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AN ACCOUNT OF THE GENUS PYTHIUM AND SOME CHYTRIDIACEÆ

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Imperial Mycologist

AGRICULTURAL RESEARCH INSTITUTE, PUSA

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PREFACE.

THE work which is included in the following paper was commenced under the guidance of Professor M. M. Hartog of Queen's College, Cork, and continued at several places since, in part in the intervals of other work which left little time for systematic study.

My thanks are due to the late M. Maxime Cornu and M. Ph. van Tieghem of the Muséum d' Histoire Naturelle, Paris, to Prof. Fr. Oltmanns of Freiburg i. Br. and to Dr. Scott, Director of the Jodrell Laboratory, Kew, for kind permission to work in their laboratories. But I am most indebted to Prof. Hartog and to M. Georges Poirault, Director of the Jardin d' Acclimatisation of the Villa Thuret, Antibes, for valuable advice and assistance freely rendered while working in the laboratories under their control.

AGRICULTURAL RESEARCH INSTITUTE,
PUSA, BENGAL, INDIA.
8th July 1906.

E. J. BUTLER,
Imperial Mycologist.

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By E. J. BUTLER.

PART I.

AN ACCOUNT OF THE GENUS PYTHIUM.

INTRODUCTION.

The genus *Pythium*, a small group of primitive fungi with both aquatic and terrestrial forms, is of considerable interest to students of the lower fungi. Originally described from aquatic species and included, with the family *Saprolegniaceæ*, in the algæ, the genus was transferred by de Bary to the *Peronosporaceæ* at the commencement of his main line of the fungi. It was again included in the *Saprolegniaceæ* by Berlese and de Toni (1888). Schröter (1897) created a special family, the *Pythiaceæ*, included in the *Saprolegniaceæ*, and divided the species into two genera, *Nematosporangium* and *Pythium*. All the species are, however, usually regarded as constituting the single genus, *Pythium*. Neither Fischer (1892) nor Schröter (1897) accept the primitive position assigned to the genus by de Bary; while the majority of recent workers agree with him in including it in the *Peronosporaceæ*.

The *Pythiaceæ* belong to the class Phycomycetes—the "algæ-like" fungi. As in the majority of these, the thallus is unsegmented while young. The absence of chlorophyll is the main distinguishing point from some families of the algæ. In place of the sexual free-swimming gametes of the latter, fertilisation of an oogonial cell takes place by means of a tube protruded from a fixed antheridial cell, which penetrates the oogonium and liberates a nucleus and cytoplasm into the ooplasm. The asexual reproduction is effected by swimming zoospores liberated from a zoosporangium, which is undifferentiated from the vegetative thallus in the lower forms, but cut off as an enlarged, more or less spherical, cell in the higher. As the genus invaded the land, the swimming spores were gradually suppressed, and in the higher forms the sporangium germinates as a whole by putting out a germ filament which grows into a new plant.

Attention was first directed to the economic importance of the genus by the work of Hesse (1874), on *Pythium de Baryanum.* This destructive parasite of seedlings has been frequently observed since, and the list of plants which it is known to attack is now considerable. A number of other species have been since described as active parasites, and it is improbable that our knowledge of the parasitic forms and the diseases which they produce is at all complete.

The genus is well known as a favourite laboratory type for exhibiting the phenomena of sexuality in fungi. Antheridium and oogonium are formed in a simple manner permitting of the readiest examination in the living state. In spite of the vast increase in recent years of our knowledge of the forms of reproduction in fungi, *Pythium* is likely to hold its own in this respect. Although sexuality or, probably, an equivalent process is now known to exist very generally even in the higher fungi, it is often difficult either to see or to interpret. In the *Peronosporaceæ*, for instance, the sexual organs are formed in the interior of the tissues of higher plants, and the details of fertilisation cannot easily be followed in the living condition. In the *Uredineæ* Blackman (1904) has shown that fertilisation of a reduced sort occurs in the base of the *Æcidium*, but the process is not completed by fusion of the nuclei until the greater part of the life cycle has been completed. This indicates that in the higher fungi, such as the *Basidiomycetes* and higher *Ascomycetes*, where a nuclear fusion is known to occur at a definite stage, the actual fertilisation, if one exists, is to be looked for much earlier, and these groups require re-investigation. In any case their sexual processes, if any, are infinitely more difficult to follow than in the simple type of the genus under consideration. For this reason *Pythium* is perhaps the best known, as it was one of the first, of the types showing a true fecundation.

The systematic position of the genus is of extreme interest. So marked are its affinities to three of the five main groups usually included under the Phycomycetes that a knowledge of the relationships of this single genus is essential in any attempt at understanding those of the lower fungi as a whole.

A further, and scarcely less interesting, point is that strong indications occur in the group of that transition from water to land which probably took place in several instances amongst the fungi. Its least differentiated forms are aquatic, its most differentiated terrestrial, and some of the adaptations acquired by aquatic forms when they colonised the land are beautifully shown.

In spite of the importance which attaches to the genus, especially from the phylogenetic point of view, its study has been somewhat neglected. Since the early work of de Bary, to whom we owe so much of our knowledge of the Phycomycetes, no general survey has been made, including any considerable number of species, excepting that of Ward (1883). Berlese in his "Monograph of the Peronosporaceæ" (1898), though including *Pythium* under that head, does not deal with the genus at all. The study of Schröter in Engler and Prantl's "Pflanzenfamilien" contains nothing new. Alone the cytology of the reproductive processes has been the subject of some recent investigations [Trow (1901), Miyake (1901)], which have been of much value from the point of view of the affinities of the group. Partly as a result of this neglect, and partly from the imperfections of some of the original descriptions, the identification of particular species is attended with much difficulty. Herbarium specimens are practically useless for this purpose, and are, besides, exceedingly rare. Another reason which doubtless has tended to check work is the supposed difficulty of obtaining material. Yet I believe there are few fungi more widely distributed than the *Pythiaceæ*, though the actual number of species appears to be small. They merely require to be sought for definitely, since their presence is not disclosed by a casual observation. A simple process will usually give three or four species almost anywhere that humus-rich soil and pond water are available, yet without this their presence is almost certain to escape detection. The following method has rarely failed to give me satisfactory results. Garden earth, preferably from the neighbourhood of the roots of higher plants, is taken three to six inches below the surface of the soil and placed in a large shallow vessel to which enough tap water is added to leave a layer free above the earth. In this the substratum is floated. Numerous trials have shown that the nature of the latter is by no means a matter of indifference. Flies and meal-worms, so much used for the growth of aquatic fungi, are exceedingly liable to attract minute protozoa and also zooglœa-forming bacteria which may interfere seriously with subsequent work. By far the best medium which I have found for general use is boiled sliced root of *Abutilon*[1] either plain or steeped in decoction of flies. For an animal substratum centipedes were found much cleaner than flies. For small cultures ants are very satisfactory.[2] Small softened twigs of elm and various other trees which have lain in water for some time are also suitable in some cases. So also are cress seedlings (for *P. de Baryanum*) potato, slabs of Basidiomycetes (*Agaricus*, *Clathrus*, &c.) and various other substances. Working in the tropics I have found it very useful to acidify slightly with citric acid the water used during the first step in the isolation from earth, in order to keep down bacteria which are capable of strongly inhibiting the growth of fungi. The acid often, however, interferes with spore-formation, so that it is necessary to shorten the time in it as much as possible. After twenty-four hours, the culture substratum is removed and placed in a relatively large quantity of

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[1] None of the other *Malvaceæ* which I have tried gave as good results.

[2] Species of *Aphanomyces* seem to have a special liking for ants.

water, and after another twenty-four hours examined. Algæ and vegetable debris from ponds or streams also generally afford one or more species. They are treated in exactly the same way as above described, being covered with water in which fresh food for the fungus is floated. The mere placing of pieces of twigs, decomposing leaves, &c., from the pond in fresh water cannot be relied on to give any results. The usually poor development of *Pythium* in nature is due to the competition of other forms. By supplying a substratum on which *Pythium* grows rapidly, while the others do badly, a start is given to it, and a sufficient quantity may be obtained to provide successively purer cultures. By floating the chosen culture medium near the surface of the liquid, the chance of picking up wandering zoospores from whatever part of the vessel they may have been liberated is largely increased, since these zoospores generally seek the surface film of the liquid. The *Saprolegniaceæ*, whose zoospores behave in a similar manner, can be easily eliminated, since they are almost invariably of slower growth than *Pythium*. Different species of the latter can then be isolated in the usual manner and pure cultures obtained. They may be grown in watch glasses, Petri dishes, sporulating dishes, &c., according to size, and for examination in a living state I have found a Zeiss D* water-immersion objective ("plankton searcher") most useful, since it can be lowered directly into the water. Hanging-drop cultures are of limited application, for the formation of zoospores requires frequent changes of water. Open slide cultures are better and can be obtained in greatest perfection when placed under a continuous drip of water from a burette or siphon, the slide being tilted slightly against the stream to prevent the culture washing off. By these means the cultures are maintained almost free from bacteria.

The study of living cultures permits of some remarkable glimpses into the processes of intra-cellular life. In the maturation of the sporangium very interesting indications are to be had of the part played by osmotic action and of the need for the expulsion of some of the cell water before the differentiation of the zoospores can be completed. The discharge of the latter appears to be dependent on chemotactic forces, and the function of the discharging process or beak is probably explained on this view. The manner in which sporangia pass into conidia, and the one or two observations which lead to the supposition that oogonia and conidia are not always differentiated in their early stages, illustrate the close connection which must exist between the three classes of reproductive bodies.

Distribution and Habitat.

The group is probably of world-wide distribution, but, as in the case of most aquatic fungi, information is scanty. I have obtained *P. intermedium* from soil in the neighbourhood of the roots of *Abutilon* in five widely separated localities in the British Isles, France and Germany, and it has also been reported from the United States. *P. gracile* Schenk occurs in Europe commonly, and also in India. *P. vexans* occurs in England, the south of France and Germany. *P. monospermum* in Europe and Asia (Java). *P. proliferum* is as common in pond water in India as it is in Europe. Endemic species are probably rare. In pond water in India the same species occur as in England, or the south of France.

In habitat they appear to occupy the soil as frequently as they do water. As a rule they are found in connection with decomposing organic matter, but the parasitic species are exceptions, while *P. Indigoferæ* was found in the waxy coating of the leaves of *Indigofera arrecta*, and *P. palmivorum* is a parasite of the tops of palms. The species on *Indigofera* is thus remarkable for its epiphytic growth, the only case so far in the genus.

The aquatic species are chiefly of the sub-genus *Aphragmium* with the proliferous members of the sub-genus *Sphærosporangium*. The terrestrial include the non-proliferous members of the *Sphærosporangia* with some of the *Aphragmia*, as *P. Indigoferæ*. *P. Anguillulæ aceti*, found on the vinegar eel, belongs to *Sphærosporangia*.

This general distinction between the aquatic and terrestrial species leads at once to the enquiry which is the more primitive section. Several indications occur that the aquatic species are to be so considered. The most simple members are those of the sub-genus *Aphragmium*, where an undifferentiated portion of the mycelium becomes emptied as zoospores, while conidia are unknown. These are, with few exceptions, aquatic, even those which are to be found in the soil being much oftener met with in water, *P. Indigoferæ* being the only truly terrestrial one. It is easy to see how in colonising the land such forms would be at a severe disadvantage. Motile spores would no longer be an efficient mode of propagation where a watery environment was rare, and sexual reproduction or vegetative propagation by the mycelium would alone serve to perpetuate the species. It is remarkable that in the one species of this section which has been found out of the reach of frequent submersion, *viz.*, *P. Indigoferæ*, zoospores are very rarely formed, and the reproduction is chiefly carried on by the somewhat wasteful method of oospore formation. Proliferous species of the sub-genus *Sphærosporangium*, which are without conidia but with definite sporangia, through which, after emptying, the basal hypha continues to grow, also are all aquatic. The transformation of their sporangia into conidia, in conditions where zoospore production has become of less use, is readily conceivable. Either of these sections or both may show the ancestral characters of the genus. That is to say, the fore-runner of the land forms existing at the present day, which mostly form conidia, may have been a non-conidial species without any differentiation of its thallus into vegetative and asexual reproductive parts as in the *Aphragmia*, or with such a differentiation similar to *P. proliferum*, or both of these may have invaded the land and evolved conidia independently. *P. monospermum* and *P. gracile* sometimes show a tendency to gemma formation which indicates a possible road by which the evolution of conidial forms from the *Aphragmia* may have taken place (Plate I, Fig. 1). Too much weight must not be attached to this. There is abundant evidence that conidia and sporangia are of common origin, the conidium in *Pythium* representing merely a sporangium which has failed to form zoospores and which germinates directly. Thus in *P. intermedium*, where both conidia and sporangia are ordinarily freely produced, they not alone present no difference to the eye, but their behaviour is wholly a matter of environment. A given spore may, if appropriately treated, empty its contents as zoospores, or put out a vegetative hypha from which a new plant arises; and even after one or the other of these two methods of propagation has commenced, some change in the conditions may cause the other to be the final result of germination. A striking example is figured on Plate VI, Fig. 5. Here the spore had begun by putting out a tube similar to those formed by the sporangia of this species, and the apex of this tube had even begun to show modifications such as usually precede its protrusion as the bladder in which the zoospores are matured. Then a check occurred from some unknown cause. After some hours of quiescence development continued, but now by a hypha put out laterally from below the apex of the tube. This grew and ramified as a new plant. Indeed, the change from sporangia into conidia is probably exceedingly common. A young culture of *P. intermedium* or *P. rostratum*, surrounded with a large quantity of fresh water, will discharge a great number of zoospores, whereas a perfectly similar culture left to stand in unchanged water for several days will have almost lost the power of forming sporangia at all, though morphologically equal spores are present on both occasions. Many of the conidia found in the latter case must have been originally potential sporangia, but their power of germination as such is gradually lost and the simpler process of direct germination substituted.

The gemma, on the other hand, is clearly a secondary formation by no means of equivalent morphological value to the sporangium. It is a vegetative bud representing a portion of mycelium containing within itself a sufficient store of reserve nutriment to enable it to withstand isolation from the plant without losing its power of renewed growth. The gemmæ of the *Saprolegniaceæ* are not of this category, for they are rather conidia representing modified sporangia (Maurizio, 1894).

It is, then, probable that the proliferous species of the *Sphærosporangia* must be accepted as representing the type which first invaded the land, while the *Aphragmia* are to be considered as a blind offshoot probably from the most primitive aquatic forms.

Some of the land forms appear to have lost together the power of zoospore formation. A well-marked series of forms exists from *P. rostratum* with numerous sporangia, through *P. vexans*, where they are rare, to *P. ultimum*, in which Trow (1901) failed to obtain their formation at all. It is not impossible that they may be formed occasionally even in *P. ultimum*, for de Bary never found them in his cultures of *P. vexans* in which, nevertheless, they do sometimes occur.

This persistence in the land forms of the aquatic type of spore—the zoospore, added to the rarity of the terrestrial organ —the conidium—amongst the aquatic species, tells all against the possibility of the group being a degenerate one, progressing in the opposite direction from land to water with an accompanying simplification of type. Besides, to account for the *Aphragmium* section on this view would be a matter of extreme difficulty.

In general the aquatic forms are characterised by a great spore production in comparison to the size of the thallus. A culture of *P. gracile* may discharge almost the whole contents of its mycelium as zoospores, most of the hyphæ opening at the tip or by a lateral branch and liberating their protoplasm as free swimming spores into the water. *P. proliferum* and its allies attain the same result in a different manner. Their hyphæ are often scanty, sparingly branched and short, and the sporangia are formed terminally. After discharge fresh sporangia are produced, one after another, in the cavity of the first or just beyond it, the growth of the vegetative hyphæ being thus reduced as far as possible. Hence these species attain a comparatively high degree of zoospore formation at the expense of very little growth of their free sterile parts, a clear advantage in life passed in a liquid medium. On the land, however, the sterile thallus is of greater importance. Distribution by zoospores is here comparatively rare, and much of the spread of the organism is effected by vegetative hyphæ which ramify through the soil in search of fresh nutriment. Most of the land forms have, consequently, a much greater mycelial development in nature than the aquatic ones and less copious reproduction. It may even be that the curious sterile fungus which assists in forming the endotrophic mycorhizas of the majority of phanerogamous plants in the tropics, and of a large number in temperate climates, is a case of a *Pythium* with complete suppression of the reproductive parts and a remarkable development of the vegetative.[1]

The only other important difference between the land and water forms is that the former appear to produce their sexual organs in any position, whereas the latter, as a rule, bear them in the interior of the tissues of the substratum on which they are growing. *P. monospermum* is an exception to this, according to Ward (1883), as its oospores occur extra as well as intra-matrically; *P. gracile* can be induced to form oogonia outside the algal filament in which it grows, though I have not seen fertilisation occur in these conditions. The intra-matrical position is probably for protection, free organs in water being more exposed to injury than similar ones in the soil.

PARASITISM.

All the species which have been investigated are capable of living saprophytically. Many are able, in addition, to attack and destroy living tissues; but they are hemi-saprophytes, not true parasites, for even the most destructive, *P. de Baryanum* Hesse, attains its maximum development and reproductive activity when cultivated saprophytically. This species is parasitic on a number of Cruciferous and other plants. It has been described as attacking *Camelina sativa*, *Lepidium sativum*, *Trifolium repens*, *Spergula arvensis*, *Panicummiliaceum*, *Zea Mays*, *Siuapis alba*, *Beta*

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[1] This fungus, which is ubiquitous in cultivated soils, is, unlike the entophyte of the *Orchidaceæ*, probably a Phycomycete, as the mycelium is unseptate except in old hyphæ and the branching tends to be dichotomous. Though I have failed to find true spores after several years' search, I believe it to be at least related to the *Pythiaceæ*.

vulgaris, *Stanhopea saccata*, *Curcumis sativus*, &c., and in most cases with fatal results As it is the best known of the parasitic members of the genus from the work of Hesse (1874), Ward (1883), and others, its mode of action may be described at some length.

The plants attacked are usually seedlings, and the series of changes produced, designated by the phrase "damping off," is quite definite when considered from the pathological side, though other fungi besides *Pythium* are capable of causing it.

The species is a not uncommon one in garden soils. In fact, de Bary considered it was always present in such soils in some parts of Europe. However this may be, it is certain that in many localities any considerable series of pot experiments with several of the plants above-mentioned will show a number of seedlings "damped off" from its presence. Darwin and Acton (1894) also remark on the frequency in water cultures of attacks by *Pythium*, causing the plants to damp off at the collar and, though they do not mention the species, it is probable that the present one is at least in part responsible.

This (the collar or its vicinity) is the usual seat of attack. The hyphæ enter directly into the base of the hypocotyl or the upper part of the roots, across any cell of the epidermis. Here their development is rapid. They bore both through and between the cells, quickly infecting the whole of the cortex in the neighbourhood of the point where they enter. In boring through the cell walls there is evidence of the action of a cellulose-softening ferment, but this must be extremely localised since there is never any trace of an opening larger than the contained hypha, or of any alteration in the wall around the opening. Cells thus attacked lose their turgescence, their protoplasm is destroyed, chlorophyll, if any be present, disappears, and the walls collapse. Prof. Marshall Ward (1883) mentions that starch is not diminished in cells of the potato whose other contents have been completely destroyed by *P. de Baryanum*, and that inulin in tubers of the dahlia similarly does not seem to be affected. On the other hand, Roze and Cornu (1870) state that in plants of *Lemna arrhiza* attacked by *P. Cystosiphon*, starch is completely absent, while found in quantity in healthy plants. They do not mention whether this occurs early in the attack or not—a point of importance, since both Hesse and Ward found that the final dissolution of tissues killed by *P. de Baryanum* is accomplished by hosts of saprophytes entering in the train of the parasite. It is possible that the disappearance of starch in *Lemna arrhiza* was due to these. But in the ætiology of such diseases it is a mistake to attach too much weight to the action of saprophytic followers. There is no doubt that the *Pythium* by itself is quite capable of causing such a softening and destruction of tissues as to lead to the collapse of the seedling. Once they are cut off from the roots, the further reduction of the dead parts by saprophytes is no more than the common fate of dead organic matter. The same happens frequently in the well-known potato disease. The uncomplicated action of the parasite *Phytopthora infestans* appears to lead to a dry rot unaccompanied by any unpleasant smell, whereas the normal condition in the disease is a wet rot accompanied by swarms of butyric acid-producing bacteria and other saprophytes, which give rise to the familiar smell usually associated with it. But the dry form is not less destructive than the other. An interesting point distinguishes *Pythium* in its subsequent behaviour from *Phytopthora.* Having killed the cells, it still continues to develop in the dead material, and having begun as a parasite, lives on the decomposition products of the cells which has itself killed in truly saprophytic wise.

Between the cells the hyphæ occupy intercellular spaces or force their way along the middle lamellæ, growing chiefly along the axis of the shoot. They never lead to complete isolation of tissue elements, such as happens in the case of some wood-destroying fungi. This is another reason for believing that the cellulose altering ferment produced is localised, probably at the tip of the hyphæ. The tissues are rapidly killed, no time being given for protective tissue formation. Externally, the resulting effects are shrinkage and softening of the hypocotyl due to the collapse of the cells, and discoloration of green parts, when these are invaded, from the loss of chlorophyll.

The disease is usually quite localised near the collar, and in such cases the cotyledons and upper parts of the seedling remain unchanged until collapse ensues. Hence *P. de Baryanum* belongs to the simplest group of parasites, those whose destructive power is exercised only on the tissues in their immediate vicinity, and whose action on these tissues is wholly inimical. The cells are killed very shortly after the fungus reaches them, and there is no attempt to react in any way to the invading organism ; no hypertrophy appears nor any attempt at cell division. There is also no evidence whatever of any remote poisoning action, such as occurs in several other plant diseases, as the silver leaf disease of *Prunus* (Percival, 1902), and probably potato blight. Nothing appears to be excreted by the parasite capable of entering the circulation and acting injuriously on remote tissues. None the less the effect on the whole organism is, as a rule, fatal. The rot, produced at a part of the plant least calculated to stand any weakening, generally progresses rapidly until it softens the tissues sufficiently to bring down the entire superstructure. There is a second factor, important in animal diseases, which may also play a part here. It is possible that in plants as in animals, though perhaps in a lesser degree, a local destruction of tissues may lead to the death of the whole owing to interference with nutrition or to the phenomenon of " shock." We have no evidence of shock conditions in plants, but their possibility must be borne in mind.

P. de Baryanum though, as described above, a vigorous parasite, is only so when conditions are favourable for its development. It requires, as a rule, plenty of moisture to enable it to get a start sufficient to cause an epidemic. Seedlings of a few days' growth are also usually the only ones killed by it. Older plants either escape entirely, or the damage is slight and confined to small browned patches on the stem, sometimes sunken in slightly, but whose presence in no way interferes with the further development of the plant.

Pythium palmivorum, though imperfectly studied, undoubtedly merits mention here. The species is parasitic on palms, causing a destructive disease which has been responsible for considerable loss in one locality in India. *Borassus flabellifer*, *Cocos nucifera* and possibly *Areca Catechu*, three of the most valuable economic trees of India, are attacked. The mycelium enters through the leaf sheaths which form a dense envelope to the terminal bud. The affected locality shows a depressed area, brownish in colour, and surrounded by a dark ring separating the diseased from healthy tissues. This is usually found on the outer sheaths, overlying similar spots in the next innermost, and in this way the spots may be traced in through a dozen or more sheaths towards the centre of the bud. Eventually the terminal growing point itself is reached and destroyed, death resulting since the growing point of palms is single. As the disease advances, spots appear in various positions, sometimes on the base of the leaves and flower stalks, but usually only on the sheaths; the mycelium sometimes extending longitudinally in the inner layers of the bud and bursting out again to form a new spot, above or below the first, on the outer sheaths.

At an early period the necrosed spot is occupied by the mycelium of the *Pythium* only. Later on swarms of saprophytic fungi and bacteria follow and a wet rot ensues, resulting in the conversion of the whole apex of the tree into a foul smelling putrid mass. Hence, except in the early stages, the actual presence of the *Pythium* is apt to be overlooked. Inoculation experiments have not been undertaken and the further history of this disease remains to be worked out.

Two species of *Pythium*, *P. megalacanthum* de Bary and *P. intermedium* de Bary, are parasitic on prothalli, the first on *Todea africana*, the second on *Todea*, *Equisetum* and *Ceratopteris* (de Bary, 1881). *P. megalacanthum* is a weak parasite capable of attacking only some of the prothalli infected with it, but *P. intermedium* causes a "damping off" similar to the seedling disease described above (Atkinson, 1895). It will be seen below that this species often occurs on the roots of certain plants, but I have not observed it causing any damage.

P. gracile Schenk, *P. dictyospermum* Raciborski and *P. tenue* Gobi are parasites in various algæ, *Spirogyra*, *Vaucheria*, &c.

There is some confusion between the members of these algal parasites, which resemble each other closely. The species which I have considered as identical with Schenk's *P. gracile* is a weak parasite when grown artificially, being with difficulty induced to penetrate living filaments of *Vaucheria* grown on slides. As soon, however, as these begin to show signs of lowered vitality from growth in unfavourable conditions, the *Pythium* enters and, passing from cell to cell, eventually may occupy a considerable length of the filaments with its mycelium. Unlike *P. de Baryanum*, the first effects are slight. The parasite may even be found developed sufficiently to form oospores within the cells before any harmful results of its presence are visible. The first sign of the alga's failure is usually a contracting of the green contents into masses towards the centre of the cell. Then the chlorophyll is destroyed, and a brownish mass with numerous loose granules occupies its place. From the many instances in which fresh, healthy-looking and strongly-growing filaments were found to contain a few threads of the parasite, it appears probable that, in nature, it experiences little difficulty in entering normal plants of the host. But in my cultures on flies inoculated from infected *Vaucheria*, I had great trouble in obtaining fresh infections. They were only got when distinct signs were visible that the alga's vitality was at an ebb. There appears to be here an attenuation of virulence such as has been shown by Laurent (1899) to result from artificial cultures in the case of some other facultative parasites. That the *Pythium* was not happy in its environment in this particular series of experiments was evident from the fact that, although large masses of mycelium were obtained around the flies, they remained for the most part sterile. When then brought from these uncongenial conditions into contact with healthy cells of the host from which they had originally come, they were unable to return immediately to the parasitic life, and only did so when its vital resistance was sufficiently lowered.

A species which I cannot separate from *P. gracile* was found as a parasite on phanerogamous plants on two occasions in India.

In the first of these it was associated with some members of the eelworm family in a disease of cultivated ginger (*Zingiber officinale*) in Bombay Presidency. The parts played by worm and fungus in the disease were not determined, but the filaments of the latter were found extending into perfectly healthy tissues and were clearly parasitic. The other case was interesting, as showing a degree of weak parasitism comparable to that observed in the algal parasite in artificial cultures. Some water cultures of *Ricinus communis* were observed to be doing badly, and on examination the roots were found suffering from a soft rot. The cause of this was traced to *P. gracile*, or a near ally, whose mycelium had permeated many of the roots. The cultures had previously been inoculated with soil organisms from a particular plot. In this plot a number of healthy *Ricinus* plants, planted about the time that the water cultures were commenced, grew nearly to maturity without showing any disease. The *Pythium* appeared to have succeeded in damaging the delicate plants which resulted from a not very flourishing set of water cultures, while unable to harm strong ones growing in the soil.

Raciborski (1900) mentions similar instances of weak parasitism in two species "*P. complens* Fischer" (*P. monospermum*) and (?) *P. vexans* de Bary, which sometimes attack overcrowded or weak tobacco plants in Java. The former is especially found on plants already infected with *Phytophthora Nicotianæ*. but infection of healthy plants by the *Pythium* alone could not be obtained.

The next parasite to be mentioned is *P. Cystosiphon* (Roze and Cornu) Lind., found in several water plants, *Lemna arrhiza*, *L. minor*, *L. gibba* and *Riccia fluitans*. It has not since been recorded. There are no peculiarities calling for special attention in its parasitism, except that it causes the tissues of the invaded plants t become water-logged, so that the whole plant sinks to the bottom.

P. Anguillulæ aceti Sadebeck occurs as an animal parasite, th only one known in the genus. It attacks and kills in a few hour the vinegar eel, *Anguillula aceti*, and then continues to live saprophytically on the dead body. This power, common to all th parasitic members of the genus, of passing directly to a saprophytic life under the conditions found in nature, is indeed the most noticeable one possessed by these forms. It provides them with a means, independent of the production of resting conditions of spores or mycelium, of tiding over long intervals of time during which they may be deprived of suitable living food-material. Scarcely a fallen leaf or twig can be found in soil where *Pythium* occurs that has not been permeated by its mycelium. As this is normally transient it is not easy to detect; but it can be seen in pot cultures of *Abutilon* containing *P. intermedium* and subjected to frequent examination, how one after another each mass of humus or fragment of moss becomes a prey to the fungus. The importance of the genus as soil organisms has been entirely overlooked.

MYCELIUM.

The mycelium of all the species I have examined is both intra- and extra-matrical. There is not, however, any formation of rhizoids such as occurs in the *Saprolegniaceæ.* The thallus is quite undifferentiated. Branching is free, and is irregularly lateral except in *P. intermedium*, where it is dichotomous. All the species studied were examined on small pieces of solid substances floating in aqueous media; but in nature many ramify in the decomposing vegetable matter of the soil. In hanging-drop cultures the hyphæ often spread far beyond the limits of the drop, but this they do by carrying water with them as they grow. In aqueous cultures the hyphæ rarely rise above the surface of the liquid, though I have seen them do so in the case of *P. gracile.* Trow (1901) has shown that in these cases massive aerial cultures may easily be obtained on such materials as potato, carrot, etc. Ward (1883) had previously noted potato as a satisfactory substratum for *P. de Baryanum. P. Indigoferæ* and *P. palmivorum* are the only species which I have observed with an aerial habitat in nature. They were obtained respectively from young twigs of *Indigofera arrecta* Hochst, growing in the Royal Botanic Garden, Calcutta, at a height of about four feet from the ground, and attacking the buds of palms fifty feet or more in height in the Godavery delta. The oospores alone were observed in fresh material of the former, but a mycelium developed, when the shoots were placed in water, in a few days. This formed large quantities of oogonia both extra and intramatrically, as well as a few sporangia. *P. palmivorum* produces both sporangia and oospores in abundance.

The hyphæ are at first always unseptate and crowded with a dense protoplasm. Fat is abundant and leads to considerable blackening of material fixed in Flemming's solution. Glycogen also occurs in highly refractive droplets, whose resemblance to the Chytridiaceous parasites of the genus is remarkable. At a later period septation usually occurs. The fact that this genus forms septa, distinct from those which serve to isolate the reproductive organs, is not even yet fully recognised. Treub (1884) withdrew from his opinion that the endophyte of Lycopodium prothalli is a *Pythium* mainly because he had observed septa in the hyphæ. Prillieux (1895) also describes *P. de Baryanum* as possessing hyphæ which are never septate before the appearance of the reproductive organs. All the species, however, are capable of forming septa without any connection whatever with the latter. The period of their formation depends entirely on the supply of nutrition available. If this be scanty the basal part of the hyphæ, during their growth, becomes emptied of protoplasm, which passes on into the apical portion. Septa are formed to cut off the full from the emptied segments. The hypha represented in Fig. 5, Plate I, was watched while three such septa were formed. In each case as the protoplasm grew scanty, an accumulation of granules was observed a little behind that portion of the hypha that was still well filled. These granules obscured the minute details of the formation of the septum, which appeared in their midst. Some of them remained in the basal segment cut off by the septum from the active part of the hypha. These soon disappeared. As the hypha continued to increase in length, another portion became emptied, and a new septum cut this off from the full portion as before. In this way the old mycelium of almost any culture will be found to be irregularly septate, though in some species the hyphæ are transient and emptied ones rapidly disappear.

Besides these septa the reproductive organs are always cut off from the mycelium by transverse walls, except in the case of the sub-genus *Aphragmium*, where septa are not found in connection with the sporangia, and in one case (*P. tenue* Gobi) are absent in the anthridia.

In size and thickness the hyphæ of *Pythium* do not approach those of either the *Saprolegniaceæ* or the *Peronosporaceæ*. I have rarely seen hyphæ exceeding 7μ in breadth, while the majority of species form filaments of 3 or 4μ only. In *P. Indigoferæ*, which has the largest hyphæ of any species which I have seen, the diameter is less than 8μ. The hyphæ are usually regular, but the members of the sub-genus *Aphragmium* examined by me, habitually form peculiar bud-like lateral processes which appear to have been observed previously only by Ward (1883). Some of these are figured in Figs. 3 and 4, Plate I. Ward who observed them on *P. monospermum* supposed that they serve as reservoirs of protoplasm either for the immediate needs of the mycelium or for the formation of oogonia. They rather appear to me to represent rudimentary buds, comparable to those toruloid structures so often formed by other fungi, and whose function is to carry on a vegetative propagation of the species. It is true that oogonia are formed in these buds in *P. Indigoferæ* after the manner described by Ward. But I have never seen such a formation in *P. gracile*, and here, so far from their being used up to supply the needs of the mycelium, the buds appear to be nourished rather at the expense of the normal hyphæ. They become crowded with dense protoplasm, and are probably capable of preserving their vitality under conditions which would be fatal to the much more slenderly nourished hyphæ. I have often observed them continue their growth as figured in Plate I, Fig. 1 *d*, by putting out a hypha.

The wall in *Pythium* is usually assumed to consist of cellulose. However, as Trow has observed in the case of *P. ultimum*, the cellulose reaction is often difficult to obtain.

The blueing with chloriodide of zinc is often faint or only got after long treatment. The explanation of this is, so far, unknown.

GEMMÆ.

Gemmæ, resembling in their external features those of the *Saprolegniaceæ* [conidia of Maurizio (1894)], are formed in *P. rostratum* (Plate V, Fig. 15). I have not observed them elsewhere, though the vegetative buds mentioned above are probably allied bodies.

THE SPORANGIUM.

Sporangia are formed in the great majority of the *Pythiaceæ*. *P. ultimum* is the only species so far described in which they do not occur; and as *P. vexans* for a long time occupied a similar position, whereas I have found an occasional sporangium in my cultures, it is not improbable that they may yet be described for this species also. They may be either terminal or intercalar. In *P. proliferum*, *P. palmivorum* and *P. diacarpum*, terminal sporangia alone are formed. All the other species examined by me present both terminal and intercalar ones.

The sub-genus *Aphragmium* is distinguished from the rest by possessing filamentous sporangia, not differing in any way from the sterile hyphæ. These are usually terminal, but may be intercalar, in which case they discharge by a lateral branch. Their length may be considerable, and a single sporangium may involve a main hypha and some of its branches, so that a branched sporangium results. In discharging, a certain length of the hypha may give out its contents as zoospores, while the rest, not marked off in any way, remains behind and may in its turn discharge through some lateral branch as a new sporangium. In this way almost the whole mycelium may be converted into sporangia. It sometimes happens in badly nourished cultures that a hypha becomes septate while its contents may still be capable of forming zoospores. In these conditions a false appearance is got of sporangia marked off from the rest of the mycelium by septa. The distinction which has been drawn between the species *P. monospermum* founded by Pringsheim (1858) and *P. gracile* of de Bary (*P. complens* Fischer) from this delimitation of sporangia by septa is hence probably not valid. It is not maintained in the systematic description below.

In the sub-genus *Sphærosporangium*, sporangia are formed either terminally or intercalarly by an accumulation of granular protoplasm which, when fully formed, is cut off by a wall, or by two when intercalar. When mature, discharge of zoospores occurs almost invariably while still connected to the hypha. The shape of the sporangium in this sub-genus is usually spherical, oval or pyriform.

Several of the species, chiefly aquatic forms, are distinguished by the innovation growing through the emptied sporangium. To this condition the term 'proliferation' is applied. In *P. proliferum* (Plate III) three conditions result. The new hypha starts its growth after a sporangium has discharged by pushing up the floor of the sporangium and entering its cavity. Here it may form a new sporangium within the first, as shown in Fig. 9 *a*. Three or more may thus be formed one within another. Or, as in Fig. 4, the new hypha may grow out through the emptied sporangium and form the fresh sporangium just beyond the first. In this way a series of four or five may be got, all but the terminal remaining as a set of collar-like formations round the distal end of the hypha. A third condition is got when the new hypha does not immediately form another sporangium, but continues to grow, or even to branch, after it has passed across the cavity of the first sporangium. Eventually a new sporangium is formed at the apex (Fig. 2 *bb*). But it often happens in these proliferous species that the power of renewing growth across an emptied sporangium is lost. This especially occurs in cultures, where a form distinctly proliferous when first obtained may be renewed for several generations without any of the sporangia appearing proliferous. In this case the innovation invariably arises from just beneath the sporangium and grows out more or less laterally. A very characteristic appearance is thus obtained (Fig. 2 *a*). In some cases this subsporangial branching is renewed several times as sporangia are formed very close together, and a curious appearance of sporangial heads, as shown in Fig. 6 and the accompanying diagram, results. It is clear that this subsporangial branching takes the place of proliferation. It is especially to be expected where growth is vigorous, but the conditions are not such as favour zoospore formation. The hypha, having formed a sporangium terminally, finds its growth checked. It cannot pass on through the sporangium until the latter has discharged. A lateral process is therefore formed just under the sporangium at the point where the effort of growth is greatest. Into it the accumulating protoplasm passes. Even when a sporangium has discharged, the renewal of growth may be subsporangial, owing probably to some condition which causes the apex of the hypha to lose its power of penetrating the septum at the base of the sporangium. The details of this penetration and of the formation of a new sporangium within the old are shown in Figs. 4 and 8. After discharge the septum is seen to project into the sporangium in a dome-like fashion (Fig. 8). It has acquired a highly refractive clear appearance, such as may be noted when a hyphal end is engaged in boring through a cellulose wall. It may probably be interpreted as indicating the action of a cellulose dissolving ferment. Beneath the bright band of the septum is a clear space without granules, probably of hyaloplasm. As the hypha grows on, it does not appear to bore right through the septum but rather to soften the latter so that it is carried on by the growing apex and gradually merges in the new sporangium wall. The apex of the hypha soon swells up, and the new sporangium is formed in the usual way. When ripe, a tube of discharge is formed which does not leave the sheath formed by the old sporangium through the previous opening, but forces a way for itself across the membrane opposite the point of its formation. This power of the tube of discharge of the sporangium to bore through cell walls is still more strongly shown in the case of *P. Cystosiphon* as described by Roze and Cornu (1870). Here the sporangia are formed in the peripheral cells of *Lemna arrhiza*, and the tube of discharge bores right through the external walls to liberate the zoospores into the surrounding water. This it must do by ferment action, as neither here, nor in the proliferous species, is there any tendency to a displacement of the sporangium in the cavity in which it lies, such as would result from pressure exercised by the beak.

