6, quite variable in size even in the same oogonium, 17–38μ in diameter (not rarely some very small ones as little as 13μ thick), most about 21–27μ, centric.

Antheridial branches quite slender and soon becoming very inconspicuous, arising from any of the main branches, usually from the proximal half, and running to oogonia on other branches than the one from which they arise (diclinous); oogonial branches often give rise to antheridial branches lower down. Antheridia cylindrical or tuberous, present on all oogonia, usually several to many, when young well filled with protoplasm, in age apparently empty; antheridial tubes formed in most cases and remaining visible for some time after the eggs are formed. Gemmae more or less numerous or rather few, usually spherical, sometimes pear-shaped or tuberous and of other shapes, usually in short or long chains, easily becoming sporangia on change of conditions, emptying by a proliferating tube. Often there may be several proliferation tubes, but only one opens for the escape of the spores.

Rare, and found only three times, twice in Chapel Hill collections, from Arboretum branch (No. 12 of January 30, 1913), and from a marshy sink opposite the cemetery (No. 2 of January 2, 1917), and once in Wilmington, N. C., in a ditch at the golf links, this last a form with somewhat larger oogonia and more numerous eggs. No. 12 of January 30, 1913, was cultivated for about four months from a single spore and No. 2 of January 2, 1917, was cultivated for a year and a half, part of the time from a single spore. The species is distinguished by its characteristic sporangia, two or more sizes of spores, and diclinous antheridia on every oogonium.

The frequent appearance of two or three sizes of spores recalls *S. anisospora*, and we have become convinced that our plant is that species. But for the centric eggs there is no great difference to be noted in deBary's description.* In fact, the small oogonia with unpitted walls, the small number of eggs, the numerous and conspicuous diclinous antheridia on each oogonium, and the very variable spores form so striking a set of similar characters, particularly as in this group of characters both species are well separated from all others, that one is inclined to suspect that deBary, who rarely made a mistake, was in this case wrong in

*DeBary's description of the oogonia as club-pear-shaped is evidently a distinct exaggeration of the tendency to that shape. His figures show oogonia that are spherical or slightly pear-shaped.

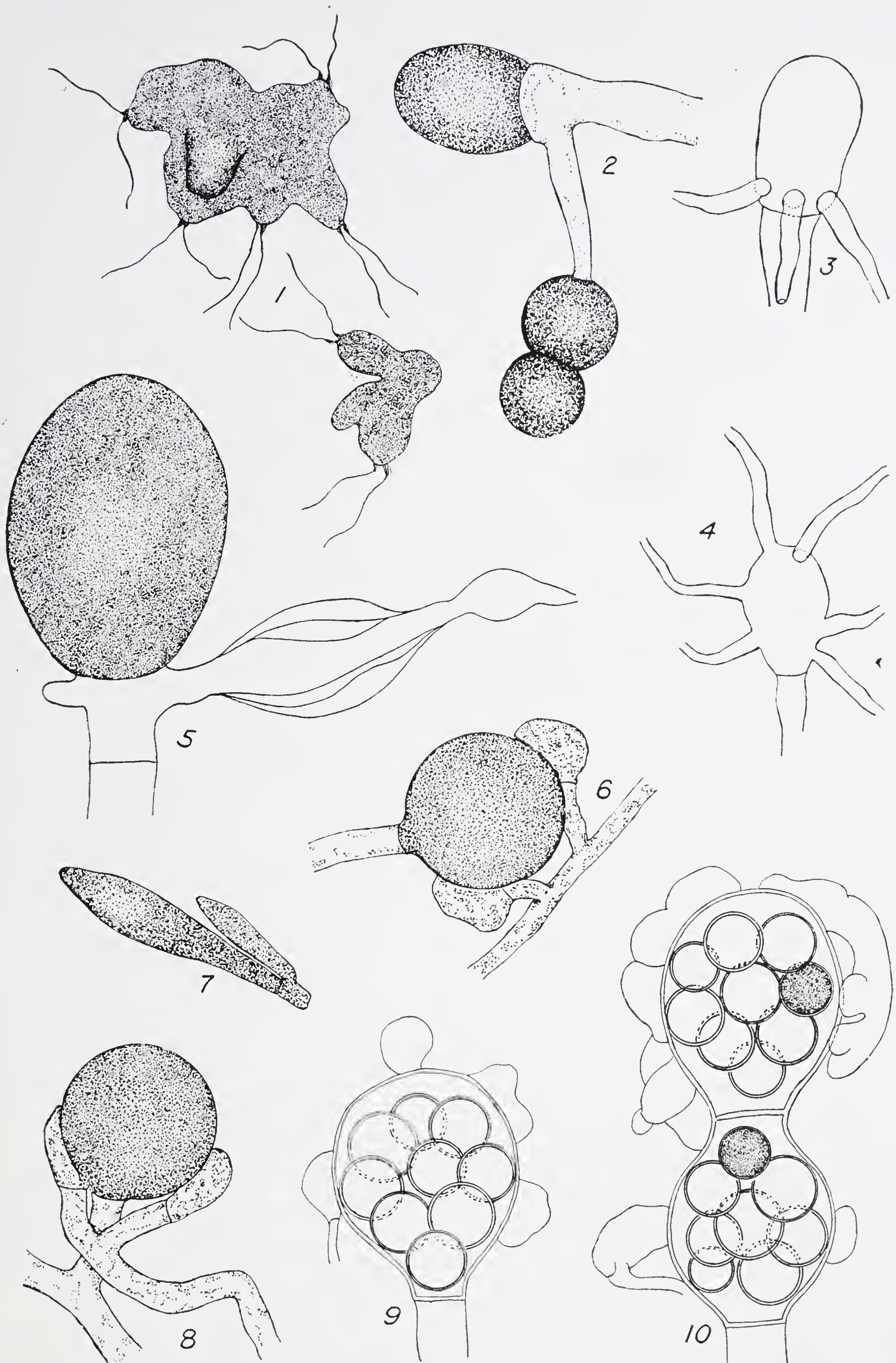
PLATE 7

PLATE 7

Saprolegnia anisospora

Fig. 1. Spore masses which came out of sporangia without breaking up. × 810.
Fig. 2. Gemmae. × 188.
Fig. 3. Gemma with three papillae. × 188.
Fig. 4. Gemma with six papillae. × 188.
Fig. 5. Gemma and sporangia. × 503
Fig. 6. Young oogonium. × 503.
Fig. 7. Resting sporangia. × 188.
Fig. 8. Young oogonium. × 503.
Figs. 9, 10. Oogonia containing many eggs. × 503.

PLATE 7.



SAPROLEGNIA ANISOSPORA

PLATE 8

PLATE 8

SAPROLEGNIA ANISOSPORA

Figs. 1–3. Various forms of sporangia. × 188.
Fig. 4. Gemma which has become a sporangium with small spores. × 503.
Figs. 5 and 6. Forms of sporangia. × 188.
Fig. 7. Large spores in amoeboid state. × 810.
Fig. 8. Large spore encysted. × 810.
Fig. 9. Large spore with cilia. × 810.
Fig. 10. Sporangium in act of emptying large spores. × 188.
Fig. 11. Sporangium containing large spores. × 503.
Fig. 12. Sporangia. × 188.
Fig. 13. Gemma with long papilla, emptying spores. × 503.
Fig. 14. Small spore encysted. × 810.
Fig. 15. Small spore coming out of cyst. × 810.
Fig. 16. Three small spores in first swimming stage. × 810.

PLATE 8

SAPROLEGNIA ANISOSPORA.

PLATE 9

PLATE 9

Saprolegnia anisospora

Fig. 1. Proliferating sporangia. × 278.
Fig. 2. Intercalary oogonium. × 188.
Fig. 3. Young oogonia with antheridia, showing habit. × 188.
Fig. 4. Oogonium with two ripe normal eggs and two others going to pieces. × 503.
Fig. 5. Sessile oogonium. × 503.
Fig. 6. Oogonium in old sporangium. × 503.
Fig. 7. Oogonia in a chain. × 188.
Fig. 8. Chains of gemmae with oogonia on tip in two cases; the oogonium on the right with all its contents rounded up into a single mass which formed a wall, but did not take on the typical internal structure of an egg. × 188.
Fig. 9. Intercalary oogonium with eggs of varied size. × 278.
Fig. 10. Gemma emptied by a long papilla. × 278.
Fig. 11. Odd-shaped oogonium with a single egg. × 503.
Fig. 12. Intercalary oogonium containing large and small egg. × 503.
Fig. 13. Cluster of *Pythiopsis*-like sporangia. × 188.

SAPROLEGNIA ANISOSPORA.

PLATE 10

PLATE 10

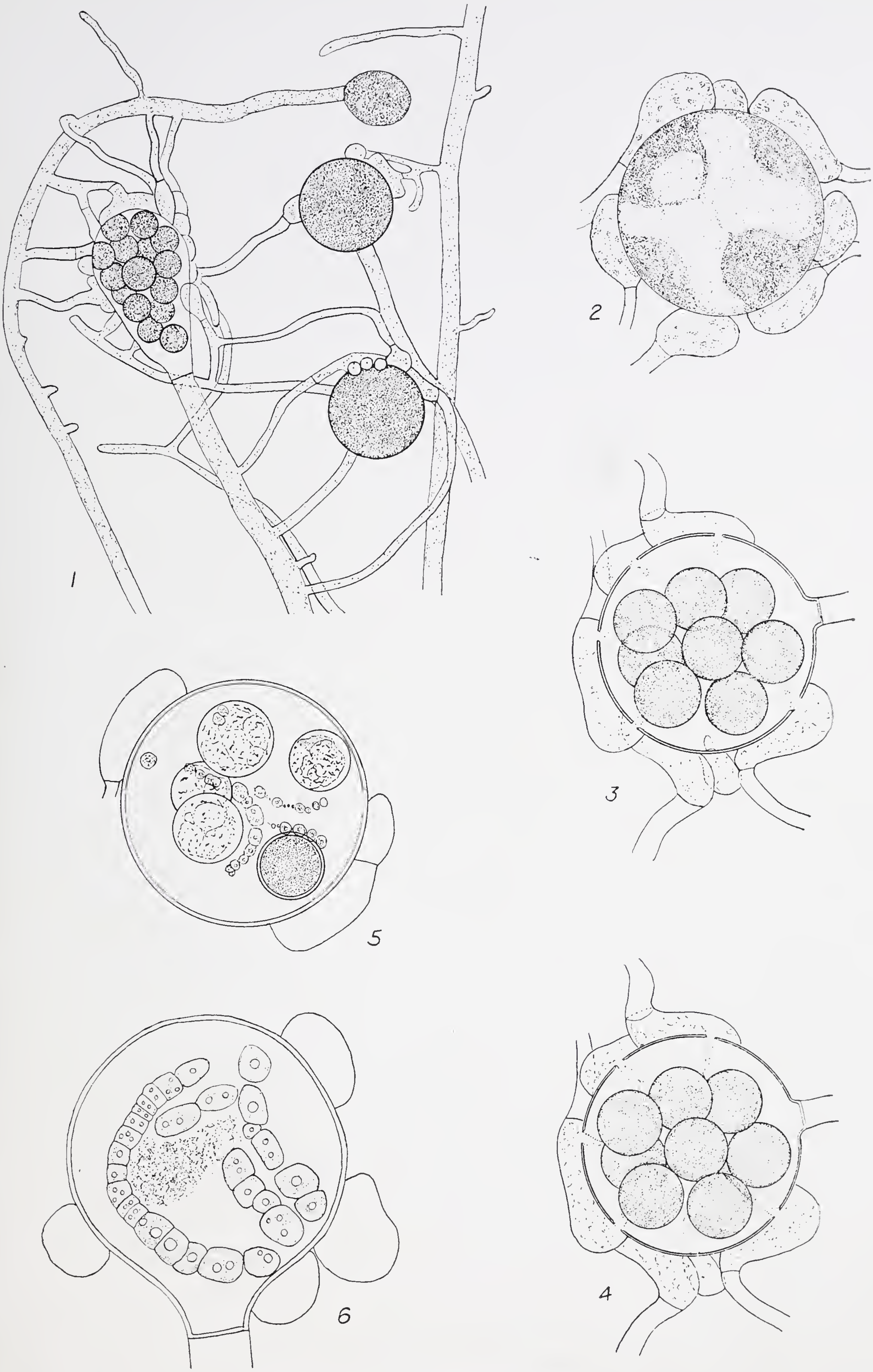
SAPROLEGNIA ANISOSPORA

Fig. 1. Habit of oogonia and antheridia. × 188.

Figs. 2, 3, and 4. Formation of eggs and growth of antheridial tubes in the same oogonium. No. 4 was drawn 15 minutes later than No. 3. × 536.

Figs. 5 and 6. Oogonia attacked by a parasite that seems to be a blue-green alga. × 503 and 810.

PLATE 10



SAPROLEGNIA ANISOSPORA. FORM B.

thinking the normal eggs eccentric. His figures clearly show eccentric eggs, but may they not have been breaking down? This seems the more likely as no other *Saprolegnia* has an eccentric egg. Moreover, deBary found his plant only once (in a small swamp at Strassbourg) and had it in cultivation only two months before losing it. He grew cultures from a single large spore and found them to produce both large and small spores. Our pure cultures were made from single spores, but whether large or small was unfortunately not noted. They produced both oogonia and antheridia, as described above. This proves that at least one of the two sorts of spores is bisexual. Fischer ('92, p. 337) suggests that the small spores may be male (and, by implication, the large ones female).

Since deBary's day no one seems to have found the plant except Obel, who casually refers to it in his paper on oogonia formation in *Achlya decorata* (Ann. Mycologici **8**: 422. 1910), saying that "Cultures of this species grown up from the great [larger] zoospores may be low and crowded with oogonia, while cultures grown up from old gemmae may be large and quite free from oogonia generation after generation." He does not further enlighten us on the important point as to whether the cultures from gemmae ever do form oogonia.

This species is apparently nearest *S. diclina*, as indicated by the strictly diclinous antheridia on every oogonium, the unpitted wall of the oogonia, and the apical or intercalary position of the oogonia. It differs from that species in the spores of two or more sizes, in the smaller, more spherical oogonia (apical oogonia of *S. diclina* are mostly oval), with fewer eggs, which are abundantly borne at room temperature, in the more conspicuous antheridial branches which do not disappear so quickly, in the large and tuberous antheridia, and in the absence of the abundant long gemmae of *S. diclina*. *Saprolegnia Kauffmaniana* differs in about the same respects.

In the appearance of the culture, the abundance and size of the oogonia and in number of eggs the species is like *S. delica*, but is easily distinguished from it by the unpitted oogonial walls, the many intercalary oogonia, and by constantly diclinous antheridia. In the several collections that we have measured the oogonia of *S. anisospora* average about $\frac{1}{5}$ to $\frac{1}{10}$ larger than in *S. delica* on the same medium.

A rather frequent abnormality is that shown in the oogonium on pl. 9, fig. 8. The protoplasm, instead of dividing into several eggs, rounds itself up into a single dense mass, the size of the combined eggs of a normal oogonium, the mass remaining homogeneous, as in a gemma, but denser, and not taking on the centric structure of a normal egg. Such masses might be considered as modified gemmae rather than eggs.

In the Wilmington plant, that we will call Form *B*, the oogonia and the number of eggs run distinctly larger, all other characters the same. For example, two cultures of each were compared, one of each on a piece of corn grain, and one of each on a mushroom grub. On the corn the Chapel Hill plant showed oogonia 33–81µ; eggs 1–8, mostly 2–6, many single; while Form *B* showed oogonia 65–93µ, eggs 4–20, mostly 8–12 in an oogonium. On a grub the former showed oogonia 40–64µ, eggs 1–5, mostly 2–4, while Form *B* showed oogonia 48–70µ, eggs 2–8, mostly 4–6. In Form *B* the first cultures which were made in the ditch water with algae, etc., in which it was taken, showed a remarkable abnormality (pl 10, figs. 5 and 6). A good many of the oogonia were much swollen to as much as twice the average size (up to 150µ), while the eggs, which were no greater in number than usual, were in great part disorganized, often only one or two maturing. The disorganized eggs would usually reach the point of forming a thin wall, and would then go to pieces inside. This condition was not thoroughly realized until after the culture had been purified on agar and the abnormality thus arrested. It was then found from slides that the enlarged oogonia contained a parasitic organism with the exact appearance of a blue-green alga, which ran among the eggs. There was not the least resemblance to the ordinary fungal parasites that attack the water molds, and it is to be regretted that the parasite was lost before it could be more thoroughly studied. It is of interest to note the close resemblance of this parasite to the one shown by Reinsch ('78) in his fig. 11, pl. 14. He considered the parasite a species of *Saprolegnia*, but gives no reason for thinking so.

The following observations were made on the Wilmington Form *B* by Mr. J. N. Couch, Instructor in Botany: "The oogonial wall is thickened just before the protoplasm draws away to form the egg initials and as the eggs become rounded the fertilizing tubes appear. They grow rapidly and in fifteen minutes have reached the eggs. Granules were observed to pass slowly down the tubes and some of them seemed to disappear into the eggs." The notes given below all refer to the Chapel Hill form.

Repeated cultures in spring water show that growth through old sporangia is much more common in spring water than in distilled water, where there is a strong tendency to proliferate as in *Achlya*.

On emergence the large spores are elongated and often bent, squarish at one end. They change this shape to pear-shape in a few seconds. The ciliated end usually, if not always, emerges last. The opposite end is often indented on emergence. Both large and small spores appeared in large numbers in a hanging drop of sporangia and gemmae in pure water. The spores are certainly diplanetic: some sporangia were put on a slide in water in a moist chamber to test this again. Many spores were formed and coming to rest on the slide emerged again, leaving a large number of cysts.

The following notes were made on the development of the spores: At one o'clock the outlines could be seen. These would appear and then almost disappear in different parts of the sporangium. This happened several times. At 1:16 the whole sporangium rapidly became homogeneous. After about 4 minutes the spore outlines began again to appear and became pretty plain in five minutes. They continued in this condition, the outlines getting gradually more distinct, for about 15 minutes without change. All this time the spores (or protoplasm) were firmly touching the wall, but now, about 4 minutes before emerging, there could be seen a narrow space filled with sap between the spores and the wall. The spores emerged at 1:42, popping out rapidly at first under strong pressure and gradually reducing the pace. As soon as the sporangium opened the column of spores shrank from the wall in proportion as the escape permitted, the last ones forming a narrow column right in the center. On emptying the sporangium became slightly narrower and about 8μ longer. Again: Small spores liberated at 12:37 were nearly all at rest by 12:50, all by 12:56 (in spring water at room temperature of 18° C. a single sporangium was separated just before discharge and the spores were liberated in a drop on a slide.) On emerging the spores swim slowly and aimlessly, changing direction momently and coming to rest in about 15 minutes. After several hours they emerge and swim very actively (more than three times as fast as in first stage) and with a definite aim, revolving slowly. They are slightly smaller than in the first stage and have a slight groove down most of one side.

By examination of a large number of sporangia of all shapes and sizes we find that in the majority of cases the large sporangia produce the large spores and small sporangia (gemmae) produce small spores. This is subject to numerous exceptions. As a rule the spores of a single sporangium are all of one size, but in several cases we have seen the two sizes in the same sporangium. Moreover, there are intermediate sizes, but these are not nearly so numerous as the extremes.

All the following cultures were made from a single spore culture (size of spore not known) from No. 12 of January 30, 1913:

On corn meal agar. Growth good. A good many gemmae in chains of two to four, a condition not found in water cultures except very rarely. No sexual reproduction.

On white of egg in distilled water. Growth delicate. A very few gemmae formed, soon dying. Apparently no sporangia.

On egg yolk in distilled water. Growth extensive. A few small gemmae. No sporangia, apparently. This experiment was repeated and the growth was very strong. A good many sporangia of usual shape. Very many gemmae of all shapes, most in chains—some very long chain-forms, exactly as in *S. ferax*.

In equal parts maltose 5% and peptone .01% solution. Growth very extensive, but entirely sterile. No gemmae, sporangia or oogonia. Many threads were spirally curled.

On corn meal egg yolk agar. Remarkable growth, different from any other water mold. Agar was covered and an aerial growth followed that hid the agar under a dense pure white cottony mantle ½ cm. thick, some fibers reaching still higher and touching the cover. In the aerial mass were a good number of pear-shaped or nearly spherical gemmae of smaller size than usual. Growth inside the agar only vegetative.

On corn meal agar. Fine growth filling dish. Many gemmae of usual shape. No oogonia.

In egg yolk broth. Growth good, but not over an inch in diameter. Many sporangia were formed, but very few formed spores. Most of them became resting sporangia with undifferentiated protoplasm. Many gemmae. These resting parts were left free by the disappearance of the threads. All parts smaller than usual.

In egg yolk broth. Growth very strong and extensive. Many large gemmae and sporangia. No oogonia. Sporangia emptying normally.

On yolk of egg and ant in distilled water. Very strong growth, nearly filling dish. Many large gemmae. Also a good many oogonia were formed, but only near the ant.

On white of egg and ant in distilled water. Growth abundant, but more delicate than in above. Many gemmae, smaller than above, and a good many sporangia. No oogonia.

On ants alone in distilled water. Growth strong, many sporangia and gemmae, about as in above culture. No oogonia.

On egg yolk in distilled water. Strong growth and many spores, both large and small. Many gemmae. No oogonia. Culture soon going to pieces.

On corn meal egg yolk agar. Grew well but did not send up any conspicuous growth in air. Many oval, round, and pear-shaped gemmae formed. No other reproduction.

On mushroom grub in distilled water. Grew well. Many gemmae of all usual shapes and many sporangia. All spores formed were small ones. A few very large undivided masses had been ejected as in most cultures.

On mushroom grub in distilled water. Two cultures were made. One showed apparently no large spores, thousands of small ones. The other showed a good many large spores also. Nearly all sporangia proliferated as in *Achlya*. Many gemmae in each as usual. Two other cultures made a little later gave the same result except all spores small.

In 5% maltose in distilled water. Growth vigorous, but entirely vegetative. Threads small. Later a fair number of spherical gemmae were formed. Another culture gave the same result except that it went to pieces without forming any gemmae.

On mushroom grub in spring water. Growth and behavior as in distilled water except that many sporangia proliferated repeatedly from within old ones, exactly as usual in *Saprolegnia*. A second culture gave the same results. There were a few large spores—not more than 1 in 100. Four years later (in 1918) repeated experiments were made with No. 1 of April 4, 1918, to test again the effect of different water on sporangia proliferation. The results were the same. In distilled water most of the sporangia proliferate by lateral branching below as in *Achlya*, while in spring water most of the proliferation is internal as usual in *Saprolegnia*.

The cultures in the following solutions were all made on termites:

In .1% KNO_3. Gemmae and sporangia present. No oogonia or antheridia.

In .1% KH_2PO_4. Growth limited, and soon dying.

In .1% Na_2HPO_4. Growth good. Not many sporangia, only a few liberating spores. A good many gemmae.

In .1% of K_2SO_4. Growth good. Gemmae plentiful; sporangia few, some discharging, some not.

In .1% $Ca_3(PO_4)_2$. Growth good. Many gemmae. Few sporangia; some not discharging.

In .1% $Ca(NO_3)_2$. Growth limited. Few gemmae. No sporangia.

The following cultures were made in a solution consisting of equal parts of a .2% solution of the salts indicated and a maltose-peptone solution made by adding equal parts of 5% maltose and .01% peptone solutions. Bacteria limited the growth in most cases.

In $Ca(NO_3)_2$ + maltose-peptone solution. Growth about ½ inch in diameter. No reproduction.

In KNO_3 + maltose-peptone solution. Same result as in preceding.

In $Ca_3(PO_4)_2$ + maltose-peptone solution. Growth delicate and rather more extensive than in preceding. A few sporangia formed. A number of threads spirally wound.

In KH_2PO_4 + maltose-peptone solution. Delicate and extensive growth (2 inches in diameter). No reproduction.

In Na_2HPO_4 + maltose-peptone solution. No growth.

In K_2SO_4 + maltose-peptone solution. Growth about 1 inch in diameter. A very few gemmae. No other reproduction.

In ½ maltose-peptone solution and ½ egg yolk broth. Dense growth of fine even threads 1½ inches in diameter. No reproduction.

The following experiments were made to find the best method of preserving live cultures:

Culture put in vial on corn meal agar March 18, 1913, was found to be dead, December 1, 1913.

Culture put in aquarium jar with algae, etc., on laboratory table on March 3, 1913. No growth could be obtained from it when test was made September 18, 1917.

The following cultures were made from No. 2 of January 2, 1917:

On corn meal agar. Growth vigorous, covering the plate. A few sporangia, which did not empty their spores. Many oogonia with eggs. Antheridia present in varying numbers on every oogonium, all diclinous, so far as seen. This experiment was repeated several times with varying results. A few sporangia were always formed, but often there was no sign of any sexual reproduction.

In haemoglobin solution. Vigorous growth, but no eggs and no sporangia. Repeated this experiment. Vigorous growth. Abundance of oogonia formed, but eggs went to pieces as soon as formed. A few sporangia and some large and small spores given off in early stage of growth. Antheridia present on all oogonia.

5. **Saprolegnia ferax** (Gruith.) Thuret. Ann. Sci. Nat. Bot., Series 3, **14**: 214, pl. 22. 1850.

Achlya prolifera Pringsh. Nova Acta Acad. C. L. C. N. C. **23**: 395, pls. 46–50. 1851.

Saprolegnia dioica Pringsh. Jahrb. f. wiss. Bot. **2**: 206, pl. 22, figs. 1–6. 1860.

Saprolegnia dioica var. *racemosa* de la Rue. Bull. Soc. Imp. Nat. Moscow **42**, **1**: 469. 1869 (see Fischer, p. 336).

Saprolegnia Thureti deBary. Abh. Senckenb. naturf. Ges. **12**: 326, pl. 5, figs. 1–10. 1881. Also in Morph. u. Phys. der Pilze, IV Riehe: 102. 1881.

Saprolegnia bodanica Maurizio. Jahrb. f. wiss. Bot. **29**: 107, pl. 2, figs. 52–59a. 1896.

? *Saprolegnia esocina* Maurizio. Jahrb. f. wiss. Bot. **29**: 82, pl. 1, figs. 4–17. 1896.

Plates 11 and 12

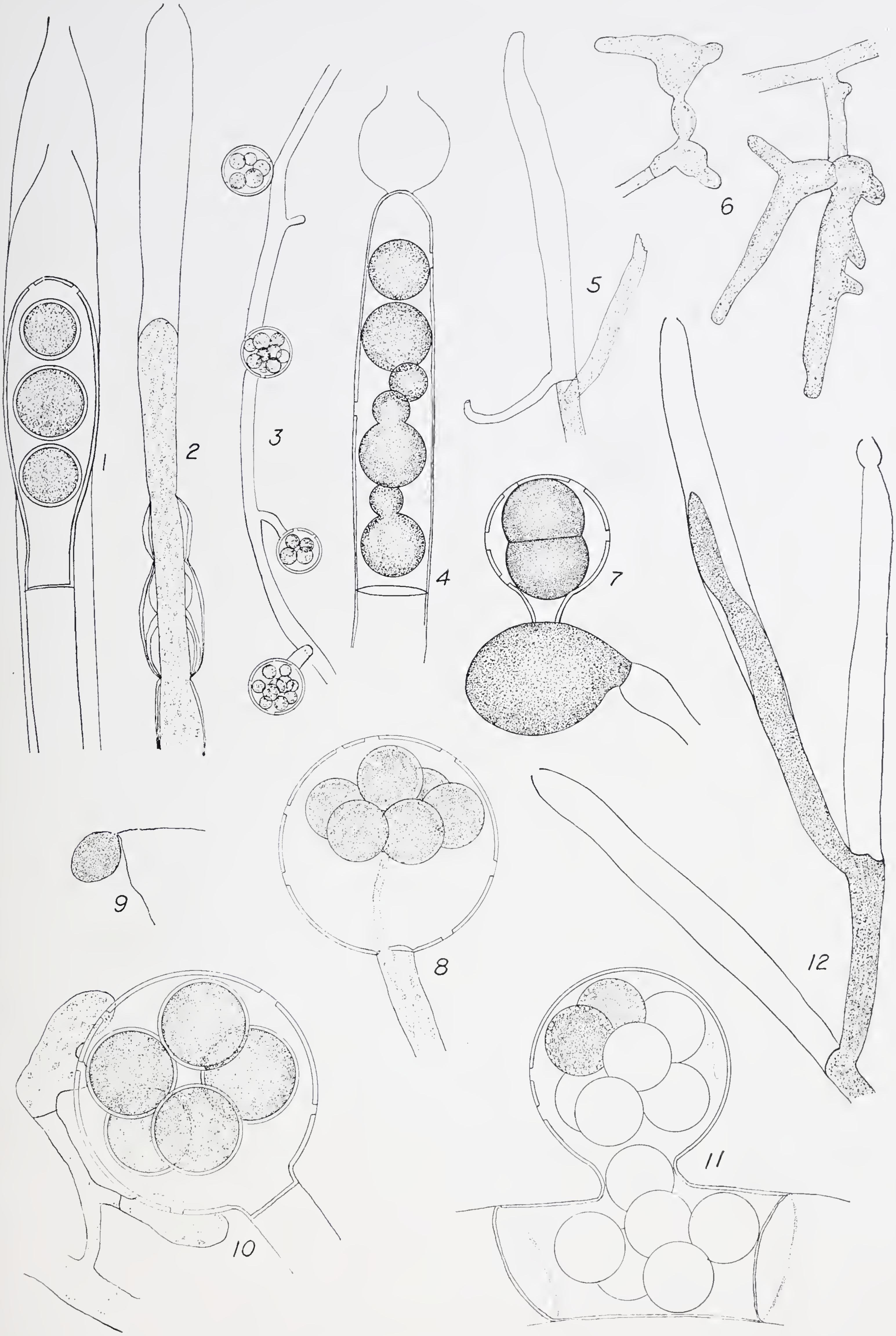
Hyphae moderately stout and vigorous, irregular, sparingly branched below. Sporangia plentiful, only slightly enlarged, typically wavy and bent and of unequal diameter, often tapering upward, rarely almost cylindrical, often proliferating laterally from below old ones; zoospores about 9μ in diameter. Gemmae not very abundant, more or less elongated usually, but varying to bulbous, or pyriform and sometimes jointed. There is a strong tendency to the formation of long tapering tips on the ends of stout threads, the ends of which are later cut off as rejected tips of irregularly tapering gemmae below. In such cases the gemma opens later by basal protuberance as is also usually the case even when no tip is cut off. Oogonia numerous, varying in diameter from 37 to 97μ, the wall only about 1.3–1.6μ thick, but with numerous conspicuous pits, which are about 4.5–5.5μ in diameter; either lateral on stalks which are usually short and frequently curved, or terminal on the main branches, sometimes intercalary, but not in chains; spherical to slightly oval with a basal neck, which is often curved; not rarely formed inside of empty sporangia, and then cylindrical; thread-like extensions of the oogonia, containing a single row of elliptic eggs, are not rare. Eggs centric, 1–20, mostly 4–16, the diameter 24–30.5μ, (rarely as small as 14.8μ or as large as 33.8μ), the greater number about 26μ, extremes sometimes occurring in the same oogonium. Antheridial branches short, stout, mostly androgynous, present in nearly all cultures in varying number, usually on about 10–15% of the oogonia, but the number of oogonia furnished with them varies from none to 98%, depending on the medium used. The antheridia arise as a rule from the same main strands that bear their oogonia, but often also from the oogonial stalks, and in the latter case usually applying themselves not to their own but to nearby oogonia, either from the same or another strand. Antheridia usually cut off, short and tuberous, not more dense than the threads; fertilizing tubes suppressed or very rare.

PLATE 11

PLATE 11

Saprolegnia ferax

Fig. 1. Sporangia containing a cylindrical oogonium. × 447.
Fig. 2. Proliferation through old sporangia. × 247.
Fig. 3. Habit of fruiting. The stalks of the two upper oogonia are hidden. × 108.
Fig. 4. Cylindrical oogonium, with some eggs of abnormal shape. × 447.
Fig. 5. Sporangium with long papilla. × 167.
Fig. 6. Gemmae. × 102.
Fig. 7. Gemma with an oogonium attached. × 447.
Fig. 8. Oogonium partly filled with ripe eggs, and showing an ingrowing tube from below. × 447.
Fig. 9. Spore in first swimming stage. × 720.
Fig. 10. Oogonium with antheridia. × 417.
Fig. 11. A cylindrical oogonium with a spherical protuberance. × 447.
Fig. 12. Sporangia renewed both as in *Achlya* and in *Saprolegnia*. × 167.



SAPROLEGNIA FERAX.

PLATE 12

SAPROLEGNIA FERAX. × 6.

Distinguished by conspicuous and mostly numerous pits, by the occurrence in most cultures of cylindrical oogonia, by the frequently curved stalks and necks of the oogonia, and by the scarcity of antheridia, which are mostly androgynous and borne on short, simple or little branched stalks.

Common in springs and branches, as Arboretum spring and brook, Battle's branch, etc., appearing in 12.5% of all Chapel Hill collections between February 15, 1912, and December 12, 1913 (see table); also many times since.

Distribution: Chapel Hill, N. C., Kentucky, Missouri, Massachusetts, Wisconsin, Michigan.

For other illustrations see Pringsheim ('73), pl. 18, figs. 5 and 11; W. G. Smith, ('78), 2 unnumbered plates; Minden ('12), figs. 1b–c on p. 520; Maurizio ('96), pl. 1, figs. 28–36; Humphrey ('92), pl. 16, figs. 43–45; Lechmere ('11a), figs. 2–4; Klebs ('99), figs. 1–2 (or *S. mixta*); Rothert ('88), pl. 10, figs. 1–13; Istvanffi ('95), pl. 35, figs. 14–18; Dangeard ('90), pl. 5, figs. 6–27.

Minden ('12, p. 521) has described two forms of *S. ferax* as follows:

Form *1*. "Sporangia more inflated or spindleform than cylindrical, often irregular in sections and with tapering tips; oogonia terminal, often on long, bent stalks, typically spherical, if indeed at times with a cylindrical neck, very rarely cylindrical; antheridia very rare, but no more so than in the typical form.

Form *2*. "Like the typical except that the oogonia are borne mostly on the ends of very short side branches in regular racemose arrangement, which according to Fischer should not be the case in the typical form. Many of the large oogonia, which contain numerous eggs, are cylindrical in empty sporangia."

It is obvious that this Form *2* is very like our Chapel Hill plant, and it is also probably not different from Fischer's form in spite of supposed discrepancies. Humphrey's slides show a plant similar to ours. It is also to be noted that neither Humphrey nor we find the large number of eggs, up to 40–50, in an oogonium which are recorded by deBary and other European writers who seem to have copied from him (Fischer, Minden). Pringsheim's figures 5, pl. 17; and 3, pl. 18, in his Jahrb. f. wiss. Bot. 9, 1873, look suspiciously like our *S. ferax*, though labelled by him *Achlya polyandra*.

Saprolegnia esocina Maurizio is so near this species as perhaps to fall well within its range of variation. It is described as differing from the typical *S. Thureti* in the size of the eggs, which are 21.5–25μ in diameter, one to 30 in an oogonium, and in absence of oogonia in chains.* Antheridia scarce, only two seen and these diclinous. Found on a living pike in Switzerland. This may be the same as *S. lapponica* (see p. 73), but that is said rarely to have oogonia terminating the main branches.

Saprolegnia bodanica Maurizio is another plant that seems almost exactly like our Chapel Hill *S. ferax:* Threads slender, main ones 31–47μ thick, often with cellulose grains which may stop up the small threads as in *Leptomitus*. Oogonia racemose, rather seldom intercalary, never in chains; wall thin, colorless, with numerous small pits; oogonia 54–93μ thick, the oblong ones 88×108μ. Eggs 23.5–31μ thick, 4–30+ in an oogonium. No antheridia.

Saprolegnia ferax cannot be limited to forms without antheridia. DeBary admits the presence of some antheridia as does Humphrey also, but it would appear from the work of Pieters and myself that antheridia are frequently present on as many as 10–15% of the oogonia. Pieters (Mycologia 7: 310. 1915)† has found that in his strains from Ann Arbor grown on fly at room temperature and in cooler temperature (12°–15° C.) the number of oogonia with antheridia varied from 0–19% (see his pl. 170, fig. 1). For other experimental work in this species see Pieters ('15b). In his studies on the physiology of *Saprolegniaceae* (Ann. Bot 22: 361. 1908) Kauffman supposes a strain of his from Ann Arbor to be *S. mixta* because it contained a few antheridia, 1 or 2 per cent normally. This must be considered *S. ferax* if the latter and *S. mixta* are to be kept separated at all. In experiments with his strain he increased the proportion of antheridia-bearing oogonia to 25% both in leucin + potassium sulphate and in haemoglobin + potassium nitrate. In haemoglobin + calcium nitrate, on the other hand, there were no antheridia on the abundant oogonia. For cytological data see Dangeard ('90), p. 101 and ('16), p. 87.

Trow has shown that in material that he calls *S. Thureti* or *S. mixta*, depending on the absence or presence of antheridia, the unfertilized eggs are uninucleate as in other species and are without nuclear fusions in his opinion ('95, p. 637). In all respects except fertilization the cytological phenomena are alike. See *S. mixta* for some detail.

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*Of the typical form of *S. ferax* Maurizio says the oogonia are often in chains, eggs 3 to about 50, 23–26.5μ in diameter (Jahrb. f. wiss Bot. **29**: 93. 1896). Minden says (l. c. p. 521) the oogonia are seldom intercalary in chains, the eggs 20–27μ in diameter, not rarely over 40–50 in an oogonium. Oogonia in chains are not mentioned by deBary, Pringsheim, Humphrey or Fischer (the latter giving the eggs as 20–27μ thick). To separate new species on absence of such chains would seem, therefore, to be poor judgment. It may be said that Maurizio's work gives many indications of inferiority.

†Also listed in Ann. Mich. Acad. Sci. **17**: 195. 1915, as *S. Thureti*.

Davis ('03) worked with a plant that would better have been called *S. ferax* than *S. mixta*, as it bore no antheridia at the time he was putting up his preparations. His results were in general the same as Claussen's for *S. monoica* and Trow's for *S. mixta* and *S. diclina*, aside from fertilization phenomena (which were of course absent) and with the exception of certain details of the structure and division of the nucleus, in which he corrected Trow in several points. His attacks on Trow's conclusion that fertilization did occur where antheridia were present have proved since to be entirely unjustified. Davis found four chromosomes in the division in the oogonium, a matter of much interest in this parthenogenetic form, as Claussen found the number to be 10–14 in *S. monoica* where fertilization occurs. The spindle is entirely intranuclear. The eggs are frequently binucleate and rarely trinucleate.

The pioneer work in the physiology of this group was done by Klebs who worked on a plant that normally on flies in water showed only 10–20% of oogonia with antheridia. He considered the plant *S. mixta*, but according to our rule it would fall under *S. ferax*. For this reason we refer here to his conclusions although we do not know that his plant really was *S. ferax*. The important results of his work are summarized by him as follows (translated) ('99, p. 582):*

1. Uninterrupted continuous growth:—in all good nutrient media, so long as fresh unaltered nutrient is present, e. g., in water with peas, in weak meat extract (1–2%), in gelatin with peptone, in mixtures of water with albumen, casein, etc.
2. Prompt and complete transformation of the mycelium into sporangia and zoospores:—by placing a well-nourished mycelium in fresh water.
3. Growth with continuous formation of zoospores:—in very weak solution of certain nutrients, *e. g.*, 0.005% haemoglobin, also in mycelium on agar-albumen jelly that is put in running water.
4. Active formation of oogonia with limited growth:—by putting a well nourished mycelium in agar-agar.
5. Active growth, then active formation of oogonia:—
   (a) oogonia with antheridia:—in solution of leucin (0.1%) with tricalcium phosphate (0.1%).
   (b) oogonia without antheridia:—in solution of haemoglobin (0.05–0.1%).
6. Growth, then formation of sporangia, then of oogonia:—by placing the mycelium in water from gelatin-meat extract; or by culture on dead insect in water.
7. Growth and simultaneous formation of sporangia and oogonia:—in water with some fibrin or syntonin.
8. Growth, then formation of oogonia and later sporangia:—after strong nutrition of the mycelium transfer to 0.01% haemoglobin.
9. Active formation of gemmae:—by putting a well nourished mycelium in 0.6% tricalcium phosphate, or 1% sodium chloride, etc.

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*An even more condensed and somewhat modified restatement of his results is made by Klebs in his Willkurrliche Entwickelungsänderungen bei Pflanzen, p. 41. Jena, 1903.

10. Growth with sporangia, then gemmae; or growth with oogonia, then gemmae; or growth with sporangia and oogonia and then gemmae:—in the items 3, 5 and 6 above, when the culture is continued to the complete exhaustion of the nutrient material.

General conclusions in regard to conditions for the formation of oogonia and antheridia are summarized as follows (l. c., p. 566):

1. In a mycelium which is constantly given fresh nourishment no oogonia are ever formed. Young, just formed oogonia were, by means of fresh, soluble food, induced to vegetative growth. The old were, however, killed.
2. If a strongly nourished mycelium is changed to a medium of low nutritive value (in which the formation of sporangia is rare or absent) oogonia are formed in a few days.
3. In a good soluble food, preferably at such concentration that the sporangia cannot be formed, the mycelium begins to form oogonia as soon as the solution is chemically changed on account of its growth, and has lost its nutritive value.
4. The formation of oogonia is particularly encouraged through phosphate, which is likewise necessary to the formation of the antheridia. In a soluble food that is poor in phosphate oogonia are formed, but no antheridia; particularly abundant are such oogonia in a pure solution of haemoglobin.
5. In many soluble foods, for example in peptone, gelatine, etc., are excreted certain products of assimilation of the mycelium which hinder the formation of oogonia.

All of the following cultures were made by us from a single spore culture of No. 10 of January 15, 1913:

In equal parts of 5% maltose and .01% peptone. The solution was inoculated with swimming spores on February 14, 1913. By the 18th or 19th the culture had covered the petri dish, and had formed a large number of oogonia with perfect eggs. Oogonia were somewhat inflated with eggs not filling the cavity; eggs of usual number, varying in size from 15.5–27.7μ, averaging about 24.4μ. This is smaller on average than other cultures. Of scores of oogonia examined only one or two had antheridial branches attached, and no antheridia were cut off. No sporangia were formed, and therefore all oogonia were spherical. This is the only *Saprolegnia* that forms normal oogonia and eggs in this medium.

On ant in rain water. Sporangia abundant. Oogonia plentiful, heavily pitted, nearly all spherical, and containing 1–6 eggs, most with 2 or 4 eggs. Many oogonia do not perfect the eggs. Scattering gemmae. Repeated this experiment with same results except that there were more cylindrical oogonia. Almost no antheridia. It is evident that the purer the water the fewer the antheridia.

On ant in spring water. Abundant sporangia. Oogonia very abundant, nearly all spherical, a few cylindrical; pits abundant: oogonia very healthy, all maturing the eggs, which vary from 1–12, mostly 1–6. Antheridia on probably 33% of the oogonia. Very few gemmae. Repeated this experiment with same results, except that there were more cylindrical oogonia.

On ant in distilled water. Growth shorter and stouter than in the two preceding cultures. Sporangia abundant and stout. Oogonia very abundant and large, with thick, heavily pitted walls, spherical or cylindrical, the proportion of cylindrical ones being much larger than usual, comprising about ¼ of all, very healthy and maturing all eggs, cylindrical ones generally with 4 eggs, often with 2 or 3, spherical ones sometimes with 12 eggs, but generally from 1–8. The eggs of this culture average larger than in maltose-peptone, varying from 14.8–33.3μ, averaging about 25.8μ. The few eggs that reach the largest size are only one to the oogonium. The smallest size may occur in an oogonium with much larger ones. Apparently no antheridia. Repeated above culture with same results.

On yolk of egg. Growth abundant and extensive. A good many sporangia and a vast number of oogonia formed. A very few oogonia (probably not more than 1 in 100) had an antheridium attached. Antheridia androgynous or diclinous. No oogonia inside sporangia, hence no cylindrical ones.

On corn meal agar. The whole petri dish covered and within the agar a great number of fine, healthy oogonia were formed, with perfect eggs of normal size. No antheridia. A few small gemmae formed. This is our only *Saprolegnia* that forms oogonia in this medium except *S. monoica* and in that case many of the oogonia are inflated and with no eggs.

On corn meal egg yolk agar. Fine growth, covering agar. Very many oogonia, with fine eggs. Antheridia on not more than 5%.

In the following cultures a .1% solution of the salts in distilled water was used, and the food material is yolk of egg, unless otherwise indicated:

In KNO_3. Growth delicate, but healthy. Many normal but small oogonia, about one-third with diclinous antheridial branches. A few sporangia formed.

In KH_2PO_4. Growth strong and healthy. Sporangia present, but scattering, their spores emerging and dispersing slowly. Many normal oogonia, none cylindrical, the great majority with one or more antheridial branches about them, which are nearly all diclinous (not one in a hundred androgynous), no antheridia cut off from the branches.

In Na_2HPO_4. Many sporangia, and very many normal oogonia, nearly all (at least 98%) with antheridial branches, of diclinous origin; occasionally an antheridium was cut off.

In K_2SO_4. Growth strong and healthy. Sporangia few, emptying as usual. Oogonia very abundant, with normal and good eggs. In the main mass of the culture there were antheridial threads on at least 90% of the oogonia, a good many with antheridia cut off. No gemmae.

In $Ca_3(PO_4)_2$. Growth strong and vigorous. A good many sporangia. Very abundant oogonia, maturing all eggs. Those in bulk of culture with 95% or more furnished with antheridia; on margin a much smaller proportion have antheridia. Scattering gemmae.

In $Ca(NO_3)_2$. Growth more delicate than in others of this series, but about same extent. Oogonia much fewer than in preceding, all with good eggs. Even in the denser mass of culture not more than 10% with antheridia and very few with antheridia near the periphery. Many oogonia-like gemmae.

The following experiments were made to test the vitality of the eggs:

A culture on corn meal agar with fine oogonia and eggs was allowed to dry out to a sufficient extent to collapse the gemmae. Water was put on and it was examined at intervals. The eggs went to pieces soon. They had been killed by the desiccation.

Eggs which had been resting for about four months were put in fly extract on September 13, 1912, in two watch glasses. One had a cover glass put over the eggs to exclude air. Kept until September 24th. Neither showed germination though the eggs seemed perfectly good.

Experiments to test best method of preserving live cultures:

Culture put in vial on corn meal agar, March 18, 1913, was found to be dead December 1, 1913.

Culture put in aquarium jar with algae in laboratory on March 3, 1913, was tested in jar on September 18, 1917, but no growth appeared.

6. **Saprolegnia mixta** deBary. Bot. Zeit. **41**: 38 and 54. 1883.

? *S. heterandra* Maurizio. Jahrb. f. wiss. Bot. **29**: 87, pl. 1, figs. 18–27. 1896.

? *S. dioica* Schroet. Jahrb. d. Schles. Gesell. f. vaterl. Cultur, 1869, **47**: 143. 1870.

As originally defined by deBary this species was placed in the *Ferax* group and separated from *S. ferax* on the one hand by having antheridia on about fifty per cent of the oogonia instead of none or a very few, by the swollen oogonia with fewer eggs, and by the more delicate mycelium; on the other hand it was distinguished from *S. monoica* by fewer antheridia, more numerous and often larger pits in the oogonia, and by the weaker mycelium. *Saprolegnia hypogyna*, with very similar oogonia, is easily separated from all others by the sub-oogonial cell.

In recent years there has been great confusion in regard to *S. mixta*, and subsequent collections have shown pretty clearly that this species and probably also *S. ferax* are composed of a number of forms, themselves variable, of which deBary had only one. In a very sensible discussion of this subject Pieters (Mycologia 7: 307. 1915) has concluded that from what is known at present it is best to consider as *S. mixta* those forms with weak mycelium and with antheridia on one-half or more of the oogonia, while to *S. ferax* should be referred those with stronger mycelium and only a small number of antheridia on fly cultures at a temperature of 12–15 degrees centigrade. *Saprolegnia monoica* will still include only those forms with antheridia, usually androgynous, on every oogonium in all ordinary natural media. To these conclusions we agree (if *S. mixta* is to be retained at all), but it is not to be supposed that this convenient arrangement will put an end to all confusion in so various a set of forms, or that it adequately expresses the complexity of the group.

To show still further the frail basis on which this species stands we have but to refer to the most recent monographs of the family by Minden, who says that "*S. mixta* would, indeed, have to be united with *S. Thureti* [*S. ferax*] had not deBary asserted its constancy over a long period." (Krypt. Fl. Mark B. 5: 519. 1912). From our own experience we would not hesitate to reduce *S. mixta*, and retain it only in deference to the opinion of deBary and Pieters (but see remarks below as to the plant Pieters observed).

By applying the above rules we find that we have secured several times in Chapel Hill and once in Hartsville, S. C., forms that can be referred to *S. mixta*. We do not illustrate these forms, as the drawings could not be distinguished from similar ones taken from *S. ferax*. Of these, No. 1 of November 2, 1916, may be described as follows:

Growth moderately strong (not so delicate and more extensive than in *S. delica*), sporangia long, cylindrical, repeatedly proliferating, spores 10–12μ thick. Gemmae typically elongated rod-shaped, sausage-shaped, pyriform, or oval, not often spherical, usually in chains. Oogonia spherical to oval, moderately plentiful (not nearly so abundant as in *S. delica*), borne on short lateral stalks, or terminal, or rather infrequently intercalary, with or without a conspicuous neck; wall not very thick, about 1.8μ, with numerous conspicuous pits (the small ones with few) which are about 4.5–6.5μ in diameter; eggs centric, 20–30μ thick, most about 24–25μ, often oval from pressure, 1–20, mostly 4–10, not so numerous or so nearly filling the oogonium as is usual in *S. ferax*. Antheridial branches short, arising usually from main branches near the oogonia and running to nearby oogonia either on the same thread or on others near, occurring on about 40% or more (not on all) of the oogonia on flies or grubs.

Found three or four times in Chapel Hill, as in Arboretum spring (No. 1 of November 2, 1916), and from Bowlin's Creek, under bridge on Durham road (Nos. 7 and 8 of November 16, 1917).

Occurrence in America (of the species in all reported forms): Chapel Hill, N. C., Hartsville, S. C., Mississippi, Pennsylvania, Louisiana, Michigan. DeBary once found the species on sick fish (1888, p. 617). For other illustrations see Humphrey, pl. 16, figs. 40–42; Minden ('12), figs. 1d and 1i on p. 520.

As will be seen from above, our form agrees as well with deBary's description as could be expected. Especially is this true in regard to the rather few eggs which do not so nearly fill the oogonium as usual in *S. ferax* (but the latter also varies to similar oogonia). In our Nos. 7 and 8 of November 16, 1917, the number of oogonia with antheridia when grown on grubs was about 40%, while in No. 1 of November 2, 1916, the number was around 90%.

Humphrey describes his plants as having antheridia "absent from a part, sometimes from a large part of the oogonia."

Kauffman has found in Michigan a form of this species which he cultivated in various media and has described as follows (Ann. Bot. 22: 367. 1908):*

"Hyphae rather slender; zoosporangia nearly cylindrical. Oogonia with rather thick walls, terminal, intercalary or lateral, flask-shaped, rarely spherical, the lateral on short oogonial branches; pits medium to large, rather numerous but not easily seen; antheridial branches usually androgynous when the oogonium is lateral, diclinous when the oogonia are terminal or intercalary, long and slender when diclinous, short and slender and not coiled when androgynous; antheridia on 75 per cent to 90 per cent of the oogonia, long, subcylindrical, not very profuse on a

*Also noted in Ann. Mich. Acad. Sci. **8**: 27. 1905.

single oogonium, often only one present. Oospores up to 15 and 20 in an oogonium, average diameter 24 microns, with rather thick walls."

Pieters (l. c.) found that in two collections, one from Germany and one from Ann Arbor that he considered *S. mixta*, there were antheridia, usually of diclinous origin, on at least 90% of the oogonia; the mycelium being flaccid and delicate, the pits while present less prominent than in *S. ferax*. These characters are suspiciously like our *S. delica* and it seems to us rather likely that Pieters had that species and not *S. mixta* as here considered. We cannot think that *S. delica* is deBary's *S. mixta*, as he emphasizes the close resemblance of the latter to *S. ferax* and *S. monoica*, and they are very different from *S. delica*. Particularly is this true in regard to the oogonia, which are thin-walled and with few and inconspicuous pits in the latter, while in the *Ferax* group the walls are thicker and with far more abundant and conspicuous pits. Most conspicuously is this true for *S. ferax*, and deBary says that the oogonial structure of *S. mixta* is most like that of *S. ferax*, with the pits often very large. This would never do for *S. delica*. Moreover, the often very long and preponderatingly diclinous antheridial branches of *S. delica* are very unlike those of the *Ferax* group.

Trow ('95) finds fertilization to occur in case antheridia are present. He worked on material not certainly pure and called it *S. ferax* when antheridia were absent and *S. mixta* when they were in part present. His conclusions covering work on both *S. mixta* and *S. diclina* (*S. dioica*) may be condensed as follows (in so far as we think them correct): The vegetative nucleus has a distinct membrane, with chromatin material and a linin network; it divides repeatedly and when sporangia are formed enough nuclei enter to furnish one for each spore, no division or fusion taking place in the sporangia. The oogonium receives many nuclei which divide once within it and form about 20 times more than are necessary to supply the eggs with one each; the excess degenerate. Most of the nuclei in the antheridia and fertilizing tubes also degenerate. A single male nucleus enters the egg and approaches the egg nucleus, but does not fuse with it completely until a late stage. On germination the fusion nucleus divides to form a number which become the nuclei of the resulting zoospores. Trow was misled, in this paper, as to the structure of the nucleus, the number of chromosomes and the method of division. See under *S. ferax* for Davis's work on a plant closely related to, if not the same as, *S. mixta*.

Maurizio describes in some detail a plant he took for *S. mixta* (1895, p. 11, figs. 1–3), but in it the antheridia, which were present on about a third of the oogonia, were borne always (?) on stalks arising from the oogonial stalks. The oogonia were heavily pitted, about 35.5×108.5µ thick; the eggs up to 40, with a diameter of 22.5–27.5µ.

In his pioneer work on the response to various media in this group Klebs studied a plant that he regarded as *S. mixta*, although on a natural substratum, as flies in water, it produced constantly only 10–20% of oogonia with antheridia. For a summary of his important results see under *S. ferax*, where, according to our rule, it is to be presumed that Klebs's strain should be placed. For experiments on the behavior of the spores of *S. mixta* under various chemical stimuli see Müller ('11).

Saprolegnia heterandra Maurizio is so near *S. mixta* as to be best treated as a form of it, although Maurizio places it nearest *S. torulosa*. It may be briefly described as follows: Antheridia on about half the oogonia, androgynous or diclinous. Eggs 23.5–28µ in diameter, 1–40 in an oogonium, usually 4–10; germinating in 60 days by a short germ tube; oogonia arranged very variously but not in chains; pits not numerous and of medium size.

According to Humphrey, what Schroeter ('89) took to be *S. ferax* is more like his (Humphrey's) *S. mixta*. In such case *S. dioica* Schroeter ('69, p. 143), listed without description, is also *S. mixta*, as Schroeter later ('89) quotes it as a synonym of his *S. ferax*.

7. **Saprolegnia monoica** Pringsheim. Jahrb. f. wiss. Bot. **1**: 292, pls. 19 and 20. 1858.

? *S. dioica* Pringsh. Jahrb. f. wiss. Bot. **2**: 266. 1860. (This may be *S. ferax*. See Fischer ('93), p. 336.)

Achlya intermedia Bail. Naturf-Ver. Königsb., p. 5. 1861.

Diplanes saprolegnioides Leitgeb. Jahrb. f. wiss. Bot. **7**: 374, pl. 24. 1869.

Saprolegnia semidioica Petersen. Bot. Tidssk. **29**: 378. 1909; also Ann. Myc. **8**: 519. 1910.

The typical form of this species as understood by European botanists has not been recognized in any of our collections, and its presence in America is somewhat doubtful. The form reported by Humphrey seems to be the var. *glomerata*, and it does not appear what form Atkinson had from Alabama. We include the species as American from the Michigan record by Pieters (Mycologia 7: 307, pl. 170, fig. 2. 1915; also listed in Ann. Mich. Acad. Sci. **17**: 195. 1915) who, while he does not give the necessary data to determine his form, does show in his drawing an oogonium with at least eight eggs which by measuring and reducing to scale seem to be about 15–18µ thick, a size in accordance with the type as understood by Fischer.

Pieters has shown ('15a, p. 312) that in 0.05% haemoglobin the number of oogonia with antheridia is reduced to 0–17%. For other experiments by Pieters on this species in various media see Am. Jour. Bot. 2: 529. 1915. Pieters also writes us of an interesting observation of his on this species, that in plates of pea agar that had become infected with bacteria the oogonia were very abundantly produced on the hyphae along which the bacteria were thickest, but not along the hyphae where the bacteria were few.

Next to Pringsheim's, which is ill-defined, the most authentic description of the species may be taken as that by deBary ('88, p. 616), who writes as follows (translation):

"Main threads straight, tense. Primary sporangia slender, clavate-cylindric. Antheridial branches androgynous, forming antheridia on all the oogonia, almost always arising near and springing from the same stalks as the oogonia to which they are attached or from neighboring ones. Oogonia usually borne on racemosely arranged, bent or straight short branches which are about as long as the diameter of the oogonia; the main hyphae from which these spring ending in an oogonium, or a sporangium, or a sterile point. Oogonia spherical, smooth, with several large pits in the membrane. Oospores from one to over 30, mostly 5–10 in an oogonium, centric. Antheridia bent-clavate, with the concave side applied to the oogonium."

Fischer gives the eggs as 16–22μ thick. Minden ('12, p. 608) thinks that *S. semidioica* of Petersen is the same as *S. monoica*, and to all appearances he is right. According to Lindstedt ('72, p. 64) *Achlya intermedia* Bail ('61) is a synonym of *Diplanes saprolegnioides*. For other illustrations of *S. monoica* see Reinke ('69), pl. 12; deBary ('81), pl. 5, figs. 11–19, and pl. 6, figs. 1–2; Pringsheim ('58), pls. 19 and 20; Ward ('83), pl. 22, figs. 11–22; Rothert ('88), pl. 10, fig. 14; Massee ('91), pl. 5, figs. 91–93; also Dangeard and Claussen as cited below.

Fertilization has been convincingly proved for this species by Claussen ('08). His results may be summarized as follows: Young oogonia are full of protoplasm and contain many nuclei; degeneration of both protoplasm and nuclei takes place from the center outward until there remains only a rather thin layer of peripheral protoplasm containing comparatively few nuclei; these remaining nuclei now divide once mytotically at the same time, showing about 10–14 chromosomes; of the resulting nuclei all degenerate except one for each egg. A centrosome with radiating fibers is observed with the nucleus in this division in the oogonium, and it remains visible in the nucleus of the young egg. It is not to be observed at time of fusion of the egg and sperm nuclei. The antheridia contain a varying number of nuclei which divide simultaneously with those of the oogonia; fertilizing tubes are formed which are simple or much branched. One male nucleus is discharged into each egg and soon reaches the egg nucleus, the two remaining pressed together for a long time before completely fusing and losing their identity. No reduction of chromosome number takes place in the antheridia and oogonia before fertilization; though not observed, such reduction probably occurrs in the germinating egg. For other cytological data see Dangeard ('90), p. 111, pl. 6, figs. 1–5.

8. **Saprolegnia monoica** var. **glomerata** Tiesenhausen. Arch. f. Hydrobiologie und Planktonkunde **7:** 277, figs. 6–8. 1912.

PLATES 4 AND 13

The typical Chapel Hill strain of this variety may be described as follows (No. 7 of April 3, 1913):

Growth moderately extensive, the hyphae not very robust; sporangia abundant, cylindrical or long club-shaped, later ones more irregular, proliferating from within or not rarely from one side also, varying greatly in size, rarely so small as to have only a single row of spores; spores 10–11μ in diameter; gemmae abundant or few, often in moniliform chains, pear-shaped or irregularly club-shaped, often nodulated or branched, quickly forming spores when brought into fresh water; oogonia abundant, usually lateral on short stalks which are mostly a quarter to equally as long as the diameter of the oogonia, rarely intercalary, occasionally terminal and then usually cylindrical in old sporangia; wall colorless, moderately thick, the pits few or numerous in the same culture, and rather conspicuous, 5.5–7μ in diameter. Eggs centric, generally one, two, or four, occasionally six or eight, rarely 20 (or more?), diameter 24–31μ; usually about 25–27μ. Antheridial branches short, typically clustered and contorted, often branched, arising androgynously from the main branches near the oogonia or at times from the oogonial stalks, not rarely reaching also to nearby oogonia on other threads (diclinous); antheridia pear-shaped or tuberous, one or more on every oogonium; antheridial tubes formed.

The plant is evidently rare in Chapel Hill, as we have recognized it with certainty only once—in the brook in Battle's grove (No. 7 of April 3, 1912). Also reported by Humphrey from Cambridge and Amherst, Massachusetts, as *S. monoica* (see below). It is easily distinguished from *S. ferax*, which seems nearest, by the short, clustered, androgynous antheridial branches with antheridia on every oogonium. The antheridia are usually several, sometimes numerous and of both androgynous and diclinous origin, nearly always one or more androgynous ones on an oogonium and very often diclinous ones also.

There are important differences between this plant and European interpretations of the typical *S. monoica*, these appearing in the larger size and fewer number of the eggs in the American form on the average. Humphrey refers to *S. monoica* a plant with eggs about 26μ on the average, agreeing with ours, and he gives the number as "commonly not above 10, rarely numerous" (see his figures 37–39, pl. 16). See under *S. monoica* for note on the Michigan plant found by Pieters. There seems no doubt that our Chapel Hill plant and Humphrey's plant are the same as Tiesenhausen's var. *glomerata*, which he describes as follows (translation):

"Turf delicate, up to 1 cm. broad. The side branches are thickened, branched and contorted to form small knots, some of which are sterile and mixed with similar ones bearing oogonia. Sporangia as usual, with addition of conidia-like sporangia of various shapes, as pyriform or round. Oogonia on principal and side branches, 37–80µ thick, stalks variously contorted and bent, often knee-shaped or knotted, much branched, wall as a rule without pits, seldom with several. Eggs 22–24µ thick; 1–21, mostly 3–6 in an oogonium. Antheridia always present, springing from the oogonial stalk or main thread or also from nearby threads." Found in a small lake near Zermatt, Switzerland.

Continuing the discussion the author brings out the fact that his variety differs from *S. monoica* in the peculiar contorted clumps of side branches and stalks and also in the larger eggs (10–22µ in *S. monoica* as he finds). Oogonial initials that are already provided with antheridia may halt and become sporangia. It is plain that this is our form of *S. monoica*, the figures also agreeing well. It would appear from Maurizio's description of his *S. floccosa* that it is very near if not the same as the above (see p. 74).

Rarely one or more very small eggs about half the normal size are found in our plant mixed with the others, and oogonia have been observed which contained only such subnormal eggs (fig. 10). Dwarfed sporangia with a single row of spores are met with, as are also dictiosporangia. Thick clusters of short, distorted branches that seem to be antheridial branches are not rarely seen in some cultures without the near presence of oogonia. This seems to be an abnormality induced by the medium. In cases where the cylindrical oogonia are found inside emptied sporangia there may be diclinous antheridia wrapped about the sporangium, and at times one may enter the sporangium tip and run down inside to the oogonium. Androgynous antheridia are usually also present in such cases, running up inside from below.

The following experiments were made with single spore cultures from No. 7 of April 3, 1912:

In equal parts of 5% maltose and .01% peptone solution. Completely filled the petri dish with very delicate threads. No gemmae, sporangia, or oogonia of good shape, but a few bladdery initials were found which later sprouted by one or more tubes from any point on the surface. This experiment was twice repeated with the same results, except that there were more initials formed. The stalks of these bodies were often twisted and curved.

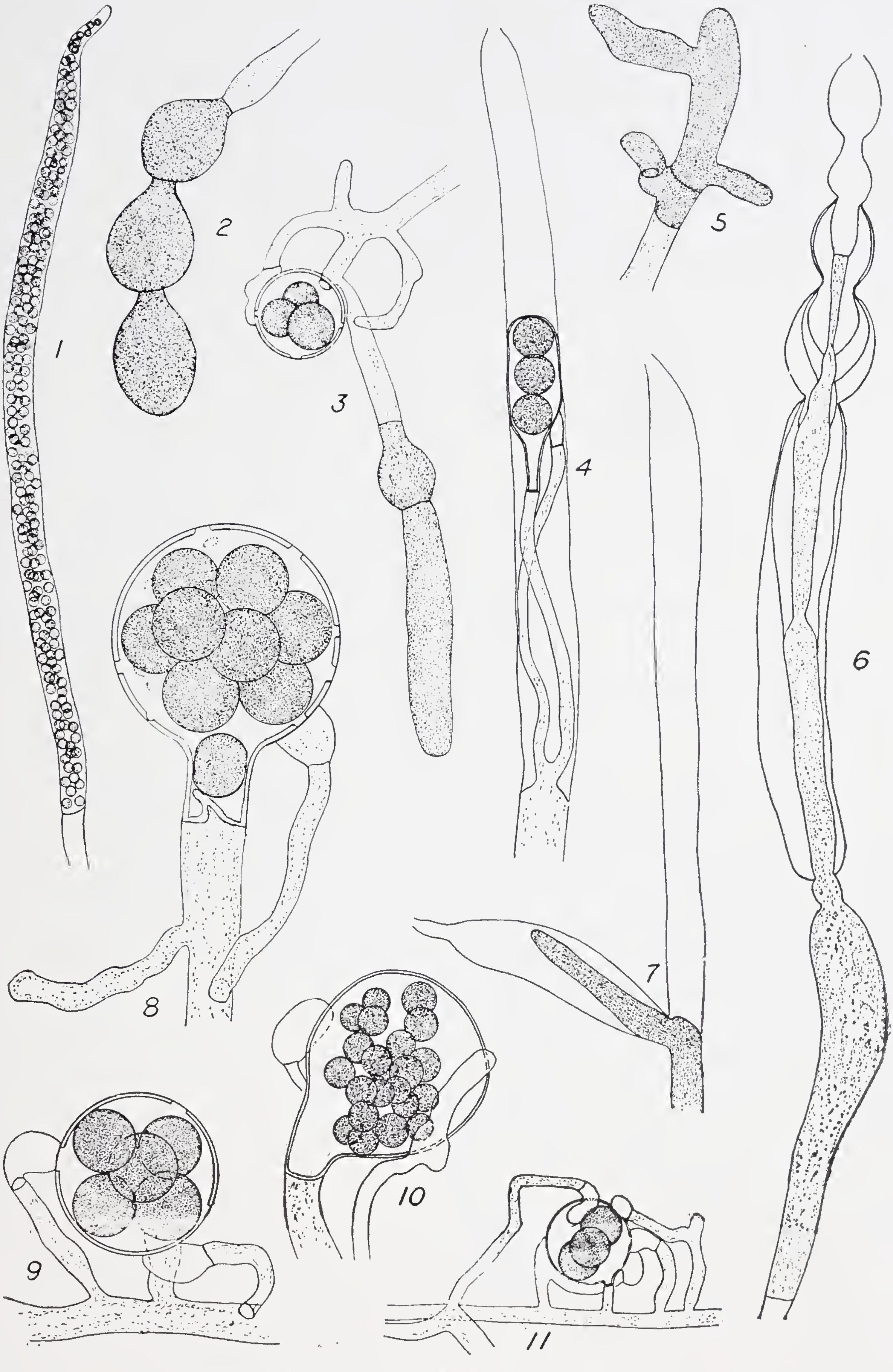
PLATE 13

PLATE 13

Saprolegnia monoica var. glomerata

Fig. 1. Spores encysted within a typical sporangium. × 167.
Fig. 2. Gemmae in a chain. × 247.
Fig. 3. Oogonium with gemmae growing out from beneath it. × 247.
Fig. 4. Empty sporangium containing a cylindrical oogonium and an antheridium. × 247.
Fig. 5. Gemmae. × 247.
Fig. 6. Proliferating sporangia. × 447.
Fig. 7. Sporangium growing from beneath an old one as in *Achlya*. × 247.
Fig. 8. Apical oogonium with conspicuous pits and with a thick-walled outgrowth from below. × 447.
Fig. 9. A typical oogonium with antheridia. × 447.
Fig. 10. Oogonium with abnormally small eggs. × 447.
Fig. 11. Oogonium with androgynous and diclinous antheridia. × 247.

PLATE 13



SAPROLEGNIA MONOICA VAR. GLOMERATA.

PLATE 14

ACHLYA RACEMOSA [ABOVE]. × 6.
SAPROLEGNIA DICLINA [BELOW]. MAGNIFIED.

In pea broth, February 28, 1913. Extensive growth. Large number of gemmae. No sporangia or oogonia. The culture remained healthy and alive in this broth for about two months.

In corn meal agar. Grew vigorously and formed an immense number of oogonia. Most were normal with good eggs and with antheridia. Many were inflated and no eggs formed. Many gemmae were also present. This is the only *Saprolegnia* except *S. ferax* that forms eggs in this medium, and the oogonia here are not so perfect as in that species. Culture repeated twice with same results.

In ½ corn meal + ½ egg yolk agar, March 4, 1913. Vigorous growth covering dish. An immense number of good oogonia on rather longer stalks than usual. The stalks bear at a little distance below the oogonium (about the middle) a forest of short lateral threads, often branched, which may or may not reach the oogonium. When near enough they will also apply themselves to other oogonia. This, so far, is the medium that produces the most striking characters of this species. No gemmae.

On whole egg agar, March 13, 1913. Strong growth. A good many sporangia. An immense number of oogonia with average of 4–6 eggs. Antheridia on every oogonium, generally several; almost always some androgynous ones, and perhaps 2% of the oogonia with diclinous ones in addition. Many short branches here and there like abortive antheridia.

On a bit of whole egg agar in distilled water. Oogonia nearly always on short lateral branches (95% or more), occasionally terminating main hyphae (5% or less). Eggs generally 4, rarely 6.

The following tests (single spore culture of No. 7 of April 3, 1912) were made to find the best way to preserve the life of cultures:

(a) Two cultures were made January 15, 1912, in sterile bottles containing pea broth (50 peas boiled an hour or more in 500 cc. water). Growth was good, and when examined after a month showed results as follows: In pea broth alone, there was only vegetative growth and all was dead; on ant larva in pea broth there were formed plenty of oogonia and perfect eggs that were still alive, other parts dead. (b) In May, 1913, a culture was put in a vial of water by cutting out a piece of corn meal agar on which it was growing. An ant larva was also dropped in at the same time. The vial was closed with a plug of cotton and left in a dark place in the laboratory over summer. A test for life was made in December, 1913, by dropping a mushroom grub in the vial, and renewed growth resulted. The old eggs were alive (as shown by their normal structure) both in the agar and on the ant that was put in at the same time with the agar, but none had sprouted. (c) Culture was put in vial on corn meal agar March 18, 1913, and found to be dead December 1, 1913. (d) A culture on an insect was put in an aquarium jar with algae on March 3, 1913. Test for life was made in September, 1917, by dropping in mushroom grubs but no growth appeared.

9. **Saprolegnia monoica** var. **vexans** Pieters. Bot. Gaz. **60**: 489. 1915.

We have not found this, and the following is taken from the original description by Pieters:

"This was secured from algal material collected at Sukey Lake, near Ann Arbor, Michigan. The vegetative growth, sporangial characters, and the formation and shape of gemmae do not differ in any particular from those present in *S. monoica*, *S. ferax*, or any other species of that group except *S. mixta*, which has weaker hyphae. The material was cultivated for nearly a year and a half on flies, in agar, and by transfer from a strong culture medium such as pea decoction or peptone, into haemoglobin, leucin, peptone, or other solution. During all this time no oogonia were produced. Toward the end of this time a series of tests was made with several cultures by transferring vigorous mycelium to leucin to which various sugars and salts had been added. Among other combinations there was used leucin $\frac{M}{200}$+levulose $\frac{M}{200}$, and in this a mycelium out of pea extract produced an abundance of oogonia. When these were examined they proved to be indistinguishable from the oogonia and antheridia of *S. monoica* Pringsh. Rarely an oogonium was found on which there was no antheridium, but in some solutions this may also be the case with *S. monoica*.

"The fact that cultures of *S. monoica* were going on at the same time suggested the possibility of contamination. Check cultures were made, therefore, by taking mycelium from the dish in which the oogonia were formed and growing this on fly. Had the mycelium producing oogonia been that of *S. monoica* (No. 79c of my series), plenty of oogonia would have been produced. In fact, no oogonia were formed on the fly culture, but a fresh culture from this fly through pea decoction into leucin and levulose again produced oogonia as before.

"We seem to have here, therefore, the remarkable case of a variety of *S. monoica* having lost sexuality, but recovering it under stimulus of this special combination, leucin and levulose in concentration $\frac{M}{200}$ each.

"The gemmae of this form are perhaps a little more varied in shape than is the case with the species, but the shape of these organs is so variable in most species that they are of no value for systematic purposes.

"Had time permitted, it would have been interesting to cultivate this form for many generations in leucin-levulose solutions to determine whether the vigorous production of oogonia which characterizes such forms of *S. monoica* as my 79c would be regained by this variety.

"The forms described in this paper are remarkable examples of the intimate dependence of the members of this group on external conditions."

10. Saprolegnia litoralis n. sp.

Plates 15 and 16

Growth about as in *Saprolegnia ferax*, more vigorous, extensive and irregular than in *Saprolegnia delica*, the hyphae reaching a length of 1–1.5 cm. on a mushroom grub. Sporangia not abundant, far less so than in *Saprolegnia diclina*, early ones nearly cylindrical, or more often

PLATE 15

PLATE 15

Saprolegnia litoralis

Fig. 1. An oogonium with an apical papilla. × 278.
Fig. 2. Gemma with 3 papillae which has emptied. × 278.
Fig. 3. Sporangium with long papilla. × 278.
Fig. 4. Proliferating sporangia. × 278.
Figs. 5 and 6. Gemmae. × 278.
Fig. 7. Gemma with 2 papillae which have formed spores and emptied. × 278.
Fig. 8. Sporangia and gemmae. × 278.
Fig. 9. Empty gemmae with two papillae from the same spot. × 278.
Fig. 10. Gemmae. × 278.