The stages of maturation and discharge of the sporangium were observed in several species, notably *P. proliferum*, *P. rostratum*, *P. intermedium* and *P. gracile*. *P. proliferum* first clearly showed the incompleteness of the description of these processes hitherto given.

The ripe sporangium of *P. proliferum* invariably possesses one or more large vacuoles, usually of an irregular shape (Plate III, Fig. 8 ; Plate IV, Fig. 1). It will often remain quiescent without germinating for a considerable period unless appropriately treated. But while thus capable of passing a long time (I have kept them for a month, and Ward (1883) for many months) in a dormant state, its structure shows no alteration from that of a newly-matured spore. The striking feature is still the large vacuoles. Zoospore production may be induced by bringing the mature spore into a large body of fresh water, well aerated and exposed to the light. A well washed slide culture exposed to the strong light of the Abbe condenser is under favourable conditions for observation. The details of the process which I am about to describe were made on a vigorous culture on a species of *Cryptops* four days old. At 3-45 P.M. the culture was washed with tap water. None of its sporangia had discharged or were provided with beaks, though all were more or less vacuolated. At 6-15 P.M. a few had already discharged. Many were beaked. Almost all had large vacuoles, sometimes single, sometimes three or four. From time to time fresh water was added, the old being drawn off with blotting paper. At 8-30 a large proportion had discharged. At 9 a sporangium with two oval vacuoles was placed under observation (Plate IV, Fig. 1 *a*). The beak was already fully formed on the side of the sporangium furthest away from the larger of the two vacuoles. Its length was (as is usual in this species) about one quarter of the diameter of the sporangium.

The apex was double contoured and highly refractive. Beneath this there was a small clear space, and the base of the tube contained a little finely granular protoplasm with some highly refractive globules showing a faint reddish tint. As this species is unsatisfactory for observing the changes that occur in the beak, owing to the small size of the latter, they will be more fully described in *P. rostratum* below. At 9-20 a few faint scattered vacuoles appeared outside the two main ones which were enlarging and were quite clear, without granules. A moment later the two had coalesced forming a large vacuole more than half the size of the cell and irregularly triangular in shape (Fig. 1 *b*). At 9-30 the whole beak was quite clear and the apex very bright. The great vacuole approached the wall at several places, being separated from it by a thin peripheral layer of protoplasm. At 9-35 the vacuole became rounded in its portion nearest the beak. This rounding gradually extended to the rest, and at 9-40 it was absolutely spherical, in position opposite to the beak, and separated from the wall at this side by a thin layer of protoplasm (Fig. 1 *c*). It had increased in size within the last minute. Thirty seconds later it discharged to the outside across the protoplasmic layer, flattening gradually from the top, *i.e.*, the part nearest the beak, and closely resembling a contractile vacuole in systole (Fig. 1 *d*). The discharge was at first thought to be by a definite passage, since certain granules which had appeared floating in the vacuolar contents in the previous ten minutes seemed to be shot out into the surrounding water. The opening was not visible though in another instance, to be described below, the protoplasmic layer was distinctly divided by a crack at the moment of discharge. Further observation failed to detect an opening in the sporangial wall in any instance, and it is possible that the granules noticed in the water during systole were a precipitate of some substance held in solution in the sap of the vacuole. The granules which appeared to come from the vacuole, together with others which seemed to be bacteria from the water around executed a vigorous dance in the vicinity of the sporangium. This at once recalls the descriptions given by Hartog (1887) and

Rother (1887) of the processes which occur in the formation of the zoospores of the *Saprolegniaceæ*. These observers have noticed the attraction, not alone of bacteria, but also of *Saprolegnia* zoospores, by the discharged cell-sap during the production of shifting vacuoles in the homogeneous stage of the process, in this family.

While the vacuole was discharging, the beak collapsed somewhat, and a portion of its contents was drawn back into the sporangium, forming a vacuole near the insertion of the beak (Fig. 1 *d*). When discharge was quite completed the protoplasm appeared more finely granular and homogeneous than before, and changed a little in colour and transparency, showing a paler tint of the faint blue under artificial light which it previously had (Fig. 1 *e*). The beak soon filled out again, and the sporangia, protoplasm showed here and there sharp dark dots surrounded by a clear area. These (nuclei ?) remained distinct to the end. Three minutes later, at 9-43, discharge commenced and lasted four minutes (Fig. 1 *f*). The apex of the tube of discharge was blown up into a thin bladder into which the contents of the tubular vacuole passed followed by the rest of the protoplasm. In the early stages the vesicle was blown up faster than the protoplasm flowed into it. Hence a considerable space was left between the wall of the vesicle and the contained protoplasm. Towards the end the protoplasm flowed out faster than the vesicle grew, so that the space was diminished. The flow resembled that of porridge forced through a hole, but with one difference. The granule directly opposite the opening was not always the first to escape. It was sometimes shoved aside by one to the right or left. From this I have been led to suppose that even at this stage the spore-origins are definitely formed and that, though fused into a mass in which individual spores cannot be made out, yet each nucleus has obtained a hold on a certain mass of cytoplasm which passes out with that nucleus. Hence in passing out, when the nucleus engages in the tube it draws its cytoplasm with it, whether this be directly in the centre or to one side of the opening. As will be seen below there is an appearance of spore-origins already forming earlier than this stage. As the protoplasm passed out it rotated slightly except that part actually in the tube. The unescaped part remained in a diminishing, more or less spherical, mass, pressed against the opening of the tube until the last portion passed out. The subsequent differentiation and liberation of the zoospores presented nothing differing from the descriptions given by Ward (1883) and others (Fig. 1 *g* and *h*).

The above case has been described at length, since it illustrates in a single continuous observation most of the essential features in the maturation of the sporangium.

A consecutive account of the process as studied in a very large number of cases belonging to many species will now be given. Some of the observations have been made on certain occasions only, as attention was concentrated on a particular point. All, however, have been verified several times.

A ripe sporangium ready to discharge is always vacuolated. One or more vacuoles may occur, and their size is variable, small usually in *P. intermedium* and *P. vexans*, larger in *P. rostratum* and *P. proliferum*. The beak does not usually occupy any definite position, but its length as measured in terms of the diameter of the sporangium is fairly constant. Thus in *P. intermedium* it is about one quarter of the sporangium diameter, in *P. proliferum* rather less, in *P. rostratum* about equal to the diameter of the sporangium, in *P. diacarpum* at least twice the diameter. In the *Aphragmium* sub-genus the place of the beak is taken by the apex of a hypha, but the changes which occur in it are precisely similar to those in the other cases.

The first indication that zoospore formation is about to occur appears in the apex of the beak. It consists in the formation of a cap of hyaline refractive substance, replacing the endosporal wall of which the rest of the beak consists, and probably formed from it. The bright appearance closely resembles that of a cell-wall at the point where a fungus hypha is boring through it. It may be interpreted as indicating the existence of a cellulose softening ferment, in support of which is the ease as already mentioned with which the beak perforates cellulose walls when formed within a cell.

The ripening of the sporangium is accompanied by changes in the beak, in the sporangial protoplasm and in the vacuoles. Whether one or more of the latter are at first visible, the eventual condition is one in which the sporangium contains a single irregular vacuole.

The protoplasm of the sporangium outside this is in a state of slow rotation. While the beak is forming it becomes lumped into heaps around the periphery of the sporangium. These are sometimes very apparent, as in *P. intermedium*, sometimes difficult to see. They are separated from one another by thinnings in the protoplasm. I believe they are constant in position, though, owing to the rotation of the protoplasm, they appear to be continually changing both shape and position. In number they appear fewer than the number of spores to be produced, though this may result from difficulties of observation due to the spherical shape of the sporangium. They remain visible up to just previous to the discharge of the vacuole, after which they are no longer to be seen. I have never been able to detect any relationship between them and the small secondary vacuoles that are sometimes present; and there is certainly never anything like what occurs in *Peronospora*, where each spore-origin is marked by a distinct vacuole occupying its centre. Indeed it usually happens that there is no other vacuole visible besides the conspicuous one already mentioned. This shows a continual change of shape and increase in size. Finally it becomes spherical, usually moves to the side of the sporangium opposite to the beak and discharges its contents to the outside. Fig. 11, Plate VI, shows these changes in the vacuole very distinctly. In *a* the centre of the sporangium is occupied by an irregular vacuole. Ten minutes later the condition shown in *b* was apparent. The vacuole is almost spherical. Five minutes later the spherical shape was absolute and a few granules were visible in its contents. Cracks appeared in the protoplasm at various points, two of which are shown in *c*, as though the vacuole were endeavouring to discharge its contents.

These appeared and disappeared. Finally two were formed at a point where the protoplasmic lining between the vacuole and the sporangial wall was thinnest. One of these opened to the outside and the contents escaped, and immediately granules appeared which were seen to dance vigorously close to the sporangium, as if in response to violent diffusion currents. The sporangium was now, as shown in *d*, filled with finely granular contents. On actual measurement the diameter was less by about one-tenth of its previous size.

The decrease in diameter resulting from the emptying of the vacuole, when once noticed, was found to occur always. A similar result follows the discharge of the vacuoles in *Saprolegnia* [Hartog (1887), Rothert (1887-8)]. Discharge of the sporangial contents occurs at a variable period after the emptying of the vacuoles. An interval of about five minutes occurred in several cases.

Meanwhile the beak exhibits changes *pari passu* with those in the sporangial contents. The beak itself is a protrusion of the endosporal lining of the sporangial wall. Its wall is usually thin. The hyaline cap is not formed until the changes which are to end in the discharge of the zoospores have commenced. In one instance, however, shown in Plate VI, Fig. 5, a sporangium of *P. intermedium* with a tube showing a distinct hyaline cap failed to form zoospores, and after a few hours germinated by putting out a hypha from the side of the tube. This case affords absolute proof that the one spore may give rise to zoospores or to a germ-hypha, according to circumstances. It further shows that the change from one to the other mode may occur even after the commencement of the germinative changes.

The alterations in the beak previous to discharge may be studied in Fig. 6, Plate I. In Fig. 6 *a* a hypha of *P. gracile* is shown after it has reached its full growth, but before it has commenced to form zoospores. A few linear vacuoles may be observed in the hypha, which is filled with granular protoplasm almost to the apex. In *b* the sub-apicular vacuole, which is invariably formed as the sporangium commences the processes leading to zoospore formation, has appeared. The apex is now clothed with a hyaline cap showing a double contour. The vacuoles, which were noticed by Wahrlich (1887, Fig. 1 *a*), though the regular spacing figured by that author is far from occurring in the majority of cases, are more distinct. In *c* the sub-apicular vacuole is larger. At this stage granules of a bright oily appearance move up and disappear in it. The true nature of the vacuole, which might at first sight be taken for the ordinary accumulation of hyaloplasma in the apex of a growing hypha, is apparent in fixed and stained preparations. The study of well-fixed and stained preparations is of great assistance in interpreting the conditions ; the uniformity of refraction enables a clear idea to be formed where in the living state the relationships are often indistinct. In Flemming's solution the double contour of the hyaline cap of the sporangial beak is lost, and the sub-apicular vacuole is sharply defined from the underlying protoplasm. In *d* discharge has just commenced. The hyaline cap is enlarging and its double contour disappearing. A forward rush of protoplasm into the sub-apicular vacuole has begun. In *e* the vesicle is being rapidly blown up, faster than the protoplasm can flow into it. *f*, *g*, and *h* represent stages in the differentiation of the zoospores. The cleavage lines shown in *f* are, in reality, nothing but lighter areas between the spore-origins. The dark appearance figured is due to the optical effect of hollows bounded by rows of granules at the margins of the origins, and extending in from the surface to join with the cleavage spaces in the inner part of the mass. The appearance and interpretation are the same as in the *Saprolegniaceæ* (Hartog, 1887 and 1888), but the spaces are less defined and the cleavage appears to extend in from the outside.

Two or three minutes after the outflow of the sporangium contents, the mass begins to move as a whole within its bladder. This happens about the same time that the cleavage areas appear. In the meantime vacuoles may be formed in the mass, which grow and then disappear. They are by no means of constant occurrence and one large one or several small may appear. Their formation is probably due to a too rapid leakage of water into the vesicle. This penetrates the protoplasm and is expelled again by contractile vacuoles.

The movement is at first a shaking or rolling one and precedes the appearance of cilia. It has been already noted that a slight rolling movement may be visible before the protoplasm has completely left the sporangium. It is, of course, possible that it is due to the appearance of the rudiments of the cilia, but I have never been able to detect them at this stage. They appear rather suddenly 5 to 10 minutes after the flow, and before the zoospores are completely separated.

As the zoospores are more fully defined they pass through a short amœboid stage. Pseudopodia are put out and again retracted. Soon after the motion changes its character, and becomes oscillatory and more vigorous than before. The zoospores now acquire their definite shape, and separate partially from each other, though still remaining in close contact by a portion of their periphery. A swimming motion replaces the oscillatory one and each zoospore moves independently for a short period, until the bladder ruptures, and they at once fly off in all directions.

Fig. 13 *a*, *b*, *c*, Plate V, shows the corresponding changes in the sporangium of *P. rostratum*.

One observation would seem to show that the cilia are really formed for some time before they are visible. A sporangium discharged quite normally. The bladder wall, however, was broken about five minutes after its formation, possibly by the knock of some passing infusorian. Part of the protoplasm at once streamed out and the portions both within and without the opening were seen to possess cilia. This occurred at the moment after motion had begun and before the zoospores were at all separated, a stage in which cilia are not usually visible. The remainder of the protoplasm eventually escaped and all swam away in a mass by means of the cilia, and was lost to sight.

The above description of the formation and liberation of zoospores in the *Pythiaceæ* differs considerably from previous accounts. It was indeed to be anticipated, from a comparison with the other Phycomycetes, that in *Pythium* as in the other cases, the earlier accounts would prove incorrect. These are to be found chiefly in the papers of Hesse (1874), and Büsgen (1882). The more recent work of Hartog (1887 and 1895), and Rothert (1887-88) on the *Saprolegniaceæ*, and of Harper (1899), on *Synchytrium*, etc., made it probable that the cell-plates described by Büsgen would prove as non-existent here as in the other cases. But whereas in *Saprolegnia*, and still more in *Synchytrium*, structures are apparent in the sporangium which might readily be mistaken for cell-plates, I have not detected anything in the sporangium of *Pythium* at all resembling them. The heaped-up masses of protoplasm described above as often visible before the discharge of the sporangial vacuole, can scarcely have been what Büsgen described as "Spuren eines Kornernetzes" (*l. c.* p. 24), and Hesse as "helle Grenzstreifen," (*l. c.*, p. 20). The equidistant vacuoles mentioned by Büsgen are certainly of rare formation, if indeed they ever occur once the large sporangial vacuole is formed. On the other hand the significance and behaviour of this latter escaped the observation not only of Büsgen and Hesse but also of the other students of the genus. Yet the part played by the vacuole is clearly of considerable importance in the preparation of the sporangium for discharge, and I have never found it wanting. Furthermore the apicular vacuole was mistaken by Büsgen and Hesse for an accumulation of a jelly-like substance, engaged, according to Hesse, in building the bladder at the mouth of the tube, whereas the broad cap of highly-refractive (? cellulose) substance, which actually forms the latter, was not perceived.

Closer observation has shown that the processes in *Pythium* may be brought into line with those in the *Saprolegniaceæ* so fully described by Hartog (1887 and 1895).

In *Saprolegnia* four stages in the differentiation of the zoospores occur. The large sporangia of this genus possess when ripe either a large central vacuole ("normal" and "poor" sporangia of Hartog), or a ziz-zag cavity or linear lacunæ ("full" sporangia of Hartog). The first stage, that of *preliminary division*, is characterised by an accumulation of protoplasm into spore-origins around each nucleus, a linear vacuole separating each spore-origin from the next. This is followed by the *homogeneous* stage, in which the spore-origins swell up and appear to fuse, a homogeneous condition resulting. Immediately afterwards, and scarcely to be separated as a distinct stage, *vacuolation* occurs, a number of minute vacuoles appearing in the sporangial protoplasm. These grow, fuse and disappear, discharging their contents to the outside. During both these stages cell sap passes out and attracts bacteria, which dance vigorously around the sporangium. The last stage is the *final fashioning* of the zoospores by grooves running in from the periphery and joining the network of linear vacuoles, which are formed afresh. The homogeneous condition of the second stage has been shown by Rothert to be due to a rupture of the protoplasmic layer lining the sporangial wall. During this stage Hartog found that the protoplasm took up dilute aqueous solution of eosin, indicating that the osmotic layers bounding the zoosporal protoplasm had lost continuity. The result of the destruction of the inner osmotic membranes of the cell is a loss of turgescence and the taking up of water by the now naked spore-origins, as all naked protoplasm will do under similar circumstances. Part of this water is expelled by the contractile vacuoles of the third stage. With the loss of resistance to exosmosis during both these stages cell sap passes out, as is made evident by the attraction of bacteria.

In *Pythium* the first stage is represented by the accumulations of protoplasm, which may usually be seen in the sporangium as the latter matures. If Hesse's observations be correct, there is a separation of spore-origins by linear vacuoles at this stage, as in *Saprolegnia*, but I have never seen anything but the peripheral lumpings referred to. In any case the first stage is much less marked than in *Saprolegnia*. It is clear that the irregular shape and continual change of form of the vacuole are due to this tendency of the protoplasm to accumulate in heaps, accompanied by its rotation. The second stage follows and leads to the rounding off and enlargement of the vacuole, and its subsequent discharge. This must be due to a *temporary* rupture of the protoplasmic investment between the spore origins, as in the *Saprolegniaceæ*, but owing to the density and spherical shape of the sporangium I have not been able to see clearly the rupture. Water is taken up into the cell and enters the vacuole. This enlarges as a result of the inflow, until its tension overcomes the resistance of the cell wall, and it bursts to the outside. At the same time the diluted cell sap enters the protoplasm of the spore origins, determining their swelling, so as to fill the sporangium with a homogeneous mass. A few small contractile vacuoles are sometimes visible at this period, which corresponds to the third stage in the *Saprolegniaceæ*, but their constant formation, if it occurs, has escaped my attention. After discharge of the vacuole the sporangium is much diminished in size, its turgescence is lost and its contents are homogeneous and more watery than before. In this condition it remains until discharge of its contents into the vesicle takes place, and here the fourth stage, the final fashioning of the zoospores occurs.

It may be asked why the vacuole should not discharge its cell sap at the moment of rupture of the protoplasmic lining. To this the answer is not difficult. The cell sap is known to contain substances of high osmotic value, and there is evidence that these increase during maturation of the zoospores, since there is an increase of tension in the vacuole, marked by its becoming spherical some time before its enlargement takes place. As long as the hautschicht and vacuolar wall persist, the osmotic flow is strictly regulated, for, as Pfeffer has shown, these are the chief osmotic membranes of the cell. When rupture of these occurs, there is only left the cell wall, whose function is less that of an osmotic membrane than to form part of the mechanism regulating turgor, for it gives support to the protoplasmic lining which would otherwise rupture at a comparatively low degree of turgidity, and also by its elasticity allows of turgor being steadily maintained. But rupture of the protoplasmic investment takes place chiefly as a result of the tendency of the protoplasm, at a certain stage, to segregate together into masses around the spore origins, or groups of these, and is hence not an indication of the amount of pressure in the cell. We have only to assume that, at the moment of rupture of the osmotic membranes, turgor is not at its maximum, to see that the immediate result must be an inflow of water into the cell. It is this which causes the vacuole suddenly to enlarge. The inflow will go on until the tension in the cell just surpasses the resistance of the cell wall, when the flow will now be in the opposite direction. Part of the cell sap is, therefore, expelled, while part—now diluted with water—enters the spore origin protoplasm and leads to their swelling, so as completely to fill the sporangium with a homogeneous mass. The accounts of Hartog and Rothert for *Saprolegnia* would indicate that, in this genus, turgor is in excess at the moment of rupture of the protoplasmic layers, so that an immediate outward flow of cell sap occurs. No enlargement of the vacuolar spaces immediately preceding the homogeneous stage was observed. Hartog, however, states (*l.c.* 1895, footnote to p. 676) that it is obvious that the cell sap, which is an "indifferent fluid," cannot alone determine the appearance of the contractile vacuoles; to do this it must be diluted by water from without. Hence an inflow of water must occur at some period shortly before the vacuolar stage. The period of this inflow is clearly visible in *Pythium* and, as will be seen below, the process in the Chytridiacean sporangium is entirely similar to that of *Pythium*.

While the sporangial contents are still in the homogeneous stage, about five minutes after the discharge of the vacuole, the beak blows up into its vesicle. The latter is formed from the wall of the apex of the beak, altered probably by the action of a cellulose-softening ferment. The protoplasm flows out into the vesicle, usually, at a steady, uniform rate. To explain the escape of the zoospores in the *Saprolegniaceæ* into the surrounding water, Hartog assumed either a positive aerotaxy, or a negative pneumotaxy, to exist, *i.e.*, that the spores were either attracted by the aerated water outside, or expelled by a tendency to escape from the products of their own metabolism.

The conditions in *Pythium* are better explained by supposing that the protoplasm is attracted out by the formation of a chemotactic substance in the sub-apicular vacuole.

The first thing to consider is why there should be a beak formed at all. If the mere presence of aerated water outside, or of metabolic substances within, the sporangium, be sufficient to cause discharge, then the beak is a superfluous structure and the sporangium might just as well empty through a hole in its wall, as some of the *Chytridiaceæ* do. But the beak is of constant occurrence, and it is always occupied at its upper part by the sub-apicular vacuole. It has already been shown by Rothert and Hartog that the ordinary contents of the sporangial vacuole are attractive to *Saprolegnia* zoospores, in the second phase of their activity, since, besides bacteria, these zoospores also dance around the sporangium during the homogeneous stage. What is more probable than that the contents of the sub-apicular vacuole are equally attractive to the spore protoplasm of *Pythium* sporangia, in its undifferentiated state as the vesicle is formed? The use of the beak and of its vesicle would then be fully evident. They would serve to hold the chemotactically active substance, and to prevent its diffusion into the surrounding water. A stronger stimulus is required to bring about the discharge of the *Pythium* sporangium than of the Saprolegniaceous one, since the emerging protoplasm, being unprovided with cilia, is unable to give any assistance in the matter. In *Saprolegnia* and *Achlya* cilia assist; in *Aphanomyce* the spores themselves (as also in *Achlya*) attract each other, so that the escape of the first, brought about by the tension in the sporangium, or possibly by a chemotactic substance as in *Pythium*, leads to the discharge of a second, and so on.

In the vesicle the *final fashioning* of the zoospores occurs, corresponding to the fourth stage in the *Saprolegniaceæ*.

THE ZOOSPORE.

The zoospore is kidney-shaped, with two cilia arising near the hilum. Atkinson (1895), however, has pointed out that there exists some slight confusion in the literature on this point, but whether due to imperfect observation, or to actual divergences, he was unable to decide. Thus the zoospore of *P. de Baryanum* was described by Hesse as uniciliate, and his figures are often found in text-books to the present day. *P. proliferum*, when first described by de Bary, was considered to possess uniciliate zoospores. In other species again, as *P. Cystosiphon*, *P. Equiseti*, two cilia have been described or figured, arising from the concave side of the kidney-shaped spore, near the extremities instead of near the hilum. Finally de Bary (1860) and Atkinson (1895) have both described, the one for *P. proliferum*, the other for *P. intermedium*, an anomalous condition in which the zoospores are liberated with two cilia, and subsequently divide into two uniciliate oval spores which, in Atkinson's observation, germinated normally.

As regards *P de Baryanum*, *P. intermedium* and *P. proliferum*, there is little doubt that divergence from the normal zoosporal form of the genus is rare. De Bary does not mention it in his later observations, from which we may gather that he doubted the accuracy of his early description of *P. proliferum*. I have always found the zoospore perfectly normal in this species, and also in *P. intermedium*. Hesse's figures of *P. de Baryanum* have not been confirmed by later observers. It is possible that his figures were drawn from a spore which had arisen from the division of a bi-ciliate spore, as described by Atkinson. It is quite certain that similar divergences from the usual condition occur in the zoospores of other fungi. They are to be explained either as morbid phenomena, or, as seems more likely in some cases, as a process on a par with diplanetism, and an account of some cases in the *Chytridiaceæ* will be found in the second part of this paper.

The form of zoospore figured by Roze and Cornu, and by Sadebeck for *P. Cystosiphon* and "*P. Equiseti*" (*P. de Baryanum*) respectively, is probably to be explained by the difficulty of determining the exact point of insertion of the cilia in the living spore; besides the author's figure in the former case does not agree with their description. The use of re-agents is generally necessary to determine the insertion of the cilia. I have never observed any other than the kidney-shaped spore, with two cilia attached near the hilum, in any member of the genus I have examined.

The above deviations, then, do not lend any support to the view that the zoospore is an essentially variable organ. The variations, so far as they do not rest on errors of observation, are probably either useful adaptations, on a par with diplanetism, or else morbid changes.

True diplanetism, however, does occur in the *Pythiaceæ*. It was first mentioned by Cornu (1872, p. 14), for the zoospores of " *P. proliferum* and its varieties ", and has not since been signalled by any observer so far as I am aware. I have observed its occurrence in *P. diacarpum*, and it is figured in figs. 12—17, plate II. In fig. 14, a group of zoospores is shown, which have come to rest and put out tubes. One has already discharged its contents. The vacuolation of the others is comparable with that of a ripe sporangium before discharge. In fig. 15, the tube has opened and the contents form a round mass at the orifice. The most careful examination has failed to show the existence of a vesicle surrounding the extruded mass at this stage, though a film is prolonged from the boundary of the orifice to its base. The movements, while still in position at the mouth of the tube, would seem to show that some sort of a vesicle is formed, but it must be exceedingly delicate, as the spore often breaks away before it is fully formed. At 16, the new spore, similar to the first, is almost complete and has developed its vacuole. At 17, it is swimming away. It has acquired a new lease of existence.

After finally coming to rest, the zoospore rounds off and clothes itself with a membrane. In the algal parasites, germination takes place usually only in such spores as settle on a filament of the host plant. In *P. dictyospermum* and *P. tenue* a fine tube is now put out, which pierces the wall of the host and expands into a hypha inside. Into this the contents of the zoospore pass, leaving the empty cyst outside.

In the other species the zoospore germinates by putting out an ordinary branched hypha.

The Conidium.

Conidia are found in *P. de Baryanum, vexans, rostratum, ultimum, Anguillulæ aceti, intermedium* and *Cystosiphon.* They do not occur in the species of the subgenus *Nematosporangium,* nor in the proliferous species, *P. proliferum, ferax, megalacanthum* and *diacarpum,* and have not been observed in *P. palmivorum.* Their formation corresponds entirely with the description already given of the early stages of sporangium formation.

Morphologically, as already stated, the conidium is to be considered as a derivative of the sporangium. Apart from the evidence offered by the series of forms, from species with only sporangia, through those, such as *P. rostratum,* in which sporangia and conidia, outwardly indistinguishable, are often formed in almost equal numbers, to *P. ultimum* in which sporangia are absent or at least unknown, there are more direct proofs that the two bodies are morphologically similar. Thus, as already explained, it is merely a question of environment whether the spores of a given culture of *P. rostratum* or *P. intermedium* will give rise mainly to zoospores or germinate by hyphæ. The change from one to the other form of germination may be brought about even after the spores are cut off from their parent mycelium; the case (pl. VI, fig. 5) where a spore which had formed the discharging tube characteristic of the sporangium, afterwards germinated by a hypha, has already been referred to. I have several times divided a single culture into two parts after the spores were mature, and by transferring the one to running water, while the other was left undisturbed, obtained in the former free zoospore formation, and in the latter for the most part direct germination. Intermediate conditions between zoospore building and direct germination have been observed by Sadebeck (1875). The sporangium empties its contents in the manner usual in zoospore formation, but the emptied mass, instead of breaking up into zoospores, remains for a time at the mouth of the sporangium, and then germinates by putting out a hypha. Exactly the same condition occurs normally in species of *Plasmopara.* In the genus *Peronospora,* de Bary (1863) observed the gradual transformation from sporangia to conidia-bearing forms, and the true significance of this was recognised by Brefeld ("Untersuchungen," Vol. VIII, page 242). In *Phytophthora infestans* the customary sporangia may also germinate directly. In *Saprolegnia rhætica*, as described by Maurizio (1894), the conidia, or "sporangiumanalagen," instead of germinating directly, often give rise to zoospores. Here also the derivation of the one from the other is clear. In this species also, and in the later investigated *S. paradoxa* and *S. furcata* (Maurizio 1899), the conidia are sometimes transformed into oogonia, and even antheridia are to be included in the cycle of transformations of the "sporangium-analagen." Such a change from a conidium or sporangium to sexual organs has never been observed in *Pythium*. This is possibly because it would be difficult to detect, since conidia and oogonia usually are much alike until mature. In *P. ferax*, de Bary observed that oospores are sometimes developed in the empty sporangia cases by proliferation, as occurs also in *Saprolegnia Thureti* (de Bary and Woronin 1881, taf. V., fig. 8). Such a position, though taken by Maurizio as one of the arguments in tracing the change from sporangia to oogonia in *Saprolegnia*, is by itself too slender a circumstance on which to base a real morphological relationship, and there is no further evidence of any such relationship in *Pythium*. So far, therefore, while it is clear that *Pythium* offers one of the most convincing arguments in favour of the views on the morphology of the conidium in the higher fungi, developed by Brefeld throughout his great work (see "Untersuchungen," Vol. III, page 199, Vol. IV, page 171, Vol. VIII, page 241 *et seq.*), there is nothing in the genus to indicate the earlier relationship between sexual and asexual sporangia. The consideration of such forms as *Monoblepharis* can scarcely fail to suggest the derivation of sexual from asexual sporangia, and the view has been several times suggested [see, particularly, von Tavel (1892, p. 2, and appendix "Grundplan des natürlichen Systems der Fadenpilze") also Dangeard (1898, p. 263 *et seq.*), Trow (1901)]. But the change from the one to the other type of reproduction must have been brought about early in the history of the ancestors of the fungi, far earlier than the other great change from sporangia and gametangia to conidia and oogonia, and no trace of it has, so far, been found in the *Pythiaceæ.*

The conidia usually germinate rapidly. In some cases, however, they have the power of lying dormant, under certain conditions, and can germinate after many months' rest (*P. de Baryanum, intermedium, etc.*). These are sometimes termed resting-conidia ("*dauer-conidien*"), a name which is scarcely correct, if by it is meant a comparison with such asexual resting spores as those of the *Chytridiaceæ.* In *Pythium* the only structural peculiarity of these spores, distinguishing them from the ordinary conidia, is a somewhat thicker wall (pl. VI, fig. 9), which is not constant; and the character seems insufficient to justify a special term attaching to them. As already mentioned, "resting" sporangia occur in *P. proliferum.*

THE SEXUAL REPRODUCTION.

Sexual reproduction probably exists in all the members of the genus. In a few cases oospores are rarely produced or have so far escaped observation.

Our knowledge of the process is largely based on the work of de Bary and Woronin (1881). Ward (1883) and Atkinson (1895) have since observed in detail the living phenomena, while Trow (1901) and Miyake (1901) have recently studied the cytological details.

As a rule the sexual organs appear at a later stage than the sporangia or conidia. Sometimes, however, no relationship in time can be detected (occasionally in *P. de Baryanum*), or the sexual may be the only method of reproduction found (*P. Artotrogus*, some cultures of *P. Indigoferæ*). They may be formed either within or outside the substratum, thus differing from the *Peronosporaceæ* proper, where the oogonia are always intramatrical.

The oospore is single. Sadebeck (1895) has described cases where two oogonia result from a single swelling of a hypha, which is divided into two compartments by a septum. Wahrlich (1887) figures cases of multiple-spored oogonia. How far this is a pathological condition is uncertain, but it is probable, from fig. 2 of Wahrlich's species, that his cultures were attacked by Chytridiacean parasites.

In position on the mycelium, and in the early stages of their formation, the oogonia closely resemble conidia. It is, indeed, often far from easy to tell if a given spore is about to become an oogonium or a conidium. In most cases, however, the incipient oogonia may be recognised by its somewhat denser contents, and by a slightly different, often more pallid tinge by transmitted light. The formation is exactly similar to that described for sporangia and conidia. When terminal, one wall separates the oogonium from the general mycelium, when intercalar two.

There are some observations which seem to show that the sexuality of incipient oogonia remains in doubt up to a late period. Thus Ward (1883) observed oogonia which showed a strong tendency to revert to the vegetative condition either before or after they displayed distinct oogonial characters. Similarly, Wahrlich (1887) described in his "*P. fecundum*" non-fertilized oogonia which continued their growth by vegetative hyphæ. How far such a change in development depends, in all cases, on the absence of an antheridium, is not clear.

A few observations show that the appearance of sexual or asexual spores depends, to some extent, on the nutrition provided and some other factors. Thus Sadebeck (1887) noticed that a certain degree of concentration of vinegar inhibited conidium formation in *P. Anguillulæ aceti*, while oospores were still produced. Trow (1901) also observed that cultures of *P. ultimum* on cabbage leaves normally produced oospores only.

As the oogonium reaches its full size, the antheridium appears. It may arise in three ways :—1, from the hypha bearing the oogonium, somewhat below the latter (pl. II, figs. 5 and 6); 2, from a neighbouring branch (pl. I, fig. 11) ; 3, by the cutting off of a segment of the oogonial hypha itself, immediately adjacent to the oogonium ("hypogynal" antheridium) (pl. V, fig. 16). The form 3 appears to be merely a reduced form of 1 ; pl. V, fig. 19, showing a state intermediate between the two. In the species to which this figure belongs (*P. rostratum*), 1 and 3 are common, and the case figured shows an antheridium formed of a hypogynal cell which has, however, grown out laterally to form a curved outgrowth by which fertilization is effected. Pl. V, fig. 16, is interesting as showing a series of three oogonia formed on a single hypha, alternating with three hypogynal antheridia. It seems probable that, in this case, male and female nuclei had their origin from nuclei occupying contiguous protoplasmic areas and perhaps nearly related. In *P. Artotrogus* the antheridia are always hypogynal.

The antheridia are sometimes typically single (*P. monospermum, ultimum*), sometimes multiple (*P. de Baryanum, proliferum,* &c.). Up to five have been observed by de Bary in *P. proliferum* (1881, p. 18). When multiple, all may empty their contents into the oogonium, but the cytology of this condition has not been investigated, and it is doubtful if multiple fertilization really occurs. Atkinson (1895) and Gobi (1899) figure cases in which only one of two antheridia is functional.

In *P. tenue*, Gobi states that the antheridium is not cut off from the mycelium by a septum. This is the only case of the sort known.

Fertilization in the genus was first observed by Pringsheim (1858), who, however, believed that the antheridium, after being closely applied to the oogonium, set free antherozoids which entered the latter and effected its fertilization. Prior to fertilization the oogonial contents contract into a ball towards the centre, leaving a free space inside the oogonial wall. Roze and Cornu (1870), and Cornu (1872), correctly interpreted the process as consisting in the penetration of the oogonial wall by a fertilizing process from the antheridium, through which the antheridial protoplasm is evacuated into the oogonium. The latter figured the steps in a species which he termed "*P. gracile* Schenk" in Van Tieghem's Traité de Botanique (1874). Both Hesse (1874) and Sadebeck (1875) confirmed the absence of antherozoids, though the former believed that, at least during the early stages of fertilization, the process remained closed, a passage of matter by diosmosis occurring ; and the latter held the act to be one of contact only, without any discharge from the antheridium into the oogonium. De Bary and Woronin (1881) showed clearly the correctness of Cornu's interpretation, and extended our knowledge of the details of fertilization. They found that in several species a differentiation of antheridial protoplasm into a functional portion, the gonoplasm, and a sterile remainder, the periplasm, occurs. When the antheridium reaches the wall of the oogonium, it sends the fertilizing tube across the wall to reach the contracted oosphere. The tube then opens and the gonoplasm passes into the oosphere, the periplasm eventually degenerating (*l. c.*, pl. I, figs. 1-7, 21, 25, pl. II, figs. 6-15). A portion of the oogonial protoplasm does not take part in the contraction which occurs in the egg contents prior to fertilization, but remains as a faint mass in the space between the wall and the oosphere. This de Bary interpreted as a periplasm similar to that thrown off in the *Peronosporaceæ*, a view which the cytological investigations have not confirmed. The true periplasm is closely applied to the ooplasm and invisible in living material.

The cytological study of the genus was long neglected. In 1889-1900, I made several attempts at the Jardin d' Acclimatation at Antibes under the generous advice and guidance of the Director, Dr. Georges Poirault, to find a suitable species for examination, but without success. In 1900, Dr. Trow read to the British Association an account of the cytology of a new species which he named *P. ultimum*, and the detailed paper was published in the Annals of Botany in June 1901. In the same Journal, Miyake published the results of his study of *P. de Baryanum* in December of the same year. The two accounts are essentially similar, differing only in matters of detail. That of Dr. Trow is the fuller.

Both oogonium and antheridium are multinucleate. As the former ripens, its nuclei pass to the periphery and arrange themselves in a hollow sphere. Here they divide once. A differentiation into periplasm and ooplasm is clearly marked, the former being so closely applied to the ooplasm as to be indistinguishable in the living state. Hence the description given by de Bary and others, of the periplasm as filling part of the space between the oogonial wall and the condensed mass, is erroneous, the true periplasm forming the outer layer of the condensed mass. The part seen by de Bary must have been protoplasm which does not take part in the contraction ; that this subsequently degenerates without assisting in the formation of the walls of the oospore, I have several times observed. Into the true periplasm the nuclei move, one remaining behind and occupying the centre of the oosphere according to Trow (*P. ultimum*), or moving there from the periplasm according to Miyake (*P. de Baryanum*). Near this nucleus, in the centre of the ooplasm of *P. ultimum*, a coenocentrum appears, which is interpreted by Trow as rather an expression of forces acting in the centre, than as a distinct organ. This was not observed by Miyake. A receptive papilla is also formed where the antheridium touches the oogonial wall.

Meanwhile in the antheridium the nuclei also divide once, and all degenerate except one. The fertilization tube penetrates the wall, passing through the periplasm to reach the ooplasm where it opens and emits the male nucleus. The greater part of the cytoplasmic contents of the antheridium also pass over into the oosphere, most going, probably, into the periplasm according to Trow. He observed no differentiation of gonoplasm and periplasm in the antheridium, as described by de Bary. Miyake merely states that the greater part of the contents pass over. Fusion of the sexual nuclei is delayed in *P. ultimum*, but early in *P. de Baryanum*. As the oospore matures, its wall thickens, being formed in two or more layers. The outer, the exospore, is formed probably from the periplasm, according to Miyake, or, according to Trow, from the ooplasm, the periplasm being entirely digested by the growing oospore. The inner layers described by Trow (endospore of Miyake) are doubtless formed from the oosplasm.

The oospores germinate when ripe, or after a period of rest. Trow obtained both conditions in *P. ultimum.* In *P. vexans* and *Indigoferæ* free germination can be got a few days after the maturation of the oospores. *P. monospermum* germinates after several months, as does also *P. de Baryanum.* On germination some species give rise immediately to zoospores (*P. vexans*), others (*P. ultimum, de Baryanum, Anguillulae aceti, Artotrogus, Indigoferæ*) germinate with a hypha. In *P. proliferum*, sometimes in *P. monospermum*, and in those cases of *P. palmivorum* which have been observed, the hypha which results from germination grows but a short distance and then gives rise to sporangia. This condition may be likened to the formation of a promycelium and is intermediate between the others.

HISTORICAL AND PHYLOGENETIC.

Pringsheim (1858), by whom the genus *Pythium* was founded, placed it amongst the *Saprolegniaceæ.* His example was followed by Cornu (1872). De Bary (1881) showed its close relationships to *Phytophthora*, and classed it with the *Peronosporaceæ.* Zopf (1890), Fischer (1892) and most other mycologists have accepted this view. Berlese and de Toni in Saccardo's Sylloge Fungorum (1888) have, however, returned to Pringsheim's classification, and more recently Schröter in Engler's Pflanzenfamilien (1897) adopts the same view, while making the *Pythiaceæ* a family joined to the *Saprolegniaceæ* and *Leptomitaceæ* to form the *Saprolegniineæ.* Trow (1901) and Miyake (1901), the most recent workers in the genus, consider that de Bary's view is that which has most to recommend it. It is impossible, indeed, to avoid coming to this conclusion when the characters of the two groups are actually studied. The similarity to the *Saprolegniaceæ* is seen to depend on characters of little value, chiefly due to the aquatic mode of life shared by some members of the *Pythiaceæ* with the former group.

Pythium shows relationships more or less distinctly with the *Chytridiaceæ, Saprolegniaceæ, Leptomitaceæ* and *Peronosporaceæ.*

Hence it is in a position of extreme interest amongst the groups of the Phycomycetes.[1]

Before dealing with its individual relationships, we may consider some of the characters which are likely to be of phylogenetic importance. As is generally admitted, characters likely to arise from adaptations necessitated by a particular habitat, are of little value. Such organs as conidia may have been many times separately acquired, as aquatic forms colonised the land The characters of the vegetative mycelium are also not of great importance, though the presence or absence of true segmentation appears to be of considerable morphological value. The reproductive processes are those on which relationships must be chiefly based; for while we must, in the main, regard the aggregate of the characters as the final test of affinity, the proportionate weight to be attached to each individual character must vary considerably. Both theoretical considerations and facts indicate that the reproductive parts and processes are, on the whole, more constant than the vegetative, and therefore more valuable in classification. Another character of importance is the shape of the zoospore, and the number and position of its cilia. Leaving aside the *Chytridiaceæ*, two types of zoospore occur in the three families to which *Pythium* is most nearly related. One in which the zoospore is egg-shaped and with two cilia attached to its narrow end, and the other with kidney-shaped spores and two cilia arising from near the hilum. These two types are connected together in the *Saprolegniaceæ* and *Leptomitaceæ*, in a remarkable manner, through the phenomenon of diplanetism. A spore liberated as the first type swims for a period and comes to rest. After a time it gives rise to a new spore of the second type. In *Aphanomyces* a peculiar condition obtains. The

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[1] Thaxter (1896) has shown that the *Leptomitaceæ* must either be included under the *Pythiaceæ* or else elevated into a separate family, as their reproductive processes separate them from the *Saprolegniaceæ*. As placing them with the *Pythiaceæ* involves including them under the *Peronosporineæ*, with which the genus *Pythium* offers too great a relationship to be ignored, and would lead to placing such forms as *Cystopus* and *Apodya* (*Leptomitus*) in the one family it is much better to keep this group separate, as King (1903) suggests.

protoplasm of the sporangium escapes in lumps, unprovided with cilia, which encyst at the mouth of the sporangium. From these cysts the second type of zoospore arises. This would appear to indicate that the first type of spore (egg-shaped with terminal cilia) is a recent interposition, the kidney-shaped spore being the primitive form. In some cases, as in *Pythiopsis*, the recent form has supplanted the older. In *Apodya* also, the egg-shaped is the ordinary form and the existence of diplanetism is doubtful; but in the great majority of cases, including the whole of the *Peronosporaceæ*, the kidney-shaped spore alone is found. In the *Chytridiaceæ* great variation in the zoospore occurs. But this group differs from the others in being a heterogeneous mass of organisms, whose affinities are often obscure. It is not improbable that forms are included in it which have no relationship with one another. The group itself is probably (as regards some of its members at least) of high antiquity, since it appears to be connected with the Myxomycetes and the Monadines on the border line between the animal and vegetable kingdoms [Dangeard (1889), Fischer (1892)]. Hence, it must contain species whose relationships date from a very distant time. In these circumstances, variations in characters constant in other groups, need cause no surprise. The greater the antiquity of the group, the more they are to be looked for. Within the limits, however, of the genera of the *Holochytriaceæ* (*Ancylistaceæ*), with which *Pythium* shows relationship (excluding *Achlyogeton* and *Resticularia* which are aberrant in many respects) the shape of the zoospore is constant, and is that characteristic of the *Peronosporaceæ.*

To deal now with the relationships to each of these groups in detail. With the *Chytridiaceæ*, affinities are strongly marked with the family of the *Holochytriaceæ* or *Ancylistaceæ.* In the three genera *Myzocytium*, *Lagenidium* and *Ancylistes*, the vegetative body is segmented, and the whole of each segment becomes transformed into either a sporangium or an oogonium, or (in *Ancylistes*) passes into a tube of infection by means of which dissemination is effected. An indication of this complete transformation of the vegetative thallus into reproductive organs occurs in *Pythium gracile*, where in good cultures almost the whole contents of the mycelium become transformed into zoospores. The reproductive processes, so far as they are known, correspond closely with those of *Pythium*, except that a periplasm is not thrown off in the oogone and that the oospores in *Ancylistes* are multinucleate even when ripe [Dangeard (1890)]. These features, as well as the segmentation of the thallus, serve to separate the two families sharply. But the formation of *Pythium*-like zoospores in a bladder at the apex of the tube of the sporangium, together with the true sexuality, resembling, with the exception of the absence of a periplasm and the multinucleate oospore, the corresponding process in *Pythium*, are characters showing a decided affinity. In *Ancylistes*, as Fischer (1892) has pointed out, the transformation of sporangium into conidium is foreshadowed. It is unfortunate that the cytology of the *Holochytriaceæ* is so little known, as it may be expected to have an important bearing on the question of their affinities. It is certainly unsafe in the light of our present knowledge to draw any conclusions whatever as to the place occupied by the *Chytridiaceæ*, in the series of the Phycomycetes, from cytological evidence alone. The multinucleate oospore of *Ancylistes* cannot by itself lead us to deny relationship with *Pythium*. Stevens' researches on *Albugo* (*Cystopus*) (1899), have shown that a remarkable degree of diversity may occur within the limits of a single genus, in the type of fertilization. It is not unreasonable to suppose that as great diversities will be found in some of the other groups, as our knowledge of them increases, so far as a study of cytological conditions affords positive evidence of affinities, its importance cannot be overestimated. Negative evidence is for the present valueless.

With the *Leptomitaceæ* the affinities of *Pythium* are still more distinct, though here also a fuller knowledge of the cytology is much to be desired. The work of Thaxter (1894-'95-'96) on the exceedingly interesting members of this family, has shown that a series of forms occurs, connecting with *Pythium* on the one hand and, though less evidently, with the *Saprolegniaceæ* on the other. The vegetative body is segmented, recalling the *Holochytriaceæ* in this respect. Whole segments are transformed into sporangia, but the thallus itself is not entirely used up in the process. Zoospores are formed within the sporangium, but, in the genera *Rhipidium*, *Araiospora* and *Sapromyces*, escape in a mass surrounded by a bladder, which is more transient than that formed in *Pythium*. The zoospore is *Pythium*-like in the three genera mentioned above, while in *Apodachlya* diplanetism occurs similar to that in *Achlya*, and in *Apodya* (*Leptomitus*) the *Pythium*-like spore has been replaced by the other form mentioned above. Pringsheim (1860) states that diplanetism does not occur in this species, but Humphrey (1892) considers that it does.

The sexual processes are similar to those of *Pythium* in all important respects, periplasm being formed in abundance in *Rhipidium* and *Araiospora*, while it is scanty in *Sapromyces*. The cytology is unknown excepting in one genus, *Araiospora* (King 1903), where the processes of fertilization are shown to be related to those in *Pythium* and the *Peronosporaceæ*. In *Apodachlya* resting spores are formed, resembling the resting conidia of some species of *Pythium*, and in neither this genus, excepting a doubtful observation of Humphrey (1892), nor in *Apodya* have sexual organs been observed.

Hence the main character which serves to separate the *Leptomitaceæ* from *Pythium* is the segmentation of the thallus, a character possessed by the *Leptomitaceæ* in common with the *Holochytriceæ*. Species are found within the family illustrating the passage from *Pythium* and *Rhipidium* with abundant periplasm, through *Sapromyces*, where it is scanty, to the *Holochytriaceæ*, where its absence is complete.

With the *Saprolegniaceæ*, in spite of the fact that *Pythium* is still often included in the group, relationships are much less marked. The reasons which have led to the inclusion of *Pythium* in the *Saprolegniaceæ* may be shortly discussed. These are chiefly based on habit, and are largely the outcome of the erroneous idea that the genus is predominantly aquatic. This, as has already been said, is not the case. The land forms are quite numerous, and they lead by gradual steps very definitely to the

Peronosporaceæ. Habit, and the peculiarities of structure which may result from a particular habitat, are very unsafe guides in this connection. It is true that the aquatic forms are very similar to the *Saprolegniaceæ*, but two of the chief characters to which this similarity is due are, in all probability, secondary adaptations of little value from our point of view. They are the proliferation of sporangia, common to both *Saprolegnia* and *Pythium*, and the transformation of ordinary hyphæ wholly into sporangia as occurs in *Aphanomyces* and the sub-genus *Aphragmium* of *Pythium.* These are both, as pointed out in discussing the modifications induced by the invasion of the land, probably adaptations acquired by aquatic forms to ensure rapid dissemination, without wasting nutriment on useless hyphæ. It is true the habit of the vegetative mycelium is very similar, especially in *Aphanomyces,* but the character of the mycelium, except in so far as segmentation is concerned, is in itself of little importance in fungi. Segmentation is absent in both cases.

As far as the thallus is concerned, and in certain of the cruder details of reproduction, the aquatic species of *Pythium* approach the *Saprolegniaceæ*, particularly *Pythiopsis* and to a less degree *Aphanomyces.* But these resemblances are superficial and are probably partly connected with the environment. In the essential points in which affinities must be looked for, the divergence is considerable. Thus, the zoospores are never liberated in a bladder as always occurs in *Pythium.* The spores themselves are often diplanetic and dimorphic, a form (egg-shaped with two anterior cilia) not found in *Pythium,* being set free first, from which afterwards a *Pythium*-like spore arises. This, as already mentioned, is found also in *Apodachlya* and probably also *Apodya* amongst the *Leptomitaceæ.* A periplasm is not differentiated in the oogonium, which is usually many-egged, and fertilization is inconstant, most species being apogamous.

Cytologically the processes of oogenesis differ considerably. There is a withdrawal of protoplasm from the centre of the oogonium during the differentiation of the oospheres. No receptive papilla is formed (Trow, 1904). In *Pythium,* on the other hand, the centre of the oogonium is not denuded of protoplasm, and there is a receptive papilla. Other important differences may be found in the papers of Trow (1901, 1904).

Relationships, however, may be found hinted at in the majority of these characters. Thus, the *Pythium*-like zoospore is probably the primitive form in the *Saprolegniaceæ*, for there is evidence to show that the first type of spore is an interpolation. In *Aphanomyces* the spores escape as irregular lumps of protoplasm unprovided with cilia. They encyst at the mouth of the sporangium, forming a globular spore mass. From these cysts *Pythium*-like spores are liberated. In *Achlya* the process is similar, but the first spores reach the mouth as fully formed zoospores of the first type. In *Saprolegnia,* finally, these first spores swarm freely in the surrounding water for some time before coming to rest and giving rise to the second form. In long filamentous sporangia, such as those of *Aphanomyces*, chemotactic forces will be far more effective in emptying the sporangium than in the case of broad sporangia. Hence, in this genus, the spores emerge as unformed lumps requiring no propulsory organs to assist their escape. In *Achlya* cilia are evolved to help in the liberation, since the sporangium is broad and chemotactic force exerted to less advantage. *Saprolegnia* is merely a further advance on *Achlya*. Hence, the cilia of the first type of spore appears to have been evolved to replace a wholly passive mode of dissemination, by one partly passive, partly active. From the consideration of these three genera, the *Pythium*-like spore appears to be the primitive one in the *Saprolegniaceæ*.

Again, though a true periplasm is not formed in the *Saprolegniaceæ*, there are indications of a rudiment of it. In *Achlya* and *Saprolegnia* portions of the protoplasm are thrown off as the oospheres round off. Some of these are re-absorbed, but others degenerate (de Bary and Woronin 1881, Hartog 1895). De Bary has noted the probable homology of these with the periplasm of *Peronospora* (1881, p. 122). Also in *Pythiopsis* the same observer found (1888) that in cultures made at the beginning of winter a sort of outer layer was added to the wall of the oospore, though at other times of the year nothing of the sort was noticed. Although he did not observe the origin of this layer, he considered it resembled the throwing off of a periplasm.

Further, the central massing of protoplasm in the oogonium before fertilization is less marked in *Pythium* than it is in, for instance, *Cystopus*. The *Pythiaceæ*, therefore, are intermediate in this respect between the *Peronosporaceæ* and the *Saprolegniaceæ*.

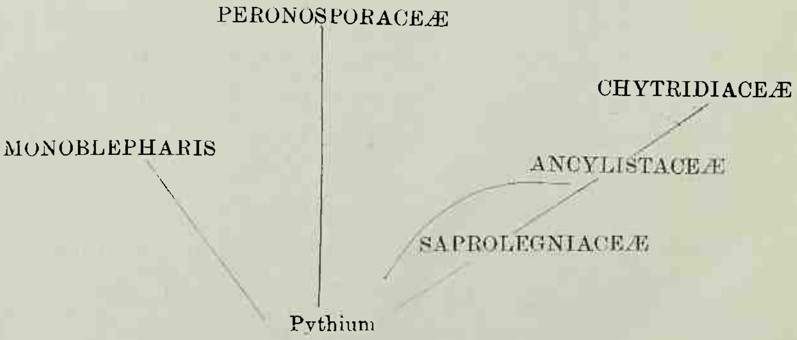
As to the question of fertilization in the *Saprolegniaceæ*, doubt still remains. Trow (1899, 1904) believes that it occurs normally in several species, and his last paper seems to me to be conclusive for one of them. Hartog (1899) and Davis (1903) have, however, not accepted Trow's earlier work. In *Aphanomyces*, which seems to have closer affinities to *Pythium* than most of the other *Saprolegniaceæ*, I have had an opportunity of observing the formation of the oospore in a species corresponding to *A. lævis* de Bary. There is certainly no fertilizing tube in this case. The antheridium closely encircles the oogonium, but does not send a process into its interior. Nevertheless, a receptive papilla is formed, before the contents recede from the oogonial wall, and it is possible that a nucleus may be transferred from the oogonium at this moment. This possibility is strengthened by King's investigations of *Araiospora* (1903). Here the receptive papilla is the active agent in establishing communication between antheridium and oogonium, as by it the oogonial wall is perforated. No antheridial tube is formed, but a nucleus and cytoplasm pass from the antheridium through the opening provided by the receptive papilla, down a tube formed by the oogonium itself. When the contents in the oogonium of *Aphanomyces* have receded, it is easy to convince oneself that no breach of continuity in the wall exists, so that if an opening is formed, it must be rapidly closed again. It is quite certain that many of the *Saprolegniaceæ* have completely lost their functional sexuality. Still the morphology of the sexual organs in *Pythiopsis*, *Leptolegnia* and *Aphanomyces* shows a distinct affinity with *Pythium*. *Pythiopsis* and *Aphanomyces* may represent two distinct, if distant, ines of relationship between *Pythium* and the *Saprolegniaceæ*.

We come now to the *Peronosporaceæ*, into which group *Pythium* seems most naturally to fall. It would appear that the differences between *Pythium* and the other members of the group are sufficient to justify Schroter's formation of a special family—the *Pythiaceæ*. This may be joined with the *Peronosporaceæ* to form an order, the *Peronosporineæ*. A perfectly natural series of forms occur in the genus *Pythium* leading up from the aquatic forms through *P. Cystosiphon*, *P. de Baryanum*, *P. vexans* and *P. intermedium* to the higher forms of the *Peronosporineæ*. But the genus *Pythium* is separated from all the rest by liberating its zoospores in an imperfectly differentiated state into a bladder at the mouth of the sporange, in which differentiation is completed. There are other minor differences, such as the aerial habitat and parasitism of the *Peronosporaceæ*, the formation of haustoria correlative with the latter, &c. None of these differences are absolute. In *Cystopus* the oospore germinates by liberating imperfectly differentiated zoospores in a mass into a bladder formed from the endospore, in which differentiation is completed, exactly as occurs in *P. vexans*. *P. palmivorum* is an example of a *Pythium* with the endogenous habit and parasitism of a *Peronospora*. An intercellular mycelium and haustoria are formed in this species, which is separated from *Phytophthora* by no other character than the manner of zoospore formation. Other characters, such as the formation of conidia in chains, are common to both groups (*P. Anguillulæ aceti*, *P. intermedium*, *Cystopus*). For a discussion of the points of similarity in the sexual processes, de Bary's work (1881) needs no supplementing. The recent cytological researches of Trow (1901) and Miyake (1901) have emphasized the relationships to the *Peronosporaceæ*. The details of fertilization are almost identical with those described by Wager (1900) in *Peronospora parasitica*. They are of value in showing that the relationship is closer to *Peronospora* than to *Cystopus*. This is especially seen in the mode of "zonation" which, as Wager (*l. c.*, page 271) has pointed out, differs somewhat in the two genera. The type in *Pythium* clearly approaches that of *Peronospora*.

The *Peronosporaceæ* are, then, connected through the *Pythiaceæ* with the *Leptomitaceæ* and through these, more distantly, with the *Chytridiaceæ* and *Saprolegniaceæ*. The question at once arises in the line a continuous one, whether progressive as held by Fischer (1892), or in part retrogressive, as de Bary (1881) and Brefeld ("Das natürliche System der Fadenpilze," Unters. VIII) thought, or have the extremes diverged from an ancestral form intermediate in type? Are the *Chytridiaceæ* or the *Peronosporaceæ* the forms from which the others have arisen, or have they come from a third type intermediate between these?

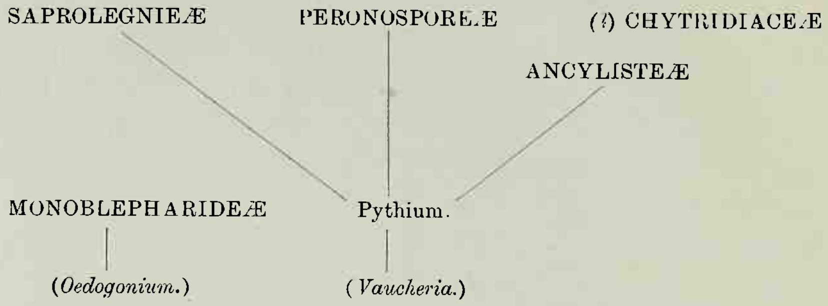
The question hinges on the relationships of the various groups to the algæ from which there is scarcely a doubt they have arisen. There can be little hesitation in admitting, from the affinities which have been shown above to exist between the four groups of the Oomycetes—the *Leptomitaceæ*, *Holochytriaceæ*, *Saprolegniaceæ* and *Peronosporaceæ*—that they have a common origin. Have we anything to show where this must be sought?

On de Bary's view (1881, 1884, p. 142 *et seq.*), the *Peronosporaceæ* (with *Pythium*, the *Ancylistaceæ* and *Monoblepharis*) stand at the beginning of the main line of the fungi. *Pythium* is the link which connects the group with the *Chlorophyceæ*, especially *Vaucheria*. Connected also with *Pythium* are the *Saprolegniaceæ* as a side branch. From the *Peronosporaceæ*, either direct or through the *Saprolegniaceæ*, have diverged the *Chytridiaceæ* in a line with marked degenerate tendencies. The *Ancylistaceæ* show affinities both with *Pythium* and the *Chytridiaceæ*. The arrangement can be shown somewhat in this manner:—



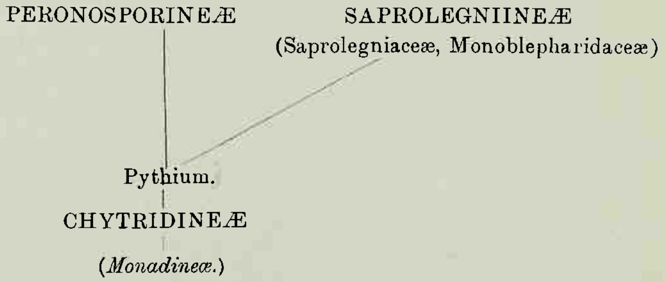
But this view assigns to *Pythium* a more primitive place amongst the Oomycetes than appears to be justified by the characters of the genus. The *Peronosporaceæ* are almost universally considered to be the most highly developed of the group, and we have seen that *Pythium* is very closely connected with them. Whether in their asexual or sexual reproduction, the facts are not such as would lead us to derive them very directly from any algal form ; and no intermediate forms are known which would mark the transition to *Vaucheria*.

Brefeld, since he did not deal specially with the groups allied to *Pythium*, has not expressed definite views regarding their relationships further than to state that the *Chytridiaceæ* are a degenerate series, in which the vegetative body is more and more lost, until finally the whole is reduced to a sporangium ("Das natürliche System der Fadenpilze", Unters. VIII). His follower, Von Tavel (1892), is more explicit. *Monoblepharis* may be looked on as a chlorophyll-free alga. The sexual organs are sporangia (gametangia). In *Pythium* and the vegetatively degenerate *Ancylistaceæ*, the antheridium has lost its sporangial character, but still empties the whole of its contents, which pass into the oogonium. In *Phytophthora* only a small part is thus emptied. In the other Oomycetes, the reduction of the male sporangium goes still further ; in the remaining *Peronosporaceæ* and the *Saprolegniaceæ* the antheridium is functionless, or even in many of the latter no longer formed. (This is, of course, erroneous as the more recent cytological work of Wager, Stevens, Trow and others has shown.) The oogonium retains its character as a sporangium. It encloses in the *Saprolegniaceæ* several, in the others only one egg-cell. Only in the *Entomophthoraceæ* does it become as reduced as the antheridium. The asexual sporangia are usually, as in the algæ, zoosporangia. They undergo a reduction in the *Peronosporaceæ* to conidia. Two distinct lines of divergence from the algæ are indicated ; the first from *Oedogonium* to *Monoblepharis* ; the second from *Vaucheria* through *Pythium* to the *Peronosporaceæ*, *Ancylistaceæ* and *Saprolegniaceæ*. The *Chrytridiaceæ* occupy an uncertain place. The arrangement may be indicated schematically, as far as such is possible, as follows :—



This assigns the same primitive position to *Pythium* as de Bary's view, and is hence equally unsatisfactory.

According to Fischer (1892), the *Chytridiaceæ* are the most primitive group, derived from the Mycetozoa and giving rise not only to the Oomycetes through the *Holochytriaceæ* (*Ancylistaceæ*), but also to the Zygomycetes through *Resticularia*, if Dangeard's (1890) observations be correct. The *Peronosporineæ* and *Saprolegniineæ* are two co-ordinate groups linked by *Pythium*. On this view the relationships may be thus represented :—



The derivation of the Oomycetes from mycetozoan rather than algal ancestors has little to recommend it, even though we should admit that some so called "*Chytridiaceæ*" show a transition to the *Monadineæ*. The affinities of *Monoblepharis* and *Pythium* with the algæ are too strong to be ignored. It seems more reasonable here to admit a line of evolution for the primitive *Chytridiaceæ* distinct from that of the Oomycetes.

Schröter (1897) assigns to the *Chytridineæ* relationships with the *Monadineæ* and *Protococcoideæ*. From them are derived the *Saprolegniaceæ* through the *Ancylistaceæ.* To *Pythium* (included by Schröter in the *Saprolegniineæ*) are related the *Peronosporineæ.* The *Monoblepharidineæ* come near the *Saprolegniineæ.* The relationships are roughly as follows :—

PERONOSPORINEÆ.

SAPROLEGNIINEÆ. —MONOBLEPHARIDINEÆ.
(Saprolegniaceæ, Pythiaceæ, Leptomitaceæ.)

CHYTRIDINEÆ.

(*Monadineæ and Protococcoideæ.*)

This agrees with Fischer's classification except as regards the *Saprolegniineæ,* in which *Pythium* is included, and which is regarded as a more primitive group than the *Peronosporineæ.* The *Saprolegniineæ* are made to include three orders, the *Saprolegniaceæ, Leptomitaceæ* and *Pythiaceæ.*

The position of *Monoblepharis,* with its obvious algal affinities, in the classifications of Fischer and Schröter, is anomalous. Their views may perhaps have been influenced to some extent by a desire to represent the Phycomycetes as a single series, derived from a common ancestor. That the Oomycetes, as above grouped, including the *Ancylistaceæ,* but not necessarily the other "*Chytridiaceæ,*" form a natural series, appears probable, and if *Monoblepharis* is to be included in the Oomycetes at all, as I think cannot be avoided, it must occupy the most primitive position, indicating the direction through which the whole group diverged from the algæ. Unfortunately its relationships to the other Oomycetes are not entirely clear. A form described by Reinsch (1878) as *Saprolegnia siliquaeformis,* and subsequently stated by Cornu (1877) to be identical with his *Monoblepharis prolifera* may, however, represent a type intermediate between the *Monoblepharidaceæ* and the *Leptomitaceæ.* Fischer (1892) afterwards formed the genus *Gonaodya* for this form, and Thaxter (1895) added a second species, *Gonapodya polymorpha.* Though Cornu (1877) mentioned that the sexual process in his species resembled those of *Monoblepharis,* Thaxter considers it possible that Cornu may have been misled in his observations, and figures a different form of oospore found in connection with *G. polymorpha,* but not proved to be actually belonging to it. He assigns to the fungus a doubtful place amongst the *Leptomitaceæ.* Should the observations of Cornu have been correct, the genus is undoubtedly closely connected with the *Monoblepharidaceæ* and shows also a relationship to the *Leptomitaceæ.* To the former it corresponds by its reproductive processes and zoospores, and to the latter by the segmented mycelium. Even if Cornu's account of the sexual processes be incorrect, affinity to the *Monoblepharidaceæ* seems to be indicated. The asexual reproductive processes are almost identical, and are peculiar amongst the Phycomycetes. Lagerheim (1900) has since described two imperfectly observed fungi, which he refers to the *Monoblepharidaceæ* as *M. regignans* and *M. ovigera.* His reasons for this are chiefly the character of the contents of the vegetative hyphæ, a distinguishing mark of the *Monoblepharidaceæ,* and the formation and liberation of the uniciliate zoospore. Their sexual form was not observed. But these two species recall *Gonapodya* in the shape and arrangement of their sporangia, and in the latter being proliferous. It is true they are not distinctly segmented, but Thaxter has remarked that *Gonapodya polymorpha* sometimes loses this character. On the whole the discovery of these two species increases the probability of a relationship between *Monoblepharis* and *Gonapodya,* and through the latter with the *Leptomitaceæ.* If this be so, and the matter must remain in doubt until the discovery of the sexual organs of *Gonapodya* and the study of the process of fertilization, especially in its cytological aspects, the *Leptomitaceæ* are the group from which *Holochytriaceæ*, *Saprolegniaceæ* and *Peronosporaceæ* have diverged. They are derived through the *Monoblepharidaceæ* from the *Chlorophyceæ* or green algæ, probably from a form allied to *Oedogonium.* The line would be a fairly direct one for the *Leptomitaceæ, Pythiaceæ* and *Peronosporaceæ*, while the *Saprolegniaceæ* would be a group with somewhat degenerate tendencies, and degeneration would be strongly marked in the *Holochytriaceæ*.

A totally different suggestion as to the ancestry of the Oomycetes has been offered by Davis (1900). He has been led to imagine a development from Zygomycetal ancestors. There appears to be little to support this view except a doubtful resemblance between the formation of the zygospore of the *Mucorineæ* and the fertilization of the "compound oosphere" of *Cystopus Bliti*. But as Trow (1901) has pointed out, Stevens' observations on *Cystopus Bliti* are far more readily explicable on the basis of the homology indicated by Von Tavel (1892), and Dangeard (1898), of the sexual organs with the sporangium. Just as the multinucleated conidium of the *Pythiaceæ* is merely a sporangium, whose zoospores have failed to individualise, and is therefore a "compound" spore, so the "compound oosphere" is a sexual sporangium (gametangium), whose gametes remain united. The female gametangium of *Monoblepharis* contains a single gamete. Its oosphere is then simple in the strictest sense of the word. But it is doubtful how far the oospheres of the other members of the Oomycetes can be considered "simple." They are uninucleate, it is true, in the majority of cases, but they have reached this condition by the suppression of the individuality of numerous gametes. The nuclei of all but the favoured gamete, together with a part of their cytoplasm, have been pushed out to the periphery and there degenerate, or are used in the formation of the wall of the egg. The successful gamete acquires a portion of their cytoplasm and is probably benefited thereby. But it is clear that the term simple as opposed to compound in this case, is something of a misnomer. The condition is intermediate between the truly simple oosphere of *Monoblepharis*, and the truly compound oosphere of *Cystopus Bliti*.

Since the derivation of the sexual from the asexual sporangium must have occurred at an early period in the evolution of the algæ, so the appearance in the genus *Cystopus* of numerous functional gametes offers no aid in tracing its immediate descent.

The condition which *Cystopus Bliti* still represents, is a primitive one, though as a whole it is certainly not a primitive Phycomycete. It has merely preserved a degree of functional activity in its gametes which the majority of the Oomycetes have lost. The occurrence of the conditions described by Harper (1900) in *Pyronema*, shows that it is not necessary to assume that the multinucleate oosphere indicates a primitive position. The algal forms, amongst which it appears that we must look for the ancestors of the Oomycetes, have themselves already reached the uninucleate condition. *Monoblepharis*, clearly a very primitive Phycomycete, has a uninucleate oosphere. But its occurrence in these cases does not invalidate the proposition that the gametangium was the earlier form. It only leads to the conclusion that the transition from gametangium to oogonium took place before the Oomycetes diverged from the algæ.

There are still many gaps to be filled up before we can come to a definite conclusion as to the ancestry of the Oomycetes. In particular a fuller knowledge of the cytology of the *Leptomitaceæ* and *Chytridiaceæ* is to be desired. Still, I think, we are justified in considering that a derivation of the main families through *Monoblepharis* and the *Leptomitaceæ* has most in its favour. The discussion might be held premature at the present stage of our knowledge, were it not for a tendency to base classification on a single character, particularly as regards cytological details, which is sometimes noticeable. It is said in the "Origin of species," (Chapter XIV) that "a classification founded on a single character has always failed." If the consideration of the broad affinities of *Pythium* detailed above, emphasises the need for caution in regard to the value of particular characters, it will not be useless.

SYSTEMATIC.

Fischer (1892) divides *Pythium* into three subgenera : *Aphragmium* characterised by the absence of specially marked off sporangia ; *Nematosporangium*, containing the single species *P. monospermum* Prings., with filamentous sporangia cut off by a septum from the vegetative mycelium ; and *Sphærosporangium* with specialized more or less spherical sporangia or conidia. The validity of the character on which the subgenus *Nematosporangium* was founded in *P. monospermum* is, however, as will be seen below, doubtful. Under the circumstances it seems unnecessary to separate this species from the *Aphragmia*. The classification which I propose to adopt is as follows :—

I. SUB-GENUS *Aphragmium*.

Sporangia filamentous, resembling the vegetative hyphæ, not separated off from the vegetative mycelium by septa, often branched and very unequal in size. Conidia unknown. Species predominantly aquatic.

A. Antheridia (perhaps also oogonia) not cut off from the mycelium by septa.

1. *P. tenue* Gobi.

B. Antheridia and oogonia cut off by septa.

I. OOSPORES SMOOTH.

2. Parasitic in algæ and saprophytic in vegetable debris. Oospore not filling oogonium. *P. gracile* Schenk.
3. Saprophytic on dead insects and in soil. Oospore filling oogonium. *P. monospermum* Prings.
4. Epiphytic on leaves of *Indigofera*. Oospore not filling oogonium. Mycelium toruloid. *P. Indigoferæ n. sp.*

II. OOSPORES RETICULATE.

5. Parasitic in algæ. *P. dictyospermum* Racib.

II. SUB-GENUS *Sphærosporangium*.

Sporangia spherical, oval, etc. (not filamentous), cut off by septa from the vegetative mycelium.

A. Sporangia proliferous, conidia unknown.

I. OOGONIA SMOOTH.

6. Antheridia club-shaped, usually from a distinct branch. Sporangia normally vacuolated. *P. proliferum* de Bary.

7. Antheridia cylindrical, usually hypogynal. Sporangia usually not vacuolated. *P. ferax* de Bary.

II. Oogonia spiny.

8. *P. megalacanthum*, de Bary.

III. Sexual reproduction unknown.

9. Hyphæ very slender, up to $1\frac{1}{2}\mu$. Sporangial tube broader and very long. *P. diacarpum n. sp.*

B. Sporangia not proliferous, often transformed into conidia.

I. Oogonia and oospores smooth.

10. Parasitic on palms. Mycelium intercellular. Sporangia pear-shaped. Conidia not known. *P. palmivorum n. sp.*

11. Saprophytic. Sporangia numerous, subspherical. Oospore filling oogonium. *P. rostratum n. sp.*

12. Parasitic in seedlings. Sporangia subspherical. Oospore not filling oogonium. *P. de Baryanum* Hesse.

13. Saprophytic in soil. Sporangia rare. Conidia irregular. Oospore not filling oogonium. *P. vexans* de Bary.

14. Saprophytic. Sporangia not known. Conidia subspherical. Oospore not filling oogonium. *P. ultimum* Trow.

15. Parasitic in *Anguillula aceti*. Sporangia and conidia often catenulate. All spores very small. *P. Anguillulæ aceti* Sadebeck.

16. Parasitic in prothalli and saprophytic in soil. Sporangia and conidia catenulate. Sexual reproduction unknown. *P. intermedium* de Bary.

II. Oogonia smooth. Oospores spiny.

17. Parasitic in water plants (*Lemna*, &c.). Sporangia spherical. *P. Cystosiphon* (Roze and Cornu) Linstedt.

III. Oogonia spiny. Oospores smooth.

18. Asexual reproduction unknown. Antheridia always hypogynal. *P. Artotrogus* (Mont.) de Bary.

I. Sub-genus Aphragmium.

There is great confusion apparent in the literature of the members of this subgenus. The most recent paper which deals with the subject, that of Gobi (1899), does not appreciably help to lighten the task of those who would follow their relationships.

Schenk (1859) described as *P. gracile* a form found parasitic in green algæ (*Spirogyra*, &c.). The mycelium was fine : sporangia unmarked from the rest of the thallus in any way, discharged zoospores to the outside of the algal filament, and were the only reproductive bodies observed. Later, de Bary (1860) described as *P. reptans* a similar form, but he also failed to obtain its sexual condition. A variety found saprophytic on dead insects in water was included with this species at first, but afterwards considered by de Bary to belong rather to that next mentioned. This was described (de Bary 1881) under the name *P. gracile*. Morphologically it was indistinguishable from Schenk's *P. gracile*, except that it formed oospores, but physiologically it differed in refusing to attack living algæ. De Bary left the question of the real identity of these two forms open, while admitting that the algal parasite which he had previously called *P. reptans* was probably Schenk's species, and the name *P. reptans* should therefore be suppressed. So far two forms had been described under the same name, *P. gracile*, one parasitic and one saprophytic, and their relationship had been left unsettled.

Two years later, Ward (1883) described fully, a form which he considered to be de Bary's species, calling it *P. gracile* de Bary. It differed in the minor details of forming its oospores extramatrically, instead of within the substratum and in possessing lateral buds on the filaments. These differences were probably cultural, and there can be little doubt that the identification was correct. In the course of the same research, Ward also found a *Pythium* with oospores differing from those of the above and formed within the cells of a *Spirogyra*. Though the sporangia were not observed, it at once suggested itself that these were the long-sought-for oospores of Schenk's species, which would therefore be quite distinct from de Bary's *P. gracile*. It is not now possible to be certain that this is so. For other species of *Pythium* which live as parasites within green algæ have since been found, which resemble Schenk's and Ward's species in their vegetative mycelium and sporangia, but differ from the latter in their sexual state. Hence, Schenk's species may be represented by any of these, or be a quite distinct species whose sexual state has never been observed. The algal parasite which I have studied agrees entirely with the partially observed one of Ward. The other forms have both been provided with distinct names, *P. dictyospermum* Raciborski, and *P. tenue* Gobi, and I propose therefore to retain the name *P. gracile* Schenk, for the form observed by Ward and myself, even though we can never be sure what fungus Schenk actually saw. This appears to be more satisfactory than to add another to the names of the *Pythium* parasites of algæ.

The three algal parasites, therefore, which are now fully known, are :—

1. The species which I have identified with *P. gracile* Schenk, and which was also partly studied by Ward, with a smooth oospore not filling the oogonium.
2. *P. dictyospermum* Raciborski, with a reticulately walled oospore.
3. *P. tenue* Gobi, with the antheridium not cut off by a septum.

Of the saprophytic forms of this subgenus the first was described by Pringsheim (1858), as *P. monospermum*. In this the sporangium was said to be cut off from the thallus by a basal septum. *P. gracile* de Bary (1881) (not Schenk) differed from the last, only in having sporangia not separated from the thallus. For it, since Schenk's *P. gracile* takes precedence, Fischer (1892) proposed the name *P. complens.* As will be seen, I have been unable to separate these two forms, and have included *P. complens* under *P. monospermum.* The second saprophytic species is that which I have named *P. Indigoferæ*, which differs from the last in having an oospore not filling the oogonium

Gobi (1899) has discussed the relationships of some of the above forms, and proposed a classification based on the presence or absence of septa cutting off the reproductive parts from the thallus. It is as follows :—

SUB-GENUS *Nematosporangium.*

Sporangia filamentous, not thicker than the mycelium, single or branched, formed from mycelial segments of irregular size.

(*a*) Zoosporangia and antheridia (perhaps also oogonia) not cut off by septa from the mycelium.
P. tenue, Gobi.
(*b*) Zoosporangia not cut off, antheridia and oogonia cut off.
P. distyospermum, Racib.
(*c*) All reproductive organs, asexual and sexual, cut off by septa from the mycelium.
P. monospermum, Prings.
P. reptans, de Bary (*P. complens*, A. Fischer).