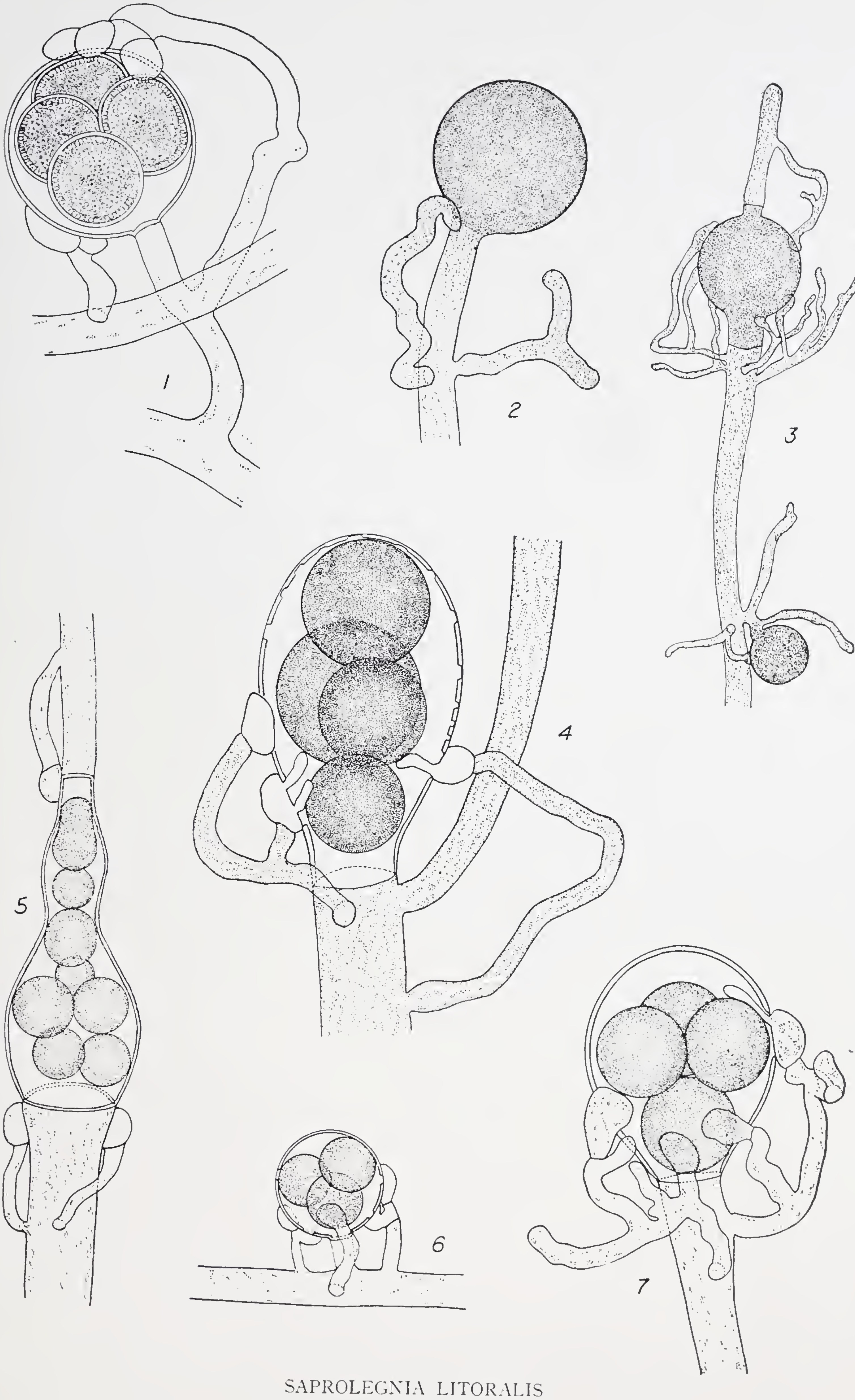
SAPROLEGNIA LITORALIS.

PLATE 16

PLATE 16

Saprolegnia litoralis

Fig. 1. Oogonium with mature eggs. × 503.
Fig. 2. Young oogonium. × 503.
Fig. 3. Young oogonia with typical antheridia. × 503.
Fig. 4. Oogonium with antheridial tubes after the antheridia have emptied. × 503.
Fig. 5. Intercalary oogonium. × 278.
Fig. 6. A short-stalked oogonium. × 278.
Fig. 7. Apical oogonium with unemptied antheridia. × 503.



SAPROLEGNIA LITORALIS

irregular in diameter, usually curved, repeatedly proliferating, later ones more irregular and often pointed; spores 10–12μ in diameter. Gemmae very abundant, spherical, pyriform, clavate, etc., often in chains, the terminal one very often with an elongated papilla. Oogonia plentiful as a rule, about as much so as in *Saprolegnia ferax*, but at times not found on grubs in distilled water (more scattered than in *Saprolegnia delica* and *Saprolegnia anisospora*), about 35–80μ thick, the larger number terminal on main hyphae, others (usually appearing later) on short lateral branches; shape spherical, or if borne on the ends of main threads usually oval, the latter frequently with a slender, more or less lengthy terminal extension, which when short may be included in the cavity of the oogonium, but which is often extended into a thread 2.8–3μ thick, thus making the oogonium intercalary; furnished with rather few, very conspicuous and usually large pits, up to 11μ across. Eggs centric, large and dark, 1–20, mostly 2–6 in an oogonium, their diameter 20–40μ, most about 30–33μ, often elliptic from pressure. Antheridia on every oogonium (one to several), androgynous on short branches which usually arise very near the oogonium, frequently, when the oogonium is on a short stalk, arising from immediately below it. In addition to the androgynous antheridia a few diclinous ones may arise rarely from other nearby threads.

We have not found this in Chapel Hill, our only collections having been taken by us in fresh water with algae in a roadside ditch near Southport, N. C. (No. 3 of April 6, 1918), and in a ditch at the golf links, Wilmington, N. C., Dec. 27, 1922.

There seems little doubt that this is near the European *Saprolegnia paradoxa* of Maurizio* (see p. 75) which agrees in the characteristic antheridial branches and in the apical extension on some of the oogonia. We would refer our plant to this species except for the much smaller eggs and frequent presence of an ingrowing process from below in the oogonia of the latter.

The present species is obviously near *Saprolegnia monoica*, but is clearly distinct in the much larger eggs, the absence of an ingrowing tube from the oogonial wall below, in the presence of a slender extension on the tips of many of the oogonia and gemmae, and in the typically more abundant and more branched antheridial branches. These latter, when the oogonium is apical on a main thread, may be so abundant and branched as to give the effect of a basket holding the oogonium. It is possible that this is the plant Minden found and describes as *S. spiralis* Cornu and thinks is the same as *S. retorta* Horn. If his plant is indeed the same as ours it is not *S. retorta*, which is easily different, nor does our plant correspond to Cornu's imperfect description of *S. spiralis*.

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*Not of Petersen, which was published about ten years later: see under *Aplanes Treleaseanus* (p. 79).

The following cultures were made from No. 3 of April 6, 1918:

On corn meal agar. Growth vigorous, covering agar plate; sporangia rare; gemmae present in chains; fair number of oogonia produced; antheridia on every oogonium.

On boiled corn grain. Growth very vigorous; sporangia as normally produced; many gemmae; a good many oogonia of normal shape and appearance. This culture was repeated and it was observed that the gemmae became sporangia, emptying their spores by a quite lengthened papilla upon change of conditions, such as pouring on fresh water or transference from ice box to open.

Many other cultures were made, of course, on insects, grubs, flies, etc., with results considered normal as described.

11. Saprolegnia megasperma n. sp.

Plate 17

Mycelium on grubs and vegetable media about as vigorous as in *Saprolegnia ferax* or *S. litoralis*; threads on mushroom grubs or termites, 9–35μ thick, most about 15–20μ thick, reaching a length of 0.5–0.7 cm.; threads straight to wavy, usually wavy on termites. Sporangia abundant on grubs, termites, and vegetable media, apical, 15–45 × 100–400μ, variable in shape: the first ones usually long and distinctly swollen at the distal end, later ones usually smaller and more or less irregular in outline; emptying normally for the genus; renewed by internal proliferation or rarely by cymose branching as in *Pythiopsis* or *Achlya*. Not rarely in cultures slightly infected with bacteria the sporangia may break away from the threads as in *Dictyuchus*, such sporangia emptying normally after a long or short rest. Spores diplanetic, biciliate, 11μ thick when encysted. Gemmae abundant, round to oval or very irregular, emptying upon the addition of fresh water by one or more long papillae. Oogonia produced in fair abundance, inversely in proportion to the number of sporangia and gemmae, 40–100μ thick, wall smooth (rarely with a papilla), not thick, without pits or rarely with a few small ones; usually borne on short racemose branches which in length are as a rule less than the diameter of the oogonia; not rarely borne singly or in clusters of several on the ends of main threads in cultures in which sporangia are sparingly produced. Eggs 1–10, single in over 50% of the oogonia in most cultures (not rarely running considerably above or below this per cent); 30–52μ thick, usually about 38μ thick; subcentric (one row of oil droplets on one side, two on the other), not filling the oogonia. Antheridia present on all oogonia, applied by their ends, seldom by their sides; antheridial walls thick, easily visible even in old cultures; antheridial branches usually of androgynous origin but quite often diclinous, usually simple and unbranched; antheridial tubes developed and easily visible.

Found near Wilmington, N. C., on December 30, 1921, in water, trash and a little green algae collected from a branch by the old Atlantic Coast Line Railroad bed.

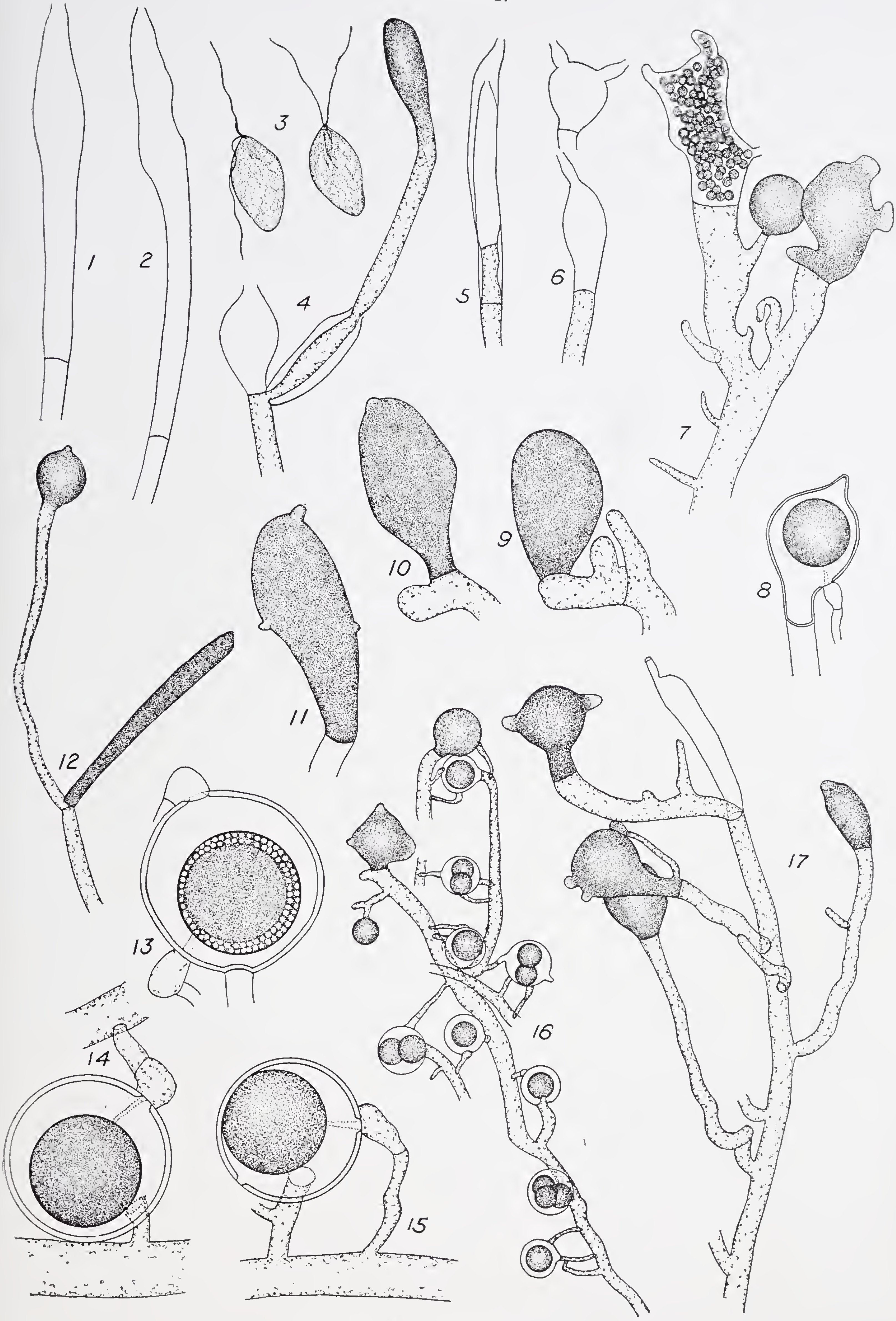
In its general habit the above species might be confused on hasty study with *S. litoralis*, which also was found near Wilmington. But

PLATE 17

PLATE 17

SAPROLEGNIA MEGASPERMA n. sp.

Fig. 1–2. Typical sporangia. × 167.
Fig. 3. Spores stained, showing cilia. × 900.
Fig. 4. Sporangia, one proliferating internally. × 167.
Fig. 5. Sporangia showing internal proliferation. × 167.
Fig. 6. Gemmae which have become sporangia and emptied. × 167.
Fig. 7. Typical forms and position of gemmae, one from which the spores failed to discharge completely. × 167.
Fig. 8. Oogonium with a single egg and an apiculus. × 247.
Fig. 9–11. Stages of sprouting gemmae. Each gemmae shows three pits from which sprouts are appearin' in two cases. × 247
Fig. 12. A gemma breaking away from the hypha as in *Dictyuchus*. × 167.
Fig. 13. Oogonium with a single ripe egg, and two diclinous antheridia. × 500.
Fig. 14. Oogonium with a single egg and a diclinous antheridium showing an antheridial tube. × 500.
Fig. 15. Oogonium with androgynous antheridia. × 447.
Fig. 16. Habit sketch showing oogonia, antheridia and gemmae. × 108.
Fig. 17. Habit sketch showing a sporangium and several gemmae, one of which has developed an abortive antheridium. × 167.



SAPROLEGNIA MEGASPERMA.

the former is obviously distinct from *S. litoralis* in the smaller number and larger size of the eggs, in the simple, unbranched antheridial stalks, in the fewer antheridia, in the absence of intercalary oogonia, in the greater abundance of sporangia, and in the extremely distorted gemmae.

The present species can be readily recognized after the formation of eggs by the large number of oogonia with single eggs (usually over 50%); by the simple, unbranched antheridial stalks, and the antheridia which apply themselves to the oogonia by their ends instead of by their sides. The above characters will serve in identifying the plant, but the number of oogonia and even the number of oogonia with single eggs varies considerably under different conditions of culture. These facts will be brought out in the following general summary of results of experiments.

It has been found that the number of oogonia produced varies inversely with the number of sporangia produced. When cultures were made in the ordinary way and left to grow for about five days, only a fairly good number of sporangia were formed while a very large number of oogonia were produced, oogonia often being borne in clusters at the ends of hyphae in addition to the usual number of racemose ones; on the other hand when fresh water was added to the cultures sporangia were formed on the ends of practically all the hyphae, the oogonia present being racemosely borne. On grubs and termites the oogonial walls are unpitted, but on bits of corn grain the walls of about 10 to 20% of the oogonia show a few conspicuous pits.

Quite often, especially when bacteria were present, clusters of gemmae were formed on the ends of the threads, the gemmae resting until the addition of fresh water. When the plant is cultivated on termites the threads are spirally twisted and the oogonia are mostly borne in elaborate clusters on the ends of threads, about 75% of the oogonia containing single eggs, the number in the rest of the oogonia being two, three or four or very rarely more. On mushroom grubs the threads are usually nearly straight and clusters of oogonia are rare, about 50% of the oogonia containing single eggs.

12. **Saprolegnia parasitica** n. sp.

PLATE 18

Growth rather delicate on insects and other usual media, moderately dense, not long, rarely reaching 1 cm. on a mushroom grub. Gemmae abundant, size and shape very variable; often in chains, mostly terminating hyphae, but sometimes intercalary. Sporangia variable, but usually bent and irregular, at times up to 0.7 mm. long, very often proliferating from the side below as in *Achlya;* when growing through others sometimes discharging spores through the side wall of the old sporangium; spores 9–11.5μ thick, in our form swimming in two stages as usual in the genus (not swimming in Huxley's form—see below). Sexual reproduction not observed so far in our form and very rarely observed by others (Huxley). Received by us from the fish hatchery at Wytheville, Va., and from the aquarium of the U. S. Fish Commission in Washington, in each case growing as a parasite on fish.

There is every reason to think that this is the same as the sterile *Saprolegnia* that has been reported so often as causing (or appearing with) a well-known diseased condition of fish. The disease is apt to appear at any time on young fingerlings in fish hatcheries or on the eggs and may at times be a serious pest. It is also apt to appear now and then on gold fish and in fish pools and aquaria and sometimes causes (or accompanies) epidemics of disease which result in great mortality of salmon, trout, etc., in the free waters of rivers and lakes.

That the species is almost or quite without oogonial reproduction is evidenced by the fact that only very rarely have oogonia been reported (and then without proof that they belonged to the parasite), notwithstanding the fact that this species has been more studied perhaps than any other of the family. The plant was for a long time carelessly spoken of as *S. ferax*, but in the few cases of sexual reproduction observed there is a close similarity to that of *S. monoica*. This resemblance is so close in fact that Huxley, who was the first to report the oogonia (Quart. Jour. Mic. Sci. **22**: 311. 1882), considered it *S. monoica* (*S. ferax* var. *monoica*, he called it). Nevertheless we do not think we are justified in considering the (at least practically) sterile parasite in question the same as the well-known and abundantly fruitful saprophytic *S. monoica*, even though they are much alike in other respects. The tendency to proliferate from the side below, as in *Achlya*, is from our observations even more strong in this species than in *S. monoica*. Huxley reports that in his plant the spores *did not swim* on emerging but floated passively. Our plant does not show this character (if indeed it is a permanent character in any form, which is more than doubtful), the spores swimming as usual in the genus. It is interesting to note here the similar suppression of a swimming stage in *Achlya aplanes* which is otherwise very like *A. prolifera*.

For comparison it will be of interest to refer to Rothert's remarks on a *Saprolegnia* found by him on fish eggs (called *S.* sp. I) and probably the same as this. He says (1888, p. 295) that although the plant was quite free from parasites and was cultivated for a half year in various conditions he was not able to induce the formation of oogonia. That so far as

PLATE 18

PLATE 18

Saprolegnia parasitica

Figs. 1, 2, 3 and 4. Various forms of sporangia. × 447.
Figs. 5. 6. 7, and 8. Gemmae. × 447.
Fig. 9. Spores in first swimming stage. × 720.
Figs. 10, 11, and 12. Various forms of sporangia showing proliferation. × 447.

SAPROLEGNIA PARASITICA.

seen it was so like *S. monoica* that it was possibly identical with it; but it might also be a distinct form quite without oogonia, somewhat like (in this respect) *Leptomitus lacteus*. Hine's ('78) figures and description indicate the present species (pls. 4–5), particularly in the proliferation of sporangia at times by lateral growth from below, as in *Achlya* (p. 95). He records the natural infection of uninjured tritons (*Minobranchus*) in an aquarium at Cornell University and also experimental infections of injured animals which died in about three or four days. Dictiosporangia were also observed; but no sexual reproduction. As to remedies Hine notes the curing by Mr. Gage of an eel by sponging it with a ten per cent solution of carbolic acid, and of *Minobranchus* by sponging with camphorated water. He also quotes from Rev. M. J. Berkeley, who says in his Treasury of Botany that carbonate of soda, and probably also bisulphate of potash, will prevent the growth of these parasites.

The sterile species described by Lindstedt in his Synopsis (p. 48) may possibly be this, but is more probably a very sterile strain of *S. diclina*, as he obtained it on flies from water in which had grown *Chara*, and the drawings look more like the latter species.

Radais (1898) found a sterile *Saprolegnia* in a conduit and referred it with some doubt to *S. ferax*, which it could hardly be.

Tempère (1904) calls a parasite on fish that he found *S. ferax*, although oogonia were not observed. It was probably *S. parasitica*.

Hardy (1911) found a sterile *Saprolegnia* infecting several species of young trout and associated with the alga *Myxonema*, the latter with branched filaments about 1 cm. long and, apparently, more conspicuous than the fungus. The fungus was always present with the alga, but itself occurred alone at times. Hardy had previously noted this alga on goldfish and incidentally mentioned the presence of a fungus (1907), and Minakata had found this alga on fish in Japan, but did not mention a fungus associate (Nature 79: 99. 1908).

Miss Collins (1920) describes considerable variations in behavior of the sporangia and spores in a sterile species found by her in South Australia. It may be the same as ours.

Huxley (1882) has shown that the *Saprolegnia* he studied could advance into sound tissue from infected areas and was thus capable of being a parasite. It is of course not an obligate parasite, and Hardy (l. c.) suggests that its attacks on fish may be prepared for by parasitic bacteria such as *Bacillus salmonis pestis* or *B. piscicidus bipolaris*.

To test for length of life a pure culture was put in a jar of distilled water in March, 1917. Grubs placed in contact with this culture several times between Sept. 14 and 25, 1917, failed to show growth.

13. **Saprolegnia hypogyna** Pringsheim. Jahrb. f. wiss. Bot. **9**: 191, pl. 18, figs. 5, 9, 10. 1873.

Saprolegnia intermedia Maurizio. Jahrb. f. wiss. Bot. **29**: 97, pl. 2, figs. 37–51a. 1896.

First described briefly by Pringsheim as a variety of *S. ferax* (but referred to as *S. hypogyna* in the description of the figures), this plant was later recognized as a species by deBary (Bot. Zeit. **46**: 615. 1888). It is defined by Pringsheim and deBary as having no antheridial branches, but with an antheridial cell cut off, usually just below the oogonium, from which an antheridial tube pushes up through the wall in most cases. As even the more typical Michigan form departs in certain respects from the European, we add below deBary's description for comparison:

"Threads delicate, strict; primary sporangia repeatedly proliferating from within. Oogonia terminal and then mostly round or pear-shaped, or intercalary and then broadly barrel-shaped, not rarely two or more in a row; wall smooth, moderately thick, with not very numerous large pits. Egg usually about 5–10 (1–40), centric as in *S. monoica*. Antheridial branches absent; antheridia usually present in the form of a cylindrical or clavate-cylindrical cell, which is cut off just below the oogonium; antheridial tubes usually entering the oogonia through the basal wall, often branched and not rarely absent. Intercalary oogonia when single frequently have an antheridium cut off at each end with antheridial tubes present or absent. A small proportion of the oogonia remain without antheridia even to full maturity of the eggs."

A form of this species approaching the typical has been found in America heretofore only by Kauffman in Michigan (Ann. Bot. **22**: 361, pl. 23. 1908. Also noted in Ann. Rep. Mich. Acad. Sci. **8**: 27. 1905). He does not describe in detail the normal appearance of his plant on insects in pure water, but as he does not mention the absence of the suboogonial cell in such conditions it is to be presumed that it is regularly present. In a set of experiments with haemoglobin or peptone with or without the addition of various salts Kauffman found that in case oogonia were produced the antheridial cell was always present in most of the media, and partly absent in a few. Side branches from the antheridial cell were often present, and attached themselves to the oogonium above. In K_3PO_4 and to a less extent in KNO_3 and Na_2HPO_4 the normal behavior could be so modified that there were formed numerous antheridial branches, mostly of diclinous origin, much as in *S. mixta* and *S. diclina*. Several of Kauffman's drawings (figs. 5, 6, 7, 14, 15 and 16) show androgynous antheridial branches running up around the oogonium from immediately below it, as in var. III of Maurizio (see below). These two forms, therefore, approach nearest to *Aplanes Treleas-*

eanus, but differ sharply in the presence of an abstricted hypogynal cell. They may be considered as connecting links between *A. Treleaseanus* and the typical *S. hypogyna*. That *S. hypogyna* is very variable is shown, not only in Kauffman's results, but also by the fact that Maurizio has described six forms, or varieties, and one so-called species (*S. intermedia*), which should be reduced to a form, or variety, and is so reduced by Minden. For other illustrations see Minden ('12), fig. 1f on p. 520; Tiesenhausen ('12), figs. 2 and 3; and Maurizio as cited below.

It is certain that in *S. hypogyna* and its forms the upgrowths into the oogonia are in most cases at least quite functionless as antheridial tubes, and are of no more significance than in many other species, *e. g.*, in the *Ferax* group, where something of the kind is often seen. In such forms, however, as *S. intermedia* and Form V, where the upgrowths have thin walls and dense protoplasmic contents, it is still to be shown that they are without function. For views on this point in practical agreement with the above see Maurizio (Flora **79**: 149. 1894) and Kauffman (Ann. Bot. **22**: 382. 1908). The species is reported from Lapland by Gäumann (1918) who gives the eggs as 17–20μ thick.

For the use of students it is desirable to give a more or less abbreviated description of the seven varieties (exclusive of Kauffman's form) as understood by Maurizio. Five of these (I–V below) were first described as varieties in Flora (**79**: 109, pl. 4–5. 1894).* Next was published *S. intermedia* (Jahrb. f. wiss. Bot. **29**: 97. 1896); and then var. *Coregoni* (Mitt. d. Deutsch. Fischerei-Vereins **7**:55. 1899). We think it best, with our present knowledge (or rather lack of it) to consider all these variants as forms, giving them consecutive numbers up to 8, thus:

Form 1—Var. I of Maurizio (pl. 4–5, figs. 5–12).
Form 2—Var. II " " (pl. 4–5, figs. 13–16).
Form 3—Var. III " " (pl. 4–5, figs. 17–20a).
Form 4—Var. IV " " (pl. 4–5, figs. 21–23).
Form 5—Var. V " " (pl. 4–5, figs. 24–27).
Form 6—*S. intermedia* Maurizio.
Form 7—Var. *Coregoni* Maurizio.
Form 8—Kauffman's form from Michigan.

In Krypt. Fl. Mark B. (5: 528. 1912) Minden gives a key to Maurizio's first five varieties (he has overlooked the var. *Coregoni*) which is copied below. On account of the easily accessible originals by Maurizio

* The plate is double and cannot be separated into two. It should be referred to as plate 4–5.

in Flora, we shall not here describe these five forms further than is done in the following key (the plate references are omitted):

Antheridial cell very rarely cut off, but more or less thread-like projections enter the oogonia from below. Stalks of oogonia much bent. Pits small, numerous and conspicuous. Eggs mostly 3–6, rarely 12–20; diameter 20–30μ. Germination period 70–80 days Var. I

Antheridial cells rarely absent under the oogonia.

Pits numerous. Germination period of eggs 70–80 days.

Antheridial cells very rarely absent; under these usually still other cells cut off. Eggs 2 to over 30; diameter 18–22.5μ. . . . . Var. II

Antheridial cells always present, but no other cells cut off beneath them. Not rarely are formed lateral outgrowths from the hypogynous cell, which lay themselves against the oogonia, and appear like androgynous antheridia. Eggs 1 to over 40, diameter 15–25μ. . . . . Var. III

Pits fewer, not over 4. Germination period 39–50 days. Diameter of eggs 18–23μ.

Wall of oogonium very thin, by slight pressure becoming flattened. No extra cells beneath the antheridial cell. Eggs often polygonal from pressure, mostly 2–10, but up to 35–50 in an oogonium. . . . . Var. IV

Wall of oogonium stronger; additional cells cut off under the antheridial cell. Antheridial tubes 1–2, very long, bent, often filled with plasma. "Stärker als bei irgend einer der anderen" varieties. Eggs mostly 3–12, never more than 12. Var. V

Saprolegnia intermedia (Form 6 above) is said by Maurizio to be nearest his Var. V, which agrees in the almost constant presence of the antheridial cell, the well developed antheridial tubes with thin walls and abundant protoplasm, and in the small number of pits. In the thin wall, few pits and number of eggs, he says it approaches his Var. IV. This sixth form (*S. intermedia*) may be briefly defined thus:

Antheridial cell almost always present; antheridial tubes usually well developed, single or several, simple or branched, thin-walled, with distinct protoplasmic contents, oogonia in groups on short or long stalks, or in chains or intercalary, spherical or pear- or barrel-shaped; membrane thin, colorless, in small oogonia without pits, in larger ones with 2–5 pits, which are not easily seen except with high power. Eggs 6–10, or up to 40 in large oogonia; diameter 19–23μ. Germination period 20 days.

Saprolegnia hypogyna var. *Coregoni* Maurizio. Mitt. d. Deutsch. Fischerei-Vereins 7: 55. 1899 (Form 7 above). On account of the inaccessibility of the original, we give below a practically complete translation of Maurizio's description (there are no figures):

"Isolated from an egg of *Coregonus* [pike] which came from a fish hatchery on the Hallwylersee, Kt. Aargau. Cultivated for over a year. Turf up to 1 cm. long. Sporangia as usual. Oogonia intercalary in chains, or in sympodial arrangement, or finally rather rarely in racemes. Stalk of moderate length and frequently remarkably slender; when arrangement is racemose the stalk is often so long as to obscure the arrangement; spherical (when in racemes), elongated or barrel-shaped in other arrangements; wall thin, a little yellowish, pits small, not numerous; diameter 44–77μ or 40–72μ broad by 50–97μ long. Eggs 3 (rarely), mostly 12–18, wall thick, clear yellow, 19.5–21μ. Hypogynous antheridial cell always present, no other antheridia, and no other cells below it; under small oogonia it is about 14.5×20μ, usually, however, about 17–20μ broad by 24.5–68μ long; fertilizing tube (which is often absent) 5–10μ long, or up to as long as the antheridial cell, simple or branched. Conidia abundant, spherical or elongated, and produced like the oogonia, except that racemes are less distinct. The conversion of conidia into oogonia is a common occurrence."

14. **Saprolegnia torulosa** deBary. Beitr. zur Morph. und Phys. der Pilze, IV Reihe: 31, pl. 6, figs. 3–17. 1881. Also see Bot. Zeit. **46**: 618. 1888.

Saprolegnia sp. Lindstedt. Synopsis d. Saproleg., p. 48, pl. 4. 1872.

This is reported by Humphrey from Massachusetts, New Hampshire and Louisiana, but it is doubtful if he had the true species (see remarks under *Isoachlya toruloides*). DeBary's description in Bot. Zeitung is as follows:

"Primary sporangia slender, cylindrical, claviform; oogonia irregularly spherical, elongated, pyriform or cylindric, seldom egg-shaped, almost always appearing in torulose rows of two to several by constriction of the main hyphae; after ripening remaining firmly attached to each other. Oogonial wall with few or no pits. Oospores centric. Antheridial branches and antheridia usually completely absent. In the rare cases where present either androgynous or diclinous. Antheridia with or without a fertilizing tube.

"Up to the formation of primary sporangia the species cannot be distinguished from *S. monoica*. Later, as already fully described (Beitr. IV, p. 102), the main branches become divided by cross walls into structures, the outer of which occupy the position to be taken by the oogonia. Between these initials the threads are constricted so as to appear torulose. Such appearances are also not rare in old examples of other species, as in the formation of the sporangial rows described by Pringsheim and of resting cells [gemmae]. In the present species certain of these structures become such sporangia and gemmae; others, however, in special position and number become oogonia, and indeed the oogonia here are almost entirely formed from such chained initials. Single oogonia terminating the vegetative threads are occasional and such are often ovate or pyriform. Significant details are fully described in Beitr. IV.

"The species appears rare. Found in a stream near Strassbourg in April, 1879, and cultivated until 1884. Also doubtfully seen in slime from the Todtensee near the Grimsel."

The species has been found only a few times. Recently Häyrén has reported it from Finland and Gäumann from Lapland. Fischer has a good description with measurements ('92, p. 340, fig. 52b). He gives the eggs as up to a good many in an oogonium, 14–22µ thick, round or at times irregular; antheridia mostly absent, when present androgynous or diclinous. He thinks that Lindstedt's plate 4 ('72) of an unnamed species is really this. For another supposed figure of this see Lechmere ('11a), fig. 1. Lechmere found that the plant he studied formed no oogonia on egg albumen.

15. **Saprolegnia asterophora** deBary. Jahrb. f. wiss. Bot. 2: 189, pl. 20, figs. 25–27. 1860.

PLATE 19

Mycelium extensive, but thin and delicate. Hyphae slender, uneven, much or little branched, about 5–11µ thick, rapidly thickening towards the sporangia which are typically very scarce and often entirely absent in cultures on insects. They are up to 40µ thick, sub-cylindrical to clavate, and proliferate from within, or rarely laterally from below as in *Achlya*. Spores 14–15µ in diameter, emerging and swimming slowly and aimlessly in the neighborhood for two or three minutes, then encysting and after a few hours emerging in the usual form and in a more active state. Gemmae not abundant, often absent on insects, peculiar, shaped like the sporangia or pear-shaped, tuberous, knotted, etc. Oogonia numerous, usually thickly set with blunt papillae which are usually 2–4µ, rarely up to 8µ, long; oogonia about 30–57µ thick, including the papillae, most about 37–45µ, borne on even more slender lateral branches of small ordinary hyphae (the stalks rather long), or occasionally intercalary or terminal; walls thin and unpitted. Eggs one or often two, rarely three (very rarely 4 or 5—deBary), 18–35µ in diameter, dark, often a large and a small one together; structure subcentric, *i. e.*, with the protoplasm completely surrounded by small oil drops, which are in a double layer on one side and a single layer on the other. Antheridial branches varying greatly in abundance, often nearly absent at low temperature, appearing close to the oogonium and usually from its stalk, rarely from neighboring hyphae, often branched and several arising in a twiggy group, but only one or two becoming fully developed. Antheridia short-tuberous or pear-shaped; antheridial tubes not seen.

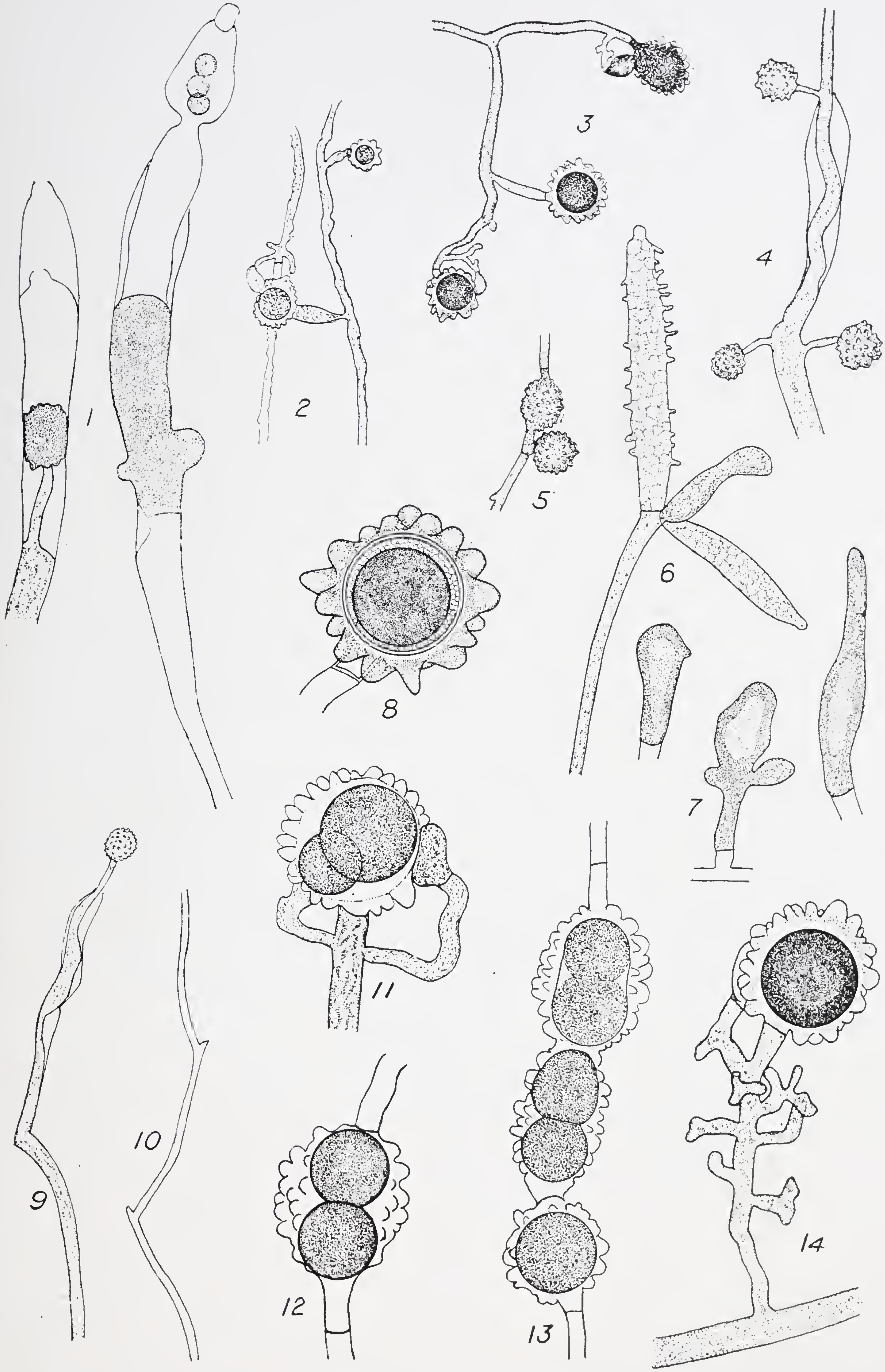
Found only three times out of more than two thousand collections in Chapel Hill, twice on the same day (Nos. 4 and 5, February 14, 1918) in two places on New Hope Creek margin south of the Durham bridge; again in a wet weather branch in Strowd's lowgrounds, February 8, 1922. For other illustrations see deBary ('81), pl. 6, figs. 18–29; Humphrey ('92), pl. 17, figs. 54 and 55; Minden ('12), fig. 1f on p. 520; Istvanffi ('95), pl. 35, figs. 19–21, and pl. 36, fig. 22.

PLATE 19

PLATE 19

Saprolegnia asterophora

Fig. 1. Emptied sporangia one on left containing young oogonium. × 278.
Fig. 2. Irregular hypha with oogonia. × 188.
Fig. 3. Cluster of oogonia. × 188.
Fig. 4. Oogonia and sporangia. × 188.
Fig. 5. Young oogonia. × 188.
Fig. 6. Spores sprouting in sporangium. × 188.
Fig. 7. Three gemmae. × 188.
Fig. 8. Oogonium with ripe egg. × 810.
Fig. 9. Proliferation through an old sporangium forming an oogonium. × 115.
Fig. 10. Zigzag course of hypha caused by death of tips. × 115.
Fig. 11. Oogonium with antheridia. × 503.
Fig. 12. Intercalary oogonium. × 503.
Fig. 13. Chain of intercalary oogonia. × 503.
Fig. 14. Oogonia with an antheridium and numerous aborted antheridial branches. × 503.



SAPROLEGNIA ASTEROPHORA.

This rare plant has been reported heretofore in America only by Humphrey and by Pieters. The former did not see the living plant, but recorded it from preparations by Trelease, who found it in eastern Massachusetts (Cambridge and Woods Hole). Pieters found the plant near Ann Arbor, Michigan, and its occurrence is noted in Ann. Rep. Mich. Acad. Sci. 17: 195. 1915. He has kindly allowed us to examine his unpublished notes and drawings. The species has been reported from several places in Germany, but seems to be rare there also (Fischer, '92, p. 343).

This species is distinctly set off from the other members of the genus by its fine, irregular hyphae and few-egged, papillate oogonia. No other species of *Saprolegnia* has an average of so few eggs. If it were not for the sporangia one would place it near *Aphanomyces stellatus*. Its actual relationships are, however, probably with the *Racemosa* group of *Achlya*, particularly with *A. hypogyna*. The tips of the hyphae are blunt and very hyaline, and sometimes die without apparent cause, the hyphae being continued by a lateral branch just below, as in certain species of *Achlya*. The mycelium grows to a greater diameter than in other species under similar conditions, but is more thin and sparse than others. The threads are rarely cylindrical, but are wavy and knotted.

It is interesting to find that Pieters has also observed the "slow, deliberate movement" of the discharging spores and the great scarcity of the sporangia (unpublished notes). This shows that these characters are not casual or accidental. In his notes Pieters says that in all respects save only in the method of discharge of the sporangia *Achlya racemosa* and *Saprolegnia asterophora* are quite similar and the question arises whether the latter is not more closely related to some species of *Achlya* than it is to other species of *Saprolegnia*. He bases this suggestion in part on the apparent absence of gemmae in his cultures of *Saprolegnia asterophora*. We also find them absent or scarce in many cultures, while in others there are not a few. There is no doubt, however, of the striking similarity noted by Pieters, and his suggestion may correspond with the facts. He makes the following interesting observations on this species: "Physiologically this species differs from all other species of *Saprolegnia* studied. It thrives on extremely small quantities of food in solution. If a piece of mycelium grown in pea water is transferred to water, and care is not taken to wash thoroughly, no sporangia are produced but only oogonia and growth. In no other *Saprolegnia* studied have I ever gotten oogonia from a mycelium in water. If a good culture of

Saprolegnia asterophora be transferred to haemoglobin, 0.1% or 0.05%, no oogonia are produced; only a vigorous growth results, though *Saprolegnia ferax* will have many after being in 0.05% solution for three days. In 0.01% *Saprolegnia asterophora* produces a few oogonia after four days but not nearly so many as are produced in water in the same time. The explanation for this is merely that *Saprolegnia asterophora* does not need so much food for growth as *Saprolegnia ferax* does, and that therefore the small amount of food in 0.05% haemoglobin is enough for vigorous growth and as long as there is food enough for vigorous growth no oogonia will be produced. If 0.05% of total salts KH_2PO_4, $MgSO_4$, and KNO_3 in equal parts be added to the 0.01% haemoglobin no oogonia are produced, but in 0.01% haemoglobin and 0.01% salts a few are found after four days.

"It is of interest to add that *Achlya racemosa* which resembles *Saprolegnia asterophora* in the number of oospores and in the position of the antheridia, and which is sometimes slightly spiny, will also produce oogonia in water."

The ease with which the number of antheridia can be made to vary would make this a favorable species for experiment on the causes for such variation. DeBary has noted that in *Saprolegnia asterophora* oogonia without antheridia appear when the growth is old, *i. e.*, poorly nourished (on insects).

The production of sporangia is erratic, always limited, and often entirely suppressed, even for long intervals on different media. In our 1922 collection no sporangia ever appeared, although cultivation was continued on various insects and vegetable media for several months.

Among many cultures made from No. 4 of February 14, 1918, we note the following:

On corn meal agar. Growth slower than any other *Saprolegnia* on agar, but threads are thick in the agar and rather stout; no sporangia, but a few oogonia produced after the culture was several days old. Repeated: no sporangia; oogonia formed in abundance; a good many antheridia.

On pieces of boiled corn. Growth vigorous. Sporangia produced but not in abundance; a considerable number of oogonia, about half of which had antheridia.

At low temperature. Three young cultures on grubs put in ice box. Growth about as usual; very few sporangia in any one culture; oogonia scattered and very few antheridia present.

EUROPEAN SPECIES NOT YET RECOGNIZED IN AMERICA.

Saprolegnia rhaetica Maurizio. Flora oder Allgemeine Botanische Zeitung 79: 109, pl. 3, figs. 1–16, and pl. 4, figs. 1–4. 1894.

This species is apparently near *S. torulosa* and possibly the same. The next species (*S. variabilis*) also can scarcely be separated from these by the description. From *S. torulosa* and *Isoachlya toruloides* this series differs most obviously in the reputed absence of antheridia. Maurizio's description follows:

"Turf not very thick, up to 1.5 cm. long, with straight, slender, fragile and unbranched filaments. Sporangia apical, seldom intercalary. Primary sporangia small, clavate, producing later through-growths of varying lengths which usually tilt back over the empty cases and take a variety of forms.

"The fungus forms gemmae ['conidia' is the word used here and below], which may be arranged in rows or as rolls and screws. There are also complicated arrangements of gemmae in no definable system. In the empty sporangia gemmae may form by constriction (usually) or by cross division in basipetal succession. Corresponding to this great variation in the gemmae noted is a similar variation in the gemmae found inside the empty sporangia. If the conidia are formed by abstriction at the ends of hyphae the single cross walls, which for some time are common to two gemmae, finally separate into two thin lamellae, one of which surrounds the conidia, the other forming a connecting sheath. By such a sheath are connected often a number of gemmae in a row. After some time the connections between the gemmae break down and the single gemmae lie free in the water by hundreds.

"There are gradations between sporangia and gemmae in that a part of the gemmae become sporangia. Also a part of the gemmae may become oogonia. The formation and emptying of the zoospores is the same in the sporangia and the gemmae. The spores swim more than one-half hour. The small oogonia are mostly spherical, and when apical and intercalary somewhat elongated. When they develop from gemmae they stand in rows and may develop in the same row with sporangia, otherwise they are arranged in clusters on short stalks. Their diameter is 48–61.5μ. Wall is thin, colorless, then yellowish and has 2, seldom 3, pits. From the wall below the oogonium there generally grows up into the oogonium a protuberance that may remain hollow or may be closed with a cellulose plug. These growths are not antheridia, which in this species are not present and were not even seen. Oospores up to 12 in number, but mostly one to five. They have only a moderately thick membrane and a centric structure. Their diameter varies between 19 and 27.5μ. Germination not seen."

Saprolegnia variabilis Minden. Krypt. Flora Mark B. 5: 524. 1912.

"Turf thick, formed of rather thin, slender, little branched or simple threads 22–27μ thick. Primary sporangia variable in size, spindle, or club-shaped or spherical, formed in large numbers, mostly on smaller threads, *e. g.*, 60×80μ or 57×70μ. Later the threads fall into more or less numerous structures in rows, which become sporangia, oogonia or gemmae, so that chains of oogonia or sporangia pure or mixed result. There occur also structures in more or less sympodial arrangement; often on the ends of hyphae are swollen sporangia or oogonia or both; oogonia also occur in empty sporangia. Form of secondary sporangia very various; often when in rows there is a lower stalk piece and an upper swollen part; or they may be spherical with a slender discharge neck. Oogonia spherical or seldom more or less elongated with rather thick membrane and a few not conspicuous pits, often with a solid projection from the wall below; diameter 50–70μ, or also 70×90μ or 40×60μ. Eggs spherical 1–15, mostly 4–8, 23–26μ thick; position of the oogonia as various as the sporangia; antheridia never observed.

"Hamburg; on ant eggs in a swamp; several times found and long cultivated pure. The species is related to *S. torulosa* as well as to *S. monilifera*. When the oogonia occur in chains they remind one of the last species where such an appearance is typical. We can state certainly, however, that this species is different." He goes on to say that this is very near perhaps identical with *S. rhaetica* Maurizio, of which he can not get a clear idea from description and figures. Minden gives no figures.

In one form observed by Minden certain branched twigs occur rather numerously and appear like antheridial branches, but do not apply themselves to oogonia.

Saprolegnia crustosa Maurizio. (A group species.) Mitt. d. Deutsch. Fischerei-Vereins 7: 52. 1899.

This species includes three varieties, none of which is indicated as the typical. It is therefore a question if Maurizio's arrangement can stand as he has it. He calls this "*Sammelspecies der Saprolegnia crustosa* sp. nov." There are no figures. He says: "We could consider the three varieties here described as good species, were we not already familiar with such numerous representatives of this genus that are so closely related. Sooner or later, the extension of our knowledge of the water fungi will lead to a more systematic arrangement. The discovery of conidia in the genus *Saprolegnia* looks necessarily to a change in the classification of deBary. The fungus here described, which also bears conidia, cannot be in any way placed in the system of deBary or of Fischer in Rabenhorst's Krypt. Flora. I refer to *S. paradoxa* sp. nov., *S. furcata* sp. nov. and the earlier described species with conidia. In spite of the authoritative work of Fischer, it is my opinion that we have here a new group.*

*This kind of talk is a good illustration of Maurizio's unbalanced judgment. There is nothing to his great claim of having found conidia in the *Saprolegniaceae*.

"The three varieties show an identical arrangement of the oogonia and are distinguished by diclinous antheridia, which are either constantly present, or are more or less abundant. They show, however, no agreement in the diameter of the oogonia and eggs, or in the thickness of the oogonial wall. According to Fischer's principle of classification, *i. e.*, from the presence of diclinous antheridia, we must place the three species [meaning his three varieties] in the neighborhood of *S. dioica*, though in this species the diameter of the oogonia is not given: however, in the egg diameter my No. III alone comes near, 22–29μ against 25–30μ for *S. dioica*. This is, however, only of limited significance. Again, the position of the oogonia—here racemed to clustered, terminal or intercalary, simple or in rows—is still another common character that associates the fungi in question. The name given is intended to show the fact that two of these fungi were isolated from the crust of fungi that covered water pipes."

The following descriptions are condensed from Maurizio:

Saprolegnia crustosa var. I. p. 52.

Isolated from a varied growth on the thin shell of a freshly caught mussel (*Anodonta mutabilis*) and cultivated for a year. Growth dense, 2 cm. long. Sporangia as usual. Oogonia racemose or clustered, also intercalary; and also in simple sympodia of 2 or 4; spherical or elongated at times, if intercalary; stalk short, straight, of moderate thickness, membrane somewhat yellowish, of medium thickness, with pits of medium size that are not numerous; frequently an upgrowth enters the oogonium from the wall below; diameter of oogonia 31.5–60μ or when elongated 33–41μ broad by 50–55μ long. Eggs 4–25, various in size, mostly 19.5–22μ, smallest 12μ; wall moderately thick and yellowish, Antheridia mostly present, diclinous, coming from a distance, and plentifully enveloping the oogonia, not observed to branch. In this species, as in all the species examined by me, conidia are present, in rows or chains, also in complicated sympodia, rarely single, becoming either sporangia or oogonia, or resting. There are also present in the conidia the tubes from the cross wall below, as in the oogonia.

Saprolegnia crustosa var. II. p. 53.

As with *S. furcata* and the following variety, this was found in the layer of fungi that covered the inside of the outlet pipe of an aspirator. Cultivated for a year. The dense, if rather slender, threads about 1.5 cm. long on a mealworm. Hyphae and sporangia not peculiar. Oogonia in racemes and clusters, mostly spherical, 39–104.5μ thick, stalks moderately slender. The elongated intercalary ones were not measured because so rare; membrane very thin and colorless, pits not numerous, moderately broad, not deep; wall yellowish, after long standing in water. Eggs 1–5 in small oogonia, 9–20 in large ones, 17–27μ thick, almost filling the oogonia, their membrane thick and somewhat yellowish. Antheridia numerous, diclinous; entire thread-ends break up into antheridial branches, which then plainly enwrap the oogonia. Here and there fertilizing tubes to be seen: conidia produced in abundance in chains or rows, or racemes, with manifold side branches, also in empty sporangia; many chains visible to naked eye, some 1–1.5 mm. long.

Saprolegnia crustosa var. III. p. 54.

Found in same place as var. II. A somewhat denser turf than in var. II distinguish the two nearly related species; hyphae 1.5 cm. long; rather flexible, but standing out straight. Sporangia as usual. In cultures on mealworms and ant larvae there occur at the beginning neither the sporangia nor oogonia. Slowly the fungus strengthens itself in the culture and forms the turf, which does not disappear. Oogonia racemose, or also terminal or intercalary, stalks very unlike, and the arrangement thus of very different appearance. They are short, or at times so long as scarcely to be recognized as oogonial stalks. Oogonia mostly spherical, seldom elongated, membrane of medium thickness; pits numerous, small, sharply defined. Diameter of oogonia 73–121.5μ, rather regular (small ones with 1–3 eggs are rare). Hollow tubes not rarely enter the oogonia from below. Eggs 20–50, wall thin, almost colorless, thickness nearest that of *S. dioica*, being 22–29μ. Antheridia diclinous, only on about one-third of the oogonia. Conidia various in position and form; spherical, barrel-shaped to long-compressed and thread-like, often difficult to describe, enormously various in size, 30 to 300 or 500μ thick. Position of the conidia very various, in chains with sporangia, also with side-branches, also sympodial or irregularly tangled, and thus most resembling *S. rhaetica*, often in empty sporangia. Oogonia may also be formed at times (not often) in empty sporangia.

Saprolegnia stagnalis Tiesenhausen. Arch. f. Hydrobiol. und Planktonkunde 7: 276. 1912.

Found in a ditch near St. Moritz, in Oberengadin, Switzerland.

"Turf delicate, about 2 cm. broad, sporangia usually rather slender, *e. g.*, 260×29μ, 500×20μ, 720×45μ, or even 80×20μ in size. The secondary through-growing sporangia are constricted (*i. e.*, grow through the older ones and are pinched in by their mouths). Oogonia 25–80μ in diameter, terminal on the principal branches and on side branches, sometimes on branches of the second order, also inside empty sporangia or in a row by twos (zu zweien); wall with numerous pits that are even visible in surface view; stalks short and often bent. Eggs as a rule concentric, 12–22.5μ, exceptionally 32.5×15μ; 1–12 in an oogonium. One or two eggs in an oogonium occur frequently. Antheridia always diclinous."

The author remarks that the species differs from *S. dioica* (*S. diclina*) principally in four points: (1) The constricted sporangia (the sporangia of *S. dioica* do not grow beyond the older ones). (See deBary, '88, p. 619, pl. 10, fig. 13.) (2) In *S. dioica* the oogonia appear only on the tips of the main branches, while in *S. stagnalis* they also occur on side branches. (3) The oogonial wall in *S. dioica* has only a few, or *no* pits, in *S. stagnalis* there are many large pits visible at 80 times magnified. (4) The eggs are smaller (12–22.5μ) than those of *S. dioica* (25–30μ). There are no illustrations.

Saprolegnia spiralis Cornu. Ann. Sci. Nat., Series 5, **15**: 10, pl. 6, figs. 10–12 and 15–17; pl. 7, figs. 1–4 and 10. 1872.
? *Saprolegnia retorta* Horn. Ann. Myc. **2**: 233, fig. 21. 1904.

Minden thinks he has found Cornu's imperfectly described species and that it is the same as Horn's *S. retorta*. Horn's figure is good, but as Minden's description is more complete, we append a translation of the latter (Krypt. Fl. Mark B. **5**: 517. 1912):

"Turf thick, with long, slender threads 11–16μ thick, which are more or less crooked or bent and have slightly granular contents. Sporangia of various shapes, cylindrical, spindleform, spherical, etc., about 30–40μ thick and 200–300μ long; secondary sporangia few, growing through the old or formed also by cymose branching, oogonia either on the tips of main threads or on short side branches which are mostly bent, or spiral or sinuous; not rarely the oogonia are also intercalary and then elongated; oogonia rarely quite spherical, mostly elliptic or ovate, at times with a blunt extension at the tip, usually with a small basal neck, wall moderately thick, slightly or not at all pitted; on the average 50×65μ, or also for example 38×46μ. Antheridia always present and usually numerous, irregularly cylindric to clavate, touching the oogonium with the entire length or only with their tips, borne on branches which spring either from the stalk of the oogonium or from the main thread, often numerous and much branched, and after maturity quickly becoming inconspicuous. Eggs mostly 1–2, seldom 3 or even 4, smooth, spherical or when several more or less elongated, at maturity with one or many, mostly lateral fat drops and therefore more or less plainly eccentric; diameter 26μ or for example 34×45μ.

"Found in spring in several places near Hamburg, Germany, mostly in company with other species of *Saprolegnia*. Resembles *S. monoica* in androgynous antheridia, but differs so clearly in the position of the oogonia, the small number of eggs and the crooked stalks as to be easily distinct. Its identity with *S. spiralis*, while not certain, is made very probable by the few eggs, the crooked stalk, and the opinion of Cornu that his plant was near *S. monoica*."

Cornu refers to Braun's pl. 5, fig. 22 ('55), as representing his plant, His own figures show only parasitized parts.

Horn states that in his plant the oogonia are very numerous, unpitted, 30–75μ thick; the eggs 1–8 in an oogonium, 20–25μ thick, with a central fat drop. This plant may be different from the one Minden describes above. (See note under *S. litoralis*.)

Saprolegnia furcata Maurizio. Mitt d. Deutsch. Fischerei-Vereins 7: 48, figs. 13–15. 1899.

The following is a complete translation except for one or two sentences of no importance. By the "conidia" he refers to what are properly known as gemmae or chlamydospores:

"Found in a crust inside the outlet pipe of an aspirator in the laboratory, and cultivated for over a year. Turf thin, only 0.5 cm. long on a mealworm, hyphae 5–18µ thick, frequently as slender or more so than the stalks of the oogonia, *e. g.*, in several cases the stalks of the entire oogonial group were only 7–12µ; stalks with short, lateral outgrowths that give them a knotted appearance. Sporangia proliferating as usual. Conidia present. Frequently the zoospores do not escape, but sprout inside, through the sporangial wall. Oogonia in racemes, or one-sided racemes, or they may be reduced to one; not in a true fascicle. A peculiarity of this species is that the branch of the always present antheridium, after it has enwrapped an oogonium, may divide and bear an oogonium on its end. Such an oogonium can, for its part, have an antheridium of its own. It is usually, but not always, a solitary oogonium that shows this peculiarity. If the oogonial and antheridial stalks are equally thick, it appears as if the hypha forked, carrying an oogonium on one branch and an antheridium on the other which does not form a fertilizing tube. Instead of a fertilizing tube, the antheridium forms an oogonium on its end. Oogonia small, 24.5–41.5µ thick, almost always round, intercalary elongated ones rare; borne on slender, short or rather long, much bent or wound stalks. Wall yellowish, pits small, not very obvious or projecting. A tube often enters from the wall below. Eggs 1–9, wall thin, clear yellow, 19.5–22µ in diameter. Antheridia on almost all oogonia, androgynous, springing either from the stalk of the oogonium or the thread that bears it. They branch frequently and attach themselves to the oogonia. Conidia occur here as described for *S. paradoxa*, and have no marked peculiarities. On the conidia undeveloped antheridia were observed.

"The species shows several similarities to *S. mixta*. The latter is an intermediate form between *S. Thureti* and *S. monoica*, and it must be emphasized that our species is decidedly not related to *S. Thureti*. *Saprolegnia furcata* has distinctly smaller oogonia than *S. monoica* and differs from it further in the occurrence of the forks which bear an antheridium and an oogonium. As this is a constant peculiarity found in all cultures, it must allow this species to be distinguished as new, and it may be known as *S. furcata*."

Saprolegnia curvata Minden. Krypt. Flora Mark B. 5: 609. 1915.

The following is a much condensed description of this interesting species, made from the rather long original in German. The sluggish spores and oogonia in chains would suggest that this species might more properly fall in the genus *Isoachlya*;

Found on the egg mass of a snail (*Bunynia*) at Hamburg, Germany. It is peculiar in having the sporangia spiral or bent, in the very large (25µ) spores with a very short and sluggish first swimming stage, and in the oogonia being borne inside the substratum (egg mass). Oogonia terminal or mostly intercalary, at times many in a row, seldom approaching spherical, mostly only swollen places in the hyphae and very irregular and unsymmetrical with bent in or projecting places in the membrane. Antheridia numerous, borne on long, sinuous, much-branched threads (whether androgynous or diclinous is not stated). Eggs up to 10, mostly 3–5, at times only one [size not given].

Saprolegnia lapponica Gäumann. Botaniska Notiser, p. 156. 1918.

This species is near *S. esocina* and *S. ferax.* As described it differs from the latter in the smaller eggs and in oogonia rarely ever terminating the main hyphae. The following is a free translation from the original (there are no figures):

"From a puddle at the foot of the Nuolja [Lapland], I isolated a form which belongs in the *S. Thureti* group, but which cannot be identified with any of the so-far described species. While the typical *Thureti,* as Fischer correctly observed, has terminal oogonia, those of *lapponica* are borne laterally and are mostly at a considerable distance from one another and from the ends of the hyphae. The latter can be identified (even with the unaided eye) by the pretty turf, in which the oogonia occupying the inner zone cause a white field to stand out; later, in old exhausted cultures, this character is obliterated, in that oogonia may be found farther out and occasionally even terminal as in the typical form. Yet this character of the oogonia being (normally) borne laterally on the hyphae has remained constant through nine generations.

"Over against the two other species of the *Thureti* group, namely, *S. esocina* and *S. bodanica,* the demarcation is harder to make good. As to the size of the oospores, *S. lapponica* agrees strikingly with *S. esocina,* in that the majority of the oospores (91 out of 100) are 21–24µ thick. It can readily be separated, however, from *S. esocina* by the brown (not colorless) oospore membrane and by the absence of oogonia in chains." [Gäumann is mistaken here, for Maurizio says oogonia in chains are *not* found in his species.]

"On the other hand *S. lapponica,* through its lateral oogonia, reminds one of *S. bodanica.* I purposely at this place avoid the expression of a racemose oogonial order, since it could give an incorrect conception of its appearance in regard to *S. monoica.* The oogonia are less numerous, averaging three to five on each hypha; often situated far apart and occasionally even on the same side. While, furthermore, the oogonia of *S. bodanica* are often borne on long stalks (see Maurizio, pl. 2, fig. 52. 1896), in *S. lapponica* the oogonia are mostly borne on short stalks or are even sessile on the main hyphae. Furthermore, and this is the main point of distinction, the oospores of *S. bodanica* measure in general 23.5–31µ, while *S. lapponica,* as noticed above, vary normally from 21–24µ.

"These grounds caused me to separate the form in question as an individual species, which perhaps may be synonymous with the *S. Thureti* form V, of Minden (1912, p. 523). By all means this complicated and often unclear systematic situation summons one (as was the opinion of deBary) to investigate again the extent of variability of the species of the *Saprolegniaceae.* The diagnosis of the species follows:

"Turf delicate, extending 1–2 cm. on a fly; hyphae slender, straight, sparingly branched, 8–14μ thick; zoosporangia slightly greater in diameter than the hyphae, terminal; antheridia absent; oogonia lateral, borne on a short stalk or sessile, a tube-like growth from the oogonial stalk often proliferating up into the oogonium; diameter of oogonia 45–75μ; pits distinct, of unequal frequency (6–18 in oogonia on same hypha); eggs globose or broadly ellipsoid, tawny-colored, 19–31μ, usually 21–24μ, in diameter, 1–18 in each oogonium."

Saprolegnia mixta var. **Asplundii** Gäumann. Botaniska Notiser, p. 155. 1918.

This is said to differ from the typical form (in the sense of Maurizio, 1895) only in the smaller oospores, which vary from 15–21μ, mostly 16–19μ, thick (no figures).

Saprolegnia floccosa Maurizio. Mitt. d. Deutsch. Fischerei-Vereins **7**: 50, figs. 16 and 17. 1899.

The following is a translation from the original, complete except for the omission of a few unimportant words:

"This species, like *S. paradoxa*, was isolated from eggs of the American brook trout at Munchhausen hatchery, in the winter of 1895. Cultivated for 1½ years. Turf very delicate, and 1.5–2 cm. long. It spreads out and floats in a flocculent manner on the water. This quality suggested the name. Sporangia growing through as usual, and not peculiar. Oogonia in racemes or intercalary; there also occur sympodia of a few oogonia; spherical, only the intercalary and terminal ones are elongated or barrel-shaped: the flattened or angular appearance of small oogonia I attribute to the sharply projecting pits. The mostly short oogonial stalks are contracted at the base; they are straight or a little bent, never wound; membrane only moderately thick; small projections enter the oogonia from below; pits numerous, small, sharply springing outward, to this fact is due the angles and depressions of the oogonia; diameter 45.5–73μ. Eggs 1–15, usually 4–9, wall thin, diameter 24–25.6μ. Antheridia on about three-quarters of the oogonia, arising from their stalks or from the hyphae near them, thickly applied to the oogonia. Conidia [meaning gemmae] present, in chains or complicated, often also more or less racemose with the oogonia, therefore they may be considered the original fruit-form from which the oogonia sprang, often with a slender projection; form various, *e.g.*, often narrow in a thread-like way. In the system the species falls in the *Ferax* group and also approaches in many points *S. dioica*. It is distinguished from it by the racemose oogonia and by the androgynous antheridia. Eggs somewhat smaller than in *S. dioica*. The diameter of the oogonia agrees pretty well with that of S. *monoica*, but the latter has distinctly smaller eggs. In *S. floccosa* we have, therefore, a new species of the *Ferax* group."

It is evident from the above that this species is very near if not the same as *S. monoica* var. *glomerata*, about the only apparent difference being the bulging out of the egg membrane at the pits.

Saprolegnia monoica var **montana** deBary. Bot. Zeit. **46**: 617. 1888.

DeBary says: "This differs from the typical form in the abundant, irregularly arranged and longer stalks of the oogonia; in the longer and more slender branches, and in the somewhat thicker oogonial wall with few or no pits."

Saprolegnia paradoxa Maurizio. Mitt. d. Deutsch. Fischerei-Vereins 7, heft I: 46, figs. 10, 11, 12. 1899.
Not *S. paradoxa* Petersen.

The typical form, found on eggs of sea trout in the fish hatchery at Munchhausen, Reg. Bez., Cassel, has androgynous antheridia in a group below the oogonia; eggs 9–30 in an oogonium, 25–27μ thick, or in the small oogonia 17–22μ; oogonia terminal or intercalary, more or less racemosely borne, spherical to long barrel-shaped or even thread-like with a row of eggs (thread-like, egg-containing extension of oogonia are not rare in *S. ferax*); often with a solid or hollow process running in from the wall below as so frequently happens in the *Ferax-Monoica* group; wall of oogonium not very thick, pits distinct. A very great oddity is the fact that the antheridial branches, which not rarely spring from the long, neck-like extension of the oogonia, may in such cases contain eggs.

This species is obviously near *S. monoica*, from which it seems to differ in the apical extension on many of the oogonia and possibly in other ways. It is much like our *S. litoralis*, in which such extensions are frequent, but the latter has much larger eggs.

EXCLUDED OR DOUBTFUL SPECIES NOT MENTIONED IN THE TEXT

Saprolegnia xylophila Kütz. Phycologia Generalis. Lipsiae, 1843. No information is given that could define the species.

S. minor Kütz. (l. c., 1843) is probably an *Empusa*.

S. deBaryi Walz. Bot. Zeit. **28**: 537. 1870. Probably a *Pythium;* not a *Saprolegnia*.

S. siliquaeformis Reinsch. Jahrb. f. wiss. Bot. **11**:293. 1878. This is *Monoblepharis prolifera*, according to Cornu and Fischer.

S. Schachtii Frank. Krankheiten der Pilzen, p. 384. Berlin, 1881. This is probably *Pythium deBaryanum*.

S. Libertiae (Bory) Kütz. Species Algarum. Lipsiae, 1849. This is thought by Fischer to be *Apodya lactea*. Too imperfectly described to distinguish are *S. candida*, *S. tenuis* and *S. saccata* published at the same time by Kützing.

S. mucophaga Smith. Gardeners Chron. **22**: 245, fig. 50. 1884. Not a *Saprolegnia*; possibly a *Pythium*.

S. philomukes Smith. Gardeners Chron. **22**: 245. 1884. Illustrated in the same, **20**: 781, fig. 140. 1883. Not a *Saprolegnia*; possibly a *Pythium*.

S. corcagiensis Hartog. Quart. Jour. Mic. Sci. **27**: 429. 1887. Said to be constricted as in *Leptomitus* and with similar sporangia, but with oogonia. Needs further study.

S. quisquiliarum Roumeg. Fungi Selecti Exsiccati. Cent **60**, No. 5932. 1891. Humphrey states that the specimen fails to show anything in this family.

S. elongata Massee. British Fungi, p. 217. 1891. Apparently based on mixed material, in part a *Pythium* and in part a *Saprolegnia*.

S. sp. (Pieters No. 66.) Bot. Gaz. **60**: 483. 1915. This is a sterile *Saprolegnia* from Michigan studied by Pieters for eighteen months. It yielded abundant harvests of round single gemmae but no oogonia.

APLANES deBary, 1888, p. 613.

This genus was established by deBary on a single character, the absence of any swimming stage in the spores, which remain in the sporangium and sprout there by germ tubes through the sporangial wall; otherwise as in *Achlya*. There has always been some uncertainty about the sporangia, as Reinsch's *Achlya Braunii* which was considered the same as *Aplanes* by deBary was described as having dictiosporangia, the spores emerging singly through the sporangial wall and leaving behind a "cell net" which quickly disappeared. Reinsch writes in a confused way about the sporangia and one of his figures (as well as the explanation of figures) distinctly contradicts his statements, as it shows two sporangia with distinct papillae of emergence as in *Saprolegnia* or *Achlya*. Still further confusion is caused by his reference under *A. Braunii* in the text to a figure (pl. 14, fig. 9) showing four empty sporangia emptying as in *Achlya*, but in the explanation of figures putting it under *Saprolegnia* sp. No. 2. On page 287 he says that the sporangia are usually borne on threads that do not bear oogonia; on page 297 that they are borne on different plants. It has been assumed that because of mixed material or from some other cause his observations were not correct.

Fortunately the only species heretofore assigned to the genus has strongly marked characters and Fischer and others have extended the generic description so as to include a number of the characters of the species. We are now convinced that *Saprolegnia Treleaseana* is co-generic with *Aplanes Braunii* (*A. androgynus*) and that the genus should be redefined so as to include it, as neither species can be properly assigned to any other genus. It is also obvious that these species are related to *Saprolegnia hypogyna* and it may in the end be best to transfer it also to *Aplanes*. However, as its sporangia and spores are well known to be of the *Saprolegnia* type and behavior, so that it may be retained in that genus without violence, we think it best to let it alone for the present at least. *Saprolegnia litoralis* is a connecting link with the *Ferax* group.

As modified to include *S. Treleaseana* the genus *Aplanes* may be defined as follows (adapted in part from Fischer):

Mycelium as in *Achlya*. Sporangia extremely scarce, often entirely absent for long periods in culture, cylindrical, renewed as in *Saprolegnia* and perhaps also as in *Achlya*; spores at times escaping, at times retained in the sporangium and sprouting there, their behavior not well known. Oogonia abundant, in chains or single and terminal, barrel-shaped, spherical or pyriform, their walls very thick (more so than in other water molds) and heavily pitted. Antheridial branches arising from immediately below the oogonia, or when the oogonia are in chains arising from the top of one oogonium and attached to the next above, simple or branched, the antheridia with their sides attached to the oogonia. Eggs centric, at times elliptic from pressure.

1. **Aplanes androgynus** (Archer) Humphrey. Trans. Am. Phil. Soc. **17**: 134. 1892 [1893].

Saprolegnia androgyna Archer. Quart. Jour. Mic. Sci. **7** n. s.: 123, pl. 6, fig. 1. 1867.

Achlya Braunii Reinsch. Jahrb. f. wiss. Bot. **11**: 284, pl. 14, figs. 1–6. 1878.

Aplanes Braunii deBary. Bot. Zeit. **46**: 650, pl. 9, fig. 2. 1888.

This has been reported five times: by Archer from England, by Petersen ('10, p. 526) from Denmark, and by Reinsch, deBary and Minden from Germany. It is easily recognized by the very scarce sporangia, by the peculiar oogonia which are often in chains, their walls thick and very strongly pitted, their shape subspherical or more often spindle-shaped or pyriform or barrel-shaped, and by the antheridial branches which are slender, often branched, springing from immediately beneath each oogonium and pressed against it, with small antheridia on their tips. Eggs numerous, centric, 22–34μ thick (Minden). Oogonia about 65–90 $\times$ 120–160μ.

Archer describes his plant thus:

"Plant monoecious; oogonia large, barrel-shaped or elliptic, mostly in an uninterrupted terminal series, though occasionally interstitial; the terminal oogonium the oldest in a series, the oogonia thus showing gradually different degrees of development down to the basal one which is the youngest; the lateral male branches, with the exception of those fertilizing the lowest oogonium of a series, are not derived either from the principal stem of the plant or from any neighboring portion of the general plant, but these are given off from the oogonium itself, which is next immediately beneath the oogonium which is fertilized by them, and so on down to the lowest or basal oogonium of a series, to which last are given off lateral male branchlets from the original filament or stem immediately thereunder. The tube or cavity of each lateral male branchlet becomes shut off by a septum formed a short distance above its origin, the portion of the contents above the septum being developed into the male element—that portion of the contents below the septum retaining its characters, and being returned back into the oogonium whence it originated in time to become employed, with the remainder of the contents, in the formation of the oospores. Oospores large, about $\frac{1}{830}$ of an inch in diameter, mostly numerous, but very variable in number, sometimes, though rarely, as few as even one. They occasionally exhibit what appears to be a roundish eccentric vacuole. The whole plant large and coarse as compared with other described forms in this family."

In an introductory discussion Archer states that he saw no zoospores but is led to place the species in *Saprolegnia* because he once found a group of three empty sporangia, one within the other, each with a terminal opening. The one good figure shows four barrel-shaped oogonia in a row, the apical one with a terminal papilla. Thickness of wall or pits not shown.

Humphrey points out that *Saprolegnia androgyna* Archer is the same in all probability as *Achlya Braunii* Reinsch and as *Aplanes Braunii* deBary. DeBary, with slight reservation, thought his plant the same as Reinsch's, but overlooked the similarity of *S. androgyna*. Reinsch states positively that sporangia occur which show cell nets after the escape of the spores. He also says that in most cases after the emergence of the spores the cell nets are not visible, indicating that they disappear soon. His implication throughout is that the spores always escape as in *Dictyuchus*, and one of his figures (fig. 5, pl. 14) clearly shows this method. However, in fig. 2 he shows two sporangia attached to an oogonium which are empty and show distinct openings for the discharge of the spores. In fact Reinsch did not observe at all the "*Aplanes* type" of spore germination, as deBary later described it (Bot. Zeit. **46**: 651. 1888). When we remember that deBary speaks of the sporangia as of great rarity, it seems to us that we are entirely unjustified in asserting that the spores of *Aplanes* have no swimming stage. All of Reinsch's testimony, as well as Archer's, is the other way, and as Fischer says ('92, p. 367) there can be no doubt that Reinsch's plant and deBary's are the same. In his description of the genus Fischer admits that net sporangia (as in *Dictyuchus*) seem also to occur occasionally.

Minden finds a plant (1912, p. 573) that he thinks is *Achlya Braunii* of Reinsch (*Aplanes* of deBary) and he gives one figure of the oogonia (fig. 8, p. 556) showing two long oogonia, one apical and one intercalary, each with long antheridia wrapped about it. He found the plant after it was a little old and was not able to follow the behavior of the spores, but his preparations showed a few sporangia. These were like those described by deBary with spores sprouting into threads from within the sporangium. He did not observe dictiosporangia. It may be, of course, that there are two forms of this species differing from each other in the behavior of the spores.

2. **Aplanes Treleaseanus** (Humphrey) n. comb.

Saprolegnia Treleaseana Humphrey. Trans. Am. Phil. Soc. **17**: 111, pl. 17, figs. 56–59. 1892 [1893].

Saprolegnia sp. 2 Reinsch. Jahrb. f. wiss. Bot. **11**: 295, pl. 14, figs. 7, 8, 11, 12, 13. 1878.

Saprolegnia paradoxa Petersen. Bot. Tidssk. **29**: 379. 1909; also Ann. Myc. **8**: 520, fig. 1 d and e. 1910.

Not *S. paradoxa* Maurizio. Mitt. d. Deutsch. Fischerei-Vereins 7, heft 2: 46. 1899.

Saprolegnia monoica var. *turfosa* Minden. Krypt. Flora Mark Brandenburg **5**: 516. 1912.

Saprolegnia turfosa (Minden) Gäumann. Botaniska Notiser, 1918, p. 154.

Achlya Treleaseana (Humph.) Kauffman. Ann. Rept. Mich. Acad. Sci. **8**: 27. 1905.

PLATE 20

Growth moderately stout, threads about 15–25μ thick; sporangia very scarce, usually entirely absent, cylindrical, rounded at the tip, proliferating internally in the few we have seen; spores about 11μ in diameter. Gemmae fairly plentiful (not nearly so abundant as in *S. litoralis*), the great majority rod-shaped and in chains exactly as in *Achlya deBaryana*, only here and there one fusiform or oval, etc. Oogonia spherical (without a neck), or rarely oblong or pyriform, smooth or at times papillate-warted, 27–90μ in diameter, nearly always racemosely borne on short stalks (no intercalary or cylindrical ones seen); wall hyaline, varying in thickness with the size of the oogonia; in small ones as little as 1.2μ thick, in large ones thicker than in any species of *Saprolegnia* or *Achlya*, and reaching up to 4μ; pits numerous and very conspicuous (few and less conspicuous in small oogonia). Eggs 1–30, mostly 6–20 in an oogonium, 20–26μ thick, often elliptic or block-shaped from pressure, and usually well filling or even crowding the oogonium, centric; wall thick. Antheridia on all the oogonia, very peculiar, arising from short stalks which spring laterally from immediately beneath the oogonia, an antheridial cell not being cut off from the oogonial stalk except in very few cases; tubes from the partition wall into the oogonium lacking. The whole of the antheridial thread may be cut off as an antheridium or only a part of it; at times also no wall cutting off an antheridium can be seen.