It will be noticed here that Gobi states that the sporangia of the saprophytic *P. reptans* de Bary, which is the *P. gracile* of de Bary (1881) and Ward (1883) (*P. complens*, Fischer) are cut off from the rest of the mycelium by septa. He supports this (since he has not himself studied the species) by a quotation from de Bary's paper of 1860. It is possible, however, from his 1881 paper, that de Bary himself did not adhere to this original opinion. He is, in this, emphatic about the morphological identity of Schenk's *P. gracile* and his own *P. reptans* and *P. gracile.* I have had what is certainly de Bary's *P. gracile*, as well as the species which I have identified with Schenk's, in culture for many months, and have never observed the sporangia to be cut off, though the accidental appearance of this, as described under *P. monospermum*, was not rare. Fischer also (1892, p. 398) agrees with Schenk that septa are absent. It is possible, with de Bary, as with Pringsheim, that the purely vegetative formation of septa may have led to error in the earlier observations. Until further observations show clearly that there are species parasitic in algæ with filamentous sporangia separated off by septa, it is impossible to accept the earlier observations. It is certain that de Bary did not lay special stress on the point.

The whole of this subgenus has been raised to generic rank, under the name of *Nematosporangium*, by Schröter in Engler's Pflanzenfamilien.

P. TENUE *Gobi.*

Entwickelungsgeschichte des Pythium tenue nov. sp. "Scriptis Botanicis Horti Univers. Imper. Petropolitanae." Fasc. XV, 1899, plates IV, V.

P. entophytum Schenk (1859) non Pringsheim.

Mycelium of very fine hyphæ of even diameter, often richly branched and with a tendency to form coiled masses in the host cells, mostly intramatrical, but also extending outside in the surrounding water and passing across to infect new plants ("infection hyphæ"). Branching irregular, sometimes falsely dichotomous. Contents at first finely granular, afterwards collected into dense areas separated by clearer spaces. Sporangia irregular in size, single or branched, extending to the exterior of the host cell by short straight branches arising laterally from the intra-cellular mycelium, rarely more than two to four times as long as broad. These branches are usually swollen just below the cell wall. Zoospores 2—60 from each sporangium, germination only observed in those which come to rest on a cell of the host. These penetrate the cell wall by a fine process through which the spore contents flow, leaving the zoospore wall behind as an empty sphere. Oogonia only within the host cells, terminal, 12μ in diameter, not completely filled by the oospore. Antheridia usually one, sometimes two, club-shaped, not cut off by septa, arising from the oogonial hypha or from a neighbouring one, oospores smooth, 8-9μ in diameter, germination not observed.

The sporangial contents sometimes fail to divide up into zoospores after escape from the sporangium. Gobi considers this is a transitional stage between sporangia and conidia. In *P. equiseti*, Sadebeck observed a similar phenomenon, and states that the extruded mass can germinate by putting out a hypha. This recalls the germination of *Plasmopara* where the transition is certainly indicated.

Gobi identified his fungus with the species called *P. entophytum* Pringsheim by Schenk (1859). He points out that Schenk's *P. entophytum* was certainly not Pringsheim's, since the former occurred in the vegetative cells, the latter only in the zygospores of *Spirogyra*. Pringsheim's species is, as was shown by Zopf (1884), a *Lagenidium* and no *Pythium*.

This species differs from *P. gracile* Schenk, as observed by Schenk, in several points. In the latter the hyphal contents are not marked into dense and light areas. The sporangia show a larger swelling below the point where they pierce the wall The zoospore on germination enters as a whole into the host cell It differs from the other algal parasites of the genus chiefly in its sexual reproduction.

Parasitic in *Vaucheria sessilis* DC. and *Mesocarpus sp.* from Finland (Gobi).

P. GRACILE *Schenk.*

Verhandl. d. phys. med. Gesel. in Würzburg, IX, 1859, p. 12, plate I, figs. 1—6.

P. reptans de Bary, *pr. p.*, Jahr. wiss. Bot. II, 1860, plate XXI, figs. 38—41.

P. sp. Marshall Ward, Q. J. M. S., Vol. XXIII, n. ser., 1883, Plate, XXXVI, figs. 37—39.

Nematosporangium gracile (Schenk) Schröter, de Wildeman, notes Mycologiques VI. Ann. Soc. Belge Micros., T. XIX, 1895, p. 207.

Mycelium intra-matrical and also extending freely outside into the surrounding water. In the saprophytic form it grows out as a fine cloud surrounding the substratum. Hyphæ from extremely fine, up to 4μ in diameter, often with numerous thickened lateral processes, branching irregular; sporangia single or branched, in the parasitic forms opening to the exterior by nearly straight, rather short, branches, usually swollen below where they pierce the cell wall, length very variable, up to 1 mm. Zoospores from a few, to 40 or more. Oogonia formed usually only intramatrically, 15-33μ in diameter. Antheridia single, from a neighbouring branch, oospores smooth 13-24μ, never filling the oogonia. Germination not observed.

The following varieties are included in the above description :—

(*a*) Parasitic in green algæ, oogonia 25-30μ, oospores 20-24μ, hyphæ about 1·5 to 2μ.

(*b*) Saprophytic in soil, oogonia 15-22μ oospores, 13-20μ, hyphæ very fine.

(*c*) Parasitic in the roots and base of stem of *Zingiber officinale* in the field, and in roots of *Ricinus communis* in water culture. Oogonia only found in former, 27-33μ, oospores 23-27μ, hyphæ up to 4μ.

The algal form was found in *Vaucheria aversa* from running water in the Mooswald near Freiburg i. Br. It was cultivated saprophytically, but under these conditions zoospore formation was reduced. It was not, indeed, until after six successive cutting cultures on *Abutilon* root that I obtained a few sporangia. In slide cultures of the infected alga a considerable extra-matrical growth occurred, but few sporangia. Within the host cells a distinct tendency to the formation of matted hyphal clumps, such as are commonly found in the saprophytic cultures, was observed. The chlorophyll was collected into a mass in the centre of the cells, and in and around this the hyphæ formed a coiled interwoven mass (plate I, fig. 13). This condition was rarely seen in infected filaments when first gathered, but appeared in those secondarily attacked from these, in the laboratory. In nature it is usual to find long branched filaments ramifying from cell to cell without much coiling. Oospores were found both in the freshly gathered material and also in those secondarily infected in the laboratory. In slide cultures, however, they were not matured, though large numbers of oogonia were formed within and outside the host cells. These were not fertilized, though antheridia were formed, and not a single case was observed in which the oospore was developed. They, however, permitted of a study of the positions of oogonia and antheridia (plate I, fig. 11). When cultivated saprophytically on *Abutilon* root and insects, no oogonia appeared. Re-infection of healthy *Vaucheria* from these saprophytic cultures was difficult and it was only obtained when eventually the *Vaucheria* filaments showed signs of failing health. I afterwards found that most of the water, both distilled and tap, which was used, contained traces of copper. It is possible that the unsatisfactory results were in part due to this, but on the whole the evidence pointed to the fungus being a weak parasite. As a parasite, when once established, it is certainly capable of vigorous growth and reproduction. Infection from filament to filament in this condition is easy to obtain. Saprophytic cultures taken direct from the parasitic plants gave good vegetative growth but poor reproduction, the latter, however, appearing to increase with subsequent sub-cultures. Having thus got the fungus habituated to the saprophytic life, it was found difficult to obtain a return to parasitism. Hence, the fungus appears to be one which, according to its mode of life, may be either a facultative parasite or a facultative saprophyte. In the former case its virulence requires to be accentuated by growth for some time on specimens of the host plant whose resistance has been diminished, before it can freely attack quite healthy plants.[1] Having become established on healthy plants, it can then readily infect new ones, and in these cases the hyphæ extend rapidly from cell to cell with less branching than when grown on weakened plants, and without the formation of hyphal clumps. I have in this manner infected a considerable proportion of a large quantity of the alga kept in a large vessel, and obtained mature oospores.

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[1] For some interesting observations on accentuation of virulence, sec Laurent, E. " Récherches Expérimentales sur les maladies des Plantes " Anns. Inst. Pasteur, T. XIII, No. 1. 1899.

This form quite agrees with that partially studied by Ward (1883, p. 511, plate XXXVI, figs. 37—39).

I have often found a species parasitic on *Spirogyra* with the slender hyphæ and short sporangial branches of Schenk's fungus. As, however, I never obtained sexual reproduction in this host, and as both *P. tenue* and *P. dictyospermum* are found in the same plant, it is not certain to what species these forms belong. The same, perhaps, applies to de Wildeman's species under this name.

The form found saprophytic in soil was found at Antibes and again at Calcutta In the former case it was characterized by very free zoospore production and by the formation of large hyphal clumps (plate I, figs. 3 and 4). The latter were found chiefly in older cultures which had been left in unchanged water for some weeks. The oospores were formed entirely within the substratum (*Abutilon* root), and their position on the hyphæ could not be detected, nor the origin of the antheridia. In Calcutta it was found on decaying *Marchantia* plants, and differed from the above only in being generally smaller.

The parasite of *Zingiber* and *Ricinus* was found in Surat on the former and in Dehra Dun on the latter, and could not be morphologically separated, though oospores were only obtained on the former host. It is the cause of a serious disease of ginger. Affected plants can be recognized by a whitening of the leaves and stalks and are softened at the collar, though not to such a degree as to make them fall over. Once the disease becomes established, no new rhizomes are formed. The fungus is found in the true roots, rhizomes and base of the stem, the hyphæ penetrating the scales for a considerable distance. Sporangia were freely formed in culture. The oospores appear chiefly on the scales at the base of the stem, below ground.

On *Ricinus* this fungus appeared in some water cultures, attacking the root, and causing their loss. Plants grown at the same time in natural soil were not attacked, though the infective material which gained entrance to the water cultures came from the same plot, the cultures having been treated with the washings of a portion of this soil, in order to supply them with common soil organisms. This indicates that the fungus acted as a weak parasite similar to the algal form. It was capable of causing injury to plants grown under the artificial conditions of water culture, but not similar ones grown in natural soil. No oogonia were formed, so its identity is doubtful.

Parasitic in *Vaucheria aversa* Hass. Freiburg i. Br.; *Zingiber officinale*, Surat, India, and (?) *Ricinus communis*, Dehra Dun, India. Saprophytic in soil, decomposing plant remains, etc., Antibes and Calcutta.

Parasitic in *Spirogyra sp.*, Great Britain (Ward).

Parasitic in *Spirogyra* and *Cladophora*, France and Belgium (de Wildeman).

Parasitic in *Spirogyra nitida* and *Heeriana*, and in *Cladophora*, Germany (Schenk).

Parasitic in *Vaucheria* and in *Bangia atro-purpurea*, Germany (de Bary).

PLATE I; PLATE II, FIG. 1.

P. MONOSPERMUM *Pringsheim.*

Jhar. wiss. Bot. I, 1858, p. 288, plate XXI, figs. 2—16.

Dangeard, Recherches histologiques sur les Champignons. Le Botaniste II, 1890, p. 122, plate VI, figs. 32—38.

P. reptans de Bary *pr. p.*, Jahr. wiss. Bot. II, 1860, plate XXI.

P. gracile de Bary non Schenk, Abh. Senck. Ges. XII, 1881, p. 19, plate II, figs. 6—15, and Bot. Zeit 1881, p. 569, plate V, figs. 25—28.

Marshall Ward Q. J. M. S., Vol. XXIII, n. ser., 1883, p. 503, plates XXXV, XXXVI, figs. 22—36.

P. fecundum Wahrlich, Ber. deutsch. bot. Gesel., V, 1887, p. 242, plate X, figs. 1—12.

P. complens A. Fischer, Rabenhorst's Kryptogamenflora, I, Ab. IV, 1892, p. 398.

Mycelium forming a cloud around the substratum. Hyphæ irregularly branched, up to 7μ in diameter, often with numerous bud-like outgrowths laterally. Sporangia and zoospores as in *P. gracile*, but general habit larger. Oogonia within and outside the substratum, terminal or intercalar or formed in the lateral buds. Antheridia one or more, club-shaped, arising from the oogonial or from a distinct hypha. Oospores smooth, completely filling oogonium, the wall of which is often difficult to define, 12—15μ in diameter, germination after a rest, which may be several months in duration, by a hypha which is quickly transformed into a sporangium.

This species was doubtfully separated from Schenk's *P. gracile* by de Bary (1881), owing to its failure to infect living algæ. It differs also in its much greater mycelial development and in the fact that its oospores completely fill the oogonia. The multiple-spored oogonia described by Wahrlich are probably only a further development of the oosporal formation in the bud-like processes described by Ward. It is not possible to separate Wahrlich's species from this. Wahrlich's figures 3 and 8 seem also to indicate that his cultures were attacked by parasitic *Chytridiaceæ*. The accumulation of dense protoplasm in the centre of the oogonium is quite unlike any normal stage in oospore formation and resembles closely the early stage of spore formation in *Pseudolpidium* (see plate VII, figs. 1 and 9).

The saprophytic form of de Bary's earlier *P. reptans* doubtless belongs to this species also.

The name *P. complens* was given by Fischer (1892) to include de Bary's *P. gracile* and saprophytic form of *P. reptans*. It cannot be separated from Pringsheim's *P. monospermum* by any character of specific value in our present knowledge of these forms. The only distinguishing mark, the presence of a septum cutting off the unbranched sporangium from the vegetative mycelium, in Pringsheim's species, is of too doubtful value to be admitted in classification. It is so universal to find septation in the saprophytic forms of *Pythium*, that we must suppose Pringsheim's form was similarly septate. From the nature of the sporangium the discharge of the contents of a hypha down to such a vegetative septum is likely at times to occur. Similar septa are common in what is undoubtedly de Bary's *P. gracile*. In *P. Indigoferæ* the same occurs frequently. In certain cases this would give rise to the appearance of a definite sporangium as described by Pringsheim. But the absence of any mention of a species in which this septation is part of the reproductive stage since Pringsheim's time, joined to the frequency in nature of the form in which the sporangia are not differentiated, makes it at least probable that Pringsheim was misled by a purely vegetative occurrence. Ward (1883, p. 503) also considers that with the evidence at hand these forms cannot be definitely distinguished.

The species described by Raciborski (1900) as *P. complens* Fischer, is a facultative parasite, according to the author. If the identification be correct, this is the only mention we have of parasitism in this species. It is not, however, definitely stated that the oospore completely fills the oogonium, though this is implied in the author's remark that his fungus agrees quite with de Bary's description. Should the oospore be smaller than the oogonium, this would probably be *P. gracile*, in a condition similar to that found on *Zingiber* and *Ricinus*. The fungus was found in tobacco plants attacked by the "bibitziekte," *Phytophthora Nicotianæ*, Br. d. Ha., and grew vigorously on cut green tobacco stems, but failed to infect healthy plants. It occurred in the roots and base of the stem, producing freely both sporangia, without a basal septum, and oogonia.

Saprophytic in soil, Cork.

Saprophytic on insects in water, Germany (Pringsheim, de Bary, Wahrlich), England (Ward), France (Dangeard).

Plate II, fig. 2.

P. INDIGOFERÆ *n. sp.*

Mycelium scanty on the leaves, dense in water culture. Hyphæ very irregular in size, up to 8μ in diameter, with large lateral outgrowths, roundish or cylindrical, up to 12μ in diameter. Sporangia not frequent, branched, often small, opening laterally by short straight branches. Zoospores from 4—20. Oogonia terminal on rather short lateral branches or in the bud-like lateral outgrowths, very constant in size, 18—20μ in diameter. Antheridia, when filamentous, always single and arising below the oogonium from the same hypha, very rarely from another. When formed from the lateral processes, it is possible that more than one may be provided. The antheridial stalk is nearly straight, but that of the oogonium is strongly curved towards the antheridium producing a characteristic appearance. Oospore not filling oogonium, smooth 14—16μ or, less commonly, 13—18μ in diameter, germination rapid, by a hypha, not by zoospores.

This species, which is of interest because of its peculiar habitat in the waxy covering of leaves of *Indigofera arrecta*, must, I think, be separated from any of those above described. It is related to *P. gracile* in the structure of the sexual organs, but differs in the origin of the antheridium from the oogonial stalk. The mycelium is much larger in culture, and the lateral buds more copious and highly developed, than those of *P. gracile*. In some cultures almost the whole of the mycelium becomes transformed into these toruloid buds. The sporangia are smaller and less frequent than those of *P. gracile*, and oospores are more numerous. Their formation in the lateral buds resembles that described by Ward (1883) in *P. monospermum*.

In some cultures much of the mycelium becomes denuded of protoplasm which collects in the lateral buds. In these cases septation is common, and I have seen sometimes short lengths of mycelium separated off by two septa from the emptied parts and discharging the whole of their contents down to the septa, though a lateral branch, as zoospores. Such cases, if alone observed, would suggest the formation of definite sporangia cut off from the vegetative thallus, but the examination of numerous cultures indicates the true value of this septation as a purely vegetative process, and not essentially connected with sporangium differentiation.

Epiphytic on the leaves of *Indigofera arrecta* Hochst., and cultivated on boiled stems of the same plant in water, Royal Botanic Garden, Calcutta.

PLATE II, FIGS. 3—7.

P. DICTYOSPERMUM *Raciborski.*

Proceedings Cracow Academy of Sciences, XXIV, 1892, plate I (first described by the author under the name *P. dictyosporum* in International Bulletin of the Academy of Sciences, Cracow, 1891, No. 8, p. 283).

Nematosporangium dictyosporum (Rac.) Schrot., de Wildeman, Notes mycologiques VI, Ann. Soc. Belge Micros., T. XIX, 1895.

The description of this fungus is in Polish, and I have been, therefore, obliged to interpret it mainly from the author's figures. A very brief account is given by Fischer in Rabenhorst's Kryptogamenflora I, 4, p. 490, and a fuller one by de Wildeman, *l. c.*

The hyphæ are 3-4μ in diameter, crowded in the cells of *spirogyra nitida*, the walls of which they perforate to pass from cell to cell. Short lateral branches bore through the outside walls, with a slight or no swelling under the point of contact, become somewhat enlarged at the tip, and empty their contents with those of a portion of the underlying mycelium as zoospores. These sporangia are not cut off from the thallus by septa. Infection takes place by the zoospores, which come to rest near the host filaments, and put out a fine tube through which the contents of the spore pass over into the host cell. This is similar to what occurs in *P. tenue*. Oogonia, 25-35μ in diameter, are formed (within the substratum) on lateral branches and are fertilized by one or two antheridia from the oogonial stalk, or from a neighbouring branch. Antheridia curved, cylindrical, and cut off by septa. Oospores not filling oogonia, with a very thick exospore, the surface of which is sculptured into a network of raised, more or less distinct, bands resembling the exospore of some *Peronsporaceæ.*

This species is sufficiently separated from all others by the reticulate exospore. Whether it is Schenk's *P. gracile*, as suggested by Fischer, cannot now be ascertained (see above, p. 64).

Parasitic in *Spirogyra nitida.* Cracow (Raciborski).

Parasitic in *Spirogyra sp.* France (de Wildeman).

II SUB-GENUS *Sphærosporangium.*

This subgenus falls into two natural groups: A, predominantly aquatic forms, with proliferous sporangia, and without conidia (*P. proliferum ferax, megalacanthum* and *diacarpum*); and B, predominantly soil forms, with non-proliferous sporangia or none, and usually with conidia. (*P. palmivorum, rostratum, de Baryanum, vexans, ultimum, Anguillulæ aceti, intermedium, Cystosiphon* and *Artotrogus*).

P. PROLIFERUM *de Bary.*

Jahr. wiss. Bot. II, 1860, p. 182, plate XXI. Abh. Senck. Ges. XII, 1881, p. 18, plate I, figs. 20—21. Bot. Zeit., 1881, p. 558, plate V, figs. 17—24.

Marshall Ward, Q. J. M. S., Vol. XXII n. ser., 1883, p. 497, plates XXXIV-V, figs. 11—21.

Dangeard, Recherches histologiques sur les Champignons, Le Botaniste, II, 1890, p. 123, plate VI, figs. 39—48.

Mycelium, in water culture, fine. Hyphæ uniform 4-5μ broad, branching lateral and sparing in young cultures. The origin of a lateral branch may be somewhat swollen, and rarely, fusiform swellings occur in the course of the hyphæ. Sporangia terminal, spherical, rarely oval, vacuolated, very variable in size, 30-58μ in diameter, with a short tube of discharge, rarely equalling one-fourth of the diameter of the sporangium, placed in any position, but usually opposite the stalk. After discharge, growth of the supporting hypha occurs through the emptied sporangium, or immediately below it, laterally, new sporangia being formed within the empty sporangium, or beyond it, in the first case. Zoospores large, 3 to numerous. Conidia unknown. Oogonia within and outside the substratum, terminal or often intercalar, 19-36μ in diameter. Antheridia 1—3 or more, usually more than one, from neighbouring branches and, less frequently, from the oogonial stalk. Oospores spherical, not filling oogonium, 16-27μ in diameter. Germination, after a rest of several months, by a hypha which usually branches, the branches being short and clustered and bearing one or rarely two sporangia, sometimes, particularly in small spores, by an unbranched hypha which soon gives rise to a sporangium.

The form found on decomposing vegetable matter in the soil differed from that ordinarily met with on dead insects in water in exhibiting a much more irregular manner of growth and more marked proliferation (plate III, fig. 4). The sporangia were always terminal, the bearing hyphæ being short and swollen (up to 7μ). The new growth, after discharge, was generally through the old sporangium, fresh sporangia being formed (*a*) within the old, or (*b*) just beyond it, five such in a chain being observed, or (*c*) at a considerable distance beyond. When the hypha grows on through the emptied sporangium, it is often much contorted and swollen. It usually emerges through the tube of discharge, presenting in this a marked difference to the apex of the new tube of discharge in those cases where the sporangium is formed within the old case. The apex of the tube of discharge possesses, as has already been pointed out, the power of dissolving cellulose, and hence grows through whatever part of the wall it happens to come in contact with. It would appear that the apex of the vegetative hyphæ of *P. proliferum* does not possess this power. This is in accordance with the observed fact that this species is a true saprophyte, as the hyphæ of the parasite members of the genus are capable of readily boring cell walls. Oospores were obtained in the legs of flies on which the fungus were growing, and outside, in vigorous cultures. De Bary states (1881) that the antheridia are mostly from the oogonial stalk. In my cultures, however, the majority arose from a neighbouring hypha. Hypogynal antheridia occur, and in general the antheridium is short and little curved.

In young cultures branching is sparse, and the sporangia are produced at a more or less regular distance from the substratum, giving the cultures a more regular appearance than in any other I have seen. As the first formed sporangia discharge, the growth becomes more uneven and branching more free. In some cultures subsporangial branching predominates, in others the innovation always grows through the emptied case.

The vacuolation of the sporangia is more marked in this species than in any other which I have seen. Sometimes, a single large vacuole, which may be $\frac{4}{5}$ths the diameter of the sporangium, occurs; at others, two or three smaller ones. The vacuole often appears to be bounded by a denser layer of protoplasm than the rest.

The sporangia may preserve their power of germination for a long time if kept moist. Oogonia are often not formed.

Saprophytic on dead insects in water and vegetable debris in the soil, Antibes, Calcutta, Dehra Dun.

Saprophytic on dead insects in water, Germany (de Bary, Schröter), England (Ward), France (Cornu, Dangeard).

P. proliferum Schenk (*P. globosum* Schenk) is *Myzocytium proliferum* and not a *Pythium*.

PLATE III ; PLATE IV, FIG. 1.

P. FERAX *de Bary*.

BOT. ZEIT. 1881, p. 562.

This species, which has not been observed since de Bary's time, stands very near *P. proliferum*. The points of difference are, however, according to its author, sufficiently marked. The hyphæ are smaller, the sporangia unvacuolated, and the oogonia chiefly formed extra-matrically. The latter are sometimes inter calar, more commonly terminal, either single, or several one behind another, separated by the hypogynal antheridia. They are often formed either by prolification within an emptied sporangium or just below the latter. In large sporangia, two oogonia may be formed serially. The antheridia are chiefly hypogynal, usually single but also double. They are short, usually not longer than broad, and often applied to the oogonium apophysial-like. They are straight or curved on themselves, often so much so in hypogynal cases, that the oogonium comes to be at right angles to the stalk. Germination of the oospores was not observed. The sporangia do not long preserve their vitality, and in opposition to *P. proliferum* the propagation of the fungus becomes difficult once the oospores ripen.

Habitat not stated. Germany (de Bary).

P. MEGALACANTHUM *de Bary,*

Abh. Senck. Ges. XII, p. 19, plate II, figs. 3—5.
Bot. Zeit. 1881, p. 539, plate V, figs. 8—13.

This is another of de Bary's species which has not since been described, though Schröter (1889) states that he believes that he found it in Breslau on rotting stems of *Veronica hederæfolia.*

The mycelium is intra- and extra-matrical and composed of fine hyphæ, which may reach a length of a millimeter, and are irregularly branched. Shorter hyphæ may be swollen into spindle-shaped thickenings. The sporangia are formed in the epidermal cells of the substratum or outside on free hyphæ. In the former case they are spherical or elongated to the shape of the epidermal cell, in the latter spherical, elipsoidal or pear-shaped. They are usually terminal, sometimes intercalar, and rarely two serially arranged at the end of the hypha. They are irregular in size and shape, provided with a tube of discharge which may be several times the diameter of the sporangium, or shorter, and may be terminal or lateral. After discharge, proliferation occurs as in *P. proliferum*. The zoospores are very large, up to 18-20μ in diameter after coming to rest. Conidia unknown. Oogonia terminal or intercalar, at first smooth, then with numerous radial conical processes about one-quarter of the diameter of the oogonium in length, without these 36-45μ in diameter, processes 6-9μ long. Antheridia usually several, from neighbouring hyphæ not from the oogonial stalk. Oospores about 27μ in diameter, smooth. Germination after a long rest, up to 11 months, type of germination not observed.

The oogonia are usually intra-matrical, but in some cultures extra-matrical. In weak cultures antheridia sometimes are wanting, and the oogonia in such cases do not produce oospores, but the contents either degenerate or else grow out by the swelling of one or more of the oogonial processes into hyphæ, which may bear sporangia.

Parasitic in certain cases in prothalli of *Todea africana*, and saprophytic in vegetable debris from the soil, Germany (de Bary, (?) Schröter).

PYTHIUM DIACARPUM *n. sp.*

Mycelium of exceedingly slow growth, intra- and extra-matrical. Hyphæ very slender, less than 1½μ in diameter, straight or curved, sparingly branched. Sporangia spherical, about 30μ in diameter, formed terminally, at a more or less uniform distance from the substratum, never very far away. Tube of discharge long, up to several times the diameter of the sporangium, often spirally coiled or wavy, at least twice the diameter of the hyphæ in thickness. The apex on discharge expands into an urn-shaped vesicle which is, at least in some cases, open at the distal end. In these cases much of the sporangial contents flows out, so as to lie at the mouth of this, and here segmentation occurs in the usual way, though the vesicle is absent. Zoospores large, early coming to rest and then rounding off, and after a pause giving rise to new zoospores similar to the first (diplanetism). Conidia and sexual organs not observed.

Sporangia-bearing cultures of this species are very characteristic, the large sporangium borne on an exceedingly fine stalk and with a long, curved, broad tube of discharge, being quite different from anything I have seen in other species.

Diplanetism is figured for the first time, so far as I am aware, in *Pythium*, in plate II, figs. 12—17. The zoospore, after coming to rest, loses its cilia and becomes surrounded by a wall. After a short period a vacuole appears, and at the same time a short tube is put out from the wall of the spore. This may be rather more than the diameter of the spore in length. The changes in the vacuole and tube appear to correspond to those which occur in the ripe sporangium, but were difficult to observe owing to the small size of the object. The apex of the tube opens, and the contents flow out and lie at the mouth of the tube. Whether they are retained here by a vesicle similar to that of the sporangium was not determined. The extruded plasma gradually acquires the usual kidney shape, becomes free from the end of the tube, develops cilia and swims away. Cornu (1872, p. 14) stated that *Pythium* was capable of forming diplanetic zoospores, mentioning as examples " *P. proliferum* and its varieties. " Raciborski described in *P. dictyospermum*, an intermediate condition, in which the germ filament from the zoospore, in cases where it failed to reach a filament of the host, formed a sporangium at the tip, which opened to emit one zoospore only.

This species is possibly the "*P. imperfectum*" of Cornu (1872, p. 13). The description of the latter is, however, so meagre that its identification is impossible.

Saprophytic on fallen plant remains in a pond, Black Forest, near Freiburg i. Br., Germany.

PLATE II, FIGS. 8—17.

PYTHIUM PALMIVORUM *n. sp.*

This species is the cause of a very destructive palm disease, which has recently broken out in the Godavery Delta, on the East Coast of the Indian Peninsula.[1] It attacks the leaf sheaths, penetrating them until the apical bud is reached, when it may also extend to the upper part of the stem. The mycelium is intercellular, only haustoria passing into the cells. This is the only instance of the sort known in the genus. The hyphæ are large, often irregularly swollen, up to 7μ in diameter and ramify between the cells of the parenchyma, never penetrating the fibro-vascular bundles. Branched finger-shaped haustoria enter the cells, which are rapidly killed and turned brown, the mode of attack resembling that of *Phytophthora.* Brown, dry, sunken spots are at first formed, but these rapidly undergo a wet rot, due to the entry of putrefying organisms. The spots underlie each other on the successive leaf sheaths, tracing the progress of the fungus from the exterior into the bud. In the softer layers inside, extension is usually more rapid than on the outer sheaths, and here the fungus reaches its greatest development. After ramifying through the tissues of the sheaths, it comes out on their surfaces to fructify, forming large white mycelial felts on the inner surfaces of the sheaths. In these, sporangia are formed in the web, not raised on special sporangiophores (plate IV, fig. 8). The sporangia are *Phytophthora*-like, inverted pear-shaped or, more rarely, round, and always terminal. They germinate readily in water, giving rise to rather large zoospores, formed in the manner typical of *Pythium.* The sporangia measure 50 × 35μ, on an average (extremes 38—72 × 33—42), and the zoospores, after coming to rest, 8 to 10μ.

In some of the mycelial webs large numbers of thick-walled oospores were found, always extra-matrical. Their development

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[1] See Butler, E. J. "Some diseases of Palms." Agricultural Journal of India, Vol I, pt. 4, Oct. 1906.

was not seen, and an oogonial wall could not be made out, possibly because the spore may have completely filled the oogonium. The origin and nature of the antheridia could not be determined with certainty, but some cases were seen where the antheridium appeared to arise from a neighbouring branch, and to coil round the oogonium. The oospores are spherical, measuring 35-45μ in diameter, the walls being up to 4μ thick. They germinate readily, giving rise to a short branch on which sporangia are early formed (plate IV, fig. 7).

The symptoms of the disease are characteristic, and the cultivators are able to distinguish the attack at an early period. The first sign that a tree is infected, is the whitening of one of the leaves of the crown, usually one of those recently expanded, towards the centre of the head. This leaf soon withers, while others become gradually paler in colour. Leaf after leaf withers until the apical bud is reached, when the whole top is killed and, after some months, falls off. No case of recovery, once the leaves commence to wither, was encountered.

In the earlier spots only the *Pythium* is found. Later on it is followed by putrefying organisms, which give rise to a wet rot, converting the whole terminal bud into a foul-smelling mass. As the spots extend, the large unseptate hyphæ can be traced into their growing margins. The constantly inter-cellular course of the hyphæ, and the presence of haustoria, point to its being a vigorous parasite, and there is no doubt it is the cause of the disease.

Morphologically, this species is of considerable interest. Its vegetative mycelium, haustoria and sporangia resemble those of *Phytophthora infestans* (see G. Delacroix, "Sur la Pourriture des Pommes de terre," Bull. Soc. Mycol. de France, Vol. XIX, 1903, p. 356). In fact the sole point of difference between this *Pythium* and the genus *Phytopthora* is the liberation of the undifferentiated zoospores into a bladder. It forms a connecting link with one genus of the *Peronosporaceæ,* as *P. intermedium* perhaps does with another—*Cystopus*. From the other *Pythiaceæ* it is somewhat divergent.

Parasitic on *Borassus flabellilfer*, *Cocos nucifera*, and (?) *Areca Catechu*, Godavery, India.

PLATE IV, FIGS. 2—10.

P. ROSTRATUM *n. sp.*

This species was obtained from humus-rich garden soil, in the Jardin d'Acclimatation at the Villa Thuret, Antibes, France. It is a pure saprophyte, growing vigorously on boiled *Abutilon* root, flies, centipedes, etc.

The mycelium in water cultures is large, but as individual hyphæ extend to very variable distances from the substratum, it is never as regular in appearance as that of *P. intermedium* or *P. proliferum*. The hyphæ are often very large, up to 6, or even 8μ in diameter, and tapering gradually at the ends, but never prolonged into fine filaments, as in *P. vexans*, nor showing intercalar irregularities, as in several other species. Branching is irregularly racemose. When old, the mycelium is, as usual, sparingly septate.

Spore formation begins about two days after inoculation, the first form to appear being the sporangia. These may be either terminal or intercalar, being almost regularly spherical in the first case, but often oval in the later. They average about 28μ in diameter, ranging from 23 to 34μ. Zoospores are normally liberated while the sporangium remains attached to its bearing hypha. The tube of discharge is very large and broad, usually about equal to the diameter of the sporangium, and thickened about half way in its length in a characteristic fashion. It is usually lateral in position. Intercalar sporangia are formed in two ways. Sometimes the hypha swells up in its course, so as to form an oval enlargement, which is then cut off by two septa and germinates by a tube put out laterally. More rarely a terminal swelling appears on a hypha, but, before reaching maturity, growth continues, either in a prolongation of the course of the original hypha, or in a new direction. After the new hypha has grown to some length, the swelling becomes cut off, as before, by two septa. This must be distinguished from those cases of direct germination of conidia *in situ*, which also occurs in this species, as in this case the spore is cut off by a septum, before it puts out its new hypha. The latter is a case of true germination, the former of vegetative growth, the spore in this case maturing after the hypha has grown out, and then germinating independently. A rare condition occurs when a still immature terminal spore puts out a bud, which grows immediately into a second spore, usually seated laterally on the first (plate V, fig. 14).

Conidia are rarely as frequent as sporangia, and appear usually later. They resemble the sporangia in all respects, except the mode of germination.

Gemmæ were produced on one occasion on an old fly culture, and, inoculated on a fresh fly, gave a typical culture. They were pear-shaped buds, arranged in a zig-zag manner, each succeeding one arising from the side of the previous (fig. 15). They resemble the gemmæ of the *Saprolegniaceæ* but have not the same morphological value (see above, p. 8). This is the first occasion, so far as I am aware, of their observation in the *Pythiaceæ.*

Sexual organs appear several days later than sporangia. They are usually intercalar or lateral (in short lateral diverticula from the hyphæ), and are formed extra-matrically. The oogonia measure about 21μ in their transverse diameter and rather more longitudinally. They are completely, or nearly completely, filled by the oospores, but the wall of the oogonium can usually be made out. The antheridium is generally single, and usually arises from the hypha bearing the oogonium (fig. 17). It is often extremely short, and may be reduced to a hypogynal cell, or a short lateral process, arising from a cell cut off immediately below the oogonium ; the cell and process together forming the antheridium (fig. 18).

The oospores are spherical, smooth and measure on an average 21μ, the extremes being 12 to 26μ. They are hence distinctly smaller than the sporangia. Interesting are small oospores, 12μ or so in diameter, formed in the lumen of the hypha, which becomes swollen to contain them, and cut off by two septa. In these cases, they usually lie somewhat laterally (fig. 21). A series of two or three oogonia, separated by short lengths of hypha, is not uncommon, and even sporangia may be included in the series (fig. 16). Germination was not obtained.

This species differs from *P. de Baryanum* in only a few, but nevertheless important, points. The prominent beak of the sporangium, with its curved walls, differs from the straight-sided beak figured by Hesse and de Bary. Sporangia are more frequent than conidia, the reverse of what happens in *P. de Baryanum*. The chief character is, however, the fact that the oogonium is completely filled by the oospore, whereas in *P. de Baryanum* a considerable space is left unoccupied. This is a character of specific value in the *Pythiaceæ*, so far as the species already studied show. The size of the oogonia and spores is also greater in *P. rostratum*. From a comparison of the two species, I have no doubt of their distinctness.

Saprophytic in garden soil, Antibes, France.

PLATE V, FIGS. 11—22.

P. DE BARYANUM *Hesse.*

Hesse, R. Ueber Pythium de Baryanum, ein endophytischer Schmarotzer Inaugr. Dissert., Halle, 1874, plates I and II.

De Bary, Abh. Senck. Ges, Vol. XII, 1881, p. 11, plate I, figs. 1—19. Bot. Zeit., 1881, p. 522, plate V, figs. 1-2.

Marshall Ward, Q.J.M.S., Vol. XXII, n. ser., 1883, p. 487, plate XXXIV, figs. 1—10.

P. Equiseti Sadebeck, Sitzungsb. d. bot. Verein d. Prov. Brandenburg, 1874, p. 116. Unters. über P. Equiseti, Cohn's Beiträge, III, 1875, p. 117, plates III and IV.

W. G. Smith, Gardners' Chronicle, n. ser. Vol. V, 1876, p. 656, fig. 117.

P. autumnale Sadebeck, Tageblatt d. 49. Vers. deutsch. Naturf. u. Aerzte 1876.

Lucidium pythioides Lohde, Tagebl. d. 47. Vers. deutsch. Naturf. u. Aerzte zu Breslau, 1874.

Artotrogus debaryanus (Hesse) Atkinson, " Damping off," Bulletin 94, Cornell University Agric. Exper. Station, 1895, plate I.

Mycelium rather coarse, intra- and extra-matrical. Hyphæ large, branching irregular and free, septate in old cultures. Sporangia spherical or oval, chiefly extra-matrical, terminal and intercalar; supporting hypha usually emptied of its contents for a variable distance below the sporangium, and separated by septa from the full portion of the hypha, and from the sporangium. Tube of discharge lateral, about the diameter of the sporangium in length. Proliferation absent. Conidia usually numerous, intra- and extra-matrical, 15—25μ in diameter, round, oval, or somewhat irregular in shape and size in old cultures, may germinate at once, but more usually do so after a short rest if transferred into fresh water. Kept moist, they preserve their vitality for months, and even, according to de Bary, stand drying and freezing without being killed. Oogonia usually numerous, intra- and extra-matrical, sometimes formed very early in culture, 20—25μ in diameter, spherical, terminal or intercalar. Antheridia up to three in number, from the same or another hypha as the oogonium, often formed close below the latter, and not seldom hypogynal. Oospores 14—18μ in diameter, not filling the oogonium, spherical, smooth, germinating after a rest of some months by a branching hypha.