Found by us twice, first near Southport, N. C., in a ditch with algae, April 6, 1918; again from a little pond near Trenton, N. C., February 24, 1922 (Mrs. Matherly, coll.). Reported from Michigan by Kauffman (l.c.).

Humphrey described his species from Trelease's notes and material and never saw the living plant. He describes the oogonia as having rather scattered blunt outgrowths and no pits. Our plant has such outgrowths on only a part of the oogonia and very conspicuous pits, but these apparent discrepancies do not weigh very heavily with us, as Humphrey's material was probably scanty and the pits do not appear in areas where the projections are moderately frequent. The positive characters are too convincing for us to doubt the identity of the two plants.

This species has had a curious history. It is certainly the same as *S. monoica* var. *turfosa* of Minden, which he later (Krypt. Fl. Mark B. 5: 608. 1912) recognizes as the previously described species *S. paradoxa* Petersen, and admits to be of good specific rank. This name cannot stand, however, as it had been used for a different plant ten years earlier by Maurizio, a fact overlooked by Minden. Minden's description fits our plant perfectly except that he says the oogonial walls are yellowish and does not mention the scarcity of sporangia. Petersen says of his species that the oogonial walls are dilute yellow to hyaline. Minden also refers to Reinsch's unnamed "*Sap.* No. 2" as being the same, evidently, as shown from the latter's good figures (a fact that we had also noted for our plant before seeing Minden's work). Two of Reinsch's figs. show cylindrical oogonia, a condition not recorded by others. (See note under *S. anisospora* on the parasite shown in Reinsch's fig. 11). Gäumann finds the species in Lapland and calls it *S. turfosa*. It is significant that both Reinsch and Minden found the present species associated with *Aplanes androgynus*.

The plant is near *S. hypogyna* but is sharply marked by its very thick oogonial wall, racemosely borne oogonia on short stalks, and by the peculiar origin of the antheridial branches, resembling in the latter respect *Aplanes androgynus* and at times even *Pythiopsis cymosa*. A similar origin of these branches is also found at times in Forms 3 and 8 of *S. hypogyna*, but they differ easily in the hypogynous cell, in thinner walls, etc. Kauffman gives the sporangia as abundant in his form (Form 8) of *S. hypogyna*.

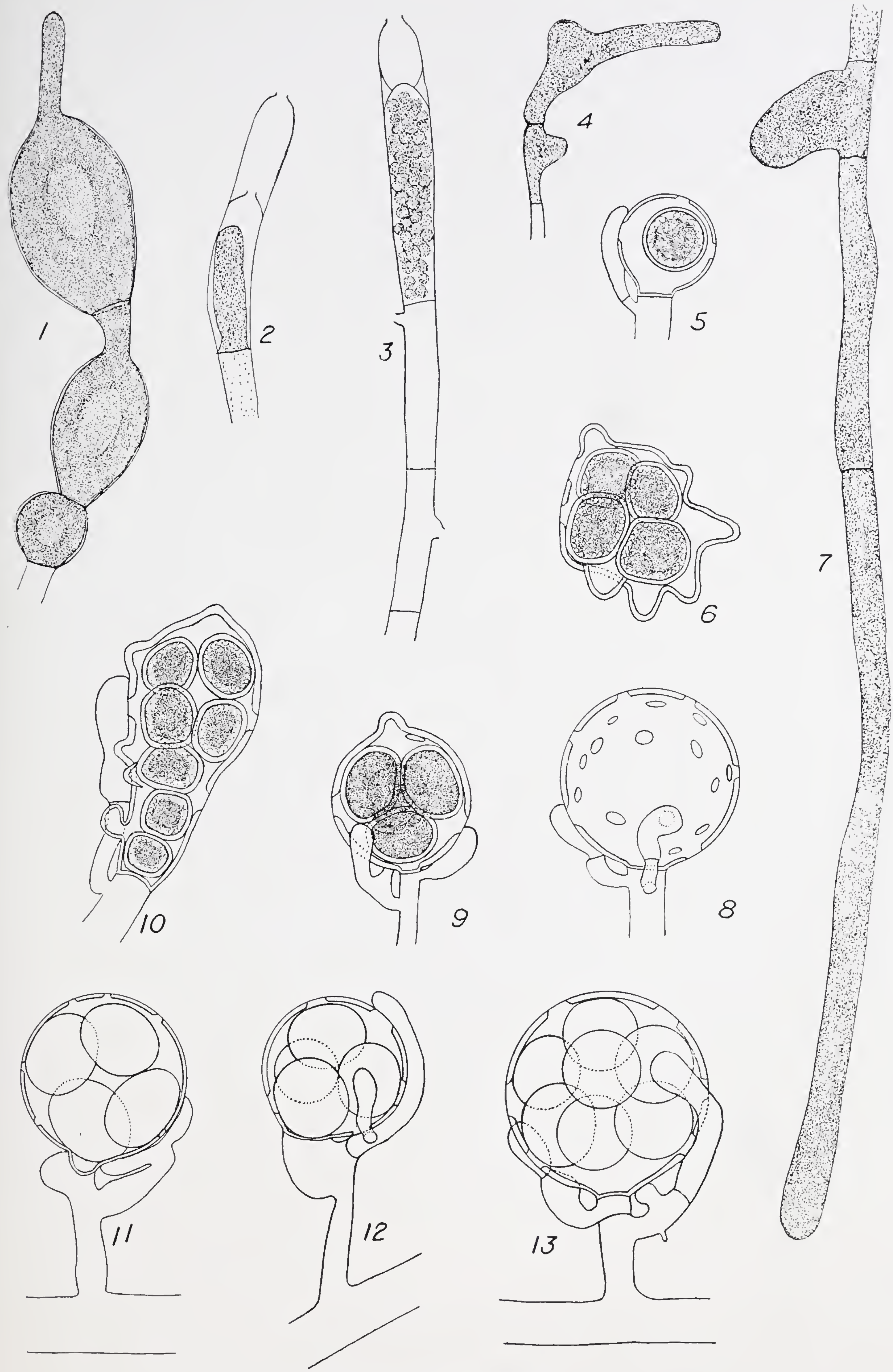
Petersen says of his plant that the walls of the hyphae are very thick, and suggests that this may be due to a parasite. Minden does not mention this in his first description of *S. monoica* var. *turfosa*, but later (p. 608) he notes that the hyphae (walls ?) are thick and yellow, and the ends are often splintered. This last peculiarity must refer to old, dead hyphae. The hyphal walls in our plant are not particularly thick, so far as they

PLATE 20

PLATE 20

Aplanes Treleaseanus

Fig. 1. Gemmae. × 503.
Fig. 2. Proliferating sporangia. × 278.
Fig. 3. Sporangia in basipetal arrangement. × 278.
Fig. 4. Gemmae. × 278.
Fig. 5. Oogonium with a single ripe egg. × 503.
Fig. 6. Oogonia with large papillae. × 503.
Fig. 7. Gemmae in basipetal arrangement. × 278.
Fig. 8. Empty oogonium (optical section and surface view combined). × 503.
Fig. 9. Oogonium with a single papilla. × 503.
Fig. 10. Irregularly shaped oogonium with ripe eggs. × 503.
Figs. 11, 12 and 13. Various forms of oogonia and antheridia. All × 503.



APLANES TRELEASEANUS.

have appeared in our cultures, and we are inclined to think that such hyphae, with such thick walls, are only occasional in the European plants. They are not shown or mentioned by Reinsch.

The sporangia and spores of this species are scarcely better known or more consistently described than in *A. androgynus.* Humphrey saw no sporangia, but placed the species in *Saprolegnia* because Trelease saw one sporangium which he said was like those of *Saprolegnia* and which he drew empty with an open tip (Humphrey, '92, pl. 17, fig. 56). Reinsch (pl. 14) gives three figures showing external proliferation through empty sporangia, but gives no detail as to the behavior of the spores. Petersen ('10, p. 520) refers his plant to *Saprolegnia* with much doubt and solely on the proliferation of the sporangia. He does not mention the spores or their behavior, a fact which indicates that the sporangia were rare. Minden (l.c.) does not mention the sporangia and spores in the body of his description of his var. *turfosa*, but says the plant cannot be *Achlya racemosa* because of the method of spore escape and the long, internally proliferating sporangia. Kauffman ('05, p. 27) finds the species in Michigan and transfers it to *Achlya* as "the sporangia emptied in the manner of *Achlya;* in every other respect it agreed with *Saprolegnia Treleaseana* Humphrey, and is no doubt the same plant."

As to ourselves, we can add little to clear up the uncertainty. The very few sporangia we have seen looked like those of *Saprolegnia* and had emptied at the tip and proliferated internally (figs. 2 and 3; emptied gemmae are also shown). We have not seen the spores emerge. Our experience emphasizes the very great rarity of the sporangia. The ones shown were from our first collection (1918) which was unfortunately lost during the summer vacation. The other collection (1922) yielded not a single sporangium, although cultivated for about three months on many sorts of media and in different temperatures with this object in view. Media used were flies, termites, mushroom grubs, corn grain, beans, etc.

ISOACHLYA Kauffman, 1921, p. 231.

Hyphae rather stout or slender. Zoosporangia formed from their tips, oval, pyriform, ventricose-clavate, elongated pyriform to clavate or cylindrical-clavate; the later ones (secondary) arising either by cymose or pseudo-cymose arrangement, as in *Achlya*, or by internal proliferation as in *Saprolegnia*, both modes occurring earlier or later in the development of one and the same species, or frequently on the same main hypha. Zoospores diplanetic, as in *Saprolegnia*, escaping and swarming separately, and after encystment swarming the second time before the formation of a germ-tube. Oogonia terminal or torulose, occasionally intercalary. Oospores with centric or eccentric contents, the spores filling the oogonium incompletely. Antheridia present or few to none.

The genus is characterized and distinguished, in the main, by the presence of the cymose or *Achlya* mode of formation of secondary sporangia, coupled with diplanetic zoospores.

The above is copied without change from the original description except for the addition of the words "or eccentric" in speaking of the oospores, so as to admit our new species, *I. eccentrica.* It should also be added that the genus is characterized by the great scarcity of antheridia, which are absent in three of the species and few in the other one. In his new genus Kauffman proposed to include *I. toruloides*, our *Achlya paradoxa* and *Saprolegnia monilifera.* After looking over the matter carefully we have concluded that while *I. toruloides* and *S. monilifera* should be put here, together with our two new species, *I. unispora* and *I. eccentrica*, it would not be well to add to them *A. paradoxa*, which differs materially. For the latter we propose the genus *Protoachlya.* It is quite possible that other species of *Saprolegnia*, such as *S. curvata, S. torulosa, S. rhaetica, S. variabilis* and *S. bodanica*, might be referred to *Isoachlya* if better known.

Key to the Species

Antheridia present on some (less than half) of the oogonia........... *I. toruloides* (1)
Antheridia absent
  Eggs usually 1–2 in an oogonium, centric.......................... *I. unispora* (2)
  Eggs usually 1–2 in an oogonium, eccentric........................ *I. eccentrica* (3)
  Eggs usually 2–6, centric... *I. monilifera* (4)

1. **Isoachlya toruloides** Kauffman and Coker. Am. Journ. Bot. **8**: 231, pls. 13 and 14. 1921.

Plate 21

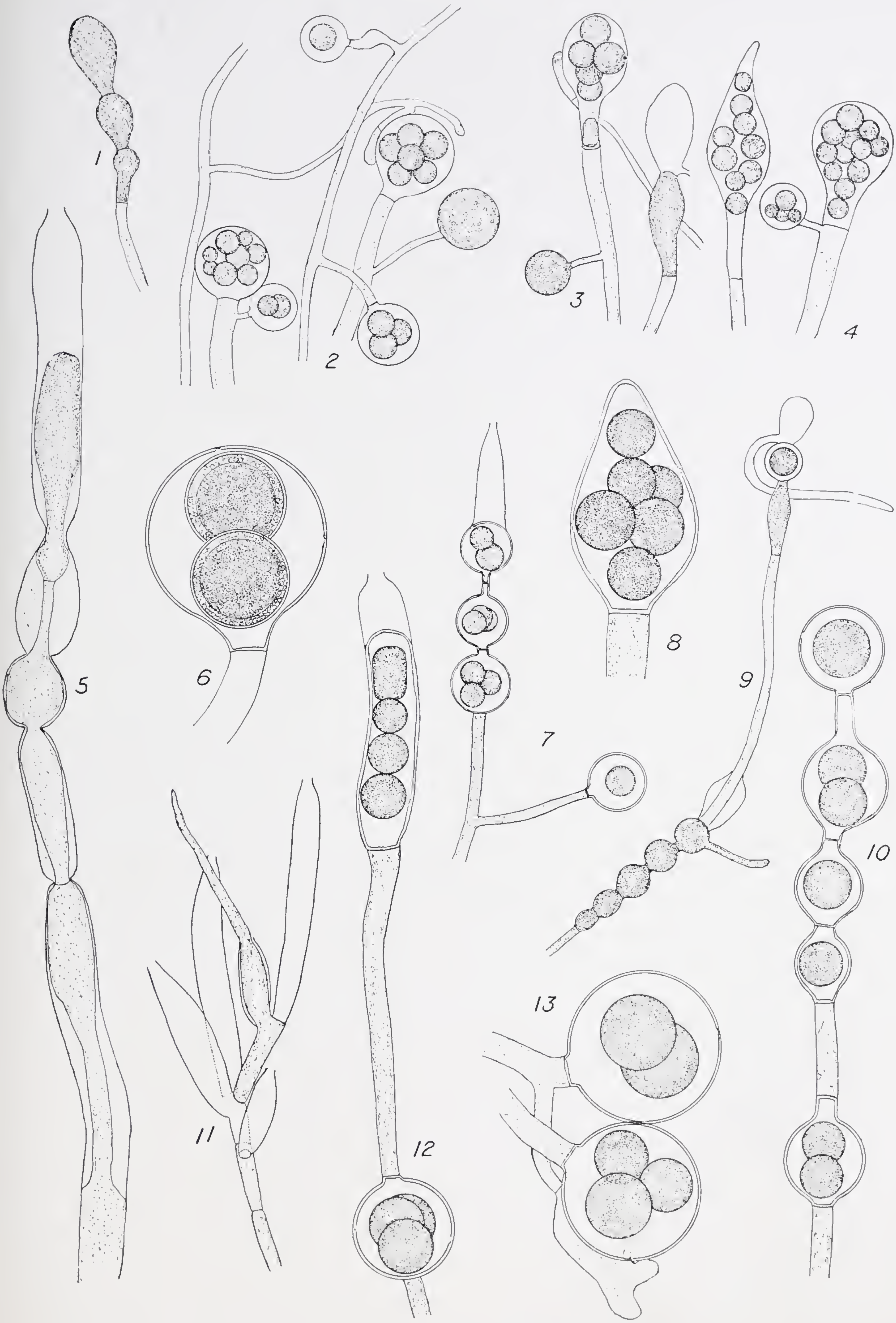
Threads delicate but vigorous, moderately branched, thickened towards the sporangia, reaching a length of about 6–8 mm. on a mushroom grub. Sporangia nearly cylindric to clavate or irregular, proliferating from within, or less often laterally as in *Achlya*, usually broadest near the papillate tip, moderately abundant throughout the growth of the culture. Spores diplanetic, 11.5–12.5μ in diameter during the first resting stage, at times sprouting through the wall of the sporangium as in *Aplanes* (net-sporangia not seen). Oogonia abundant, mostly spherical, not rarely oval or pyriform even though apical, at times pointed or otherwise irregular and rarely cylindrical in empty sporangia; with or without a neck; in young cultures mostly borne singly at the tips of main hyphae, later appearing throughout the culture on bent or crooked lateral branches about 1–3 times the length of their diameter, these stalks often sending out near the oogonium a lateral branch which bears another oogonium on its tip; in older cultures often intercalary (single) or two or three in a row (moniliform); at times a good many may be borne singly just outside the mouth of empty sporangia by the through growth of the threads that bear them; walls thin, about 1.3μ thick, colorless,

PLATE 21

PLATE 21

Isoachlya toruloides

Fig. 1. Gemmae. × 167.
Fig. 2. Oogonia with and without antheridia. × 167.
Fig. 3. Oogonia and gemmae. × 167.
Fig. 4. Oogonia. × 167.
Fig. 5. Proliferation through old sporangia. × 250.
Fig. 6. Oogonia with ripe eggs. × 417.
Fig. 7. Oogonia in chain with an old sporangium on tip of chain. × 167
Fig. 8. Oogonium. × 250.
Fig. 9. Gemmae, sporangia, and an oogonium on same hypha. × 167.
Fig. 10. Oogonia in a chain. × 250.
Fig. 11. A group of sporangia of *Achlya*-like origin, one showing internal proliferation.
Fig. 12. A spherical oogonium and a cylindrical oogonium in old sporangium on same hypha. × 250.
Fig. 13. Oogonia, one with a antheridium. × 417.



ISOACHLYA TORULOIDES.

with a few, usually 1 or 2, inconspicuous pits, or often with no pits visible. Antheridial branches diclinous, usually 'ateral from small threads in the neighborhood of oogonia, sometimes from branches which bear oogonia, very clear and hyaline, delicate, quickly disappearing after the formation of the antheridia; antheridia absent on most of the first oogonia, present on a varying proportion of the later ones, usually few, varying from almost none (less than ½ of 1%) to about 10% or 15% or even 45% on grubs, cylindrical or tuberous, laterally applied and partly embracing the oogonia, not wrapping them about, easily visible after their threads disappear; antheridial tubes formed. Eggs usually 1–6, rarely 8 or 12, centric, very variable in size, 11–33μ in diameter, most about 22–30μ, extremes often mixed in the same oogonium. Gemmae not very abundant, sub-spherical or pyriform or irregularly rod-shaped, often in moniliform chains.

Rare; found in Chapel Hill only four times: in the marsh opposite the cemetery, January 12, 1917, and again on April 30, 1918; in the branch at west end of Battle's Park, February 2, 1918; and among decaying leaves in a little branch behind Dr. Archibald Henderson's house, March 8, 1918.

Kauffman found his plant at Ann Arbor, Mich., November 23, 1920, in shallow water over peat-like organic remains, shore of First Sister Lake and in a pool of sphagnum near by. He read a diagnosis of his plant as a species under *Isoachlya*, but without specific name, before the Botanical Society of America, and I found on receiving the manuscript from him that his plant and mine were the same with perhaps a varietal difference. We then decided to publish this jointly. The diagnosis as given above by me is from the Chapel Hill form. It will be noted that the two forms differ in no important respect except in the maximum number and size of the eggs in the Chapel Hill form.

This is easily distinct from our other North Carolina species, and outside of its own genus seems nearest *S. torulosa*, as described by de-Bary and by Fischer. It differs from the latter in the much smaller proportion of chained to single oogonia, in the antheridia being only diclinous and apparently more numerous, and in the larger average size of the eggs. In *S. torulosa* the oogonia are said to be almost always in torulose rows, while in our plant the single ones are far more common. Humphrey says of the oogonia "commonly in torulose series," but of two slides of his labelled *S. torulosa* one shows only a few such rows, most of the oogonia being single as in our plant, and with a good many antheridia, while the other looks more like *I. monilifera*, with brown-walled oogonia in chains that are separating from each other. The first mentioned slide may be the present species but the oogonia have many more pits, and its identity is doubtful. It is probable that on more careful study *S. torulosa* will be found to belong to this genus. See *S. rhaetica* and *S. variabilis* which are also near.

In the character and extent of its mycelium and in some other characters also this species is most like *S. delica*. The few and always diclinous antheridia are the most striking character distinguishing this from *S. delica*. Another difference is the more hyaline and more quickly disappearing antheridial branches, which are more difficult to trace than in *S. delica* and *S. anisospora*. Moreover, the antheridia when present are rarely over one or two and are never numerous enough to cover or almost cover the oogonium, as is so often the case in *S. delica* and *S. anisospora*. Nearly all the antheridia are found in the denser, central part of the culture, the long and conspicuous antheridial branches, winding about the periphery in *S. delica*, being absent. Another difference from the latter is found in the frequent appearance of oogonia on short branches from just below the terminal ones, and also in the rather constant appearance of a number of oval or pointed or otherwise irregular oogonia in each culture. From *S. diclina* it is easily separated by the few antheridia, fewer eggs and much less abundant gemmae. From *S. ferax*, which may have about the same proportion of antheridia, it differs in having smaller oogonia, with fewer and far less conspicuous pits, much fewer cylindrical oogonia, diclinous antheridia and fewer eggs. In the same way it differs from *S. mixta*, if that is a good species.

The oogonia have a pitted appearance when the protoplasm is preparing to divide, just as in many other species. These spots are apparently oil drops or vacuoles just below a surface layer of protoplasm, and have no correspondence to the real pits that appear later.

At temperature of 79° F. the spores are expelled with force in a semitorpid state, gather strength after a few seconds of slow movement in an open cluster and then swim off. They are popped out with violence a distance of one-fourth to one-half the length of the sporangium, although almost dormant—a sure evidence of internal pressure being the cause of emergence.

The following cultures were made from No. 8 of March 8, 1918:

In .05% haemoglobin solution on house fly the growth was only fairly vigorous. Many oogonia, but few antheridia. Few sporangia. The oogonia initials form readily enough in haemoglobin solution, but the eggs do not round up until the growth is transferred to pure water.

In equal parts of .05% haemoglobin solution and .25% NaH_2PO_4 on fly. Growth quite vigorous, more so than in any other of the solutions tried, many oogonia formed and many antheridial branches, but few, in comparison to the ones formed, were applied to oogonia. A good many empty sporangia seen. As in above experiment no eggs were formed until culture was transferred to pure water.

In equal parts of .05% haemoglobin solution, .25% NaH_2PO_4, and .25% K_3PO_4 on fly. Growth slender, a fair number of oogonial initials, few antheridia. Upon transferring the culture to pure water many sporangia were formed, and a good many oogonia with eggs and with antheridia attached to them.

In equal parts of .05% haemoglobin, .25% NaH_2PO_4, .25% K_3PO_4, and .25% $Ca_3(PO_4)_2$, growth vigorous, many oogonia (but few with eggs) and many antheridial branches, but only a few applied to oogonia. In this culture many hyphae gave rise to numerous antheridial branches all along the hypha from the base almost to the tip. Many of these resembled branched and tortuous root hairs. The culture transferred to pure water and left standing for a few days and then examined. It contained many oogonia with eggs and many empty sporangia and gemmae. The antheridial branches had disappeared.

On piece of boiled corn grain cut from the germ but also having some of the hard and soft endosperm of grain. Growth vigorous, sporangia produced in fair number during youth of culture and all of them emptying. Oogonia produced in great abundance, varying much in size and shape and in the number of eggs. Antheridia very scarce, only 2 oogonia with antheridia having been seen in the whole culture. In cases where antheridia were seen antheridial tubes were developed.

On piece of boiled corn grain cut from the germ. Growth vigorous, a fair number of sporangia present. Oogonia produced in great abundance, varying considerably in shape from spherical to cylindrical. Antheridia almost totally absent, only one oogonium with antheridium having been seen.

On corn meal agar, growth fairly rapid, vigorous, covering the agar in petri dish in about two days. No sporangia. Oogonia produced in great abundance, a large majority of them containing perfectly formed eggs. About 5% of oogonia with antheridia. Some of the oogonia very irregular in shape.

2. Isoachlya unispora Coker & Couch n. sp.

PLATES 22 AND 23

Mycelium vigorous, more extensive than in most species of *Saprolegnia;* hyphae irregular, not straight or cylindrical, normally little branched, about 10–35μ thick, and usually largest toward the periphery; sporangia typically scarce, frequently almost none, often quite irregular; primary ones elongated, varying from sub-cylindrical and slightly if at all thicker than the hyphae to shorter, thicker, and more flask-shaped; secondary sporangia arising by cymose branching, and also not rarely growing through the empty ones, but in such cases the new sporangia forming entirely outside the mouth of the old ones; spores diplanetic, 9.3–13.7μ, most about 10.5–11.5μ thick at rest; emptying as in *Saprolegnia* and swimming rather sluggishly and aimlessly, some coming quickly to rest: on emerging from the cysts they swim longer and more actively. Not rarely the spores remain in the sporangia and sprout there as in most other species at times. Gemmae plentiful or few, typically spherical, with or without a neck, usually in chains, the distal member of which is not rarely an oogonium; emptying on changed conditions by an elongated papilla. Oogonia abundant, mostly spherical, rarely pyriform, usually with a distinct neck and borne on lateral branches, quite often terminal on small hyphae, and in strong cultures frequently in clusters, with the arrangement of a scorpioid cyme, not rarely intercalary or in chains of two or three, sometimes cylindrical inside old sporangia; diameter 24–75μ, most about 50μ, wall clear at first, distinctly yellowish in age, about 2.8μ thick, with few (usually 2 or 3 visible) very large and conspicuous pits. Eggs few, usually one, often two, rarely three and very rarely four, 18.5–43μ in diameter, most about 32–35μ when two in one oogonium or 40–45μ when only one; centric. Antheridia never developed.

Found four times by Mr. J. N. Couch, April 25, 1918, and June 22, 1918, in a drinking trough for horses about 2½ miles east of Chapel Hill; and April 30, 1918, and February 18, 1921, in the marshy place opposite cemetery in Battle's Park.

Differs from *I. toruloides* in larger and fewer eggs, more scorpioid arrangement of oogonia at times, and absence of antheridia. This peculiar species has a decided resemblance to *Pythiopsis Humphreyana* in its few large eggs, appearance of the oogonia, cymose branching, etc., but the latter, as well as *P. cymosa*, has monoplanetic spores. There is a tendency, though not a strong one, for the oogonia when in chains to become separated from each other; in this respect, as well as in the yellowish walls, approaching *S. monilifera*. There are formed not rarely at the base of the oogonia ingrowing tubes through the partition wall. These usually reach the egg and may indeed run along the egg surface and approach the distal side of the oogonium. Such tubes do not seem to be in any sense antheridia, as they not only do not discharge but become thick-walled and often nodulated. In an ordinary culture in distilled water spores were seen to emerge at 10:04 A. M., and swim sluggishly and aimlessly; some came to rest in less than a minute, all in about 30 minutes, at temperature of 86° F. A similar observation was made on another collection about three years later. Spores swam from one-half minute to thirty minutes, most coming to rest after twenty minutes. It is remarkable that after repeated renewal of cultures in the laboratory the color in the oogonial walls tends to disappear. This is true also of *Achlya colorata*.

Maurizio described a new species, *Saprolegnia bodanica*, which is apparently in the same group as *S. monilifera*, and *S. torulosa*. *S. bodanica* has no antheridia and has a somewhat irregular habit of growth. In these two ways it is similar to *Isoachlya unispora*. But the two species are distinct in that the latter never has over 4 eggs in an oogonium, and usually has only one, while none of Maurizio's figures show an oogonium with one egg, and most of them have over four; the oogonia in *Isoachlya unispora* have from one to several (usually 2 or 3) large pits, while the oogonia of *S. bodanica* have (in Maurizio's figures) 9–11 pits.

PLATE 22

PLATE 22

Isoachlya unispora

Fig. 1. Oogonium in an old sporangium. × 278.

Figs. 2–6. Various forms of sporangia and proliferation. 3, 5 and 6 × 278; 2 and 4 × 188.

Fig. 7. Proliferation as in *Saprolegnia*. × 278.

Fig. 8. Proliferation through old sporangial wall. × 278.

Fig. 9. *a* Two spores stained upon emerging to show cilia; *b* two double spores with 4 cilia and one double spore encysted; *c* three encysted spores and one emerging from cyst; *d* and *e* masses of protoplasm escaped from sporangium without forming into spores. All × 810.

Fig. 10. Gemmae in a chain with an oogonium at tip of chain. × 278.

Fig. 11. Sporangia renewed as in *Achlya*. × 278.

Fig. 12. Sporangia renewed as in *Pythium*. × 278.

Fig. 13. Sporangia renewed as in *Achlya* and in *Pythium*. Culture on corn meal agar square in H_2O. × 278.

Fig. 14. Showing hypha growing through wall and forming sporangium as in 8. On corn meal agar square in distilled H_2O. × 278.

ISOACHLYA UNISPORA.

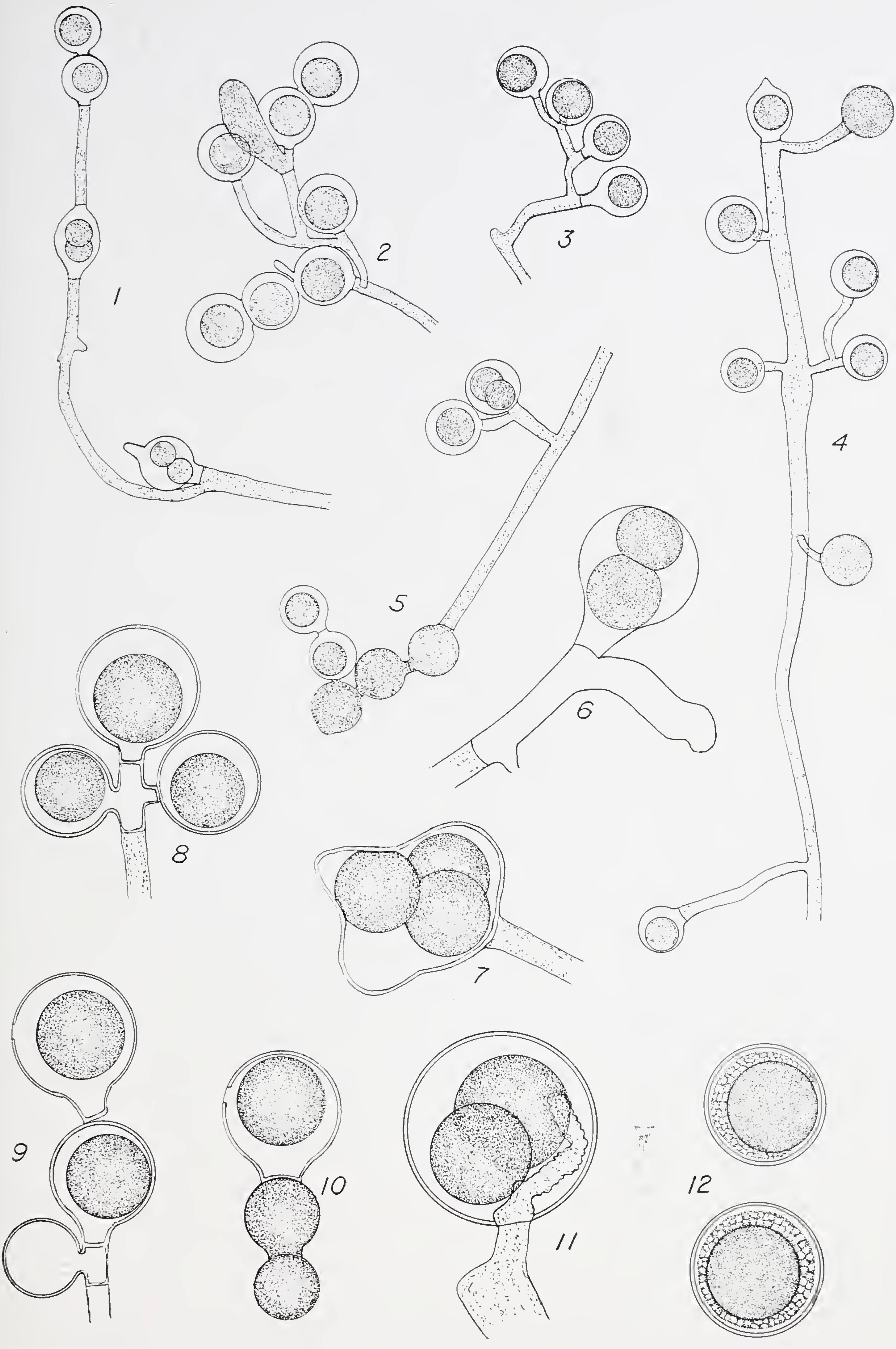
PLATE 23

PLATE 23

Isoachlya unispora

Fig. 1. Intercalary and apical oogonia. × 188.
Figs. 2 and 3. Typical clusters of oogonia. × 188.
Fig. 4. Habit of oogonia. × 188.
Fig. 5. Habit of oogonia showing some in a chain. × 188.
Fig. 6. Oogonium with sporangium formed in part of hypha just beneath it. × 281.
Fig. 7. Irregular shaped oogonium. × 281.
Fig. 8. Three oogonia in form of a cross. × 281.
Fig. 9. Oogonia in a chain, the one at base empty. × 281.
Fig. 10. Oogonium in a chain with gemmae. × 281.
Fig. 11. Oogonium with basal ingrowth. × 281.
Fig. 12. Two ripe eggs, showing structure. × 503.

PLATE 23



ISOACHLYA UNISPORA

Cultures of No. 1 of April 25, 1918:

On pieces of boiled corn grain (repeated several times): Growth fairly vigorous to very vigorous—over a centimeter in length; hyphae somewhat wavy and curled; sporangia produced normally as on grubs; many oogonia with eggs formed.

On grubs in pure water in ice box: Invariably produced many sporangia and spores. Check cultures were kept going out in the laboratory and though the room was quite hot (30° C.) they were very vigorous. They produced no sporangia, however.

On corn meal agar: Growth fairly vigorous; sporangia present; many oogonia with eggs; no bacteria visibly present. Same result with No. 1 of June 22, 1918.

3. **Isoachlya eccentrica** n. sp.

Plate 24

Threads long, slender, little branched, 6–18.5μ thick, most about 12–15μ, growth vigorous on termite ants, bits of boiled corn grain and on agar plates; length on corn grain up to 8mm., on a termite ant up to 4mm. tips pointed and clear. On corn grain many or most of the tips become sporangia of a regular cylindrical shape, with a distinct papilla, 30–45 × 142–400μ, at times broader in the middle, again near the tips. On termite ants the sporangia are less regular, with several apertures in most cases. Proliferation of sporangia not common, when present never internal as in *Saprolegnia*, but irregularly from below, as in *Achlya* and *Pythiopsis*. Dictiosporangia observed a few times. Spores diplanetic, 10–11μ thick when at rest, emerging rather slowly with the cilia directed backward, then reversing and swimming sluggishly for a very short time, many coming to rest in the immediate neighborhood of the sporangium; shape and contents as in *Saprolegnia*. Gemmae plentiful, following the sporangia, very irregular in size and shape, after a time forming spores. Oogonia spherical as a rule, seldom oval, 15–40μ, most about 30–35μ thick, usually single, at times in chains of four or five; commonly borne on short lateral stalks which are from one-half to twice as long as the diameter of the oogonia; often as well on the tips of threads which have proliferated through empty sporangia, and in such cases not rarely formed inside the sporangia; sections of old threads may also become oogonia. Wall colorless, smooth, many without pits, some with a few large, conspicuous ones. Eggs usually one, often two, rarely three or four; 12–31μ thick, most about 20–25μ, eccentric, with a single large oil drop at maturity. Antheridia none.

Found but once, without intermixture of other forms, in a small shallow pool with decaying leaves, behind the power house, Chapel Hill, N. C., December 2, 1921 (F. A. Grant, coll.).

The present plant is remarkable as being the only species of the family with a typically eccentric egg, in which the spores swim away on emerging (see remarks under *Saprolegnia anisospora*). Except for the eccentric egg all other characters are those of *Isoachlya* and we therefore refer it to that genus. The nearest relative seems to be *I. unispora* which easily differs in the smaller oogonia and smaller, centric eggs, as well as in other less conspicuous characters. It will be noted that this species as well as *I. unispora* differs from *Achlya* not only in the motility of the primary spores, but also in the frequent extension of threads through empty sporangia, although this does not lead to the formation of nested sporangia.

4. **Isoachlya monilifera** (deBary) Kauffman. Am. Journn. Bot. **8**: 231. 1921.

Saprolegnia monilifera deB. Bot. Zeit. **46**: 629, pl. 9, fig. 6. 1888.

PLATES 25 AND 50

Vegetative growth short; main hyphae 13–22μ in diameter near base. Sporangia scarce, often entirely absent, short or moderately long, usually largest near the tip; in older cultures the sporangia often proliferating laterally below as in *Achlya*. Spores 11–11.8μ in diameter. Oogonia abundantly produced in a very dense zone immediately surrounding the substratum, appearing before the sporangia, mostly in chains, the lower elements of the chain usually smaller and sometimes remaining as gemmae, commonly spherical with or without a basal neck, rarely elongated inside old sporangia (in collection No. 1 of January 15, 1913, on corn meal agar, some of the oogonia were irregular in outline); diameter about 40–93μ, most about 50–65μ, a large part of them breaking off more or less completely from the hyphae and from each other after the maturity of the eggs; walls yellowish-brown when old, smooth, slightly or not at all pitted. Eggs 1–12 in an oogonium, mostly 2–6, 17.7–33.5μ in diameter, average about 23–25μ, extremes sometimes occurring in the same oogonium, yellowish-brown, centric, with two rows of small droplets all the way around or subcentric, with one row on one side and two on the other. Gemmae abundant in all cultures, spherical, pear- or club-shaped, very often borne in chains; upon change of condition they may become sporangia, discharging their spores through a lateral papilla. Antheridia not developed.

Easily distinguished from all others by the yellowish-brown oogonia borne in chains which separate wholly or in part from each other at an early stage, and by the absence of antheridia. DeBary's figure does not show the structure of the eggs. The species has not been recognized heretofore in America.

Not rare in ponds, meadows, and small streams, such as pond across road in front of cemetery, branch back of athletic field, and Glen Burnie meadow. Collected in Chapel Hill 26 times between February 29, 1912, and December 16, 1913 (see table, p. 14). Found several times since.

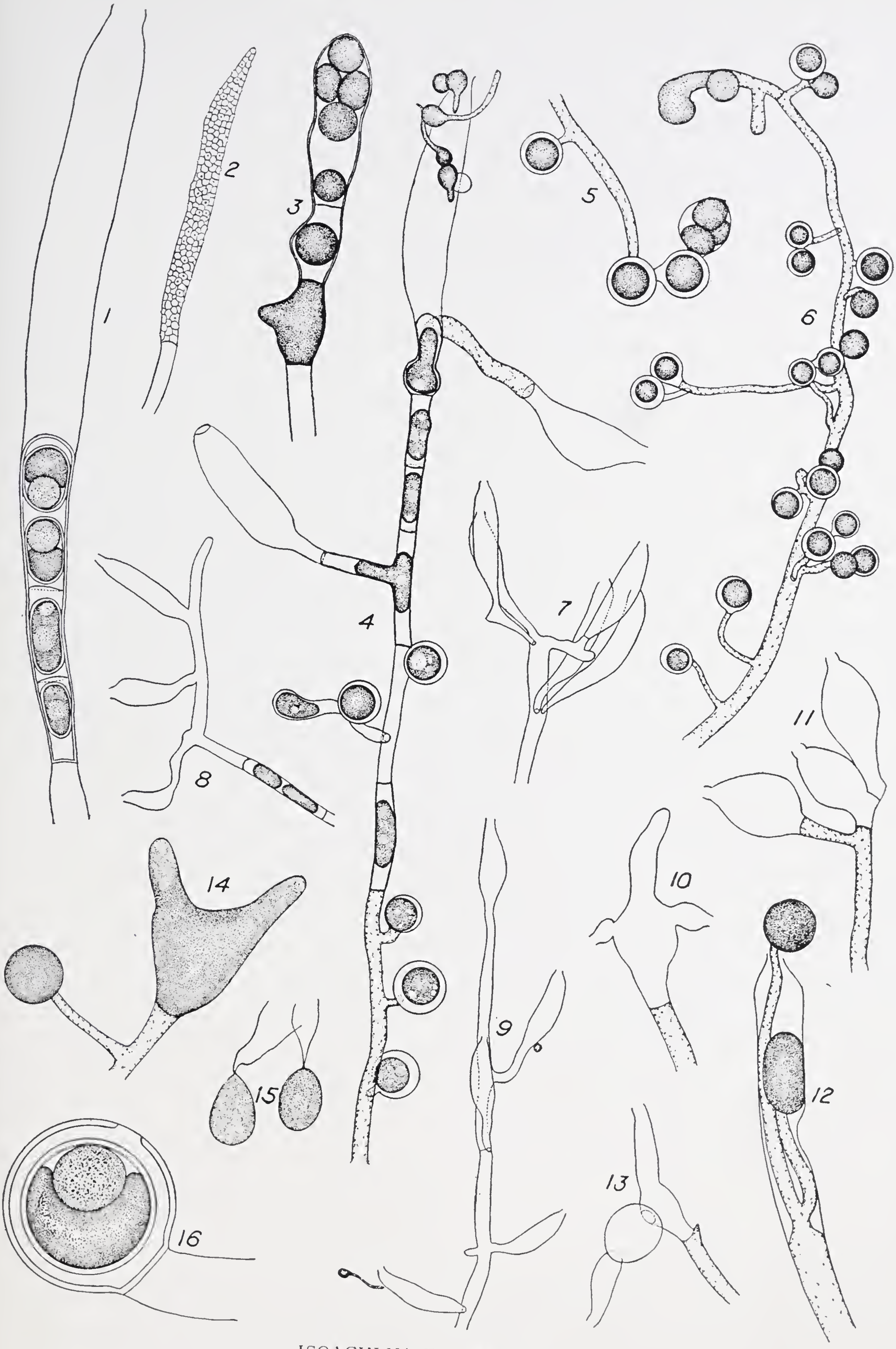
This is undoubtedly the true *S. monilifera*, and agrees in all essentials with deBary's description. In one of our collections (marsh at foot of Lone Pine hill, February 17, 1921) a good many of the oogonia with young eggs were parasitized by the penetration of a slender, non-septate fungus which destroyed the eggs and completely filled the oogonia with

PLATE 24

PLATE 24

Isoachlya eccentrica

Fig. 1. Cylindrical oogonium with four eggs inside an empty sporangium. × 447.
Fig. 2. An empty dictiosporangium. × 167.
Fig. 3. Irregular oogonia. × 247.
Fig. 4. A thread showing empty sporangia, sprouting spores and one empty cyst near top, oogonia on lateral stalks and intercalary oogonia. × 247.
Fig. 5. Part of a thread showing a single oogonium and a chain of three. × 247.
Fig. 6. A thread showing oogonia before and after eggs are formed and a gemma at the end. × 167.
Fig. 7. A cluster of empty sporangia, × 167.
Fig. 8. A compound sporangium. × 167.
Fig. 9. A group of empty sporangia. × 167.
Fig. 10. A gemma after emptying as a sporangium. × 247.
Fig. 11. Three empty sporangia. × 247.
Fig. 12. An empty sporangium proliferating internally by two threads which bear young oogonia. × 247.
Fig. 13. Two empty sporangia from transformed gemmae. × 247.
Fig. 14. An immature oogonium and a forked gemma. × 247.
Fig. 15. Two spores showing cilia in first swimming stage. × 720.
Fig. 16. An oogonium showing one pit and a mature eccentric egg. × 720.



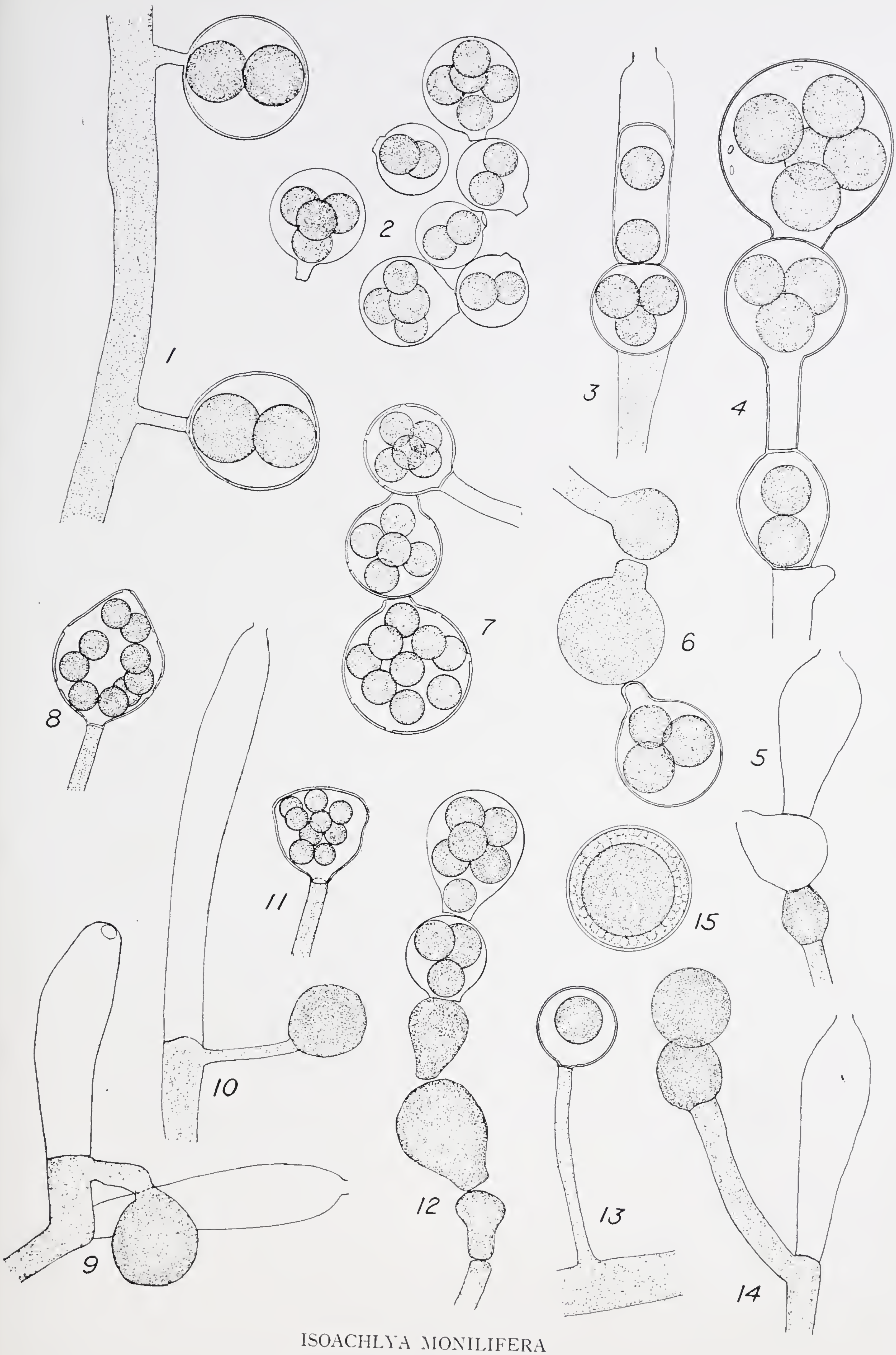
ISOACHLYA ECCENTRICA.

PLATE 25

PLATE 25

Isoachlya monilifera

Fig. 1. Stalked oogonia. × 503.
Fig. 2. Oogonia which were in a chain but have now fallen apart. × 278.
Fig. 3. Two oogonia, one inside an empty sporangium. × 319.
Fig. 4. Oogonia in a chain. × 503.
Fig. 5. Sporangia and gemma. × 278.
Eig. 6. Oogonia and gemmae. × 278.
Fig. 7. Oogonia in a chain. × 278.
Fig. 8. Oogonium of odd shape. × 188.
Fig. 9. Sporangia and young oogonium. × 278.
Fig. 10. Sporangium and young oogonium. × 278.
Fig. 11. Oogonium of odd shape. × 188.
Fig. 12. Oogonia and gemmae in chain, × 278.
Fig. 13. Oogonium with a long stalk. × 278.
Fig. 14. Sporangium and young oogonia. × 278.
Fig. 15. A mature egg. × 810.



ISOACHLYA MONILIFERA

a compacted complicated growth, making them appear somewhat like sporangia with spores. The parasite recalls a *Pythium*, but no reproductive stages were seen. In Humphrey's reference to the species (which he had not found) he speaks of the separation of the oogonia or their initials as if it always occurred. This is incorrect, as they perhaps more often hang together loosely by an angle of the stalk after bending partly to one side. DeBary's figures (pl. 9, fig. 6) show the oogonia still in chains though the eggs are fully formed. It is also not the case, as Humphrey supposed, that all the elements of a chain of initials necessarily have the same fate. Both oogonia and gemmae are often combined in a chain. The tendency to constriction is sometimes shown even in the sporangia, as in pl. 50, fig. 10. For other illustration see Minden ('12), fig.1g on p. 520. As in *I. unispora* the spores emerge slowly from the sporangium with the cilia end directed backward. After emergence most of the spores show a very sluggish movement close to the tip for a minute or so and then swim more actively away. Not rarely a few encyst without active swimming.

Isoachlya toruloides easily differs in the presence of antheridia (though often in small numbers), in the much less frequent occurrence of chained oogonia and in the paler walls of the oogonia. See remarks under that species.

As in *S. asterophora* and *Aplanes Treleaseanus* the sporangia are as a rule exceedingly scarce or absent. In one of our collections made in 1921 the numerous cultures, continued for months on various media, failed to produce a single sporangium.

The following four cultures were made from No. 1 of January 15, 1913:

On corn meal agar. Good growth and many fine gemmae. No oogonia. (No. 2 of February 17, 1921, produced many oogonia with good eggs, also gemmae).

In equal parts maltose 5% and peptone .01%. Good growth about one inch in diameter. The only reproduction was a very abnormal attempt to form gemmae or eggs. The tips swelled and only the apical half of the swelling was cut off. These tip cells generally remained filled with moderately dense protoplasm, but in a few cases eggs were formed in them.

In egg yolk broth. Good dense growth. Many inflated oogonia, about ½ with good eggs, a good many enlargements as in above experiment, with cross wall in middle or nearer tip of base. Some of these had eggs in tip cell. No other reproduction.

On corn meal egg yolk agar. Growth strong, covering the agar, many oogonia, nearly all with good eggs. Many gemmae. Oogonia almost always single and terminal. When chains were formed all except the tip one remained as gemmae. A good many oogonia were irregular in shape and so far this is the only medium in which this was the case. See figs. 8 and 11.

The next two cultures were made from No. 6 of March 27, 1914:

On an ant in distilled water. Good, but rather small growth, many sporangia, no eggs and few gemmae.

On corn meal agar. Good growth and many fine gemmae, three or four oogonia were found, the eggs not very normal looking and some going to pieces.

The following experiments were made to determine the best method of preserving live cultures:

Culture (No 1 of January 15, 1913) put in vial on corn meal agar on March 18, 1913. and was found to be dead December 1, 1913.

Culture (No. 1 of January 15, 1913) was put in jar on March 3, 1913. Test for life was made September 18, 1917, by dropping in mushroom grub, but no growth appeared.

PROTOACHLYA n. genus

This genus is established on a species collected at Chapel Hill and previously described as *Achlya*. It may be defined as follows:

Hyphae more delicate than in *Achlya;* sporangia subcylindrical to clavate or flask-shaped, blunt and usually thickest beyond the middle, proliferating like a cyme, as in *Achlya*, and also less frequently by growth through the empty sporangia, as in *Saprolegnia*. Spores diplanetic, on emerging ciliated and all or some showing sluggish or less often active motion, some remaining attached in an irregular clump to the tip of the sporangium. Oogonia borne singly, the great majority on short lateral stalks from the main hyphae and with or without a few pits; eggs usually few, centric. Antheridia androgynous or diclinous, typically pyriform with their tips applied to the oogonia. Gemmae spherical to pyriform or elongated. Vegetative behavior not noticeably different from other genera.

The genus is of great interest as indicating a possible common ancestor of several existing groups. It seems almost exactly intermediate between *Saprolegnia* and *Achlya*. The genus normally exhibits not only characters of several genera, but combines what have been considered antithetic characters, as both cymose and inter-sporangial proliferation of sporangia and motile and motionless spores on emerging. When *Achlya paradoxa* was first described (Mycologia **6**: 285. 1914) we were reluctant to introduce a new genus based on one species, and so retained it in *Achlya*, with considerable violence to the elasticity of that genus. Further study of the species has convinced us that it should be separated from *Achlya*. Kauffman ('21, p. 231) transfers it to *Isoachlya*, but this we feel sure is a mistake.

Protoachlya differs from *Achlya* in the motility of some or all of the spores on emerging, the not infrequent internal proliferation of the sporangia and their thick rounded tips, the presence of spherical or pyriform gemmae, and, from all except the *Racemosa* group, in the egg structure. It differs from *Saprolegnia* in the non-motility of some of the spores on emerging, in the predominant sympodial proliferation of the sporangia, and in the frequent occurrence of dictiosporangia. From *Isoachlya* it differs in the non-motility of some of the spores on emerging, in the frequent occurrence of dictiosporangia, in the absence of chained oogonia and in the presence of antheridia on all oogonia (normally). The relationships of the genus are not obvious. Either *Dictyuchus* or the *Racemosa* group of *Achlya* seems nearest.

1. **Protoachlya paradoxa** n. comb.*

Achlya paradoxa Coker. Mycologia **6**: 285, pl. 146. 1914.

Isoachlya paradoxa (Coker) Kauff. Amer. Journ. Bot. **8**: 231. 1921.

PLATES 26, 27 AND 28

Plant delicate; hyphae straight, slender, and little branched, the larger threads having a diameter of about 37μ below on a mushroom grub; many much smaller, the average being about 10–15μ; sporangia plentiful at all stages, narrowly club-shaped and largest at the distal end, which is about 20–30μ in diameter, rounded at the tip, and furnished with a distinct but short papilla; secondary sporangia formed usually by cymose branching beneath the old ones, but occasionally also by proliferation through the empty ones, exactly as in *Saprolegnia*, except that the new sporangia are formed entirely outside the old ones; dictiosporangia not rare. Spores diplanetic, formed in several rows as in *Saprolegnia* and *Achlya*, on emerging all ciliated, but varying greatly in behavior—some swimming away as a rule, the others remaining attached in an irregular group to the tip of the sporangium. Oogonia produced on the tips of short lateral branches, usually near the base of the main hyphae, sometimes intercalary; spherical, the diameter 32–80μ (one seen 100μ thick), sometimes elongated or flask-shaped especially when intercalary; their walls smooth and usually with a few pits (two or three often visible in addition to the thin places where the antheridia are attached);† eggs centric, usually two or four, often six, and rarely one or eight or ten (twelve seen once); their diameter from 22–37μ, averaging about 30μ; antheridia always present, generally several and sometimes so numerous as

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* Most of the data given herewith for this species is taken from the original publication, as is also plate 24 in part.

†In the original description the walls were erroneously said to be without pits. This is true only for many of the smaller ones.

completely to cover the oogonia, short, club-shaped, or often tuberous and branched, terminating slender branches of diclinous or rarely androgynous origin which at times show a tendency to twine about the oogonial branches; antheridial tubes enter the oogonia, run among the eggs, and probably fertilize them.

Found 35 times in branches, marsh borders, etc., often with algae, such as *Vaucheria,* as in brook near east gate of the University campus, Arboretum branch, etc.

In fig. 1 the sporangia are shown in a group, after the manner of *Achlya.* The bending of the sporangia that is quite noticeable in this figure is characteristic, though not always so pronounced.

In figs. 2 and 3 are shown the proliferation of sporangia by both the *Achlya* and *Saprolegnia* methods on the same thread. The latter method is rare.*

The behavior of the spores on emerging is remarkable and very variable. The usual behavior is for some of the spores, perhaps a half or a third, to swim slowly away on emerging, the others remaining attached to the sporangium mouth and encysting there. In regard to their action we give the following quotation somewhat modified from notes in Mycologia (l. c., p. 286) made at the moment of observation:

> The spores emerge somewhat elongated and may be seen to bend backward at the ends and fuse into a pear-shaped spore, as is the case in *Leptolegnia.* The spores as a rule move sluggishly and most of them soon settle to the bottom near the sporangium mouth and encyst, also a lot of inactive ones are often left in a group that sticks to the sporangium mouth, giving the effect of *Achlya.*
>
> We find cases where all, or nearly all, of the spores group themselves at the mouth exactly as is typical of *Achlya.* When conditions are unfavorable the spores sometimes do not emerge at all and in such cases they sprout in position.
>
> Noticed two sporangia empty near together. In one the spores grouped themselves at the mouth of the sporangium just as in *Achlya,* except that a few of the outermost gently rocked themselves away a little distance from the main mass and then settled down. The spores that remained in a mass at the tip of the sporangium also showed a very slight rocking movement, thus proving the presence of cilia, but in a couple of minutes they became quite still. In the other sporangium the spores charged out with great rapidity and every one dashed rapidly away.
>
> A sporangium emptied and all the spores sank slowly to the bottom of the dish, separating themselves considerably by a gentle rocking motion. After settling individuals would move spasmodically at intervals, turning and jerking, but none swam actively or any distance. This interrupted movement continued for at least a half hour after emergence.
>
> A sporangium emptied (temperature 89° F.) and all the spores were expelled to some distance from the opening, all remaining in a group as if held lightly together, and all

*We know of no reference in the literature to internal proliferation of any kind in *Achlya,* except by Petersen (Ann. Mycologici **8**: 520. 1910), who says: "Thus I have seen zoosporangia which had proliferated in undoubted species of *Achlya.*" We have found such proliferation twice in *A. imperfecta.*

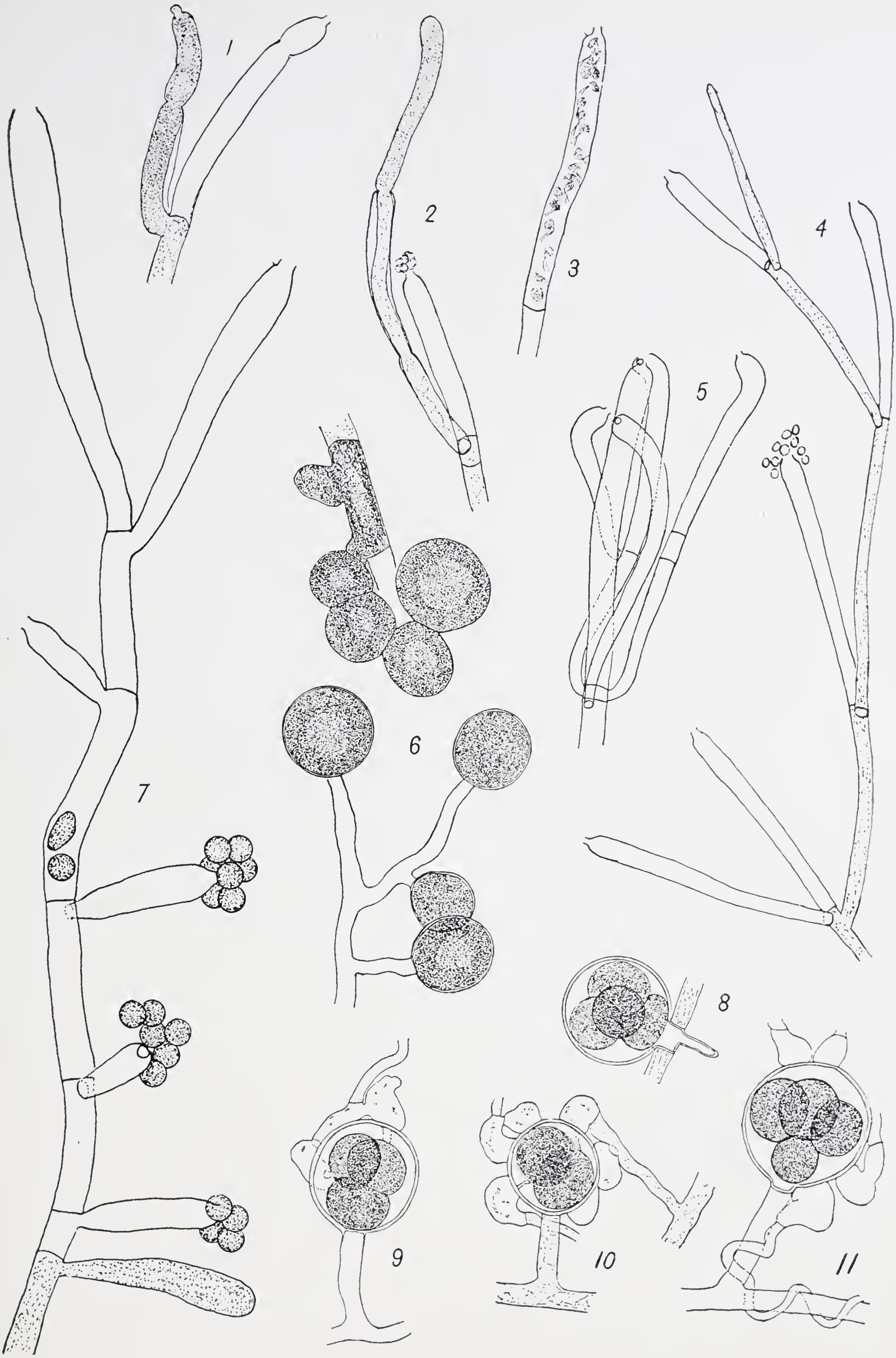
PLATE 26

PLATE 26

(All figs. except 4 and 7 are from Mycologia **6:** Pl. 146. 1914).

PROTOACHLYA PARADOXA

Fig. 1. A group of sporangia showing both lateral and internal proliferation. × 335.
Fig. 2. Another example of the above. × 335.
Fig. 3. A sporangium with spores killed during their exit, showing cilia. × 335.
Fig. 4. A group of sporangia, produced as in *Achlya*. × 162.
Fig. 5. A group of empty sporangia of normal appearance. × 335.
Fig. 6. Gemmae. × 335.
Fig. 7. Sporangial formation peculiar to this species. × 370.
Fig. 8. An oogonium with base intercalated in a hypha. The projection below is not usual; an antheridium was present, but it is not shown. × 335.
Figs. 9, 10 and 11. Typical oogonia with antheridia and fertilization tubes. × 335.



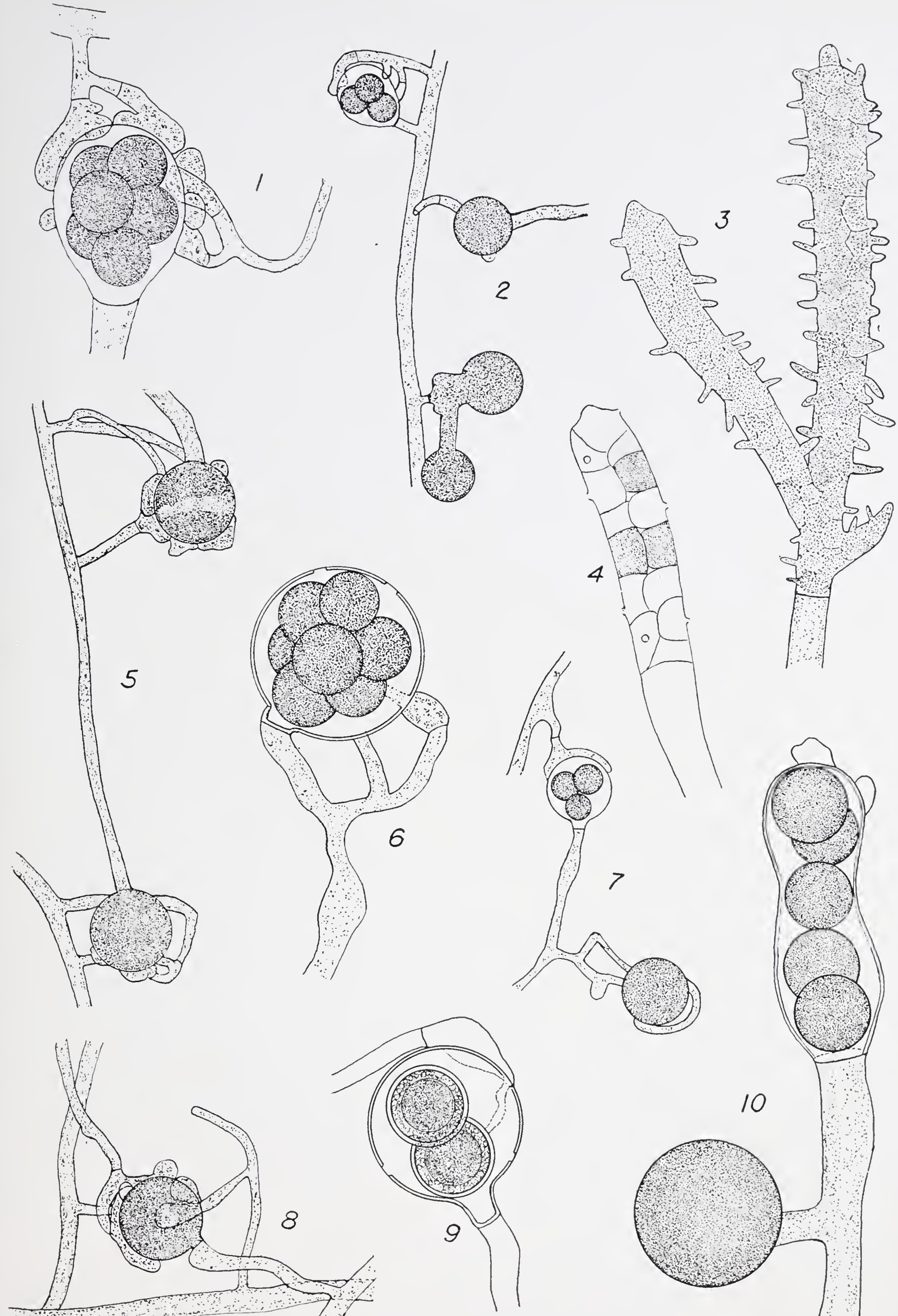
PROTOACHLYA PARADOXA.

PLATE 27

PLATE 27

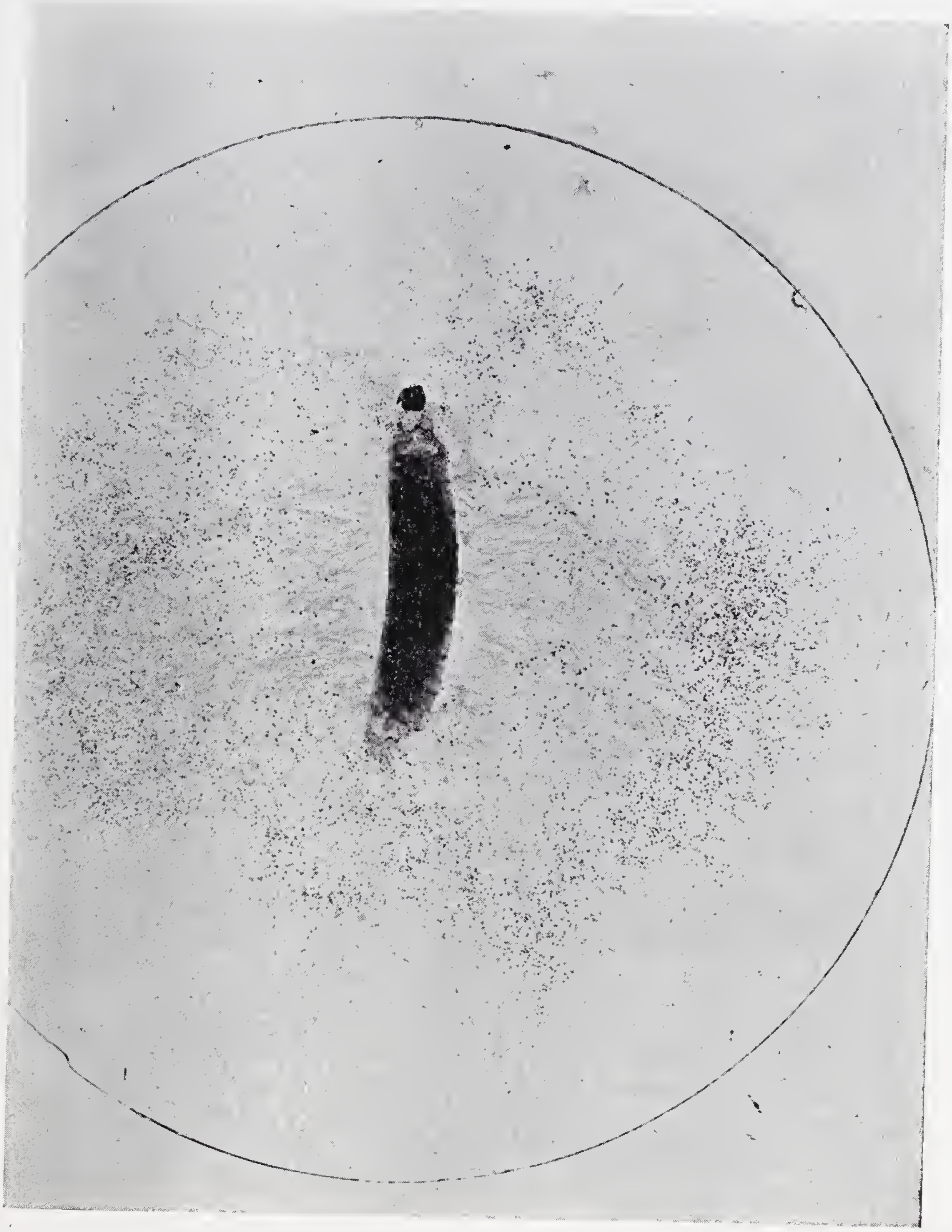
Protoachlya paradoxa

Fig. 1. Subspherical oogonium with typical antheridia. × 281.
Fig. 2. Oogonia with androgynous and diclinous antheridia, and gemmae. × 188.
Fig. 3. Sporangia with spores sprouting in position. × 281.
Fig. 4. A dictiosporangium. × 503.
Fig. 5. Oogonia with diclinous antheridia. × 188.
Fig. 6. An oogonium with an androgynous antheridium. × 281.
Fig. 7. Oogonia with androgynous and diclinous antheridia. × 188.
Fig. 8. An oogonium with antheridia arising from three different hyphae. × 278.
Fig. 9. An oogonium showing antheridial tube clearly, and two ripe eggs. × 503.
Fig. 10. An unusual shape of oogonium. × 281.



PROTOACHLYA PARADOXA.

PLATE 28



PROTOACHLYA PARADOXA. × 6.

coming to rest at once in a group. This behavior is only another proof of the fact that spores are expelled by sporangial turgor in this family and not by their own power.

The existence of cilia thus indicated by the behavior of the spores was demonstrated by treatment with potassium iodide solution at the moment of emergence. A sporangium so treated is shown in plate 26, fig. 3. All the spores can be distinctly seen to have cilia. From these observations it will be seen that the behavior of the spores has no normal parallel in the genus *Achlya*.

Oogonia without stalks and with their bases formed from a section of a hypha are not at all rare. Slender upgrowths into the oogonium from the partition below are occasionally seen. Such growths often appear in other species of *Saprolegnia* and *Achlya*, but, while they give the effect of antheridial tubes, they are apparently quite functionless except perhaps in the cases where a hypogynous antheridium is cut off (*A. hypogyna* and *S. hypogyna*). The antheridia themselves are peculiar. They are sudden enlargements of the tips of the antheridial branches and are short, thick and tuber-like. They often proliferate, and usually by growth from near the base of the antheridial cells themselves. These outgrowths are then cut off as separate antheridia. When first formed the antheridia are well filled with protoplasm and contrast strongly with the almost colorless branches that bear them. Later the antheridia appear almost empty as if they had discharged their contents into the eggs. This, however, was not actually observed. Gemmae, unlike the oogonia, are not rare, but appear plentifully, though not densely, in almost all cultures. The majority terminate short branches and approximate the oogonia in size, shape and position (plate 26, fig. 6); others are arranged in chains (fig. 6) which are usually curved or contorted. Elongated and irregular forms are also produced from somewhat swollen and knotted segments of the hyphae. Under ordinary cultural conditions, such as on flies, termite ants, gnats, mushroom grubs, etc., in water, there is usually no sexual reproduction. Out of a hundred cultures perhaps one would show a few oogonia, and their appearance seems to be entirely whimsical at times. For example, in a series of cultures made from the same original, one of the cultures on a mushroom grub produced a large number of oogonia, while others on the same as well as other media produced none. On the other hand, the oogonia appear rather frequently in cultures first appearing in the water brought in with the collection. This fact is illustrated in the following note by Mr. J. N. Couch.

"On July 25, 1918, I brought in ten glasses of water containing trash, leaves, sticks, etc. The material was put into crystallizing dishes and in order to cover the bottom well more water was added from the spigot, and four or five grubs were dropped into each dish. A few days later it was found that 7 out of the 10 collections had a fine growth of *A. paradoxa* with abundant oogonia and a fine lot of typical sporangia. As a rule *A. paradoxa* resists quite stubbornly all efforts to produce oogonia by artificial methods. Sub-cultures from the above have been made on grubs in pure water and few cultures produced any oogonia at all."

A number of experiments have been made to induce the formation of oogonia. The results of some of these are as follows:

On a bit of whole egg agar in distilled water. Growth vigorous and healthy. Sporangia abundant, emptying normally and proliferating laterally from below. No oogonia or gemmae.

On a bit of whole egg agar in distilled water. Growth vigorous, reaching a diameter of 4 cm. Sporangia slow to form, but after full growth appearing rather plentifully. Gemmae of usual shape present, but not plentiful. No oogonia.

On a bit of hard boiled egg yolk in distilled water. Growth vigorous, reaching a diameter of 4 cm. Sporangia abundant and formed earlier than in culture above. Gemmae plentiful. Oogonia present, but scarce. Antheridia of diclinous origin. As this was a culture from a single spore, the presence of antheridia proves that the plant is not dioecious.

On a bit of whole egg agar in spring water. Growth vigorous and strong. Many gemmae. No sexual reproduction.

On fly in spring water. Growth vigorous. Many sporangia, all proliferating from side below as in *Achlya*. No gemmae.

On corn meal agar. Growth extensive, filling dish. Aerial branches nearly reaching cover, but not dense. Only gemmae present.

In 5 per cent maltose + 0.1 per cent peptone solutions mixed half and half. Growth vigorous and healthy. A few small sporangia were formed, but the spores were only imperfectly discharged. Also a few of the characteristic knob-like gemmae.

On corn meal agar in tightly stopped sterile bottle. Growth vigorous, extending across bottle and making a mold-like aerial growth an inch high. On examination there were found only single gemmae, most of which were quite empty, they having sprouted by a slender thread about 3μ in diameter. In fact all the growth was remarkably slender (3μ in diameter), enlarging to normal size only just below the gemmae.

On corn meal and egg yolk agar. Growth very strong, covering dish and developing abundant aerial hyphae that reached the cover. No reproduction of any kind.

The following six cultures were all made on termites in distilled water with the salt added as indicated:

In 0.1 per cent KNO_3. Growth good. Normal sporangia, discharging and spores taking second swim, as was the case in all except the last of these six cultures. Many good gemmae of usual shape, the larger ones having a tendency to form the cross wall some way up from the base. No sexual reproduction.

In 0.1 per cent KH_2PO_4. Growth good. Many gemmae. No sexual reproduction.

In 0.1 per cent Na_2HPO_4. Growth good. A good many gemmae, but not so numerous as in the preceding cultures. No sexual reproduction.

In 0.1 per cent $Ca_3(PO_4)_2$. Growth good. Many gemmae of usual shape. No sexual reproduction.

In 0.1 per cent $Ca(NO_3)_2$. Growth good. Many gemmae. No sexual reproduction.

In 0.1 per cent K_2SO_4. Growth slight. Culture infested with fungus. Sporangia formed but not discharging. A few gemmae. No sexual reproduction.

The following seven cultures were all made on hard boiled egg yolk in distilled water with the chemical added as indicated:

In 0.1 per cent KNO_3. Strong growth. No sporangia. A few good gemmae. No sexual reproduction.

In 0.1 per cent KH_2PO_4. Growth good. A very few sporangia with normal discharge. No gemmae or sexual reproduction.

In 0.1 per cent Na_2HPO_4. Strong growth. Abundant sporangia proliferating repeatedly and discharging normally. A very few gemmae. No sexual reproduction.

In 0.1 per cent K_2SO_4. Strong growth. Sporangia plentiful. Gemmae abundant. No sexual reproduction. One sporangia was seen discharging. The emergence was rather slow, and the last few spores were very slow and showed obvious swimming movements in the sporangium on escaping. About a dozen clung to the tip of the sporangium. The others spread in a l ose flock, showing slow movements, and every now and then one would swim briskly away.

In 0.1 per cent $Ca_3(PO_4)_2$. Strong growth. Many sporangia, quite normal. A very few gemmae. No sexual reproduction. Several sporangia seen to dis harge. In two cases six spores detached themselves and moved away, in another case four.

ACHLYA Nees v. Esenbeck, 1823, p. 514.

Resembling *Saprolegnia* essentially in size, growth and appearance of vegetative parts and as now constituted approaching that genus closely in some species. Sporangia typically (except in the *Racemosa* group) broadest in the middle or towards the base, gradually pointed, not increased from within others but by lateral branching from below the older ones, at times in close clusters, again in more interrupted sympodial arrangement. Spores on leaving the sporangium coming to rest at once, or after a short period of slow rocking, in a hollow sphere or irregular cluster (in several species at least furnished with cilia during emergence), encysting there and after a few hours swimming again as in *Saprolegnia*. Oogonia borne variously as in *Saprolegnia*, with or without pits or papillae. Eggs formed of all the contents of the oogonia and not completely filling them, one to many; varying in structure with the different groups. Antheridia of near or distant origin, androgynous or diclinous, in a few species absent; fertilizing tubes usually present. Fertilization has been demonstrated in *A. americana* var. *cambrica* and *A. polyandra* Hildb. by Trow, and in *A. deBaryana* by Trow and by Mücke (see these species for details). We have observed the passage of living material from the antheridium into the egg of *A. Orion*, and it is very probable that fertilization occurs in a number of other species.

The species of *Achlya* can be divided very naturally into several distinct groups or sub-genera which are separated by the internal structure of the eggs and the form of the antheridial branches and antheridia. One of the species heretofore included we have removed to our new genus *Protoachlya*. As it remains after this removal, the genus *Achlya* contains four primary groups that we are considering as sub-genera, under the more typical of which one may recognize two well-defined assemblies. One of the sub-genera (*Centroachlya*) is so sharply delimited as easily to qualify for a new genus were one inclined to make it. As small and numerous genera are a nuisance to everyone except the most extreme specialist (and a nuisance to him except in his own field) we gladly refrain from increasing the number any further than seems to us necessary in order to retain a clear conception and definition of the old genus *Achlya*. *Achlya paradoxa*, which we are removing to *Protoachlya*, is not a good *Achlya*, as a part of its spores swim away on emerging.

As to the correct position of *A. glomerata* there is some doubt. Its odd little oogonia, single egg, and antheridia as in the *Racemosa* group seem to set it off distinctly. It may be a lead toward *Aphanomyces*.

As the words centric and eccentric have both been used to cover quite different organizations of the eggs, one must await more careful study to be sure of the position of certain European species in which the eggs are described as centric or weakly eccentric.

In attempting to put into order the members of the *Prolifera-deBaryana* group we meet with some of the most perplexing problems in the entire family. Either deBary was entirely too rigid in his descriptions of *A. prolifera*, and *A. deBaryana* (his *A. polyandra*) and failed to allow for a certain amount of normal variation, or there are more than five times as many species or sub-species (or what not) in the close group represented by these two species than he described, a fact not surprising in itself if it were not for certain strange inconsistencies in their distribution.

With the great majority of the good species of the older European botanists already known to occur in America with little or no discrepancies, it is odd indeed that *A. prolifera* and *A. deBaryana*, the two most common European Achlyas (according to deBary, Fischer, Minden and others), should so far not have been recognized in America. Instead we have several species (or forms) very close to them, indeed, but failing to agree with them in one or more characters most emphasized in their descriptions. *Achlya proliferoides* is just like *A. prolifera* except that the antheridial branches are not always diclinous and do not wrap themselves so extensively about the oogonia, and the number of eggs in an oogonium is small, nearly always two to six. On the other hand, among their many striking resemblances they are the only two species we have seen that show the conspicuous winding of the antheridial branches about the hyphae.* *Achlya imperfecta* is just like *A. proliferoides* except that it has as many androgynous as diclinous antheridia and that the antheridial branches do not wind about the hyphae. These two also show the odd peculiarity (shared to a less extent by *A. flagellata*) of failing to develop the great majority of their eggs to maturity. It would seem that such a character could not fail to attract remark if observed in related European plants,† but of this we cannot be sure. This peculiarity and the great variability of this group suggest the possibility of a hybrid origin. See Science, April 4, 1914, The Mutation Myth.

The main difficulty that led Humphrey to set up his *A. americana* was the supposed absence of pits in the European plant, as stated by all authors until recently. In 1910 Petersen ('10, p. 524) reported a plant from Denmark like *A. polyandra* deBary except that the oogonium wall was pitted, and lists it as *A. polyandra* forma *Americana*, supposing it to be Humphrey's plant and reducing it to a variety (egg measurements not given). He did not find a similar plant without pits. As the pits are very inconspicuous and are absent on many of the oogonia it is easy to see how deBary might have overlooked the pits if they were absent on certain oogonia especially examined for this point. The pits are as whimsical as in *A. flagellata* and are sometimes absent over a large part of a culture and present in another part. Most authors since deBary, in following his authority, might easily have been misled. The pits are as a rule practically invisible under low power and are not so obvious under any power as in many Saprolegnias. Horn found ('04, p. 227) that while his plant was without pits in normal media, obvious pits appeared when cultivated in peptone and in grape or cane sugar. In such cases pitted and unpitted oogonia appeared on the same hyphae, as is normally the case in our *A. imperfecta*, *A. flagellata*, and *A. conspicua*.

The following brief diagnosis of known forms of the *Prolifera* group showing the principal differences may be of interest:

Achlya americana: Wall pitted; antheridial branches practically all androgynous, branched, but short; eggs small, of moderate number.

Achlya americana var. *cambrica*: Wall conspicuously pitted; antheridial branches all (?) androgynous, on all oogonia, short and rather simple; eggs of medium size (23–26μ) and moderate number (usually 3–8).

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* Coiling may occur to a slight extent in almost any species, as in *P. paradoxa* (pl. 26, fig. 11). See also similar coiling in *A. polyandra* as shown by Cornu ('72, pl. 1).

†Horn shows proliferating oogonia and abnormal empty ones in a plant he took to be a form of *A. deBaryana* ('04, fig. 19).

Achlya deBaryana (*A. polyandra* deB.), typical form: Wall not pitted: antheridial branches almost always androgynous, but never arising from oogonial stalks, much branched, not winding about the hyphae; eggs small (18–26μ) and numerous.

Achlya deBaryana, Horn's form: Wall not pitted (except in certain chemicals); antheridial branches mostly diclinous, but also androgynous, long and branched; eggs small, few (1–5, usually 2 or 3 normally: in peptone up to 20). This is nearest *A. imperfecta* and is probably the same.

Achlya Orion: Antheridia practically all androgynous, present on about 75% of the oogonia; eggs usually 1 or 2, most about 33–36μ thick.

Achlya prolifera: Wall pitted; antheridial branches all diclinous, much branched, winding about hyphae; eggs small, rather numerous.

Achlya aplanes: Wall with or without pits; antheridial branches long and branched, all diclinous; eggs rather few, large (24–31.5μ); spores emerging as usual or remaining in the sporangium, in either case *without a swimming stage.*

Achlya proliferoides: Wall pitted; antheridial branches mostly diclinous, long and much branched, winding about hyphae; eggs small, few, mostly failing to mature.

Achlya flagellata: Wall pitted; antheridial branches more often diclinous than androgynous, perhaps about three times as often but varying in this respect, long and much branched; eggs large, of moderate number.

Achlya imperfecta: Wall pitted; antheridial branches as often androgynous as diclinous, frequently long and much branched, not winding about the hyphae, often arising from the oogonial stalks; eggs few, small, mostly failing to mature in our form (this point not mentioned by Minden).

Achlya Klebsiana: Wall not pitted; antheridial branches all diclinous, long and much branched (apparently), not wrapped about the hyphae; eggs of moderate size and number.

Achlya caroliniana: Wall not pitted; antheridial branches absent; eggs few, small.

Natural key to the Species of Achlya*

Sub-genus Centroachlya: Eggs truly centric with a complete circle of small droplets surrounding the protoplasm in *A. racemosa*, *A. colorata*, and *A. hypogyna*, subcentric in *A. radiosa*.† Antheridia present in all but one species, androgynous, borne on short and usually simple or little branched threads which arise in great part from the oogonial stalks (except in one species). Oogonial walls yellow. Sporangia typically cylindrical or slightly thicker near the tip; spores not forming a hollow sphere on emerging (in Chapel Hill species).

Racemosa Group: The only group of the sub-genus and similarly defined

Oogonia smooth; eggs commonly about 22μ thick............... *A. racemosa* (2)

Oogonia more or less thickly set with blunt or short papillae or spines (a part of the oogonia sometimes smooth)

Hypogynal cell often present........................ *A. hypogyna* (1)

Hypogynal cell absent

Antheridia on all the oogonia, and nearly always arising from the stalk, eggs usually 1–4, their diameter as a rule 30–37μ....... *A. colorata* (3)

Antheridia on all the oogonia, arising from their stalks or from the main threads; eggs 1 (rarely 2 or 3), their diameter usually 34–37μ *A. radiosa* (p. 139)

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*The position of *A. Hoferi* is entirely conjectural until the structure of the eggs is known. It is therefore omitted from the following scheme. For artificial key see p.104. American species are followed by a number, European by a page reference.

† The structure is not accurately known in the other species.

Antheridia usually on all oogonia, and arising as a rule from the main threads; eggs 4–6, their diameter about 25μ. . . . .*A. papillosa* (4)

Antheridia present on about half the oogonia, arising from just below the oogonium; eggs 1 or 2. . . . .*A. spinosa* (p. 138)

Antheridia absent. . . . .*A. cornuta* (5)

SUB-GENUS EUACHLYA: Eggs eccentric, with a large oil drop on the periphery outside of the protoplasm, or subcentric, with the protoplasm nearer one side than the other and surrounded or nearly so by small droplets of oil. Antheridial branches androgynous or diclinous, usually branched and often long; antheridia elongated, their sides applied to the oogonia; often branched. Oogonial walls hyaline or rarely yellowish. Sporangia typically narrowed toward the tip; spores with a strong tendency to form a hollow sphere on emerging.

Prolifera Group: Eggs eccentric with a single large oil drop on one side

Antheridia present; oogonial wall normally without papillae or warts

Antheridia practically all androgynous; present on all or a majority of the oogonia; eggs usually more than two

Antheridia on all oogonia, arising mainly from the main hyphae, but also at times from the *very short* oogonial stalk; oogonial wall hyaline, pitted but the pits not very conspicuous. . . . .*A. americana* (6)

Antheridia on all oogonia, nearly always arising from the main hyphae; oogonial wall yellow, with very conspicuous pits; oogonial stalk about as long as the diameter of the oogonium; eggs 23–26μ thick *A. americana* var. *cambrica* (p. 139)

Antheridia on all oogonia, arising only from the main hyphae; oogonial walls unpitted, their stalks one to three times as long as the oogonial diameter; eggs 18–25μ thick. . . . .*A. deBaryana* (p. 141)

Antheridia practically all androgynous, present on about 75% of the oogonia; eggs usually 1 or 2, mostly 33–36μ thick. . . . .*A. Orion* (7)

Antheridia always diclinous or both diclinous and androgynous, usually longer and more branched than in the above four species; present on all or nearly all the oogonia; eggs usually more than two

Oogonial wall pitted

Antheridial branches always diclinous, much branched and winding like a parasite about the oogonia; eggs small (20–26μ), numerous *A. prolifera* (p. 143)

Like the above, but eggs larger (24–31.5μ) and spores said to be without a swimming stage. . . . .*A. aplanes* (p.143)

Antheridial branches mostly diclinous, in many cases winding themselves about the main hyphae; eggs 18–24μ thick *A. proliferoides* (8)

Antheridia more often diclinous than androgynous, not winding about the hyphae and never arising from the oogonial stalks; eggs about 26–35μ thick. . . . .*A. flagellata* (9)

Antheridial branches about equally androgynous and diclinous, usually long and much branched but not winding about the hyphae, not rarely arising from the oogonial stalks; eggs 17–23μ thick . . . .*A. imperfecta* (10)

Oogonial wall unpitted (except in one case in certain chemicals); antheridia nearly all diclinous. . . . .*A. Klebsiana* (11)

Antheridia absent; eggs usually 1 or 2; oogonia smooth or with a few papillate outgrowths . . . . *A. caroliniana* (12)

Apiculata Group: Eggs in all American species not filling the oogonium and often far from filling it, subcentric, with one or more rows of inconspicuous droplets all or most of the way around the globule of protoplasm which is nearer one side than the other;* oogonial wall unpitted except in *A. conspicua*

Eggs few (usually 1–5), most about 36μ thick; oogonia often with an apiculus; antheridia in greater part androgynous . . . . *A. apiculata* (13)

Eggs few (usually 1 or 2), most about 45μ thick; otherwise as in *A. apiculata* *A. apiculata* var. *prolifica* (14)

Eggs usually 4–6, about 45μ thick; oogonium without an apiculus *A. megasperma* (15)

Eggs more numerous and smaller

Oogonia smooth, antheridia androgynous . . . . *A. polyandra* (16)

Oogonia smooth, antheridia both androgynous and diclinous *A. conspicua* (17)

Oogonia smooth, antheridia diclinous

Oogonia subspherical to short pyriform, their stalks long *A. oblongata* (18)

Oogonia spherical, their stalks short . . . . . *A. oblongata* var. *globosa* (19)

Oogonia papillate; antheridia diclinous and androgynous

Oogonia with 1–10 papillate projections; eggs usually 4–8 *A. oligacantha* (p. 144)

Oogonia with more numerous papillate projections; eggs usually about 10 *A. recurva* (p. 144)

Sub-genus Glomeroachlya: Oogonia often borne in branched glomeruli, very small, their unpitted walls thickly covered with blunt warts; eggs single, eccentric; antheridial branches androgynous and arising near the oogonium, but at times a little branched; antheridia as in *Centroachlya*. There is but one species in the sub-genus. *A. glomerata* (20)

Sub-genus Thraustoachlya: Eggs eccentric with a single large oil drop outside of the protoplasm at full maturity. Antheridia all diclinous and present on all oogonia. Oogonial wall yellowish. Sporangia often releasing the spores by disintegration as in *Thraustotheca*. There is but one species in the sub-genus . . . . *A. dubia* (21)

Position doubtful; oogonia absent . . . . *Achlya* sp. (22)

Artificial key to the Species of Achlya†

1. Oogonia absent . . . . *Achlya* sp. (22)
1. Oogonia without spines or papillate outgrowths or only a few of them with such projections 2
1. Oogonia mostly with spines or papillate outgrowths 11
2. Antheridial branches almost always androgynous 3
2. Antheridial branches diclinous or both diclinous and androgynous 6

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* The exact structure of the eggs in *A. oligacantha* and *A. polyandra* is not known. The eggs of the first are described as "centric" by deBary and those of *A. gracilipes*, which may be the same as the second, he also describes as centric, and we have no data on the egg structure of *A. recurva*. Other characters, however, point to their inclusion here.

† American species are followed by a number; European by a page reference.

3. Eggs small, averaging less than 23μ in diameter 4
3. Eggs larger, averaging more than 23μ in diameter 5
4. Oogonial walls pitted; antheridial branches arising from the main hyphae between and near the oogonial branches.....................................*A. americana* (6)
4. Oogonial walls unpitted (except where the antheridia touch); antheridial branches arising from the oogonial branches, sometimes from the base of the oogonium; eggs centric...*A. racemosa* (2)
4. Oogonial branches not long, oogonial wall unpitted; eggs eccentric, about 18–25μ thick, usually 3–10; antheridial branches rather long and branched, arising as a rule from the main hyphae (rarely from the oogonial stalks also); antheridia on all oogonia *A. deBaryana* (p. 141)
5. Oogonial branches about as long to twice as long as the diameter of the oogonia or some even longer; oogonial wall strongly pitted; most eggs about 25μ thick; antheridia on all or nearly all oogonia....................................*A. conspicua* (17)
5. Oogonial branches much longer than the diameter of the oogonia; oogonial wall unpitted; eggs subcentric (?), about 27μ thick, 5 to 25 in an oogonium; antheridia on all oogonia from branches which arise as a rule from the oogonial stalk....*A. polyandra* (16)
5. Oogonial branches much longer than the diameter of the oogonia; oogonial wall unpitted; eggs eccentric, usually 1–2 in an oogonium, most about 33–36μ thick *A. Orion* (7)
6. Oogonial wall pitted...7
6. Oogonial wall unpitted (except in one case in certain chemicals)....................9
7. Antheridial branches always diclinous, much branched and winding like a parasite about the oogonia; eggs small (20–26μ), numerous ....*A. prolifera* (143)
7. Like the above, but eggs larger (24–31.5μ) and spores said to be without a swimming stage...*A. aplanes* (p. 143)
7. Antheridial branches diclinous and androgynous..................................8
8. Antheridial branches mostly diclinous, in many cases winding themselves about the main hyphae...*A. proliferoides* (8)
8. Antheridia more often diclinous than androgynous, not winding about the hyphae and never arising from the oogonial stalks............................*A. flagellata* (9)
8. Antheridial branches about equally androgynous and diclinous, usually long and much branched but not winding about the hyphae, not rarely arising from the oogonial stalks...*A. imperfecta* (10)
9. Antheridial branches all diclinous; oogonial wall unpitted; eggs numerous........10
9. Antheridial branches all diclinous; oogonial wall unpitted; eggs few; sporangial wall often disintegrating...*A. dubia* (21)
9. Antheridial branches mostly androgynous; eggs 2–8.............*A. megasperma* (15)
10. Oogonia oval to pyriform (in a variety globular); eggs numerous, usually about 15, averaging 23μ thick (27μ in Humphrey's form)...................*A. oblongata* (18)
10. Oogonia round, their stalks short; eggs averaging 10–15 in an oogonium, and 27μ thick *A. oblongata* var. *globosa* (19)
10. Oogonia round or slightly oval, the stalks short; eggs 4–10 in an oogonium, averaging about 25μ thick...*A. Klebsiana* (11)
11. Oogonia without antheridia...12
11. Oogonia (at least in part) with antheridia...................................13
12. Oogonia spherical or elliptical; eggs 1–4, about 29μ thick...........*A. cornuta* (5)
12. Oogonia oval; eggs numerous, generally about 20, averaging 20μ thick; parasitic on fish..*A. Hoferi* (p. 145)
12. Oogonia spherical; eggs few, usually 1–2, not rarely 4, 22μ thick....*A. caroliniana* (12)

13. Antheridia both androgynous and diclinous; oogonia with a single apiculus; eggs 1–5 in an oogonium, large, averaging about 36μ thick ....*A. apiculata* (13)
13. As in *A. apiculata* except eggs usually 1–2 and oogonia smaller *A. apiculata* var. *prolifica* (14)
13. Antheridia both androgynous and diclinous, oogonia usually with several blunt papillae; eggs 4–8 (seldom up to 12 or more) in an oogonium, small (15–25μ) *A. oligacantha* (p. 144)
13. As above, but eggs usually about 10 (1–25), 22–27μ thick; papillae more numerous, and oogonial stalks recurved above..........................*A. recurva* (p. 144)
13. Antheridia all or almost all androgynous.......................................14
14. Antheridia often arising from a hypogynal cell; eggs commonly 3–5 in an oogonium, averaging 27–28μ thick ....*A. hypogyna* (1)
14. Antheridia not formed as above...15
15. Eggs commonly one or rarely two or three in an oogonium.......................16
15. Eggs commonly 4–6 in an oogonium, averaging 25μ thick..........*A. papillosa* (4)
15. Eggs commonly 1–4 in an oogonium averaging 30–37μ thick.........*A. colorata* (3)
16. Oogonium densely covered with sharp spines; eggs one, rarely two or three, commonly 34–36.5μ thick..*A. radiosa* (p. 139)
16. Oogonia covered with sharp or blunt spines, mostly barrel-shaped with a large apical point..*A. spinosa* (p. 138)
16. Oogonia covered with blunt warts, roughly spherical and with a distinct point; eggs one or rarely two, averaging about 20μ thick..................*A. glomerata* (20)

1. **Achlya hypogyna** Coker and Pemberton. Bot. Gaz. 45: 194, figs. 1–6. 1908.

Plates 29 and 30

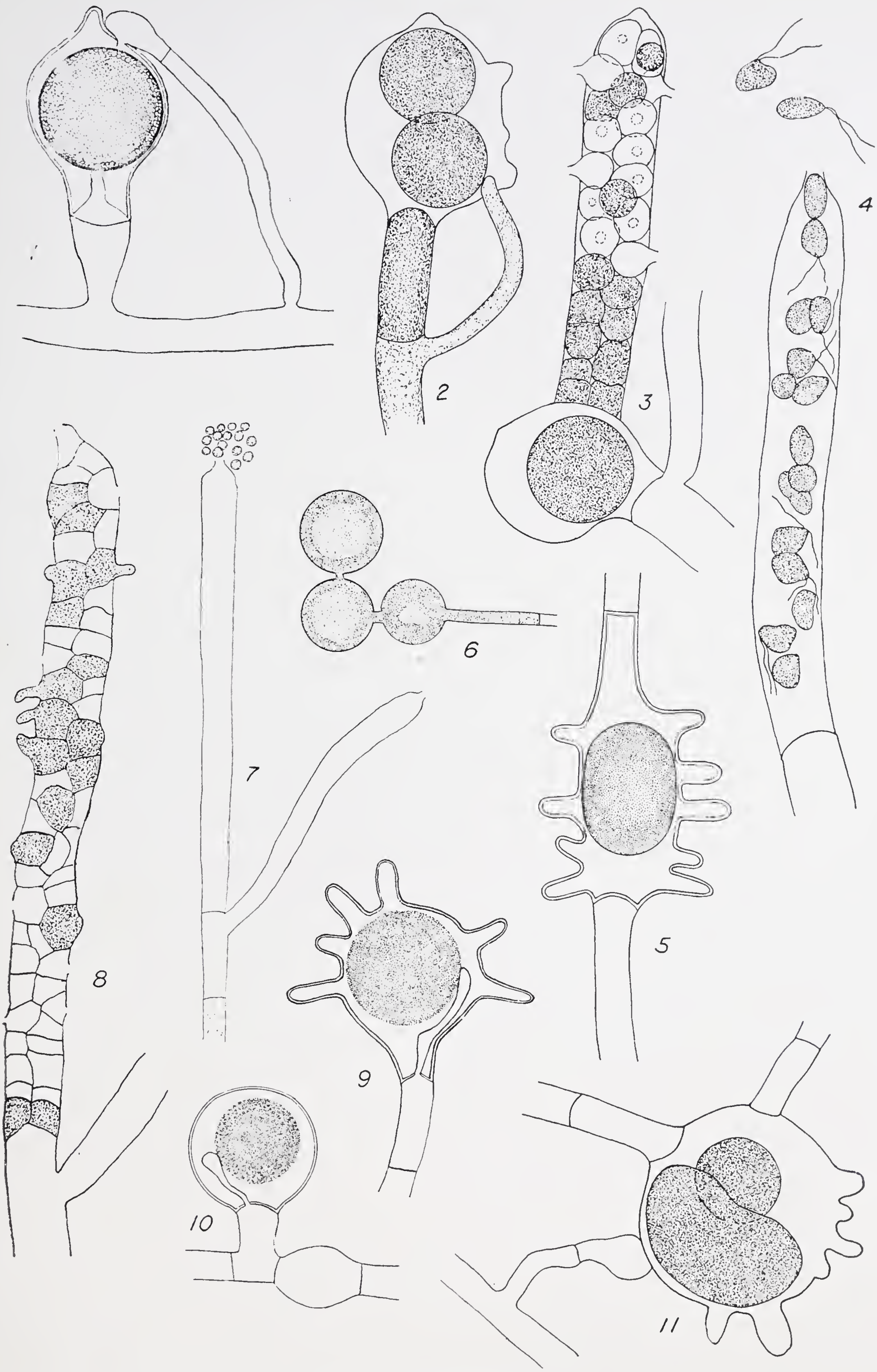
Hyphae slender, tapering gradually toward the apex, at base about 35μ in diameter, at or near tip about 8μ, in vigorous cultures reaching a length of 1 cm. Sporangia rather plentiful or few, nearly cylindrical, a little larger at the rounded and papillate distal end, usually curved, somewhat like those of *Protoachlya paradoxa;* dictiosporangia common, sometimes more abundant than the typical sort; spores on emerging ciliated, a part usually dropping to the bottom and showing a little motion from the sluggish cilia. Gemmae at times abundant, again few, pyriform or flask-shaped, less often spherical, often in chains of two, three or four; long, rod-shaped gemmae are also formed by segmentation of the hyphae. Oogonia generally borne on short branches, racemosely arranged on the main hyphae, but occasionally terminating a main hypha, and very rarely intercalary; globular or rarely oblong, the walls not pitted, more or less abundantly producing short or long rounded outgrowths, or a varying proportion smooth; yellow when old; diameter 26–83μ without the papillae which are up to 30μ long, the longest at times on the smallest oogonia. Eggs 1–7 (commonly 3–5), centric, diameter 20–36μ, averaging 27–28μ; not rarely elliptic and then up to 45 × 57μ. Antheridia cut off from oogonial branches just below the oogonia, very rarely absent; simple antheridial branches with one or

PLATE 29

PLATE 29

Achlya hypogyna

Fig. 1. Oogonium with apical papillae and single egg. × 503.
Fig. 2. Oogonium with papillae on side of wall. × 503.
Fig. 3. Oogonium with a dictiosporangium attached to it. × 503.
Fig. 4. Sporangium emptying, the spores with cilia. × 503.
Fig. 5. Intercalary oogonium with single egg and many papillae. × 503.
Fig. 6. Gemmae. × 278.
Fig. 7. Sporangia. × 188.
Fig. 8. A dictiosporangium. × 503.
Fig. 9. Apical oogonium with basal antheridium and egg with no definite wall yet formed. × 503.
Fig. 10. Smooth oogonium with egg as in 9. × 503.
Fig 11. Oogonium with diclinous antheridium. × 503.



ACHLYA HYPOGYNA.

PLATE 30

PLATE 30

ACHLYA HYPOGYNA

Fig. 1. Gemmae and sporangium. × 167.

Fig. 2. Gemmae. × 247.

Fig. 3. Two empty sporangia, one with an internal proliferation that is bearing an oogonium on its tip which is just outside the sporangium. × 247.

Fig. 4. A small oogonium with two fertilizing tubes. × 247.

Fig. 5. Habit of sporangia. × 167.

Fig. 6. Oogonium with a hypogynous antheridium and another of more distant origin. × 447.

Fig. 7. Small immature oogonium covered with long papillae. × 447.

Fig. 8. Oogonium with hypogynous and lateral antheridia. × 247.

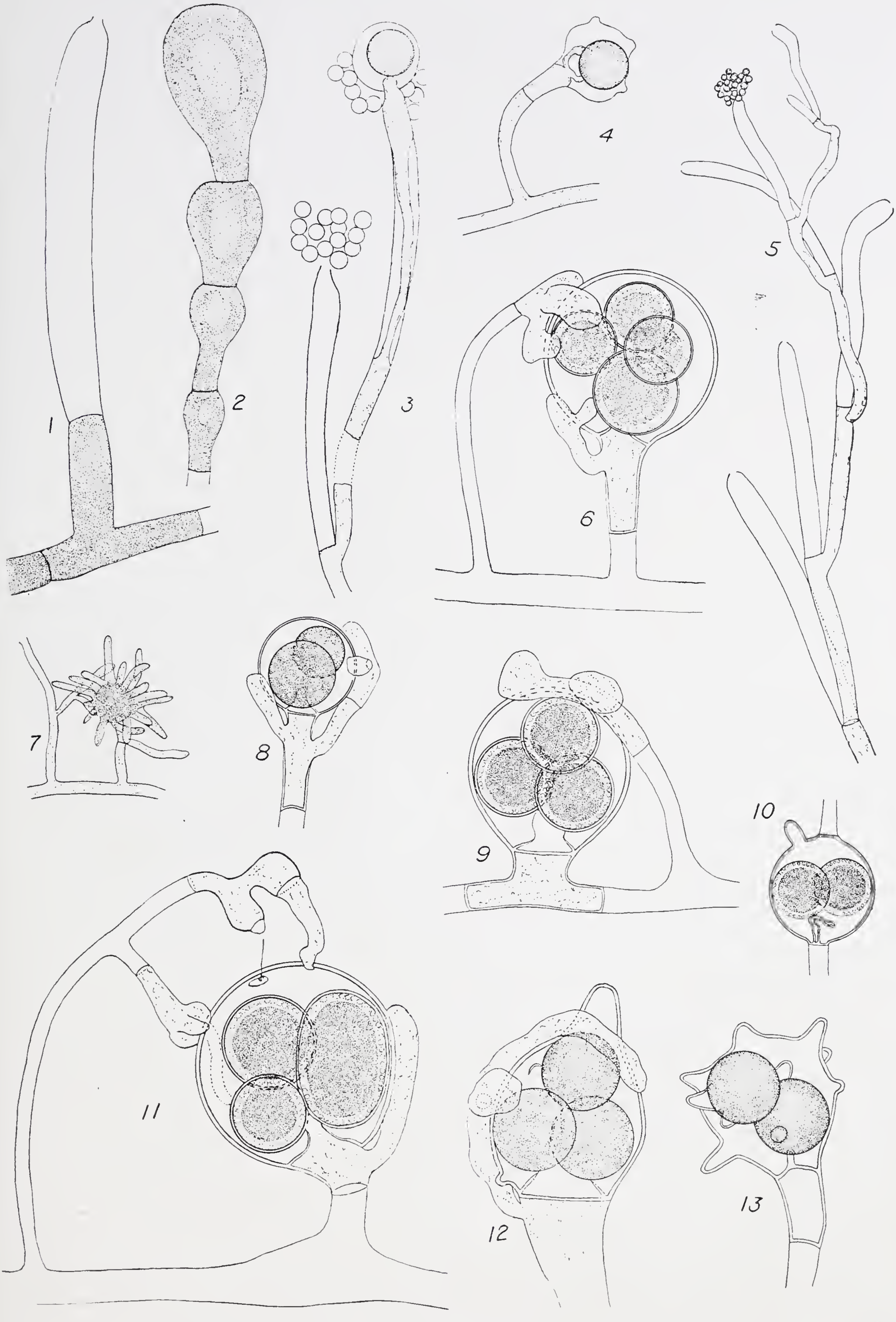
Fig. 9. Oogonium with an intercalary basal antheridium. × 447.

Fig. 10. Intercalary oogonium without antheridia but with a thick-walled ingrowth from one end. × 247.

Fig. 11. An oogonium with ripe eggs, and showing a hypogynous antheridium with a lateral branch; also antheridia of more distant origin. One of the latter with its fertilizing tubes pulled out by tension in mounting. × 447.

Fig. 12. Oogonium with a stout papilla. × 447.

Fig. 13. A typical oogonium and antheridium. × 447.



ACHLYA HYPOGYNA.

more branched, tuberous, antheridia also present at times and arising from the suboogonial cell or below it or even from the main hypha; in the latter case rarely diclinous. Fertilizing tubes arising through the common septa from the suboogonial cell and penetrating the oogonia from below (hypogynous), also from the other antheridia when present.

Compared with other species it may be said to have medium occurrence, appearing mostly in fall in such places as brook south of athletic field, Arboretum branch, branch on south edge of Glen Burnie meadow.

An extremely variable plant. The smallest or largest oogonia may be quite smooth or heavily spined or with every possible variation between; they may be quite free of antheridial cells or branches or either or both may be present, and all these different conditions may be found in a single culture on ordinary media, as a mushroom grub or gnat.

The presence of a hypogynous antheridial cell and the origin of the fertilizing tubes from the septa separating this cell from the oogonium distinguish this from all other species of *Saprolegniaceae* except the *Hypogyna* group in *Saprolegnia*. It is hardly probable that the presence of a hypogynous cell is anything more than an adventitious resemblance to *S. hypogyna*. In other essential points the two species are very different. In the latter the oogonia are borne in long chains or are intercalary and their walls are strongly pitted; the sporangia are of different shape and proliferate abundantly internally, and the spores all swarm at once. It is evident that *Achlya hypogyna* is related to *Achlya colorata* and *A. racemosa*. The general habit, the structure of the oogonia, and the sub-oogonial antheridia are very much the same as in *A. colorata;* but the slender hyphae, the hypogynal cell, colorless oogonial walls, and the smaller eggs easily distinguish the present species.

Maurizio suggests that sub-oogonial antheridia with hypogynous tubes may be of generic value in *Saprolegnia* (1899), but such a suggestion can scarcely be followed when we find this character present in only one of a group of related forms, not to mention the variability of that character in *S. hypogyna* itself.

In a very few cases the oogonia are oblong and some intercalary ones are occasionally seen. When the stalk is very short the antheridium may extend some distance into the main hypha. Oogonia with smooth walls are usually very few or none on ordinary media; occasionally, however, a culture on insects in water may contain a good many such.

Maurizio (Flora **79**: 149. 1894) and Kauffman (Ann. Bot. **22**: 382. 1908) think that the fertilizing tube in *Saprolegnia hypogyna* is not such, "but represents a tendency to produce secondary growth, as in sporangia." It is important to note in this connection that there are two kinds of growths into the oogonium. One kind is thin-walled and full of protoplasm and comes only from the true antheridial cell either below or on lateral stalks; the other is thick-walled and knotted and very soon without protoplasm and grows in from below only when no antheridial cell is cut off. This is exactly like the tubes that enter so often the oogonia in the *Ferax* group in *Saprolegnia* and the *Prolifera* group in *Achlya*. The last kind is certainly functionless; while the first-mentioned are probably functional.

The following 20 observations were made on cultures from No. 2 of September 10, 1912:

On ant in rain water. Sporangium seen to discharge at 10:55. Spores emerged rapidly, and expanded into an open cluster. All the more distant ones fell away and dropped at once to the bottom, at rest, where they lay in an open cluster, some at a distance from others, some in contact. The other half remained attached to tip of sporangium in an irregular, apparently solid, mass. There was no motion, except at very moment of escape, when the separate ones on periphery were seen to rock a little. The spores were watched, and they began to emerge from their cysts at 1:05, showing a rest of 2 hours and 10 minutes.

On egg yolk in distilled water. Spores emerged rapidly, and behaved exactly as described in preceding experiment, except that about ¾ of the spores fell at once to bottom, and that there was a very obvious movement in all the spores that could be seen individually. This movement consisted in a rocking and slight change of place and readjustment. This lasted only a few seconds.

On egg yolk in .1 % $Ca_3(PO_4)_2$. Three sporangia were seen to discharge. Two dropped all spores to bottom, and slight swimming movements were noticeable in outer ones. One sporangium was put on glass slide and then discharged. The spores spread at once in a loose colony, and most of them showed a decided rocking and change of place, far more plain than in the other cases. They could readily be watched in the very shallow water, and their movement was unmistakable.

In nearly sterile pea broth. Grew beautifully through the broth and formed a dense mat on the surface. In this mat were formed many normal oogonia. None were formed below the mat. This culture was transferred from the test tube in which it was originally made to a petri dish and more pea broth added. Soon the whole mycelium was plentifully dotted with oogonia that were quite healthy though bacteria had by this time become abundant. The great majority of the oogonia were either with hypogynous antheridia or none, but several were found with antheridia coming from a distance, as in figs. 6 and 11, pl. 30.

On corn meal agar. Extensive growth. Many gemmae and very many oogonia. These were larger than usual and less spiny. About half contained 3, 4 or 6 eggs, while in other conditions, such as on ants and in pea broth, a great majority contained only one or two eggs. No antheridia were present, and the spines were very much reduced. No inflated or abnormal oogonia.

In pea broth. Good growth, but did not fill dish. Many normal sporangia, and very many oogonia, these quite spiny and the vast majority with only one or two eggs No antheridia seen. The only resting bodies seemed to be arrested oogonia.

In maltose 5% peptone .01%. Vigorous growth, filling dish. Many inflated oogonia, some without spines, but irregular in outline. No good eggs and no antheridial tubes. No sporangia or gemmae. Protoplasm of the hyphae became segregated into dense little bits at intervals. Most of the oogonia have good long spines. A part of this culture was put in fresh maltose-peptone to see if the bits of protoplasm were alive. No growth resulted.

On corn meal, egg yolk agar. Growth very vigorous, soon covering the dish and many long aerial hyphae reaching cover (¼ inch or more long). Very abundant oogonia with hypogynal tube nearly always present, but not more than 5% with antheridial cell cut off. Spines reduced to low prominences and angles in nearly all cases. No other reproduction.

The following six cultures were on lumps of egg yolk in distilled water with the salt added:

In .1% KNO_3. Growth vigorous. No sporangia visible. Many oogonia, not more than half maturing eggs. A good many intercalary. Sub-oogonial antheridial cell not cut off in more than ⅓ cases, and no antheridial tubes seen. One case was seen of a diclinous antheridium.

In .1% KH_2PO_4. Growth vigorous. Many sporangia, discharging normally. Many oogonia, only a small number maturing eggs, Antheridial cell cut off below in great majority of cases, but no tubes formed. About 14 cases seen of outside antheridial branches with club-shaped antheridia, nearly all diclinous. This shows the effect of this salt in antheridial formation.

In .1% NaH_2PO_4. Growth vigorous. A moderate number of sporangia, discharging normally. Many oogonia, very few maturing eggs, and almost no antheridial cells.

In .1% K_2SO_4. Growth vigorous. A few sporangia. Many oogonia and a good many good eggs. Antheridial cells cut off in scarcely half the cases, but hypogynal tubes present in most cases where there were antheridial cells. Several cases seen of outside antheridial branches.

In .1% $Ca_3(PO_4)_2$. Growth vigorous. Many sporangia. In discharging about ½ scatter away from the tip with very obvious swimming motion, but slowly, and fall in an open group. A drop of iodine on an emerging group of spores showed the cilia clearly at the tip (pl. 29, fig. 4). Many oogonia, about one-third with good eggs, and these mostly with antheridial cell cut off and with hypogynal tube. The most singular thing about this culture is the occurrence frequently of rows of long eggs in sections of hyphae which might be continuations of an oogonial cavity or might not. That is, long sections of the hyphae are cut off as oogonia and these are quite smooth,— not spiny like ordinary oogonia.

In 1% K_3PO_4. Slight growth. A good many inflated and abnormal oogonia without eggs or antheridial cells.

The following six cultures were made on lumps of egg agar in distilled water with the medium added (infected from pure spore culture on corn meal agar):

In .1% KNO_3. Vigorous growth. Many oogonia, hardly half with antheridial cells, and many of the latter with antheridial tubes. Out of more than 1000 examined 9 had antheridia from outside, most of them androgynous. Oogonia normally spiny. No sporangia to be seen.

In .1% KH_2PO_4. Vigorous growth. Many normal sporangia and many normal oogonia; antheridial cell and hypogynous tube nearly always present. At least 5% of the oogonia with additional external antheridia which are either androgynous or diclinous.

In .1% NaH_2PO_4. Vigorous growth. Many normal sporangia. Three sporangia were observed while discharging. The spores were killed on slide and all showed cilia very plainly, both in and out of sporangium. Distinct swimming noticed before killing.

Oogonia abundant, the majority with antheridial cells and tubes. A good many, not over 2%, with outside antheridia.

In .1% K_2SO_4. Vigorous growth. Very few sporangia. Many oogonia, nearly all with antheridial cells and tubes and at least 5% with extra outside antheridia.

In .1% $Ca_3(PO_4)_2$. Rather sparse growth. Very many sporangia. Many oogonia, a good many extending into the threads, which also contained eggs. Antheridial cell cut off in most cases, and tube formed. Not more than 1 or 2% of outside extra antheridia.

In .1% K_3PO_4. Growth moderate. No sporangia. Many oogonia, nearly all failing to mature eggs. Antheridia cut off on only a small number.

The following two experiments were made to test the best method of preserving live cultures:

Culture from No. 2 of September 10, 1912, put in vial on corn meal agar on March 18, 1913. Found to be dead when tested on December 1, 1913.

Culture on corn meal agar put in vial of water which was then closed with a plug of cotton and put in a dark place in May, 1913. Test for life was made with a mushroom grub in December, 1913, and new growth resulted. The eggs, however, were nearly all dead and disorganized. Scarcely one in 100 was alive.

2. **Achlya racemosa** Hildebrand. Jahrb. f. wiss. Bot. **6**: 249, pl. 15. 1867.
? *Achlya lignicola* Hildb. Jahrb. f. wiss. Bot. **6**: 255, pl. 16, figs. 1–6a. 1867.

Plates 14 and 31

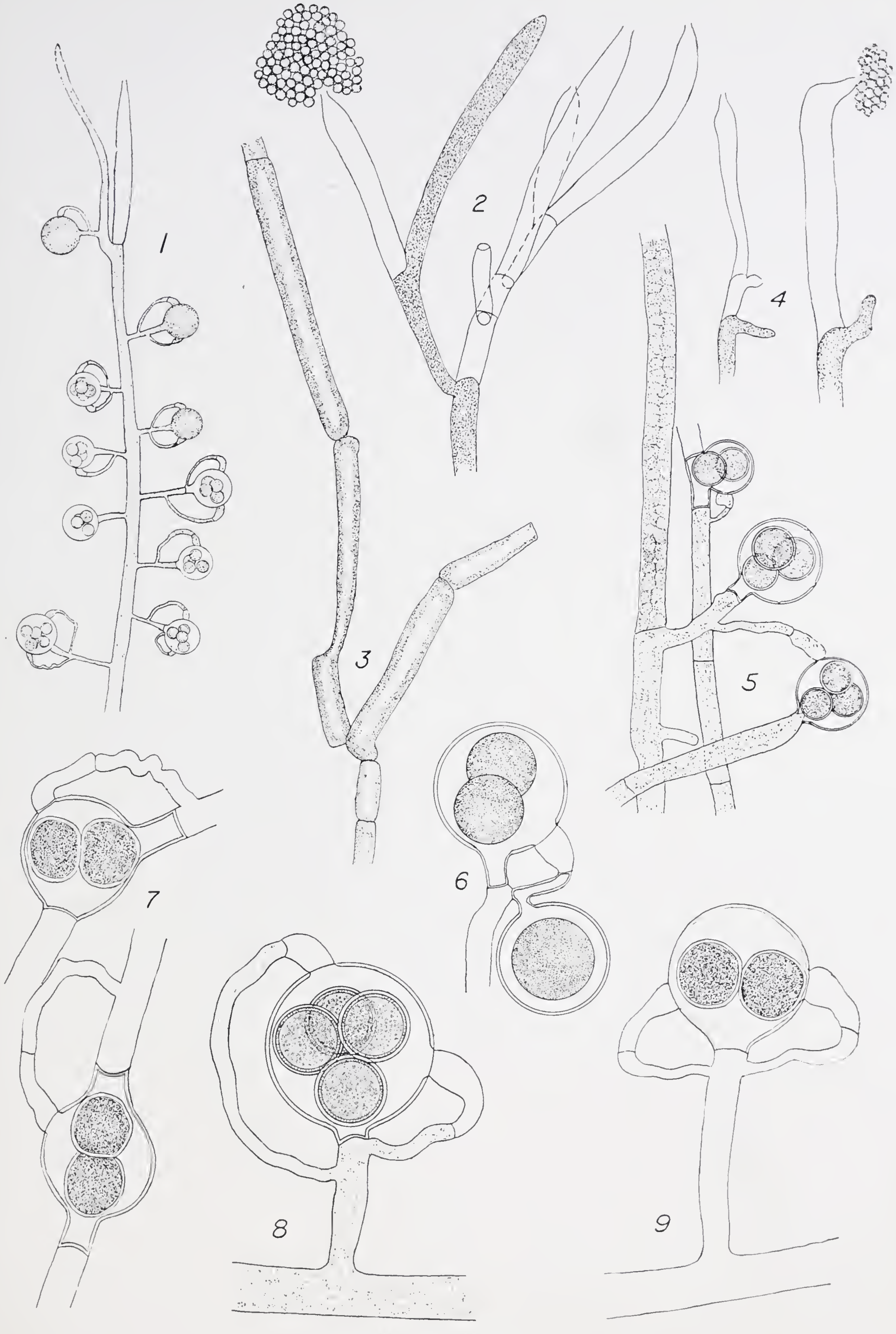
Hyphae stout, usually 25–36μ thick at base. Sporangia long, almost cylindrical, rounded or tapering at the tips, about the size of the hyphae bearing them or sometimes slightly larger, sometimes twisted like a cork-screw. Spores 9–11μ in diameter; on emerging forming an irregular cluster or imperfect sphere which slowly expands as if embedded in jelly so that the spores become more or less separated singly or in groups. Gemmae usually few, formed by the distal parts of hyphae becoming divided into joints after being densely filled with protoplasm. Oogonia racemosely borne on short lateral branches, rarely intercalary, plentifully developed in all cultures, rather small, 40–70μ in diameter; wall distinctly yellowish at maturity, smooth and unpitted except where antheridia touch. Eggs variable in size, 16.6–27.7μ in diameter, most about 22μ, centric, 1–8 in an oogonium (Humphrey says 1–10), in most cases 2–5, centric, the wall thick (about 3.5μ). Antheridial branches short, arising from oogonial branches near the basal walls of the oogonia, or as often from the neck-shaped base of the oogonium or even from its curved surface, rarely from the main hyphae. Antheridia one or two, sometimes more, to each oogonium; short-clavate, usually bent and applied by their tips to the oogonia.

PLATE 31

PLATE 31

Achlya racemosa

Fig. 1. Habit of fruiting. × 115.
Fig. 2. A sporangial cluster. × 188.
Fig. 3. Gemmae. × 122.
Fig. 4. Forms of sporangia. × 188.
Fig. 5. Habit, showing part of a sporangium and several oogonia, one intercalary and of peculiar shape, another with a diclinous antheridium. × 278.
Fig. 6. Two apical oogonia on the same hypha, an antheridium arising from the base of one and going to the other oogonium. × 503.
Fig. 7. Intercalary oogonia. × 503.
Fig. 8. An oogonium with two characteristic antheridia and ripe eggs. × 503.
Fig. 9. An oogonium with typical antheridia. × 503.



ACHLYA RACEMOSA.

Not rare in cool or cold weather in springs, branches, and edges of marshes, as in Arboretum spring and branch, and in Glen Burnie marsh. Collected 24 times in Chapel Hill between February 15, 1912, and December 15, 1913 (see table on p. 14), and many times since. Also found near Yadkin College, N. C., by Totten, and reported from Amherst, Mass., by Humphrey, and from Washington County, Michigan, by Kauffman ('15, p. 195). For other illustrations see Cornu ('72), pl. 1, figs. 2–8; Humphrey ('92), pl. 19, figs. 92–95; Pringsheim ('73), pl. 19, figs. 1–15; pl. 21, figs. 1, 2, 13; pl. 22, figs. 1–3; also ('83a), pl. 7, figs. 10–20; Zopf ('84), pl. 20, figs. 1–9 (oogonia parasitized by *Rhizidiomyces*); Petersen ('10), fig. 3i; Minden ('12), fig. 2a on p. 520.

Easily distinguished from all other species, except *A. colorata*, *A. spinosa* and *A. radiosa*, by the position and behavior of the antheridial branches, and remarkable in being the only species except *A. colorata* in which the antheridial branches may come from the oogonia themselves. Easily distinguished from *A. colorata* by the always smooth oogonial wall, and by the much smaller eggs which reach a higher number. For experiments with this species in various media see Pieters ('15b, p. 529).

Achlya lignicola is considered a synonym of *A. racemosa* by Fischer, Humphrey, Minden, and others, but Hildebrand's ('67) careful observations on apparently abundant material indicate a variety that we will call var. *lignicola* (Hildb.), which is distinguished by the more slender and less branched mycelium (growing on wood); and by the smaller oogonia which are borne ordinarily on the end of long slender hyphae, less often on short, lateral branches, but even then not racemosely arranged.

Hine ('78) describes a variety (without name, p. 140, pl. 6, figs. 1–14) which seems to differ from others in the frequent appearance of cylindrical intercalary oogonia with a single row of eggs.

Minden treats *Achlya colorata* as a variety or form *stelligera* of *A. racemosa*, and adds two other forms of his own (the second we take to be a form of *A. colorata*), as follows ('12, p. 548):

(a) *Forma maxima.*

Oogonial wall smooth or very seldom with a single projection; up to 8 antheridia and 12 eggs. This form, in its large number of antheridia, resembles *A. lignicola.*

(b) *Forma Pringsheimii.*

Oogonial walls very thick and yellow-brown, mostly with few projections which may give the oogonia an angular appearance; oogonial stalk often very short; antheridial branches often springing from the oogonia themselves, and at times from the main hyphae. With few, often only 1–3 eggs.

3. **Achlya colorata** Pringsh.* Sitzungsber. der Akad. der Wissensch. zu Berlin, 1882, p. 855, pl. 14, figs. 12, 15–31.

Achlya racemosa var. *stelligera* Cornu. Ann. Sci. Nat. Bot. **15**: 22. 1872.

PLATE 32

Hyphae stout, 25–50μ in diameter at base. Sporangia long, almost cylindrical, or slightly tapering toward the end, very little or not at all larger than the hyphae bearing them. Spores 11μ in diameter, emerging and behaving as in *A. racemosa*. In neither species is any spontaneous movement shown before encystment. Oogonia varying greatly in size, 41–90μ in diameter, rarely as much as 107μ, commonly 55–66μ, racemosely borne on short lateral branches and also at times on the tips of main branches; the yellow walls producing short, blunt outgrowths in varying number or rarely almost smooth. Eggs mostly 1–4, rather rarely 5 and very rarely 6, 26–39μ in diameter, mostly about 30–37μ, centric, the wall very thick. Antheridial branches short, arising from the oogonial branches near the basal wall of the oogonium, and, as in the typical *A. racemosa*, often from the neck-shaped base of the oogonium itself, rarely from the main hyphae. Antheridia 1–4 on each oogonium, commonly 2, short-clavate, usually bent and applying their tips to the oogonium. Gemmae formed at the maturity of the culture in large numbers. They are scarcely enlarged sections of hyphae arranged in rows of rarely over 5, one end often projecting to one side below the partition and somewhat thickened. They do not form all the way to the substratum, but only near the ends of the hyphae. When brought into fresh water they sprout by tubes or become sporangia.

Not rare in winter and spring in branches, outlets of springs, etc., as in Arboretum spring and brook, and branch by Raleigh road beyond cemetery. Collected 29 times before December 15, 1913 (see table), and many times since. For other illustrations see Pringsheim ('73), pl. 19, figs. 1–15; pl. 21, figs. 1–3 and 13; pl. 22, figs. 1–3; Pringsheim ('83a) pl. 7, figs. 10–20; Hine ('78), pl. 6, figs. 1–14; Humphrey ('92), pl. 19, figs. 96–98; Petersen ('10), fig. 3d.

This good species has been masquerading since its first discovery as a variety or form of *A. racemosa*, and Fischer (Rabenhorst's Flora, p. 351), finding papillate and smooth oogonia on the same thread, considers it the same as *A. racemosa*. It is true that smooth or nearly smooth oogonia may appear rarely in this species, but they are not the oogonia of *A. racemosa*, which are always easily recognized by their much smaller eggs. We have never seen a perfectly smooth oogonium

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*This name was first used by Pringsheim in a footnote in 1874 (Jahrb. f. wiss. Bot. **9**: 205) as a name he was using in his notes. In the body of the paper and in the plates he still uses *A. racemosa*.

PLATE 32

PLATE 32

Achlya colorata

Fig. 1. Habit of fruiting. × 115.

Fig. 2. Sporangia, one with spores sprouting as in *Aplanes* (aplanosporangium). × 115.

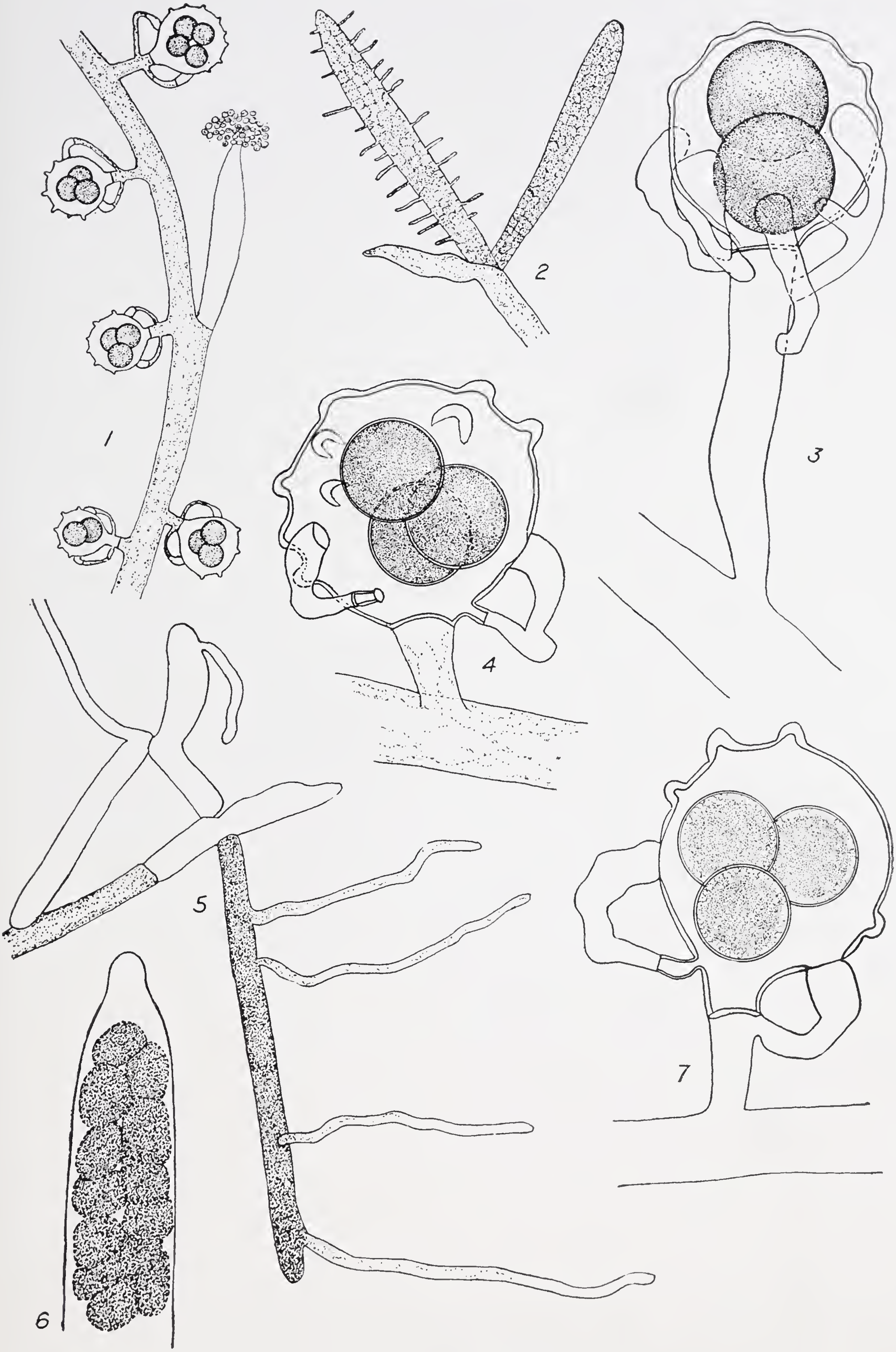
Fig. 3. Oogonium with four antheridia. × 503.

Fig. 4. Oogonium with antheridia arising from the oogonial wall. × 503.

Fig. 5. Gemmae sprouting to filaments. × 113.

Fig. 6. Enlarged tip of sporangium showing spores shrunken about 10μ away from wall before escaping. Showing that there is pressure from gelatinization of inner part of sporangial wall. × 810.

Fig. 7. Oogonium with ripe eggs and two antheridia, one arising from the oogonial wall. × 503.



ACHLYA COLORATA.

in *A. colorata* or a papillate one in *A. racemosa*. It is easily distinguished from the typical *A. racemosa* by the oogonia bearing short blunt outgrowths and by the fewer and larger eggs, which are rarely more than five to an oogonium. As in *Isoachlya unispora* the color in the oogonial walls becomes much fainter after repeated culture renewals. Both dictio- and aplanosporangia occur.

On mushroom grub in distilled water. Showed a great many oogonia (probably ¼ of all) with antheridia arising from the oogonial neck (No. 6 of Nov. 15, 1913).

On corn meal agar. Growth normal but delicate; oogonia not abundant, eggs maturing or going to pieces; antheridia absent from some oogonia; papillae irregularly produced and on some oogonia only a few in a group on one side (No. 4 of Feb. 14, 1918).

Experiment to test best method of preserving live cultures:

Culture put in aquarium jar with algae in laboratory on May 17, 1911. When tested on September 22, 1917, no growth occurred.

4 **Achlya papillosa** Humphrey. Trans. Amer. Phil. Soc. **17**: 125, pl. 20, figs. 99–102. 1892 [1893].

We have not found this and its relationship must be considered doubtful. The following is from Humphrey:

"Hyphae rather slender, long. Zoosporangia sparingly developed, cylindrical, little larger than the hyphae. Oogonia terminal on main threads or on short lateral branches, or sometimes intercalary, oval or ovate, rarely globular, thickly studded with short, blunt, wart-like outgrowths of their unpitted walls, often with a marked apiculus. Antheridial branches usually developed with each oogonium, fine and branching, arising near it from the main thread, or rarely from the oogonial branch. Antheridia imperfectly formed. Oospores as many as twelve in an oogonium, oftenest four to six, centric, their average diameter about 25µ.

Massachusetts—Amherst.

"This plant, which seems to be sufficiently distinct from previously described species, has been obtained in several cultures, but from only a single source; namely, the very prolific mossy pool in Amherst, already mentioned. It may be recognized by its long hyphae, finer than those of most *Achlyae*, and its oogonia with warty, rather than spiny, walls, and several oospores in each. I have never seen well-differentiated antheridia or fertilization-tubes, although the ends of the antheridial branches are applied to the oogonia.

"While bearing no near resemblance to any species heretofore figured, this plant may be somewhat closely related to the next [*A. recurva* Cornu], if the latter is well founded."

5. **Achlya cornuta** Archer. Quart. Jour. Mic. Sci. 7: 126, pl. 6, figs. 2–6. 1867.

? *Achlya stellata* deBary. Bot. Zeit. 46: 648, pl. 10, figs. 10 and 11. 1888.

This species has been found by Humphrey at Amherst and this is the only American record. His description follows ('92, p. 126. See also his pl. 20, figs. 103 and 104):

"Hyphae of medium size, short. Zoosporangia rare, cylindric. Oogonial branches rarely long, straight or flexuous, racemosely arranged. Oogonia terminal, globular or elliptical, densely beset with rather long, blunt outgrowths of their unpitted walls, the apical one often larger and forming an evident apiculus. Antheridial branches and antheridia wanting. Oospores from one to four in an oogonium, globular or slightly flattened, centric, their average diameter about 29µ.

"Massachusetts—Amherst. Europe.

"The same culture which yielded *A. megasperma* for the first time contained a small amount, all I have seen, of this form. It has been referred with some doubt to Archer's species, since it fails to show at all a feature which one would suppose, from that author's account and figures, to be very characteristic of his plant; namely, the development of several oogonia in a series from a single hypha. In other respects, however, it corresponds too closely with his description to justify one in regarding it as distinct. Archer saw no sporangia, probably not, as he thought, because he found it too late, but because of their rarity. In species which produce sporangia abundantly, one can always find empty ones on plants with mature oospores. In the limited material at my disposal, I have been able to find but a single one, and that only long after it was emptied. From below its base arose a branch bearing an oogonium. This, so far as it goes, supports Archer's conclusion that the plant is an *Achlya*, which seems almost certainly correct. The oogonial branches sometimes show the incurving mentioned by Archer, and are often less definitely bent. This writer states that an oogonium may contain as many as eight or ten oospores; but I have never seen more than four, and his figures show no more than three. He describes no special antheridial branches, but says that the antheridia are like those of *A. dioica* Pringsh. As these latter are not antheridia at all, one would expect to find, as is the case with American specimens, that the species has no true male organs. As will be seen from the figures, the spines could hardly be more closely set, and their form is more cylindrical than conical.

"This and the next species [*A. stellata* deBary] seem to be closely related, the more so if the American form here described proves to be more typical than Archer's."

The species is very near *A. spinosa* deBary, and Fischer considers it as the same, both being characterized by very few sporangia (Archer found none). However, as that species has antheridia on about half the oogonia it would seem unjustifiable at present not to separate them. Humphrey regards *A. cornuta* as nearest *A. stellata* deBary, which he says is almost too similar. The latter has no antheridia, thus agreeing with *A. cornuta* in this respect. As there seem to be no differences of any consequence between the last two species we are treating them as probably the same.

6. **Achlya americana** Humphrey. Trans. Amer. Phil. Soc. **17**: 116, pl. 14, figs. 7, 9, 10; pl. 15, figs. 24, 25, 29; pl. 16, figs. 30–36; pl. 18, figs. 69–73. 1892 [1893].

PLATES 33 AND 34

Growth not dense, consisting of stout hyphae with more slender ones intermingled, the largest up to 100μ thick at base, the tips pointed. Sporangia long, slender, usually more or less fusiform (one of about average size measured 22 × 370μ); emptying normally, the spores furnished with cilia as they emerge (Humphrey), 10.5μ thick. Gemmae very few, not peculiar, elongated and formed by segmenting hyphae, single or two or three in a row. Oogonia numerous, racemosely borne from the base to the tip of main hyphae on short stalks which are usually straight and much shorter than the diameter of the oogonia (rarely oogonial stalks may be several times longer than diameter of oogonia); not rarely apical on main threads (fig. 2), no intercalary ones seen (rarely intercalary, Humphrey); spherical, rarely distorted, 40–90μ, most about 50–60μ thick; walls hyaline, rather thin, pits numerous and obvious. Eggs varying little in size, 18.5–25μ, the great majority about 22μ thick, rarely a very small one about half size occurs with the normal ones, 3–30 or even more, usually 6–12, in an oogonium, eccentric. Antheridial branches androgynous, occasionally one from an adjoining strand, one or two, seldom more on each oogonium; arising from the main hyphae near the oogonia or rarely from the oogonial stalk. Antheridia elongated and closely applied to the oogonia, antheridial tubes developed and clearly visible.

Our cultures were obtained from two sources in vials of water with a little trash kindly sent us in June, 1920, from Woods Hole, Mass., by Mr. George M. Gray, Curator of the Marine Biological Laboratory. In one of these collections there were a good many oogonial stalks that were as long as or even longer than the oogonial diameter, though such were greatly in the minority; in the other strain only a very few, perhaps one or two in a culture, were of such length. It is strange that the species has never appeared in any of our North Carolina collections. Humphrey reports it from Massachusetts, Pennsylvania, Alabama, and Louisiana, and he speaks of it as "our most abundant member of this genus, and indeed of this family" so far as he has observed. The only foreign records of this species we have met with are by Petersen ('10, p. 524), who reduces it to a form of *A. polyandra* de B. and calls it "forma *Americana*," and by Minden ('12, p. 645) who reduces it to a variety of *A. deBaryana*. The species differs easily from our interpretation of *A. deBaryana* in the short androgynous antheridial branches, short-stalked oogonia and smaller eggs. See under the genus and under *A. imperfecta* for comparisons and discussions.

This is the species that Humphrey studied with sections to determine the presence or absence of sexual fusion, and he came to the conclusion that no fertilization occurs ('92, p. 94). This conclusion is, however, in all probability erroneous, as an examination of his own figures will show (see Trow '95, p. 638; and '99, p. 163). We have examined Humphrey's slides (not the cytological preparations) generously lent by Dr. D. S. Johnson, and find his plant to be identical with ours from Woods Hole. In two of Humphrey's figures (29a and 29b) germinating eggs are shown.

An Irish plant, considered a variety of this by Trow, has not been found in America (see p. 139). It is probably of specific rank.

7. **Achlya Orion** Coker and Couch. Journ. E. Mitchell Sci. Soc. **36**: 100. 1920.

Plates 34 and 35

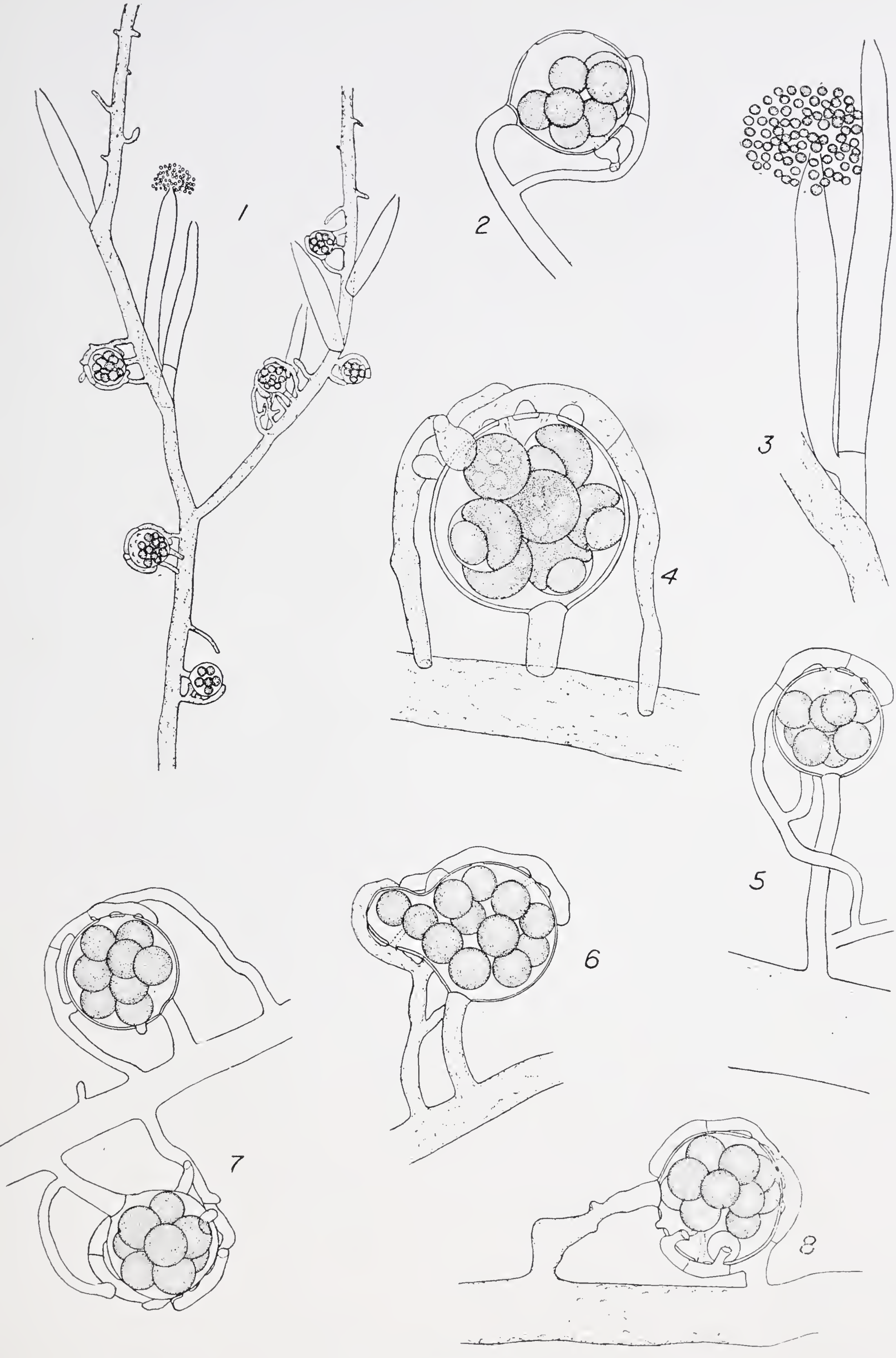
Hyphal threads long, reaching a length of 1.5 cm. on house-flies, more slender than in most Achlyas, from 10–40μ thick close to base, rarely up to 85μ thick, often wavy; usually little branched and pointed at tips when young; becoming considerably branched with age. Sporangia abundant, cylindrical, usually borne singly on the tips of the main hyphae in young cultures, renewed by cymose branching, often forming several clusters at regular intervals on the same hypha, irregular and wavy in old cultures, 12–37 × 36–600μ (rarely up to 900μ). Spores 9–10μ thick, emerging as usual in *Achlya*, but often falling to the bottom in an open group instead of forming a sphere at the sporangium mouth. Oogonia abundant on flies, grubs, and vegetable media, spread over the entire culture from the bases of hyphae to tips, giving the culture a lacy interwoven or net-work appearance; the diameter 30–60μ, commonly 32–48μ; usually borne singly on long, crooked, recurved stalks which arise racemosely from main hyphae and which vary in length from 2–10 times the diameter of the oogonia; often oogonial stalks may branch bearing two oogonia, and rarely oogonia may be borne on a stalk which arises directly from another oogonial wall; very rarely intercalary; oogonial wall usually without pits (except where the antheridial tubes enter) when grown on flies or grubs, but as a rule with pits when grown on boiled corn. Eggs 1–8, usually 1 or 2 in each oogonium; 25–45μ in diameter, most 33–36μ, eccentric when ripe, with one large oil drop; usually spherical, but often elliptical from pressure. Antheridial branches

PLATE 33

PLATE 33

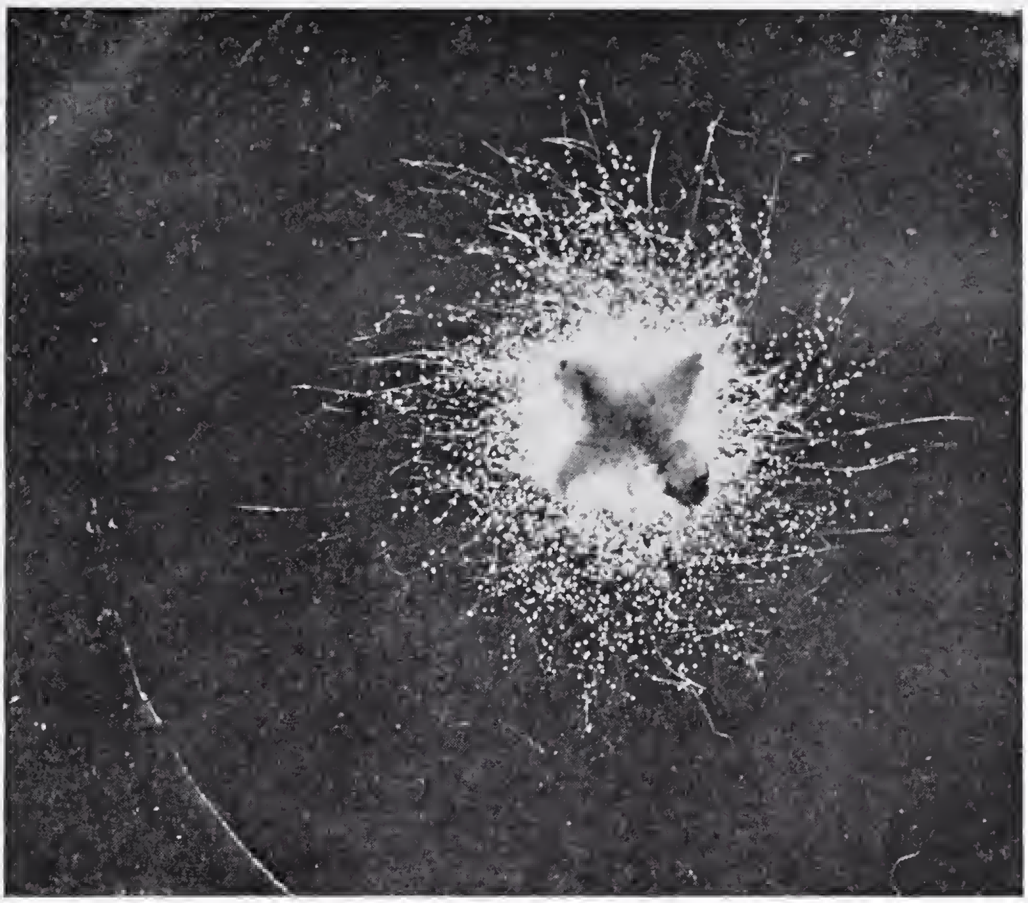
Achlya americana

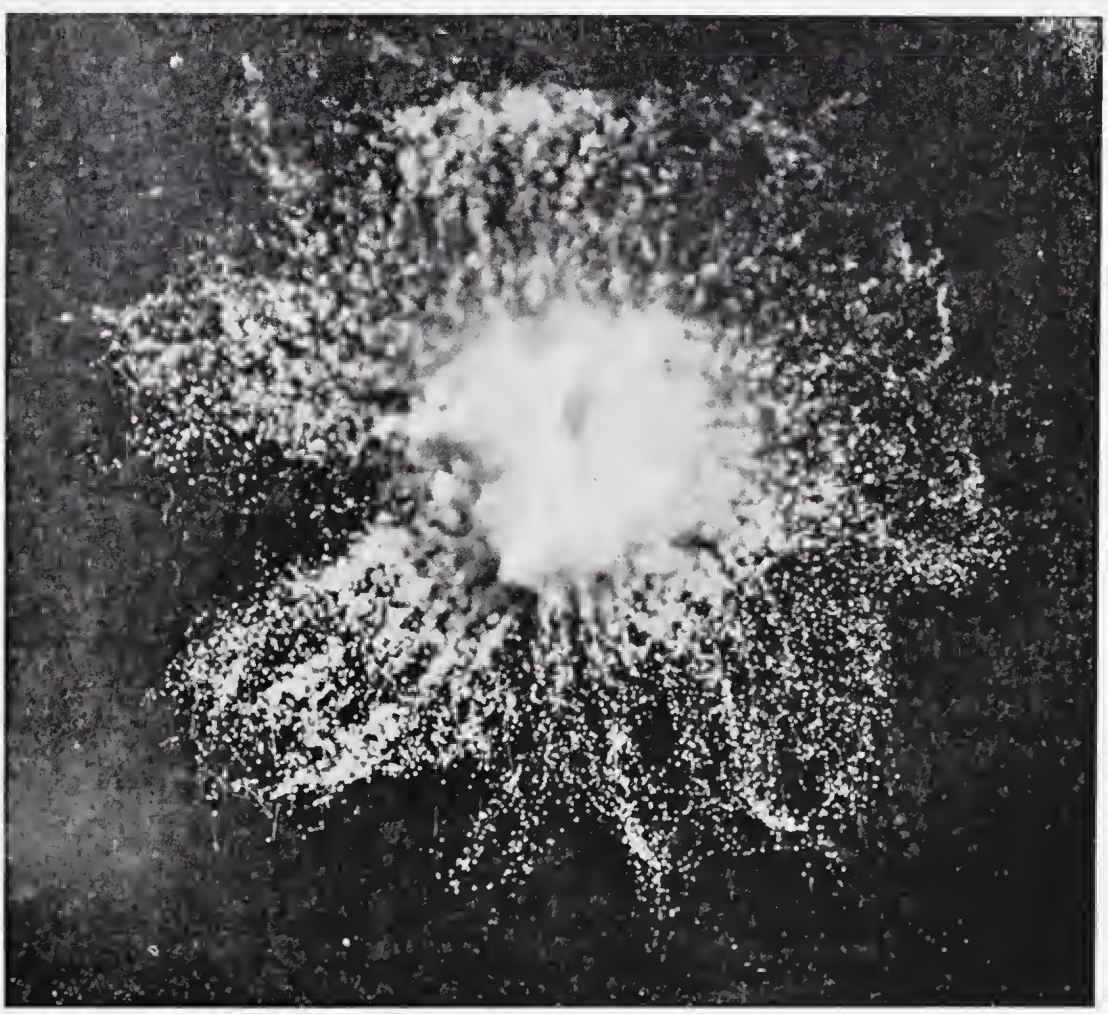
Fig. 1. Habit of plant. × 68.
Fig. 2. Apical oogonium (only one of its kind seen). × 278.
Fig. 3. Typical sporangia. × 188.
Fig. 4. Typical oogonium and antheridia. × 503.
Fig. 5. Oogonium with diclinous antheridium and an unusually long oogonial stalk. × 278.
Fig. 6. Irregularly-shaped oogonium. × 278.
Fig. 7. Two typical oogonia, one with a slight protuberance. × 278.
Fig. 8. Oogonium with a crooked and irregular stalk, also with a slight protuberance as in fig. 7. × 278.