The mycelium is parasitic in living plants, chiefly seedlings, and also saprophytic in garden soils. It is extraordinarily common in many European soils, and has been several times reported in the United States, but has not yet been found in India, nor have I seen any mention of it in the East. The hyphæ ramify through and between the cells of the host plant, not penetrating the fibro-vascular bundles, and being, usually, much constricted where a cell wall is bored through. Their course is chiefly longitudinal, but the branches from the main hyphæ, which usually show a progressive attenuation, extend in every direction. In water or damp soils, the mycelium spreads copiously outside the host plant, and in culture most of the organs of propagation are formed extra-matrically. In Atkinson's paper (1895), very clear descriptions and figures are given of the sexual organs. He separates the resting-conidia from ordinary conidia, while stating that the only morphological difference is a slightly thicker wall. There seems no good reason for making two distinct classes of conidia, for they show all sorts of intermediate conditions In many cases sporangia have not been found. Thus Sadebeck (1875), obtained them but rarely. Ward (1883), and Atkinson (1895), not at all, and I have not been more fortunate. Unlike *P. proliferum* they only give zoospores when sown at once or within a few days, in fresh water. The several spore forms are not produced in any regular succession.

P. Equiseti Sadebeck cannot be separated from this species. The identity was first pointed out by de Bary (1881). Sadebeck has figured a case where two oogonia were produced from the one swelling, without any intervening segment of the hypha. The "*Pythium Equiseti*" of W. G. Smith (1876), obtained from *E. arvense* in England, differs somewhat from Sadebeck's species in the larger oospores, almost filling the oogonia, and in the germination of the oospore with emission of zoospores. *P. autumnale* Sadebeck is only distinguished by the mostly parthenogenetically formed oospores This is hardly a sufficient ground for separating the species, and Fischer includes it in *P. de Baryanum.*

Lucidium pythioides Lohde is, from its parasitic habitat on *Lepidium sativum*, as well as from the description of the author, doubtless this species. It has been included here by de Bary and Fischer. The description agrees absolutely, except for certain abnormal forms of oogonium. The author states that oogonia are formed both terminally and intercalarly, the latter being seldom fertilized. Oogonia, which have not been fertilized, may give rise to zoospores. It is clear, from the author's description, that the cultures were attacked by one of the *Chytridiaceæ* parasitic on *Pythium*. He states that the contents of these "unfertilized oogonia" divide into numerous small parts, which commence to move, the movement being at first chiefly in the centre. Then "die Membran riss in einer kleinen offnung auf und entliess einzeln die kleinen, mit *einer* Cilie versehenen Schwärmer." This is clearly the sporangium of a Chytridian. Two other forms of reproduction were obtained in sugar cultures, which equally appear to represent *Chytridiaceæ* parasites. The first corresponds to the resting spore of a species such as *Pleolpidium Pythii*, the second to the multiple spores of *Pseudolpidium*. They are certainly totally distinct from any stage in the development of a *Pythium*.

The species described as *Saprolegnia Schachtii* by Frank ("Krankheiten der Pflanzen," 1st ed., 1880, p. 384), is of doubtful position. Fischer (1892) includes it under *P. de Baryanum*, an identification which Frank (*ib.*, 2nd ed., 1896, Vol. II, p. 91) does not accept. From the observations of Frank on the probable symbiosis of this species, with the plant in which it was found (*Pellia epiphylla*), it is possibly the common symbiont of endotrophic mycorhizas, which is present in many *Hepaticæ*, and which has been several times taken for a *Pythium*.

The losses caused by this species are very considerable. The literature is scattered, but the following are some of the plants which it is known to attack.

It was described first by Hesse from *Camelina sativa* and also found by him on *Trifolium repens*, *Spergula arvensis*, *Panicum miliaceum* and *Zea Mays*, grown at Halle in Strassburg soil.

The form described as *P. Equiseti*, was found by Sadebeck on prothalli of *Equisetum arvense* in Berlin (1874). The same author (1876) found it to be the cause of a potato disease near Coblentz, which was at first supposed to be due to *Phytophthora infestans*. The latter was, however, entirely absent from some of the attacked fields.

The species described as *P. autumnale* by Sadebeck (1876), attacked prothalli of *Equisetum palustre* and *E. limosum*.

De Bary (1881) cultivated it on living tubers of potato, and found it also attacking, besides some of the seedlings mentioned by Hesse, *Lepidium sativum*, *Capsella* and *Amaranthus* species. Algæ, such as *Spirogyra* and *Vaucheria*, are immune.

Lohde (1874) found his *Lucidium pythioides* on *Lepidium sativum*, *Beta vulgaris*, *Sinapis* and *Stanhopea saccata*.

Rostrup (1893-4) observed it on *Beta vulgaris* in Denmark.

Humphrey (1892) described the damping off of seedlings of *Curcumis sativus* from this species in Massachusetts. Schoyen found a similar outbreak in Norway (1897), and the same disease is mentioned in England by W. G. Smith (1900) and in Kentucky by Garman (1901).

Speschneff has observed the fungus on young maize in the Caucasus (1896-7).

In 1893, Halsted described a disease of seedling peas in the Eastern United States, in which *P. de Baryamum* was associated with other organisms. In Connecticut a similar disease was ascribed to this fungus by Sturgis (1898). Carruthers (1899) also describes an attack on peas in England.

Pim observed it on *Impatiens Sultani* in England (1888).

In Italy, Peglion (1900) mentions that it is at least one cause of the damping off of seedlings, but can be checked by heating the soil or applying carbon bisulphide, or 2-3 per cent. formaline.

The *Pythium* found by Halsted (1892), causing the collapse of young egg plants (*Solanum melongena*) in the Eastern United States, is referred doubtfully to this species.

According to Atkinson (1895), Galloway found it on seedlings of *Gilia*, *Viscaria*, *Lobelia*, etc., in the Botanic Garden of Harvard University.

P. de Baryanum occurs commonly in the disease of sugar beet known as "Wurzelbrand." Stocklasa (1898) found it accompanying several other organisms, but the part played by it in the disease does not appear to have been clearly established.

Ward (1883) cultivated it successfully on young buds of carrot, tubers of potato and dahlia, and cut pelargonium stems.

My specimens were obtained by growing cress seedlings in damp sawdust and garden earth at Freiburg i. Br. A number of the seedlings damped off in the usual manner. Conidia and oospores were formed in abundance both extramatrically and within the rotted tissues. No sporangia were seen. Nothing was observed to add to the accounts already published, the species being by far the most carefully studied of the genus.

In hanging drop cultures, violent protoplasmic streaming was several times seen, in drops which had been kept in the dark and then transferred into strong light for examination. The whole of the contents of some of the larger hyphæ, set up a rapid flow towards the distal end, passing into the numerous younger branches, which became densely packed with protoplasm. The movement was rapid and steady, lasting for about half an hour. It was apparently brought about by the stimulus of light, no injury being possible in the closed chamber of the drop. The same occurrence was seen occasionally in *P. vexans* and *P. intermedium.*

Parasitic on *Lepidium sativum,* Freiburg i Br., Germany.

Parasitic on a number of seedling Phanerogams and on prothalli of ferns and *Equisetum,* Europe, United States.

Saprophytic in garden soils, Europe, United States.

P. VEXANS *de Bary.*

Journal of Botany, V, 1876, p. 119. Journal Royal Agricultural Society, England, Vol. XII, 2nd ser., 1876, p. 339, fig. 6. Bot. Zeit., 1881, p. 537, plate V, figs. 3—7.

The mycelium is slender, finer than that of *P. de Baryanum* or *P. rostratum,* but resembling *P. intermedium,* both in the thickness of the hyphæ and the size of the thallus in water cultures. The hyphæ taper at the ends, particularly in the lateral branches, which are given off in a very irregular manner (plate V, fig. 1). The branches of the secondary or tertiary order, often extend far beyond the primary hyphæ, tapering into very fine filaments at the ends. This character distinguishes it from any other species which I have seen.

Sporangia and conidia are developed on two to three days' old cultures. The former are rare. They were not obtained by de Bary, and did not appear in some of my cultures. They occur both terminally and, more rarely, intercalarly, and are scarcely ever spherical or oval, but usually irregularly pear-shaped, ovoid or sub-angular. The sporangial tube of discharge is short, and zoospores are given only when the sporangia are sown immediately in fresh water. The sporangia and conidia measure 17—24μ in diameter, averaging about 21μ. The conidia germinate usually after they have fallen, but sometimes while still attached. To induce germination, change of water is often necessary. They preserve their vitality for a considerable time, at least some months. The conidia are filled with very dense protoplasm, vacuolation being rare. They are distinctly denser in appearance than those of *P. de Baryanum*. They are more variable in shape than any other species which I have seen. A second conidium is sometimes budded off from the first by a continuation of growth, before the first is cut off by its basal septum (plate V, fig. 6). Irregular intercalar swellings, from which one or more branches arise are not rare.

The oogonia are 22—25μ in diameter, and are formed about the fourth or fifth day in culture, on the extra-matrical mycelium. They always arise laterally, usually on short branches from the main hyphæ, or sessile on the latter. The oogonium is usually inserted on its stalk by a broad base. The antheridia usually arise from the oogonial stalk and are sometimes hypogynal. Usually, there is only one to each oogonium, rarely two. The antheridial cell is club-shaped, or rounded, and large in relation to the oogonium. In every case seen it was closely applied to the oogonial wall, so as to fuse with the latter in a large part of its circumference. As a result of this, oogonia, such as that figured in plate V, figs. 9 and 10, are common, the antheridium appearing as the basal section of a large pear-shaped cell, divided into two parts, an oogonial and an antheridial. Careful observation showed that this cell was formed as described in two distinct parts on separate stalks, the fusion being secondary. The appearance might easily be mistaken for a hypogynal antheridium, in ripe spores. The broad insertion of the oogonium and the peculiar attachment of the antheridium are characteristic of this species. Oospores free in the oogonium, but larger in relation to it than in *P. de Baryanum*, 20—22μ in diameter, smooth, round. Germination, in recent spores, often by giving zoospores directly, a thick tube being put out which, after growing to about the length of the diameter of the oospore, blows up at the apex into a bladder in which the contents of the oospore are divided into zoospores. In older spores (5—6 months) germination only by a branched hypha. I have only observed the latter form, after about a month.

In de Bary's description the oospores are given as 12—15μ in diameter and formed intra-matrically. Mine were formed outside, possibly because the dense texture of the substratum (*Abutilon* root) was unfavourable for intra-matrical development. The larger size of my spores is probably correlated with this position.

The sexual organs are formed in abundance, and are very suitable for observation since they are often formed just at the surface of the water, and can be observed without any disturbance, an important matter, as any one who has tried to follow the stages of fertilization knows. Fig. 7, plate V, shows an arrangement which is fairly common. Here a short lateral branch arises from the hypha *a*. The oogonium is formed in a diverticulum of this, which swells up into a spherical cell, which is cut off by a septum *b*, giving the oogonium a broad basal attachment. The rest of the branch coils, putting out other small diverticula many of which are cut off by septa, and eventually the antheridial cell is cut off at the tip. This bends round to reach the oogonium by its apex, and the fertilizing tube enters the oogonium at this point.

In spite of the larger oospores and their extra-matrical position, this species is undoubtedly the same as de Bary's. The peculiar insertion of the oogonium and shape of the antheridium are distinct from those of any other species.

P. vexans has not been encountered previously since de Bary's description was published. That referred here doubtfully by Raciborski (1900, I, p. 9) from Java is unlikely to have been really this species. Only conidia were seen, and these are described as roundish, whereas the normal conidia are hardly ever round. The fungus was, besides, parasitic on tobacco seedlings, whereas *P. vexans* is, so far as is known, a saprophyte. It is possible however that the *Pythium* found by Cunningham (1897), in India, on living potato plants, was this species, with which it agrees in the broad attachment of the oogonium, figured in plate I, fig. 14, and other points. De Bary failed entirely to cause *P. vexans* to attack living potato, and this fact makes it a matter of considerable doubt what species Cunningham's really was.

P. vexans was first found by de Bary during his classical researches into the nature of the potato disease fungus, and was studied with some care, in order to establish its connection or otherwise with *Phytophthora infestans*, for a sexual stage of which it might have been, and probably was by some observers, taken. It occurred only on potato already killed by *Phytophthora*, and attempts at infection clearly showed its saprophytic nature. I have found it not uncommonly in soil.

Saprophytic in garden soil, Cork, Kew, Great Britain; Antibes, France.

Saprophytic in potatoes, Germany (de Bary).

PLATE V, FIGS. 1—10.

P. ULTIMUM *Trow.*

Observations on the Biology and Cytology of Pythium ultimum n. sp. Annals of Botany, XV, 1901, p. 269, plates XV and XVI.

Mycelium intra- and extra-matrical, with vigorous snow-white aerial growth on potato, etc., hyphæ very long and slender, from 1·7 to 6·5μ in diameter, septate in old cultures. Branching irregularly monopodial. Conidia are formed in about two days in warm weather. They are both terminal and intercalar, averaging 20μ in diameter with extremes of 12 or less to 28μ. Germination is always direct, never by zoospores. Later on oogonia appear. They resemble the conidia, but are very rarely intercalar. Diameter 16·9 to 22·9μ, average 20·6μ, smooth. Antheridia generally single, arising from the oogonial stalk immediately below its boundary wall, curved. When two antheridia occur, they often arise from a neighbouring hypha, and are then frequently straight. Oospores spherical, smooth, 14·7 to 18·3μ average 16·3μ, with a central reserve globule. Germination immediate, or after a rest up to seven months, always by one or more hyphæ.

This species is very fully described by Trow, both as regards its biology and cytology. It is most nearly related to *P. vexans,* from which it is, however, certainly distinct. It differs in the more usually spherical conidia, and in the insertion and mutual relationships of the oogonia and antheridia. Though sporangia were not observed by Trow, it is possible that they may sometimes appear, as in the case of *P. vexans,* in which sporangia were not found by de Bary, and *P. de Baryanum,* in which Ward, Atkinson and myself have only observed conidia.

Saprophytic in *Lepidium sativum,* and cultivated on various substances ; does not infect living plants, Cardiff, England (Trow).

P. ANGUILLULÆ ACETI *Sadebeck.*

Botanische Centralblatt, XXIX, 1887, p. 318. (Gesellschaft für Botanik zu Hamburg, 1886).

Mycelium parasitic in the vinegar eelworm, *Anguillula aceti,* which it rapidly kills, then continuing to grow as a saprophyte on the dead body. Hyphæ very fine, filling the body of the eelworm with a dense mass. Sporangia, conidia and oogonia formed at the same time, not in succession. Sporangia rare. Conidia numerous, produced in series one behind another, or in clusters, when ripe usually falling of, and germinating directly by a hypha, only about 6μ in diameter. Oogonia numerous, very small, to 6μ in diameter. Oospores spherical, very small, at most 6μ in diameter, germinate by a hypha [Fischer (1892)].

Sadebeck found that an increased concentration of vinegar of 4—5 per cent., inhibited conidia formation. One of 10 per cent.

prevented oogonium formation and the growth of the mycelium, but oogonia which had previously been matured retained their power of germination, when again returned to very dilute solutions. The epidemic which he describes was found in unusually weak vinegar from a Hamburg manufactory.

Parasitic on the vinegar eelworm, *Anguillula aceti*, Hamburg, Germany (Sadebeck).

P. INTERMEDIUM *de Bary.*

Bot. Zeit., 1881, p. 554, plate V, figs. 14-16.

Marshall Ward, Q. J. M. S., 1883, p. 514, plate XXXVI, 45-6.

Artotrogus intermedius Atkinson, Bull. 94, Cornell University Agric. Expt. Station, Ithaca, 1895, p. 247, plate II, figs. 10—25.

Mycelium extra- and intra-matrical, forming a regular fine haze around the substratum in water culture. Hyphæ very numerous, up to 6μ thick, regular, without intercalar swellings. Branching often at right angles, sometimes dichotomous, more usually lateral. In old cultures septa, with a distinct double contour, are not uncommon. The tips of all free branches usually end in spores. These measure 18—24μ in diameter, and are normally arranged in chains, up to thirteen in a single chain having been observed. When ripe they fall off readily, and can germinate immediately in fresh water. Growth may continue from the hypha immediately under the spore, which is gradually pushed to one side, as in *Phytophthora infestans* (plate VI, fig. 6). The new hypha may arise so as to leave the lateral spore supported on a short basidium, or sometimes from a swollen part, immediately under the spore, which is very often present, and in this case the lateral spore lies sessile. Sometimes the new hypha arises further down, leaving the spore or chain of spores supported on a lateral stalk, which may itself give out branches and support new chains of spores. The chains are formed basipetally, the end spore being the oldest. The spores in the chain are usually spherical, and divided from each other by short stalks, which may persist as a tiny process, rectangular in outline on the fallen spore. Sometimes however they are pear-shaped, in which case the narrow end of each arises directly from that below. No other shape but spherical and pear-shaped occurs, and, in the latter, the divergence from the spherical is never great. The spores of a chain may germinate as sporangia or as conidia, both forms occurring in the same chain. Even young sporangia are often vacuolated, a single vacuole variable in size, shape and position being present in quite two-thirds of the cases.

In young cultures large numbers of sporangia occur, and discharge zoospores on the addition of fresh water. In older ones the conidia are the chief organs found. The tube of discharge is always very short, about one-fourth of the diameter of the sporangium, and appears in any position, most frequently laterally.

The conidia are often provided with thick walls, showing a distinct double contour (plate VI, fig. 9). They can preserve their vitality if kept moist, for at least 11 months, and can stand freezing. If completely air-dried, they soon die.

Sexual organs have not been observed.

The branching sometimes approaches the dichotomous mode, two branches appearing at the tip of a filament. On closer observation, however, one of these is seen to arise a little behind the other, which is the true end of the hypha. It then grows more rapidly than the other, so that at a certain stage the two are equal in length. Later on one usually outstrips the other, which comes to lie laterally.

In some cultures peculiar flattened, swollen, processes appear, either at the ends of the branches, or laterally near the ends (plate VI, fig. 3). They are often branched and collected into clusters. Usually growth does not cease with their formation, but after a rest they give out new branches, often several from a single swelling, which may bear spores eventually. A single terminal swelling, after rest, often gives out two or three new hyphæ from its distal end. In other cases the swellings degenerate and become emptied of their contents. These may be compared with the similar bodies found in *P. monospermum* and *Indigoferæ*, but they appear to be never changed into spores.

It is curious that this species, which is common as a soil saprophyte, should have been so rarely observed. I have so regularly obtained it from water, in which were suspended fresh roots of species of *Abutilon*, that I at one time suspected parasitism on this plant. I, however, failed to induce it to attack living roots. De Bary equally failed to infect *Lepidium sativum* and *Amaranthus* with it. He, however, observed its parasitism on prothalli. Atkinson found that it was responsible for a disease of fern prothalli in the United States. The affected plants were soft, limp, and darker in colour than healthy ones. A high temperature, moist soil and air, and insufficient light and ventilation, are the chief predisposing factors to damping off. When the soil is infected, it should be replaced by fresh sterilized earth.

In this paper of Atkinson's, and in a previous note (Atkinson, 1904) the aberrant type of zoospores, mentioned above in the section on the zoospore, is described. The cilia are described as issuing, one from each of the recurved, rounded, ends. After coming to rest, the zoospore passes through an amœboid stage, and then divides into two uniciliate spores, which swim away, come to rest and germinate. This peculiar process did not occur in my cultures, the zoospores behaving normally in all respects.

This species shows relationships with the *Peronosporaceæ*, as pointed out by de Bary, being allied to *Cystopus* in the catenulate arrangement of the spores, and to *Phytophthora* in the lateral displacement of the latter, by the onward growth of the spore stalk.

Saprophytic in garden soil, Cork, Kew, Great Britain; Paris, Antibes, France; and Freiburg i. Br. Germany.

Parasitic in prothalli of *Equisetum*, *Todea* and *Ceratopteris*, Germany (de Bary).

Parasitic in fern prothalli not further specified, Cornell University, New York State, United States (Atkinson).

Saprophytic in rotted seedlings of *Lepidium* and *Amaranthus*, Germany (de Bary). Ward's cultures were obtained from de Bary.

PLATE VI, FIGS. 1—11.

P. CYSTOSIPHON (*Roze and Cornu*) *Lindstedt.*

Synopsis der Saprolegnieen, 1872, p. 50.

Cystosiphon Pythioides Roze and Cornu, Ann. Sc. Nat., 5th ser., XI, 1869, p. 72.

Mycelium parasitic in small water plants, hyphæ much branched, rarely septate, chiefly occupying the cells of the periphery. Sporangia intra-matrical, usually situated in the peripheral cells of the submerged portions, one usually in each cell, spherical or compressed to the shape of the cells, 20µ in diameter, vacuolate, usually maturing simultaneously. Tube of discharge boring through the outer cell wall to liberate the zoospores into the water, sometimes enormously long, up to twelve times the diameter of the sporangium, usually short. Zoospores with the cilia attached to each end (?). Conidia similar to the sporangia. Oogonia terminal or intercalar, subspherical, often with a short beak in the direction of the prolongation of the bearing hypha. Antheridia from separate hyphæ, club-shaped, usually single, sometimes two to an oogonium, very rarely three. Oospores spherical, not filling oogonia, exospore very thick, pale rose coloured, sculptured into a network, the angles of which appear as distinct spines. Germination unknown.

This species has not been observed since Roze and Cornu's description.

Parasitic on *Lemna arrhiza*, also on *L. minor*, *L. gibba* and *Riccia fluitans*. The attacked plants become water-logged and sink to the bottom. On some plants the sexual reproduction alone is found. A temperature of about 15° C. appears to augment oospore production. Bologne, France (Roze and Cornu).

P. ARTOTROGUS (*Montagne*) *de Bary*.[1]

Artotrogus hydnosporus Montagne in Berkeley, Gard. Chron., 1845, p. 640, and Journ. Royal Hort. Soc., Vol. I, 1846, p. 27, plate IV, figs. 27-29, also Sylloge generum specierumque Cryptogamarum, 1856, p. 304.

De Bary, Abh. Senck. Ges. XII, 1881, plate I.

P. hydnosporum (*Mont.*) Schroter (1897, p. 105).

Mycelium saprophytic in dead plants, intra- and extra-matrical, hyphæ richly branched. Sporangia and conidia unknown, Oogonia intra- and extra-matrical, chiefly intercalar, spherical, with radial processes from the oogonial wall, without processes 18-27μ in diameter, processes 3-6μ long. Antheridia always hypogynal, usually single, but possibly double, one being perhaps cut off above as well as below the oogonium ; usually formed from the unaltered, cut off, segment of the bearing hypha, which is very seldom swollen. Oospores smooth, spherical, sometimes lying quite loose in the oogonium, more usually almost filling it, 15 to 24μ. Germination after 3—4 months with a branched hypha.

The frequency with which this species has been found associated with other Phycomycetes (*Phytophthora, Pythium*), together with its reduced type of reproduction, suggests that it is a mycoparasite like *Piptocephalus*. This is discussed by de Bary (1881), who found that it did not grow when inoculated alone on dead cress seedlings, but did so when accompanied by *P. de Baryanum*. I found it in Calcutta, in 1902, in potato tubers attacked by *Phytophthora infestans*, and maintained it for some time in cultures that appeared to be quite free from *Phytophthora* hyphæ.

The part played by this species in the controversy regarding the sexual reproduction of *Phytophthora infestans* is well known. At least a part of the supposed oospores of the latter, described by Worthington Smith, belonged in reality to this *Pythium*. The discussion may be found in de Bary (1876, p. 256 *et seq.* and

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[1] I have included this species, more because it is customary to do so, and because it is impossible at present to place it in any other genus, than because there is any certainty that it is a *Pythium*. Its proper place cannot be known, until the asexual reproduction is discovered.

1881) and W. Smith (Diseases of Field and Garden Crops, 1884, p. 295; Grevillea, 1876, p. 18).

In rotting potato tubers attacked by *Phytophthora infestans*, Calcutta.

Saprophytic (?) in rotting *Lepidium*, potato, etc., accompanied by other fungi, France (Montagne), Germany (de Bary), England (W. G Smith).

PLATE VI, FIGS. 12—14.

SPECIES OMITTED.

P. ACTINOSPHÆRII *Brandt.*

Monatsberichte Berl. Acad., 1881, p. 399, figs. 33—53.

The original description has not been available. Fischer (1892) states that it appears not to be a *Pythium*. Only round spores were observed without mycelium. On germination these gave rise to a short, sometimes branched, hypha which emitted zoospores. Fischer suggests it may have been the swarmspores of a *Saprolegnia* which on germination gave an arrested mycelium and early zoospore formation.

Associated with *Actinosphærium Eichhornii*, Germany (Brandt).

P. ? DICHOTOMUM *Dangeard.*

Récherches sur les Organismes inférieurs. Ann. des Sc. Natur., 7th sér., Bot., T. IV, 1886.

Found in *Nitella sp.* Subsequently (1890, p. 121) Dangeard stated this fungus was probably not a *Pythium*, but a new genus. It was imperfectly observed.

P. Characearum *de Wildeman.*

Ann. Soc. Micros., T. XX, 1896, p. 116, plate VIII, figs. 4—14.

Hyphæ 3—4μ in diameter. Oogonia terminal, wall smooth, rather thick, formed in the interior of the host. Antheridia single, or 2—4. Oospores 20—30μ in diameter, not filling the oogonium. This seems to differ from some of the members already known as algal parasites only in its habitat. The asexual reproduction was not observed, only the oogonia, antheridia and oospores. It must be remembered also that the genus *Aphanomyces* occurs as an algal parasite and is difficult to distinguish from *Pythium* in its sexual state. The oospores of *Aphanomyces lævis* may be very readily mistaken for those of a *Pythium*. Further investigation is necessary before this can be accepted as a good species. De Wildeman places it in this genus because *Pythium* possesses more aquatic species than the allied genera, and considers it a new species chiefly from its habitat.

Parasitic in the oogonia of *Chara*, and saprophytic, Switzerland (de Wildeman).

P. Chlorococci *Lohde.*

Tagebl. d. 47 Vers. deutsch. Naturf. u. Ærzte zu Breslau, 1874. (See also Bot. Zeit., 1875, p. 92.)

Sporangia roundish, zoospores very small. Cannot be accepted as a good species from the meagre description. Sexual spores not observed.

Parasitic on *Chlorococcum*, Germany (Lohde).

P. Circumdans *Lohde.*

Tagebl. d. 47 Vers. deutsch. Naturf. u. Ærzte zu Breslau, 1874. (See also Hedwigia, 1875, p. 5, and Bot. Zeit., 1875, p. 88.)

Lucidium circumdans Lohde *l. c.*

Only the asexual fructification as sporangia with proliferation and conidia was observed. Further identification is impossible. Fischer (1892, p. 404) gives it as a synonym for *P. de Baryanum* which seems to be untenable.

Parasitic in fern prothalli, Germany (Lohde).

P. ENTOPHYTUM *Pringsheim.*

Jahr. für wiss. Bot., I, 1858, p. 289, pl. XXI.

This is *Lakgenidium entophytum* (Prings.) Zopf (1884, p. 154).

P. FIMBRIATUM *de la Rue.*

Bull. Soc. Impl. Natur. Moscow, XLII, I, 1869, p. 469.

Is, according to Saccardo, Sylloge, VII, p. 273, only named not described. I have not consulted the original paper.

P. ? GIBBOSUM *de Wildeman.*

Ann. Soc. Belg. Micros., T. XX, 1896, p. 119, pl. XXI. figs. 11—15.

Oogonia 21μ in diameter, wall prolonged into irregular processes. Oospores $15-18\mu$. The remarks under *P. Characearum* apply still more to this form, which de Wildeman gives with considerable doubt.

Parasitic in the oogonia of *Chara*, Switzerland (de Wildeman).

P. GLOBOSUM *Walz.*

Beitrage zur Kenntniss der Saprolegnieen. Bot. Zeit., XXVIII, 1870, p. 553.

This is, as pointed out by Zopf (1884, pp. 160-161), not a *Pythium*, but probably a composite species, including *Myzocytium proliferum* Schenk and *Lagenidium Rabenhorstii* Zopf.

Parasitic in *Spirogyra*, *Zygnema*, *Mesocarpus*, *Closterium*, and *Cladophora*, Russia, Germany (Walz).

P. Hydrodictyorum *de Wildeman.*

Ann. Soc. Belg. Micros., T. XXI, 1897, p. 22, plate II, figs. 1—5.

The remarks above under *P. Characearum* apply also here. Hypha irregularly branched, about 3µ thick. Antheridia single. Oospores 10—17µ in diameter, nearly filling oogonium. Asexual reproduction unknown.

Parasitic in cells of *Hydrodictyon utriculatum*, Java (Massart).

P. imperfectum *Cornu.*

Monographie des Saprolegniees. Ann. des. Sc. Natur., sér. V. Bot. T. XV., 1872, p. 13.

Sporangia terminal. spherical, proliferous, with a long tube of discharge. The species, which is allied to *P. proliferum* de Bary, is incapable of identification from the brief mention which the author makes of it.

Habitat not stated, France (Cornu).

P. incertum *Renny.*

Journal of Botany, 1876, Justs' Botanish. Jahresber, 1876, also W. G. Smith in Gardeners' Chronicle, 1876, vol. VI, p. 11, fig. 3.

I have not seen the author's description of this species. It is stated by Fischer (1892, p. 410) to be very uncertain and is excluded by him. The figure given by Smith is unsatisfactory.

P. polysporum *Sorokin.*

Mycological Researches (Kazan, 1872, with figs.).

Hyphæ tortuous, continuous; oogonia spherical, 40-50µ, with several spherical oospores, 7—8µ in diameter, in each oogonium. The multiple-spored oogonia make this a very doubtful *Pythium.* The author figures the zoospores as being furnished with two cilia at one end of the egg-shaped body, a form which does not occur in *Pythium.* If Sorokin's species be correctly depicted, it must be taken as the type of a new genus.

Habitat ?. Russia (Sorokin).

P. SADEBECKIANUM *Wittmack.*

Mitteilungen des Vereins zur Forderung der Moorkultur, Vol. X, 1892, p. 83.

This species occurred as a parasite on peas in Pomerania and lupines in Hamburg, doing a considerable amount of damage in 1891. The diseased peas were examined by Wittmack, who published a brief account of the pathological conditions without, however, any diagnosis of the fungus further than the measurements of bodies which resembled the oospores of a *Pythium.* Sadebeck, who saw the specimens, considered it to be a new *Pythium*, allied to *P. de Baryanum* and *P. Equiseti.* The oogonia measured 32μ in diameter. Whether it is the same as the species generally included under *P. de Baryanum*, which has been reported attacking peas in the United States, England and elsewhere is uncertain.

Parasitic in peas and lupines, Germany, Austria and Switzerland (Wittmack, Sadebeck).

P. UTRIFORME *Cornu.*

Monographie des Saprolegniees. Ann. des Sc. Natur., Bot., ser. V, T. XV, p. 13.

This is, like "*P. imperfectum*" Cornu, an allied form to *P. proliferum* de Bary, but not capable of identification from the author's note on it.

Habitat ?. France (Cornu).

The fungi which have been often described in prothalli and in the roots of phanerogamous plants as belonging to the genus

Pythium, are in many cases to be referred to the symbiont of the endotrophic mycorhizas of plants other than those of the *Orchidaceæ*. In many cases the symbiontic character of the fungus has been recognised, or at least its constant presence indicated, in others it has been taken for a parasite.[1]

Jeffrey places the fungus between the *Pythiaceæ* and *Completoria*. I have observed it very frequently and made many attempts to cultivate it. So far as my observations go (I have found it in over 50 species), the fungus is always the same, characterized by the formation of large spore-like bodies within and outside the roots. These bodies, however, are not true spores for they are not cut off in any way from the thallus. The mycelium, when young, is unseptate, and it seems clear that the fungus is a Phycomycete. It grows in soil freely, without, so far as I have discovered, ever fruiting. The systematic position of the form is, therefore, a matter of doubt. Those forming the mycorhizas of the *Orchidaceæ* are, according to the researches of Bernard (1904), entirely different to that here referred to. It is possible that some of the fossil Phycomycetes, such as *Peronosporites antiquarius* Smith, really belong to this form.

The fungus described by Walz (1870, p. 337) as *Saprolegnia de Baryi* is, as Fischer (1892) points out, probably a composite species, which may include a *Pythium*. The mycelium (plate IX, fig. 1), and the sexual state (figs. 3—5) resemble *Pythium*. But the sporangia and conidia also figured certainly do not belong to this genus. The oospores do not agree with those of any species of the sub-genus *Aphragmium*, and the further elucidation of the author's description and figures is at present impossible.

It was found parasitic in *Spirogyra densa*, in Russia (Walz).

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[1] Amongst other references, see Treub, M. "Etudes sur les *Lycopodiacees*." Ann. Jard. Bot. Buitenzorg, IV and VIII, 1884-90; Bruchmann, "das Prothallium von *Lycopodium*." Bot. Centralb, XXI, 1885, p. 310; Goebel, Bot. Zeit., 1887, p. 165; Jeffrey, E. C. "The Gametophyte of *Botrychium virginianum*." Trans. Canadian Inst., Vol. VI, 1896-7; Treub, M. "Onderzoekingen over Serehziek suikerriet," Mededeelingen uit's Lands. Plantentuin, II, 1885 (referred to also in Wakker and Went, "Ziekten van het suikerriet op. Java," p. 117, plate XXII).

PART II.

OBSERVATIONS ON SOME CHYTRIDIACEÆ

THE aquatic *Saprolegniaceæ* and *Leptomitaceæ* are, as is well known, attacked by several interesting Chytridiacean parasites. These are amongst the best known members of the group, chiefly as a result of the work of Cornu (1872), in the earlier stages of the long controversy on the sexual reproduction of the *Saprolegniaceæ*. Pringsheim (1860) stated that in certain *Saprolegniaceæ* devoid of special antheridial branches, antherozoids are produced in peculiar cells lodged within the filaments, and that these fertilize the oogonium in the same way as in the *Œdogoniaceæ*. Cornu showed that the so-called antherozoids were in reality the zoospores of parasitic *Chytridiaceæ*, of which he described several species; and A. Fischer, in a detailed study (1882), added considerably to our knowledge of their life-history.

The presence of similar parasites in *Pythium* has almost entirely escaped the attention of observers. I have been able to find only three instances described, all imperfectly. One was observed by Dangeard (1896) on an unidentified *Pythium* of peculiar habitat, living, like *Leptomitus lacteus*, on stones in a stream. The sporangial wall of the parasite was fused entirely with that of the containing filament, the whole resembling a spore belonging to the host. From each sporangium 50 to 100 zoospores escaped through a colourless papilla formed in any position in the wall. These were 1-ciliate and moved with a lively motion, often stopping dead or turning sharply in their paths in a manner not uncommon amongst similar bodies belonging to other species. They were observed to come to rest on the

Pythium filaments, but their mode of entry was not described. Resting spores were not seen. Basing himself on a supposed analogy with *Chytridium subangulosum* A. Braun, Dangeard saw in "une sorte de canal axile incolore qui part de la base du sporange" a basal rhizoid belonging to the parasite. No other argument or observation was advanced in support of the existence of this structure, and his figures (*l. c.*, fig. I. A. to F.) are distinctly against it. The axile clear channel of fig. A is common in the hyphæ of *Pythium*, especially when poorly nourished, and figs. C and D show the hypha below the occluding wall of the sporangium entirely empty. Two closely allied species, which will be described below, were carefully examined by me without showing any trace of a prolongation below the sporangia. They, as well as Dangeard's species, evidently belong to the genus *Pleolpidium* established by A. Fischer for three of Cornu's species of *Rozella*, parasitic on *Monoblepharis polymorpha*, *Rhipidium spinosum* and *Apodya brachynema* respectively. This genus is characterized by sporangia not grouped in a sorus and with a wall closely fused with that of the host, the possession of elongated zoospores with a single cilium trailed behind, and spiny resting spores. The fact that the supporting filament often becomes completely emptied of protoplasm would enable one to see an axile strand, if one existed, even in the narrow hyphæ of *Pythium;* and in the broader lumina of the hosts of Cornu's species, it is not to be imagined that such a structure would have escaped so close an observer.

The second case is *Rhizophidium Pythii* de Wildeman (1897), found on the oospores of "*Pythium complens*" (*P. monospermum*) in the tissues of aquatic plants. It differs from *Rhizophidium globosum* only in its habitat, and the species must therefore, as de Wildeman himself observes,[1] be accepted with reserve. Resting spores here also were not observed, nor the mode of infection followed.

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[1] 'Nous donnons donc notre espèce pour ce qu'elle vaut ; nous avons préféré décrire le champignon sous un nom spécifique que de le rapporter comme variété à une espèce ancienne" de Wildeman, *l. c.*

The third parasite previously found on *Pythium* is mentioned by Dangeard (1890, p. 90), who states that he found *Pseudolpidium Aphanomycis* Cornu on a *Pythium* which was not more nearly indicated. The sporangia were small, with a long tube. The identity of the species with that on *Aphanomycis* was not ascertained by inoculation, and as shown below *Ps. Aphanomycis* cannot infect *Pythium monospermum* or *P. proliferum*.

In the species to be described below, two types of life history appear, though in all the main facts are similar. Commencing as a motile zoospore, provided with one or two cilia as the case may be, and originating from a sporangium entirely imbedded in the cells of the host fungus, the first stage is passed in the free swimming condition in water. Though from this one would expect to find that the aquatic species of *Pythium* are more frequently attacked, the reverse is in fact the case. All my species were found on soil-inhabiting forms of the hosts, taken from a few inches to a foot below the surface of the soil, and I have never seen an aquatic *Pythium* bearing parasites. As the sole mode of propagation is by means of the swimming zoospores, it is remarkable that this should be the case. The invisible water present in such soils as those from which my specimens were obtained, which were well watered garden soils, must suffice for a limited degree of reproduction, and periods of drought may be tided over in the resting spore condition.

After swimming for a time the zoospore comes to rest and, unless it finds a resting place on a hypha of its host, rounds off as a mass of naked protoplasm with no wall and soon loses the cilia and breaks up without further development. For the more fortunate spores which succeed in reaching the host before losing the power of free movement, the next stage is the formation of a wall on the rounded spore. In some cases at least, a short period of amœboid motion precedes the final rounding off, but I am unable to say how far this is a constant phenomenon. After rounding off and encysting, a period of rest ensues, lasting from an hour to several hours. Then a fine process appears, joining the spore to the wall of the filament, and at this period it may be noticed that the spore wall is no longer in direct contact with the host, but is separated from it by a distinct space across which the fine joining process extends (plate VIII, fig. 9). This process bores into the interor of the filament and becomes the infection tube. It is only visible when the spore is exactly on the optical margin of a hypha. In many cases the infection does not go any further, and the parasite fails to gain an entrance. This does not appear to depend entirely on the age of the filament. It is true that the young hyphæ are usually selected by the wandering zoospores for attack. But on these, as on older ones, it is common to find zoospores which have failed to effect an entrance, and on the other hand infection of the older hyphæ has sometimes been directly observed. The difference in infective power is probably inherent in the zoospore in the majority of cases, for the number of parasites which a hypha may harbour is very variable, ranging from one to thirty or forty in the same culture and on hyphæ of about the same age. It is indeed evident that the zoospores start life with different reserves of energy, since their period of swarming varies considerably; thus some may have only enough energy to enable them to reach the host but not to enter it.