ACHLYA AMERICANA.

PLATE 34





ACHLYA AMERICANA [ABOVE]. × 6.
ACHLYA ORION [BELOW]. × 4.

PLATE 35

PLATE 35

ACHLYA ORION

Figs. 1 and 2. Oogonia with single egg and unbranched antheridia. × 233.

Fig. 3. Oogonium with branched antheridia. × 233.

Fig. 4. Oogonial stalk arising from the wall of another oogonium. × 233.

Fig. 5. Eggs showing a late stage in maturation with several oil droplets not yet united into one large drop. × 387.

Fig. 6. Oogonium with a typical long stalk. × 233.

Fig. 7. Cluster of oogonia, one of which is barrel-shaped with the eggs in distal end and a perforated wall partly separating the two ends. Grown on a bit of boiled corn grain in distilled water at room temperature. × 233.

Fig. 8. Oogonia on a very much distorted oogonial stalk, as typical when cultivated three days in electric oven with temperature of 36° centigrade. × 233.

Fig. 9. Oogonium with diclinous antheridium and ripe egg, showing oil drop. × 387.

Fig. 10. Oogonium with antheridia arising from oogonial stalk and main hyphae also. × 233.

Fig. 11. Habit sketches to show appearance of oogonia and antheridia and occasional behavior of spores. × 97.

Figs. 12 and 13. Habit of sporangia. × 97.

Fig. 14. Spores emerging from cysts. × 720.

Fig. 15. Habit of sporangia. × 97.

ACHLYA ORION.

almost always androgynous, usually arising from the oogonial stalk itself, less often from the main hypha; rarely diclinous; antheridia on about 75% of the oogonia, one or two on an oogonium, tuberous; antheridial tubes obvious, penetrating the oogonia and reaching the eggs.

The species seems to be quite rare, having been recognized only twice in considerably over two thousand collections made by us. It was found in some water and trash collected from the west branch above the Meeting of the Waters (No. 6 of September 26, 1919), and in the same kind of material from the branch in Battle's Park behind Dr. Pratt's residence (No. 4 of June 10, 1920). The description has been made from cultures descended from a single spore.

Our plant can be distinguished (with the unaided eye) from most other Chapel Hill Achlyas by the network appearance given it by the oogonia, which are evenly scattered over the entire culture from the bases of the hyphae to the tips. *Achlya racemosa* approaches this network appearance more than any other species of *Achlya*, but in it the oogonia are not nearly so abundant nor do they extend entirely to the tips of the hyphae. In some species, such as *Achlya oblongata* or *Achlya conspicua*, the oogonia are borne in a definite zone near the substratum and from half to two-thirds of the length of the hyphae from the tips backwards are without oogonia. In the *Prolifera* group the oogonia are scattered more or less over the entire culture, but the big hyphae and long sporangia dissipate the network appearance.

If we ignore the egg structure, the present species seems to be closest to *Achlya polyandra* Hildb. The two plants resemble each other in the long, racemose oogonial branches which are recurved at the tip; in the often branched antheridial stalks which arise chiefly from the oogonial branches; and in the smooth oogonial walls which are normally without pits except where the antheridia touch.* The two species are readily distinguished, however, by the difference in the number of eggs in the oogonia, and in the size and structure of the eggs. In *Achlya polyandra* the number of eggs varies from five to twenty-five, the usual number being ten to fifteen, while in *A. Orion* the number varies from one to eight, the usual number being one or two. In *Achlya polyandra* the eggs are said to be centric with an average diameter of 27μ, but in our plant they are eccentric and most with a diameter of between 33 and 36μ. There are, moreover, other differences which are more subject to variation. In *Achlya polyandra* the sporangia are reported as often not abundant, and secondary ones rare; while in our plant both primary and secondary

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*Though pits are rarely seen when the plant is cultivated on flies or grubs they are not at all unusual in cultures on a piece of boiled corn grain. Compare *A. flagellata* for similar variations.

sporangia are abundant. The species is named for the nebula in Orion, which a photograph of the magnified culture somewhat resembles (see pl. 34).

The following series of experiments were made to test the effects of different temperatures on growth and reproduction. All cultures were made from strains descended from a single spore (from No. 6 of September 26, 1919). Inoculations were uniformly made by cutting out near the periphery of the mycelium small squares of corn meal agar, on which the fungus was actively growing, and placing on these squares termite ants, flies, or vegetable media, etc., for food. Distilled water was used unless otherwise stated.

Culture on fly, healthy, and just beginning to form sporangia and oogonia was put in electric incubator, temperature 40° C. Examined twenty-three hours later and found to be dead. Repeated with same result.

Check culture on fly left in room, temperature 21.5° C. Formed many normal asexual and sexual reproductive organs. Repeated with same result.

Culture on mushroom grub in sterilized spring water was put in incubator at temperature of 36° C. Examined two days later: mycelium thick, long (1 cm. from host), mostly straight but some slightly wavy hyphae. Threads unusually densely filled with protoplasm. No sporangia. A good many oogonial initials. No harmful effects of bacteria observed. Reexamined twenty-eight hours later: mycelium still growing vigorously. No sporangia formed. Many oogonia formed, a majority of which had queer stalks which wer every long and coiled like a cork-screw (see pl. 35, fig. 8). In most cases at tips of these stalks abortive attempts to form oogonia were made, resulting, as a rule, in from one to three swellings.

Two check cultures under same conditions as above, except in room temperature of 20° C. Growth normal, many sporangia and oogonia formed during the third day.

Culture on piece of boiled corn grain embryo was put in incubator at temperature of 36° C. A few sporangia produced, some of which emptied normally, others did not empty, though spores were formed. As compared with cultures in room temperature a very small number of oogonia were produced, and about 1 out of 10 of these formed eggs which had the appearance of being normal. About a third of the oogonia had the curiously coiled stalks as cultures on grub at same temperature (above). Antheridia usually of normal shape on normal oogonia, but on oogonia with coiled stalks antheridia also coiled. Repeated with essentially the same results.

Culture on piece of boiled corn grain endosperm (horny and starchy) was put in incubator at temperature of 36° C. Sporangia produced in abundance, many emptying normally; others forming spores which encysted within the sporangium. Compared with cultures in room temperature a considerably larger number of normal oogonia with good eggs were formed, and compared with culture in incubator on termites a smaller number of oogonia with coiled stalks were formed. This culture approached far nearer in appearance the normal room cultures than any yet cultivated in oven. Repeated with same results. In one of these cultures two little bits of the starchy part of grain, size of pin head, broke off and were inoculated by spores. Both formed tiny cultures about 3 mm. across. The hyphae were very delicate, about one-third the normal diamater. The sporangia, produced in plenty, were mostly relatively as small. The spores, however, were normal size, 9–10μ in diameter. A good many oogonia produced of normal size (50–80μ), with eggs equally normal (average 33μ).

Four cultures on termite ants put in incubator at temperature of 36° C. produced many oogonia and antheridia with coiled stalks; very few good eggs.

On nutrient agar in one per cent solution of levulose (room temperature). Hyphae normal. A good many sporangia formed which emptied normally. Very few oogonia and these smaller than usual and abortively shaped. Culture washed and transferred to pure water. A great many perfectly normal oogonia formed with normal eggs.

On nutrient agar in ten per cent solution of levulose (room temperature). Hyphae normal. A large number of sporangia formed, all of which emptied. Spores sprouted immediately without coming out of cysts. Many oogonia and about one-third of these with eggs; the average number of eggs 3–4, a few oogonia with 8.

8. **Achlya proliferoides** n. sp.

Plate 36

Growth moderately dense and strong, reaching a length of about 1 cm. on a mushroom grub. Hyphae moderately branched, variable in size, usually wavy and irregular, the tips hyaline and dying back here and there as in *A. imperfecta* and *A. flagellata.* Sporangia subcylindrical, usually bent, often with several openings; about 35–45μ thick as a rule, short or long, at times up to 1425μ long. Spores 11–12μ thick, double ones not rare, often falling to the bottom in an open group on emerging. Oogonia abundant, spherical, smooth, 40–55μ in diameter, racemosely borne on stalks that are about 1–1⅔ times as long as the diameter of the oogonia; wall hyaline, not thick; pits numerous (usually), but not very conspicuous. Eggs eccentric, with a large oil drop, about 18–24μ in diameter, often elliptic, the great majority always going to pieces before maturity on ordinary media. Antheridial branches numerous, diclinous (mostly) or androgynous, usually long, contorted and much-branched, in many cases coiling themselves about certain selected hyphae which may or may not bear oogonia. Antheridia, one or several, on every oogonium, elongated, applying their sides to the oogonium or touching it by several blunt, foot-like processes.

Not nearly so common in Chapel Hill as *A. imperfecta* or as *A. flagellata*, but not rarely found with them, as in branch below Cobb's Terrace (No. 3 of July 24, 1918. Type).

As a rule very few and often none of the eggs mature in normal cultures, such as on insects or corn grain in distilled water. They fall to pieces into scattered granules or amorphous masses as soon as, or before, a thin wall is formed. The oogonia often halt in their development and send out a branch which bears another oogonium just as in *A. imperfecta.* At times, however, instead of forming another oogonium this branch may develop into an antheridial branch (fig. 3). Like *A. imperfecta* again, the stalk of the oogonium may not rarely carry a branch near its base which usually bears an oogonium. The tendency to coiling in *A. proliferoides* is shown not only by the antheridial branches wrapping themselves about the hyphae, but also by the frequent coiling of the oogonial stalk towards the end (fig. 6), and by the hyphae not rarely coiling themselves into a flat spiral like a watch spring.

The antheridia while usually in the great majority diclinous are not consistently so and are very variable in this respect. On mushroom grubs or bits of boiled corn grain very few androgynous antheridia may appear or they may be numerous, these changes appearing in consecutive cultures of the same pure strain.

9. Achlya flagellata n. sp.

Plate 37

Growth stout and moderately dense, reaching a length of about 1 cm. on a mushroom grub or ant larva. Hyphae branching, tapering outward, up to 150μ thick near the base, more or less crowded and uneven, the tips hyaline and often dying and renewed from one side below as in all members of this group. Sporangia plentiful, subcylindrical, very variable in size, often bent and at times with more than one opening, scattered or clustered. Spores often falling to the bottom in an open cluster on emerging, about 11–11.5μ thick. Gemmae abundant, usually in rows from the segmentation of the distal parts of hyphae, short or long, usually more or less cylindrical, but often pear-shaped or ten-pin-shaped or at times very irregular; usually becoming sporangia on change of medium and discharging through an elongated papilla at either end. Oogonia abundant, typically spherical, but not rarely irregular by abnormal growth on one side, and one or two papillate projections may be seen rarely; usually about 48–75μ thick, rarely up to 100μ, racemosely borne on short, slender stalks about as long usually as the diameter of the oogonia or a little shorter, rarely on longer stalks and quite rarely intercalary; wall hyaline, not thick (about 1.5μ); pits very variable, perhaps more often absent, but again numerous and rather easily seen, about 5.5μ wide. Eggs spherical, eccentric with a large oil drop, 1–10 (rarely 20) in an oogonium, mostly 2–6, diameter 26–35μ, most about 28μ, rarely small ones down to 18μ may be mixed with the others. Antheridial branches abundant, usually much branched and irregular, often so much so as to make an intricate network like a group of rhizoids, originating laterally and apically from hyphae which may or may not bear oogonia and applying themselves to oogonia on the same or on other threads or to both; more often diclinous than androgynous, perhaps about three times as often usually, but varying in this respect; the antheridial branches never arising from the stalks of the oogonia. Antheridia on nearly all oogonia, one or several, elongated with the side on the oogonium, frequently touching the oogonium with foot-like projections; antheridial tubes easily observed.

Very common in Chapel Hill in springs, brooks, ditches and creeks, as in spring near Clark's schoolhouse (No. 1 of July 25, 1918), in Arboretum spring, in Battle's branch, etc. Also found in some material sent from Chimney Rock, N. C., June 10, 1920 (Miss Hoffmann, coll.), differing from

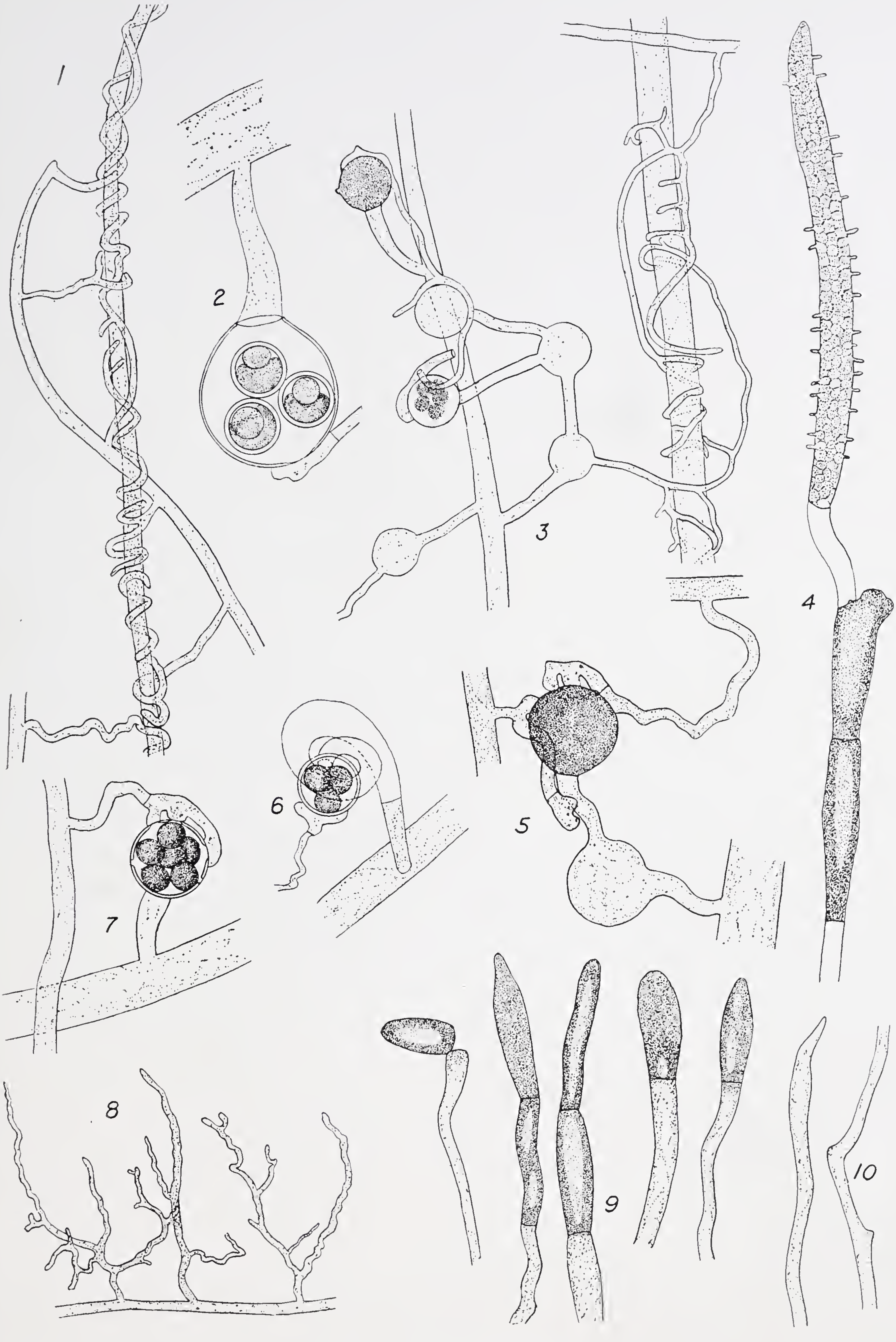
PLATE 36

PLATE 36

Achlya proliferoides

Fig. 1. Hypha with antheridial branches entwined around it. × 167.
Fig. 2. Oogonium with ripe eggs. × 447.
Fig. 3. Habit of oogonia and antheridia. × 167.
Fig. 4. Spores sprouting in sporangium and below on same hypha two gemmae. × 167.
Fig. 5. Oogonium growing from an abortive one. × 247.
Fig. 6. Spiral-shaped oogonial stalk (culture on corn grain). × 167.
Fig. 7. Oogonium. × 247.
Fig. 8. Contorted antheridial branches. × 167.
Fig. 9. Gemmae. × 108.
Fig. 10. Hyphae showing pointed tip and atrophied tips renewed from below. × 108.

PLATE 36



ACHLYA PROLIFEROIDES.

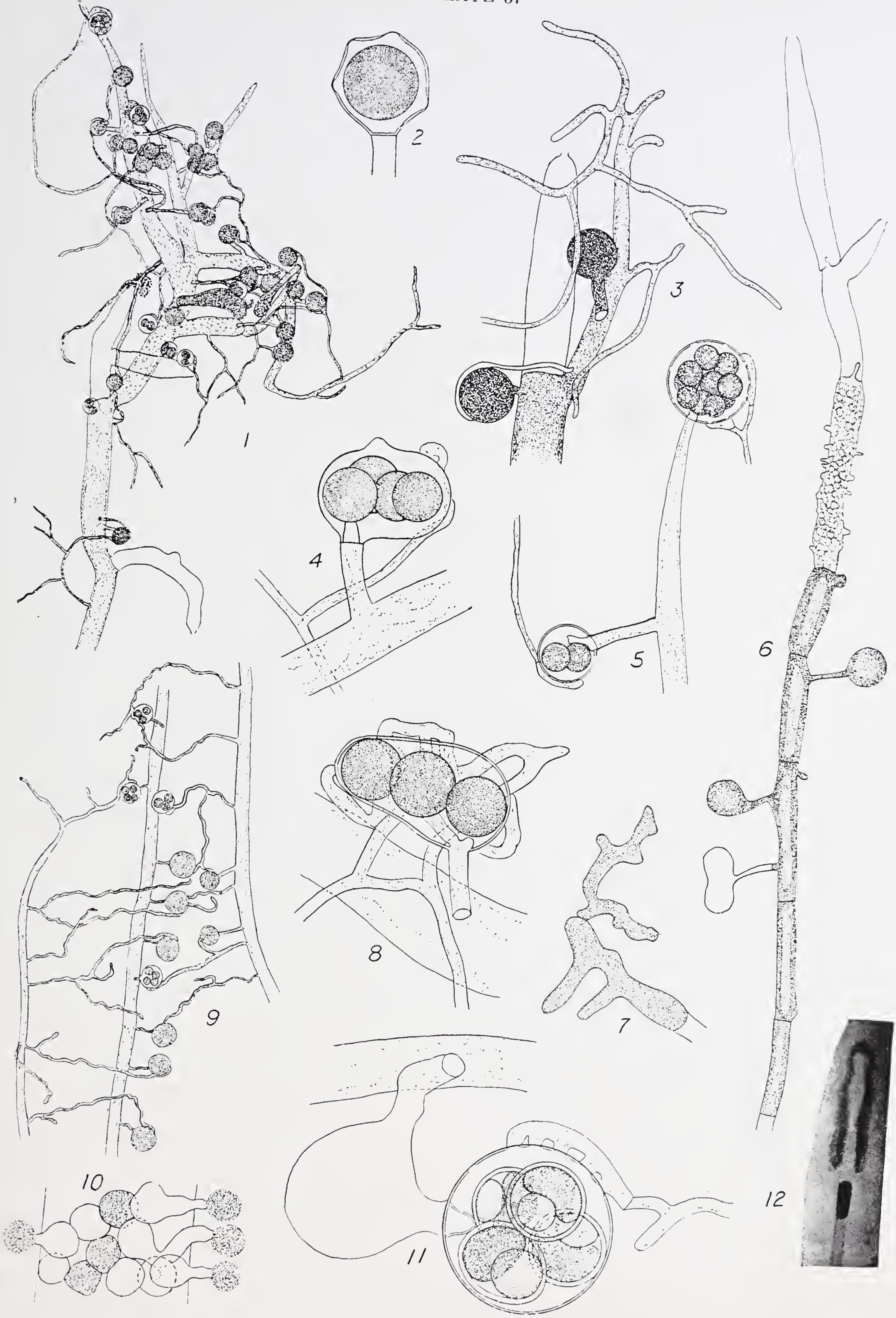
PLATE 37

PLATE 37

Achlya flagellata

Fig. 1. Habit, showing androgynous antheridia. × 41.
Fig. 2. Angular oogonium. × 447.
Fig. 3. Antheridial branches on tip of hypha curling back to oogonia. × 167.
Fig. 4. Laterally elongated oogonium with blunt papilla and ingrowth from below. × 250.
Fig. 5. Oogonia with diclinous antheridia. × 167.
Fig. 6. Empty sporangia, sporangium with sprouting spores, gemmae, and abortive oogonium. × 108.
Fig. 7. Gnarled gemmae. × 60.
Fig. 8. Laterally elongated oogonium with an ingrowth from below. × 250.
Fig. 9. Habit, showing diclinous antheridia. × 60.
Fig. 10. Part of a dictiosporangium. × 720.
Fig. 11. Oogonium with ripe eggs. × 447.
Fig. 12. Sporangium emptied in corn meal agar. × 41.

PLATE 37



ACHLYA FLAGELLATA

the Chapel Hill plants in no way except for the short stalked oogonia, more numerous eggs, and more branched antheridial branches. Again found in some material collected at Fayetteville, N. C., July 10, 1920 (Miss Holland, coll.). Distinguished from all other members of its group except *A. aplanes* by the decidedly larger eggs, and even in the latter species the eggs do not average so large and the antheridia are always diclinous. Ward's figures 1–14, plate 22 (1883), while labelled *A. polyandra* deB., look more like the present species.

This is the plant treated by us as *A. deBaryana* in Mycologia 4: 319, pl. 78, 1912, but we now think it cannot be that species. The unpitted walls of the oogonia together with the always (practically) androgynous antheridia that arise near the oogonia of the latter must exclude our plant.

The antheridial branches of *A. flagellata* are unlike all others we have seen: only *A. proliferoides* may have them so intricately branched and such branching is not so common even in that species; neither are they so complex in *A. imperfecta* (No. 1 of July 20, 1918), though often long and complex in that species; furthermore the short and simple androgynous branches of *A. americana* and at times of *A. imperfecta* are not present in *A. flagellata;* and the antheridial branches often arise from the stalks of the oogonia in *A. imperfecta* and never in this. The oogonia are much inclined to proliferate and empty their contents into a new one by an outgrowth, as is true in all members of the group, and in this species this often leads to sac-like shapes, with or without a constriction (pl. 37, fig. 11) or to other unusual forms (as said above a few papillate projections are to be seen rarely). Oogonial initials may halt after reaching full size and become gemmae, or their stalks may become part of a gemma when the main hyphae are segmented. It is also quite easy to find oogonial stalks that are branched at right angles below with a secondary oogonium on the branch, as in *A. caroliniana*, and all the other members of this group that we have seen.

In regard to the pits we have here the usual uncertainty of the group. Without apparent cause a group of oogonia here and there may show them plainly, either one or many, while the great majority in the same culture, as, *e.g.*, on a corn grain or a mushroom grub, may have none except for the easily seen thin places under the antheridia. The pits are never so large and conspicuous as in *A. conspicua* or in the *Ferax* group of *Saprolegnia*. When grown on nutrient agar the sporangia may open and discharge their spores inside the agar. In such case the spores do not make a sphere at the tip, but flow back and form a layer around it (fig. 12). The species is subject, though rarely, to the attacks of an *Olpidiopsis*.

It is also to be noted that while the gemmae in this species may become somewhat loosened at the points so as to bend back a little, they are far less inclined to this than in *A. imperfecta*, and we have never seen them fall away as so often happens in that plant. Dictiosporangia are not rarely observed and have been illustrated by us ('12, pl. 78).

10. **Achlya imperfecta** n. sp.

Achlya deBaryana var. *intermedia* Minden. Krypt. Fl. Mark B. **5**: 545. 1912.

PLATES 38 AND 39

Growth dense or rather open, not very long, many stout hyphae with more slender branches, tips hyaline, often dying and then a new growing point produced below. Sporangia plentiful, subcylindrical, little larger than the hyphae that bear them, not very long as a rule, often irregular and twisted. Spores about 10–11.5μ thick, dark, emerging as usual but often falling to the bottom in an open group instead of forming a sphere at the sporangium mouth. Gemmae formed by the segmentation of the hyphae, therefore mostly subcylindrical and in rows, but often ovate and frequently with knobs or projections at one or both ends. Any part of the culture may be segmented into gemmae even to parts of the antheridial branches. They often become loosened from each other in part, rather rarely completely separating and falling singly to the bottom. Oogonia usually abundant, spherical, 37–60μ thick, most about 40–45μ, borne racemosely on short stalks about ½–1½ times as long as the diameter of the oogonia; wall without pits, or with several to numerous small, inconspicuous ones; from the basal wall a protuberance of varying length is present in many cases, and there are rarely present one or two papillate protuberances (pl. 38, figs. 2 and 8). Eggs eccentric, with a large oil drop, 2–8 in an oogonium, commonly 4–6, diameter 17–23μ, most about 19.5–20μ, often elliptic from pressure. The great majority of the eggs go to pieces before maturity. Antheridial branches androgynous or declinous, variable in origin and length, usually branched and irregular, arising from hyphae that also bear oogonia and then applying themselves to nearby oogonia or most often by extensive growth and branching to more distant oogonia either on the same or other hyphae; or certain threads may give rise to antheridial branches only which then seek out oogonia on other threads.

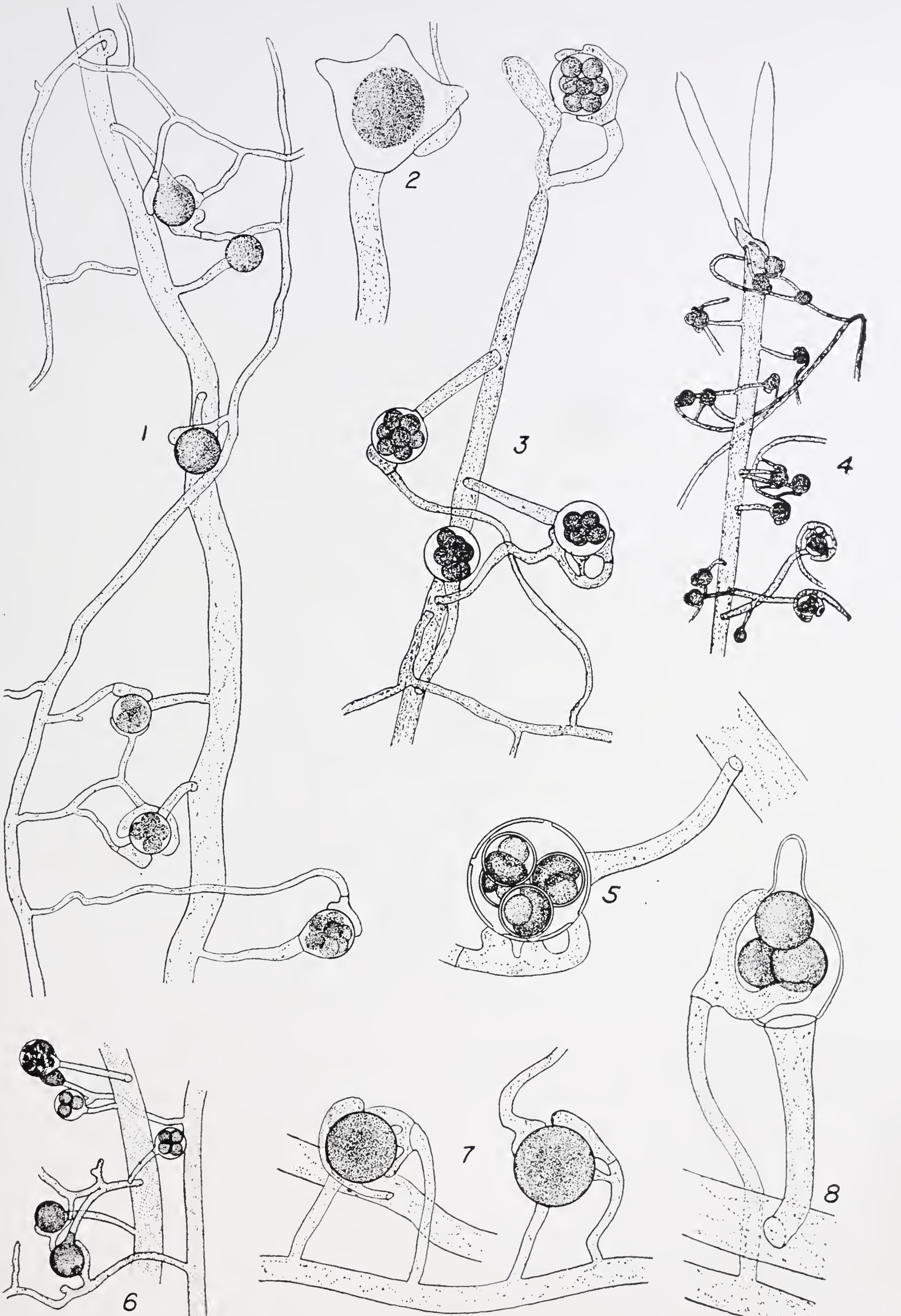
Found many times in Chapel Hill in springs, ditches, branches and creeks. Also found in material collected near Fayetteville, N. C., July 10, 1920, by Miss Holland. Pieters illustrates (unpublished notes) a species that is certainly this or *A. proliferoides*, the antheridia sometimes androgynous, prevailingly diclinous. As he shows no coiling antheridial branches we refer his plant provisionally to this species. The plant he illustrates is presumably the one he has studied in various media under the name *A. prolifera* ('15b, p. 529) as his drawings are labelled *A. prolifera*. The plant

PLATE 38

PLATE 38

Achlya imperfecta

Fig. 1. Habit of plant showing both diclinous and androgynous antheridia. × 188.
Fig. 2. Angular and papillate oogonium with an abnormally large egg. × 503.
Fig. 3. Oogonia and antheridia. × 188.
Fig. 4. Habit. × 47.
Fig. 5. Oogonium with mature eggs. × 503.
Fig. 6. Oogonia and antheridia. × 188.
Fig. 7. Oogonia and antheridia. × 278.
Fig. 8. Oogonium with blunt apical papilla. × 503.



ACHLYA IMPERFECTA.

PLATE 39

PLATE 39

Achlya imperfecta

Fig. 1. Empty sporangia with gemmae. × 113.
Fig. 2. Spores of various sizes. × 503.
Fig. 3. Spore with cilia. × 503.
Fig. 4. Spore mass which emerged from sporangium without breaking up into spores. (In maltose and peptone solution.) × 278.
Fig. 5. Oogonium in empty sporangium, only case observed. × 278.
Fig. 6. Various forms of hyphal tips. × 188.
Fig. 7. Oogonium with very small, abnormal eggs. In this culture the eggs often went to pieces. × 278.
Fig. 8. Part of a dictiosporangium. × 503.
Fig. 9. Oogonium in which eggs went to pieces. Sporangia beneath. × 278.
Fig. 10. Oogonia and antheridia of the *A. americana* type. × 47.

ACHLYA IMPERFECTA.

studied by Ward ('83) and illustrated by him as *A. polyandra* deB. may be this, but looks more like *A. flagellata.* Horn's plant, treated as *A. polyandra* deB. ('04), may also be the present species (see note under the genus).

All of the variations in the antheridial branches mentioned above are to be found in the same culture, as *e.g.*, on a mushroom grub or bit of boiled corn grain. All the oogonia of a series along a certain thread may have diclinous antheridia (pl. 38, fig. 4), another series may have only short androgynous ones (pl. 39, fig. 10), another may have long androgynous ones, and another have both androgynous or diclinous ones indiscriminately. An androgynous origin is perhaps the more common. Not at all rarely the antheridial branches arise from the stalks of the oogonia, thus differing from *A. flagellata.*

In the behavior of the contents of the sporangia many variations occur. Masses of undivided protoplasm of all sizes may be ejected with the spores and at times the entire mass, undivided, escapes as a whole (pl. 39, fig. 4). See p. 9 for references to similar cases.

It was in this species that we observed the only two cases of proliferation through empty sporangia that we have found in *Achlya.* In one case the hypha simply extended itself through the sporangium; in the other there was formed a stalked oogonium in the sporangium, the hypha extending on through beyond. We know of no reference in the literature to internal proliferation of any kind in *Achlya* except by Petersen, who says that he has seen "zoosporangia which had proliferated in undoubted species of *Achlya*" ('10, p. 520).

For a discussion of the relationships of this species to *A. proliferoides,* which is very near, and to other members of the group, see discussion under the genus. *Achlya americana* is also near, but it is certainly not the same. The antheridial branches are all short and intermingled with the oogonia in origin, the eggs are more numerous (4–20, usually 6–12), and the oogonia average a little larger (40–90μ, most about 50–60μ). The eggs, also, are a little larger (18.5–25μ, most about 22μ).

Among such a confusion of forms, or descriptions of forms, it is a pleasant relief to find one described that is apparently just like ours. This is Minden's *A. deBaryana* var. *intermedia,* which seems identical except that he does not mention the early dissolution of most of the eggs. He describes his plants as follows:

"In structure of mycelium and length of the oogonial stalk resembling the typical form. Differs in the antheridial branches arising often from the oogonial stalks as well as from the main branches and in their being longer and more bunched, and running farther, thus often attaching themselves to more distant oogonia, and then often diclinous. Pits not always obvious, and at times entirely absent. Moreover, many hyphae occur which bear only antheridial branches. Were the diclinism preponderant this would approach *A. prolifera;* other characters are like *A. polyandra.* This then occupies an intermediate position." Found in the Luneburg Heath, near Hamburg, Germany.

The species name we give the Chapel Hill plant refers to the failure of most of the eggs to reach maturity. The name *Achlya intermedia* has been used by Bail and is not available (see under *Saprolegnia monoica*).

11. **Achlya Klebsiana** Pieters. Bot. Gaz. **60**: 486, pl. 21, figs. 1–4. 1915.

PLATE 40

Threads moderately short to long, growth on termites varying from 4–14 mm. across; moderately stout, about 50–94μ thick at the base and narrowing gradually to the bluntly pointed tips; branches not very numerous, the radiating main threads obvious to the naked eye. Primary sporangia plentiful, mostly from 40–54 × 432–877μ, although some on very small hyphae are as small as 20 x 135μ; secondary sporangia abundant, usually including also a part of the hypha below the primary sporangium; at times many very small sporangia arising on slender hyphae form large gemmae. Sporangia emptying as normally in *Achlya*, but showing all the usual variations and also a unique one (see below); spores about 11–13μ thick, forming a rather loose hollow sphere about the mouth; usually some of the spores do not escape, but encyst within the sporangium. Gemmae formed abundantly by the segmentation of the old hyphae into dense, more or less irregular rods, and by the incomplete development of sporangia-like tips; after a rest becoming sporangia and emptying by a papilla of very variable length or sprouting by many threads or less often forming many very small sporangia on the ends of the sprouting threads. Oogonia plentiful, borne laterally from the main hyphae on moderately short branches which are of a length less than the diameter of the oogonium (rarely) to three times its diameter; spherical or short pyriform, usually 48–62μ thick, sometimes as small as 34μ; wall smooth, unpitted except under the antheridia. Eggs filling the oogonium, 1–8, usually 6, the diameter 18–24μ, some slightly flattened by pressure; eccentric, with one large oil drop outside the protoplasm when fully ripe; oil drop 11–14μ thick. Antheridial branches slender, practically always diclinous, never arising from the oogonial stalk though sometimes the basal wall grows up into the oogonium giving the appearance of a thick-walled hypogynal antheridium; simple or sparingly branched, sometimes branching before reaching the oogonium and the branches clasping different oogonia. Antheridia clearly abstricted, elongated and usually touching the oogonia with foot-like projections; at least one, usually more, on every oogonium.

Found once in Buzzard Spring, a shallow spring at edge of bottom land of Yadkin River, near Yadkin College, N. C., March 29, 1921. (H. R. Totten, coll.)

This well-marked species, known until now only from Michigan, is apparently nearest *A. americana*, from which it clearly differs in the

PLATE 40

PLATE 40

Achlya Klebsiana

Fig. 1. Sporangia which have emptied. × 167.

Fig. 2. Sporangium emptying by two mouths, some spores having emerged from their cysts inside and swimming actively (not half the swimming spores present are shown). × 247.

Fig. 3. A gemma sprouted into threads which formed small sporangia on their tips. × 73.

Fig. 4. Gemmae, one of which has sprouted directly into threads and one has formed spores and emptied. ×167.

Fig. 5. Oogonium with antheridium. × 167.

Fig. 6. Sporangia, one of which has emptied as typical in *Achlya*, the other only partially by numerous mouths. × 108.

Fig. 7. Habit of sporangia, the internodes longer than usual. × 60.

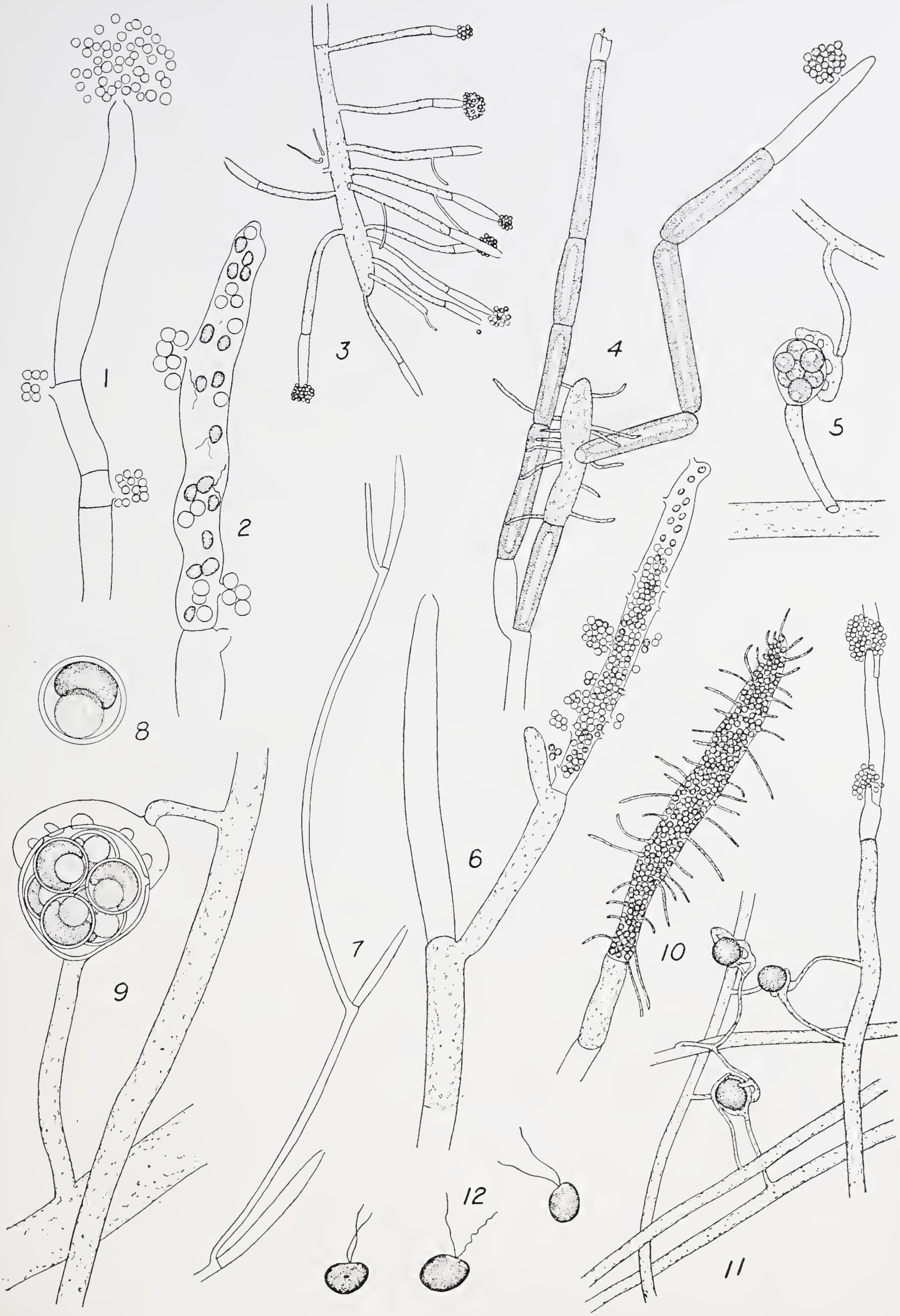
Fig. 8. Mature egg. × 720.

Fig. 9. Oogonium with antheridium and ripe eggs. × 447.

Fig. 10. Sporangium with spores sprouting in position. × 108.

Fig. 11. Habit sketch, also showing emptied gemmae. × 108.

Fig. 12. Swimming spores. × 720.



ACHLYA KLEBSIANA.

diclinous antheridia, longer oogonial stalks, smaller average number of eggs and unpitted oogonial walls (except where the antheridia touch). With Pieters's description our plant agrees unusually well and the identity of our plants is obvious. Neither on flies nor on termites do we find a pronounced tendency to produce all the oogonia near the insect (as Pieters finds in the case of his form on flies), and on corn meal agar our form produces copious oogonia and antheridia with normal eggs. Pieters says that oogonia were not formed on the agar he used (corn meal agar not mentioned). In fact, he found oogonia to be formed only on flies and in one case on a sterilized pea. The oogonia are not very densely set and, while more numerous near the substratum, occur throughout the culture except for a peripheral zone where the sporangia are being formed. The species is a very strong and rapid grower, is healthy and reliable in ordinary media and forms oogonia regularly. At room temperature a culture made on a fly at 1 p. m. was discharging spores at 6 p. m. the following day. On corn meal agar in a petri dish at room temperature (65–75° F.) the growth is at the rate of nearly a centimeter a day. When sporangia discharge in agar they do so only in part and the spores are forced back in a sheath around the sporangium just as is shown for *A. flagellata* (plate 37, fig. 12). Six cultures made to test the effect of low temperature showed that in the ice box (about 10–11° C.) in all cases oogonia were formed in immense quantities throughout the culture, while sporangia were very rare. In check cultures at room temperature there were produced large numbers of sporangia and far less numerous oogonia.

The spores vary in behavior as usual in the genus; very often only a part emerge and not rarely all are retained. When the spores escape typically they emerge from their cysts and swim as usual, but some or all of them are not rarely seen to sprout in position. There is a strong tendency in this species for the spores to emerge from more than one mouth; (for other such cases see *A. caroliniana*, *A. proliferoides* and *A. flagellata*). Sporangia with multiple mouths are of constant occurrence and are not to be confused with dictiosporangia (see fig.6). In such cases some of the spores are nearly always left inside. One remarkable condition was seen scores of times and has not appeared so strikingly in any other *Achlya*. The retained spores emerged from their cysts inside the sporangium and swam very actively inside it, none ever getting out even though the opening was free. In a number of cases in which nearly all the spores were retained they behaved in this way and the sporangia presented a novel sight with hundreds of spores swimming rapidly in a crowded swarm. In such cases the empty cysts were soon dissolved, leaving the space free. After coming to rest inside the sporangium many of these spores sprout in position, most of the sprouts remaining inside the sporangium, others emerging through the opening or directly through the wall. No well-developed dictiosporangia have been seen in our cultures, but when the spores are all retained in the sporangium a few of the peripheral ones may emerge exactly as in *Dictyuchus*, the majority emerging from their cysts to swim inside as described above. Sporangia of the *Aplanes* type with all the spores retained and sprouting with germ tubes through the sporangium wall are not rare (fig.10), but the tubes are not vigorous in such cases and soon die.

12. **Achlya caroliniana** Coker. Bot. Gaz. **50**: 381. 1910.

PLATE 41

Hyphae rather stout, about 48μ thick at the base and 20μ near the tip, in strong cultures reaching a length of 1.5 cm. Sporangia irregularly cylindrical, about 20–30μ in diameter, often discharging by several openings, sometimes remaining closed and emptying as in *Dictyuchus*, ciliated on emerging but behaving as in other Achlyas. Spores 11–12μ in diameter, most about 11.2μ. Oogonia abundant, very small, 24–55μ thick, most about 30–37μ, spherical when terminal, wall smooth, or not rarely with one or two papillae or angles, thin, not pitted, light yellow in age, terminating short or moderately long, slender branches, which are racemosely borne on the strong main hyphae, or rather rarely intercalary and elongated, at times filiform with several elongated eggs in a row. Oogonial branches generally simple, but often giving off near the base, or sometimes near the oogonia, one or two branches which also terminate in oogonia, and, as a rule, are curved downward. Eggs generally 1–2, not rarely 4, eccentric, with a large oil globule, 18.5–23μ in diameter, averaging about 22μ, often elongated by pressure. Antheridia absent. A papilla, thick-walled and soon empty, often grows into the oogonium through the basal partition exactly as in other members of the *Prolifera* group and in *A. hypogyna.*

Distinguished by the absence of antheridia and by the small, unpitted oogonia with a few small eggs.

Rare in pools and small streams, such as Arboretum branch, Battle's branch, small pools in pasture below Purefoy's Mill, etc. Appeared five times in Chapel Hill collections before December 15, 1913 (see table on p. 14).

This species is particularly interesting in that it is the only species in the *Prolifera* group without antheridia. It is almost exactly like *A. imperfecta* in other ways (except for absence of pits).

The cultures in the following experiments were made from a pure culture on corn meal agar of No. 10 of March 20, 1913, using egg yolk as the food material:

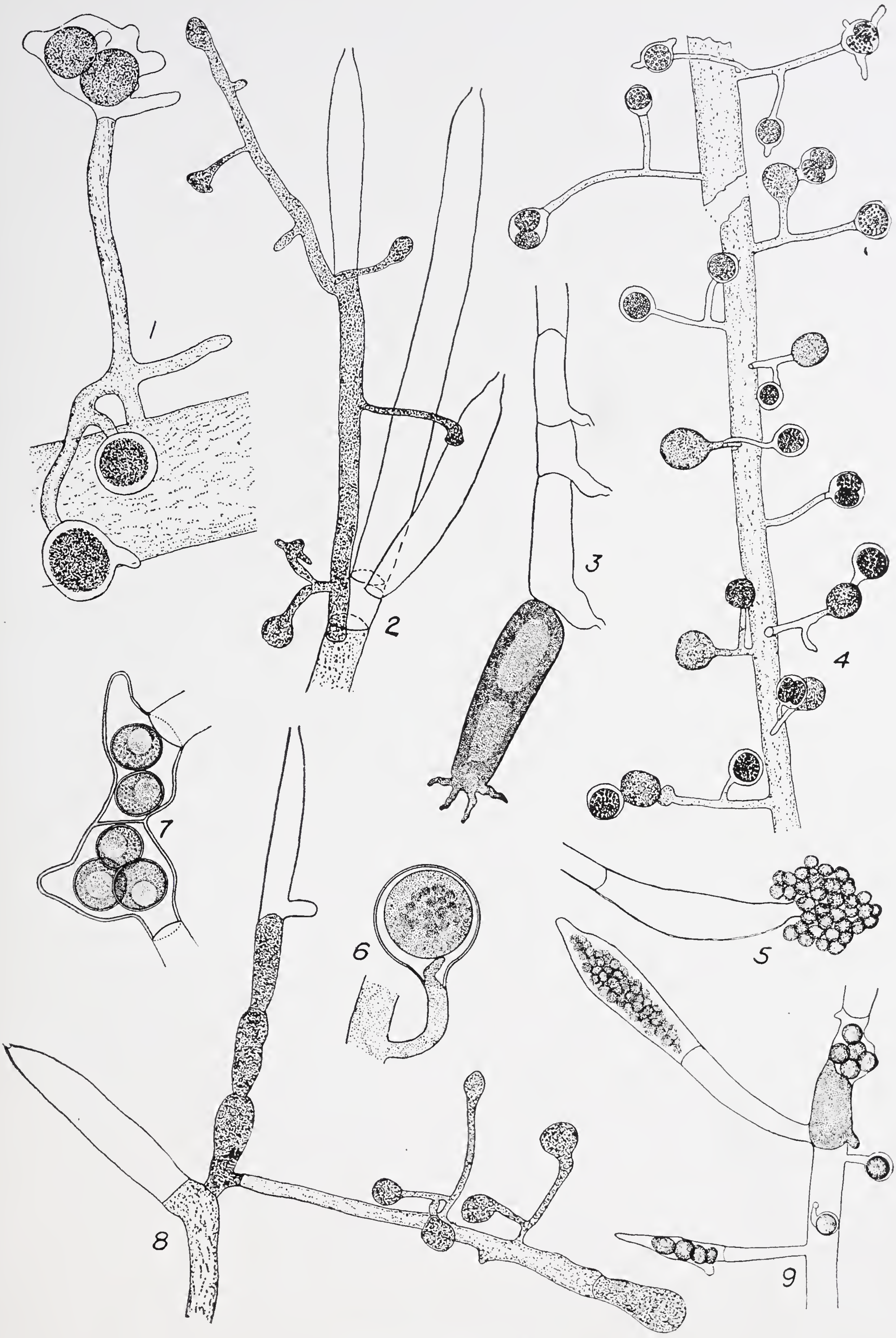
In .1% KNO_3. Limited growth of rather coarse, strong hyphae, as in the following three experiments. Sporangia present. Oogonia on smaller hyphae, but eggs not maturing.

PLATE 41

PLATE 41

Achlya caroliniana

Fig. 1. Oogonia with papillae. × 503.
Fig. 2. Young oogonia and sporangia. × 188.
Fig. 3. Gemma and three sporangia which were gemmae. × 122.
Fig. 4. Habit of oogonia. × 188.
Fig. 5. Sporangium with spores clustered at tip. × 251.
Fig. 6. Oogonium with an ingrowth from below. × 503.
Fig. 7. Two odd-shaped intercalary oogonia containing ripe eggs. × 503.
Fig. 8. Empty sporangia, gemmae and young oogonia. × 188.
Fig. 9. Sporangium partly filled with spores and odd-shaped oogonia. × 188.



ACHLYA CAROLINIANA.

In .1% KH_2PO_4. Rather short, but very stout growth. A good number of large and irregular and often branched sporangia, forming very many spores, and some generally remaining inside. The spores on emerging do not stick to sporangium, but fall to bottom in open order.

In .1% NaH_2PO_4. Strong and very stout growth. A good many large sporangia, often irregular and with more than one mouth, usually some spores remain inside. Many oogonial initials, most of which were smooth, but some spiny; none matured eggs. Some of the hyphae had a diameter of 96μ. Some of the large threads were segmented up into dense, rather short fragments.

In .1% K_2SO_4. About 1 inch growth. Hyphae very strong, as in preceding. No sporangia. A number of oogonia, but no good eggs maturing.

In .1% $Ca_3(PO_4)_2$. About 1 inch growth. Very large hyphae. Immense number of oogonia, with 1–3 or 4 eggs each. Sporangia present, but not numerous. The great majority of the oogonia were quite spherical and without spines. Some, however, had distinct and even numerous spines. More large oogonia with 4 eggs than before seen.

In .1% $Ca(NO_3)_2$. Stout growth, but not so extensive as in others. Almost no sporangia. Many oogonia remarkable for their very long stalks, 2, 3 or 4 times longer than usual, giving the culture a very peculiar appearance. The stalks massive, simple and straight. A good many good eggs formed.

In .1 % K_3PO_4. No growth.

On corn meal agar. Growth vigorous, covering dish. Many enlarged and denser ends ot stout hyphae becoming cut up in rows of sections to form gemmae. Locally there were found also a good many oogonia of perfectly normal appearance and with 1 or 2 good eggs, almost all spiny.

The following experiments were made to test the best method of preserving live cultures:

Culture put in vial on corn meal agar, vial closed with a plug of cotton and placed in a dark chamber in May, 1913. When tested in December, 1913, it was found to be alive.

Culture put in distilled water November 18, 1909. When tested on September 22, 1917, it was found to be dead.

13. **Achlya apiculata** deBary. Bot. Zeit. **46**: 635, pl. 10, figs. 3–5. 1888.

Plates 42 and 43

Vegetative growth ample and abundant, but not so stout as in *A. oblongata* or in the *Prolifera* group. The main filaments mostly about 40–60μ thick, tips rounded; breaking up soon after maturity into segments with little or no change in the appearance of the threads, each segment becoming a gemma and resting indefinitely until the conditions change, then forming spores like sporangia. Sporangia moderately plentiful, long or short, usually somewhat larger than the threads and gradually pointed towards the end, emptying as usual for an *Achlya*, or often remaining closed and emptying as in *Dictyuchus*. Spores ciliated on emerging and capable of swimming under certain conditions (see notes under experiments), 12.5–14.5μ in diameter or at times larger. Oogonia not formed regularly or abundantly except at low temperatures, racemosely borne on the tips of short or rather long branches which are usually bent and sometimes make a complete turn, rarely intercalary, ovate, short pyriform or spherical, at low temperatures very rarely formed within empty sporangia (as in *Saprolegnia ferax*), typically with (but often without) a more or less prominent apiculus; 60–119μ thick, most about 80μ; walls thin, smooth, unpitted. Eggs few, large, very dark, subcentric, 1–5, usually 2 or 3 (rarely 10), 25–40μ thick, sometimes larger, average about 36μ. Antheridial branches usually androgynous, but often diclinous, arising from the main hyphae or from the oogonial branches, soon becoming inconspicuous. Antheridia small, tuberous or cylindrical, usually one or more to each oogonium.

The species is plentiful, especially in winter and spring, in branches, outlets of springs, edges of meadows, etc., as in Arboretum spring and branch, Battle's spring and branch, Glen Burnie meadow. We find it also at Tarboro, N. C. (March 7, 1921). Collected 94 times between February 15, 1912, and December 12, 1913 (see table on p. 14), and often since. Reported heretofore in America only by Humphrey from Massachusetts and by Atkinson from Alabama. For other illustrations see Ward ('83), pl. 22, figs. 15 and 16; Humphrey ('92), pl. 15, figs. 26 and 27; pl. 19, figs 82–86.

A single antheridial branch may, on reaching the oogonium, branch so extensively as to net the whole surface, but frequently no proper antheridium is cut off, and the embracing processes are not very densely filled with protoplasm. As in *A. oblongata* the eggs go to pieces so soon that it is exceptional that the final structure of maturity is reached. The homogeneous and almost black appearance of youth (transmitted light) gradually becomes more granular and lighter. Just before disorganizing the structure is that of a large central globule nearer one side than the other and apparently protoplasmic, completely surrounded by darker, rather dimly defined fatty globules. This agrees very well with deBary's description and figure except that he considered the central mass as a fat globule. In both this species and its variety *prolifica* the oogonial apiculus when present is not the result of an outgrowth from a spherical oogonial initial, but represents the unswollen tip of the original branch which produced the oogonium by an inflation below. If the tip is rather long the oogonia tend to be somewhat oval.

The spores develop exactly as described for *Saprolegnia anisospora.* The spore units appear, then disappear again about five or ten minutes before emergence, then gradually reappear, but with less clear outline, then the entire spore mass is slightly drawn away from the walls (most noticeable in *A. apiculata* in basal part, but to be made out all over), and the discharge occurs in a few minutes. The whole spore mass holds closely together and makes a central column, emerging as one body, with no individual motion visible, thus showing without any doubt

PLATE 42

PLATE 42

Achlya apiculata

Fig. 1. Cylindrical and spherical oogonia. × 167.
Fig. 2. A dictiosporangium, with one of the spores sprouting into a filament. × 447.
Fig. 3. Habit of oogonia and antheridia. × 102.
Fig. 4. Sporangia, several in serial arrangement in same hypha. × 100.
Fig. 5. Spore emerging from cyst. × 720.
Fig. 6. A mature egg. × 447.
Fig. 7. Gemmae. × 107.
Fig. 8. A small dictiosporangium. × 167.
Fig. 9. Peculiar case in which an oogonial initial was halted and sent off an antheridial branch. × 167.
Fig. 10. Oogonia. × 247.
Fig. 11. Sporangia, one on left a dictiosporangium with the spores emerged but not swimming; one in center containing four sprouting spores. × 60.
Fig. 12. An intercalary oogonium. × 247.
Fig. 13. Sporangia and oogonium with androgynous antheridium. × 167.
Fig. 14. An intercalary oogonium with a papilla. × 247.

ACHLYA APICULATA

PLATE 43

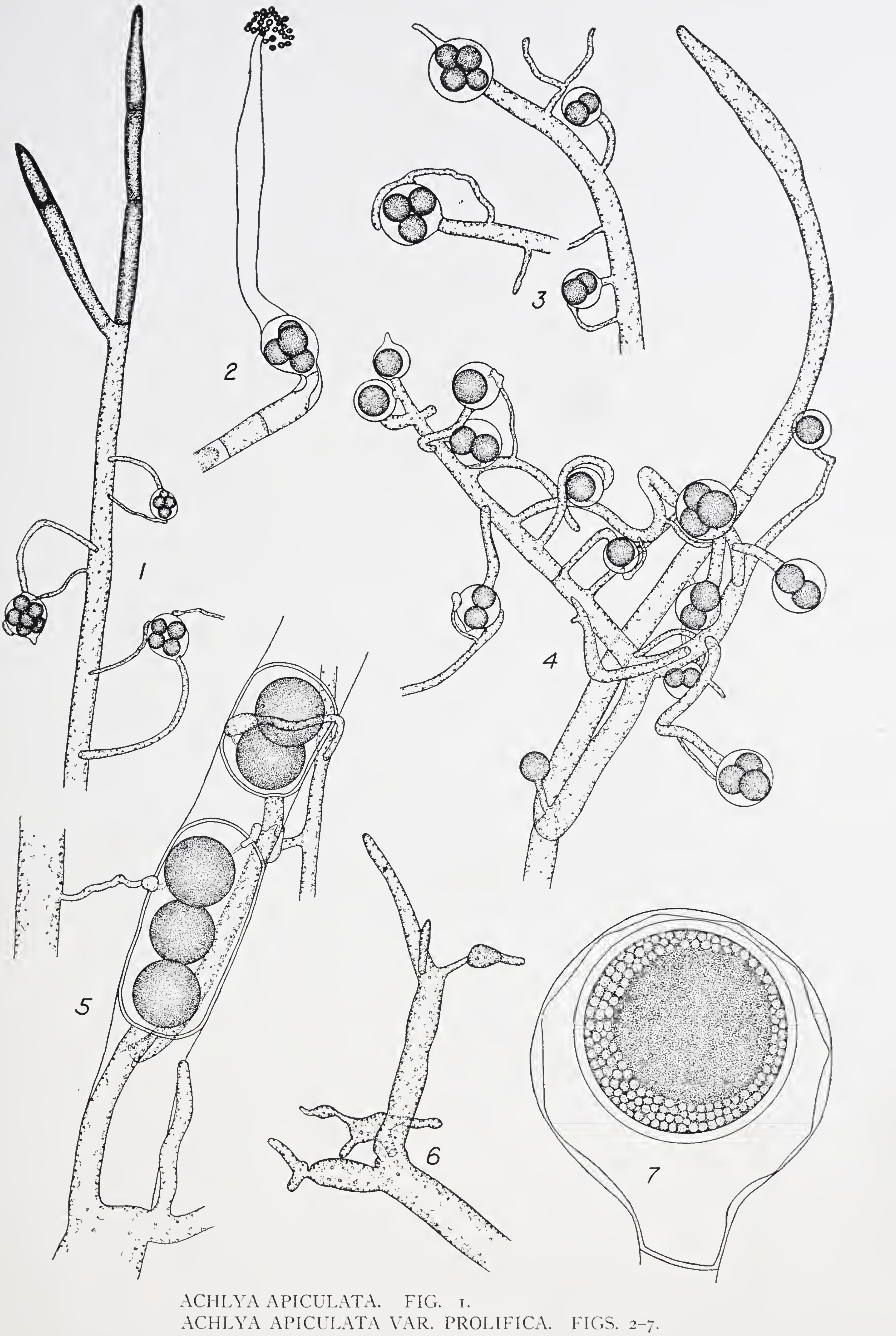
PLATE 43

ACHLYA APICULATA

Fig. 1. *A. apiculata*, showing usual habit of oogonia. (Compare Fig. 4 below). × 68.

ACHLYA APICULATA var. PROLIFICA

Fig. 2. Oogonium inside sporangium. × 122.
Fig. 3. Oogonia and antheridia. × 117.
Fig. 4. Habit of oogonia and antheridia, showing relative number as compared with *A. apiculata*. × 117.
Fig. 5. Cylindrical oogonia in a sporangium. × 278.
Fig. 6. Peculiar and characteristic tips of threads of vegetative hyphae. × 112.
Fig. 7. Egg structure and peculiar internal wall. Oogonium with unevenly thickened wall and single mature egg of typical structure. × 1012.



ACHLYA APICULATA. FIG. 1.
ACHLYA APICULATA VAR. PROLIFICA. FIGS. 2–7.

that the spores are expelled by internal pressure. In some cultures the spores will run larger in a good many of the sporangia without apparent cause, for example, in No. 2 of December 12, 1913, they were at times 14.8–16.6μ in diameter.

Dictiosporangia have been seen a number of times, *e.g.*, in No. 6 of January 7, 1914. In some cases oogonia are attached immediately below the dictiosporangia (pl. 42, fig. 10).

Oogonial reproduction is rarely either frequent or abundant in this species, and in some collections this tendency towards sterility is carried to an extreme (see under experiments below).

In a culture on a mushroom grub in water from the Arboretum spring a sporangium was made to discharge under a cover glass. The spore mass broke up in part and a good many of the outer spores were carried to a little distance and scattered, and their slight individual rocking motion could much more easily be seen. Iodine when added clearly demonstrated the cilia.

At one time spores were seen to emerge from their cysts at room temperature (about 72° F.) in about 5 hours after discharge, the emergence occupying about 2 minutes. Most of the cysts were about 13.5μ in diameter, often with larger ones in the same sporangium, the latter mostly about 22.5μ in diameter, and apparently the bulk of four ordinary spores. These latter on emerging have several sets of cilia. After emerging the spores scarcely rock for several minutes, then slowly rock, and finally after more active rocking get away rather sluggishly after about 5–8 minutes. Most are about 12 × 14.5μ in last stage.

The eleven following cultures were made from No. 4 of November 25, 1912:

In agaricus broth. Made a good start, but soon destroyed by an immense growth of bacteria.

In egg yolk broth. A delicate growth, the hyphae soon losing their contents except for the cut off tips, which look like small resting sporangia. Hyphae encrusted with amorphous granules. Bacteria not noticeable.

In equal parts maltose 5% and peptone .01%. Grew rather slowly to about ⅔ inch in diameter, then became unhealthy and died. No reproductive bodies.

On fly in jar of distilled water. Th · water was about one inch deep and the fly sank to the bottom. Growth was very stout and bold—one inch in diameter. The hyphae branched very little and all ended in sporangia, very few of which discharged their spores completely. The majority did not open at all. Many of the spores sprouted in position, and grew to some length. No other reproductive bodies.

On corn meal agar. Grew well, covering agar, but no reproductive bodies were formed. Hyphae gradually became colorless, and formed cross walls at places, segregating segments of denser material. After about one month a test was made and the culture found to be dead, even the denser sections.

On corn meal egg yolk agar. Grew vigorously but did not cover dish. A pretty dense growth of aerial hyphae was formed and reached the lid—looked much like *Mucor*. No reproduction.

In the following five experiments equal parts of a .2% solution in distilled water of the salts indicated was used; the food material being yolk of egg:

In KH_2PO_4. Stout, healthy growth. No reproduction.

In NaH_2PO_4. Good growth. No reproduction.

In K_2SO_4. Poor growth. No reproduction. Hyphae incrusted with amorphous granules.

In $Ca_3(PO_4)_2$. Strong, vigorous growth. No reproduction.

In $Ca(NO_3)_2$. Strong but limited growth. A good many sporangia, some emptying normally, most with spores sprouting in position.

The six following cultures were made from No. 12 of March 6, 1913:

On egg yolk in distilled water + 1 drop of lactic acid to 100 c.c. Growth very strong and healthy, consisting of large and little branched hyphae. Sporangia sparingly produced, long and pointed, discharging in most cases. Spores sprouting in position in some cases. No other reproduction. No noticeable bacteria.

On egg yolk in distilled water + 1 drop of lactic acid in 200 c.c. Growth as in the preceding experiment, except not quite so strong and no sporangia or any other reproduction. No noticeable bacteria.

On boiled potato tuber and sprout in potato broth. No growth.

On boiled potato tuber and sprout in distilled water. Fair growth of limited extent, bacteria present and apparently interfering. Tips of hyphae often dying and the thread extended by growth from below, as is frequent in this species. No sporangia or other reproduction.

On egg yolk in distilled water. Growth fairly good, but no sporangia or other reproduction. Not foul with bacteria.

On egg yolk in 3% cane sugar in distilled water. Growth strong, somewhat contorted, only a few sporangia, and spores sprouting at the tip.

The following experiment was made to test the resistance to cold:

A strong culture was left outside on window sill and was frozen hard (temperature 23°F.). The culture was killed.

The following experiments were made to test the best method of preserving live cultures:

A culture (No. 6 of April 19, 1913) put in vial on corn meal agar in the spring of 1913 was found to be dead December 1, 1913.

Pure culture (No. 4 of November 25th, 1912) was put in an aquarium jar with algae on laboratory table on February 19, 1913. No growth resulted when tested on September 18, 1917.

The following experiment is typical of many made to determine the effect of cold on the formation of reproductive organs:

On piece of boiled corn grain in sterile well water in ice box temperature from 12° to 20° C. Growth good and many normal oogonia formed, but only about a fifth as many as in the var. *prolifica* in the same circumstances; no sporangia.

14. **Achlya apiculata** var. **prolifica** Coker and Couch n. var.

PLATES 43 AND 50

Growth fairly dense but only moderately long, reaching a length of 0.3–0.4 cm. (on termite ants or mushroom grubs). Main hyphae branching considerably, rarely up to 90μ close to base; the tips of hyphae pointed and hyaline when young, drying or becoming rounded with maturity. In summer (room temperature of 21–32.5° C.) the ends of the hyphae become elaborately branched and the tips considerably swollen. Sporangia, spores and gemmae as in *A. apiculata*, but the gemmae less numerous. Oogonia produced in all cultures, at low temperature (12–20° C.) in great abundance, but at room temperature (21–30° C.) usually few and sometimes none; diameter 40–90μ, in most 55–65μ, spherical, less often oblong or rarely cylindrical in old sporangia as in *Saprolegnia ferax*, quite often provided with a short apiculus, rarely with a long one. Walls smooth and unpitted except where the antheridia touch; however, in old cultures on corn grain the wall may be considerably roughened but not typically pitted (pl. 43, fig. 7). Oogonial stalks as in *A. apiculata* except more branched. Eggs usually 1 or 2, rarely as many as 5; antheridial branches, antheridia and the structure of the egg the same as in the species.

This plant appeared once (No. 2 of February 28, 1921, in branch in Latta's Woods) among more than 300 collections made around Charlotte N. C., by J. N. Couch.

The present variety in vegetative growth and in the shape and size of the sporangia and spores is indistinguishable from the species. The oogonial stalks of both plants are quite similar, though the stalks of the variety show a stronger tendency to branch than in the species. Apiculate oogonia are found in both plants but oogonia with such outgrowths are more abundant in the variety. The outstanding differences between the two plants are found, first, in the regularity with which our present plant bears sexual fruits even in room temperature (17–24° C.), while *A. apiculata* fruits very poorly in the laboratory; second, in the relative number of oogonia produced under the optimum temperature for both plants, the number being about five times greater in the variety than in *A. apiculata*; third, in the comparative size of the oogonia, the average diameter in the variety being between 55 and 65μ while in the species the average diameter is between 70 and 80μ; and fourth, in the relative number of eggs in the oogonia, the usual number in the variety being 1 or 2, rarely 5, while in *A. apiculata* the usual number is between 3 and 5, rarely 10. This variety is the only member of the *Saprolegniaceae* in which we have been able to observe sprouting eggs. Old eggs from a culture made August 15, 1921, were put on corn meal agar in February, 1922. In forty-eight hours they had sprouted to form long, branched threads.

The following experiments are typical of many made to determine the effects of temperature on the production of sexual organs.

On termite ants in sterile well water in ice box. Temperature ranging from 12° C. to 20° C. Growth good, 5 cm. long. A good many sporangia formed and emptied, and a good many oogonia, some of them in empty sporangia as in several previous cultures on ants in ice box. Oogonia with very long stalks.

On termite ants in sterile well water in room. Temperature ranging from 17°–22° C. Growth good, the ends of hyphae considerably branched, swollen and contorted; many sporangia and a fair number of oogonia.

On termite ants in sterile well water in room. Temperature ranging from 21° to 32.5° C. Growth very poor, bacteria bad. Two days later dead.

On piece of boiled corn grain in sterile well water in ice box. Temperature from 12°–20° C. Growth very good, mycelium dense, threads much branched and curled. Many sporangia, a large number of which had emptied; very many oogonia close to periphery of mycelium, oogonial stalks much curled and branched as also the antheridial stalks. Oogonia average by count about five times as many in a given field as in the species.

On piece of boiled corn grain in sterile well water in room. Temperature 17°–22° C. Growth good, the ends of hyphae considerably branched, swollen and contorted; many sporangia, and a fair number of oogonia.

On piece of boiled corn grain in sterile well water in room. Temperature 21°–32.5° C. Growth good, but soon interfered with and killed by bacteria.

15. **Achlya megasperma** Humphrey. Trans. Amer. Phil. Soc. **17**: 118. pl. 18, figs. 74–77. 1892 [1893].

Plate 44

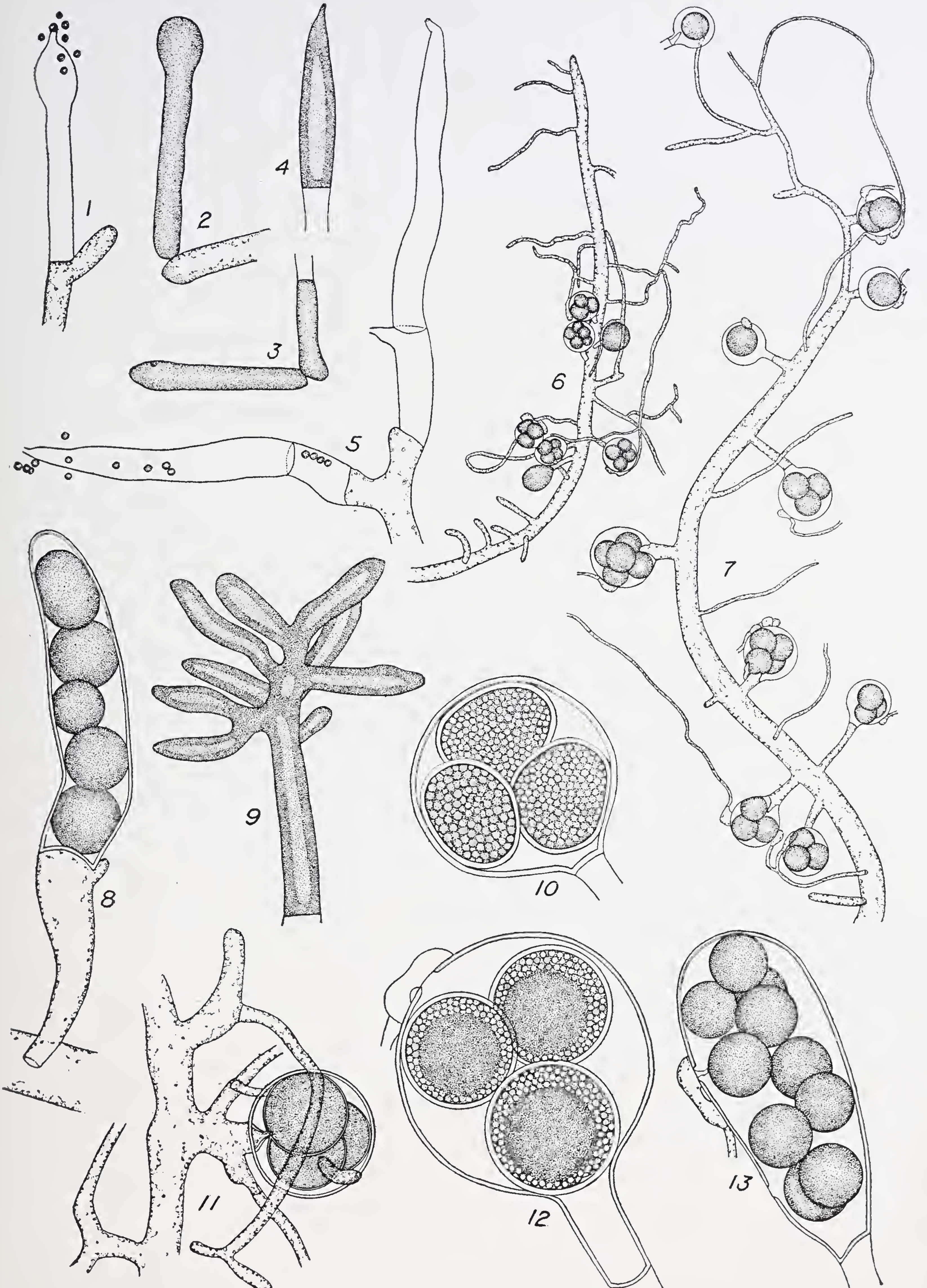
Mycelium slenderer than in most Achlyas. Sporangia very abundant, of the typical *Achlya* type, borne singly or in clusters (often as many as eight) on the ends of hyphae, varying much in shape from the long, slender, tapering sporangia of *Achlya apiculata* to a club-shaped form swollen at the distal end; 100-1000μ long, most between 300 and 400μ. Spores 11μ in diameter. Gemmae developed in considerable abundance, either single and shaped like a sporangium with pointed tip or very elaborately branched; when solitary often separating from the hypha and falling to the bottom. Oogonia racemosely borne on branches which are about as long as or shorter than the thickness of the oogonia; rarely the oogonial branches may be longer. Oogonia without an apiculus, usually spherical, occasionally oblong, rarely cylindrical, 60-119μ thick, usually between 70 and 80μ, oogonial wall thickened and without pits except for thin places under the antheridia. Eggs 1-10 or rarely more, usually 2-5, almost or entirely filling the oogonium, often elliptic from pressure; 39-66μ thick, usually between 42 and 52μ (in an oogonium in which there were 9 eggs the average size was 44.1μ); structure subcentric and exactly as in *A. apiculata*; walls 3-4.6μ thick. Antheridial branches diclinous or androgynous but never arising from the oogonial stalk, usually diclinous, often much branched and not applied to oogonia; long and very slender, becoming barely visible after the eggs are formed. Antheridia tuberous and fairly conspicuous; usually one or two on each oogonium; not rarely absent.

PLATE 44

PLATE 44

Achlya megasperma

Figs. , 2, 3, 4. Gemmae, one of which has become a sporangium. × 103.
Fig. 5. Normal sporangium. × 103.
Fig. 6. Habit sketch. × 55.
Fig. 7. Habit sketch. × 103.
Fig. 8. A cylindrical oogonium. × 247.
Fig. 9. An elaborately branched gemma. × 103.
Fig. 10. Oogonium showing surface view of eggs. × 433.
Fig. 11. Oogonium with peculiar stalk. × 247.
Fig. 12. Oogonium showing optical section of eggs. × 433.
Fig. 13. Large oogonium containing ten eggs. × 247.



ACHLYA MEGASPERMA

This plant with its large eggs reminds one of *A. apiculata*, but by careful observations and measurements it is found to differ from the latter in the following ways: the eggs average about 44μ thick; the oogonial stalks are short and straight; the oogonial walls are thickened and without an apiculus, and the oogonia are closely packed with eggs. In *A. apiculata* the eggs average about 36μ; the oogonial stalks are long and curved; the oogonial walls thin; and the oogonia only about half filled with eggs. These characters while separating the present plant from *A. apiculata* at the same time identify it as *A. megasperma*.

Humphrey found the plant only once and then in cultures from *Spirogyra*, dead leaves, etc., taken from a boggy spot by a small brook. Our plant also has been collected but once, near Charlotte, N. C., by J. N. Couch, February 12, 1921, and from a situation similar to that in which Humphrey found it. Mr. Couch has prepared the accompanying plate.

16. **Achlya polyandra** Hildebrand. Jahrb. f. wiss. Bot. **6**: 258. pl. 16, figs. 7–11. 1867.

? *Achlya gracilipes* deBary. Bot. Zeit. **46**: 635, pl. 10, figs. 2 and 6. 1888.

Plate 53

This species has been reported in America only from Massachusetts (Am herst) by Humphrey ('92, p. 119) and from Michigan by Pieters (in Kauff man, '06). It is distinguished by the subcentric (?) eggs, the absence of pits in the oogonial walls, the rather long stalks of the oogonia, and by the androgynous antheridia which spring as a rule from the original stalks. We take the following description from Humphrey:

"Hyphae stout, long. Zoosporangia often not abundant, secondary ones rare, nearly cylindrical. Oogonial branches usually very long and often recurved at the tip, racemose. Oogonia terminal, globular, with smooth and unpitted walls. Antheridial branches arising chiefly from the oogonial branches not far from the oogonia, often branched. Antheridia one to several on each oogonium, short-clavate. Oospores 5 to 25, usually 10 to 15, in an oogonium, centric, their average diameter 27μ."

Humphrey thought that *A. gracilipes* deB. and *A. polyandra* Hildb. were the same, but Pieters suggests that his figures of the European plant (figs. 1–4) are exactly like those of *A. gracilipes* deB. and that that may be a good species, different from *A. polyandra*. (It is, however, to be observed that in Pieters's figures the original stalks are not recurved at the tips as in *A. gracilipes*.) Fischer regards Hildebrand's plant as probably intermediate between *A. gracilipes* and *A. polyandra* deB. It seems to us probable, however, that Humphrey is correct. It will be noticed that both deBary and Hildebrand, as well as Humphrey and Pieters, show the eggs not by any means filling the oogonia. Pieters does not mention or show the internal structure of the eggs in the plant he studied. For other illustrations see Massee ('19), pl. 2, fig. 31.

Fertilization occurs in this species, as Trow has shown in his 1904 paper, with about the same phenomena as he demonstrates in more detail for *A. deBaryana*. Trow, however, does not claim positively for this species a second division of nuclei in the oogonium before egg formation. It is unfortunate that he gives no new data on the structure of the mature eggs. It probably is almost like that of *A. apiculata*. DeBary describes the eggs of *A. gracilipes* as "centric," but his figures do not show the structure.

Working at least in part on *A. gracilipes*, Scerbak (1910) tested the effect on diplanetism of various external conditions and found the second swimming stage could be inhibited by osmotic pressure of the medium and under the influence of narcotics, etc. The paper is in Russian and only a short paragraph in German gives the conclusion.

We quote the following discussion of synonymy by Humphrey (p. 119):

"It [*A. polyandra* Hildb.] is especially interesting as having been the subject of a misunderstanding which has led to a confusion in synonymy that I have here attempted to correct. It was undoubtedly this species which Hildebrand described ('67) as *A. polyandra*. As has been already pointed out, deBary gave the same name ('81) to a distinct species which he recognized as differing from Hildebrand's description, but thought to be probably his species. At the time of the completion of the paper quoted deBary had probably never seen this form, as his later paper ('88) states that he first obtained it in January of 1881, the year of the publication of the earlier one. And while he did study it, he failed to notice its correspondence with Hildebrand's figures and description, and therefore named it anew *A. gracilipes*. But no one who will carefully compare the figures given by both authors will, I think, seriously question that they represent the same species. Again, Hildebrand states that secondary sporangia are not produced in his *A. polyandra*, a statement that deBary ('81) disputes as untrue for his *A. polyandra*. But in his description of *A. gracilipes* ('88), deBary says that secondary sporangia are only sparingly developed, a statement which I can corroborate for American specimens. The two descriptions agree in all other essential points, so far as they are comparable; and the evidence seems completely satisfactory that the correct synonymy of this distinct species is as above given."

17. **Achlya conspicua** n. sp.

PLATES 45 AND 46

Hyphae long and more stout than in most Achlyas, up to 166μ thick near base or some as small as 30μ, the tips often withering and the hyphae extended from a bud below as in *A. imperfecta*, etc. Sporangia abundant, secondary ones plentiful, varying from short and slender to very long and slender, or rarely stocky when short, 18–60 × 105–550μ; spores emptying and behaving as typical in *Achlya*, 10.5μ thick. Oogonia not abundant, borne laterally from the main hyphae, their stalks of moderate length, varying from about as long to twice as long as the diameter of the oogonia, or not rarely even longer; oogonia spherical or rarely oval, 51–118μ thick, most about 70μ, the walls yellowish, not thick, often strongly pitted, the pits varying much in number and about 5.5μ wide. Eggs 3–30 or more, usually 4–10, with a diameter of 22–29μ, most about 25μ, not filling the oogonium as a rule; rarely maturing and of obscure structure, apparently about like those of *A. apiculata* when in normal condition, but nearly always degenerating immediately and becoming irregularly filled with large oil drops. Antheridial branches androgynous or less often diclinous, usually simple, arising near the oogonia from the main hyphae or often from the oogonial stalks, usually one or two, rarely more, for each oogonium; antheridia on all oogonia, cylindrical or long-tuberous, usually touching the oogonia by foot-like projections, at times applied by the entire side; antheridial tubes obvious. Gemmae not peculiar, long, often in rows by the abstriction of the longer threads, frequently with prongs, emptying as sporangia under suitable conditions.

Found four times in Arboretum branch (No. 5 of July 2, 1917, No. 7 of June 29, No. 2 of July 24, 1920, and April 18, 1921).

The number of pits may vary remarkably in the same culture and even on the same hypha. In culture No. 2 of July 24, 1920, on a termite some of the oogonia in one section of the culture were without any visible pits except where the antheridia touched; while in the remainder of the culture oogonia with few to many pits were found, both occurring often on the same hypha.

Another peculiarity is the strong tendency of the oogonial stalk to flare beneath the oogonium so as to be attached by a broad disc, and this is often so exaggerated as to throw the separation wall a third of the way up the oogonium.

Achlya americana var. *cambrica* (see p. 139) is superficially somewhat like this but is easily distinguished by the smaller and eccentric eggs, which much more nearly fill the smaller oogonia, and by the origin of the antheridia in greater part from the main hyphae. The subcentric eggs place our plant near *A. polyandra* Hildebrand, as understood by Humphrey and Pieters, from which it is separated by the less numerous and much less branched antheridial stalks which spring both from the main hyphae and from the oogonial stalks, and by the presence of pits in the oogonial wall. Hildebrand's original form is described as having the antheridial branches coming from the oogonial stalks, and in this and the unpitted walls differs from *A. deBaryana*. DeBary's *A. gracilipes* is certainly a very different looking plant from the present species, and may be quite distinct from *A. polyandra* Hildebrand.

Possibly representing a peculiar form of the present species is a slide prepared by us in the fall of 1903, before we were much interested in this group. The pitted oogonia and the antheridia are borne in about the same way, but the plant seems so abnormal that we are not at all sure what it is. The walls of the hyphae, the stalks, the oogonia and even the antheridia are mostly very thick and in places so thick as almost to close the lumen, reaching a thickness in places of 7.5μ; the eggs are all going to pieces, but their walls show them to be mostly 22–24μ thick, a few up to 30μ. (The plant is not *Aplanes androgynous* or *A. Treleaseanus*, which have thicker walls than other species). The stalks flare greatly at the top and the wall separating the oogonium is often so far up as to cut off a lower section of the oogonium, thus including it in the stalk. Such queer structures can only be abnormal, and careful examination of the oogonial contents show amongst the varied detritus certain minute, spherical bodies of constant structure and appearance. They may be and probably are the parasite causing the trouble. They are not at all like any of the chytridiacean parasites of the family. May they not be the amoeboid parasite *Vampyrellidium vagans* Zopf, which has been insufficiently investigated? (Schenck's Handb. **4**: 540 and 565. See reference to this by Fischer '92, p. 325).

18. **Achlya oblongata** deBary. Bot. Zeit. **46**: 646, pl. 10, figs. 7–9. 1888.

PLATES 46 AND 47

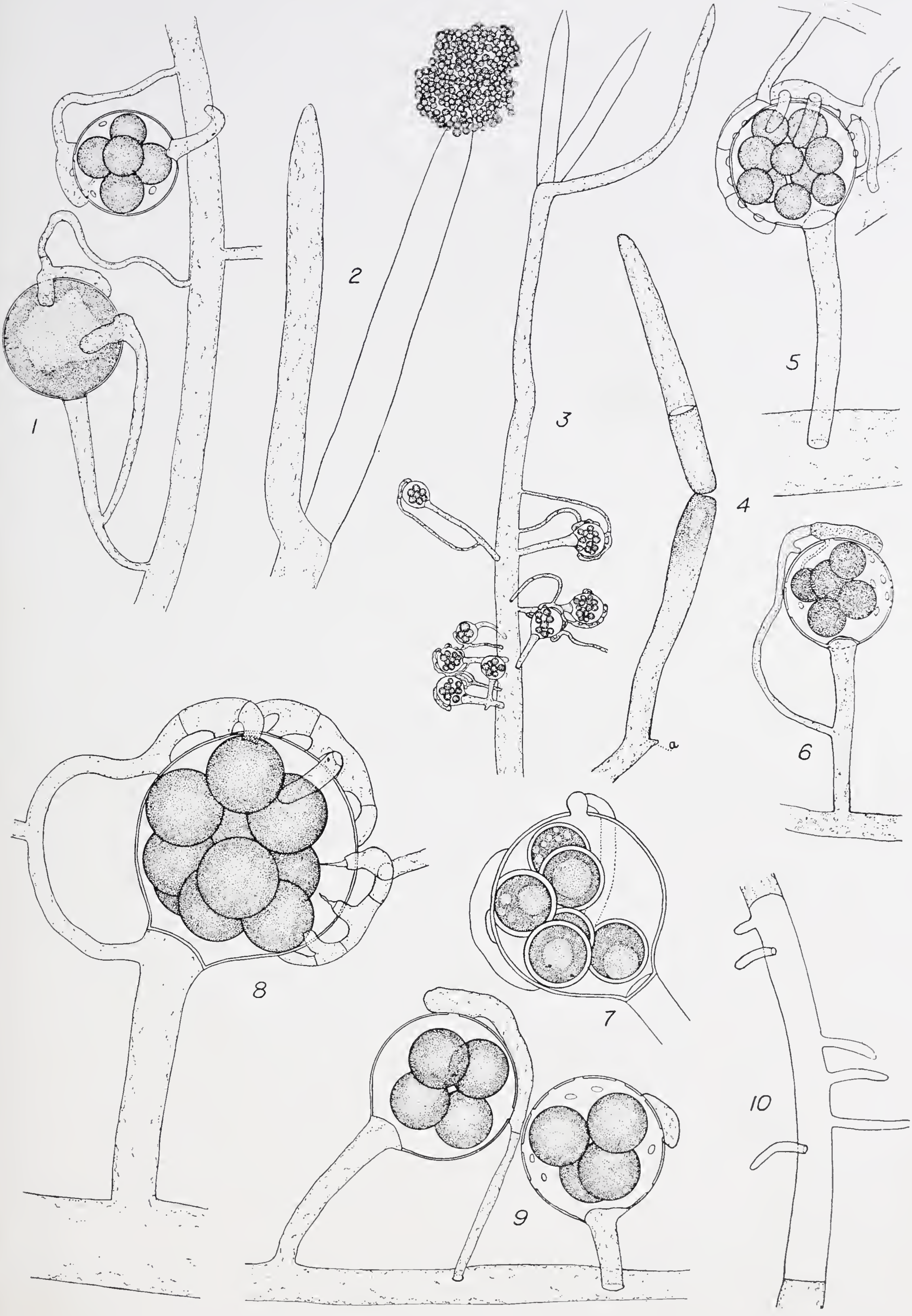
Mycelium rather stout and vigorous; sporangia subcylindric to fusiform, pointed; dictiosporangia not rare; spores small, about 9μ in diameter. Gemmae oval like the eggs or often linear by the division of the main hyphae. Oogonia very large, typically oval to pyriform, rarely nearly spherical, 40–150μ in diameter the short way, borne usually on moderately long lateral branches, at times terminal on main branches, rarely intercalary; wall thin, hyaline, without pits. Eggs 1–28 or 30, rather small, 20–30μ, most about 27μ in diameter, subcentric, with a lunate (in section) sheath of oil droplets nearly surrounding the protoplasm; usually arranged loosely in the oogonium and not filling it, at first very dark, rarely maturing. Antheridial branches delicate and slender, always diclinous and not traceable to threads bearing oogonia; antheridia on every oogonium, usually numerous, small and tuberous, no fertilizing tubes seen.

PLATE 45

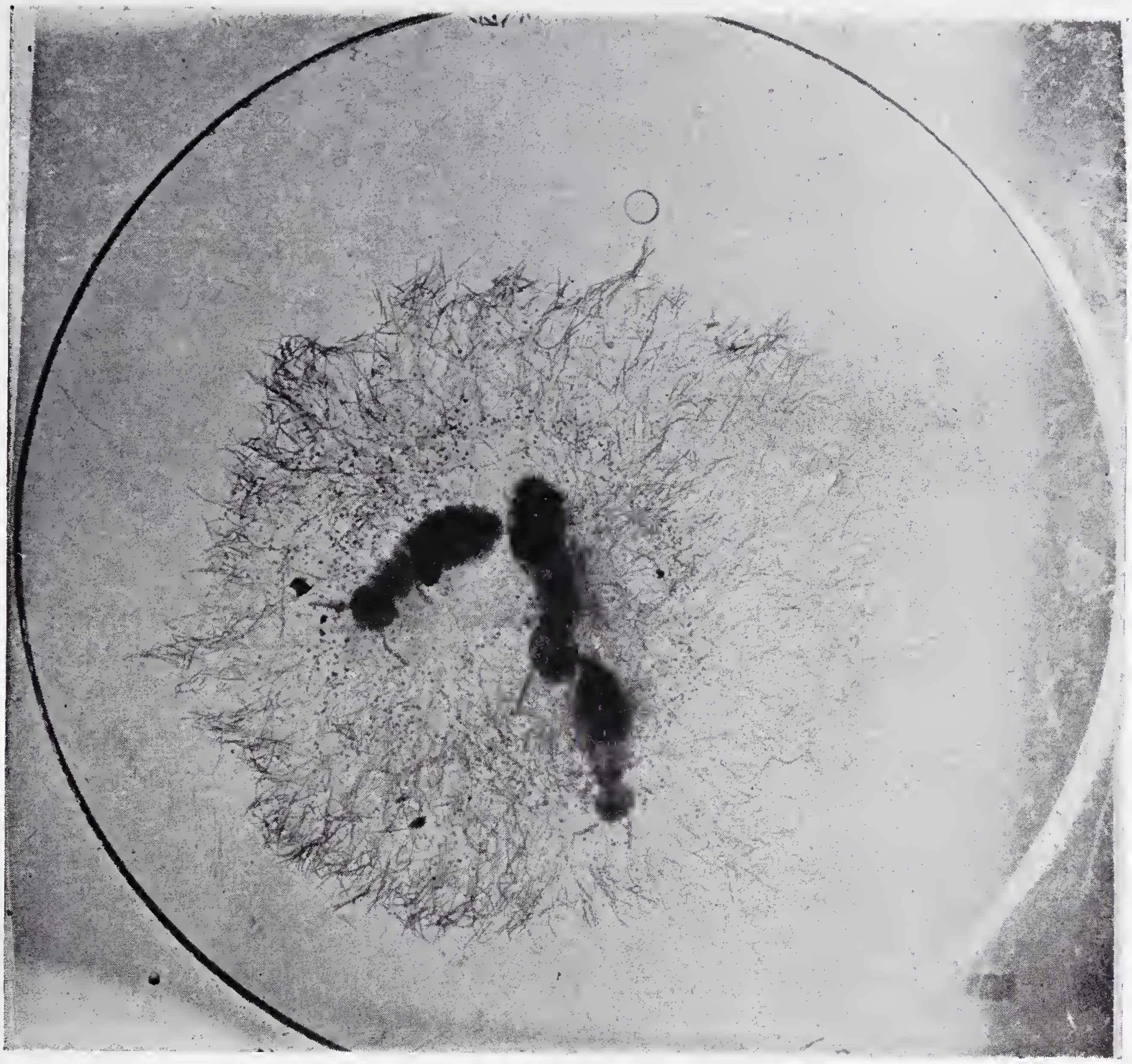
PLATE 45

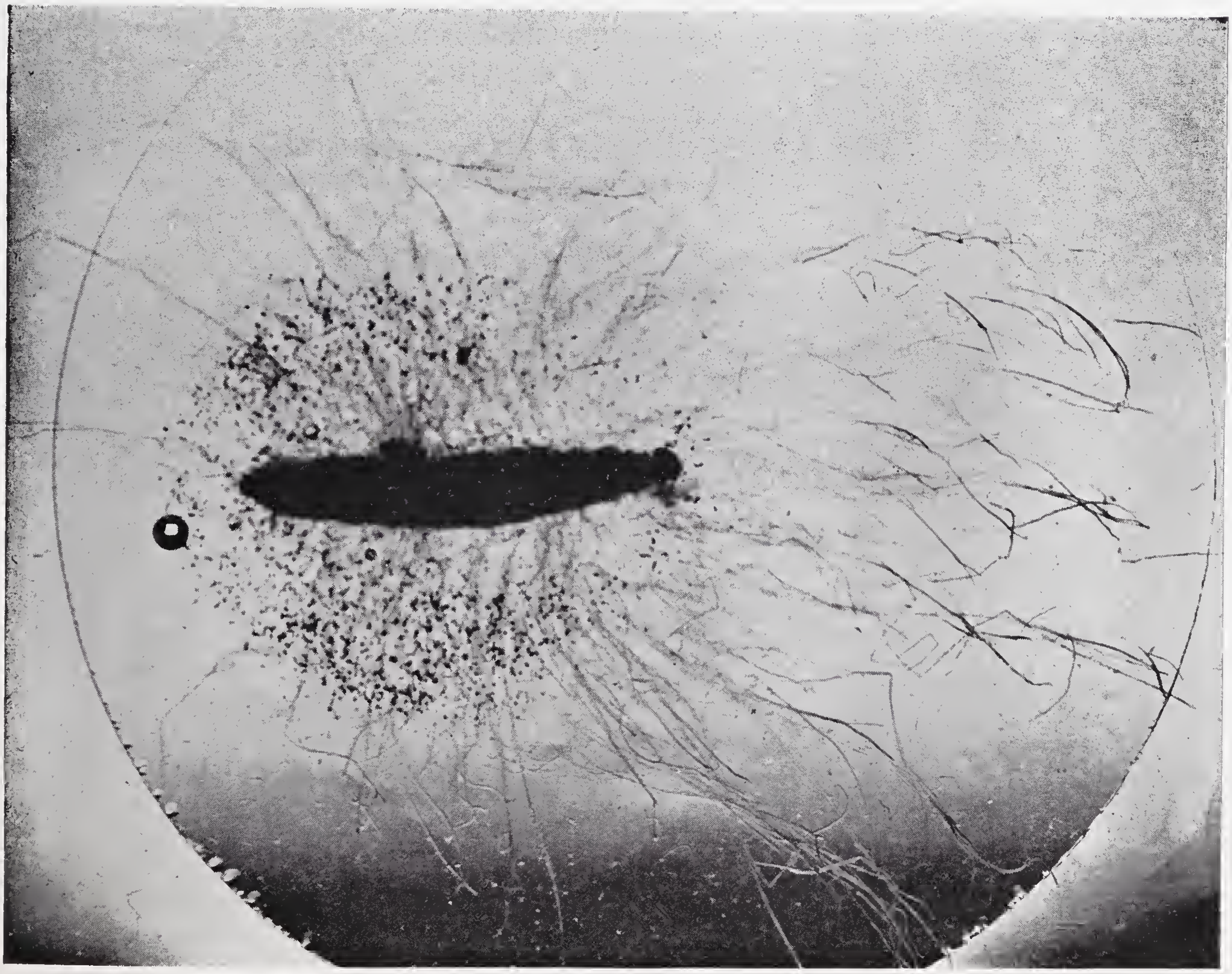
Achlya conspicua

Fig. 1. Oogonia with antheridia. × 247.
Fig. 2. Sporangia with spore cluster at tip. × 167.
Fig. 3. Habit of oogonia, antheridia and sporangia. × 60.
Fig. 4. Gemmae; also showing an atrophied growing point (a) renewed from below. × 60.
Fig. 5. Oogonium with diclinous antheridia. × 247.
Fig. 6. Oogonium with conspicuous pits. × 247.
Fig. 7. Oogonium with eggs undergoing degenerative changes. × 447.
Fig. 8. Oogonium with both diclinous and androgynous antheridia. × 447.
Fig. 9. Oogonia showing a varying number of pits. × 247.
Fig. 10. Gemma with several papillae, through the longest of which it has emptied. × 167.



ACHLYA CONSPICUA.





ACHLYA CONSPICUA [ABOVE].
ACHLYA OBLONGATA [BELOW]. × 6.

PLATE 47

PLATE 47

Achlya oblongata

Figs. 1 and 2. Sporangia with curled tips. × 247.

Fig. 3. Aplanosporangium, with the tip atrophied, the spores sprouting in the sporangium. × 247.

Fig. 4. A typical sporangium. × 100.

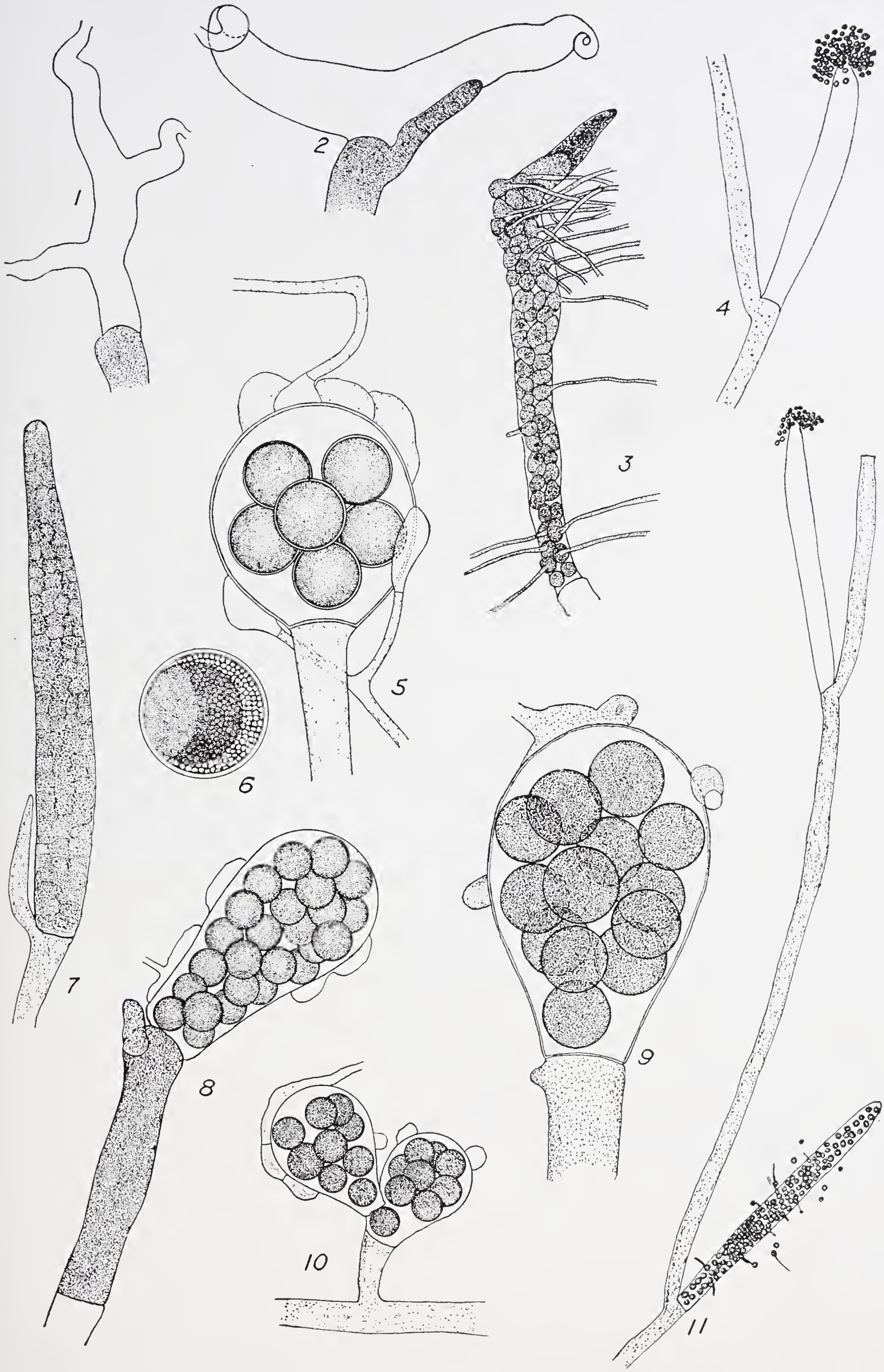
Fig. 5. An almost spherical oogonium. × 447.

Fig. 6. Egg showing structure. × 720.

Fig. 7. Sporangium with the spores forming. × 247.

Figs. 8, 9 and 10. Typical oblong oogonia. 7 and 9 × 247; 8 × 447.

Fig. 11. Showing an aplanosporangium and a typical sporangium on the same hypha. × 100.



ACHLYA OBLONGATA

Obtained first from dead sunfish fingerlings from U. S. Fish Hatchery at Bullockville, Georgia, November 13, 1916, and from this collection the plate was made. Found also at Chapel Hill in several collections from Meeting of the Waters, February 16, 1921.

Distribution: North Carolina, Georgia, Louisiana, Massachusetts. For other illustration see Humphrey ('92), pl. 19, figs. 87–89.

This is an easily distinguished species, the elongated oogonia and strictly diclinous antheridia on every oogonium defining it well. Humphrey also gives the average size of the eggs as 27μ in his form, and says they are centric, as does also deBary. The structure is, however, not truly centric, but with a lunate sheath of small droplets extending most of the way around the protoplasm (pl. 47, fig. 6). It is possible that in some eggs the protoplasm may be quite surrounded by fat drops.

19. **Achlya oblongata** var. **globosa** Humphrey. Trans. Amer. Phil. Soc. 17: 122, pl. 19, figs. 90–91. 1892 [1893].

We have not found this variety. It was obtained by Humphrey from Pennsylvania and Alabama. He defines it as like the type except that the oogonial branches are very short; the oogonia globular; the eggs reaching 25 in number, averaging 10–15. The oogonia are commonly larger than in the type and the space unoccupied by the eggs is much more marked. Very little difference appears in the descriptions of this and *A. Klebsiana*, except that it is implied that the eggs are "centric". This is an important point, and if so will put it in a different group from *A. Klebsiana*.

20. **Achlya glomerata** Coker. Mycologia 4: 325, pl. 79. 1912.

PLATE 48*

The following is copied, with few changes, from the original description:

Hyphae rather stout, branched, not long. About 40–45μ in diameter at base and tapering to slender tips about 12μ in diameter. At maturity the main hyphae strongly incline to segment into elongated sections with dense protoplasm, but the slender apical section is apt to remain almost empty (fig. 1). Sporangia almost cylindrical, inclined to be somewhat irregular and often opening by a bent papilla (fig. 2). Oogonia abundant, approximately spherical, without pits; completely covered with short, blunt, irregular warts 29–44μ thick, with the warts most about 33μ thick (fig. 3). Oogonia borne on the tips of very slender and delicate, but contorted lateral branches that are either simple, in which case there is but one oogonium (fig. 3), or more or less intricately branched, in which case there are a number of oogonia borne on the tips of the group of branches (figs. 4, 5, and 6). Eggs single or very

*This plate is a reproduction, with slight changes, of the plate in Mycologia.

rarely two in an oogonium, eccentric, their diameter 15–23μ, averaging about 20μ. Antheridia absent from a good many oogonia, when present club-shaped; borne on the tips of branches from the same glomerulus and one or several on an oogonium (fig. 5).

This species has been observed several times from two stations at Chapel Hill, North Carolina. The drawings are made from material taken from a cool spring in dense woods (Lone Pine spring) on April 30, 1912, and from the springy marsh at the foot of Lone Pine hill on February 29, 1912. Pure cultures have been continued for six months or more.

This species does not closely approach any other, but it seems to be nearest the members of the *Racemosa* group. The shape of the antheridia is like those of *A. racemosa* and its relatives, and there is considerable resemblance to the spiny oogonia of *A. colorata* and *A. radiosa*. There is also some hint of the habit of *A. glomerata* in the occasional branched oogonial threads of *A. radiosa*. In all the members of the *Racemosa* group the antheridial branches, when present, originate just below the oogonium. In *A. glomerata* they do not thus originate. This distinction with the usually bent and twisted branching habit of the oogonial hyphae separates the species sharply from any of the *Racemosa* group. As already mentioned, the oogonia are sometimes borne singly on the ends of simple branches, especially near the tips of the main hyphae, but in such cases these branches are much more delicate and longer in proportion to the oogonia than is generally the case in any member of the *Racemosa* group. *Achlya spinosa* has but one or two eggs (rarely three) in an oogonium and may be the closest relative of *A. glomerata*. It differs easily in the barrel-shaped oogonia with an apical papilla, in the antheridia arising just beneath the oogonia, and in the absence of clustered groups of oogonia.

The fruiting branches are so abundant and many of them are so elongated and extensively branched that the cultures take on a whitish, cottony appearance except near the periphery, which is usually without branches. In extreme cases this effect is so pronounced that the culture may be compared in appearance to a rug with a fringe. This reminds us of the "woolly snow-white turf" produced by deBary's *Achlya spinosa* ('88, p. 647), which species, while not in the close family circle of the *Racemosa* group, shows its relation to them by its spiny oogonia with generally one egg, and by the origin and shape of the antheridia.

So far as the sexual organs are concerned, there is a remarkably close resemblance between *Achlya glomerata* and *Saprolegnia asterophora* deBary. As in most species of *Achlya*, the spores sometimes remain in the sporangium and sprout there (Coker, '10, p. 381).

Pure cultures from No. 10 of April 30, 1912:

PLATE 48

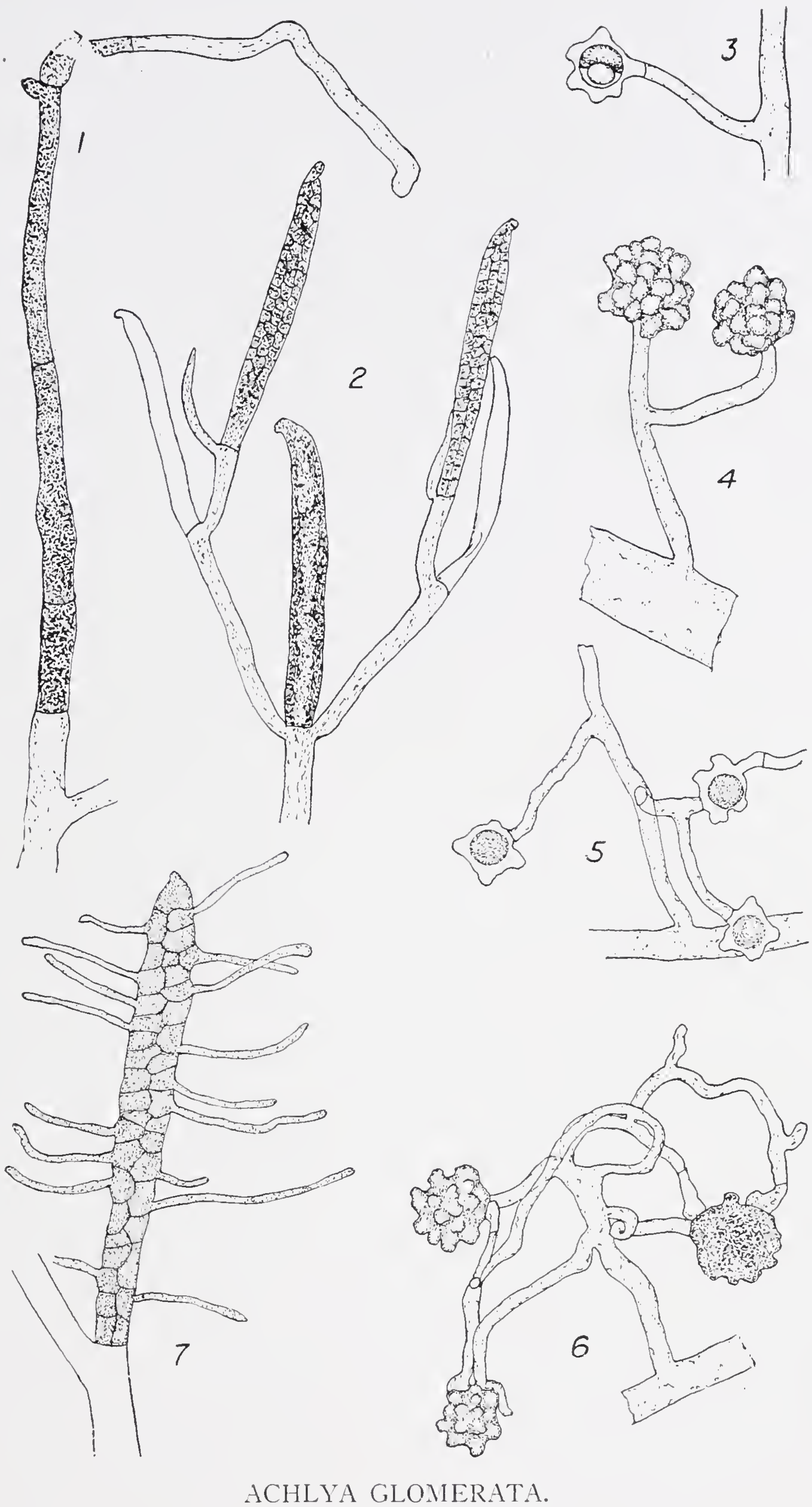
PLATE 48

(Taken with slight changes from Pl. 79 of Mycologia **4**. 1912.)

ACHLYA GLOMERATA

Fig. 1. Part of filament from an old culture, showing segmentation into gemmae. Part of one long cell is omitted. The contorted tip cell is almost empty. × 185.
Fig. 2. A group of sporangia in different stages. × 125.
Fig. 3. An oogonium containing a ripe egg. × 335.
Fig. 4. A branched oogonial filament with two oogonia. × 335.
Fig. 5. A more complex group of oogonia, not all shown. One is intercalary. × 335.
Fig. 6. A characteristic group of oogonia with antheridia. × 335.
Fig. 7. A sporangium in which the spores became encysted and sprouted in position. × 335.

PLATE 48



ACHLYA GLOMERATA.

In maltose 5 % peptone .01 %. Oogonia in fairly large number, and reaching full size with tubercles, but no antheridia and no eggs formed, contents going to pieces.

On corn meal agar. Growth limited and purely vegetative. The threads became segmented up into long sections, with dense protoplasm, as usual in the species.

In equal parts of maltose-peptone and pea broth. Growth good, about 1 inch in diameter, dense and thick. A good many oogonia, and with normal eggs in most. Antheridia on every oogonium, apparently—certainly on nearly all.

On corn meal egg yolk agar. Growth slow, but extending over about a 2-inch circle, many hyphae standing up into air ⅛ inch, but not dense. A considerable number of oogonia with good eggs. Some had antheridia.

In the following cultures equal parts of a 2% solution of the salts and of maltose-peptone solution (one part of 5% maltose+one part of .01% peptone) were used:

In $Ca(NO_3)_2$ + maltose-peptone solution. Strong, healthy vegetative growth, about 1 inch in diameter. No reproduction of any kind.

In KNO_3 + maltose-peptone solution. Growth as in preceding experiment. No reproduction.

In $Ca_3(PO_4)_2$ + maltose-peptone solution. Growth as in two preceding experiments, except somewhat more delicate. No reproduction.

In KH_2PO_4 + maltose-peptone solution. Most extensive growth of any in this series. No reproduction.

In NaH_2PO_4 + maltose-peptone solution. Growth short, contorted, and protoplasm segregated into spots in larger hyphae. No reproduction.

In K_2SO_4 + maltose-peptone solution. Growth delicate, about 1 inch in diameter. No reproduction.

Experiments to test the best method of preserving live cultures:

Culture on corn meal agar put in vial of water, which was closed with a plug of cotton and put in dark place in May, 1913. Test for life was made in December, 1913, and it was found to be alive (all old eggs were dead).

Culture as above made March 17, 1913, was found to be dead on December 1, 1913.

Culture placed in aquarium jar with algae in laboratory on March 7, 1913. No growth appeared when tested on September 18, 1917.

21. Achlya dubia n. sp.

PLATE 49

Main threads stout, little branched, only about 5 mm. long on a termite, up to about 50 or 60μ thick, tapering gradually, the rather blunt tips clear and refractive while growing. Sporangia abundant, terminating the main hyphae, long, only slightly thicker than the threads, tapering a little towards the tip, increased sparingly by growth from below as in *Achlya;* when mature discharging the spores as in *Achlya* or as in *Thraustotheca* in varying proportion, often about half and half on a termite in sterilized well water, not rarely behaving as in *Dictyuchus*. Spores normally about 11.5μ thick (but often much larger masses of protoplasm are found among them), encysting on emerging from the sporangium and escaping for a swimming stage as in *Achlya*. Gemmae abundant, formed by partitions in the threads behind the sporangia, hence subcylindrical, often bending and partly separating at the joints, soon forming spores which escape as in *Achlya* by a papilla of variable length. Oogonia borne on short lateral stalks from the main hyphae, rarely terminal on the latter, smooth, spherical, very regular in size, 50–65μ, most about 60μ, the rare terminal ones up to 90μ thick; wall rather thin, not pitted except under the antheridia, distinctly yellow-brown. Eggs few, 2–5, in large oogonia about 6 or 8, in diameter 24–33μ, most about 28–30μ, when nearly mature with several oil drops on one side, then usually with a single, lateral, conspicuous drop at full maturity (but mature oogonia not rarely still contain several or a good many oil drops). Antheridia on all oogonia, usually cylindrical and partly wrapped about the oogonia, diclinous, borne on sparingly branched threads of moderate length from main hyphae or on long threads which terminate more slender hyphae; antheridial tubes obvious and visible for a long time.

This remarkable plant has appeared but twice. It was found in a collection from a branch (Meeting of the Waters, No. 5, of February 16, 1921) where the water is somewhat contaminated by a sewer which enters about a half mile farther up. Pure cultures were made and are being continued. *Leptomitus* and *Achlya oblongata* were found at the same place. It was found again near Hartsville, S. C., August 30, 1922. In the behavior of the sporangia and spores the species is exactly intermediate between *Achlya* and *Thraustotheca*, or rather it combines in this respect the characters of both. The eggs are, however, more like the latter genus, the eggs having several conspicuous lateral droplets up to a late stage as in *T. clavata* and differing thus from the eccentric-egged Achlyas. Our figures do not show extreme condition of the late stage, in which the protoplasm draws away from the droplet as much as in *A. Orion*. The antheridial branches also remind one of those of *T. clavata* and, as in it, are always diclinous. The distinctly brown oogonial wall is also different from any *Achlya* with eccentric eggs. In such ways this seems nearer *Thraustotheca* than *Achlya*, but for various reasons we prefer to put the species in *Achlya* at present. The strongest reason is that as our cultures have gone on the *Thraustotheca*-like sporangia have grown fewer in proportion.

The majority of the *Thraustotheca*-like sporangia have the peculiar habit of cutting off a considerable area at the tip which does not form spores but contains much less dense protoplasm which usually goes to pieces but may become condensed into a ball. Other parts of the sporangium, particularly the basal part, may also fail to form spores, the protoplasm remaining undivided or imperfectly segmented into large masses. Even when the spores are discharged at the tips larger masses may be mixed with the normal spores, as is not rare in other water molds. (See *Achlya imperfecta*.) The sporangial wall may begin its disorganization at any point, but this usually shows first on one side near the cen-

PLATE 49

PLATE 49

Achlya dubia

Fig. 1. A row of gemmae, most of which have already formed and discharged spores. × 167.

Fig. 2. Two threads both bearing sporangia, one with an oogonium, the other with antheridial branches. × 167.

Fig. 3. A sporangium beginning to disorganize on one side; below it a younger sporangium cut from the main thread and with spores emptied as in *Achlya*. × 167.

Fig. 4. A sporangium (with tip cut off as in most cases), the spores sprouting through the wall. Through these tubes the spores emerged as in *Dictyuchus*. × 167.

Fig. 5. A sporangium well advanced toward dissolution. × 167.

Fig. 6. A sporangium still further disorganized attached to a younger sporangium which has discharged spores as in *Achlya*. × 167.

Fig. 7. A sporangium with the walls all gone, the tip showing two undeveloped cells. × 167.

Fig. 8. A disorganizing sporangium with two discarded and disorganizing cells at both tip and base; below a segment is developing into a sporangium. × 167.

Fig. 9. A group of liberated spores, some with abnormal size and shape. × 167.

Fig. 10. A sporangium in which disorganization of the wall began near the tip. × 167.

Fig. 11. A sporangium discharged as in *Achlya*, with some of the spores escaped from their cysts. × 167.

Fig. 12. An oogonium with fully mature (rather old) eggs, two with many oil drops, one (the more common case) with only a single drop (antheridium too far disorganized to be shown). × 447.

Fig. 13. A young oogonium with a typical antheridial branch. × 167.

Fig. 14. A dictiosporangium with an empty sporangium below. × 167.

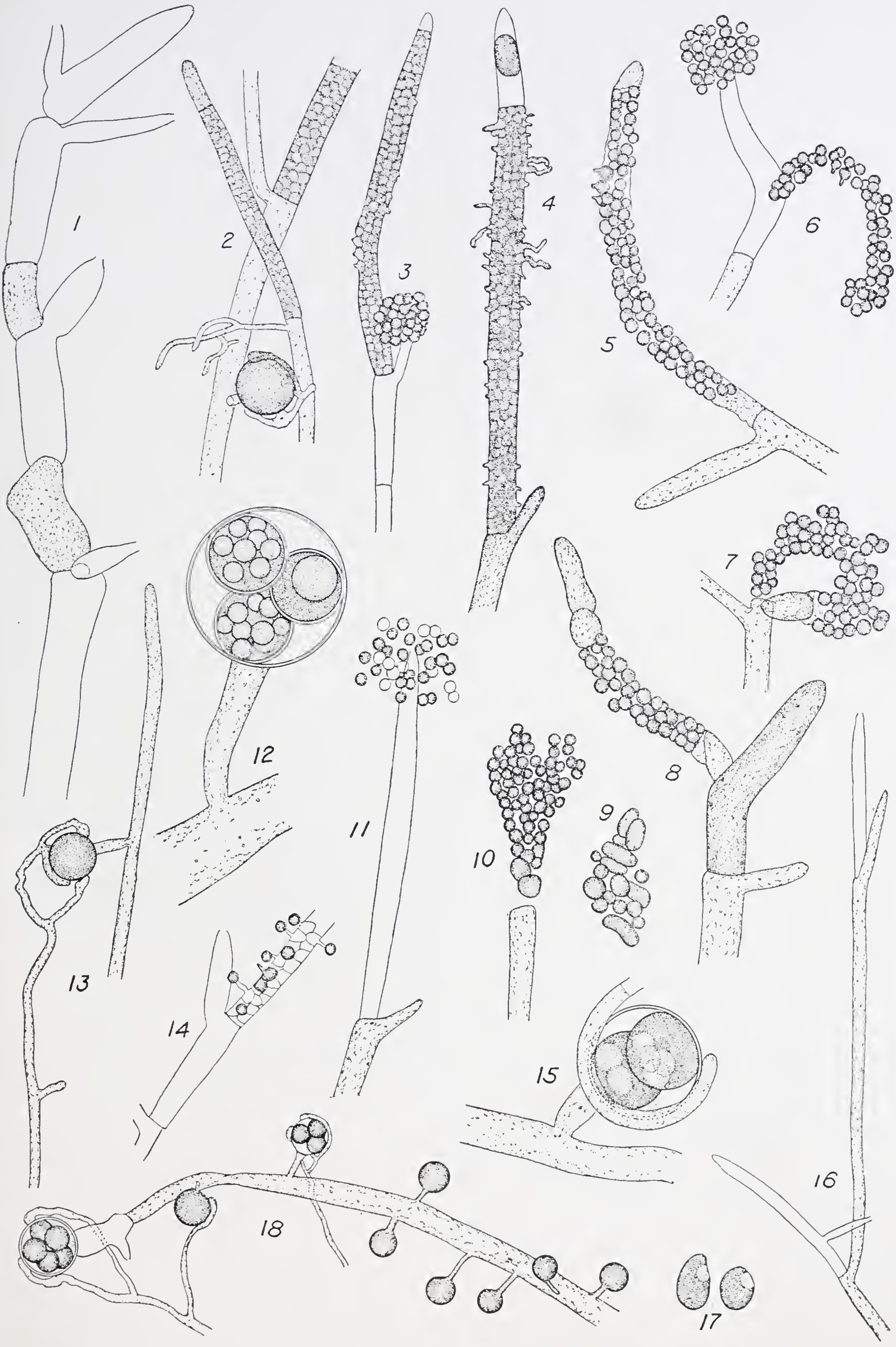
Fig. 15. An oogonium with maturing eggs, the antheridium still visible. × 447.

Fig. 16. Two *Achlya*-like sporangia, the lower emptying from one side. × 60.

Fig. 17. Spores in the swimming stage (cilia not shown). × 417.

Fig. 18. A hypha with racemosely arranged oogonia (typical) and a large terminal one. Most of the oogonia were halted in their development and did not mature. Antheridia are attached only to those which are maturing normally. × 108.

PLATE 49



ACHLYA DUBIA

ter. As the spores swell and become more separated and as the disorganization continues the curving may continue until a complete circle is formed (fig. 7). If the rupture starts near the tip the spores may spread out like a shaving brush (fig. 10). If the spores are not discharged the disorganization of the wall is not long deferred, but begins in a few hours, just as in *Thraustotheca*. In case dictiosporangia are formed the emptying tubes are often quite long, so long in fact that one often thinks the tubes are going to elongate into threads as in *Aplanes*, only to find that the spores emerge through them a little later (figs. 4 and 14).

22. **Achlya** sp.? Form without oogonia.

Plate 50

Weston ('17) found in Massachusetts a species of *Achlya* which remained sexually sterile during the entire period of culture on different media (over two years). During last August we also found a sterile *Achlya* (No. 1 of August 13, 1921) in trash and leaves collected from a branch in Strowd's low-ground pasture, which appears to be the same thing. (Compare also Tiesenhausen's sterile species mentioned on p. 148). We have cultivated this plant continually since (about 7 months, at time of writing) and in many different ways and on many media, but only sporangia and gemmae have appeared. The latter, while not very peculiar, are often in chains and are of a short, plump type, often spherical, a form that excludes many species with much elongated gemmae. The size and habit of growth of the plant and the form of the sporangia are about that of a typical *Achlya*, as *A. intermedia*. On a termite ant the threads grow about 0.5 cm. long and are about 30–70μ thick below to 20–30μ thick near the tips. The sporangia are plentiful and proliferate so as to form clusters of several. The gemmae are spherical, ovate or oblong, sometimes in chains of as many as six; their diameter runs up to as much as 110μ for the spherical ones (figs. 1–4). Results of experiments with chemicals will not be given in detail, but culture media used are given below to show the reluctance of the plant to form sexual organs:

12 cc. .05% solution of haemaglobin plus the following salts:

(1) 2 cc. 2% $Ca_3(PO_4)_2$
(2) 2 cc. 2% $Ca(NO_3)_2$
(3) 2 cc. 2% K_2SO_4
(4) 2 cc. 2% Na_2HPO_4
(5) 2 cc. 2% KH_2PO_4

25 cc. of a solution of levulose $\frac{M}{200}$ + leucine $\frac{M}{200}$. The solution being replaced by water in 60 hours. Five cultures in room temperature 21-26° C. Five cultures in ice box temperature 10–12° C.

25 cc. levulose $\frac{M}{200}$ + leucine $\frac{M}{200}$ plus the following salts:

(1) 10 drops 2% $Mg\ SO_4$
(2) 10 drops 2% KNO_3
(3) 10 drops 2% NaCl
(4) 10 drops 2% $Ca_3\ (PO_4)_2$
(5) 10 drops 2% $Ca\ (NO_3)_2$
(6) 10 drops 2% $Na_2\ HPO_4$
(7) 10 drops 2% $KH_2\ PO_4$
(8) 10 drops 2% $K_2\ SO_4$

Cochineal bugs were inoculated with the fungus and after ten days growth many of the threads had become green in color. These green threads were otherwise perfectly normal and in a healthy condition. The gemmae were almost uniformly red.

Experiments to determine the resistance of the gemmae to extreme temperatures:

(1) Resistance to cold. A culture with a number of resistant spores was placed on a block of ice in the ice box and allowed to remain there over night. The following morning the resistant spores were uninjured.

(2) Resistance to heat. The same culture used in No. 1 was transferred to a petri dish of hot agar which had just been melted in boiling water. The inoculated agar plate was left out in room temperature and after two days growth had taken place to a length of 1 cm.

EUROPEAN SPECIES NOT YET FOUND IN AMERICA

Achlya spinosa deBary. Beitr. z. Morph. u. Phys. der Pilze **4**: 54, pl. 4, figs. 13–18. 1881.

The following is translated from the fuller description of 1888 (p. 647).

"Main threads with many long interwoven side branches which produce a woolly, snow-white turf which, if well nourished, may reach a height of 2–3 cm. Reproductive organs sparingly produced. Zoosporangia small, and producing few spores; often lacking. Oogonia terminal, never intercalary [but see below], mostly barrel-shaped, densely set with numerous, broadly conic, pointed or blunt projections, only the upper and lower ends bare, the upper end with a conical point which may be extended into a long beak. Eggs 1–2, rarely 3, in an oogonium; of very various sizes, always about filling the oogonium; round or oval when ripe with a large central fat globule and a circular or interrupted row of peripheral globules [Körner]; without an obvious nuclear spot. Antheridia about as often absent as present; in the latter case always (?) only one on an oogonium; they are cylindrical-club-shaped and lay one side on the oogonium, their short stalks springing from just below the oogonial wall, rarely of diclinous origin.

"Found once in the Titisee [lake] in the Black Forest, June, 1880."

The above description does not agree with the original one in saying that the oogonia are never intercalary, as the figures show several that are. See remarks under *A. glomerata*.

PLATE 50

PLATE 50

Achlya sp.? Form without oogonia.

Figs. 1, 2, 3. Various forms of gemmae. Figs. 1, 3 × 108; 2 × 167.
Fig. 4. A chain of gemmae, the contents of which have formed spores. × 167.

Achlya apiculata var. prolifica

Fig. 5. Sprouting egg. × 100.

Isoachlya monilifera

Fig. 6. Sporangia showing internal proliferation. × 167.
Fig. 7. Internal proliferation as in *Saprolegnia* and cymose proliferation as *Achyla*. in × 167
Fig. 8. A chain of oogonia in a sporangium. × 167.
Fig. 9. Irregular oogonia in a chain. × 167.
Fig. 10. Moniliform sporangium. × 167.
Fig. 11. Sporangium renewed by cymose branching. × 167.
Fig. 12. Spore stained to show cilia. × 720.

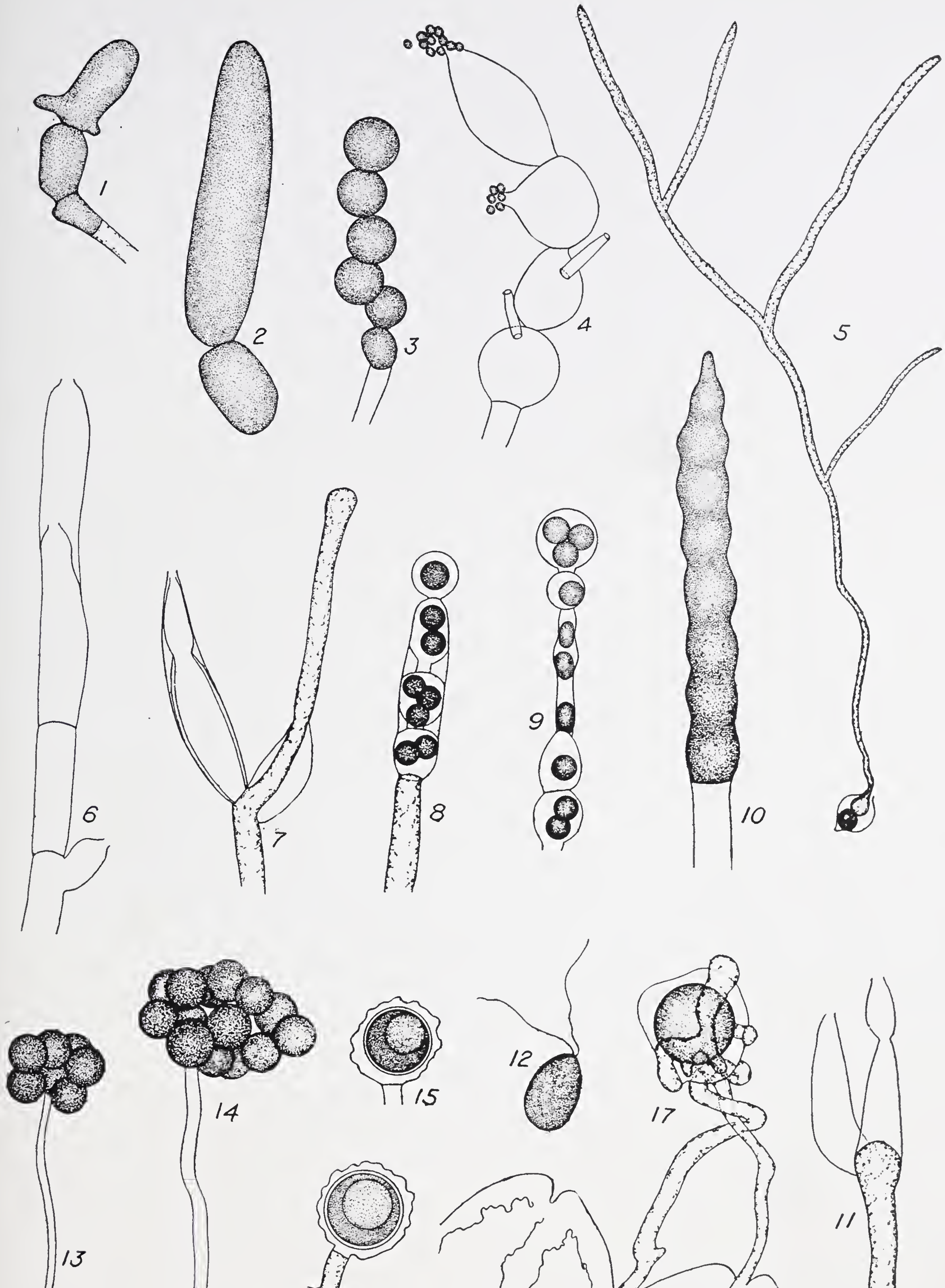
Aphanomyces scaber

Fig. 13, 14 Sporangia and encysted spores. × 720.
Fig. 15, 16. Oogonia with ripe eggs. × 720.

Aphanomyces laevis.

Fig. 17. The fungus shown growing parasitically on a chain of desmids. × 447.

PLATE 50



ACHLYA SP. (?). FIGS. 1–4.
ACHLYA APICULATA VAR. PROLIFICA. FIG. 5.
ISOACHLYA MONILIFERA. FIGS. 6–12.
APHANOMYCES SCABER. FIGS. 13–16.
APHANOMYCES LAEVIS. PARASITIC FORM. FIG 17

Achlya radiosa Maurizio. Mitt. d. Deutsch. Fischerei-Vereins **7**, Heft **1**: 57, figs. 18 and 19. 1899.

A. decorata Petersen. Bot. Tidsskr. **29**: 386. 1909. Also in Ann. Myc. **8**: 522, figs. 3a and 3e. 1910.

A. asterophora Minden. Krypt. Fl. Mark B. **5**: 549, fig. 2c on p. 520. 1912.

This species, described three times in rather recent years in Europe, has not been found in America. The prior description by Maurizio may be condensed as follows:

Hyphae dense, about 1–1.5 cm. long, 14.5–49μ thick, thickened in places. Sporangia typical of the genus, cylindrical, sympodially arranged, often bearing an oogonial branch below them. Zoospores as usual. Oogonia typically racemosely borne, or also on main or secondary hyphae; spherical, with thorny, pointed warts over whole surface; wall yellowish, diameter without spines 31.5–46μ, with them 40–54.5μ. Spines 7–12μ long, 9.5–12μ thick at base. Eggs one, rarely two, or more rarely three in somewhat elongated oogonia, filling the oogonium. Egg membrane clear yellow; contents thick, with numerous large and small fat-drops [does not say whether centric or eccentric]; diameter 29–39μ, mostly 34–36.5μ. Antheridia on short bent stalks from the oogonial stalk or the main hyphae; club-shaped, present on most of the oogonia. Antheridial tubes nearly always present. In some cases sporangia halt in development and after a while drop off, and when brought into nourishing media sprout to hyphae. Typical gemmae not present. The species is nearest *A. stellata* deB., which it resembles in the one-egged, spiny oogonia. In other ways it is quite different. Found on the eggs of American brook trout, in the fish hatchery at Munchhausen Reg. Bez. Cassel.

Not only the above description, but the figures also, leave no doubt of the identity of this species with those of Petersen and Minden. It is also quite possible that the insufficiently described *S. racemosa* var. *spinosa* Cornu is the same, but there seems no way to be sure. The species is distinguished from *A. colorata* by the oogonia containing but one egg as a rule and by the dense, sharp spines. Tiesenhausen ('12, p. 283) finds two slightly different forms of *A. radiosa* and gives a good figure of the ripe egg. He shows it (fig. 13) in section with oil drops most of the way around (subcentric). For a good account of *A. decorata* with figures and the results of numerous cultures and experiments, see Obel ('10, p. 421, figs. 1–4).

Achlya americana var. **cambrica** Trow. Ann. Bot. **13**: 135, pls. 8–10. 1899.

Trow's description we append below:

"Mycelium, as developed on house-flies, with main hyphae about 1 cm. long, a maximum diameter at the base of 92μ, and tapering gradually towards the apices, where they rather suddenly narrow to fairly sharp points.

"Sporangia terminal, cylindrical, scarcely thicker than the supporting hyphae, of a length generally varying from 250μ to 368μ and of a breadth varying from 39μ to 72μ (average of six measurements—length 315μ, breadth 56μ); sometimes very small, producing in extreme cases no more than three or four zoospores; generally developed in the typical cymose order, *the main axis, however, frequently septate behind the oldest sporangium, each segment thus formed, of which there may be as many as ten, developing a short branch, the segment and branch together constituting a sporangium.*

"Spores very numerous, averaging 13μ in diameter, generally encysting at the mouth of the sporangium, occasionally, however, inside it.

"Oogonia, on short unbranched stalks, which are about as long as the diameter of the oogonium, and of an average breadth of 10μ; at first developed regularly in racemose order on the main hyphae; generally terminal and spherical, but not infrequently intercalary and barrel-shaped; of a diameter 31μ to 85μ (average of twelve measurements 60μ), with a thick, pitted, *yellowish* smooth wall; *frequently, however, provided with blunt spines, which may exceptionally even reach the length of 25μ, and are due to outgrowths through the pits.*

"Antheridia, always present under natural conditions, *few in number*, produced on branched antheridial filaments of a diameter of 6.5μ, which arise from the main hyphae side by side with the oogonial branches, or, as observed in a very few cases only, from the stalk of the oogonium; of very variable shape, but generally long and curved, and closely applied throughout to the surface of the oogonium, or opposite the pits only by means of outgrowths from the under surface; sometimes septate; of a maximum length of 65μ and maximum breadth of 7μ; emitting from points in contact with the pits one or more branched or unbranched fertilization-tubes of a diameter of 4μ.

"Oospores, one to twenty or more, mostly from three to eight (average of twelve cases six); spherical, with a *smooth, very thick two-layered wall;* eccentric, of a diameter of 23μ to 26μ, and having an oil-globule of a diameter of 15μ; germinating at once, and producing a long, thin, branched hypha, or one or more small sporangia, or passing into a resting condition and remaining capable of germination for at least four months."

A little further on (p. 137) Trow says:

"The specific type as described and figured by Humphrey has obviously rather indistinct pits; the form I am familiar with has pits almost, if not quite, as well-defined as those of the *Ferax* group of species of *Saprolegnia.* The American plants, too, apparently differ from those which I have examined in the greater number of antheridial branches and antheridia supplied to each oogonium, the color of the wall of the oogonium, the structure of the oospore wall, and the size of the oospores. Other noteworthy points of difference have been indicated by italics in the description of the variety."

Trow found that in his plant the antheridia were suppressed when bits of mycelium with young oogonia were cut off and put in a moist chamber.

It was on this variety that Trow did a good piece of cytological work and came to the well-founded conclusion that fertilization takes place. As to cytology and fertilization of the egg his conclusions may be briefly summarized as follows: The vegetative nucleus has a membrane and a central spongy body containing chromatin and nucleolar matter from which linin threads extend to the membrane; the nuclei divide in the hyphae and large numbers enter the sporangia and the oogonia; in the former no divisions take place, but in the oogonia many at least of the nuclei divide mytotically, with the number of chromosomes probably four [?]; after this division there are about ten times as many nuclei as there will be eggs and all now degenerate except the number necessary to supply each egg with a single nucleus, which becomes the egg nucleus. The antheridia are multinucleate and their nuclei "undergo exactly the same changes as those in the adjacent oogonia." In fertilization a tube containing one male nucleus touches the naked egg and discharges into it a nucleus and some protoplasm. The egg and sperm nuclei then to all appearances fuse, though the process was not followed through all stages. The ripe egg contains a single nucleus, and may germinate at once or after as long a rest as four months; the nucleus divides mytotically to produce about twenty nuclei, showing about eight chromosomes. The egg now sprouts to form sporangia or hyphae.

Achlya deBaryana Humphrey. Proc. Am. Phil. Soc. **17**: 117. 1892 [1893].

Achlya polyandra deBary. Beit. z. Morph. u. Phys. d. Pilze **4**: 49, pl. 4, figs. 5–12. 1881.

Not *Achlya polyandra* Hildb. (See Humphrey, '92, p. 118.)

This has not been recognized in America, and we take the following from deBary's condensed statement of 1888 (p. 364):

"Main hyphae stout, usually ending in primary sporangia under which the secondary ones appear in sympodial arrangement. Oogonia short-stalked, racemosely arranged, seldom intercalary and often terminal on slender hyphae; wall stout, here and there with somewhat thinner places but not pitted, occasionally with a few wart-like projections. Antheridial branches almost always androgynous, much contorted and branched, arising from the same principal axes which bear the oogonia, but never from the oogonial stalks. Antheridia on the branched tips of the antheridial branches attached by their sides to the oogonia and sending into them one or two fertilizing tubes each. Eggs varying in number, but mostly numerous, eccentric."

Fischer uses the form *A. polyandra* (Hildb.) deB., and treats it in the sense of deBary, remarking that Hildebrand's original form is an approach to *A. gracilipes* deB. and stands between it and *A. polyandra* deB. From Fischer's description we take the following additional data:

Main threads 100–150μ thick; sporangia, *e.g.*, 45 × 280μ, often very large; oogonial stalks 1–3 times as long as the diameter of the oogonia, 8–14μ thick; oogonia 45–65μ thick; antheridial branches 8–14μ thick, 1–4 for each oogonium; eggs 3–10 or more, rarely only 1 or 2, diameter 18–25μ, certainly eccentric, germinating into mycelium or a sporangium, resting period 21–37 days. Von Minden ('12) accepts Humphrey's name, and seems to take his description from Fischer. He describes the var. *intermedia* (which we find and are treating as a species).

DeBary does not give the size of the eggs, but his figures (l. c., '81) show the eggs distinctly larger than those of *A. prolifera* on the same plate, at the same magnification.

The species is apparently nearest *A. americana*. For a comparison with this and others of the same group see under the genus and under *A. flagellata*. For other illustrations see Ward ('83), pl. 22, figs. 1–14; Zopf ('90), fig. 45; Minden ('12), fig. 2e on p. 520; Horn ('04), figs. 1–21.

Trow has fully proved sexuality in this species ('04). The phenomena are about the same as shown in his earlier paper on *Saprolegnia diclina* and *S. mixta* except that he claims (probably incorrectly) that after the first division of nuclei in the oogonium a second occurs in at least some of the daughter nuclei in which the chromosome number is reduced, apparently, from eight to four. All nuclei now degenerate except one for each egg. The egg nucleus shows a centrosome with astral rays and so does the sperm nucleus soon after it enters the egg. These bodies are not to be seen after complete fusion of the nuclei. One division of at least some of the nuclei in the antheridium occurs, followed by a slow degeneration of some of them.

Mücke has since studied oogenesis in this species ('08) and fully confirmed the occurrence of fertilization, but denies that there is a second division in the oogonium or a reduction of chromosomes before fertilization. Work by Claussen and Davis on *Saprolegnia* and by Kasakanowsky on *Aphanomyces* shows only a single nuclear division in the oogonium and leads us to believe that Trow was wrong in this respect, though clearly vindicated in his hard-fought contention for sexuality in the *Saprolegniaceae*. Mücke concludes that there are more than eight chromosomes in the nuclei of the oogonia, the number being undetermined. He also claims that the centrosome is inside the nuclear membrane and not outside it, as Trow thought, and that the centrosome is not comparable, as Davis claims, to the coenocentrum of the *Peronosporeae.*

Achlya prolifera (Nees) deBary. Bot. Zeit. **10**: 473, pl. 7, figs. 1–28. 1852.

As this plant has not been found in America, at least in its typical form,* we include it here, and make the following translation of deBary's later description ('88, p. 633):

"Main threads stout, usually ending with primary sporangia, under which the secondary are formed sympodially. Oogonia racemosely arranged on short side branches of the main hyphae, as a rule terminal, round, the wall with numerous, very sharply defined and obvious pits. Eggs variable in number, mostly numerous, eccentric. Antheridial branches diclinous, much twisted and branched, winding like a parasite about the oogonia and the threads which bear them; the oogonial walls thickly enwrapped and often completely covered by these branches which bear numerous, at times intercalary, antheridia, which lay their sides against the oogonium and send out fertilizing tubes.

"The most abundant of all *Achlya* species occurring everywhere."

For a more extensive account with numerous good figures see deBary's first treatment ('81), pl. 2, figs. 1–2; pl. 4, figs. 1–4. In this he shows oogonia with eggs varying from 1 to 16 or 20, but mostly 2–3. Also see Unger ('43), pl. 4; Maurizio ('95a), figs. 4–5; Minden('12), figs. 2b, 2d on p. 520.

For remarks on this species see under *A. proliferoides* and *A. imperfecta.* According to Maurizio ('95a), *A. prolifera* is often parasitic on fish. According to Cienkowski ('55), the eggs may sprout directly into zoospores.

Achlya aplanes Maurizio. Flora 79: 135, pl. 4–5, figs. 28–31. 1894.

The following is adapted from the original:

The sporangia frequently retain the spores and in such cases they sprout to threads. No net sporangia seen. If the spores emerge they sprout at the mouth; and in *no case do they have a swimming stage.* Fig. 29, plate 5, shows the spores sprouting at the tip of the sporangia after discharge. Segments of hyphae often cut off below sporangia to form others. Oogonia racemose (as in *A. prolifera*) on short stalks or also at times terminal, intercalary or lateral just under a sporangium; spherical (42–58.5μ) or the intercalary and end ones mostly egg- to flask-shaped (56 × 60μ); wall smooth, fairly thick, clear yellowish, usually with 2 or 3 pits, or without any pits. A young oogonium often pro-

* Under the name *A. prolifera*, Pieters publishes observations on an *Achlya* from Ann Arbor in the Am. Jour. of Bot. (**2**: 529. 1915; also recorded in Ann. Rep. Mich. Acad. Sci. **8**: 27. 1905), but as his unpublished drawings, kindly submitted to us, show a plant that could better be referred to *A. imperfecta*, we have noted it under that species.

liferates to another one and empties itself into it [as is common in *A. proliferoides*]. Eggs 1 to 12, mostly 4 to 8, diameter 24–31.5µ, eccentric; germination not observed. Antheridia diclinous always, wrapping about the oogonia. They generally proceed from more slender branches that run among the oogonial branches. Fertilizing tubes were occasionally observed entering the oogonia.

Minden thinks this species should be united with *A. prolifera* if the absence of a swimming stage should prove not constant. The eggs, however, as described are too large for that species, and approach those of *A. flagellata*.

Achlya oligacantha deBary. Bot. Zeit. **46**: 647, pl. 10, fig. 1. 1888.

This species has been reported only by deBary, and the following is translated from the original:

"Main threads slender and delicate. Oogonia on short or long branches of hyphae which bear sporangia or in part terminal on slender main threads and their racemose branches, spherical in shape, without an internal upgrowth from the insertion wall of the somewhat enlarged end of the stalk, surface always with relatively large, smooth, papillae, which are separated from each other and which vary greatly in number (1 to about 16, very seldom none); also variable in size and form (short points to large, blunt projections); wall of oogonia relatively thin, colorless, without pits, except that the projections are mostly thinner than the wall between them. Eggs mostly 4–8 in an oogonium (seldom up to 12 or more), round, centric, relatively small. Antheridia always present, mostly several on each oogonium, relatively small, irregularly spherical to cylindrical with the side against the oogonium; borne one or two in a row, on the ends of partly androgynous, partly diclinous antheridial branches.

"Brought by Zacharias from a puddle at Kork (Baden) with *S. Thureti* in June, 1881, and cultivated pure until 1883."

The species seems certainly in the *Apiculata* group, but the structure of its eggs is not quite cleared up by the word "centric."

Achlya recurva Cornu. Ann. Sci. Nat. Bot., ser. 5, **15**: 22. 1872.

Since its first publication by Cornu this species has been reported only by Hartog, who mentions it without description ('88, p. 212), and by Minden, who refers to it a plant found by him and described as follows ('12, p. 543):

"Growth extending about 1 cm. from the substratum, with strong main threads about 90µ thick at base. Sporangia long, cylindric or slightly spindleform; secondary ones few. Oogonia numerous, borne terminally on certain little-branched, often slender main threads or on more or less elongated, at times very long and branched side branches which are always very slender and bent like a bow, and which spring from the sporangia-bearing main hyphae; oogonia spherical, rarely elongated by an extension of the tip, and covered with many crowded, blunt, hollow projections; diameter of oogonia 50–90µ with the spines, the latter 7–11µ long. Antheridia cylindrical to clavate, small on slender branches, which are little or not at all branched and also not looped, but mostly only bent like a bow, and which are mostly only one to three to an oogonium, and are borne in part from the stalk of the oogonium or its main thread or in part from other threads. Eggs spherical, 1–25, mostly about 10, filling the oogonium, 22–27µ thick.

"Found in swamp water at Hamburg and Frankreich, and cultivated on ant eggs. This seems to be Cornu's inadequately described and therefore doubtful species. It holds a middle place between *A. polyandra* and *A. oligacantha*. It is like the former in the bent (though not so much bent) stalk of the oogonium, and like the latter in the papillate oogonial wall. The outgrowths on the oogonia are here much more numerous than in that species, mostly of about equal length, but rarely one may be more developed than the others."

The following incomplete diagnosis is all that is given by Cornu:

"This *Achlya* is distinguished from others by its spiny oogonia, not pitted, borne on a branch bent in an arch, toward the end of which it arises singly and in general laterally; the lateral branches arising either from the branch or the axis. The number of eggs is usually from six to eight. Sometimes the oogonium bears a cylindrical portion, as in *Pythium*, at its upper end."

Achlya Hoferi Harz. Allg. Fischerei. Zeitung. **31**: 365. 1906.

The following is abstracted from the original (compare with *A. Nowickii* in doubtful species, p. 147):

"Mycelium luxuriant, penetrating *deeply into the skin* and destroying the tissue, developing on outside numerous zoosporangia and oogonia. Hyphae of very various thickness, 45–60µ in the thickest part, reaching only 1µ in the finest tips. Threads that bear the oogonia and the zoosporangia 15–18µ thick. Sporangia very various in size, 30–100–600µ long and 5–20µ broad. Zoospores occasionally one-rowed in the sporangia. Usually many-rowed.

"Oogonia oval or occasionally spherical, 75–180µ long, 45–60µ thick, with numerous, hollow, thorn-like projections which are 6µ broad, 6–11µ high, their membrane of the same thickness as that of other parts of the oogonium. Eggs spherical, 20–30µ in diameter, the number varying from several (rarely only one) to over 30; generally about 20. They are colorless, with a hard coat, and very full of fat before ripening, as are also the mycelium and stalks; after complete maturity this fat almost completely disappears.

"From the various other *Saprolegniaceae* that grow luxuriantly on live fish, as, for example, *Achlya polyandra*, *A. Hoferi* is distinguished by its deep penetration and destruction of the skin tissues [Hautgewebe]. The fungus is always accompanied by bacteria, which are probably symbiotic with it and help destroy the tissue. However, it must not be overlooked that the same bacteria and other *Saprolegniaceae* may occur on the same fish near *A. Hoferi* and not cause destruction of tissue. *Achlya Hoferi* probably secretes an enzyme which attacks the tissue and permits the penetration of the fungus threads and bacteria.

"The assistant at the station, Dr. Plehn, reported to the author as follows on the effects on the tissue caused by the disease:

'After the fungus gets a foothold on the surface it soon begins to penetrate deeper, but proceeds only slowly. Only after weeks is the epidermis [Oberhaut] penetrated. In an infected carp that had been observed for months the fungus had penetrated the entire dermis [Unterhaut], occupying, however, only the fatty connective tissue and stopping at the muscles. In the deeper tissues also indeed in the dermis, the fungus is not dense, and one sees in very old infections only isolated threads, which mostly follow the cavities of the tissue and run between the harder layers of the dermis. Strangely there is an absence of inflammation in the neighborhood of the fungus. The greater and more serious disorganization that sometimes occurs in this infection, when even the muscles are involved, is due to other causes—the myriads of bacteria that are present.'