The contents of the encysted spore are eventually emptied, in successful cases, through the infection tube into the protoplasm of the host. The tube of entry is extremely fine and I have failed to obtain a double contour clearly, even on high magnification. It is, however, certainly a tube, remaining distinctly visible after the contents of the spore have passed over into the filament. The spore cyst also remains visible for some time, attached to the tube. The case figured in plate X, fig. 1, of an emptied *Saprolegnia* capsule with parasitic spore-capsule attached shows that the tube penetrates some distance inside the wall before it opens. The passage of the parasite across from its cyst into the filament is slow, occupying about seven minutes in some cases observed.

Under favourable conditions the contents may be watched accumulating in the hypha as they leave the cyst. The two plasmas are sometimes of different degrees of refractivity, which enables them to be distinguished for a while. The *Pythium* filaments, however, usually contain a substance (? glycogen), collected in droplets of much the same appearance as the parasite, and it is not possible to follow the subsequent stages by direct observations. Fischer has shown (1882), by careful observations based on the number of sporangia or sori produced by infection with one or a few spores, that in the case of the *Saprolegnia* parasites the following conditions occur :—

(*a*) From one spore a single sporangium or resting spore is produced, without loss of individuality (*Pseudolpidium*, *Olpidiopsis*).

(*b*) From one or several spores a sorus is produced (*Woronina*).

(*c*) From one or several spores several sori are produced or a single or several resting spores (*Rozella*).

In *Woronina* and *Rozella* a plasmodium is formed. In the cases figured by Fischer in plate II, fig. 12, and plate III, fig. 26, the plasmodium appears to be a true one formed by union of the bodies of several parasites. Usually, however, Fischer considers that from each single spore one sorus or several entire sori are produced, so that there is no union of several parasites before reproduction takes place. We must, therefore, carefully distinguish between the two cases. One in which the individual zoospore gives rise to an apocytium which develops by itself and completes its development by forming a single sporangium or sorus, or several sori, as in *Pseudolpidium*, and usually in *Woronina* and *Rozella* ; and a second in which several spore bodies unite to form a plasmodium, the whole then developing into a single or several sori, or one or more resting spores as sometimes occurs in *Woronina* and *Rozella*. In the one case the individuality of the spore bodies is preserved ; in the other it is lost. A condition of much interest occurs in *Rozella*. Soon after the entry of the parasite its protoplasm becomes indistinguishable from that of the host. Fischer considers that its individuality is lost to the extent that it becomes closely mingled with the protoplasm of the host, both together forming a sort of plasmodium.[1] Cases were observed in which the presence of the parasite did not interfere with the normal life of the host, even to the extent that normal sporangia and zoospores were formed, and it was only later on, when secondary sporangia were forming, that the parasite gained the upper hand. Similar instances occurred in my cultures of the *Saprolegnia* parasite described below. The zoospores of the parasite often infected *Saprolegnia* zoospores during their first encystment without checking the process of diplanetary formation of secondary zoospores, though whether the latter developed into a mycelium was not ascertained (plate X, fig. 1). These cases are akin to that described by Eriksson in his well-known Mycoplasma theory of the origin of cereal rusts, and in spite of the opinion of Klebahn (1904), that they are not analogous, they seem to me to be so in all essentials.

In the genus *Pleolpidium* the question of plasmodium formation has not been investigated. From a number of observations, I have been led to conclude that several spores frequently unite to give rise to a single sporangium. In *Pleolpidium irregulare* the sporangium is often terminal and solitary on a filament, and infection of a young hypha by several spores has, in many cases, resulted in the formation of only a single sporangium. In *Pl. Cuculus* the sporangium is always single and terminal and, as the young hyphæ are probably often infected by more than one spore (my observations on this point are not precise, but no case is known in any of these parasites of a hypha being only capable of harbouring a single spore body), we must suppose that a plasmodium is formed or that only one spore body develops at the expense of the rest. *Pseudolpidium Pythii* and *Ps. gracile* probably agree with

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[1] "Die eingedrungene Spore giebt sehr bald ihre Individualität auf und wenn auch die directe Beobachtung uns keine weiteren Aufschlüsse geben kann, so werden wir durch die Entwicklungsgeschichte dazu geführt, eine innige Vermengung des parasitischen Protoplasmas mit demjenigen des Wirthes anzunehmen. Wahrend bei Olpidiopsis die wachsende Spore als Anziehungscentrum die Protoplasmatheilchen der Saprolegnia an sich riss, verschwindet in dem Entwicklungsgange der Rozella die spore als Individuum vollständig" (*l. c.* p. 37.)

Pseudolpidium Saprolegniæ (A. Br.) Fischer in producing a sporangium or resting spore from each spore body, since the number of the latter produced seems to be proportionate to the intensity of infection. The different stages in the development of a single spore body in the latter are depicted in plate VII, fig. 1.

There is no indication in any of the *Pythium* parasites examined by me of a sexual process.

In the period between infection and the subsequent appearance of the swelling which is to give rise to sporangia or resting spores, the parasite is lost to sight. In *Pleolpidium Cuculus*, as in Dangeard's species, the swelling itself cannot at first be distinguished from the normal spores of the host. In *Pl. irregulare* the swelling is less regular in shape and size and often intercalar, but as no normal spores of the host were seen in this case, a comparison is not possible. In *Pl. inflatum* the hypertrophy is very great and the sporangia easily distinguishable from *Pythium* spores. In all these the contents of the swelling are homogeneous, and no distinction between host and parasite plasmas is visible. The interpenetration of host protoplasm by that of the parasite is as complete as in *Rozella*. In *Pseudolpidium Pythii* and *Ps. gracile* on the other hand the parasitic protoplasm early comes to view in the swelling, and, growing at the expense of the host protoplasm, can be followed in all its stages (plate VII, figs. 1 and 9). Hence, in the species in which there is probability of a plasmodium formation, the individuality of the parasitic plasma in regard to that of the host is less marked than in those in which each spore body gives rise to a sporangium or resting spore. This agrees with Fischer's observations on the *Saprolegnia* parasites, and indicates the occurrence of two distinct types of life history in the two groups.

Another distinction between the two groups occurs in the formation of the sporangium. In the first group the parasitic plasma entirely penetrates the swelling and eventually fills it completely. The sporangium wall is then formed and becomes closely fused with the wall of the *Pythium*, which encloses the swelling. In the second group the parasitic plasma never completely fills the swelling, and sporangia are formed lying loose in its cavity, the host protoplasm of which is, however, entirely absorbed. This again agrees with the two groups of *Saprolegnia* parasites, *Pleolpidium, Rozella* and *Woronina* on the one hand, with sporangia walls fused to that of the host, and *Olpidiopsis* and *Pseudolpidium* on the other, with free sporangia.

A further distinction occurs during the maturation of the sporangium. In *Pleolpidium* a well marked vacuolar stage does not occur, while in *Pseudolpidium* the ripe stage is characterized by the formation of large vacuoles.

The stages in the maturation of the Chytridiacean sporangium have not been fully described in any of the memoirs which I have seen. As in *Pythium,* they correspond essentially to those described by Hartog and Rothert for the *Saprolegniaceæ.* I have followed all the living stages described below only in the case of *Pseudolpidium Aphanomycis* Cornu (plate IX, fig. 6), but *Pseudolpidium Pythii* and *Ps. gracile* undergo quite similar changes.

The ripe sporangium of *Pseudolpidium Aphanomycis* is filled with hyaline refringent protoplasm, containing a single large vacuole or several smaller ones. In the state of rest which most sporangia pass through before the commencement of the minute changes which ultimately result in the formation of zoospores, the vacuoles are perfectly spherical and their contour sharply marked. Cultures left in the dark in unchanged water show a large proportion of sporangia in this condition. On transference to strong light, and the addition of fresh water, zoospore formation is induced and the process may be easily watched.

As in *Pythium* the spherical vacuoles lose their definite outline as a result of heapings of the protoplasm, and here, more than in *Pythium,* it is evident that the heapings are few in number as compared with the ultimate zoospores produced. This is doubtless the stage described by Harper (1899), in *Synchytrium,* during which preliminary cleavage occurs, resulting in the separation of multinucleate, and comparatively large, masses of protoplasm. Rotation of the protoplasm occurs, leading to constant changes of shape in the vacuoles when observed from a fixed point. The vacuoles appear in fact to push out and retract processes at various points, and this is the more deceptive the more quickly the rotation proceeds. I cannot doubt, however, that this is an optical effect due to rotation of protoplasm, bearing vacuoles which are irregular in shape as a result of the protoplasmic heapings referred to. The length of time passed in this condition is very variable, ranging from under an hour to several hours ; while it sometimes happens that sporangia which have shown vacuolar changes relapse into the immobile condition, even though the tube of discharge, which appears at the same time that the vacuoles change their shape, has been formed. Such sporangia may remain for some days without discharge. In the immobile condition the vacuoles are always exactly spherical.

The next stage corresponds to the first shaping of spore origins in the sporangium. This is made evident more by the vacuolar changes than by any direct observation of cleavage lines between the origins, on account of the minute size of the latter and the density of the protoplasm. At a given moment the vacuoles, whether one or several, enlarge, become perfectly spherical and disappear, the whole process lasting from ten to thirty seconds. Turgor in the sporangium is reduced, as is shown by the collapse of the apex of the tube of discharge and withdrawal of some of the tube protoplasm into the body of the sporangium. The latter also diminishes in size by a sensible proportion. It is now filled with homogeneous protoplasm, lighter in colour and more transparent than in the previous stages. This corresponds to the homogeneous stage in the *Saprolegniaceæ.* Five or ten minutes later final fashioning of the zoospores is complete and movement commences in the sporangium. A little later the beak opens and the zoospores escape.

In the beak itself a considerable difference between the *Pythium* type and that of *Pseudolpidium* is noticeable. The sub-apicular vacuole of the former is not marked in the latter and the beak is occupied by a mass of protoplasm, up to the apex, which takes part in zoospore formation.

The explanation of these phenomena is similar to that given for *Pythium* (see above, p. 33). The tendency to grouping of protoplasm into origins and the turgor of the sporangium lead to the cleavage fissures eventually extending through from the vacuole to the sporangium wall, and rupture of the two chief osmotic membranes of the cell. Rapid endosmosis through the remaining membrane, the cell wall, is thus induced, leading at first to increase in the watery contents of the sporangium and increased tension of its wall; then, very rapidly, by the elastic recoil of the latter to loss of part of the vacuolar contents, no longer restrained from passing out by the vacuolar wall and hautschicht. At the same time the rupture of hautschicht, accompanied by loss of turgor, causes the spore-origin protoplasm to take up the remaining water, swell and fuse, so that the sporangium temporarily becomes filled with a homogeneous mass.

The sporangial protoplasm is now more charged with water than previously. This is indicated not alone by the analogy with the homogeneous stage of the *Saprolegniaceæ* where Hartog (1887) and Rothert (1888) have shown that part of the excess water is taken up and expelled by shifting vacuoles of a contractile nature in the spore origins, but also by the swelling of the protoplasm, its lighter colour and the fact that the diminution in size is less than would occur were all the vacuolar water discharged to the exterior. The reason for temporarily retaining a proportion of the water taken up at this stage is probably to be sought in the danger which would result to naked protoplasm suddenly liberated to the outside with its imbibition water at a low ebb. The appearance of large vacuoles in such protoplasm, which grow, burst and lead to diffluence of the plasmatic mass is well known; and the contractile vacuole has been explained, first by Hartog (1888 (1)) and more elaborately by Degen (1905), as an organ provided to deal with the excess of water taken up in such cases.

For the development of this power of forming contractile vacuoles, the not too violent increase in the imbibition water of the protoplasm is probably one of the essentials, and the zoospores, still protected both mechanically and, to some extent, osmotically by the sporangial wall from a violent inrush of water, have time given them for the purpose. Whether the increase in imbibition water of the zoospores reaches the stage of actual formation of discharging vacuoles, prior to the liberation of the zoospores, as in the *Saprolegniaceæ*, I have not been able to determine owing to their small size, but they will probably be found on careful search.

The above account and explanation of some of the changes occurring during the ripening of the sporangium differ considerably from those offered by Fischer (1882) and Fisch (1884 (1),) both of whom have dealt with the question. Fischer (*l. c.* pp. 28 and 43) describes the sudden disappearance of the vacuoles in *Pseudolpidium Saprolegniæ* and *Rozella* before zoospore movement begins in the sporangium. He explains the vacuolar stage itself as due to condensation of that part of the sporangial contents which is to take part in spore building, with a throwing out into vacuoles of all useless substances, a view supported by Harper's observation (*l. c.* p. 484), that there is actually a shrinkage of the spore origins during separation. His further assumption (*l. c.* p. 68), that the spores, when liberated, are still composed of protoplasm in a state of condensation and with little imbibition power, takes no account of the fact that the spore origins expand again and take up water before movement begins, a fact not observed by him. The view given above, that an increase in the imbibition water is necessary to the final fashioning of the zoospores, is supported by an independent observation. The tip of the beak of the sporangium of *Pseudolpidium Aphanomycis* shown in plate IX, fig. 6, was ruptured by manipulation during the vacuolar state, in a rapidly maturing sporangium from a vigorous culture. The apex of the beak became partially occluded by underlying protoplasm, which was evidently killed as it took no part in the subsequent processes. Just behind this a portion of the tube contents took in a limited amount of water and four masses separated out, developed prematurely and swarmed in the confined space as perfect zoospores. Behind these a new hautschicht was evidently formed rapidly, and the body of the sporangium retained its turgor, came to maturity and discharged through a new beak, meantime formed to replace the old, some forty-five minutes afterwards.

Fisch's account (*l. c.* p. 21) is tainted by the erroneous descriptions of the maturation of the Phycomycetal sporangium given by Büsgen (1882), and he describes two homogeneous stages in *Chytridium* (*Olpidium*) *Lemnæ* separated by two periods during which the spore origins are marked off by cell plates, before the vacuolar stage occurs. Cell plates have been shown by Harper to be as non-existent in Chytridiacean as in other Phycomycetal sporangia, and nothing resembling the repeated appearance and disappearance of the spore origins has been observed in *Pseudolpidium.*

On the whole the above account, given from the study of living specimens, agrees closely with the cytological study of Harper (1899) on *Synchytrium decipiens*, except that in the latter case the first formed spore origins, having become multinucleate, again divide to form the definitive spores, and that the formation of large vacuoles which eventually disappear is not described.

The sporangium discharges through a beak in those species in which it lies loose within the host, and by a papilla in such as have sporangia fused with the host wall. One function of the beak is therefore readily understood to be the provision of a passage from the sporangium to the exterior of the host plant. The tip of the beak possesses, as in *Pythium*, the power of boring through cell membranes, and observation of sporangia lying free in the host filament shows that this is effected by solvent action and not by pressure.

The zoospores escape in an active condition, by their own spontaneous motion. But the inducement offered to them to emerge is still unknown in the majority of cases. In some other groups of the Phycomycetes we have indications of the nature of this inducement. The presence of a special expulsive substance is disproved by all the recent work on the subject. In *Aphanomyces* evidence has been offered by Hartog (1895, p. 685), to show that the exit is due to a *vis à fronte*, the nature of which is left undetermined. I have suggested above that in *Pythium* the vacuole of the beak contains a chemotactic substance which is retained in the bladder formed when the apex blows up, and which serves to attract out the still passive spore origins. In *Aphanomyces* and *Achlya* (as also in *Achlyogeton* and *Achlyella*), the spores display a tendency to collect into a ball at the mouth of the sporangium, and as they are not held together by any definite substance, such as the mucus which binds the spore mass of *Chytridium gregarium*, Hartog (1888 (2)) assumed for these a form of irritability which he termed *adelphotaxy*, and which leads to mutual attraction and a tendency to place themselves with their long axes parallel. This may be sufficient, combined with chemotactic attraction or repulsion from without or inside the sporangium, to account for the escape

In many *Chytridiaceæ* and in the *Peronosporaceæ* the above factors do not come into play, and the cause of exit has yet to be suggested. My observations go to show that it is difficult to account for the escape of Chytridiacean zoospores on purely physical grounds. Thus when from any cause, such as the blocking of the tube by a zoospore, escape is checked, motion may persist in the sporangium for many (up to 48) hours and yet reopening of the passage, which I have occasionally observed after several hours, still allows the zoospores to be attracted to the outside, though less eagerly than at first. Similarly in *Pseudolpidium* it sometimes happens, in old cultures, that the tube fails to reach the outside, and liberates the zoospores into the lumen of the empty host filament; and here also movement is much more prolonged than when the zoospores escape to the outside in the normal manner. The dead wall is not likely to influence matters, especially since enclosure within either the *Saprolegnia* filament (with true cellulose walls) or the sporangium (with walls of fungus-cellulose) has the same effect. The conditions within and outside the cell are soon similar as regards gases and liquids, owing to the free diffusion of these through cellulose walls. It would seem as if the zoospore is endowed on the one hand with a tendency to continue its motion until it reaches either a host plant or the surface film of the liquid, the two positions in which it usually comes to rest, and on the other hand is repelled by some non-diffusible substance left within the sporangium when the spore origins separate.

After escape a curious phenomenon occurs in some cultures of *Pseudolpidium.* It was first observed in the *Saprolegnia* parasite under conditions favourable for continuous observation, and afterwards, in a modified form, on several occasions in *Ps. Aphanomycis.* At the time of liberation the zoospore in the former (plate X, fig. 2*a*) is elongated and provided with two cilia, one at the front and one trailing, but fixed laterally. This is the normal shape of the zoospore in this genus, according to Fischer (1892). After swimming for a short distance it comes to rest. The cilia now disappear, but at the point of insertion of the front cilium a pseudopodic flagellum appears. This is much thicker than the cilia, moves by "streaming," and sometimes branches. The rest of the zoospore loses its original shape and also exhibits amœboid movements. This condition lasts for a period of from ten to twenty minutes, and then the movements cease and the flagellum is retracted. Shortly afterwards a projection appears on one side and grows out to form a new cilium, whose position, as far as could be observed, does not correspond with that of either of the original ones. At the same time the zoospore takes on a new definite shape, becoming bluntly kite-shaped. It remains thus for about five minutes with the single cilium, attached on one of the shorter sides, beating slowly and regularly. Then a second one appears in position corresponding to the first on the other short side (fig. 2*b*), and the zoospore, now in its final shape, swims away by regular synchronous beating of the oar-like cilia.

This process was observed on filaments of *Saprolegnia,* containing one or two ripe sporangia, cut off carefully and mounted in a hanging drop or placed on a coverglass, drop upwards, on a slide In *Pseudolpidium Aphanomycis,* it was seen in a large number of cases from vigorous cultures ; but owing to the small size of the zoospores, the use of iodine was necessary to see the cilia accurately, and a continuous observation of the ciliar changes in living spores was not found possible ; so far as was determined, no alteration in position of the cilia took place during it ; they were merely absorbed during the amœboid stage and again put out. In *Ps. Pythii,* it appeared to be constant, but here also, though two distinct periods of motion occur, the ciliar changes were not completely followed.

In the *Saprolegnia* parasite a case was observed where the spores were liberated into the lumen of an emptied *Saprolegnia* hypha and, after swimming here for over an hour in the first condition, were set free into pure water by cutting the hypha, and were still found to go through the metamorphosis described above, though in slower stages, ending up as kite-shaped spores. The process as a whole may be compared to diplanetism in the *Saprolegniaceæ* and *Pythium,* and may be, as in the latter, of rare occurrence. Spores liberated with a limited amount of energy may find a sort of rejuvenescence in the amœboid stage, and by resuming the free swimming condition gain an extended area of infection.

PLEOLPIDIUM IRREGULARE *n. sp.*

This species was obtained at Kew, in 1900, from soil containing roots of *Ophioglossum vulgatum.* It grew in a *Pythium,* the host being so severely infested that none of its spores came to maturity, though many rudimentary ones were formed. Comparison with healthy cultures of *P. intermedium* and *P. vexans* obtained from the vicinity, showed that it did not agree with the former in the size and branching of the hyphæ ; while the vegetative body and position of the rudimentary spores corresponded with the latter.

Sporangia of the parasite are formed in considerable quantity, both terminally and intercalarly (plate VIII, figs. 1 and 2). They vary considerably in size, shape and position, and the attack is so general that much injury to the hosts results. The average size from a number of measurements is 23$^{\mu}$ in diameter. In the earlier cultures few resting spores were formed, but in the later ones they were much more numerous than the sporangia. The sporangium wall cannot be distinguished from that of the host, both being fused into one continuous membrane. When ripe, a papilla appears at any point of the wall, pushing up through it from below and evidently belonging to the parasite. The apex of this is refractive, and the contents clear (plate VIII, fig. 3). The zoospores swarm for a short period within the sporangium, and then, with the opening of the papilla, escape and swim off with great speed. The movement is jerky, the spore often stopping dead and turning in its tracks or flying off at an angle. Its shape is elongatedly obclavate, with the narrow end forward, and a single long cilium trailing from the back. Near the insertion of this a bright globule is visible (plate VIII, fig. 5). Where escape is checked, the zoospores may swarm for a long time within the sporangium. Those that reach the outside come to rest more rapidly, usually at the surface or margin of the drop, and may remain for twenty-four hours before breaking up. Those which reach a filament of the host plant round off and, after a period of rest, the contents pass over into the filament through a minute infection tube (plate VIII, fig. 9). Successful infection usually occurs in young hyphæ (plate VIII, figs. 8 and 9), but older ones are also attacked (fig. 6), and even swellings, such as that shown in fig. 7, which may have been an immature spore of the *Pythium*. It is probable that the spore body may either develop by itself into a sporangium, or unite with others to form a plasmodium before doing so. In the earlier and more vigorous cultures the condition shown in plate VIII, fig. 1*a*, was common, each hypha bearing a single sporangium, whereas later on, in cultures poorer in food supplies, the condition figured in fig 1*b* was more frequently seen, though there was no considerable difference in the intensity of infection

Resting spores are formed in large numbers They are 11—15μ in diameter, brown, always spherical, surrounded by a thick wall and lie free in more or less spherical swellings of the host hyphæ, which are cut off by septa (plate VIII, fig. 11). The majority of the spores were smooth-walled, but some developed spines, and this is undoubtedly the perfect condition The spines are short and regular and are laid down in a hyaline band, which appears in the earlier stages as an aureola around the wall. The resting stage often induces abnormal branching from the swelling which contain the spores (fig. 11*b*). Their germination was not observed.

This species agrees fully with the generic description of the section of Cornu's *Rozella* in which the sporangia are not grouped in a sorus, and which A. Fischer subsequently separated from those species in which a sorus is formed, as a distinct genus *Pleolpidium*. The genus has not been subsequently encountered, so far as I am aware, though, as already mentioned, Dangeard's *Chytridium simulans* (1897) agrees with it so far as the sporangia and zoospores are concerned, and even in the absence of resting spores, can hardly be placed elsewhere than here. It comes nearest to *Pl. Monoblepharidis* (Cornu) Fischer, in that the sporangia are formed in any position, whereas in *Pl. Rhipidii*, *Pl. Apodyæ* and *Pl. Cuculus*, they are terminal and occupy the sporangia of the host. If the identification of the host as *P. vexans* is correct, this distinction may be more apparent than real, since the normal spores of this species are sometimes intercalar. The diagnosis is as follows.

Pleolpidium irregulare n. sp.

Sporangia formed in the hyphæ of the host, irregular in shape, terminal and intercalar, averaging 23μ in diameter, with a single papilla ; zoospores obclavate, with a single cilium borne posteriorly; durable spores single, free in the cavity of the host-filament which is enlarged to contain them, numerous, $11\text{-}15\mu$ in diameter, spherical, of a pale yellow colour, with a moderately thick wall, provided with short regular spines; germination not observed.

Parasitic in *Pythium* ? *vexans*, Kew.

PLATE VIII, FIGS. 1—12.

PLEOLPIDIUM CUCULUS *n. sp.*

This species was found on *Pythium intermedium* at Cork and Antibes. It differs from the last in having sporangia closely resembling the normal *Pythium* spores, being regularly spherical or oval, and always terminal. I have not been able to distinguish between healthy spores of the *Pythium* and sporangia of the parasite, until just before zoospore formation. At this stage the contents appear denser than normal, and the numerous, small spore origins become visible. The papilla is single and opens in any position of the wall. Zoospores are numerous, and are indistinguishable from those of *Pleolpidium irregulare*. The locality of infection was not observed.

Resting spores are rarely produced, having been seen on two occasions only, and then in very small numbers. They were smooth-walled, brown in colour and with a large oil drop in the centre (plate VII, fig. 24). It is probable that here, as in the other members of the genus, the perfectly developed spore is provided with spines, but in *Pl. irregulare* it has already been mentioned that smooth-walled spores are common, and a sufficient number of spores of *Pl. Cuculus* was not observed to enable me to say whether spines are produced or not. The germination was not observed. The diagnosis is as follows.

Pleolpidium cuculus n. sp.

Sporangia spherical or pyriform, formed in the spores of the host from which they cannot be distinguished, terminal, regular, about 20μ in diameter, with a single papilla; zoospores as in *Pleolpidium irregulare;* durable spores rare, spherical, single, free in the cavity of the spore of the host, 12-17μ in diameter, (?) smooth, pale yellow; germination not observed.

Parasitic in *Pythium intermedium*, Cork, Antibes.

Plate VII, figs. 22—25.

PLEOLPIDIUM INFLATUM *n. sp.*

Pythium intermedium harbours another parasite, which is remarkable for the deformity which it causes, and for the size of its sporangia, which are the largest that I have seen in *Chytridiaceæ*, being sometimes visible to the naked eye. It was found at Antibes on two occasions, and is apparently much less common than *Pl. Cuculus*. Resting spores were not observed and their discovery may show that it should be considered as the type of a new genus, since the biciliate zoospores separate it from the other known species of *Pleolpidium.*

The hypertrophy caused by this species exceeds that of the two previously described, being apparent not only at the seat of sporangia formation but also in the supporting hyphæ, which are often much swollen. As in *Pl. Cuculus* sporangia are formed terminally only, and in the spores of the host. The sporangial wall is fused over all, or a great part, of its extent with that of the *Pythium*, sometimes separating towards the base and stretching across above the basal septum of the spore as a thin membrane (plate VII, fig. 1). A similar separation of the two membranes appears in *Pleolpidium Monoblepharidis* (Cornu) Fischer. The sporangia are round, oval or pear shaped, less regular in form than those of *Pl. Cuculus* and variable in size, reaching a diameter of 85μ in some cases. They can be early distinguished from normal spores of the host, through the hypertrophy induced and the dense nature of the protoplasm. In maturation a vacuolar stage is not marked, and the stages in the separation of the spore origins cannot be followed. Movement commences, as in the other species, before the papilla opens. The latter may be formed at any point, and more than one sometimes appear (plate VI, fig. 20). The movement is at first slow, but gradually quickens and ends in a vortex-like whirl. As the spores escape, the motion becomes very violent until the whole is emptied. The zoospores (of which a single sporangium was estimated to contain over 7,000) swim away directly in smooth, long curves, in marked contrast to the jerky motion of the other two species above described. The normal spores are somewhat kidney-shaped, with two cilia, one short and attached to the anterior end, and one to a side near the back (fig. 19). The latter is trailed behind. Variations in the apparent position of the cilia are common in this, as in some other species, and are due to (*a*) the varying position of the spore which, as it rolls over, hides the point of attachment of the cilia and causes them to appear as if arising from other points, and (*b*) the movements of the cilia themselves, which often bend over and appear to fuse temporarily in the proximal part of their length to the surface of the spore. I think the position above described is the typical one.

Resting spores were not observed, as the species was not under culture for any length of time.

I have preferred to keep this species in the genus *Pleolpidium*, rather than to create a new genus for it, pending the discovery of its resting spores The 2-ciliate zoospores, however, separate it widely from the other species of *Pleolpidium*, and this is a character which is almost of generic importance in the *Chytridiaceæ*. The diagnosis is as follows :—

Pleolpidium inflatum n. sp.

Sporangia spherical, oval or pyriform, formed in the spores of the host which, with the supporting hyphæ, are greatly swollen, up to 85μ in diameter, with one or more papillæ ; zoospores very numerous, elongated, unequilateral, with two cilia, one in front and the other lateral near the back ; durable spores not seen.

Parasitic in *Pythium intermedium,* Antibes.

PLATE VII, FIGS. 17—21.

PSEUDOLPIDIUM PYTHII *n. sp.*

Cultures of *Pythium monospermum, P. rostratum, P. vexans* and *P. intermedium,* obtained from garden soil at Antibes, were frequently attacked by this parasite. No difference in its structure or development was observed on any of the hosts.

The affected cultures in all cases showed many hyphæ swollen up into oval, spherical or balloon-shaped enlargements, either terminal in position or placed laterally in a diverticulum of the hypha, as occurs in the resting spore formation of *Rozella septigena* (Cornu, 1872). Like in the latter, the diverticulum is often directly continuous with the supporting hypha, no septum to cut it off being formed (plate VII, figs. 9—10). The presence of the parasite is sufficient to produce septation in some cases where the host plasma is largely absorbed, one such instance being figured in fig. 9 *s.* Here, however, the branching between the septum and the sporangia-containing diverticulum shows that the former has not been developed in order to cut off the latter from the rest of the mycelium, but is merely of the same order as the septa so often produced in exhausted cultures of *Pythium.*

Sporangia are formed either singly, or in company with other sporangia, or with resting spores. They are oval and variable in size, up to at least 35μ in the longer diameter. They lie free in the diverticula, the membrane being entirely free from that of the host. The stages of their development are exactly similar to those more fully described in the next species, some being figured in figs. 9 and 10. The parasitic plasma early comes to view, differing in this respect from that of *Pleolpidium* (fig. 9*a*)

During ripening a vacuolar stage occurs (fig. 10*a*), and the process is similar in all essentials to that of *Pseudolpidium Aphanomycis* described below. A single tube of discharge is formed which bores through the host wall and extends a little way to the outside. It opens at the tip to give exit to the zoospores, which commence their movement before the tube opens.

The zoospores are biciliate. The attachment of the cilia at the moment of leaving the sporangium was not observed. On liberation, the zoospores swarm in the vicinity of the sporangium for a very brief period, and then come to rest in a dense cluster. After a couple of minutes, motion is resumed and the zoospores, now provided with two cilia inserted laterally (plate VII, fig. 12), swim away, at first with a slow motion. This behaviour of the zoospores probably is similar to that more fully described in *Pseudolpidium Saprolegniæ* and *P. Aphanomycis* below, and like the latter is perhaps to be interpreted as a rudimentary and abbreviated diplanetism.

Resting spores are formed freely, either alone, or in company with sporangia (figs. 14—15). They are oval or spherical, and of a rich brown colour The wall is double and beset with fine, short, evenly spaced spines, not tapering from base to apex. In imperfectly developed ones, spines may not be formed, and a common condition is that figured in figs. 13 and 15, where one resting spore has developed further than another, the smaller starved one persisting, in a manner which simulates the companion cell of *Olpidiopsis*. That it is not of equivalent value to the latter is, however, apparent from the fact that it remains filled with vacuolated protoplasm and does not empty its contents into the larger cell. The spines develop in an irregular light band of protoplasm, which appears around the wall of the spore at an early stage (fig. 16*a*). In this, light and dark radiating lines appear (fig. 16*b*), produced apparently by condensation of protoplasm along definite lines. This condensation proceeds from without inwards, so that at one period the outer ends of the spines appear sharp and fully formed, while the proximal part is still partially enveloped in the protoplasmic investment (fig. 16*c*).

The germination of the resting spores was not observed. The diagnosis is as follows :—

Pseudolpidium Pythii n. sp.

Sporangia single or multiple, formed in swollen diverticula of the hyphæ of the host, oval, up to 35μ in the longer diameter, with a single tube opening to the outside and variable in length ; zoospores elongated, unequilateral, with two cilia, one from the narrow end in front, and the other lateral near the back ; durable spores numerous, single, or with sporangia or other durable spores, oval or spherical, up to 30μ in diameter, wall brown, rather thin, provided with fine, short, evenly spaced spines ; germination not observed.

Parasitic in *Pythium monospermum*, *P. rostratum*, *P. vexans* and *P. intermedium*, Antibes.

PLATE VII, FIGS. 9—16.

PSEUDOLPIDIUM GRACILE *n. sp.*

This species was also found at Antibes on *Pythium intermedium*, forming beautiful clusters of sporangia and resting spores, in localized portions of otherwise normal cultures (plate VII, fig. 2). As in the last species, spores are produced in large terminal or lateral diverticula, not usually separated from the bearing hyphæ by any partition. The diverticula are large, sometimes reaching 80 or 90μ in their longer axis, and are oval or balloon-shaped. The hyphæ beneath the diverticula are not swollen.

The sporangia lie singly or grouped in the containing capsules, as many as forty having been counted in a single swelling. They are very variable in size ; from 4μ up to 52μ having been measured. As in *Pseudolpidium Saprolegniæ*, this probably is connected with the number of zoospores attacking a single hypha, each sporangium representing the development of a single spore They are usually exactly spherical in shape.

The development of the sporangia can be followed from an early stage. The first visible manifestation occurs in the swollen diverticulum, by the appearance of a small mass of denser protoplasm than that normally found in the host (plate VII, fig. 1*a*). This increases in size and density, leading to gradual thinning of the surrounding protoplasm (fig. 1*c*. *d*.). As the parasitic protoplasm condenses at the expense of that of the host, droplets of oil are thrown out round the former ; so that at this stage the limits of the parasite are hidden in a mass of refractive globules. These gradually disappear, and the sporangia begin to assume their definite shape, and to provide themselves with a wall (fig. 1 *e*. *f*.). As the sporangia ripen, the contents of the diverticula are entirely absorbed. The ripe sporangium is vacuolated, but the vacuoles disappear shortly before the zoospores are marked off.

A peculiarity of this species is the formation of several tubes of discharge in a large proportion of sporangia, about 80 per cent. having more than one. As many as five are sometimes formed (fig. 5). As, however, in all its other points the species agrees with other species of *Pseudolpidium*, and as *Ps. Aphanomycis* and *Ps. Saprolegniæ* frequently have more than one tube to the sporangium, I do not consider that much weight need be attached to this. The tubes project a considerable way beyond the host wall, and are often contorted and swollen (fig. 4).

The zoospores swarm within the sporangium and escape through one or more of the tubes. They are elongated and curved, resembling the spores of *Pseudolpidium Saprolegniæ* as depicted by Fischer (1882). One cilium is inserted near the narrow anterior end, and the second, attached laterally, is trailed behind. The motion is peculiar, being smooth and in long curves ; a combination of revolution of the zoospore in a circle, in the direction of the hands of a watch, with rotation of the spore body on its long axis, being common.

Resting spores are early formed, often in the same capsule as sporangia. The early stages of their development are shown in plate VII, fig. 1 *b*. *g*. They are exactly spherical, with long tapering spines, and much lighter in colour than those of *Pseudolpidium Pythii.* Germination was not observed. The diagnosis is as follows :

Pseudolpidium gracile n. sp.

Sporangia single or multiple, formed in swollen diverticula of the hyphæ of the host, spherical, up to 52μ in diameter, with a single or more usually several, rather long, tubes, opening to the exterior ; zoospores as in other members of the genus ; durable spores spherical, yellowish, rather thin-walled, with long tapering spines thickly crowded ; germination not observed.

Parasitic in *Pythium intermedium,* Antibes.

PLATE VII, FIGS. 1—8.

PSEUDOLPIDIUM (? OLPIDIOPSIS) SAPROLEGNIÆ (*A. Braun*) *Fischer.*

This species was found at Antibes in an aquatic *Saprolegnia,* and kept in culture for some months. As, however, neither the host nor the parasite developed resting spores, it was impossible to identify either with accuracy. The only difference which exists, according to Fischer (1892), between *Pseudolpidium Saprolegniæ* and *Olpidiopsis Saprolegniæ* lies in the resting spore condition, the former having no companion cell and fine scattered spines, while the latter is accompanied by a companion cell and has thickly-crowded spines or warts.

The sporangia in the species now under consideration are oval, rarely longer than twice the breadth, and occupy the apex of much swollen hyphæ, or more rarely the middle or base. The sporangia mature just as in other members of the genus *Pseudolpidium* and zoospores escape through one or, more rarely, two tubes. They are elongated, about twice as long as broad and flattened in one plane. One cilium arises in front and one longer one at the side (plate X, fig. 2*a*). The movement is in long "skating" curves, which can be particularly well seen when the sporangium discharges into the lumen of the *Saprolegnia* hypha. In this position the movement may last for many hours. When the outside water is reached, either directly, or after some hours of swarming in a *Saprolegnia* hypha, the metamorphosis described above (p. 120) is passed through, and the ultimate shape which is assumed is quite different to the original one (plate X, fig. 2*b*.).

The zoospores are capable of attacking zoospores of the *Saprolegnia* in the resting condition, both during the encysted stage, in which the interval between the two stages of diplanetism is passed, and also after the second form has come to final rest. Plate X, fig. 1, shows that the presence of the parasite is not sufficient to interfere with the normal activities of the spore which may be liberated from the first cyst in the ordinary way after attack.

Plate X, figs. 1—2.

PSEUDOLPIDIUM APHANOMYCIS (*Cornu*) *Fischer*.

This species was obtained in cultures of *Aphanomyces lævis* de Bary, at Pusa, in India, and its development followed. Attempts to inoculate *Pythium monospermum* and *P. proliferum* with it failed.

Sporangia are formed in large numbers, both intercalarly (plate IX, figs. 1 and 3), and, more rarely, terminally (plate IX, fig. 1). They vary greatly in size, often appearing in small bead-like swellings of the hyphæ, containing one or two little sporangia (plate IX, fig. 3), or occurring in large swellings, not much inferior to those produced by *Pseudolpidium Pythii*.

The infecting zoospores crowd on growing hyphæ of the host (plate IX, fig. 2). Their mode of entry is similar to that of the species already described After entry their presence is first shown in irregular swellings of the hyphæ which are exceedingly variable in size and position. The dense parasitic plasma early becomes visible in these and, gradually increasing in size, leads to the disappearance of the surrounding contents. Oil drops are formed in numbers during this process, as in *Ps. gracile.* When fully formed, the sporangia lie loose in the cavity of the swelling, and it is interesting to note that their size is wholly independent of that of the hypha, well developed sporangia having the swelling sufficiently large to permit of their lying loose, while small swellings are occupied by still smaller sporangia.

The ripe sporangia are vacuolated and provided with one or, rarely, two tubes of discharge (plate IX, figs. 4—6). Fig. 6 shows the changes during the maturation of the sporangium, from a continuous observation. It will be noticed that the vacuoles, at first spherical, change both in shape and relative position—changes that are to be interpreted as resulting from the slow rotation of the protoplasm and its condensation into heaped masses. The spore origins become visible soon after the disappearance of the vacuoles, and commence to move two or three minutes later, four or five minutes before the tube opens to emit the zoospores.