"*Achlya Hoferi* is near *A. oligacantha* deBary. It is distinguished from the latter by the entire absence of antheridia, the more elongated and larger oogonia, the mostly larger and more numerous spores [meaning oospores probably].

"Occurs so far only on Bohemian mirror carp in February of this year. The inoculation was successful in the case of three carp at the Biological Station, Munich."

One good text figure on page 366 shows oogonia and blunt tipped sporangia much larger than the hyphae. The spines as shown are not sharp. A very peculiar species.

EXCLUDED OR DOUBTFUL SPECIES NOT MENTIONED IN THE TEXT

Achlya leucosperma Cornu. Ann. Soc. Nat., Series 5, **15**: 24. 1872.

This has not been sufficiently described to make its position certain. It was distinguished from other species, especially *A. prolifera*, by having only two pits in the oogonium wall, white (not brown) eggs, and cylindrical antheridia which stand in a row on the ends of the antheridial branches. These characters seem quite worthless and it is surprising that Cornu should have so defined a species. It may be *A. prolifera*. All eggs of water molds look milk-white when seen under reflected light.

Achlya contorta Cornu. Ann. Sci. Nat., Series 5, **15**: 25, pl. 1, figs. 10–15. 1872.

Like the above this is so meagerly described as to be indefinable. Oogonia smooth, borne on long, spirally twisted branches with odd-looking local outgrowths; eggs averaging 8 in an oogonium; antheridia cylindrical, branched [compare *A. proliferoides*].

Achlya dioica Pringsheim. Jahrb. f. wiss. Bot. **2**: 211, pl. 23, figs. 1–5. 1860.

This is a name given to a lot of hyphae of an *Achlya* without oogonia, and attacked by *Woronina polycystis*. It has no validity.

Achlya penetrans Duncan. Proc. Roy. Soc. London **25**: 238. 1876.

This is probably a Siphonaceous alga, as Humphrey thinks.

Achlya oidifera Horn. Ann. Myc. **2**: 231, fig. 20. 1904.

The following is adapted from the original:

Growth good on flies, mealworms, etc., and in pea extract, hyphae about 45µ thick in the latter medium; sporangia and zoospores produced in abundance, but oogonia nearly always absent—not formed in numerous cultures on various media, such as agar-agar, haemoglobin, leucin, and numerous other media that easily produce oogonia in *Achlya polyandra* and *Saprolegnia mixta*; oogonia formed only once and then in the inside of an ant pupa. They were spherical, varied in size, and had numerous pits. Eggs 2-8 in an oogonium, and with fat-droplets on one side. Antheridia not observed with certainty as the oogonia were too old when found. The egg-like gemmae [oidienartiger Zerfall] produced in chains (hence the name), and forming spores when brought into pure water; capable of growth after a long rest (over a month) as in other species.

The author suggests that on account of the formation of eggs inside the substratum this species may represent a transition towards the *Peronosporaceae*, and that only in certain peculiar conditions not yet understood will eggs be produced.

From the above it will be seen that this species is insufficiently described and in need of further study. It must be considered doubtful, as Minden remarks.

Achlya ocellata Tiesenhausen. Arch. f. Hydrobiologie und Planktonkunde **7**: 287, fig. 14. 1912.

"Turf stiff, not thick, 3 mm. broad, main threads up to 175µ thick at base. Sporangia as usual. Oogonia racemosely borne on the more or less thickened hyphae. Stalks rarely up to 62µ long, mostly shorter than the diameter of the oogonia, at times absent with oogonia sessile or intercalary; oogonia 40–118µ thick, spherical, rarely pyriform, wall smooth with evident pits of medium size. Eggs 17.5–25µ thick, with a small brightly refractive oil drop that is very characteristic, 5–20 or more in an oogonium, or rarely one. Antheridia on branches 5–8µ thick, which are androgynous or diclinous from nearby threads."

Found near Campfer, Oberengadin, Switzerland. Another form found near the same place had very short oogonial stalks, otherwise like the first.

The author states that the species is very near *A. americana* but differs in the sometimes diclinous antheridia and in the peculiar shining droplet in the eggs which is very different in appearance from the eccentric egg of *A. americana*. As the author does not mention any other member of the *Prolifera* group and gives no evidence of having seen any other of them it seems more than likely that he makes all this ado over the typical eccentric egg of the group. From his figure and description it is probable that his plant is *A. americana*.

Achlya Nowickii Raciborski. Sitzungsber. d. Krokaner Akad. d. wiss. **14**: 149–168, pl. 3. 1886.

We have not seen the original, which is in Polish, and take the following from Minden in Krypt. Fl. Mark B. **5**: 553. 1912.

"Sporangia in plain cymose arrangement, spindle-shaped, relatively broad, 36–80μ long. Oogonia 75–100μ long, 45–110μ broad, numerous, ellipsoidal in outline, but set with large, irregular, scattered, hollow projections 5–18μ long. Eggs spherical, 3.5–7μ thick [probably misprint]. Antheridia absent. With *S. monoica* on a sink carp."

Minden says that he has not seen the Polish paper, but thanks Professor Lindau for the information, and adds: "In the form of the oogonia apparently near *A. cornuta* Archer and *A. Hoferi* Harz." Under the last species he says that it may be identical with *A. Nowickii.*

Achlya sp.?

Tiesenhausen ('12, p. 288, no figures) describes a sterile form that he does not try to place. It is notable, he says, for the great variety of forms assumed by the hyphae.

THRAUSTOTHECA Humphrey, 1892 [1893], p. 131.

Main threads stout, branching; sporangia terminal, stout, typically short-clavate, liberating the spores by cracking and disintegrating; spores encysting before escaping and remaining clustered where liberated, after a rest escaping and swimming with two lateral cilia, again coming to rest and encysting before sprouting. Oogonia single, commonly terminal on short lateral stalks, spherical, smooth, weakly pitted or without pits; eggs rather few, eccentric. Antheridia diclinous, occurring on all oogonia. Fertilization probably occurs but has not been demonstrated. There is but one species (but see *Achlya dubia*).

Thraustotheca clavata (deBary) Humphrey. Trans. Am. Phil. Soc. 17: 131. 1892 [1893].

Dictyuchus clavatus deBary. Bot. Zeitung **46**: 649, pl. 9, fig. 3. 1888.*

Plate 51

Main hyphae stout, straight, reaching a length of 2 cm. in strong cultures and a thickness of 20–120μ, averaging about 37μ; profusely branching into secondary hyphae near their tips; secondary hyphae much curved and twisted, and often curiously knobbed and gnarled, as shown in fig. 1. Sporangia 37–85 × 66–370μ, terminal or rarely intercalary, proliferating as in *Achlya*, usually short, broad, and clavate, but often elongated somewhat as in *Pythiopsis* or even as in *Saprolegnia*, varying from nearly spherical to fusiform, differing from the sporangia of any other of the *Saprolegniaceae*. Spores about 12.5μ thick, encysting within the sporangium immediately after they are formed, and liberated passively and slowly by the gradual cracking and disintegration of the sporangium wall, which is probably due to internal pressure. They now emerge from their cysts and swim actively in a laterally biciliate form, encyst again and sprout. In the sporangium they are polyhedral in shape, through pressure, each having a hyaline membrane of its own.

*The species was really first published incidentally by Büsgen in 1882, p. 261, pl. 12, figs. 1–8, who in his study of the development of the sporangia described it sufficiently under the name of *Dictyuchus clavatus* deBary sp. nov.

PLATE 51

PLATE 51

(From Mycologia **4:** Pl. 63. 1912.)

THRAUSTOTHECA CLAVATA

Fig. 1. Tip of a main hypha showing the gnarled condition of the secondary hyphae. × 155.

Fig. 2. Main hypha showing sporangia and method of growth. × 155.

Fig. 3. Spores encysted within the thin-walled sporangium. × 700.

Fig. 4. Spores falling apart, the basal ring remaining. × 700.

Fig. 5. An unusually large basal cup with a few spores still remaining in it. × 700.

Fig. 6. Oogonium containing fully ripe eggs. Empty antheridia attached to the wall of the oogonium. × 700.

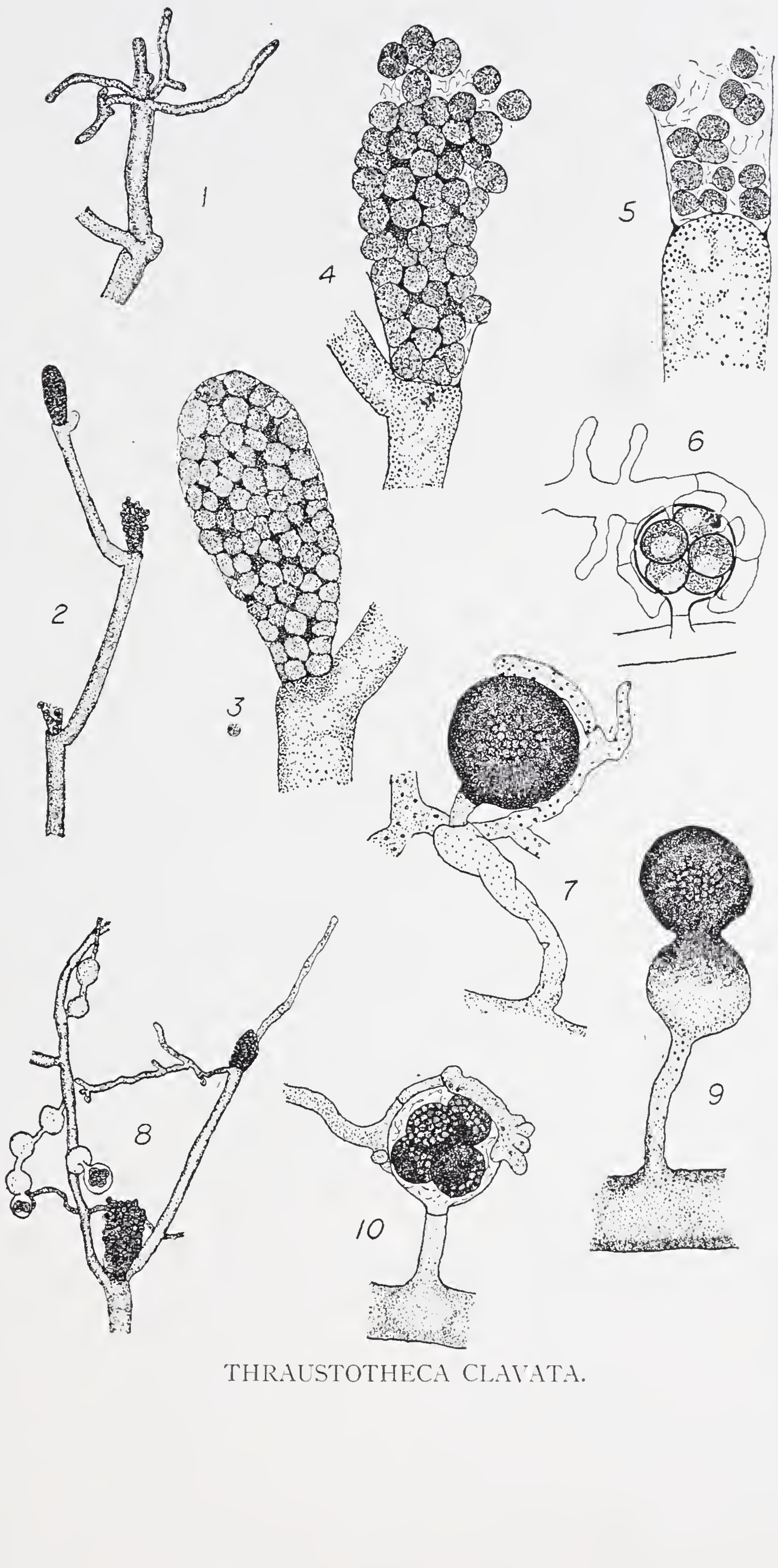
Fig. 7. Young oogonium with antheridium full of protoplasm. × 700.

Fig. 8. Showing double branching below the sporangia; antheridial branches; and proliferating oogonia. × 700.

Fig. 9. A proliferating oogonium. × 700.

Fig. 10. Oogonium with maturing eggs. × 700.

PLATE 51



THRAUSTOTHECA CLAVATA.

Occasionally among the ordinary spores large irregular spore masses are liberated. These masses slowly round up somewhat and encyst, sprouting later without a swimming stage. Gemmae small, pyriform or rarely spherical, falling into spores in suitable environment. Oogonia borne singly on short, straight, perpendicular stalks from the secondary hyphae, rarely from the primaries; 30–70μ thick, spherical, smooth, and very slightly pitted, the pits appearing only after staining with chlor-zinciodide. Eggs 1–10 or rarely 11, usually 4–6 or 8, eccentric, with a single large peripheral oil globule; size very constant, the diameter about 18–22μ. Antheridial branches diclinous, arising from the secondary hyphae, very crooked, and quite stout; antheridia club-shaped, cut off by a wall; antheridial tubes obvious.

Rare. Found in such places as spring below Cobb Terrace, Arboretum spring and branch, and in marsh on south edge of Glen Burnie meadow. Known in America from Chapel Hill, N. C., and Great Barrington, Mass. For other illustrations see Büsgen ('82), figs. 1–8; Minden ('12), fig. 7 on p. 556; and Weston ('18), pls. 4 and 5, and two text figures.

The actual fertilization of the egg has not been seen, but we have observed the antheridia to become empty during the ripening of the eggs. In no case was it found that an antheridium became attached to an oogonium arising from the same hypha as itself, but occasionally an antheridial branch may arise from the stalk of one oogonium and extend to another from a different hypha, thus proving the plant not to be dioecious. When the eggs first round up they show many oil globules situated on one side (fig. 10). These globules are at first only about 2μ in diameter, but they gradually fuse until there are only two or three larger ones from 8μ to 15μ in diameter. Finally these globules fuse into a single one, which is about 16μ in diameter, and situated at the periphery of the egg. The eggs are then ripe. In contaminated cultures an oogonium will often sprout a new one, the old being emptied into the new (fig. 9). This process may be repeated several times and the eggs be formed finally in the terminal oogonium (fig. 8). Occasionally two oogonia are produced upon one branch.

After the encysting of the spores, the sporangial wall, which has always been thin, breaks and begins to disappear, vanishing first as a rule on one side near the end, and continuing to disintegrate until nothing is left of it except a narrow circular ring at the base. This basal ring may be quite conspicuous or almost entirely absent. Except in *A. dubia* this method of dehiscence is entirely unique in water molds and is superficially like that of the mold *Mucor* and its relatives. This resemblance was remarked on at the time the plant was described, and Solms-Laubach thought he saw another point of agreement between *Mucor* and our plant in the upward bulging of the basal partition. The bulging is, however, imaginary, and if present at all is no more pronounced than in other water molds.

The species has been reported only a few times (by Büsgen, by Minden, by Weston, and by us) since its first discovery. DeBary got his material from a collection taken in 1880 by Stahl from a fresh-water lake at Vendenheim near Strassbourg, Germany, and kept it growing in his laboratory for four years.

On account of the unparalleled method of spore liberation it was suggested by Solms-Laubach, who after deBary's death arranged and edited his last paper, that this species might be considered as generically distinct from the other species of *Dictyuchus*. This was again remarked on by Fischer ('92, p. 365), and the next year Humphrey was sufficiently impressed with its distinction to give it generic rank.

For our first account of this species, from which this is taken in great part, and from which the plate is copied, see Mycologia **4**: 87, pl. 63. 1912. The most detailed study yet given to the plant is by Weston ('18). He thinks that the sporangium wall breaks from the swelling of the spores and that its fragility has been greatly over-emphasized. Weston also observed sprouting of the eggs, which may form either sporangia or an extensive mycelium, depending on the amount of nutriment available.

All the following cultures were made from No. 10 of January 30, 1913:

In equal parts of maltose 5% and peptone .01%. Extensive growth. A few sporangia formed. Spores sprouting in position. A large number of oogonia initials but none maturing eggs. Most were more or less inflated. Some sent out sprouting tubes, others went to pieces inside. Culture repeated with similar results.

On corn meal agar. Growth luxuriant. Many sporangia formed, often in rows like gemmae, but all forming spores and falling to pieces. Culture duplicated with same results.

On yolk of egg in distilled water. Strong vigorous growth. Great number of good sporangia. A good many oogonia, only a few of which matured their eggs.

On white of egg in distilled water. Vigorous growth. Only sporangia formed and they not so abundant. Some of them seemed to dissolve and become surrounded by bacteria.

In addition to these many cultures were made, of course, on insects, etc., with normal results.

A pure culture on corn meal agar was put in a vial on March 15, 1913, and was found to be dead December 1, 1913.

A pure culture was put in an aquarium jar with algae in the laboratory in March, 1913. When tested for life September 18, 1917, no growth appeared.

DICTYUCHUS Leitgeb, 1868, p. 503.

Vegetative structure and appearance as in *Achlya*, but of much more tardy development in cultures (at least in *D. sterile*); tips of hyphae rather blunt. Primary sporangia nearly cylindrical, blunt, borne typically in a zigzag sympodium with long internodes; later they are formed by the segmentation of the hyphae into long joints and in such case, after the spores are formed, rest like gemmae for a change of media before liberating the spores, and show a strong tendency to fall away from each other and from the hyphae and to lie free in the water. Spores not escaping from the sporangium as in other genera (except *Aplanes*), but remaining in the sporangium and forming there a network of walls from which they emerge, after a rest, by individual openings to the outside, where they swim by two cilia and in the form of the second swimming stage in *Saprolegnia*. They then, as a rule, sprout as usual in the family but Weston ('19) has shown that some of them may again emerge and swim before sprouting. Gemmae not represented unless the resting sporangia with spores in them be considered such. Oogonia spherical, smooth, the wall unpitted, terminal on slender branches (absent (?) in *D. sterile*). Antheridia much as in *Saprolegnia* and *Achlya*, diclinous or androgynous; fertilizing tubes observed. Eggs one to many, not filling the oogonium, at maturity containing one or a few large oil drops inside the protoplasm.

Key to the Species.*

Oogonia unknown (plant never fruiting); sporangia apt to drop off in a resting state *D. sterile* (1)

Oogonia with one egg

  Antheridia and antheridial branches not surrounding the oogonia ......*D. Magnusii* (2)

  Antheridia with the antheridial branches often thickly encircling the oogonia

    Hyphae here and there branched in a normal way; eggs centric *D. monosporus* (p. 156)

    Hyphae with irregularly arranged, mostly short, papillose outgrowths with an abnormal appearance; eggs eccentric............................ *D. carpophorus* (p. 157)

Oogonia with many eggs.................................... *D. polysporus* (p. 157)

1. Dictyuchus sterile n. sp.

Plate 52

Vegetative growth moderately stout. Main hyphae branching, up to 55μ thick, mostly 30–45μ at base, very gradually tapering towards end, the larger up to 22–37μ near tip, many much smaller. Primary sporangia borne on the tips of hyphae, later ones formed by cymose branching, but usually separated from the earlier ones by some distance by the elongation of the threads. As the culture ages the arrangement becomes more irregular and complicated and most of the threads become segmented towards the periphery into numerous sporangia in rows or

*Taken from Minden, except for the addition of the Chapel Hill plant. The American species are followed by a number, the others by a page reference.

branched groups. They are usually a little larger in the distal half, often bent, sometimes branched, of various size, in old cultures often very long, not rarely thread-like with only a single row of spores. They usually break off from the hyphae about the time the outline of the spores becomes distinct and go into a resting state which may last a few days or many weeks depending on conditions. During this time the spores are separated by walls which in this condition are scarcely visible, the individuality of the spores being indicated by the usually conspicuous vacuole that each contains. On emerging the spores escape singly and swim as normal in the genus or they often sprout in position into slender hyphae. Spores 11.8–16.6μ in diameter before sprouting, with large conspicuous vacuole. Oogonia not developed.

Plentiful in springs and small streams, such as Arboretum spring and brook, Battle's branch, branch back of athletic field, etc. Collected 63 times from February, 1912, to December, 1913 (see table on p. 14); also found many times since. The plant has a way of appearing tardily and often turns up in old cultures. In our collection notes it often may have missed being listed, because the culture was not kept long enough.

The resting stage is not of fixed length, but may be brought to an end at any time by a change of environment, such as by putting a cover glass over a resting sporangium or by a change of water. If the original culture remains undisturbed the sporangia may continue in the resting condition practically indefinitely, that is, for several months. The falling off of the sporangia begins while the plant is still in active growth, and as age approaches the protoplasm may practically all be used up in the formation of such sporangia (see also Weston, '19, p. 290). This deciduous habit is found also in *D. monosporus*, and is mentioned both by Leitgeb ('69, p. 366) and by Minden ('12, p. 566). According to the former this habit appeared only after cultivating the fungus for two months, but Minden finds it occurring regularly in the same species and does not consider it a result of degeneration. This species has been watched by us for twelve years and though grown on a great variety of media it has never been observed to form oogonia, antheridia, or gemmae other than the resting sporangia, if such might be considered gemmae.

Of the four described species in this genus our plant seems most like *D. monosporus* and *D. polysporus*, the sporangia being exactly as figured by Leitgeb for the former and by Lindstedt for the latter, and in the former, as mentioned above, they tend to fall off in a resting state. There are objections to establishing a species or variety on the basis of the vegetative and asexual reproductive characters alone, but the very positive sterility of our plant over a series of ten years of cultures in various media, representing over a hundred findings, would seem to justify naming it, if for no other reason than convenience. The same plant to all appearances is found in other places and is probably wide-spread. We take it to be the

PLATE 52

PLATE 52

DICTYUCHUS STERILE n. sp.

Fig. 1. Part of a sporangium showing net and two spores in act of emerging. × 533.
Fig. 2. Spore with two cilia. × 533.
Fig. 3. Part of a sporangium with two spores in amoeboid movement. × 533.
Fig. 4. Spores sprouting in the sporangium. × 319.
Fig. 5. Sporangia in resting state, one free, another falling off. × 188.
Fig. 6. Spores formed in small sporangia. × 188.
Fig. 7. Sporangium entirely empty of spores, thus leaving a "net sporangium." × 319.
Fig. 8. Sporangia in resting state. × 113.
Fig. 9. Showing characteristic branching of hyphae and atrophied growing points (a) renewed from below. × 188.
Fig. 10. Resting sporangium of peculiar shape. × 188.
Fig. 11. Resting sporangium from which a few spores have emerged. × 188.

DICTYUCHUS STERILE.

same as the first mentioned of the two sterile plants noted by Humphrey on p. 133 of his monograph, though he does not mention the deciduous sporangia. Dr. I. F. Lewis tells us that he has seen a similar form in Virginia, and Weston ('19) has described a second swimming stage in a sterile plant that he finds in Massachusetts. We have little doubt that his plant is the same as ours, not only from its sterility, but because a sporangium is shown nearly loosened from its hypha (fig. 19). Weston's good figures of detail in the spores and their emergence should be consulted. Both Tiesenhausen ('12, p. 289, figs. 15–18) and Minden ('12) find a sterile plant in which the sporangia fall off, which is probably the same as ours in both cases. Minden refers his sterile plant to *D. monosporus*, but this, of course, could not be convincingly done without a knowledge of the sexual organs.

The situation is indeed interesting and perplexing. Leitgeb ('69) found that sporangia appeared on both the male and female threads of *D. monosporus*; he also found a sterile plant (species unknown, perhaps the same as ours) in which only sporangia were formed during four months' cultivation. On the other hand, Lindstedt never found any sporangia on male or female threads of *D. Magnusii*, and speaks of his plant as *trioecious* for that reason (l. c., p. 16). It seems to us to be very significant that in all cases a *Dictyuchus* that started out to be sterile has remained so consistently, and thus has never been proved to be the same as a fertile species, while all fertile species have been fruitful from the first.

With the exception of *D. polysporus*, which is of more than doubtful validity, all of the species are known to be diclinous, and it may be that we have here a real dioecious plant (if so the only one in the family). In such case it might be possible that one of the sexes could come to occupy alone an extensive territory by asexual reproduction, with apparent sterility as a result. This is, however, very improbable as in no other species of the family is the presence of antheridia necessary for the appearance of the oogonial initials. We have at various times put together cultures from different sources in the Chapel Hill region in an effort to settle this point, but always without result. If sexual reproduction should ever be found in our plant and be similar to that of a known species, its extreme sterility would still entitle it to recognition as a variety or race.

Cultures from No. 13 of November 7, 1912:

In maltose 5%+ peptone .01%. Growth was fairly strong, but not over inch in diameter. The protoplasm in many of the hyphae was segregated into little clumps, as in *Achlya hypogyna* in several media. Many spores were formed. After two weeks the culture was still alive, with many hyphae of normal appearance.

On ant in spring water. Growth healthy, limited to ½ inch diameter. Many resting sporangia, which were observed to rest for nearly a month.

On ant in distilled water. As above except growth of about ¾ inch in diameter and fewer sporangia.

On ant in rain water. Exactly as in spring water above.

On corn meal egg yolk agar. Growth strong, covering dish. An immense number of sporangia.

Resting sporangia were taken from No. 1 of May 1, 1913, and put under a cover on slide to test again the emergence under such conditions. In ½ hour the spores had begun to emerge in good number. After escaping they would tremble and jerk and turn for several minutes and then swim away.

At another time the following notes were made:

Six minutes after emergence the spores began to move slightly, in 18 minutes there was violent motion and in 40 seconds more the spores tore loose and swam away. The cilia became visible in living state in 15 minutes after emergence.

In .05% haemoglobin. Vegetative growth luxuriant. Sporangia sparingly formed. Hyphae seldom branching.

In $K_6H_3(PO_4)_2$+haemoglobin. Vegetative growth was luxuriant—more than above. The hyphae twisted in their course and sent off many lateral branches almost at right angles. Sporangia exceedingly abundant. At last all protoplasm of plant seemed to be used in forming sporangia.

In KNO_3 + haemoglobin. Growth was about normal with hyphae not quite so crowded. Sporangia were formed in considerable numbers at the tips of the hyphae. Vacuoles scattered.

In KCl + haemoglobin. Growth was very sparse. Hyphae frequently branched, forming sporangia at tips. The protoplasm of the hyphae always exceedingly dense.

In $MgSO_4$ +haemoglobin. Growth very sparse. Sporangia formed. Hyphae rarely branching.

Experiments to determine the best method of preserving live cultures:

Culture of No. 13 of December 7, 1912, put in aquarium jar with algae in laboratory on February 19, 1913. When tested for life on September 18, 1917, no growth appeared.

Culture of No. 13 of December 7, 1912, put in vial on corn meal agar on March 18, 1913. When tested on December 1, 1913, it was found to be alive. It is of interest to note that of the sixteen species tested in this way at this time none was found to be alive except *Dictyuchus*.

Experiments begun January 15, 1913, show that *Dictyuchus* will not grow at all on pea-broth-gelatine, on which all species of *Saprolegnia* grow profusely; nor will *Dictyuchus* grow on acidified beef agar or on plant decoctions such as prune juice, *Baccharis* twigs, etc.

2. **Dictyuchus Magnusii** Lindst. Synopsis de Saprol., p. 7, pl. 1, figs. 1–13. 1872.*

Plate 53

This has been reported only by Humphrey ('92, p. 132, pl. 20, figs. 112–114) from preparations made by Trelease. But Pieters has found

*The condensed specific description is on page 18.

PLATE 53

PLATE 53

(Drawn by Dr. A. J. Pieters)

Achlya polyandra

Figs. 1–4. Oogonia with antheridia.

Fig. 5. Unusual form of oogonium with spines. No. 4 from Ann Arbor, Michigan; others from Heidelberg, Germany.

Dictyuchus Magnusii

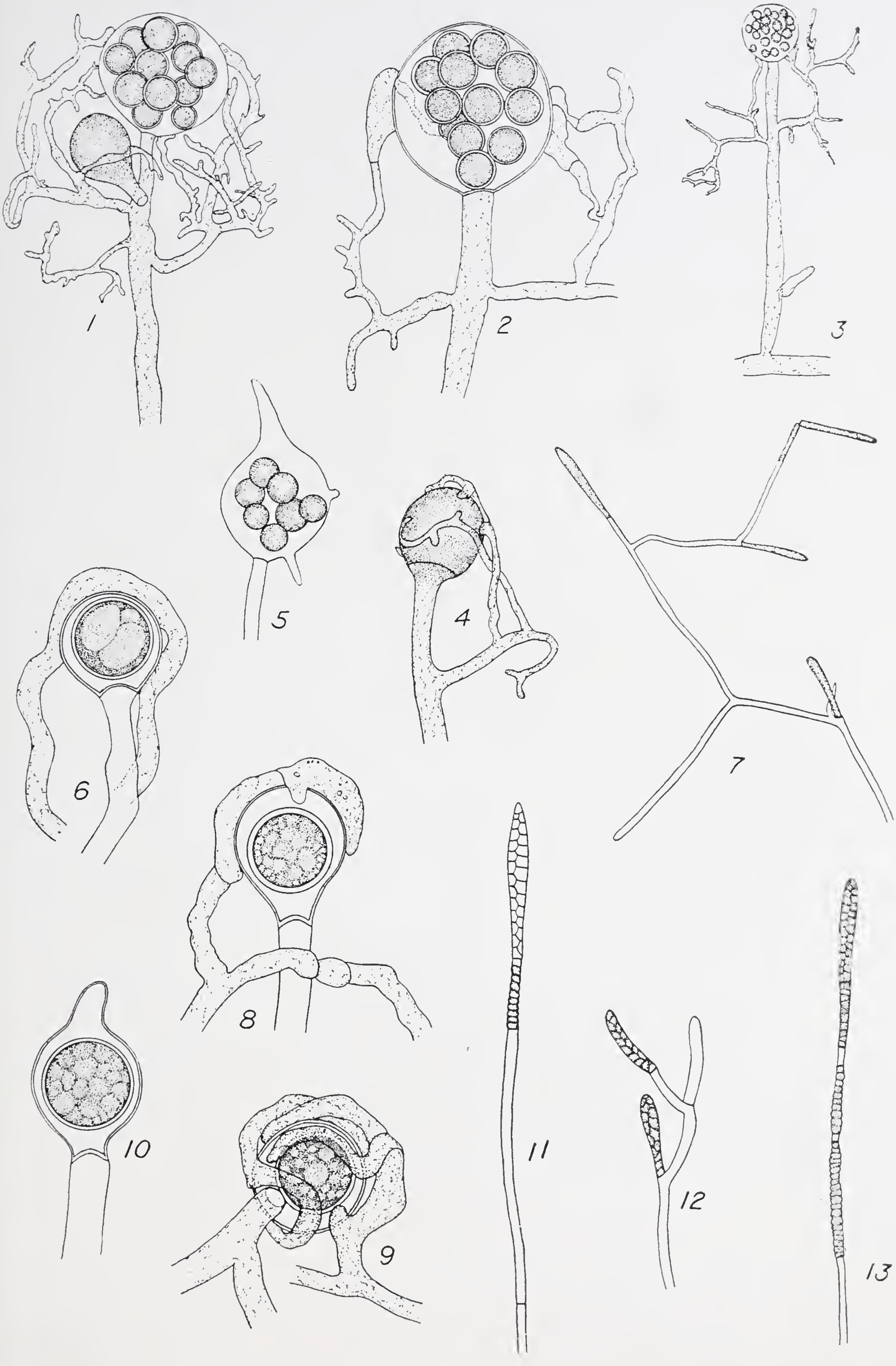
Fig. 6. Oogonium with nearly mature egg. × 525.

Fig. 7. Sporangia.

Figs. 8–9. Oogonia with antheridia and young eggs. × 525.

Fig. 10. Oogonium without antheridia. × 525.

Figs. 11–13. Sporangia. All from Ann Arbor, Michigan.



ACHLYA POLYANDRA. [FIGS. 1–5.]
DICTYUCHUS MAGNUSII. [FIGS. 6–13].

in Michigan a plant which, from his unpublished description and drawings, we take to be this species (reported as *D. monosporus* in Ann. Rep. Mich. Acad. Sci. **17**: 195. 1915). With his consent, we are using Dr. Pieters's pencil drawings in plate 53.

The following is from Humphrey:

"Hyphae rather large. Zoosporangia cylindric or fusiform. Oogonia terminal on slender branches, globular, smooth-walled, unpitted. Antheridia cylindric or slightly clavate, on all oogonia, borne on slender branches of diclinous origin. Oospores single, centric, about 25μ in diameter.

"Massachusetts—Cambridge, *Trelease*. Europe.

"Our knowledge of the occurrence of this species in America rests on the notes and preparations of Professor Trelease, who obtained it in 1881 from water in the Botanic Garden, at Cambridge. It can be confounded only with *D. monosporus*, from which it differs in its somewhat larger oogonia and less coiled antheridial branches.

"Lindstedt states that it is only in this species that the sporangia are formed from the hyphae in basipetal succession, but it seems doubtful if this is strictly true, in view of certain observations to be mentioned later." We may add to these remarks that Lindstedt also notes that the eggs after a rest sprout by a vegetative thread (l. c., p. 18).

Pieters says:

"Collected on a fly, from water with algae, in the Botanical Laboratory at Ann Arbor, November, 1913. Oogonia developed on fly at temperature 22–30° C. Antheridia always strongly diclinous, and clasp the oogonia with one or more processes. Oogonia walls thin, not pitted; eggs single, centric with two to many oil drops. Sporangia will develop in pea agar." He labels his notes *D. monosporus*, and says further: "I will admit that there may be a doubt as to whether this species is *monosporus* or *Magnusii*. I determined it as *monosporus* on the strength of the fact that antheridia coiled around the oogonia. The size of the oogonia, however, appears to be that of *Magnusii* rather than that of *monosporus*."

It is indeed extremely doubtful if *D. Magnusii* is a good species. As described by Lindstedt it differs from *D. monosporus* only in three characters, none of which is of any value unless established as constant by a series of careful cultures—a precaution that one feels pretty sure Lindstedt did not take. These three points of difference are that in *D. monosporus* the oogonia are 25μ thick, the antheridial branches wind about the oogonia, and the sporangia are in sympodia; while in *D. Magnusii* the oogonia are 30–35μ thick, the antheridial branches do not wind about the oogonia, and the sporangia are borne only in rows. As our sterile variety unites in all degrees both the above methods of sporangial arrangement, as is also the case in Humphrey's sterile plant (which is probably the same as ours), first mentioned on p. 133 of his monograph, there is little ground for taking this distinction seriously. The other differences might easily come within the limits of variation of a single species, from analogy with other *Saprolegniaceae*, and carry no conviction, especially as Lindstedt seems never to have seen *D. monosporus*, with which he compares his species.

EUROPEAN SPECIES NOT YET FOUND IN AMERICA

Dictyuchus monosporus Leitgeb. Jahrb. f. wiss. Bot. 7: 357, pl. 22, figs. 1–12, pl. 23, figs. 1–8. 1869.

This has been reported from America only by Pieters (in Kauffman, '15), but we are treating his plant as *D. Magnusii*, which see. The following is adapted from Fischer ('92, p. 362):

Growth thick, 1–1.5 cm. broad, with flaccid main hyphae up to 60μ thick. Sporangia terminal, either long, thread-shaped and little or no thicker than the threads, or long clavate, 250–950μ long, 18–37μ broad, often containing only a single row of spores; proliferating by repeated sprouting from the side below to form a sympodium. Spores bean-shaped, 9–10μ thick. Oogonia terminal, single, on long or short branches of the main threads, spherical, 25μ in diameter; the wall uneven and without pits. Antheridia always present, usually several to each oogonium, borne on slender branches of diclinous origin which often completely enwrap the oogonium. Eggs single, spherical, smooth, centric; germination not known.

Cultivated on insects, or with ease on twigs, or on bulbs of hyacinth, calchicum and tulip.

According to Cornu the plant grows well on twigs and forms very long threadlike sporangia with spores in a single row. The number of spores is often large—up to 300 (Leitgeb), up to 600 (Cornu). According to Leitgeb sporangia are borne both on the threads which bear oogonia and on the more slender male ones. Whether really dioecious or not is not stated. [Above is from Fischer].

Leitgeb had an interesting experience with this plant. For the first two months of culture the spores escaped normally while the sporangia were attached to the threads, the empty sporangia falling off afterward. After this period most of the sporangia fell off while still full, as in our *D. sterile*, and in this condition the great majority of them sprouted as in *Aplanes*. After another half month the sporangia of all cultures failed to mature normal spores, the protoplasm of the spores dying soon after they were formed. He thinks this abnormal behavior must have been due to some peculiar diseased condition that he was not able to explain.

See also Massee ('91), pl. 5, figs. 102–104.

Dictyuchus carpophorus Zopf. Beiträge z. Phys. u. Morph. n. Organismen **3**: 48, pls. 2 and 3. 1893.

This species, in spite of much elaborate description, is doubtfully distinct from *D. monosporus.* It is supposed to differ in the numerous abnormal-looking lateral outgrowths on the hyphae and the eccentric eggs in oogonia which are often thickly enwrapped with antheridia. As shown, the eggs are what we are calling eccentric, with a large oil drop in the protoplasm, and there is no evidence that the so-called centric eggs of *D. monosporus* are not of similar structure.

Dictyuchus polysporus Lindstedt. Synopsis d. Saprol., p. 19, pl. 2, figs. 1–3; pl. 3, figs. 1–7. 1872.

This species has not been found since first reported and one does not feel quite convinced of its validity. Fischer suggests that Lindstedt may have had mixed material before him. The sporangia look exactly like those of our *D. sterile* and like Leitgeb's figures of *D. monosporus,* but the antheridia are said to be androgynous and the oogonia have numerous eggs, which characters are in sharp contrast to the diclinous antheridia and single-egged oogonia of all the other species.

The species may be concisely defined thus:

Growth delicate, the threads only up to ½ cm. long. Sporangia shorter than in *D. monosporus,* multiplying by sympodial branching as in *Achlya.* Oogonia irregularly arranged, terminal or intercalary, single or several in a row, of various shapes, spherical or ovate or long flask-shaped; wall smooth, without pits. Antheridia androgynous, up to several on an oogonium or absent on some of them,* short clavate with the side attached to the oogonium, borne on usually simple branches of rather definite length which spring mostly from main hyphae which bear oogonia; fertilizing tubes present. Eggs 2–20 in an oogonium, 25–27μ thick.

LEPTOLEGNIA deBary, 1888, p. 609.

Hyphae long and delicate, sparingly branched. Sporangia long, apical, cylindrical, of the same size as the hyphae, at times multiplied by growth through empty ones, rarely branched. Spores formed in a single row, elongated on emerging, then changing their form to pip-shaped and swarming with two apical cilia, encysting and swimming

* Lindstedt does not mention their absence on some oogonia, but out of eight of his figures that show oogonia, five are without antheridia

again as in *Saprolegnia*. Gemmae absent. Oogonia borne on short lateral branches, small, smooth, subspherical, unpitted. Antheridia pyriform, diclinous. Eggs single, completely filling the oogonium, the protoplasm nearly surrounded by a surface layer of small droplets. There is but one species known.

Leptolegnia caudata deBary. Bot. Zeit. **46**: 631, pl. 9, fig. 5. 1888.

PLATE 54*

Mycelium delicate, flaccid, the hyphae little branched, about 10–18μ thick; gemmae none; sporangia filamentous, of the same size as the hyphae, often long but not so long as in *Aphanomyces*, about 15–18 × 325–880μ, sometimes branched; spores typically in a single row, irregularly angled and lobed before discharge, becoming rod-shaped when passing out; after emergence the two ends bending backward and fusing to form a pip-shaped spore with two apical cilia; diplanetic, 12.5–13.5μ in diameter in the resting state. Oogonia borne on rather short lateral branches, subspherical with a slight beak, smooth and without pits, 30–40μ thick. Eggs single with a more or less complete circle of peripheral droplets, completely filling the oogonium. Antheridia one or several on every oogonium, short-pyriform, terminating slender branches of diclinous origin.

Rather rare, first found in an aquarium jar of algae that had been brought into the laboratory from pools in the vicinity. Occurring in 0.7% of all Chapel Hill collections between February 15, 1912, and December 12, 1913, as in Terra Cotta spring, Glen Burnie farm, marsh south side of Glen Burnie meadow, Arboretum brook, etc. Later it appeared in several collections of material sent by R. S. Haltiwanger from Avon Park, Florida, and in material collected by us near Wilmington, N. C.

Found twice by deBary in mountain lakes in Germany in 1881 and 1884, the species has been reported but a few times since. Fischer ('92, p. 346) refers to a sterile plant that he thought might be this species. Minden ('12) found it often near Hamburg, Germany, and figures it (figs. 4a and 4b on p. 556), and Petersen finds it in Denmark ('10, p. 521). Dr. Roland Thaxter writes that he has seen a form without sexual reproduction that resembled *Leptolegnia*; and Dr. Pieters has unpublished drawings of the characteristic sporangia from plants found at Ann Arbor, Michigan. According to Petersen ('30, p. 511) this species is a destructive parasite on the crustacean *Leptodora Kindtii* in Denmark. He thinks that the mycelium usually enters around the opening of the mouth, and that the infection is always fatal, enveloping the mother and

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* See Mycologia **1**: 262, pl. 16, 1909, from which the plate and most of the description was taken.

PLATE 54

PLATE 54

(From Mycologia **1**: Pl. 16. 1909.)

LEPTOLEGNIA CAUDATA

Fig. 1. A branched sporangium with one opening at *a*. × 90.

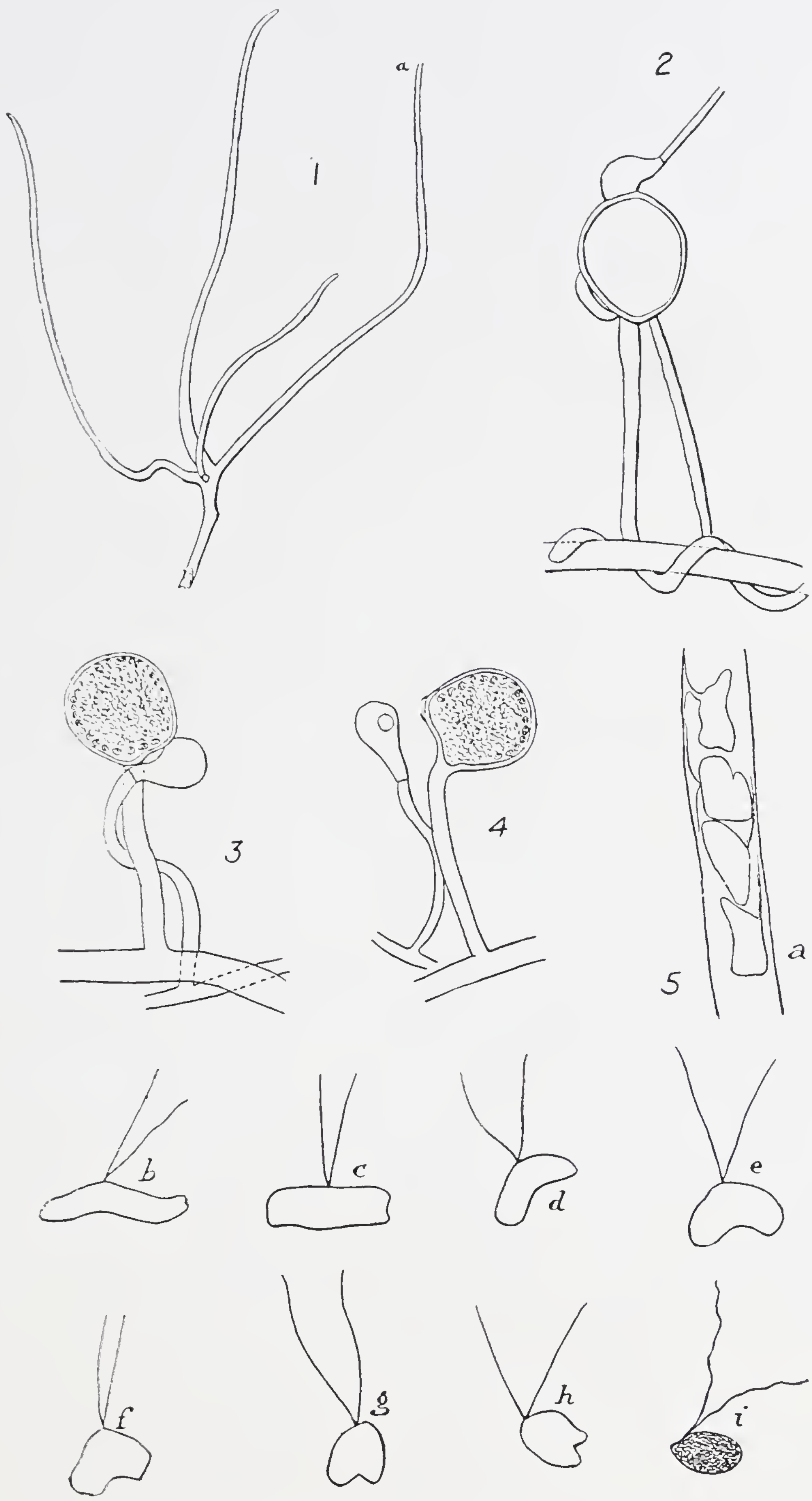
Fig. 2. Oogonium with two antheridia. × 370.

Fig. 3. Antheridium applied to an oogonium. × 370.

Fig. 4. Oogonium with antheridium pulled away leaving a distinct circular opening. × 370.

Fig. 5. *a*. Part of a sporangium, showing spores killed in the act of emerging; *b*, *c*, *d*, *e*, *f*, *g*, *h*, *i* showing spores in successive stages of rounding up in preparation for swimming off after emergence.

PLATE 54



LEPTOLEGNIA CAUDATA.

eggs in a thick mesh of hyphae. Petersen identifies this as also the parasite previously reported by Müller on the same host. The latter found ('68) that it almost exterminated the animal from a lake (Bögholm Sö) in Denmark.

At the 1909 meeting of the North Carolina Academy of Science (reported in Science **30**: 188, August 6, 1909) we referred to our plant as a new species of *Leptolegnia*, but we now think it to be nothing else than deBary's form.

Our observations on the sporangia agree with deBary's except that in old cultures the sporangia may become very complex from the extension of a single sporangium into a number of adjoining branches. In fig. 1 is shown such a sporangium that was observed before and during the discharge. All the spores emerged from the tip of one of the branches (at *a* in the figure) and the spores at the tips of the other branches had to travel all the way down these and out at *a*.

DeBary does not mention the shape or behavior of the spores, but we found them to exhibit some remarkable peculiarities ('09, p. 263). In nearly all cases they emerge from the sporangium much drawn out, as long, more or less cylindrical rods, with the cilia attached to the center on one side. As soon as they escape, the two ends of the rod begin to fold backward, away from the cilia, and fuse as they go, until by complete fusion they lose their identity and form a pear-shaped spore with the cilia near the tip, and the long axis at right angles to the original rod. By killing the spores during emergence they were caught in all stages of this transformation, as shown in fig. 5, in which *a* shows several spores that were killed in the sporangium. They become more elongated as they pass out and on emergence have the shape shown in *b* or *c*. This peculiar habit is confirmed by Petersen ('10, fig. 2). He also adds that the spores while passing out of the sporangium are linked together by their cilia (see p. 4 for later observations by Mr. Couch).

Two, three, or even more antheridia to the oogonium were common in our material. In one case five were counted. More than two are not mentioned by deBary. The antheridial branches are generally borne as rather short offshoots from a slender main branch that shows a marked tendency to twine about the larger female branches (figs. 2 and 3), but they may terminate a long branch. They are always of diclinous origin.

The antheridium is full of protoplasm when it is cut off, and is empty a little later; and the amount of protoplasm contained in it is so large that a discharge into the oogonium seems probable. When the empty antheridium is pulled from the oogonium a distinct circular opening can be seen in it and the opening in the original membrane on the oogonium can be easily made out (fig. 4).

The oogonia are very rarely found. We had cultivated the plant for about three months before the first oogonia appeared, and they were matured during the Christmas recess. They have been produced only two or three times since, and that only sparingly, notwithstanding our efforts to induce sexual reproduction by cultures on various insects and in different chemical solutions. The results of some of these experiments are as follows:

On gnat in .05% haemoglobin solution in shallow dish. Growth was about as extensive as in water but there was a much more profuse branching, especially near the ends of the hyphae. The difference was easily visible to the naked eye. No sexual reproduction.

On gnat in equal parts of haemoglobin .05% and $Ca(NO_3)_2$ .2%. About twenty oogonia, all with antheridia.

Cultures made on gnats in shallow petri dishes gave no oogonia in any of the following solutions (equal parts haemoglobin in .05% solution and chemicals in .2% solution in distilled water in each case);

Haemoglobin + KNO_3
Haemoglobin + $K_6H_3(PO_4)_2$
$Ca(NO_3)_2$
KNO_3
$K_6H_3(PO_4)_2$

Cultures on gnats, flies, wasps, mosquitoes and spiders showed no noticeable differences.

Cultures under several inches of water were unfavorable for the formation of either sexual or asexual reproductive organs.

APHANOMYCES deBary, 1860, p. 178.

Hyphae very delicate, long, sparingly branched. Sporangia formed from unchanged hyphae, long to very long, not proliferating within old ones and rarely laterally from below. Spores borne in a single row, emerging apically in elongated form, then rounding up and encysting in a clump at the end of the sporangium as in *Achlya*, then emerging and swimming again as in that genus. Specialized gemmae absent. Oogonia terminal on short or long branches, smooth or warted, wall thin and unpitted. Antheridia diclinous or androgynous, not a'ways present. Eggs single, not filling the oogonium, eccentric, with a single large fat drop in the protoplasm near one side or with a lunate disc of oil droplets on one side. Fertilization has been proved in *A. laevis*, which see for details. Spore development has been studied by deBary ('60) and Rothert ('03). The genus shows a strong tendency to parasitism.

Key to the Species

Oogonial walls smooth, not with spines or warts; eggs 16.5-26μ thick { *A. laevis* (1) / *A. helicoides* (p. 168) }

Oogonial walls uneven or tuberculate, not spiny; eggs 13–18.5μ thick.......... *A. scaber* (2)
Oogonial walls with distinct spines or papillae
  Oogonia 22–33μ thick (including papillae)........................ { *A. stellatus* (3) / *A. coniger* (p. 168) }
  Oogonia 14–22μ thick... *A. parasitica* (4)
  Oogonia 40–50μ thick, the wall hyaline.......................... *A. phycophilus* (5)
  As above, but oogonial wall brown...................... *A. norvegicus* (p. 168)

1. **Aphanomyces laevis** deBary. Jahrb. f. wiss. Bot. 2: 179, pl. 20, figs. 17–18. 1860.

Plates 50 and 55

Hyphae saprophytic or rarely parasitic on desmids and diatoms (see below), slender, much branched, about 5–7.5μ thick. Sporangia long and of the same size as the hyphae, often extending to the substratum. Spores 7.3–11μ in diameter after emerging, most about 9–10μ, rod-shaped in the sporangium. Oogonia terminal on short lateral branches, globular or nearly so, with smooth thin walls without pits, 18–33μ in diameter. Eggs single, 16.5–26μ in diameter, mostly about 19–22μ, thick-walled, eccentric, with one very large fat drop enclosed in the protoplasm and very near the surface on one side. Antheridial branches very abundant, sometimes twining around the oogonial branches in a knot, androgynous or diclinous. Antheridia large, abundant on all oogonia and extensively wrapping them about; antheridial tubes developed and plainly visible.

This species has been recognized three times in Chapel Hill collections; from Lone Pine spring (September), and from New Hope Creek. It has probably been collected at other times, but not recognized on account of its failure to fruit. It was found by Humphrey at Amherst, Massachusetts, by Pieters at Ann Arbor, Michigan (Ann. Rep. Mich. Acad. Sci. **8**: 27. 1905), and we have obtained it mixed with *A. americana* from a vial of water with a little trash sent us in June, 1920, from Woods Hole, Massachusetts, by Mr. George M. Gray, Curator of the Marine Biological Laboratory. It is easily distinguished by its smooth oogonia from all except the smoothest forms of *A. scaber*, and from it by larger eggs, and large and numerous antheridia. For other illustrations see Humphrey ('92), pl. 20, figs. 105–107; and Petersen ('10), fig. 3c.

In a collection of algae and trash from a branch near Wilmington, N. C., Dec. 30, 1921, there appeared diatoms and chains of desmids on which grew parasitically a species of *Aphanomyces* which proved to be *A. laevis* (pl. 50, fig. 17). The threads, which were 3–6.6μ thick, entered the living cells of the algae where they formed a branched and more or less contorted and undulated complex which finally destroyed the contents of its host. All reproductive parts were borne outside the algae. Sporangia typical for the genus; spores 8.5–10μ thick. Oogonia 22–32μ thick; walls sinuous but without warts or spines. Eggs single, 14–29μ thick, average about 22μ, eccentric as in the typical form. Antheridia present on all oogonia. The plant was cultivated for a short time on ants and bits of boiled corn grain but grew poorly and soon died out. It may be best to consider it a parasitic variety of the species.

Our typical form produces oogonia very sparingly. A culture was kept going in the laboratory for four months, on different media, before any oogonia were produced. They have appeared several times:—on ants in distilled water, on mushroom grubs in distilled water, and most promptly and abundantly on the protein (horny) part of corn endosperm that had been boiled for a few minutes. In each case they have appeared only after the cultures were several weeks old. The cultures are rather hardy and will last for some time, but will not go through the summer without transfer.

The eggs of our plant run smaller than deBary's measurements, which are 27–33μ. Some of the spores are often left in the sporangia, and sprout there into hyphae (pl. 55, fig. 2).

Dr. Pieters writes (unpublished notes) that "oogonia developed when a mycelium was transferred from 1% peptone to 0.05% haemoglobin, July 24, 1914. Antheridia were abundant and wrapped about the oogonia; no eggs were formed."

On this species Kasanowsky ('11) has published a good paper showing that fertilization occurs. His results are briefly as follows:

The young oogonium is filled with plasma and contains a large number of nuclei and a large central vacuole; a number of the original nuclei go to pieces, others divide simultaneously by mytosis; all the nuclei from this division go to pieces except one which becomes the egg-nucleus; there is now formed a dense mass with radiating strands called the coenocentrum, comparable to the similar body in the *Peronosporaceae*, near which lies the egg nucleus which increases in size. The antheridia contain 4–6 nuclei, which divide mytotically and then degenerate with one exception; this male nucleus is then discharged into the egg through a fertilizing tube, along with the protoplasm of the antheridium, and fuses with the egg nucleus; fat is formed in small particles which fuse to form larger ones which finally unite to form a single large drop with a distinct membrane and probably also an internal groundwork (stroma) of its own. The ripe egg is uninucleate, with the large fat drop usually in the center. After six months the egg sprouts to a tube which soon branches.

For other cytological data see Dangeard ('90), p. 113, pl. 6, figs. 6–17.

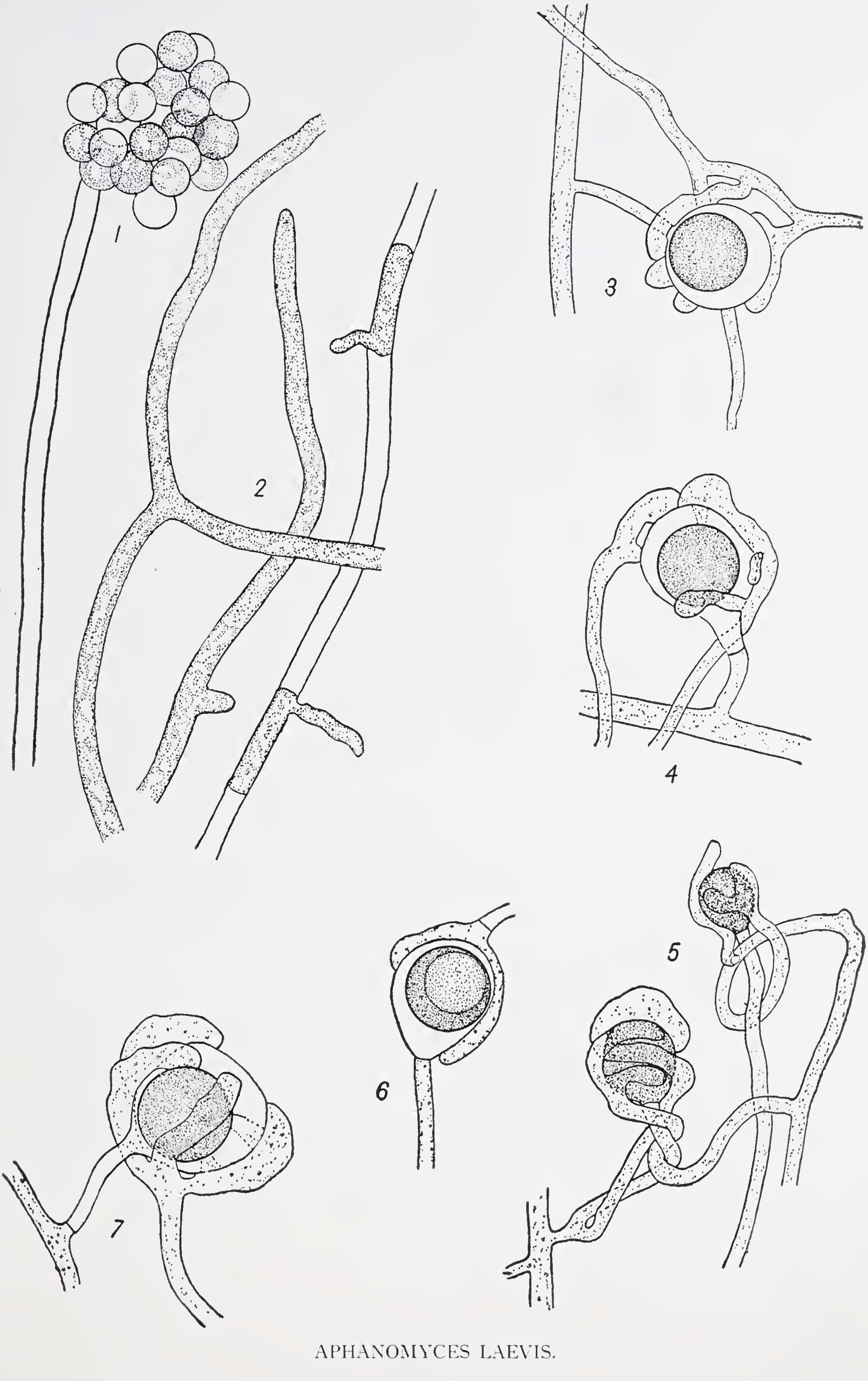
PLATE 55

PLATE 55

Aphanomyces laevis

Fig. 1. Tip of empty sporangium showing spore cluster.
Fig. 2. Vegetative threads and two spores sprouting within the sporangium.
Fig. 3. Oogonium with diclinous antheridia.
Fig. 4. Oogonium with diclinous antheridia and visible antheridial tube.
Fig. 5. Oogonia, the one on left with both diclinous and androgynous antheridia.
Fig. 6. Oogonium with mature egg showing structure.
Fig. 7. Oogonium clasped by finger-like antheridia; two antheridial tubes clearly visible.

All figures × 670.



APHANOMYCES LAEVIS.

2. **Aphanomyces scaber** deBary. Jahrb. f. wiss. Bot. 2: 178, pl. 20, figs. 14–16. 1860.

Plate 50 and 56

Hyphae delicate, branching, about 5–7.5μ thick, rarely as small as 2.5μ. Sporangia like the hyphae, of indefinite length. Spores on encysting about 9.5μ thick; narrow and elongated in the sporangium. Oogonia terminal on short or moderately long branches, very small, 15–23.7μ in diameter, averaging about 21.5μ in No. 1 of January 6, 1914, surface uneven or varying to tuberculate, but projections never so prominent as in *A. stellatus*; wall thin, not pitted. Eggs single, 13–18.5μ in diameter, averaging about 15.5μ in No. 1 of January 6, 1914, about 13.3μ in No. 8 of November 15, 1913, eccentric, a single large oil drop near one side (our figs. 8–10 are not oriented to show the full eccentricity), protoplasm small in quantity and light in color, wall rather thick. Antheridia not seen in our form; present on most of the oogonia, according to Fischer; not on all oogonia, according to Humphrey.

Not rare at Chapel Hill; occurring in brooks and springs, as brook below Howell's spring, brook behind athletic field, etc. Humphrey has reported it from Massachusetts, where he also found a more spiny form. For other illustrations see deBary ('81), pl. 6, figs. 30–36; and Humphrey ('92), pl. 20, figs. 108–111.

This species does not fruit so easily or abundantly as *A. stellatus*, but is easily distinguished from it, when fruiting does occur, by the small oogonia and eggs and by the much less papillate oogonia. From *A. laevis* it differs in smaller eggs, rougher and smaller oogonia and absence of antheridia from all (in our form) or some of the oogonia.

On corn meal and egg yolk agar a strong growth occurs, but reproduction is absent. On mushroom grubs in distilled water sporangia are produced but oogonia are rare.

3. **Aphanomyces stellatus** deBary. Jahrb. f. wiss. Bot. 2: 178, pl. 19, figs. 1–13. 1860.

Plate 56

Hyphae straight, delicate, little branched, about 5.5–6.5μ in diameter, springing abundantly from the substratum, the tips rounded. Sporangia produced from the unchanged hyphae, very long, usually reaching to the substratum. Spores when in the sporangium irregularly rod-shaped with uneven ends, on escape becoming rounded and encysting in an irregular group at the mouth of the sporangium, diameter 8–8.5μ (at times a few larger double ones 11–12μ in diameter mixed with the others), emerging and swimming actively with the usual form, the large cysts giving rise to two spores of normal size, according to deBary, and confirmed by us. Oogonia subspherical, borne on rather long or short lateral branches, normally covered more or less densely with conspicuous blunt papillae up to 5.5μ long, diameter of the oogonia, including the papillae, about 22–33μ; walls rather thin, unpitted, cavity extending into the papillae. Eggs about 16–26μ thick, most about 18.5μ, single (rarely two—deBary), contents eccentric when fully mature, with an inconspicuous lunate series of droplets on one side in optical section. Antheridial branches androgynous or also from neighboring threads, often branched. Antheridia short-tuberous, large, present on all or nearly all oogonia. Fertilization uncertain.

The species is typically saprophytic, but we have found it also parasitic on *Achlya* (see below). It is very plentiful around Chapel Hill in springs, brooks, and creeks, occurring in about 25% of all Chapel Hill collections. It has not been reported heretofore from America, but is probably widespread. For other illustrations see Sorokine ('76), pl. 7; Massee ('91), pl. 6, figs. 105–108. For cytological detail in an unnamed species which may be this, see Dangeard ('90), p. 117, pl. 6, figs. 18–23. It is easily distinguished from *A. laevis* by its conspicuously papillate oogonia and by the numerous antheridia of mostly diclinous origin. From *A. scaber* it differs in the decidedly larger eggs and oogonia. Moreover, the walls of *A. scaber*, while uneven, are much less papillate. Oogonia are abundantly produced in all fairly normal conditions, as on animal and plant bodies, nutrient agar, etc., in this respect differing from *A. laevis* and *A. scaber*, where fruiting is rare. The antheridia are formed early and reach the oogonia while the latter are still young and without their papillae. We have not observed antheridial tubes, but they would be difficult to see if present, and may be formed. The variation in the number of antheridial branches is a marked characteristic. In a collection from New Hope Creek no antheridia appeared in at least half a dozen cultures on grubs in distilled water, but appeared abundantly on at least 90% of the oogonia when grown on a lump of corn meal agar with grubs added in distilled water. On a piece of pea root growth was fairly vigorous, but even more slender than on grubs; much branched; oogonia abundant, a good many with antheridia. On solid corn meal agar growth was vigorous, with oogonia in fair amount and antheridia on many of them.

Abnormalities of the usual nature occur. Spores may be left in the sporangia, particularly a few of the last ones, and may sprout there through the wall to form a hypha or, according to Sorokine, the retained spores may form short tubes and liberate spores as in *Dictyuchus*.

We have found several times in Chapel Hill a form of this species differing from the typical only in being parasitic on species of *Achlya*. The plentiful oogonia are borne both inside and outside the *Achlya* threads.

With only the oogonia at hand, this species could easily be confused with *Saprolegnia asterophora*. In the latter, however, both oogonia and eggs average larger, antheridia are often absent, and the sporangia, of course, quite different.

PLATE 56

PLATE 56

Aphanomyces stellatus

Fig. 1. Tip of sporangium, showing spore cluster with a good many empty cysts and two spores which remainded in the sporangium.

Fig. 2. Young oogonia, one showing the beginnings of the papillae.

Fig. 3. Young oogonium without papillae.

Fig. 4. Oogonium before formation of egg.

Fig. 5. Oogonium with oval-shaped egg.

Fig. 6. Oogonium without antheridium.

Fig. 7. Oogonium with papillae and single egg.

All figures × 503.

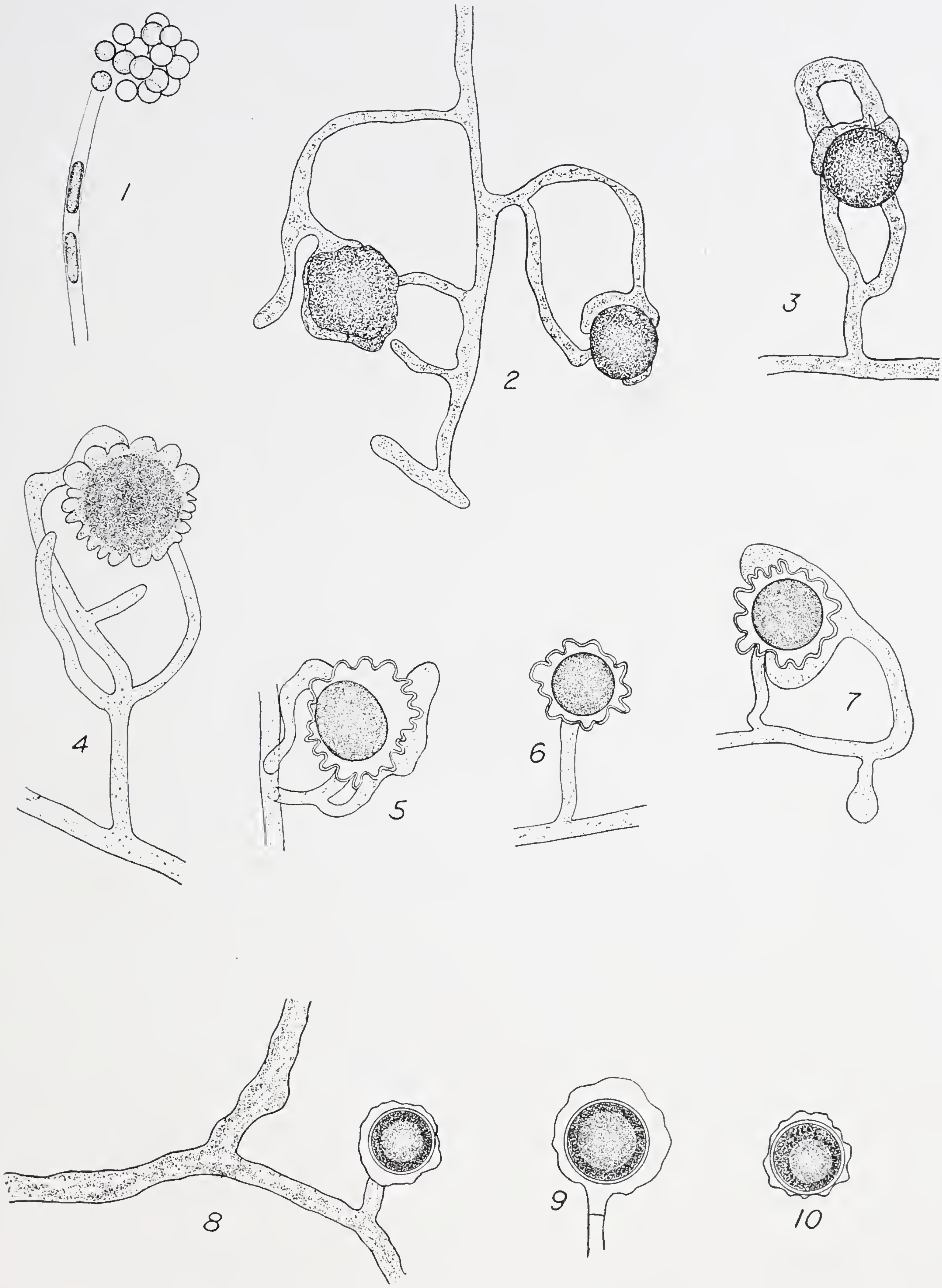
Aphanomyces scaber

Fig. 8. Oogonium with ripe egg and attached hypha.

Figs. 9 and 10. Oogonia with ripe eggs.

All figures × 810.

PLATE 56



APHANOMYCES STELLATUS [ABOVE].
APHANOMYCES SCABER [LOWER THREE].

DeBary's measurements for the eggs compared with ours are rather large, his being 27–35μ in diameter. He remarks ('60, p. 173) that in this species the spore ball at the sporangium mouth may be two spores thick around the central cavity and that the spores at the moment of emergence are held together very weakly and may be separated from each other and from the sporangium mouth by a slight current of water.

All the following cultures were made from No. 10 of January 30, 1913:

In equal parts maltose 5% + peptone .01%. Growth only about ½ inch in diameter. No reproductive bodies.

On white of egg in distilled water. Good growth and a good many scattering oogonia with antheridia.

On ant in distilled water. Good growth. An immense number of good oogonia with antheridia. Also sporangia.

On mushroom grub in distilled water. Good growth. Abundant oogonia.

On corn meal agar. Growth strong, covering dish. Oogonia quite scattering, but many in the dish, all with antheridia and good eggs and with tubercles of about usual size.

On corn meal egg yolk agar. Growth fine and strong. An immense number of fine oogonia, all with antheridia and good eggs.

Experiments to test best method of preserving live cultures:

Culture put in vial on corn meal agar on March 18, 1913. When tested on December 1, 1913, it was found to be dead.

Culture on corn meal agar put in vial of water which was closed with a plug of cotton and put in a dark place in May, 1913. When tested in December, 1913, it was found to be alive. All eggs seemed to be dead.

Culture put in aquarium jar with algae in laboratory. When tested on September 18, 1917, no growth appeared.

4. **Aphanomyces parasitica** n. sp.

Plate 57

Hyphae parasitic on vegetative threads, young sporangia and young oogonia of species of *Achlya* (*A. flagellata*, *A. Orion*, sterile *Achlya*, No. 1 of August 13, 1921), not attacking the gemmae or eggs. Vegetative hyphae endophytic at first, traversing the *Achlya* threads from base to tip, and growing out through the walls of the *Achlya* only after exhausting the threads. Threads of parasite 3–5.5μ thick, most about 4μ; straight and even at first, becoming somewhat swollen in places and distorted with age. Sporangia usually formed outside the host and emptying normally for the genus, the spores encysting at the sporangial tip. If formed within the host, which is not rarely the case, the spores do not emerge, but encyst within the sporangium. Spores 7.4–11μ thick, diplanetic. Oogonia usually borne within the *Achlya* thread, not rarely without it, on short, lateral, inconspicuous branches, 14–22μ thick, not including spines; wall warted to strongly spiny, spines rarely up to 3μ long. Eggs single, eccentric, filling the oogonium, 12.8–21.7μ thick. Antheridial branches of diclinous origin, usually long; antheridia single, irregularly oval, 11 × 14μ; applied to the base of the oogonium and often obscuring the oogonial stalk; the contents emptying completely into the oogonium.

Collected only once and then in a little wet weather branch on the north border of Strowd's low-ground meadows (No. 1 of March 8, 1922).

In some respects the present species resembles *A. scaber*. The size of the oogonia in the two plants is about the same. In *A. scaber* the oogonial walls may be set with numerous short warts or prominences, or may be merely irregularly roughened, while in the former the oogonial walls may be covered with blunt spines or with long, conspicuous, sharp spines, the walls approaching most often the latter condition. Thus the most uneven type of oogonia in *A. scaber* resembles the least spiny type of *A. parasitica*. The two plants are readily distinguished by their antheridia: in the Chapel Hill form of *A. scaber* the antheridia are absent; in the Massachusetts form (Humphrey) the antheridial branches are of androgynous or diclinous origin, the antheridia are small and are not present on all the oogonia. In *A. parasitica* the antheridial branches are of diclinous origin, the antheridia are large and are present on all oogonia.

The present form could hardly be confused with any other described species of *Aphanomyces*. In *A. stellatus* the larger oogonia are covered with thick, blunt papillae and there are several, usually androgynous, antheridia on each oogonium. In *A. laevis* spines are totally absent from the oogonial wall but quite often the wall is angular. In *A. phycophilus*, a form parasitic upon algae, the oogonia have small sharp spines, but the oogonia and eggs are much larger. Of the three species added since deBary established the genus *Aphanomyces*, all are either insufficiently known or of doubtful validity. In *A. coniger* Petersen (doubtful species) the oogonia are 30–40μ thick, and in *A. norvegicus* the oogonia are borne outside the host and have brown walls.

In its parasitic habit this plant is exacting. Several Achlyas have been successfully inoculated with the parasite, but the plant refuses to grow on *Aplanes Treleaseanus* or *Isoachlya unispora*. Four attempts have been made to grow single spores from the parasite on corn meal agar, but the spores in each case refused to sprout. Epiphytic threads of the parasite have been cut out and placed on corn meal agar, termite ants and pieces of boiled corn grain, but no growth took place. Single *Achlya* threads, apparently exhausted by the parasite, were placed on corn meal agar. The *Achlya* threads sprouted and grew but no *Aphanomyces* threads were recognizable until after the *Achlya* threads had attained a length of about two centimeters, and then the threads of the parasite were seen within the *Achlya* threads. All efforts so far to grow the plant apart from an *Achlya* have been unsuccessful.

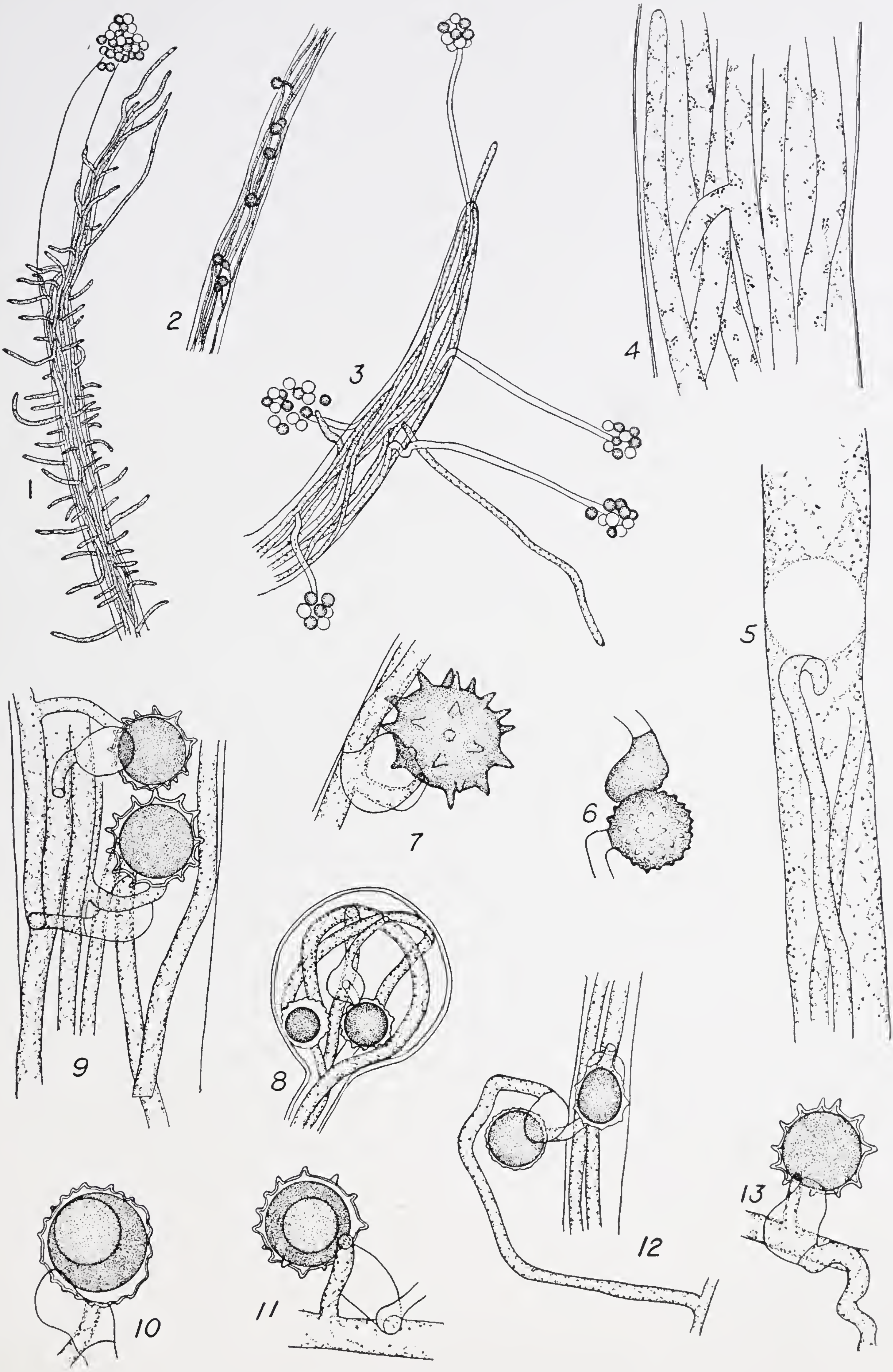
PLATE 57

PLATE 57

Aphanomyces parasitica n. sp.