The zoospores go through two periods of motion in at least a large proportion of cultures. The first lasts a very short time, and all the spores of a sporangium come to rest near together, since time has not allowed of their scattering. After a period of quiescence lasting five or four minutes, during which changes occur in the zoospores, which become highly refringent and possibly, also (from the analogy with *Ps. Saprolegniæ*), in the cilia, motion recommences. This is at first a jerky oscillation to and fro, which quickens until at last the spores dart away. The cilia cannot easily be observed in the living spore, but fig. 7 shows their number and position when fixed by iodine during the second period of their motion. They are two in number, attached laterally close together, the arrangement differing rom that of any other species observed. The cilia during the first period of motion were not observed in any case where two distinct periods occurred, but fig. 5 shows a sporangium fixed with iodine during discharge, and containing several spores with cilia arranged as in fig. 7. From this it would appear probable that there is no alteration in the position of the cilia during the two periods of motion, but it is possible that cases occur in which the first period is suppressed and the sporangium figured may have been such a case. As in *Ps. Saprolegniæ* the occurrence of two periods of motion is not, I think, a constant phenomenon.

Resting spores in this species were not observed by Cornu. The only other reference to the fungus that I can find is by Dangeard (1890, p. 90), and here the resting spores are described and figured. I have only seen a single one (plate IX, fig. 1*a*), which was spherical, thin-walled and provided with numerous fine spines. When observed, it had already germinated and the open tube found emerging to the exterior shows that it had liberated zoospores. As the culture was only about ten days old, it is evident that the spore had not gone through any lengthened period of rest before germination. That figured by Dangeard (*l. c.*, plate IV, fig. 11) has thicker walls and strong conical spines, and appears more properly fitted for the resting condition.

PLATE IX, FIGS. 1—7.

OLPIDIOPSIS MINOR *Fischer.*

This species appeared in cultures of *Achlya polyandra* de Bary, at Dehra Dun, in India. At first sporangia only were obtained, but fresh cultures infected from these gave a plentiful crop of resting spores.

The sporangia are large and spherical, occurring in groups in large dilatations of the host, just as in *Pseudolpidium Saprolegniæ*, from which they cannot be distinguished. The zoospores, which have not previously been described, are elongated and two-ciliate, having the cilia fixed one laterally and one in front.

Resting spores are produced either singly, or in company with others or with sporangia. They are spherical, yellowish-brown cells, with large, colourless, triangular spines and a smaller, colourless, smooth walled, companion cell closely attached (plate IX, fig. 10). Their germination was not observed.

Cornu (1872) confused the resting spores of this species with *Pseudolpidium fusiforme*. Fischer, however, found the true resting spores of the latter, and ascertained that they were elongated cells, of the same shape as the sporangia, provided with scattered spines and without companion cells (*l. c.* 1882, plate I, fig. 1 *b.*). The sporangia of *Olpidiopsis minor* are described by Fischer (1892, p. 39) as small and spherical, and the resting spores as much smaller than those of *Olpidiopsis Saprolegniæ*. In my cultures both were large, not much inferior to those of the latter, the resting spores being from 40 to 60μ in diameter and the sporangia reaching double this size. Contrary to Fischer's statement, an oil drop is sometimes present (fig. 10). The spines are produced on an outer membrane and being joined at their bases by prominent ridges give rise to a distinct sculptured outer wall on the spore. No fresh light was obtained on the sexuality of the resting spores which are usually regarded as resulting from fertilization by the companion cell.

PLATE IX, FIGS. 8—11.

OLPIDIOPSIS SCHENKIANA *Zopf.*

The species described under this name by Zopf (1884, p. 168, plate 4) was found at Pusa, in India, on a species of *Spirogyra*. It is the most active parasite which I have seen in the *Chytridiaceæ*. Long filaments of the host were often found with practically every cell infested; the same vigour of attack was observed by Zopf. Infected filaments are readily noticeable from their pale colour, which results from destruction of the cell chlorophyll, after it has first become collected into a mass around the parasite.

The sporangia are spherical or more usually elliptical and often somewhat curved They occurred in my cultures much less commonly than the resting spores. The latter are smaller than the sporangia, spherical or oval, pale brown and with a thick smooth wall. A companion cell closely resembling that of *O. minor* is always present, closely fixed to the wall. No additions have been made to the very full observations of Zopf.

PLATE X, FIGS. 11 – 13.

OLPIDIUM GREGARIUM (*Nowakowski*) *Schröter.*

The eggs of certain rotifers are sometimes attacked by species of *Olpidium*. One of these was described by Nowakowski (1876, p. 73) as *Chytridium gregarium*, and afterwards removed by Schröter (1889, p. 182) to the genus *Olpidium*. The rotifer attacked is not specified. I found the species producing an epidemic in the eggs of *Metopidia lepadella* in a pool in the Black Forest near Freiburg. Vessels containing large numbers of the rotifer were infected in the laboratory, with the result that the parasite multiplied to such an extent as wholly to check the reproduction of the host.

Spherical or oval sporangia are formed in quantities in the eggs (plate VIII, fig. 13). Sometimes an egg contains only a single large sporangium, at others twelve or more may be produced. Discharge takes place through a single, short, blunt tube which perforates the wall of the egg. The zoospores are liberated in mucus, which binds them together in a spherical mass at the mouth of the tube (fig. 15). The mucus quickly dissolves in water, setting free the zoospores. The latter are spherical, with a large, eccentric, refractive body and a single long cilium (fig. 16). After a short period of movement the zoospores come to rest on eggs of the host (fig. 17), and go through an amœboid stage before infection occurs.

In fig. 18 it will be observed that the sporangia and resting spores are enclosed in separate compartments of the egg, one or more being surrounded by a separate membrane within the cavity of the latter. This appearance is probably due to infection having occurred after segmentation had commenced.

The resting spores of this species were not previously known. They occurred rarely in my cultures, and are figured in fig. 18. They are spherical, thick-walled, brownish cells, the wall being smooth and showing faint evidences of striation. The germination was not observed.

Plate VIII, figs. 13—18.

NOWAKOWSKIELLA RAMOSA *n. sp.*

The genus *Nowakowskiella* was founded by Schröter (1897, p. 82), for a species described by Nowakowski (1876, p. 95), as *Cladochytrium elegans.* The chief character of the genus is that the sporangium opens by throwing off an operculum, instead of by the softening of a papilla as in *Cladochytrium.* A second species *N. endogena* was described by Constantineanu (1901), agreeing with Nowakowski's species in having an operculum to the sporangial opening, and in proliferation of the sporangia within the emptied cavity of previous ones, but having some points of resemblance to *Cladochytrium,* such as the possession of a tube for the exit of the zoospores, and the endogenous habit. Constantineanu considers his species intermediate between the two genera, and doubts the advisability of giving *Nowakowskiella* generic rank. This doubt is strengthened by Clinton's (1902) account of *Cladochytrium Alismatis.* Not only do the resting sporangia of this species open by throwing off an operculum, as indeed is known in some other species, but the temporary sporangia are proliferous. I am unable to find any important point of distinction between *Cladochytrium* and *Nowakowskiella,* in the sporangial stage. But the resting stage of the species to be described below, is so different from that of the former, as to constitute, I think, a real distinction. The position of the two species above mentioned must be a matter of some doubt, until their resting stages are known.

My species is closely related to both Constantineanu's and Nowakowski's in general habit and in the form of the sporangia. The mycelium ramifies within the tissues of the subtratum, and spreads also into the surrounding water. Sporangia are formed both within the cells, when they open by a tube, and outside, when usually the tube is absent. The opening is by an operculum, and after one sporangium has emptied, a new one is formed within the cavity. The species produces resting spores, thus differing from both of the allied species.

The fungus was obtained in decomposing plants of wheat, immersed in water to obtain the ascospores of *Erysiphe graminis*, by which the wheat was much attacked. In the earlier cultures, only sporangia were obtained, but at a later stage resting spores were formed in abundance.

The mycelium (plate X, fig. 7) is developed to an extent which is reached by few, if any, of the *Chytridiaceæ* hitherto known. The main hyphæ, up to eight or ten in number, arise from a basal, thick-walled swelling, which probably indicates the position of the original spore. They are thick-walled, hyaline and freely branched. The branches come off very irregularly, and at all angles, the junction between stem and branch being often swollen. This gives the mycelium a somewhat characteristic appearance. Anastomosis of the hyphæ is common (plate X, fig. 7). No regular decrease in the diameter of the hyphæ, as they become more distant from the spore, is observable, but the ultimate branches are extremely fine. On these, and at other points of the mycelium, irregular swellings are frequent, the swellings being, in many cases at least, in no way connected with sporangia formation. Similar swellings are known in *N. elegans*.

The sporangia are usually terminal, but sometimes also intercalar (plate X, fig. 7). At the point of sporangium formation, the hypha swells up into an elongated swelling, tapering at the proximal end. In this a septum appears, cutting off the outer, distal, cell, which swells up into a spherical or pear-shaped sporangium, from the basal part which is in direct communication with the hypha, and forms a sort of apophysis, similar to that figured by Zopf in *Amœbochytrium rhizidioides* (1884, plate XVII, fig. 7*b*). The presence of this apophysis is common, but not constant, and it is the nearest approach to the formation of a companion cell, such as is found in many *Cladochytrii* which I have seen. When intercalar, two walls may be formed in the sporangial swelling, cutting off two apophysial-like portions, from the sporangium itself (plate X, fig. 7). Sporangia are usually formed in the extra-matrical mycelium, but they may also occupy the outer cells of the tissues, and are then more or less compressed to the shape of the cell.

When ripe, a papilla forms at any part of the sporangial wall, by the protrusion of the endospore. Instead of a single point of the exospore wall being pierced, a circular portion is lifted off by the swelling endospore, and thrown aside. This is the operculum (plate X, fig. 3*o*). Before the papilla opens, the zoospores are marked off within the sporangium (plate X, fig. 3*b*) They escape either as a whole, in a mass which remains before the mouth of the sporangium for a short time, before breaking up, as in *Olpidium gregarium* or singly (plate X, fig. 3*a*). In the latter case the spore elongates, and creeps slowly out of the mouth, taking on an amœboid motion during the passage The free zoospore is spherical, with a large eccentric oil globule and a single cilium (fig. 5). After swarming for a while it comes to rest, and a period of marked amœboid motion is gone through, during which the oil globule changes its position, often eventually passing to the centre. A second period of activity sometimes follows this amœboid stage. Before germination the zoospore swells up considerably and becomes irregular in shape (fig. 6), but the subsequent stages were not followed.

The resting stage is of considerable interest. Certain hyphæ commence to proliferate, either at their ends, or laterally (figs. 9, 10), growing out into large, irregular cells. New cells are formed from these, both by proliferation, and by division of existing cells. In this manner a thin-walled mass of angular cells is formed, sometimes of considerable size. The marginal cells of this swell up into spherical bodies, which thicken their walls to become resting spores (fig. 8). As the process advances, the first formed cells are emptied of their contents, which apparently go to form the new cells. As a final result, a group of resting spores is produced, joined together by the thin-walled parenchymatous tissue of the mass. The whole formation is to be interpreted as an extreme development of the "sammelzellen" of the *Cladochytriaceæ*. In some cases two or more hyphæ or hyphal branches unite to produce the sammelzellen, and in some of these the appearance strongly suggests a fertilization. This appearance is increased by the emptying of the cells below the resting spore. That no true fertilization takes place is, I believe, the case, for, in simple examples, a single hypha can give rise to a small parenchymatous mass, bearing one or two resting spores; while in the larger masses, two or more resting spores are often united, having resulted from the swelling of two or more adjoining cells of the mass (fig. 8). It is extremely improbable that sexually produced spores would be joined together in this fashion, for the formation indicates that there is no special differentiation of the resting spores, in their early stages, from the thin-walled cells around them.

It must be admitted that the presence of true sexuality in the *Chytridiaceæ*, excluding the *Ancylistaceæ*, which have, in other respects, distinct affinities to the *Oosporeæ*, and are widely separated from the *Chytridiaceæ* proper, is far from established. The companion cells of *Olpidiopsis* and *Urophlyctis* have been interpreted as antheridia, but, until the cytological facts are known, may equally well be considered as morphologically equivalent to the emptied sammelzellen of the *Cladochytriaceæ*, or the apophyses of *Amœbochytrium* and *Rhizidiomyces*. The copulation of gametes described by Fisch in *Reessia* (1884 (1), p. 9), and by Sorokin in *Tetrachytrium*, has not been accepted by Fischer (1892) on general grounds, and certainly requires confirmation. There remains the genus *Polyphagus*, in which true sexual reproduction has been definitely established by cytological investigation (Wager (1899), Dangeard (1900)).

The *Ancylistaceæ* have undoubted relationships with the oosporal line of the Phycomycetes. *Zygochytrium*, if Sorokin's observations be accepted, is equally clearly allied to the Zygomycetes. But the remainder of the *Chytridiaceæ* is composed of several groups, whose relationships are only apparent within the group, and the most highly developed of these, the *Cladochytriaceæ*, appears to form a blind line without any trace of sexual reproduction, and connecting with none of the higher Phycomycetes. The species just described is perhaps the highest member of this line.

The diagnosis is as follows :—

NOWAKOWSKIELLA RAMOSA *n. sp.*

Mycelium copious, intra- and extra-matrical, of much branched hyphæ, irregular in size, from less than 1μ to 4 5μ in the swollen parts, old hyphæ thick walled, anastomosing; sporangia terminal or intercalar, 25—45μ in diameter, spherical or pyriform, usually with a swollen apophysis, opening by an operculum; zoospores formed before the operculum opens, escaping in a mass or singly, spherical, with one cilium and a large oil drop durable spores formed in a thin-walled pseudoparenchyma, smooth, with a moderately thick wall, yellowish, 18—25μ in diameter.

Saprophytic on rotting stems of *Triticum vulgare*, Dehra Dun.

PLATE X, FIGS. 3—10.

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PLATE I.

PYTHIUM GRACILE SCHENK.

Fig. 1. *a.* to *d.* Lateral and terminal bud-like processes on the hyphæ. In *a.* there is a resemblance to conidium formation. In *d.* the processes are continuing their growth as ordinary hyphæ. X 300.

Fig. 2. A lateral branched process. The full part of the hypha bearing the process, is cut off from the emptied proximal part by a septum. X 300.

Figs. 3—4. Matted hyphal clumps formed in the mycelium. Fig. 3, X 300 ; fig. 4, X 100.

Fig. 5. Portion of a growing hypha showing septation. Three septa were formed successively, while under observation. The septum, on each occasion, cut off the emptied proximal from the full distal part. X 300.

Fig. 6. *a.* to *h.* Zoospore formation. *a.* Mature sporangial hypha with slightly club-shaped end, but hyaline cap not yet formed. *b.* Formation of the latter, and of the sub-apical vacuole. *c.* Ripe apex of sporangial hypha. *d.* Commencement of forward protoplasmic streaming. *e.* Blowing out of the hyaline cap to form the vesicle, *f.* Vesicle fully formed. Protoplasm not completely filling it, and now showing irregular heapings, as the spore origins become defined. *g.* Spores separating off. Movement as a whole has commenced in the mass. *h.* Spores fully formed and provided with cilia. Movement now independent. X 500.

Fig. 7. A single zoospore.

Fig. 8. Fragment of vegetable tissue lying in water, permeated by the mycelium of *P. gracile.* X 300.

Figs. 9—10. Oospores obtained in culture on *Abutilon* root. Fig. 9 shows shape and position of antheridium. Fig. 9, X 500 ; fig. 10, X 260.

Fig. 11. Oogonium and antheridium from distinct hyphæ, obtained extramatrically in slide cultures of infected algæ. Fertilization did not take place. X 300.

Fig. 12. Oogonium containing ripe oospore from a *Vaucheria* cell. X 850.

Fig. 13. Portion of a *Vaucheria* filament containing *P. gracile.*

Fig. 14. Portion of decaying tissue of *Marchantia* sp. containing *P. gracile.* X 490.

PLATE II.

PYTHIUM GRACILE SCHENK, P. MONOSPERMUM PRINGS., P. INDIGOFERÆ *n. sp*, AND P. DIACARPUM *n. sp.*

Fig. 1. Portion of rhizome of *Zingiber Officinale* attacked by *P. gracile ;* showing an oospore, with antheridium arising from a different hypha. X 500.

Fig. 2. Oospores of *P. monospermum* Prings. Antheridia from a distinct hypha. X 600.

Figs. 3—7. *P. indigoferæ.*

Fig. 3. Portion of a culture of *P. Indigoferæ* with swollen hyphæ and oospores. X 260.

Fig. 4. Oospores of *P. Indigoferæ* formed in lateral swollen processes. The antheridium appears to be a cell cut off from the process, but is only distinct in the upper figure. X 500.

Fig. 5. Terminal oogonia of *P. Indigoferæ*. The oogonial stalk curves over towards the antheridium in characteristic fashion. X 500.

Fig. 6. Young oogonium and antheridium of *P. Indigoferæ.* X 500.

Fig. 7. Germination of the oospores of *P. Indigoferæ*. X 500.

Figs. 8—17. *P. diacarpum.*

Fig. 8. Part of a culture of *P. diacarpum* on a piece of rotten wood. X about 100.

Fig. 9. Stages in the discharge of a sporangium of *P. diacarpum.* Note the slender stalk and broader tube, opening into an urn-shaped vesicle at the tip. X 260.

Fig. 10. Emptied sporangium, showing the persistent collar at the top of the tube. X 260.

Fig. 11. Types of proliferation of the hypha through the sporangium. X 260.

Fig. 12. Zoospores of *P. diacarpum* immediately after emission.

Fig. 13. Zoospores encysted.

Fig. 14. Cysts with tube formed, and about to give new zoospores. On the right is one which has already emptied.

Fig. 15. Protoplasm expelled from a cyst and collected into a ball at the apex of the tube.

Fig. 16. The new zoospore formed, but cilia not yet developed.

Fig. 17. Second zoospore after escape from the cyst.

PLATE III.

PYTHIUM PROLIFERUM DE BARY.

Fig. 1. Outline showing types of branching. Sporangia almost all terminal. Sub-sporangial branching in *a*. In *b*. an immature sporangium has given out two branches terminating in spores. The hypha to the right (from an old culture) shows a degree of branching which is unusual.

Fig. 2. Part of a culture showing the regular, sparingly branched, hyphæ terminating in spherical or sub-spherical sporangia. At *a.* sub-sporangial branching. At *b. b.* proliferation. X 100.

Fig. 3. Part of a fly leg culture. X 100.

Fig. 4. A branch from a young culture showing very marked proliferation. The two lower branches have arisen from close under the emptied sporangia. In the other cases the innovation has grown on through the sporangium, swelling up at intervals, chiefly within the emptied sporangia. X 150.

Fig. 5. The innovation growing through a sporangium but unable to grow out, except through the opening of the tube of discharge. X 200.

Fig. 6. False head of spores, produced by repeated sub-sporangial branching, the new sporangia being formed close to the first. X 100.

Fig. 7. Discharge of zoospores. In the lower figure the basal septum had already commenced to push forward, while the sporangium was still full. X 200.

Fig. 8. *a.* A ripe sporangium just prior to the rounding off of the vacuole. *b.* The same after discharge showing the basal septum commencing to grow forward. Notice the thick hyaline cap, produced by the softening of the basal septum. X 300.

Fig. 9. Types of proliferation. X 200. *a.* The new sporangium formed within the old.

Fig. 10. Stages in fertilization. The oogonium is terminal. The antheridium arises from a distinct hypha, which was traced to a considerable distance, and found to originate from a branch unconnected with that bearing the oogonium. At *a.* (10 A.M.) retraction of the oogonial contents has just commenced. At *b.* (12 noon) retraction is complete, a few plasmatic masses being left behind. At *c.* (1 P.M.) fertilization is in progress. At *d.* (2 P.M.) it is nearly completed. At *e.* next morning the oosporal membranes are fully formed. The fertilizing tube is still visible. X 300.

Fig. 11. Fertilization by a hypogynal antheridium. At *a.* retraction has just commenced. At *b.* retraction is complete. Note the granules left behind (de Bary's "periplasm"). X 450.

Fig. 12. Ripe oospore. X 450.

PLATE IV.

PYTHIUM PROLIFERUM DE BARY AND P. PALMIVORUM *n. sp.*

Fig. 1. Stages in the formation and liberation of the zoospores of ***P. proliferum.*** For description see pp. 23—26. X 300.

Fig. 2. Portion of a culture of *P. palmivorum* showing terminal sporangia. X 100.

Fig. 3. Fertile hyphæ of *P. palmivorum* with terminal sporangia. In the right hand figure a branch has arisen under a sporangium. X 260.

Fig. 4. Fully formed sporangium. X 260.

Fig. 5. Discharge of a sporangium. X 260.

Fig. 6. Germination of the zoospores.

Fig. 7. Germination of an oospore. X 400.

Fig. 8. Section through a leaf sheath at a diseased spot showing the felt of mycelium.

Fig. 9. Section through a leaf sheath of *Cocos nucifera* showing intercellular hyphæ and haustoria. X 260.

Fig. 10. Haustoria of *P. palmivorum.* X 940.

PLATE V.

PYTHIUM VEXANS DE BARY and P. ROSTRATUM *n. sp.*

Figs. 1—10. *P. vexans.*

Fig. 1. Outline showing type of branching in a young culture.

Fig. 2. Portion of a culture showing irregular sporangia and conidia, terminal and intercalar. X 100.

Fig. 3. Mature sporangium. X 400.

Fig. 4. Discharge of sporangium. X 400.

Fig. 5. Conidium with thick wall. X 400.

Fig. 6. Conidium which has budded off another laterally, before it became separated from its bearing hypha by a septum. X 100.

Fig. 7. Intercalar oogonium and antheridium formed on a lateral stalk. In the left hand figure fertilization has commenced. In the right it is nearly completed. X 300.

Fig. 8. Terminal oogonium, with antheridium formed in a lateral swollen process which divides into several cells. At *a.* fertilization is commencing. At *b.* it is nearly completed. Note the fusion of antheridium with oogonial wall. X 300. (The oogonium is always divided from its hypha by septa, which have been omitted by the lithographer in two cases, in Figs. 7 and 8.)

Figs. 9 and 10. Ripe oospores, with the persistent antheridium fused with the oogonial wall to form a pear-shaped body, which is very characteristic. In Fig. 10 the oogonium is almost sessile. X 300.

Figs. 11—22. *P. rostratum.*

Fig. 11. Outline showing type of branching in a young culture.

Fig. 12. Portion of a culture with sporangia, both terminal and intercalar. X 100.

Fig. 13. Stages in the liberation of zoospores. At *a.* the sporangium is mature and provided with a large irregular vacuole. At *b.* the latter has disappeared and the sub-apicular vacuole in the tube is very distinct. At *c.* discharge is in progress. X 300.

Fig. 14. A conidium which has budded off another laterally, before being cut off from its stalk by a septum. X 260.

Fig. 15. Gemmæ. X 260.

Fig. 16. Series of three intercalar oogonia with hypogyna antheridia. X 400.

Fig. 17. Oospore with short curved antheridium, arising directly under the oogonium. X 400.

Fig. 18. Antheridium formed by a process put out from a cell cut off directly under the oogonium, the cell and process together forming the antheridium. X 400.

Fig. 19. Oogonium formed in a diverticulum from a hypha and fertilized by a hypogynal antheridium. X 940.

Fig. 20. Terminal oogonium with ripe oospore. X 400.

Fig. 21. Intercalar oogonium with the oospore lying to one side. X 400.

Fig. 22. Germinating zoospores of *P. rostratum.*

PLATE VI.

PYTHIUM INTERMEDIUM DE BARY and P. ARTOTROGUS (MONT.) DE BARY.

Figs. 1—11. *P. intermedium.*

Fig. 1. Outline showing type of branching in young cultures.

Fig. 2. Portion of a young culture. X 100.

Fig. 3. Branched finger-shaped processes produced at the ends or laterally in some cultures. X 150.

Fig. 4. Formation of chains of sporangia or conidia. X 300.

Fig. 5. Transformation of a sporangium into a conidium (see p. 8 of text). X 460.

Fig. 6. Prolongation of a hypha from directly under a sporangium, the latter being pushed to one side as in *Phytophthora infestans.* X 300.

Fig. 7. Germination of a conidium without falling off from its stalk. A new spore is produced at the tip of the germ tube. X 300.

Fig. 8. A sporangium continuing its growth before having been cut off from its stalk and producing a new spore at the tip of the prolongation. In this way a sporangium sometimes comes to lie intercalarly. X 300.

Fig. 9. Germination of a conidium with thick wall. X 400.

Fig. 10. An intercalar swelling, of rare occurrence in *P. intermedium.* X 400.

Fig. 11. Changes in the sporangial vacuole previous to zoospore formation. See text, p. 27. X 500.

Figs. 12—15. *P. Artotrogus.*

Fig. 12. Hypha with intercalar oogonium. X 500.

Fig. 13. Intercalar and terminal oogonia. Note the hypogynal antheridium. Fertilization in the terminal oogonium has not yet commenced. In the intercalar oogonium it is in progress. X 500.

Fig. 14. Oospore nearly filling the oogonium. X 500.

Fig. 15. Oospore much smaller than the oogonium. X 500.

PLATE VII.

PSEUDOLPIDIUM GRACILE *n. sp.*, PS. PYTHII *n. sp.*, PLEOLPIDIUM INFLATUM *n. sp.* AND PL. CUCULUS *n. sp.*

Figs. 1—8. *Pseudolpidium gracile n. sp.*

Fig. 1. Early stages in formation of sporangia and resting-spores. *a.* first appearance of the parasitic plasma. *c. d.* later stages. *e.* sporangium obscured by the formation of droplets of oil around it. *f.* sporangium wall visible, oil droplets fewer, host protoplasm almost absorbed. *b.* early stage in formation of a resting spore. *g.* later stage with areola, in which the spines are formed, visible. X 300.

Fig. 2. Part of a culture of *Pythium* showing infection by *Pseudolpidium gracile.* Sporangia and resting spores visible. X 300.

Fig. 3. Two diverticula containing a single emptied sporangium and a single resting spore. X 600.

Fig. 4. A ripe sporangium with four tubes of discharge, accompanied by a resting spore. X 600.

Fig. 5. An empty sporangium with five tubes of discharge. X 600.

Fig. 6. Ditto with three tubes. X 600.

Fig. 7. A diverticulum containing large numbers of sporangia and resting spores. X 300.

Fig. 8. Zoospores. X 600.

Figs. 9—16. *Pseudolpidium Pythii n. sp.*

Fig. 9. Stages in the formation of sporangia. *a.* condensation of parasitic plasma towards the centre of the swelling. *b.* the sporangia fully formed. *s.* septum in the supporting hypha. X 300.

Fig. 10. Stages in the ripening of a sporangium. *a.* vacuolar stage. *b.* final formation of the zoospores in the sporangium. Note the hypertrophy of the supporting hypha. X 300.

Fig. 11. Sporangia discharging. The zoospores are collected at the mouth of the tubes. X 100.

Fig. 12. Zoospores. X 600.

Fig. 13. Diverticulum containing two undeveloped resting spores. X 300.

Fig. 14. Ditto containing a single perfect resting spore. X 300.

Fig. 15. Sporangia and resting spores in groups. X 300.

Fig. 16. Stages in the formation of the spines of the resting spore. *a.* areola which is thrown out around the spore wall. *b.* appearance of radial markings indicating the future spines in the areola. *c.* spines becoming visible. X 600.

Figs. 17—21. *Pleolpidium inflatum n. sp.*

Fig. 17. Cluster of diverticula produced by the parasite. X 300.

Fig. 18. Sporangium just before and during discharge. X 300.

Fig. 19. Zoospores. X 600.

Fig. 20. Emptied sporangium showing two places for the emission of zoospores. X 300.

Fig. 21. Sporangium whose wall has separated from that of the diverticulum, towards the base. X 300.

Figs. 22—25. *Pleolpidium Cuculus n. sp.*

Fig. 22. Sporangium in the act of giving out zoospores. X 300.

Fig. 23. Zoospores. X 600.

Fig. 24. Resting spores found occupying the ends of hyphæ of *Pythium intermedium*, attacked by *Pleolpidium Cuculus*. X 300.

Fig. 25. Sporangia occupying the position of normal spores of *Pythium intermedium*. X 300.

PLATE VIII.

PLEOLPIDIUM IRREGULARE *n. sp.* AND OLPIDIUM GREGARIUM NOWAKOWSKI.

Figs. 1—12. *Pleolpidium irregulare n. sp.*

Fig. 1. Infected hyphæ of *Pythium* (*? vexans*), *a.* type of infection on young vigorous cultures of the host ; *b.* ditto on older cultures. X 100.

Fig. 2. Part of a badly attacked culture, showing spherical and irregular swellings. X 250.

Fig. 3. Ripe sporangium showing papilla. X 300.

Fig. 4. Irregular sporangium in the act of emitting zoospores. X 250.

Fig. 5. Zoospores. X 500.

Fig. 6. An old hypha with zoospores of the parasite attached. Some of these succeeded in penetrating. X 300.

Fig. 7. Zoospores attacking a swelling, perhaps a rudimentary spore of the host. X 500.

Fig. 8. Zoospores attacking a young hypha. X 300.

Fig. 9. A zoospore infecting. Note the vacuole of the partially emptied spore, and the accumulation of protoplasm from the latter within the hypha. X 500.

Fig. 10. A hypha, bearing diverticula in which resting spores were formed. X 300.

Fig. 11. Resting spores. Note that some have spines and others none. *b*. abnormal branching induced by the parasite. X 450.

Fig. 12. Normal branching of the host. X 250.

Figs. 13—18. *Olpidium gregarium Nowakowski.*

Fig. 13. An egg of *Metopidia lepadella* containing a large number of sporangia. X 450.

Fig. 14. Rotifer egg containing one large sporangium. X 450.

Fig. 15. A sporangium just after discharging zoospores. The latter are bound together by mucus into a sphere at the mouth of the tube. X 450.

Fig. 16. Zoospores. X 450.

Fig. 17. Zoospores attacking an egg of *Metopidia*. X 450.

Fig. 18. Resting spores and sporangia in an egg. The interior of the egg contains several chambers formed by thin walls, each enclosing one or more sporangia and resting spores. This is possibly due to infection having taken place after segmentation of the egg had commenced. X 580.

PLATE IX.

Pseudolpidium Aphanomycis (Cornu) Fischer and Olpidiopsis minor Fischer.

Figs. 1—7. *Pseudolpidium Aphanomycis (Cornu) Fischer.*

Fig. 1. Part of a culture of *Aphanomyces laevis* on a fly's leg, bearing sporangia and a resting spore, *a*. X 155 ; *b*. wall of the latter enlarged. X 912.

Fig. 2. Young hypha of *Aphanomyces*, attacked by numerous zoospores. X 500.

Fig. 3. Small sporangia forming bead-like swellings on a hypha. X 155.

Fig. 4. Two sporangia, showing the large tube of discharge. X 500.

Fig. 5. A sporangium containing a few zoospores, after fixation with iodine to show the position of the cilia. X 500.

Fig. 6. Stages in the process of zoospore formation in a mature sporangium. *a.* 10 A.M., shortly after the addition of fresh water to the culture. The vacuoles are spherical, as is usually the case until the changes which result in zoospore formation begin. *b.* 10-15 A.M., the vacuoles have lost the spherical shape and are changing in relative position. *c.* 10-20 A.M., the vacuoles show marked changes due to protoplasmic heapings within the sporangium. The beak has been ruptured accidentally and part of the contents have separated off as four rounded masses. Some of the outer protoplasm has been killed. Further in, a hautschicht has been formed, behind which the contents of the rest of the tube and the sporangium body are able to complete their development. *d.* 11 A.M., the vacuoles have suddenly blown up to a great size, gradually becoming spherical as they increase. Immediately afterwards they dwindled and disappeared, leaving the sporangium filled with a homogeneous protoplasmic mass. The four particles of the tube contents, which had separated out at the last observation, have become fully developed zoospores, which are actively trying to escape through the mouth of the tube, which is, however, partially occluded by dead remains. A new tube has been formed from the base of the first. *e.* 11-3 A.M., homogeneous stage. Note the withdrawal of some of the contents of the tube from the apex, as a result of loss of turgor in the sporangium. *f.* 11-5 A.M., the zoospores are now marked off by light bands which are too strongly marked in the drawing. In this formation the tubes participate. Three minutes later movement was observed to commence and four minutes after that, at 11-12 A.M., the spores escaped. X 500.

Figs. 8—11. *Olpidiopsis minor Fischer.*

Fig. 8. Sporangium and resting spores together, in a filament of *Achlya polyandra*. X 155.

Fig. 9. Resting spore with companion cell. The spines are joined together by raised crests, giving the wall a sculptured appearance in surface view. X 490.

Fig. 10. Resting spore in optical section, showing the manner in which the companion cell is supported by spines. The latter are borne on an

outer wall which is free from the exospore proper, except where the companion cell joins the latter. X 912.

Fig. 11. A group of sporangia. X 500.

PLATE X.

PSEUDOLPIDIUM (? OLPIDIOPSIS) SAPROLEGNIÆ (A. BRAUN) FISCHER. NOWAKOWSKIELLA RAMOSA *n. sp.*; AND OLPIDIOPSIS SCHENKIANA ZOPF.

Figs. 1—2. *Pseudolpidium (? Olpidiopsis) Saprolegniæ (A. Braun) Fischer.*

Fig. 1. Infection of *Saprolegnia* zoospores by zoospores of the parasite, in the interval between the two stages of diplanetism. The emptied capsule, with empty parasite capsules attached, shows that infection did not check the ordinary course of diplanetism. X 600.

Fig. 2. Changes which are undergone by the zoospores of this parasite after liberation. The gradual alteration of shape after the amœboid stage, with the appearance of two new cilia is shown; *a.* normal shape when first liberated; *b.* normal shape after the metamorphosis. See page 120 of text. From a continuous observation.

Figs. 3—10. *Nowakowskiella ramosa n. sp.*

Fig. 3. Various types of sporangia; *a.* "proliferation" of new sporangia into the cavity of the old; *b.* sporangium, with zoospores fully formed, before opening; *c.* division of the terminal sporangial enlargement of a hypha into sporangial and apophysial parts; *o.* operculum. X 912.

Fig. 4. Sporangium in a wheat cell, showing the tube which is formed in such sporangia. X 500.

Fig. 5. Zoospores. X 912.

Fig. 6. First stage in the germination of zoospores. Swelling into irregular masses. X 912.

Fig. 7. Mycelium showing anastomoses of the hyphæ and the irregular branches. Numerous swellings are visible in the latter. Some of these represent sporangial origins. Others persist unchanged. Below an intercalar sporangium with double apophyses. X 912.

Fig. 8. Group of resting spores, formed in a pseudoparenchymatous mass of cells, arising from one or more hyphæ. The resting spores are enlarged marginal cells of this pseudoparenchyma. Two of them are joined together, having arisen from two neighbouring cells. X 912.

Fig. 9. A small mass of pseudoparenchyma, formed from a single hypha. Part arises laterally and is sessile on the hypha; part is formed by a short branch from the same hypha. In some cases branches of this sort simulate antheridia. X 912.

Fig. 10. Early stages of resting spore formation. A single hypha only has entered into the formation of the cellular mass. X 912.

Figs. 11—13. *Olpidiopsis Schenkiana Zopf.*

Fig. 11. Part of a filament of *Spirogyra*, with every cell infected with the parasite. The cell contents are gathered into a mass around the parasite. X 155.

Fig. 12. Sporangium after discharge. X 155.

Fig. 13. Resting spore with companion cell. X 912.

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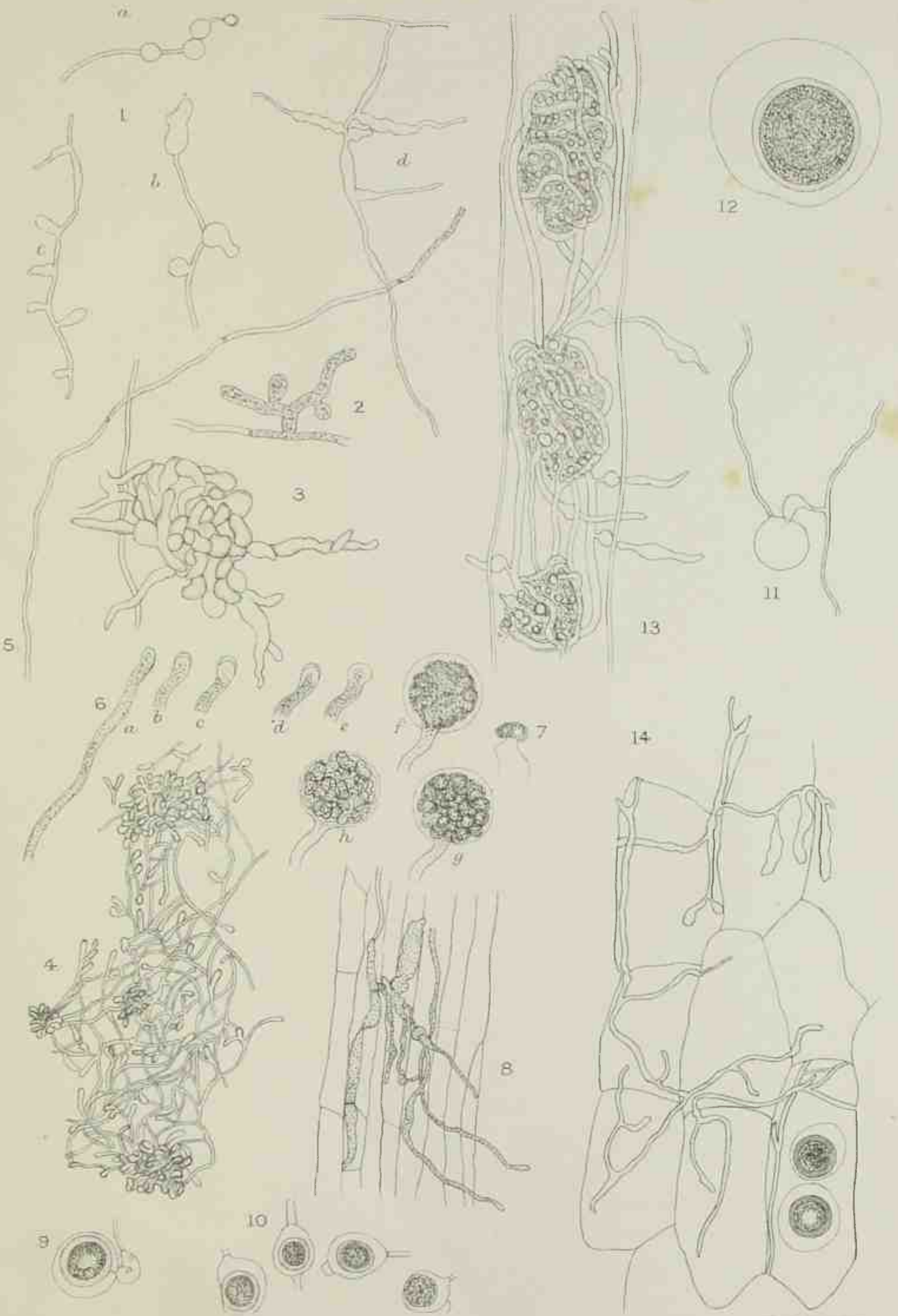
ERRATA.