Fig. 1. Threads of the parasite growing out through the walls of an *Achlya* hypha after completely filling it. × 122.
Fig. 2. Oogonia; most within, one outside the *Achlya* threads. × 122.
Fig. 3. Sporangia; formed outside the *Achlya*. × 278.
Fig. 4. Young threads of the parasite growing through an *Achlya* thread. × 1012.
Fig. 5. Hyphae of the parasite attacking an actively growing thread of *Achlya*. A vacuole appears just in front of the growing tip of a parasite thread which has been turned back thereby. × 1012.
Fig. 6. A young oogonium and antheridium. × 810.
Fig. 7. Surface view of an oogonium inside an *Achlya* thread, showing large, sharp spines. × 1012.
Fig. 8. Oogonia and antheridia within an *Achlya* oogonium. × 503.
Fig. 9. Oogonia and antheridia within an *Achlya* hypha. × 810.
Fig. 10. Oogonium with ripe egg, showing eccentric structure; antheridium attached. × 1012.
Fig. 11. Oogonium with ripe egg shown at an angle which obscures the eccentric structure: antheridium attached. × 1012.
Fig. 12. Oogonia and antheridia. × 503.
Fig. 13. Oogonia and antheridia. × 1012.

All oogonia shown, except one in Fig. 12, were formed inside the host.



APHANOMYCES PARASITICA.

It is not very surprising to find another parasitic species belonging to this genus, for all the previously known forms in it have shown parasitic habits at times. As mentioned above we have often observed *A. stellatus* growing in *Achlya* threads, and have found *Aphanomyces laevis* growing on live desmids and diatoms.

5. **Aphanomyces phycophilus** deBary. Jahrb. f. wiss. Bot. **2**: 179, pl. 20, figs. 19–24. 1860.

This interesting parasite has been reported from America by Kauffman from Michigan (Ann. Rep. Mich. Acad. Sci. **17**: 195. 1915) and by Weatherwax ('14) from Indiana. In unpublished notes, kindly submitted to us by Dr. Pieters, he says it was determined as such from an oogonium found in *Spirogyra* collected at Ann Arbor in 1913, but no culture was made, nor was it found again.

As deBary's original treatment is long we adapt the following in great part from the more condensed description by Fischer (Rabenhorst's Krypt. Flora **1**, part 4: 360. 1892):

Threads stouter than in other species, 8–15μ thick, creeping longitudinally through the host cells for some distance by penetrating the cross walls; other threads extend outside the host and these bear the reproductive organs. Sporangia threadlike, but not further described; spore formation not observed. Oogonia on the ends of short threads outside of the host, very rarely inside the cells; spherical, 40–50μ thick, the hyaline, unpitted wall thickly set with short, rather sharp spines. Eggs spherical, single; sprouting not observed. Antheridia 1–3 on each oogonium, short, club-shaped, with their tips against the oogonia, borne on the ends of short branches which arise from nearby threads.

An obligate parasite on *Spirogyra* and *Zygnema* and not to be cultivated on insects, etc.

Although deBary did not observe the asexual reproduction there is little or no doubt that he was right in referring the plant to *Aphanomyces*. In a short article by Weatherwax ('14, p. 109), he gives the diameter of the egg as about 36μ, its wall 3–4μ thick; the spines of the oogonia 5–8μ long. His plant attacked but one species of *Spirogyra* (*S. dubia*), ignoring other species in the same culture. His figures 1–6 show oogonia and threads; he did not find sporangia.

A problematic plant but almost certainly a species of *Aphanomyces* is *Achlyogeton solatium* Cornu ('70, p. 298). It is parasitic in *Oedogonium*, and may be the present species, though more apt to be distinct, as Fischer thinks. From Fischer we take the following condensed description ('92, p. 361):

"Mycelium more or less branched, boring through the cross walls of the host. Sporangia cut off by cross walls, not different from the threads, emptying by a long emission tube which runs to the exterior;

spores 3–12 in a sporangium, encysting themselves at the mouth of the sporangium; oogonia formed inside the host cells, irregularly cylindrical with perpendicular projections; eggs one or few; antheridia not observed."

EUROPEAN SPECIES NOT FOUND IN AMERICA

Aphanomyces coniger Petersen. Bot. Tidsskr. **29**: 387. 1909. Also in Ann. Myc. **8**: 525, fig. 3b and f. 1910.

This species, rather recently described, has not yet been found in America. The following is adapted from the original:

Hyphae 5–15μ thick; oogonia without pits, the wall brown and with great rounded protuberances, which are more or less conical in shape; diameter of oogonia with processes 30–40μ, processes alone about 8μ; eggs single, 16–30(?)μ thick;* antheridial branches in part androgynous, in part diclinous from a distance, in this respect resembling *A. laevis*. Resembling *A. stellatus* in the oogonial protuberances, but not to be referred to that species. Observed only once on the tegument of a nymph of one of the *Phryganeae*.

The zoospores were not seen to emerge and Petersen is in doubt in referring it to *Aphanomyces*. It seems very likely that it is an *Aphanomyces*, however, as remarked by Minden. Granting the species to be in this genus, it is not obvious to me that it differs from *A. stellatus*. The slight differences in the measurements given do not seem important, and no other discrepancies appear.

Aphanomyces norvegicus Wille. Videnskab. Skrifter. Ser. I, Math. Naturw. Klasse No. 3, p. 9, figs. 14–27. 1899.

This species, reported only from Norway, is, like *A. phycophilus*, parasitic on the *Conjugatae* (*Spirogyra*, *Zygnema*, and *Mougeotia*). It differs from that species in having the mycelium wound outside the algal threads as well as running inside, and in the brown (not hyaline) wall of the oogonium, which is strongly papillate. The oogonia are nearly always borne outside the host. For full description see Minden ('12, p. 561). This also has not been found in America, and in our opinion further study is required to establish it as certainly distinct from *A. phycophilus*. The size of the eggs is not given by Wille.

Aphanomyces helicoides Minden. Krypt. Fl. Mark B. **5**: 559. 1912.

It is very probable that this is not different from *A. laevis* as there is a strong tendency for the antheridial branches to coil in our Chapel Hill form of that species.

*The original says 16–70μ, an evident error. We guess at 30μ as that would be about right from the size of the oogonia.

The following is translated from the original (there are no figures):

"Mycelium and sporangia as in *A. laevis*. Oogonia terminal, rarely intercalary, mostly on very short side branches, more or less spherical, with smooth, moderately thick membrane; without pits; 23–38μ in diameter. Antheridia large, often long, cylindrical, usually several on an oogonium and spirally wrapping them around, as well as their stalks, later with brown, thickened membrane, and lying isolated on the oogonia on account of the disappearance of their stalks. The antheridial threads arise in part from special hyphae, in part from those that bear oogonia. They often wind themselves thickly about each other, and also about other threads, even around those that do not bear oogonia, so that they may make a thick tangle in the neighborhood of the oogonia. Eggs single, spherical, 23–27μ thick, with large fat drops, which are mostly on one side, but not always visible.

"Found near Hamburg, and cultivated on ant eggs.

"The sex organs are developed very richly with the sporangia in conditions where other *Aphanomyces* species do not develop them. This species is nearly related to *A. laevis*, and is possibly only a variety of it. However, the long antheridial branches, which often wind about the hyphae, and the often snake-like twisting of the antheridia, would seem to justify the establishment of a new species."

LEPTOMITACEAE

Filaments constricted at intervals to form a series of long or short segments; often showing conspicuous particles of material, supposed to be cellulin, which may entirely fill the constriction. Oogonia if present containing a single egg, which is surrounded by periplasm except in *Apodachlya*.

The fungi composing this family were included in the *Saprolegniaceae* until Schroeter established the family in 1893. Thaxter (1896, p. 324) has called attention to the fact that they are more related to the *Pythiaceae* than to the *Saprolegniaceae*.

Of the species now included in the family we have studied only *Leptomitus lacteus*, *Apodachlya brachynema* and *Sapromyces Reinschii*, the former of wide distribution and frequent appearance in collections. For convenience we give below a tabular view of the genera and species now recognized in the *Leptomitaceae*.

Gonapodya siliquaeformis (Reinsch) Thaxter (1895a). See also Petersen ('10, fig. 11).
Gonapodya polymorpha Thaxter (1895a). See also Petersen ('10, figs. 12–14).
Leptomitus lacteus Ag. (1824). See below.
Apodachlya pyrifera Zopf. (1888).
Apodachlya pyrifera var. *macrosporangia* Tiesenhausen (1912, p. 295, fig. 19).

Apodachlya brachynema (Hildb.) Prings. (1883). See below.
Apodachlya brachynema var. *major* Tiesenhausen (1912, p. 296, fig. 20).
Apodachlya completa Humphrey (1893). The position of this plant is quite uncertain until further observations can be made.
Rhipidium interruptum Cornu (1871).
Rhipidium continuum Cornu (1871). See also Petersen ('10, fig. 4a and e).
Rhipidium americanum Thaxter (1896).
Araiospora pulchra Thaxter (1896).
Araiospora spinosa (Cornu) Thaxter (1896).
*Sapromyces** *Reinschii* (Schroeter) Fritsch (1893). See below.
Sapromyces androgynus Thaxter (1896).
Sapromyces elongatus (Cornu) Thaxter (1896).

LEPTOMITUS Agardh, 1824, p. 47.

Hyphae delicate, sparingly branched apically and soon appearing dichotomous, constricted at intervals into distinct segments with a conspicuous cellulin plug separating them (thus differing essentially from the *Saprolegniaceae*). Sporangia apical and then in rows in basipetal succession. Spores in a single row (or nearly so), escaping as in *Saprolegnia* and of the same habit and structure (diplanetic), biciliate. Oogonia and antheridia never observed. There is but one species now recognized as good, though many have been described.

Leptomitus lacteus (Roth) Agardh. Systema Algarum, p. 47. 1824.
Apodya lactea Cornu. Ann. Sci. Nat. Bot. **15**: 5. 1872.

PLATE 58

Characters of the genus. The species may be found at almost any time by taking slime from sewers or streams contaminated with sewage. In such places it is often so abundant as to whiten all surfaces and objects in the water, as we have often seen in Chapel Hill and in Baltimore, Maryland. Huxley (Quart. Jour. Mic. Sci. **22**: 331. 1882), quoting from Geoppert, says that "refuse from a factory for making alcohol from turnips near Schweidnitz in Silesia, poured into the river Westritz, caused such a prodigious growth of *Leptomitus* that the fungus covered some 10,000 sq. ft. of the bottom with a thick white layer, compared (*sic*) to a sheep's fleece, choked up the pipes, and rendered the water of the town undrinkable." See also Bot. Zeit. **2**: 163. 1853.

Observations by Radais ('98, p. 147) on the behavior of the cellulin granules are of sufficient interest to translate. He says:

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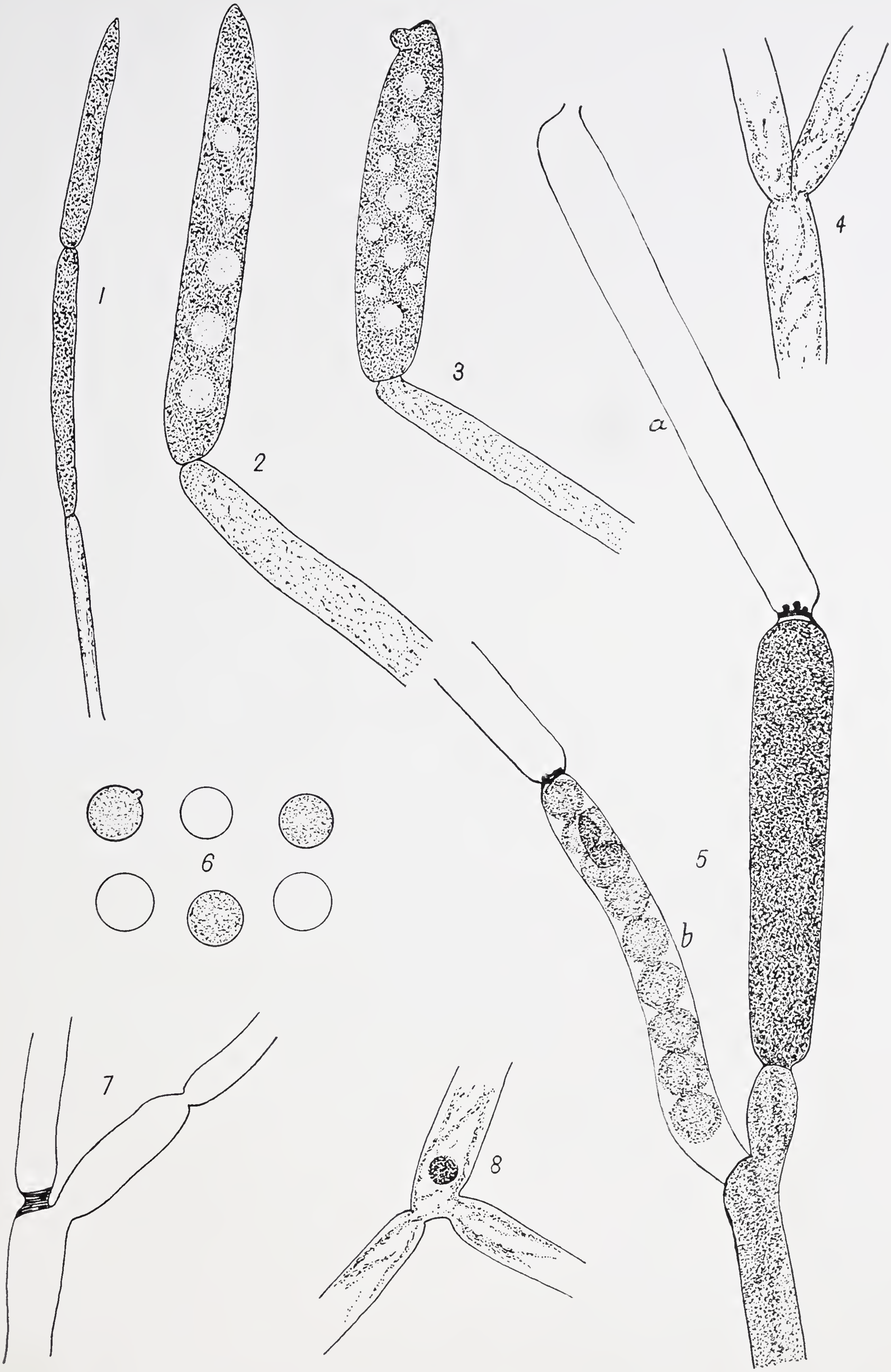
* Syn. *Naegelia* Reinsch (1878): *Naegeliella* Schroeter (1893). See Fritsch (1893, p. 420). Tiesenhausen ('12) also reports this (p. 298, figs. 21, 22).

PLATE 58

PLATE 58

Leptomitus lacteus

Figs. 1, 2, 3. Resting sporangia. 1 × 250; 2 and 3 × 670.
Fig. 4. Showing method of branching and constriction. × 670.
Fig. 5. (a) Empty sporangium; (b) spores in a sporangium. × 670.
Fig. 6. Spores and empty cysts. × 670.
Fig. 7. An old hypha with a conspicuous cellulin plug. × 670.
Fig. 8. Characteristic appearance of the particles near a constriction. × 670.



LEPTOMITUS LACTEUS

"These spheroids have been earlier studied by Pringsheim from a morphological point of view. Their number in each segment is variable, one at least always appears in a young segment; later the number may be increased. Sometimes one may notice them in some place in the body of the segment, sometimes they are localized in the constriction which they obstruct. This last situation is always noticeable when the thread has been broken at the center of the constriction which they obstruct. If the rupture is recent the cellulose granule is simply applied at the opening and obstructs it by simple contact; the protoplasmic contents are thus motionless in the hypha. If the wound is older an adhesion appears between the granules and the cellulose wall, of such a nature that in the same kind of wounds of different ages one can see all the intermediate stages between the application pure and simple of the unchanged cellulose spheroid, which closes the conical tube like a valve, and the adhesion with change of form of this spheroid, which soon forms a new tip of the hypha which may now continue its apical growth.

"This rôle of stopcock by immediate application and ultimate adhesion is carried out by the cellular granules not only opposite the opening of the apex, produced by accidental ruptures of the filaments, but also opposite all the lateral perforations which one can make intentionally with a needle.

"As to the manner in which the spheroid fulfills its function, I have been able to observe directly beneath the microscope the rapid movement of the cellulose granule as far as the opening intentionally made in the thread. It does not seem to me that this change of position is the result of an intrinsic motion. I consider it rather a mechanical force due to the protoplasmic current which determines the sudden diminution of the turgescence at the moment of the opening of the tube.

"If the preceding facts seem to me to justify the explanation which I am giving it does not follow, however, that the function of building up the membrane of the hyphae is the only one which can be attributed to the cellulose grains. Perhaps this form of carbohydrate inside of the cell is available for some other use. Whatever it may be, the special function which I have been able to observe is not sufficient to explain the larger number of spheroids in the older segments of the plant."

The appearance of a culture is more delicate and flaccid than that of a *Saprolegnia*, the threads about 8.5–16μ in thickness, and the growth is less vigorous and certain under laboratory conditions. We have found slightly boiled cow peas the best medium on which to cultivate it. The sparse branching is all done near the tips and soon presents a dichotomous appearance. The sporangia are sparingly formed from slightly enlarged segments, at first apical then often in rows, opening at the tip or on the side to discharge the actively motile, biciliate spores which are formed in a single row, or imperfectly in two rows, and are about 10.5–11μ in diameter. They are diplanetic, a fact hitherto uncertain on account of conflicting reports. Pringsheim considered them monoplanetic, but Hartog ('87) was right in stating them to be diplanetic (as *Saprolegnia corcagiensis*). Sexual reproduction has never been observed.

As a subject for the demonstration of protoplasmic rotation this plant has no superior. Unlike the *Saprolegniaceae* the strongly granular protoplasm is in constant motion and is easily observed even by inexperienced students. We have found the plant in Chapel Hill not only in sewers, etc., where it may be had at any time, but also rarely in such clean streams as Battle's brook and the brook behind the athletic field.

Kolkwitz ('03) has studied the culture requirements and physiology of this species, and has also published a condensed statement of his results ('03a, p. 147). For illustrations see Humphrey ('92), pl. 20, figs. 115–118; Pringsheim ('60), pl. 23, figs. 6–10, and pl. 25; and Büsgen ('82), figs. 9–15. For other cytological data see Dangeard ('90), p. 118, pl. 6, figs. 24–31.

APODACHLYA Pringsheim, 1883, p. 289.

Hyphae constricted into segments of variable length, more slender than in *Leptomitus*, the branching taking place from any point in a segment, but usually near the distal end. Sporangia swollen, pyriform, oval or spherical. In three species spherical resting bodies are known with the contents entirely filling them, and we regard these as true oogonia containing a single egg; in *A. completa* (probably not an *Apodachlya*) larger oogonia are formed with several eggs.

Four species have been described, but in only one, *A. pyrifera* Zopf ('88, p. 362)*, has the structure heretofore been well known. See also Petersen ('10, p. 526). The others are *A. brachynema* (see below), *A. punctata* Minden ('12, p. 586, figs. b-d on p. 580), a very doubtful species, and *A. completa* Humphrey ('93), which was referred to this genus with some doubt as the sporangia were not found. We include a description of only the species we have found. For the others see the literature cited above and, in addition, Fischer ('92, p. 373).

Key to the Species

Oogonia present, each containing several eggs............................*A. completa*

Resting bodies (oogonia?) entirely filled by the contents which is organized like a single egg.

* The name *Apodachlya pyrifera* is first used on p. 367, and the plate is labelled *Leptomitus pyrifera*.

Resting bodies in greater part borne on the tips of the main hyphae; spores usually encysting at the mouth of the pyriform to spherical sporangia.........*A. pyrifera*
As in *A. pyrifera*, but the oogonial membrane distinctly punctate (?).....*A. punctata*
Resting bodies in greater part borne on short, moniliform, lateral branches; spores usually swarming at once on leaving the pyriform to spherical sporangia..*A. brachynema*

Apodachlya brachynema (Hildb.) Pringsheim. Ber. d. Deutsch. Bot. Gesell **1**: 289. 1883.

Leptomitus brachynema Hildebrand. Jahrb. f. wiss. Bot. **6**: 261, pl. 16, figs. 12–13. 1867.

Apodya brachynema (Hildb.) Cornu. Ann. Sci. Nat., Series 5, **15**: 14. 1872.

Plate 59

Main hyphae slender, the segments about 4.5–8.5μ thick and 110–185μ long on termite ants, but 4–23.4 × 20–150μ on corn meal agar, becoming shorter near the sporangia as a rule, the protoplasm moderately dense and with small refractive drops here and there; branching rather sparsely from any point on the segments, but usually near the distal end. Sporangia terminal, single or rarely two or three in a row, swollen, pyriform or oval or spherical on termite ants, about 23–29μ thick and 23–46μ long, renewed by sympodial branching, opening by a distinct papilla formed a few minutes before discharge of spores; papilla usually apical in the longer sporangia, either apical or lateral in the short or spherical ones. Spores few, about 8–20, short-oval, in our cultures nearly always swimming sluggishly and aimlessly for a few minutes with two apical cilia on emerging, then encysting and swimming again after a rest; diameter 8.5–10μ when encysted. Resting bodies (oogonia) formed plentifully on the tips of short, lateral, jointed branches from the main hyphae, spherical or very rarely short pyriform, 23.5–29μ thick, smooth, dense, at first nearly homogeneous, then forming a number of fat droplets and finally one eccentric, conspicuous droplet as in the eccentric-egged Achlyas; wall unpitted, about 1.8μ thick; the suboogonial cell (antheridium) as a rule nearly spherical, at first denser than the other members of the chain, then discharging its contents into the oogonium and becoming quite empty before the maturation of the egg.

Found in Chapel Hill but once, in a marsh at the foot of Lone Pine hill, collection No. 2, February 17, 1921. It appeared late along with *Dictyuchus* and an invading sterile fungus with bacteria on a termite. After considerable difficulty it was got in pure culture and is being carried on. Thaxter ('96, p. 325) reports this species and *A. pyrifera* from New England.

Of *Apodachlya brachynema* Hildebrand says that in addition to the sporangia he noted at a somewhat later stage spherical cells on the ends of short side branches, these cells filled with granular stuff, and he suggests that they may be oogonia, though their further development was not noted. He also shows (fig. 23) one such body on the end of a

2-celled stalk very like the oogonia of our plant. The sporangia are also like the majority in ours and not all spherical; some are oval. He did not see the spores escape or swim, but he shows no spores at tips of sporangia and several sporangia are drawn with one or more retained inside. He says that about six are formed. He shows several sporangia (as many as four) in a close group by budding below as in ours, and as many as three in a row in one case. His figures are so nearly like ours that we must let our plant go as *A. brachynema*. Pringsheim, it is true (l. c., p. 289), says that in this species he has observed that the spores are formed not as in *Saprolegnia* but as in *Achlya*. Tiesenhausen's description and figure of the variety *major* represent our plant well, but as his variety is based on size of threads and sporangia and as our plant connects these sizes up with those of the type it is probable that this variety is only a form.

Zopf's good description and figures of *A. pyrifera* clearly exclude our plant on two main points. In the former the majority of the oogonia are borne on the tips of main threads, others not so borne are either sessile on the side of main hyphae or with a single short stalk cell. The spores are described as encysting, as a rule, in a group at the sporangium mouth, or less often swarming at once on emerging. In the latter the oogonia are nearly all borne on rather short lateral, more or less moniliform branches composed of a few short segments. A culture shows a peripheral series of sporangia with the oogonia forming in much larger numbers on laterals from the same hyphae nearer the substratum. Spherical sporangia may be distinguished from young oogonia before the spore initials appear by the longer and clearer cell below them.

Minden's species, *A. punctata*, is more than doubtful. He separates it only on the punctate membrane of the resting spore (oogonium); but, so far as his figures show, this punctation is nothing more than the emulsified contents of the young oogonium, a condition shown also by Zopf for *A. pyrifera*.

The spherical resting bodies called by Zopf "Dauersporen" or gemmae are, we believe, oogonia both in his plant and in ours. Tiesenhausen comes to the same conclusion for *A. pyrifera* (l. c., p. 298). We know of no gemmae in this or related groups which have thick walls and undergo a maturation within like an egg. Moreover, as shown below, there is good reason to believe that fertilization occurs.

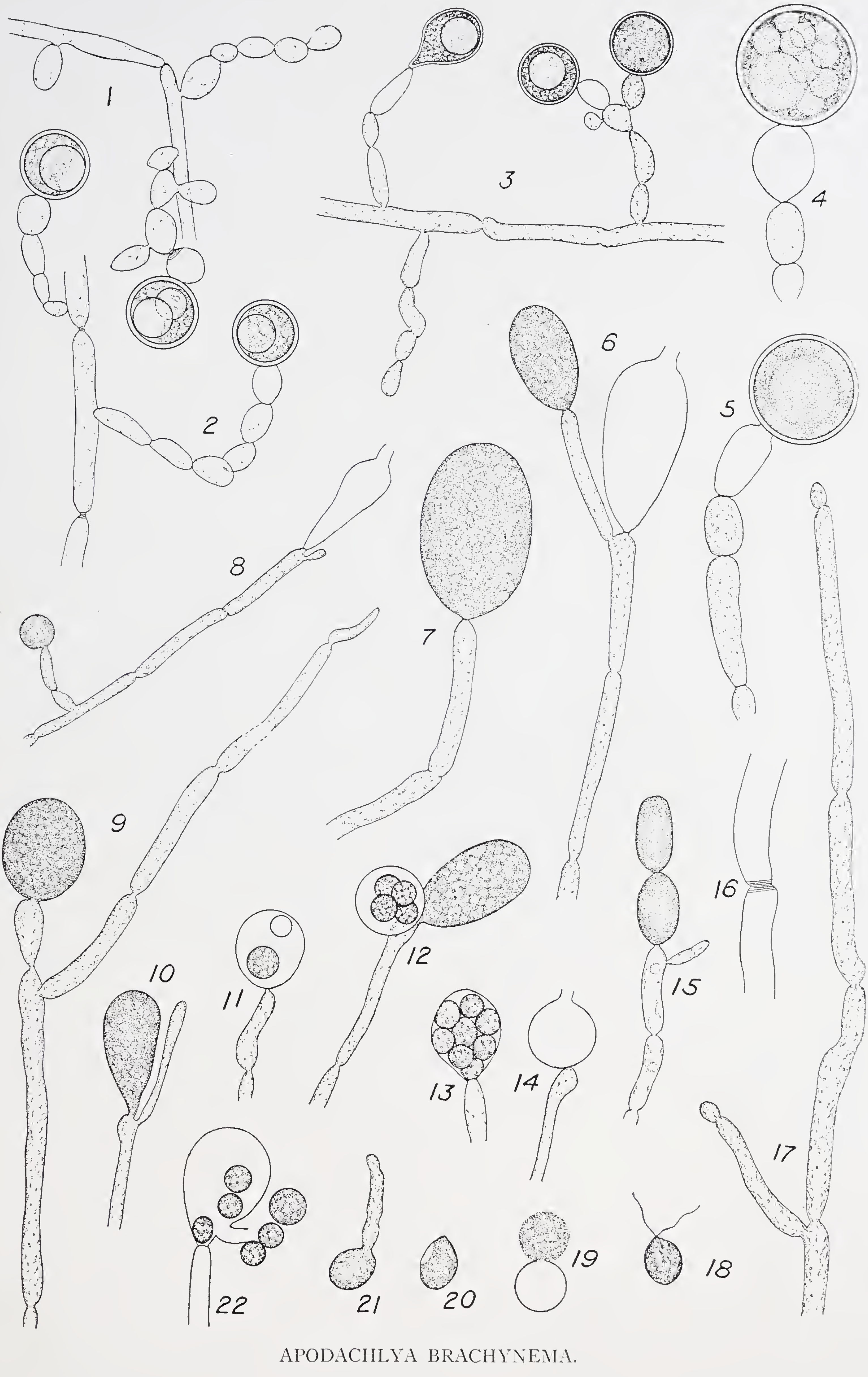
Observations of threads on a corn meal agar plate show that the suboogonial cells reach their final size before the young oogonium appears. The oogonium appears as a very small globular tip on the end of the distal stalk cell. The protoplasm can be seen passing from the hyphal segment through the rounded stalk cells into the growing oogo-

PLATE 59

PLATE 59

Apodachlya brachynema

Figs. 1, 2. Mature oogonia on their moniliform branches. One has two oil drops. × 503.
Fig. 3. Two mature oogonia and one immature. × 503.
Fig. 4. A nearly mature oogonium. × 810.
Fig. 5. An oogonium with a large vacuole in an early stage of maturation. × 810.
Fig. 6. An empty sporangium with a young one by it. × 503.
Fig. 7. A sporangium approaching maturity. × 810.
Fig. 8. A long, empty sporangium with a young spherical one on the same thread. × 278.
Figs. 9, 10. Sporangia with the hypha continued beyond. × 503.
Fig. 11. A sporangium after opening with one spore retained. × 503.
Fig. 12. A sporangium after opening with 4 spores retained and a young sporangium by it. × 503.
Fig. 13. A sporangium that failed to open, the mature spores still retained. × 503.
Fig. 14. An empty sporangium. × 503.
Fig. 15. Two sporangia in a row. × 278.
Fig. 16. A joint of an old thread with the opening stopped by a plate of cellulin. × 810.
Fig. 17. A branched hypha in active growth. × 503.
Fig. 18. A spore in first swimming stage with two apical cilia. × 810.
Fig. 19. A spore emerging from its cyst. × 810.
Fig. 20. A spore in the second swimming stage (cilia not shown). × 810.
Fig. 21. A spore sprouting after the second encystment. × 810.
Fig. 22. A sporangium with some of the spores retained, some held at the mouth (a few had swum away). × 503.



APODACHLYA BRACHYNEMA.

nium. An oogonium grows from its first visible size to its mature size in about eight hours. At the time when the oogonium reaches its mature size the contents of all the stalk cells, except the one next the oogonium, become less dense, while the oogonium and its adjoining cell are very dense and homogeneously mottled. After a short rest the contents of the dense cell pass over into the oogonium. This apparent fertilization requires from three and one-half to five hours. Then after another short rest organization of the contents begins. When the oogonium finally falls away this subspherical cell adjoining goes with it, the chain breaking below it. There is also a very significant change going on in the oogonium during the reduction of the protoplasm in the adjacent cell. A light spot appears in the proximal end in the hitherto homogeneous egg and this persists until nearly all the protoplasm has disappeared from the cell below. It then disappears as the maturation changes set in. While Zopf refers to the possibility of the cells he found attached to the gemmae being antheridia, he concludes that they are more probably resting zoospores or invading organisms of other kinds. They may well be antheridia.

Our plant does fairly well in culture on termites in boiled well water, and will grow well but slowly on corn meal agar. The sporangia are very whimsical about opening at room temperature (about 60–70° F.), but rarely fail to discharge their spores if kept in an ice box.

On corn meal agar many of the segments become dumb-bell shaped. One segment measured 23.4μ thick at proximal end, 14μ in middle, 21μ at distal end, and was 105μ long. The internodes of young threads are open, as is evidenced by the fact that protoplasmic streams can be seen passing from one segment to another, while those of old threads are closed by a cellulin plug. Usually there are two or three globules of cellulin in each segment. The globules are moved about by the action of protoplasm, either pushed along in large segments or rotated in small ones. Old segments are usually very dense with protoplasm, which moves almost imperceptibly. Occasionally a sparsely filled segment will be found between two densely filled ones; in it the protoplasm will be in active motion, limited to its own walls by the cellulin plugs at the internodes. It is on densely filled segments that oogonial branches are borne.

The spores on emerging are forced out under pressure, as usual in the water molds, but the pressure becomes so weak before all are out that several may swim around in the sporangium a few minutes before getting out, and the last one or two, rarely more, are often retained inside and encyst there after a few minutes' swim. Inside the sporangium the spores are subspherical before emerging, but in passing out of the smaller opening they become oblong, only to become short-pip-shaped again as they swim away with two apical cilia. This first swimming is very deliberate and apparently quite aimless and continues for only a few minutes. After encysting the spores emerge after a time and swim again with a clear spot on one side as usual. In this stage the swimming is much more regular, the spores going straight forward at a very steady gait. On encysting again the spores may sprout.

On a bit of corn meal in sterilized well water young threads produced oval sporangia about the same size as those on termites and pear-shaped ones about 35 × 60μ. These sporangia on agar produced long papillae, 6–7 × 50–400μ, but the spores while formed rarely escaped.

A culture on a piece of boiled corn grain in sterilized well water produced oogonia in great abundance.

SAPROMYCES Fritsch, 1893, p. 420.

Plant arising as a single, slender basal cell attached by rhizoids and branching at the tip into two or more similar segments which are constricted at the point of origin and which rebranch again one or more times in the same way. Sporangia single or in groups, apical or lateral from the continuation of the threads, elongated clavate to nearly cylindrical, the monoplanetic spores escaping by an apical papilla. Oogonia in some forms (or at some seasons?) absent, when present single or in whorls at the nodes, pyriform, often encrusted. Egg single. Antheridia borne on long or short branches which are often twisted, androgynous or diclinous, applied to the tip of the oogonia and sending a tube to the egg. Three species are known as mentioned above under the family, also a sterile plant which is thought by Thaxter to be a form of *S. Reinschii*. This sterile form was found by Mr. Couch near Wilmington and the descriptive notes were made from his observations.

Sapromyces Reinschii (Schroet.) Fritsch. Osterr. bot. Zeitschr. **43**:420. 1893.

PLATE 60

Hyphae divided into segments of unequal length by incomplete constrictions, the connection between the segments being closed by a cellulin plug; branched repeatedly, the new branches often arising in whorls, not rarely dichotomously branched; 5–15μ thick, most about 10μ. Sporangia apical or rarely lateral, single or in clusters of as many as six, very variable in shape and size, sub-cylindrical to oval, usually elongate-elliptical; 14–28 × 30–140μ, most about 25 × 125μ. Spores usually completely formed in the sporangium before emerging, and then emerging separately with the ciliated end directed backward (not rarely the entire contents of the sporangium discharged as a naked mass before the spore

PLATE 60

PLATE 60

Sapromyces Reinschii

Figs. 1, 2, 3, 4. Sporangium showing stages of spore formation.
Fig. 5. Sporangium with precipitate on wall.
Fig. 6. Habit of hyphae and sporangia.
Fig. 7. Sporangium containing encysted spores.
Fig. 8. Spore which emerged as in fig. 12 or 14.
Fig. 9. Sporangium showing emerging spores.
Fig. 10. Sporangium arising in a whorl of hyphal segments.
Fig. 11. Small sporangia; one discharging two spores.
Fig. 12. Sporangium discharging partly formed spores through a papilla, leaving precipitate.
Fig. 13. Sporangia; one containing sprouting spores.
Fig. 14. Sporangium discharging contents as an undifferentiated mass.

Figure 6 × 107; others × 447.

SAPROMYCES REINSCHII.

origins appear, and then spores, irregular in shape and size, formed out in the water); escaping through a terminal pore or not rarely through a papilla; biciliate, monoplanetic, shaped as in *Saprolegnia* or *Achlya*, 8–14μ thick, normally about 10μ. No oogonia or anthe·idia observed in our form.

Found only once by us and then growing with *Achlya* and *Saprolegnia* in a culture collected near Wilmington, N. C. (No. 3 of December 30, 1921, J. N. Couch). Reported hitherto in America only at York, Maine, by Thaxter (Bot. Gaz. **19**: 49, pl. 5. 1894) on cones and twigs of *Pinus* in a spring. Otherwise recorded from Germany by Reinsch on *Viscum* stems and on algae (Jahr. f. wiss. Bot. **11**: 298, pl. 15, figs. 1–11, 1878, as *Naegelia* species I and species II), again from Luneburg, Germany, by Minden on coniferous twigs ('12, p. 589, figs. 11a and 11b on p. 590), and from Denmark by Petersen (Ann. Myc. **8**: 527. 1910). Minden says that the plant was also found by Claussen. Thaxter found a strain of this plant which bore sporangia luxuriantly but showed no indication of any form of sexual reproduction. Later in the season he secured additional specimens from the same spring, one of which furnished fine examples of the curious oogonia and antheridia. Petersen also found both sexual and sterile individuals, and he states that the sporangia were more cylindrical in the sterile ones, suggesting that two species may be involved. Thaxter considers both forms to be conditions of a single species in which the sporangia are quite variable. There seems to be little doubt that our plant is identical with the sexually sterile strain of Thaxter and also that of Petersen.

Thaxter describes the primary axis as originating "as a single basal cell or segment which is attached by its roughened surface directly to the substratum, without rhizoidal outgrowths. It is often more or less bent and distorted but otherwise undifferentiated . . ." This basal cell was not observed in our original culture, but when the fungus was cultivated in corn juice or pea juice the basal segment became obvious and was seen to be slightly swollen and distorted.

There are certain peculiarities in the discharge of the sporangia that are worthy of comment. The contents of the sporangium may be completely formed into spores within the sporangium and the spores emerge to swim away immediately upon gaining their exit (fig. 9), or the contents of the sporangium may be discharged with the spores partly formed, to complete their formation out in the water at the sporangial mouth (fig. 12), or the sporangial contents may be discharged as an undifferentiated naked mass of protoplasm, the spores forming outside (fig. 14). All three methods of sporangial discharge may be taking place synchronously in the same culture. If the spores emerge when they are only partially differentiated a heavy precipitate is left on the walls of the sporangium (figs. 5 and 12); this precipitate is not left if the spores are completely formed before discharge or if the contents discharge as an undifferentiated mass (figs. 9 and 14). Thaxter notes only the internal formation of the spores and their escape one by one from the sporangium, swarming at once. On the other hand, Petersen states that the spores "emerge in a great vesicle which soon bursts." This is contrary both to Thaxter's observations and to ours. We find that in case the spore mass escapes before the differentiation of the spores the mass is not surrounded by a vesicle but lies naked in the water. Minden takes a somewhat intermediate position, stating that the spores escape in a bladder which, however, soon bursts so that most of the spores issue directly from the sporangium. He finds the spores to have the form shown in the second swimming stage of the *Saprolegniaceae*, with the cilia arising from a depression on one side. The scantiness of our material prevented our observing their form with certainty.

This rare and peculiar plant grows very poorly under laboratory conditions. Attempts were made to cultivate it on all the subtrata on which Achlyas and Saprolegnias ordinarily grow well, as, for example, corn meal agar, termite ants, bits of boiled corn grain, peas and beans, but without successful results. Unsuccessful attempts were also made to grow the fungus on pieces of pine cones and twigs both green and dry. The plant grows slowly in vegetable juices obtained by boiling corn grains or peas, and it was from such cultures and from the original collection that our observations were made.

For synonomy and other references see Thaxter, as above cited.

MONOBLEPHARIDACEAE*

Phycomycetes living in water and saprophytic on plant or animal remains. Mycelium slender, branched, not divided into cells or constricted at intervals. Sporangia apical; zoospores with one or two cilia. Oogonia containing only one egg each and opening at maturity to admit

*We call attention here to the remarkable genus *Myriablepharis* Thaxter (Bot. Gaz. **20**: 482, pl. 31, figs. 1-5. 1895). It is of quite uncertain position and Thaxter does not assign it to any family. According to him the zoospores are ciliated all over as in *Vaucheria*, a condition not known in any other fungus. No sexual reproduction has been observed. The plant has been found more recently in Breslau, Germany, by Minden ('12, p. 476) who confirms Thaxter's observations. He is inclined to think, however, that the remarkable spores are not those of the plant but of a parasitic protozoan, the fungus being a species of *Pythium*. Lotsy ('07, p. 125) has, apparently without sufficient reason, placed the genus in the *Monoblepharidineae*. In this place we might also refer to the genus *Rheosporangium* Edson (Journ. Agric. Research **4**: 279. 1915) which he places in the *Saprolegniaceae*. We can see no reason why it should not be considered a species of *Pythium*.

the sperms. Antheridia formed near the oogonia, producing uniciliate sperms which escape and fertilize the eggs. The fertilized egg matures within the oogonium or passes out of it and matures attached to its tip.

For many years this family contained only the remarkable genus *Monoblepharis* established by Cornu in 1871, (p. 59) and later described more fully and figured ('72). Lagerheim in 1900 (pp. 32 and 39) proposed the genus *Diblepharis* to contain the two American species described by Thaxter ('95). For other important literature see Fischer ('92, p. 378); and Woronin ('04). Tiesenhausen ('12) finds *M. polymorpha* and *M. macrandra* and gives a figure of the former. The genus *Gonapodya* of Fischer, placed by him in the *Monoblepharidaceae*, has been shown by Thaxter ('95a) to be better disposed of in the *Leptomitaceae* (which see). We have not found any species of this family, but give below an enumeration of the genera and species for the convenience of students:†

MONOBLEPHARIS Cornu, 1871, p. 59.

Zoospores with one cilium; all the contents of the sporangia and oogonia entering into their products.

Sub-genus *Eumonoblepharis* Lagerheim. Fertilized eggs maturing within the oogonia. Includes only one species.

M. sphaerica Cornu. Europe.

Sub-genus *Exoospora* Lagerheim. Fertilized eggs moving out of the oogonium and maturing outside.

M. polymorpha Cornu. Europe and New England (Thaxter).

M. polymorpha var. *macrandra* Lagerheim (l. c., p. 35) [*M. macrandra* (Lagerh.) Woronin.] Europe.

M. brachyandra Lagerheim (l. c., p. 37). Europe.

In the two following imperfectly known species the sexual reproduction has not been seen and their relationships are therefore uncertain:

M. regignens Lagerheim (p. 39).

M. ovigera Lagerheim (p. 39).

Woronin ('04) figures an intermediate form that he thinks may be a hybrid between *M. p.* var. *macrandra* and *M. sphaerica*.

DIBLEPHARIS Lagerheim, 1900, p. 39.

Zoospores with two cilia, on escaping leaving a drop of oil in the sporangium. Oogonium on opening discharging a part of its proto-

† The plant described by Hine as *Monoblepharis lateralis* (Am. Quart. Micr. Journ. **1**: 141. 1879) is evidently not of this genus but probably a *Saprolegnia* which was incorrectly observed and interpreted.

plasm into the water, the remainder (larger part) forming the egg which matures within the oogonium.

D. insignis (Thaxter) Lagerheim (p. 40). Massachusetts and Maine.

D. fasciculata (Thaxter) Lagerheim (p. 40). Massachusetts.

BLASTOCLADIACEAE

This family has been based on a single genus, *Blastocladia*, whose systematic position has been and still is somewhat doubtful. Thaxter ('96) thinks that the genus should be placed either under the *Pythiaceae* or in a new family of its own. Minden ('12, p. 506) and Petersen ('10, p. 532) recognize the family *Blastocladiaceae* and it would seem as well to follow them for the present. We are also including in the family the genus *Allomyces* Butler, placed by the author in *Leptomitaceae*. Except for a few additions made to include *Allomyces* the following diagnosis is taken with a few changes from Minden:

Saprophytic fungi living in water on substrata of plants. Mycelium unicellular, or in *Allomyces* with complete septa at the nodes, rather abundantly branched, separated usually into a main axis and secondary axes and sometimes having sterile, thin threads of unknown function. Sporangia usually ellipsoid to cylindrical in shape, often clearly forming sympodia through the shortening of the threads upon which they grow, but also thickly crowded together, seldom growing through each other. Zoospores ellipsoid to egg-shaped with broad blunt ends and one (or two?)* cilia on the broader, colorless end, the other end containing small granules; emerging with force through an apical opening in the sporangium and swimming away, then, after being surrounded by a membrane, sprouting; while entering into the resting condition there is amoeboid motion. Sexual reproduction does not take place. Instead there are formed resting bodies in the shape usually of broad ellipsoid cells that coincide in origin and position with sporangia, but have a membrane consisting of two sheaths of which the outer one is smooth and colorless while the inner one appears finely and regularly dotted (or punctured). At maturity these cells either fall away as a whole or the thin, colorless, outer sheath splits at the tip and allows the escape of the inner sheath and its contents. After a time the resting cells germinate by the cracking of the thick brown wall, so as to allow the protrusion of a delicate bladder in which the spores develop. Spore discharge, however, has not yet been observed.

BLASTOCLADIA Reinsch, 1878, p. 291.

Characters of the family except that the plant body is never septate. Four species have been described, all of which are treated in Minden's

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*Thaxter finds zoospores to have two cilia as a rule in *B. Pringsheimii.*

work. Thaxter finds *B. Pringsheimii* both at Cambridge, Mass., and at Kittery Point, Me. He also describes a new species, *B. ramosa* from Kittery Point. The four described species* we list below, with references to authors and important figures, omitting full descriptions, which may be easily found under the references:

Blastocladia Pringsheimii Reinsch '78, p. 291, pl. 16, figs. 1–13. See also Thaxter '96, p. 51, pl. 3, figs. 1–13; and Petersen '10, p. 532, fig. 10. Sporangia much elongated, resting bodies with thick and pitted wall, not slipping from a sheath at maturity; sterile, slender filaments often present among the reproductive bodies.

Blastocladia ramosa Thaxter '96, p. 50, pl. 3, figs. 14–16. Sporangia shorter; resting bodies with thin and scarcely pitted wall; sterile filaments absent.

Blastocladia rostrata Minden '12, p. 604. Much like *B. Pringsheimii*, but resting bodies slipping from sheath at maturity.

Blastocladia prolifera Minden '12, p. 604. Much like *B. ramosa*, but sporangia proliferating internally, as in *Saprolegnia:* the only species with this habit. Resting bodies slipping from a sheath at maturity.

ALLOMYCES Butler, 1911, p. 1023.†

Plant small, slender, the short or long stalk not conspicuously differentiated; branches usually dichotomous, often verticellate in groups of 3–5, separated from the nodes by distinct and complete septa, not constricted at intervals; in vigorous cultures repeating the branching in the same way to form a complex plant. Sporangia oval, terminal, sympodially arranged, not rarely in chains of several, often clustered by the shortening of the branches, which continue the stem by one or more lateral buds beneath. Spores biciliate at times, but the two cilia so closely approximated or fused as usually to appear as one. Resting bodies borne in the same way as the sporangia and of the same size and shape, at maturity enclosed in a thin, hyaline sheath out of which they finally fall through an apical slit; the wall brown and conspicuously pitted as in *Blastocladia*: the whole representing a thin-walled oogonium completely filled with a thick-walled parthenogenetic egg, or a resting sporangium as thought by Barrett ('12a, p. 365).

A saprophytic aquatic of anomalous structure and differing from all other Phycomycetes in the regular and normal septation of the plant body.

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* A fifth, *B. strangulata* Barrett, is treated here as a synonym of *Allomyces arbuscula*, which see below.

† The following treatment of the genus and species, as well as the plate, is taken with slight modification from Coker and Grant in Journ. E. Mitchell Sci. Soc. **37**: 180. 1922 (as *Septocladia dichotoma*).

Allomyces arbuscula Butler. Ann. Bot. **25**: 1027, figs. 1–18. 1911.

Blastocladia strangulata Barrett. Bot. Gaz. **54**: 353, pls. 18–20. 1912.

Septocladia dichotoma Coker and Grant. l. c.

PLATE 61

Characters of the genus. Threads extending about 3 mm. from the substratum on a termite ant, about 10–37μ thick, growing gradually more slender distally at each joint, basal joints 35–130μ long, those of central region up to about 675μ long; tips blunt, hyaline. Sporangia oval, 28–46 × 55–76μ; spores escaping singly or at times, according to Barrett, in a vesicle that soon bursts, emerging through one or two usually apical holes or short papillae, biciliate (or uniciliate by fusion of the two cilia?), oval when swimming, with the cilia apical, monoplanetic, amoeboid before encysting, 10μ thick when at rest; sprouting by a slender thread. Resting bodies appearing later than the sporangia but of the same shape, 25–39.2 × 36.3–49.2μ, the conspicuous pits apparently sunken from the outside in regular fashion as in *Blastocladia Pringsheimii*, at maturity slipping from the thin, clasping sheath; sprouting into zoospores after a rest (Barrett). The thick wall is divided into two parts, an outer layer (pitted) about 1.8μ thick and a homogeneous inner one about 1μ thick.

Found only once, October 20, 1921, on a knuckle bone of beef partly covered with water, in Sparrow's pasture, Chapel Hill, N. C. (F. A. Grant, coll.). Reported heretofore only from India (Butler, l. c.), and from Ithaca, N. Y. (Barrett, l. c.); but Dr. Weston of Harvard writes me that he has it from the Philippines.

Butler places the genus in the *Leptomitaceae*, but it seems to us that there can be no doubt of the close relationship of this plant to *Blastocladia*, in which genus it was placed by Barrett. From accounts by Butler and by Barrett there appears to be practically no difference between their plants and ours. Butler gives the maximum length of the resting spores as 60μ, but the difference is probably of little or no importance. We had unfortunately overlooked these two papers in recording our plant. Barrett gives interesting cytological detail. He found pores in the cross walls of his form. Butler does not record these nor have we found them.

In the form of the sporangia and resting cells and in the absence of sterile filaments among them our plant resembles most closely *B. ramosa* and *B. prolifera*. The remarkable resting bodies with their thick brown strongly pitted walls and peculiar habit of slipping at maturity from the closely fitting sheath are so strikingly similar in structure and habit to those of *B. rostrata* and *B. prolifera*, and in structure to those of *B. Pringsheimii*, that one is convinced of their close relationship.

In a discharging sporangium a few spores that failed to get out were observed to crawl about actively in an amoeboid fashion for a good while.

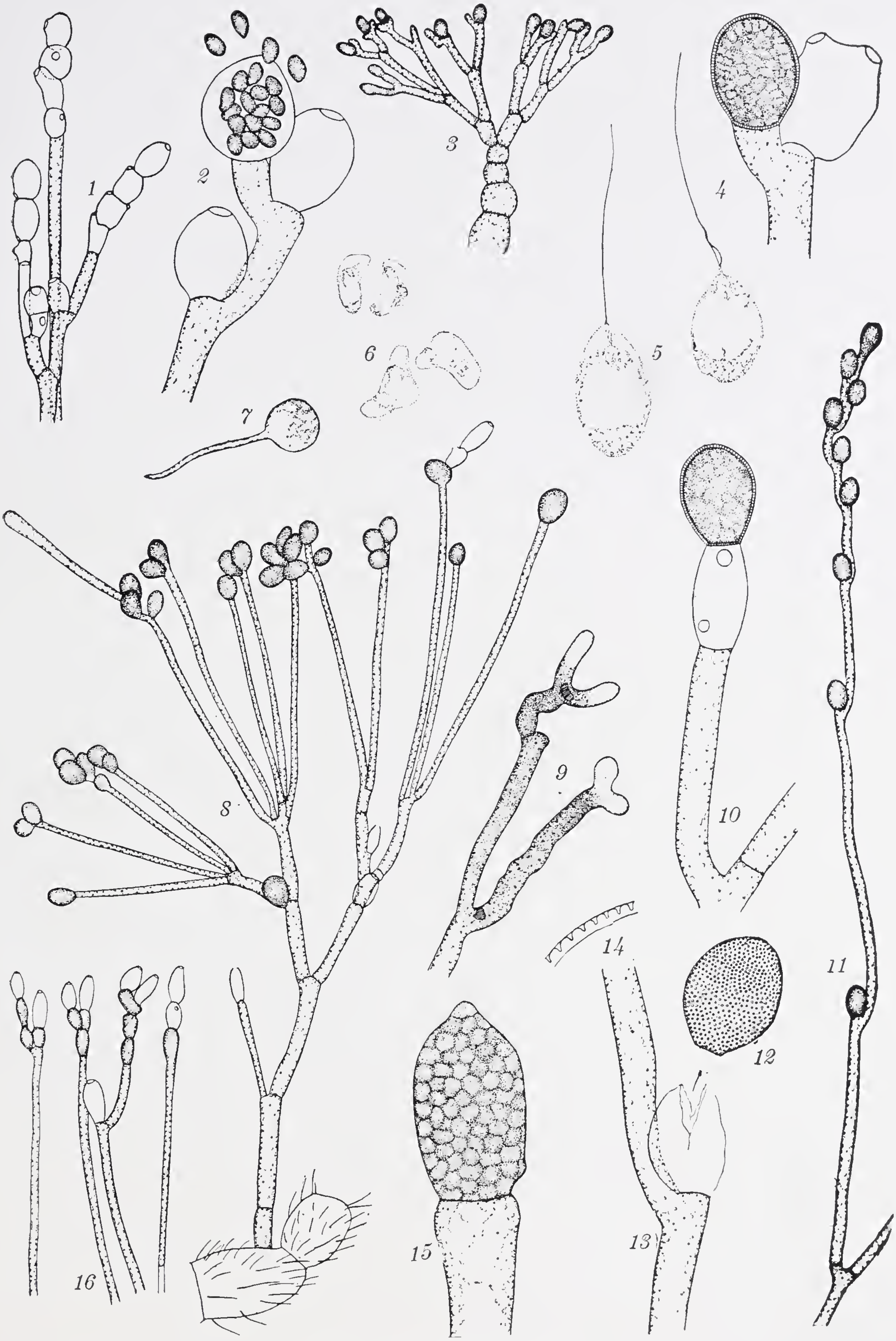
PLATE 61

PLATE 61

Allomyces arbuscula

Fig. 1. Empty sporangia in chains. × 154.
Fig. 2. Three sporangia, one discharging spores, two empty. × 420.
Fig. 3. Vegetative branch, showing short joints and young resting bodies. × 59.
Fig. 4. Optical section of resting body and empty sporangium with two apertures. × 420.
Fig. 5. Two spores showing cilia. × 1296.
Fig. 6. Spores showing amoeboid movement before encysting. × 810.
Fig. 7. Sprouting spores. × 1008.
Fig. 8. Habit sketch, showing empty sporangia and resting bodies. × 96.
Fig. 9. Vegetative tips, showing refractive bodies and clear blunt tips. × 150.
Fig. 10. Optical section of mature resting body with empty sporangium below. × 420.
Fig. 11. Long, slender thread on corn grain, showing sympodial arrangement of resting bodies. × 96.
Fig. 12. Surface view of resting body, showing pits. × 420.
Fig. 13. Part of branch, showing thin sheath out of which the resting body (Fig. 12) has slipped. × 420.
Fig. 14. Section of thick wall of the resting body, showing the pits and the sheath outside. × 1296.
Fig. 15. Mature sporangium just before discharge of spores. × 420.
Fig. 16. Group showing some sporangia before maturity and some after emptying. × 96.

PLATE 61



ALLOMYCES ARBUSCULA

After an hour they had encysted and one had sprouted. The spores are of a peculiar internal structure, resembling closely those of *B. Pringsheimii* as shown by Thaxter (l. c., pl. 3, fig. 11). Most of the protoplasm is at the end opposite the cilia, the center is almost clear and the cilia seem to extend down through the clear tip to a protoplasmic mass below, as shown in our fig. 5.

On an agar plate the plant does not do well. A few root-like threads grow out, branched and with cross-walls in the older portions, and in these older portions are found resting bodies or sporangia, sometimes fifteen or twenty of the latter in a row. The reproductive bodies are sometimes found in clusters or single on short lateral stalks.

On boiled corn agar the growth is good. The threads are about the same size as on an ant but average longer, as much as 5 mm., and the protoplasm is not as dense as when grown on ants. Threads at substratum as large as 102μ in diameter. Sporangia are produced better than on ants, and resting bodies are so abundant that the entire culture assumes a brick dust color to the unaided eye. The resting bodies are at first dark and have numerous large oil droplets. As they get older the walls assume a yellow-brown color and the contents becomes homogeneous.

PARASITES OF THE WATERMOLDS

The parasites of the water molds have been conveniently grouped by Fischer ('92, p. 149), and we adapt the following from him with the addition of two species of *Olpidiopsis* recently described. To Fischer one may also go for the literature. We are describing more fully only those species which we have had an opportunity to study in the living condition.

On hyphae of *Saprolegnia:*	*Olpidium Borzianum; Pseudolpidium Saprolegniae; Olpidiopsis Saprolegniae; Olpidiopsis echinata* (See Petersen in Ann. Myc. **8**: 540. 1910); *Olpidiopsis major**; Woronina polycystis; Rozella septigena.*
On hyphae of *Achlya:*	*Pseudolpidium fusiforme; Olpidiopsis minor; Olpidiopsis index; Rozella simulans.*
On oogonia of *Saprolegnia* and *Achlya:*	*Rhizophidium carpophilum; Rhizidiomyces apophysatus.*
On *Aphanomyces:*	*Pseudolpidium aphanomyces.*
On *Rhipidium spinosum:*	*Pleolpidium Rhipidii.*
On *Monoblepharis polymorpha:*	*Pleolpidium Monoblepharidis.*

* See Maurizio ('95), p. 15, figs. 4–9. Grows on *S. ferax* and *S. hypogyna.* This is very doubtfully distinct from *O. Saprolegniae.*

Olpidiopsis Saprolegniae (Cornu) amend. Fischer. Rabenhorst's Krypt. Fl. 1, part 4: 38, fig. 4. 1892. (2nd. ed.).

Olpidiopsis Saprolegniae Cornu, in part. Ann. Sci. Nat. Series 15, 5: 145, pl. 3, fig. 10. 1872.

PLATE 62, FIGS. 7–10

Sporangia usually elliptical but sometimes spherical, smooth, very variable in size, sometimes as much as 70μ through the shortest diameter, usually occupying intercalary swellings, but may occur in oogonia or sporangia; emptying by one or more tubes which penetrate the host's wall. Spores very minute and numerous, bean-shaped with two lateral cilia (Maurizio also finds two cilia in *O. major*), swimming rapidly, emerging through internal pressure in a slender stream and collecting at the opening in a dense irregular mass. The spores jerk rapidly and the peripheral ones free themselves by degrees until all get away. The whole process occupies several minutes and after the pressure is relieved in the sporangium a good many spores remain in the sporangium and swim rapidly there for a good while, only emerging one by one as they find the opening (apparently by chance). Oogonia up to 74μ thick; almost always elliptical. the wall rather thick and covered with protuberances which show remarkable variation from low rounded warts to long, sharp pyramidal spines. Eggs average 33μ in diameter, usually one, at times two in an oogonium which they do not quite fill. Antheridial cells about 18μ thick, varying little, elliptical to spherical, much smaller than the oogonia, thin-walled, smooth; one, as a rule, attached to each oogonium but rarely as many as three; filled with protoplasm when young, but empty when eggs are mature.

Found several times around Chapel Hill on *A. imperfecta* and *A. flagellata* in Arboretum branch.

Our plant agrees well with Fischer's description except for the thickness of the antheridia which in Fischer's plant is given as 28–30μ. In addition to figures by Fischer and Cornu cited above, see Petersen ('10), fig. 18b.

In one case material with sporangia covered with a glass was watched for half an hour. None of the sporangia emptied while the cover was on. The cover was removed and almost instantly one of the sporangia began shooting out its contents, the spores still in the initial stages of formation. The protoplasm remained at the sporangial mouth and about fifteen minutes after the sporangium had emptied some of the protoplasm began to assume the form of spores which showed a rocking movement. In ten minutes more the entire mass had formed spores, many of which were breaking from the mass and swimming away. The material was stained with iodine and two lateral cilia demonstrated on the spores.

In material stained with iodine and then preserved in glycerine which contained a few drops of eosin the sporangia stained a beautiful red,

PLATE 62

PLATE 62

Olpidiopsis saprolegniae var. levis

Fig. 1. Sporangia causing gall in tip of hypha.
Fig. 2. Sporangia sending tubes through wall of host.
Fig. 3. Two empty sporangia and one oogonium with an empty antheridium.
Fig. 4. Several small sporangia in a gall.
Fig. 5. Oogonia with antheridia.
Fig. 6. Sporangia in hypha of a species of *Aphanomyces*.

All figures × 447, and all from *Saprolegnia ferax*.

Olpidiopsis saprolegniae

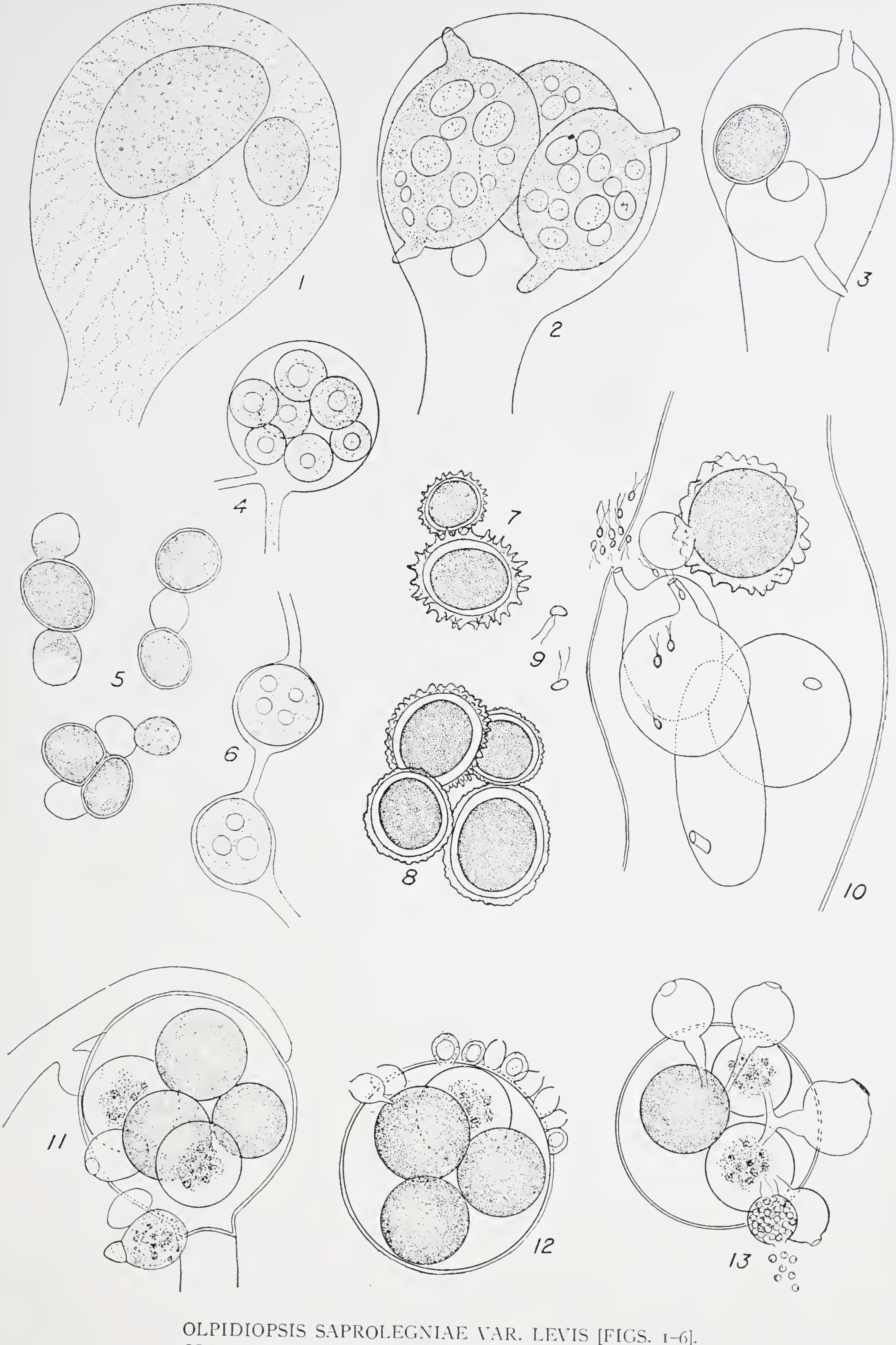
Fig. 7. Oogonia with spines.
Fig. 8. Oogonia merely rough or warted.
Fig. 9. Spores with cilia.
Fig. 10. Oogonium with antheridium, and sporangia discharging spores, all inside a hypha of *Achlya flagellata*.

Figure 9 × 613; others × 447.

Rhizophidium carpophilum

Fig. 11. Sporangia on oogonia of *Achlya apiculata*, the lower one with a short protrusion which is about to break.
Fig. 12. Smaller sporangia on oogonia of *Achlya apiculata*, some already discharged, others forming resting bodies.
Fig. 13. Sporangia on oogonia of *Achlya apiculata*, one of the sporangia in act of giving off spores.

All figures × 670.



OLPIDIOPSIS SAPROLEGNIAE VAR. LEVIS [FIGS. 1–6].
OLPIDIOPSIS SAPROLEGNIAE [FIGS. 7–10].
RHIZOPHIDIUM CARPOPHILUM [FIGS. 11–13].

while the oogonia and the few antheridia which remained partly empty were stained a greenish brown. This contrast readily enables one to distinguish sporangia from oogonia.

Antheridia in all stages were observed: some full of protoplasm and egg adjoining not formed; some partly empty and the egg beginning to show a clear space between itself and the oogonial wall, and some entirely empty with the eggs formed. An antheridium one-third empty was watched. Slowly the remainder of its contents passed into the oogonium until about one-third of the original contents was left and then a cover glass was dropped on the material. Immediately afterwards part of the contents of the oogonium shot back into the antheridium, refilling it and demonstrating an open connection.

The oogonial spines are so hyaline as to be barely visible in oogonia which have just formed eggs, but they become much more obvious after the eggs are a day old. Young oogonia stained with iodine show spines, which otherwise would have been overlooked.

Olpidiopsis Saprolegniae var. levis n. var.

Plate 62, figs. 1–6

Sporangia spherical to elliptic, smooth, very variable in size and number, usually occupying the swollen ends of hyphae but not rarely also in intercalary swellings; emptying by one or two tubes which penetrate the host's wall, but go little beyond and are usually short, at times however as long or longer than the diameter of the sporangium: spores very minute and numerous, probably with two cilia, swimming rapidly, emerging at first through internal pressure and probably showing the same sequence as described in the preceding species, but not all stages observed. Oogonia elliptic to nearly spherical, with the wall rather thick and quite smooth and even; antheridial cells smaller than the oogonia, smooth, thin-walled, one or two attached to each oogonium, their contents usually disappearing entirely by the time the oogonia are mature.

Parasitic on species of *Saprolegnia* as *S. ferax*, *S. monoica*, and probably others. Found many times in Chapel Hill, as in the small pond below Glen Burnie spring, where it may be secured almost with certainty at any time a series of collections is made.

The most important difference between this plant and *O. Saprolegniae* is that the oogonia are smooth and seem to average smaller. In the latter they are densely covered with warts or spines. This difference is striking and will make it necessary to consider our plant a variety. The two species of this genus with smooth oogonia that are so far described are parasitic on algae (see Fischer, '92, p. 37).

Rhizophidium carpophilum (Zopf) Fischer. Rabenhorst's Krypt. Fl. **1.** 4: 95. 1892. (2nd. ed.).

Rhizidium carpophilum Zopf. Nova. Acta. Acad. Leop. **47**: 200, pl. 20, figs. 8–16. 1884.

PLATE 62, FIGS. 11–13

Sporangia subspherical, seated on the surface of the oogonia of the host, varying greatly in size and number, about 10–30μ in diameter, emptying through a more or less apical, thin-walled, short beak which seems to break and then collapse and disappear; spores very minute and nearly hyaline except for one or two dots, swimming rapidly. They emerge singly and from their own efforts, and dash around madly inside the sporangium when the beak opens, seeming to find the way out purely by accident. It usually takes several minutes for all to get out; cilia number not determined (said to be one). Sporangia attached to the swollen tip of a mycelial thread which branches farther down and enters the eggs of the host and eventually destroys them. Certain of the smaller sporangia do not discharge at once, but go into a resting state, the contents becoming compacted into a dense refractive body.

Observed several times in Chapel Hill, where it is parasitic on the oogonia of *Achlya apiculata*, *A. flagellata* and *A. conspicua*. The eggs of the host become defined, in all cases we have seen, before being disorganized, and usually they are not all attacked, one or several arriving at healthy maturity amid the detritus of those destroyed.

The present plant agrees well with *R. carpophilum* as described and figured by Zopf, except that the hypha entering the oogonium is thicker than he shows. *Rhizidiomyces apophysatus* differs in the swelling just inside the oogonium wall, in the long and more persistent emptying beak and in the emergence of the spores in a bladder.

Rhizidiomyces apophysatus Zopf. Nova Acta Acad. Leop. **47**: 188. pl. 20, figs. 1–7. 1884.

PLATE 63

Sporangia spherical to subspherical, varying greatly in size, from 10 to 45μ thick; seated on the surface of the oogonia of the host and communicating with the interior of the oogonia by means of a tube-like body which may expand into an internal bladder (which may measure as much as 18.5μ thick) from which numerous rhizoid-like structures arise and penetrate the eggs or mingle with the oogonial contents. Spore initials appearing early and then disappearing by complete coalition to reappear only after discharge into an apical bladder. Spores discharged somewhat as in *Pythium*, the spore mass passing out through a thin-walled tube, one to three times as long as the diameter of the sporangium, which expands at the tip into a very delicate bladder into which the spore mass flows. Here the spore initials gradually appear again and complete their development. Before discharge they show a distinct

PLATE 63

PLATE 63

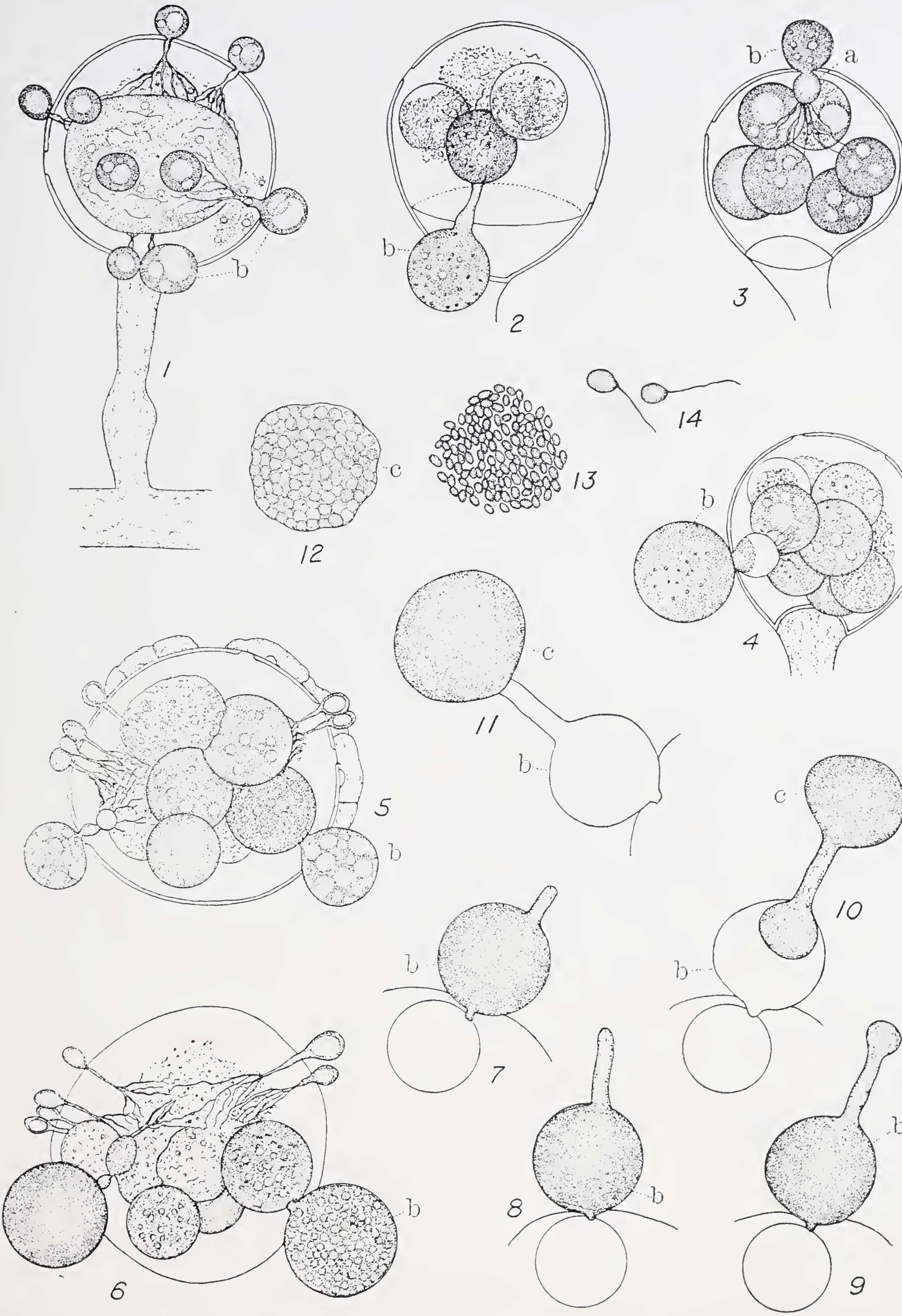
RHIZIDIOMYCES APOPHYSATUS
on
ACHLYA CONSPICUA

Figs. 1–5. Oogonia infected with the parasite to a greater or less extent. In Fig. 1 the formation of eggs has been prevented by an early attack. The parasite shows the rhizoidial rootlets entering the protoplasm of the host, the stalk passing to the exterior which is often swollen to form an apophysis (a), and the exterior swelling which becomes the sporangium (b). All × 503.

Fig. 6. The same oogonium shown in Fig. 5 drawn 16 hours later, the sporangium (b) now full size and showing the spore initials. × 503.

Figs. 7–13. The same sporangium (b) shown in Fig. 6, with the resulting spores, the figures drawn at intervals of about 10 minutes. The sac (c), in which the spores are emptied, is shown free in Fig. 12, and fully developed spores in Fig. 13, just after the disappearance of the sac membrane. All × 503.

Fig. 14. Two active spores with a single cilium each. × 1028.



RHIZIDIOMYCES APOPHYSATUS.

rocking motion which is soon followed by the appearance of a hole in the bladder out of which the spores rush rapidly one by one. Spores subspherical, very minute, 3.7μ, with one apical cilium.

Sexual reproduction unknown.

Recognized only twice at Chapel Hill, and then on oogonia of *Achlya conspicua* collected from Arboretum branch (No. 7 of June 29 and No. 1 of July 10).

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