Page	Line		
Preface	11	*for* Acclimatisation	*read* Acclimatation.
2	9 from bottom	,, poraceæ	,, poreæ.
5	15 ,, ,,	*delete* "("plankton searcher")."	
9	6	*for* together	,, altogether.
10	5 ,, ,,	,, *Panicummiliaceum*	,, *Panicum miliaceum.*
10	5 ,, ,,	,, *siuapis*	,, *sinapis.*
19	7	,, anthridia	,, antheridia.
22	3 ,, ,,	,, (1870)	,, (1869).
24	3	,, highly	,, less.
25	1	,, Rother	,, Rothert.
35	10 ,, ,,	,, *Aphanomyce*	,, *Aphanomyces.*
36	2 ,, ,,	,, author's	,, authors'.
38	4	,, *Nematosporangium*	,, *Aphragmium.*
39	6 and 12	,, Sporangiumanalagen	,, sporangiumanlagen.
41	1 from bottom	,, fig. 19,	,, fig. 17.
42	14	,, de Bary	,, de Bary & Woronin.
42	8 ,, ,,	,, (1870)	,, (1869).
48	10 ,, ,,	insert full stop before "so far".	
49	11 and 20	*for* (1892)	,, (1892, (2)).
53	11 from bottom	,, de Bary's	,, de Bary & Woronin's.
54	5	,, de Bary	,, de Bary & Woronin.
57	1 ,, ,,	,, *Gonaodya*	,, *Gnapodya.*
65	17	,, *distyospermum*	,, *dictyospermum.*
67	4 ,, ,,	,, notes	,, Notes.
71	16 ,, ,,	,, Jhar	,, Jahr.
75	10	,, mycologiques	,, Mycologiques.
75	17	,, *spirogyra*	,, *Spirogyra.*
76	9	insert comma after *proliferum*.	
90	2	*for* (1892)	,, (1892, (1)).
102	2	insert Belg. after Soc.	
102	4 ,, ,,	*for* Circumdans	,, circumdans.
103	8	,, *Lakgenidium*	,, *Lagenidium.*
103	7 ,, ,,	,, Beitrage	,, Beiträge.
104	9 ,, ,,	,, Justs' Botanish. Jahresber,	,, Just's Botanisch. Jahresber.
105	9 ,, ,,	,, Utriforme	,, utriforme.
106	1	,, symbion	,, symbiont.
142	9	insert comma after "Saccardo".	
143	13 ,, ,,	*for* and	,, und.
146	11 ,, ,,	,, Schroter	,, Schröter.
147	16	insert above "Trow, A. H." Treub, M. Etudes sur les Lycopodiacées. Anns. Jard. Bot. Buitenzorg, IV.	
147	14 ,, ,,	*for* Gesells,	*read* Gesells.,
147	9 ,, ,,	,, lersache	,, ursache.
147	8 ,, ,,	,, ver.	,, Ver.
151	10	,, hypogyna	,, hypogynal.
159	14	,, aphanomycis	,, Aphanomycis.

Plate I.



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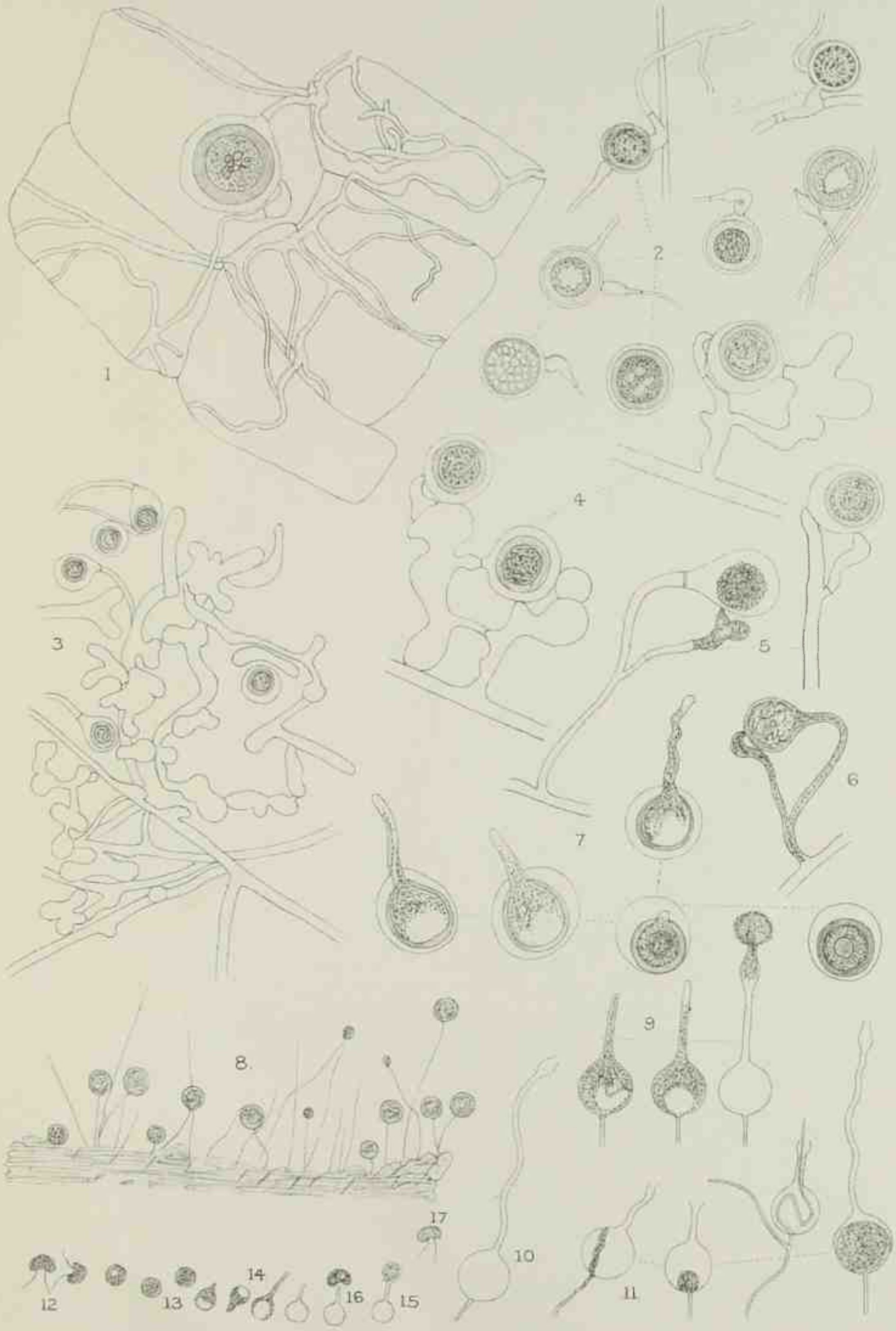
P. GRACILE SCHENK.

Plate II.



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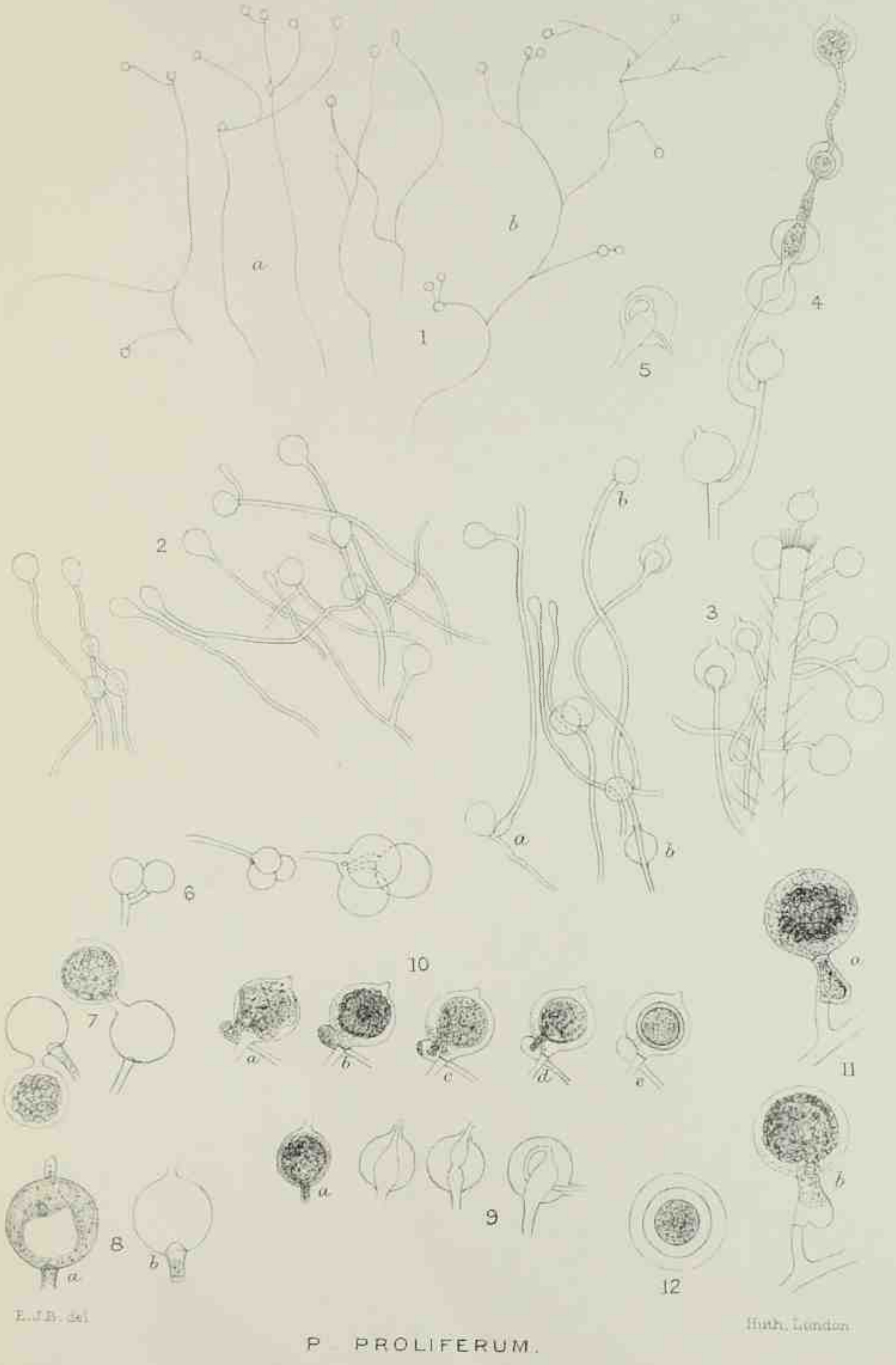
P. GRACILE (Fig. 1). P. MONOSPERMUM (Fig. 2)
P. INDIGOFERAE (Figs. 3 - 7), P. DIACARPUM (Figs 8 - 17)

Plate III.



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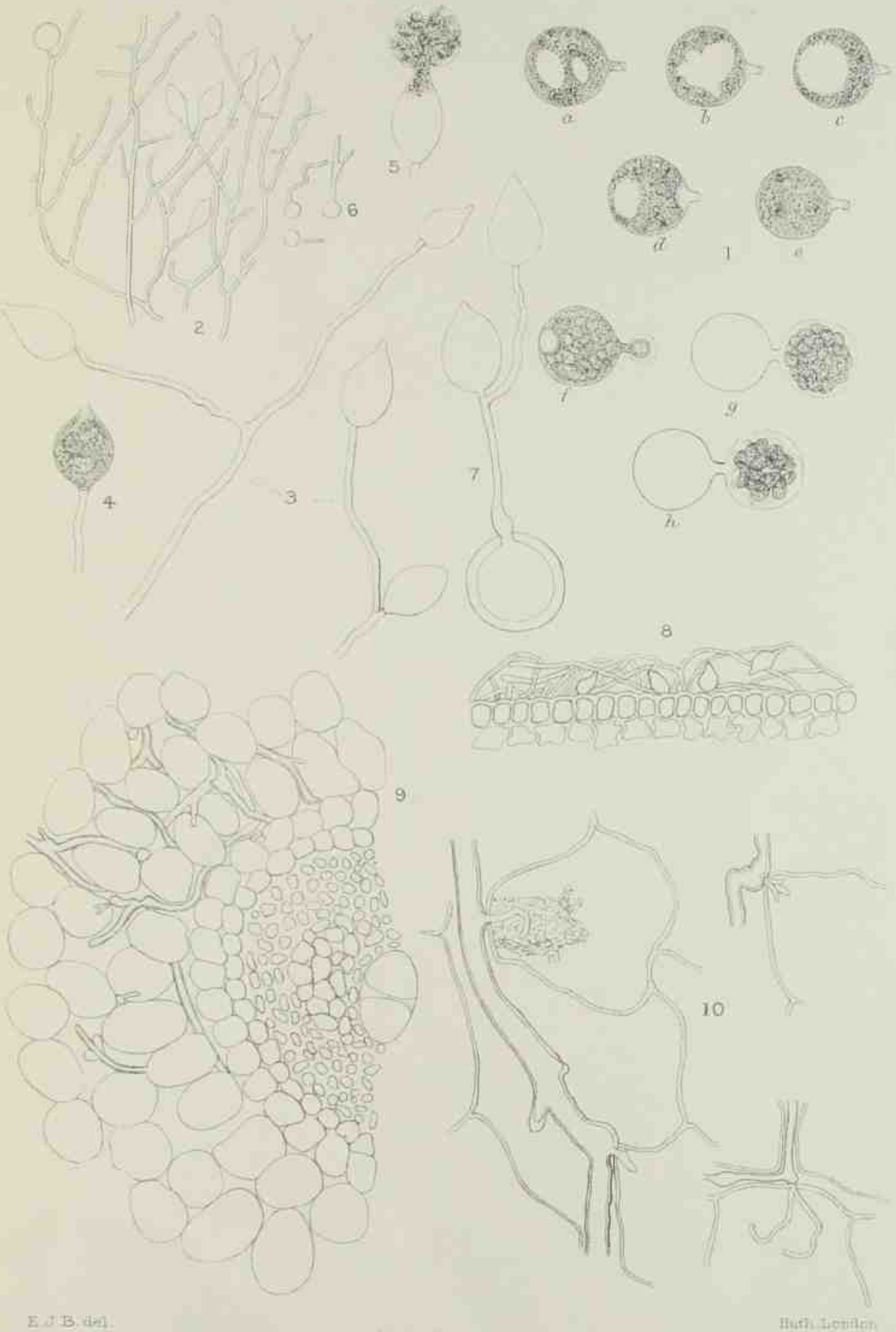
P. PROLIFERUM.

Plate IV.



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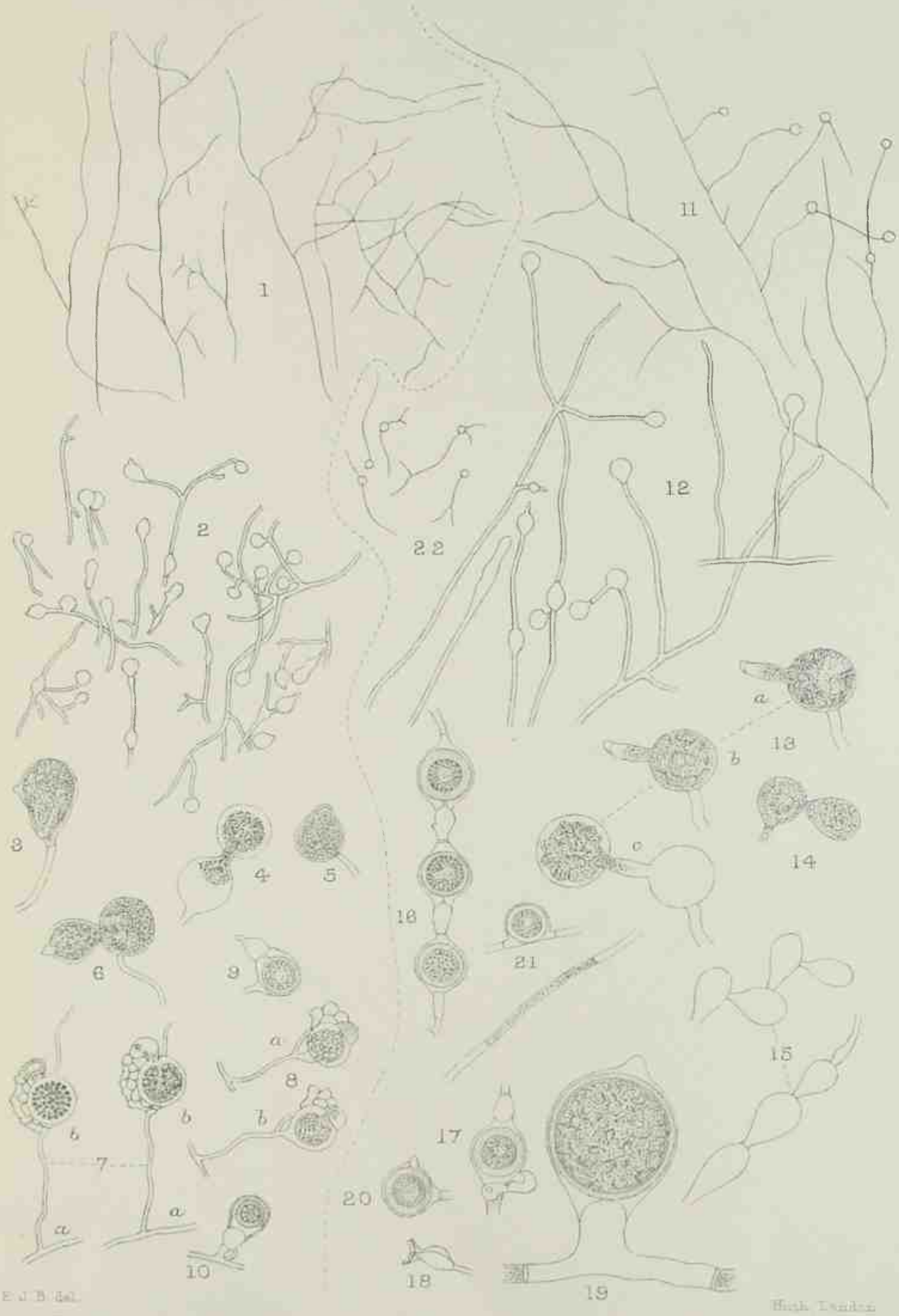
P. PROLIFERUM (Fig. 1), P. PALMIVORUM (Figs. 2–10)

Plate V.



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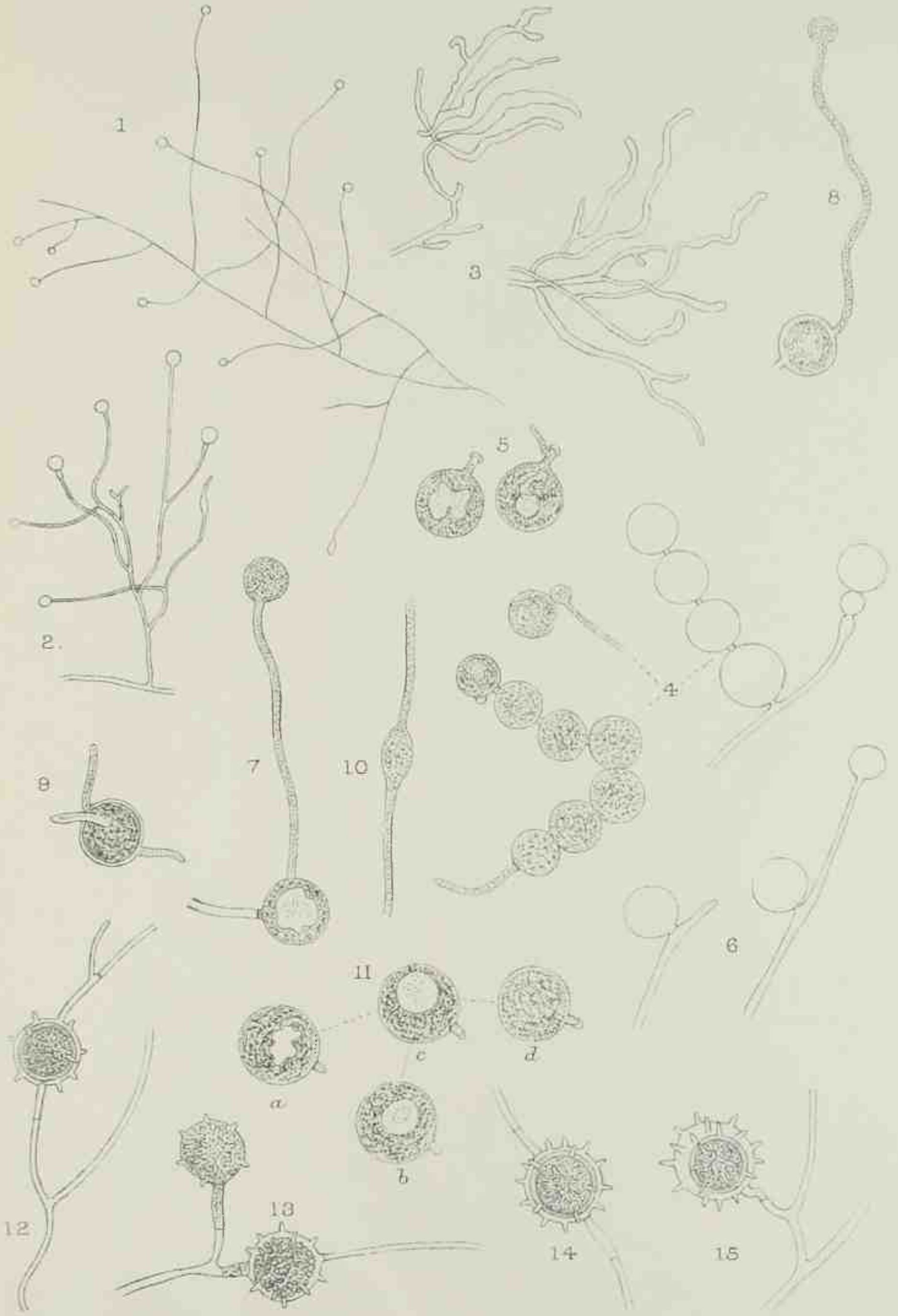
P. VEXANS (Figs. 1–10). P. ROSTRATUM (Figs. 11–22).

Plate VI



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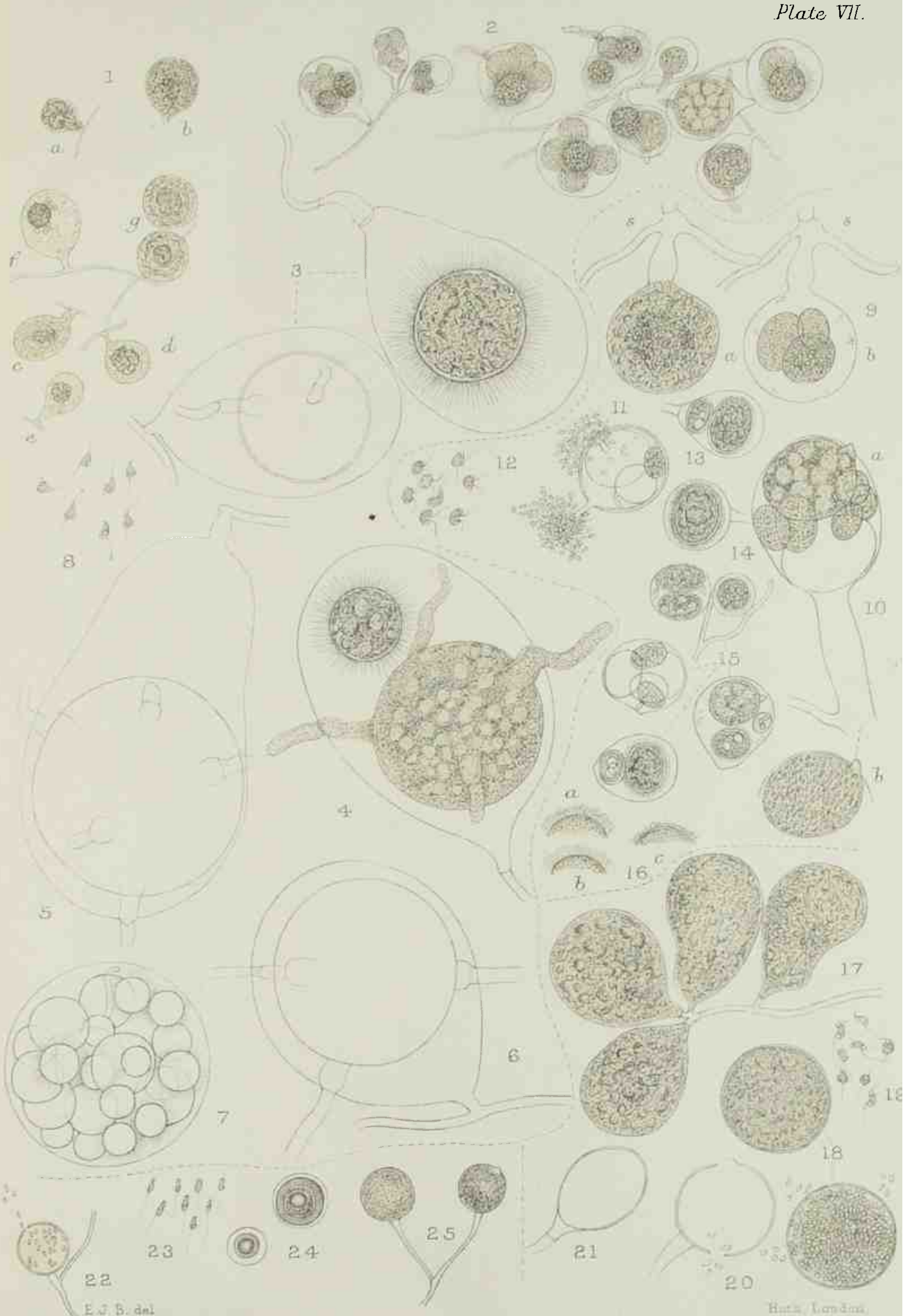
P. INTERMEDIUM (Figs. 1 – 11). P. ARTOTROGUS (Figs. 12 – 15).

Plate VII.



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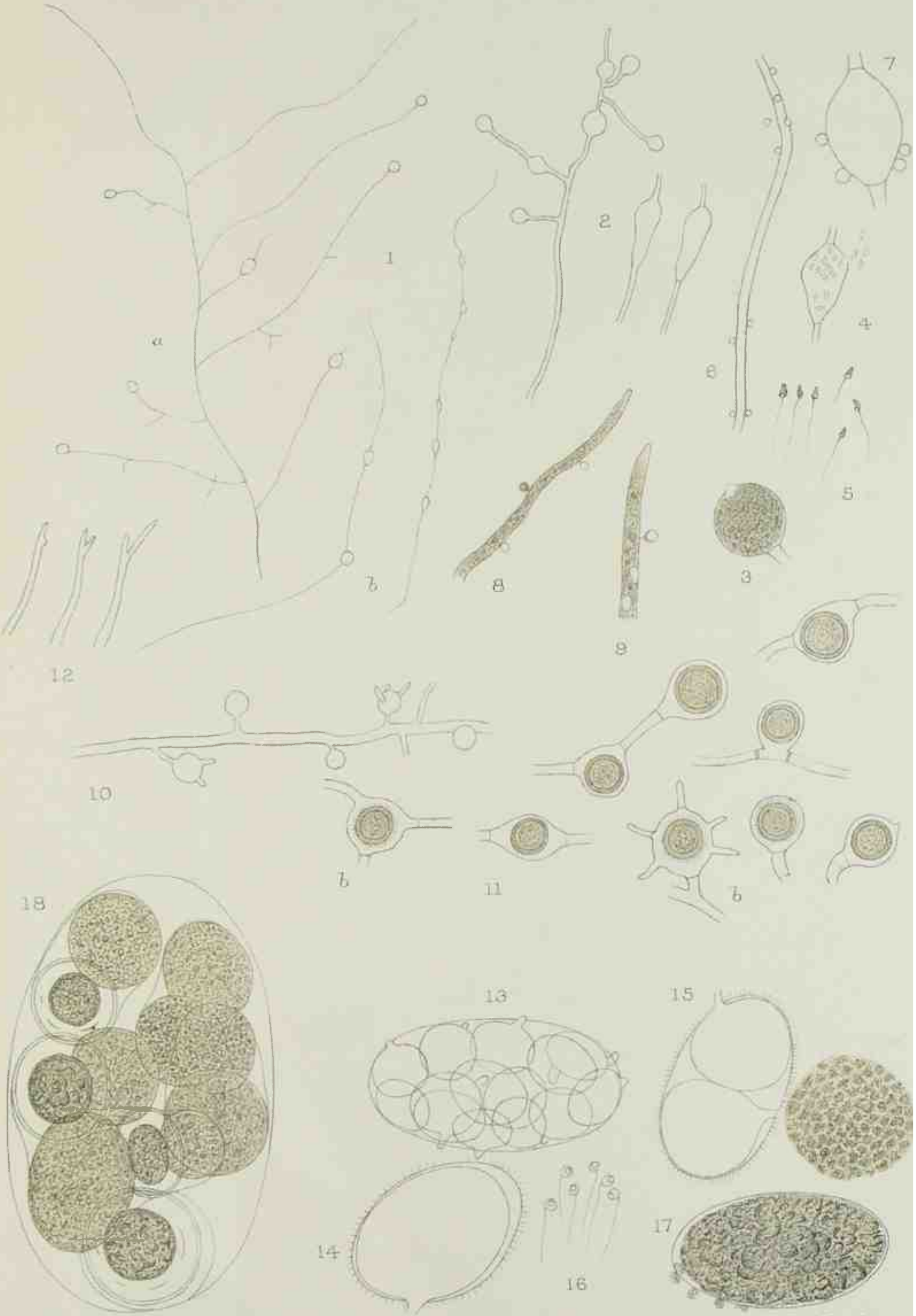
PS. GRACILE (Figs. 1–8), PS. PYTHII (Figs. 9–16), PL. INFLATUM (Figs. 17–21), PL. CUCULUS (Figs. 22–25)

Plate VIII.



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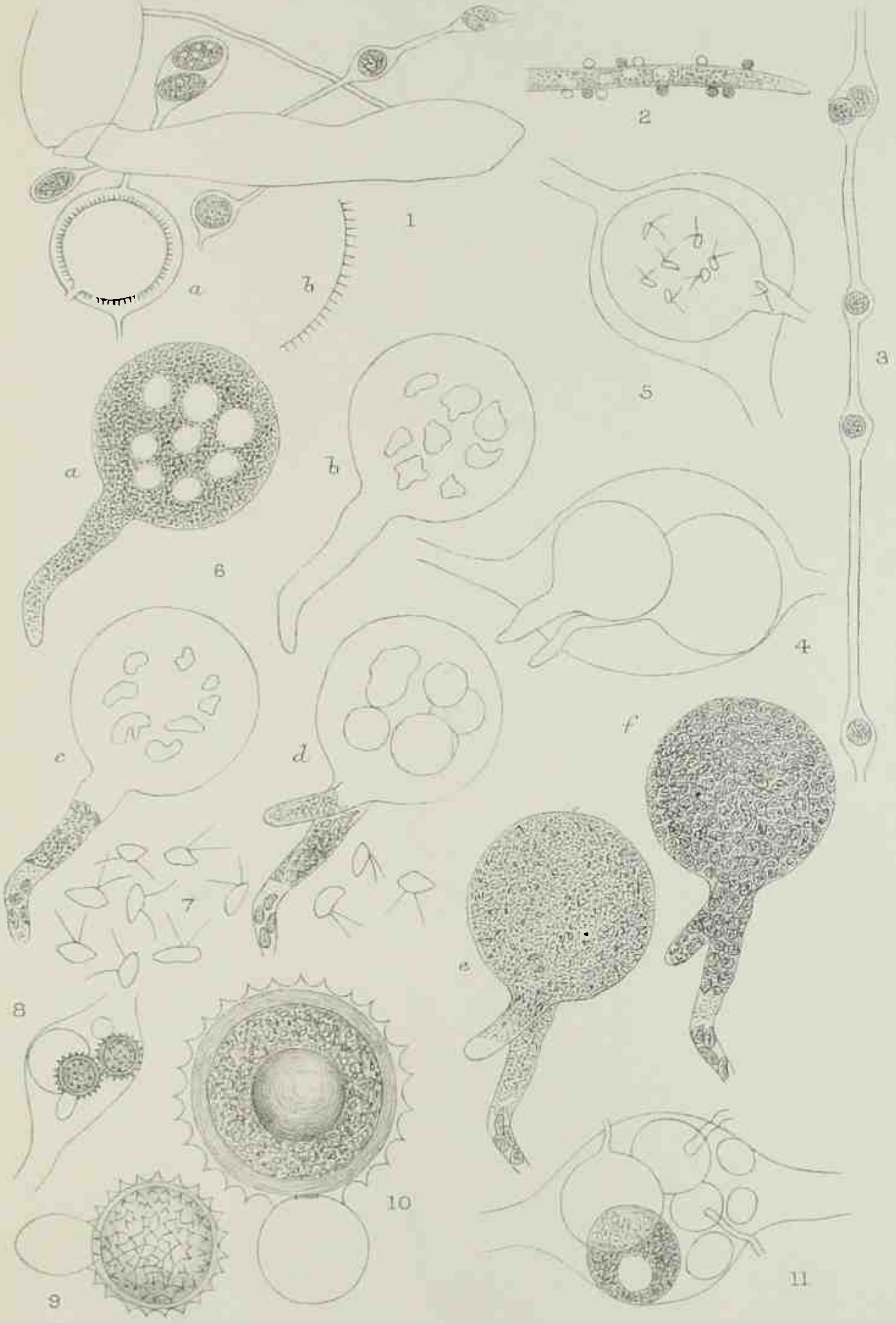
PL. IRREGULARE (Figs. 1–12). O. GREGARIUM (Figs. 13–18)

Plate IX.



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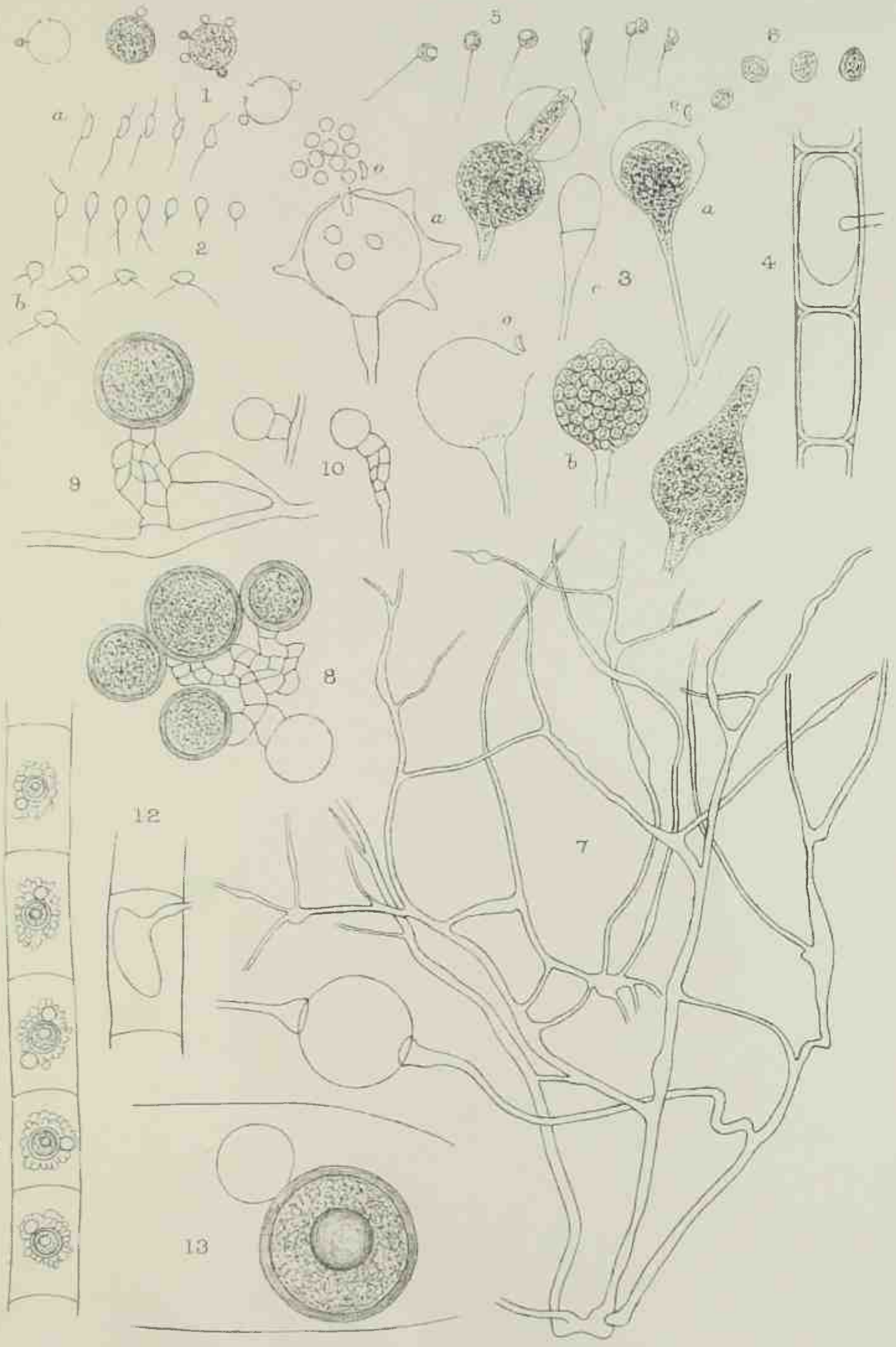
P. S. APHANOMYCIS (Figs. 1–7). OL. MINOR (Figs. 8–11)

Plate X.



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PS.(?) SAPROLEGNIAE (Figs. 1–2),
NOW. RAMOSA (Figs. 3–10). OL. SCHENKIANA (Figs. 11–13).

NOTICE.

THESE Memoirs, dealing with scientific subjects relating to Agriculture, will appear from time to time as material is available. They will be published in separate series, such as Chemistry, Botany, Entomology and the like. All contributions should be sent to the Editor, the Inspector-General of Agriculture, Nagpur, Central Provinces, India. Contributors will be given, free of charge, fifty copies of their contributions.

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