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Art. XIX.—*An Account of the Cultural and Cytological Characteristics of a New Species of Mycogala.*

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I. Introduction.

During the isolation of several fungi from Jarrah (*Eucalyptus marginata*) timber, particular interest was taken in one form which appeared to be rather unusual. On investigation, this proved to be a species of *Mycogala*. Its characters did not agree with those of any previously described members of this genus, so that it has been given the specific name, *Mycogala marginata*. An account is given here of the main cultural and structural characteristics, including the development of the perithecium, from the observations made.

II. The Fungus in Culture.

The fungus grows readily on most of the media in ordinary use. On the solid media used an important characteristic of this form is the very pronounced colour change brought about in the medium by the fungus. Generally, the growth is mainly submerged with little superficial development. The optimum temperature for growth is about 19°C.

1. *Malt Agar.*

(*a*) *Macroscopic.*—The fungus grows quickly, but aerial growth is scanty, and is usually represented by only a small amount of white, flaky mycelium at the upper end of the malt slope. With the growth of the fungus over the agar, the latter changes in colour to a light yellowish-brown which soon deepens to a reddish-brown, and, in an old culture, the medium appears almost black. Perithecia are abundantly developed on the surface of the medium, and when mature are dark and carbonaceous in appearance, and easily visible to the naked eye.

(*b*) *Microscopic.*—The hyphae of the mycelium are generally uniform in size, and contain abundant oil. Septa are developed, although usually at fairly wide intervals (Fig. 1).

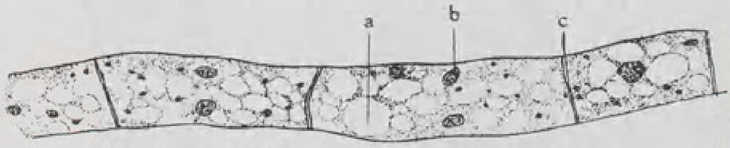


Fig. 1. Hypha of *Mycogala marginata*. × 545. (a) vacuole, (b) nucleus, (c) septum.

After about four days' growth, one type of asexual spore is found to be very abundant in the culture. These are formed on short branches of the aerial hyphae, and the branches appear to be transformed into closely septate, cylindrical, hyaline elements, which tend to break up into shorter elements—one or more septate (Fig. 2). The free cells appear, therefore, to be of the nature of "oidia" rather than of conidia, and will be discussed later.

Another type of asexual spore is soon apparent, namely chlamydospores. These may occur solitary or in chains and are formed in both intercalary and terminal positions in the hyphae (Fig. 3). At first these bodies are hyaline and appear as swollen cells, which are more closely septate than the ordinary cells of the hyphae, and they contain abundant oil globules, as shown by the red colour with Sudan III. As they mature, they increase in size, become dark brown in colour, and a thick wall is developed. They are approximately spherical in shape, and have a range in diameter of 6–18μ.

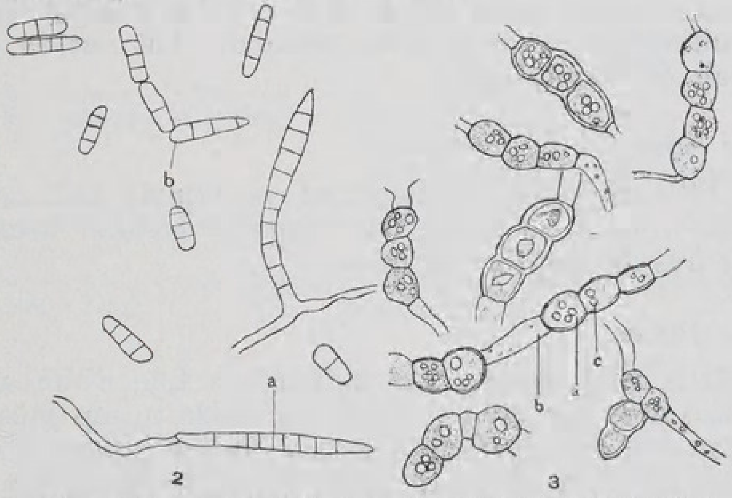


Fig. 2. Spores of oidia type, formed on aerial hyphae × 260. (a) aerial branch showing early stage in spore formation, (b) free spores. Fig. 3, Chlamydospores formed in an intercalary or terminal position in the hyphae. × 260. (a) chlamydospore, (b) hyphae, (c) oil globules.

After about five or six days at 19°C., the mycelium shows the first indications of sexual reproductive structures in the form of perithecium initials, and ripe perithecia are visible in three to four-weeks-old cultures. The fruiting-bodies are developed superficially on the medium, and may be solitary or two or three massed together. They are spherical in shape, with a range in diameter of 50–150μ, and are dark coloured when mature. No ostiole is developed, but the fruiting-body dehisces by an irregular splitting of the wall.

If the perithecia are crushed before they are quite ripe, colorless asci containing eight ascospores are apparent. The asci are sub-spherical and very delicate, and as they appear to rupture when the spores are ripe, one rarely sees asci on crushing the mature perithecium.

The ascospores are spherical, smooth and hyaline, with a diameter of 2–4.5μ. They are often seen massed together, when their shape is slightly modified, and they tend to be hexagonal in outline.

2. *Oatmeal Agar.*

The fungus causes a colour change in the medium similar to that noted on malt agar, and little aerial growth occurs. The microscopic characters of the fungus also agree with those already noted but the perithecia appear to mature somewhat more quickly.

3. *Maize-meal Agar.*

Growth is not as vigorous as on the previous media. At the end of three weeks oidia and chlamydospores were abundant in the culture, but perithecia were few and immature. Later on, these fruiting-bodies were more abundant, but not as plentiful as on malt or oatmeal agar.

4. *Dox's Agar.*

Very little mycelial development is found, and no colour change is brought about by the fungus. Neither asexual nor sexual spores develop in this case.

5. *Prune Juice.*

Growth is very sparse, and although a fine white mycelium develops, it does not form a dense mat as in turnip juice. Oidia and chlamydospores develop to a very limited extent. Perithecia are occasionally found and are easily visible as dark carbonaceous objects in the white mycelium.

6. *Turnip Juice.*

After a few days, a dense mat of hyphae is formed below the surface. Oidia are found but are not as abundant as on previous media. Chlamydospores were noted after some days. Perithecia do not seem to develop consistently in this medium, as in some cases only very few are developed, while in other instances, they are numerous.

III. Comparison with Related Forms.

From a study of the characters of the fungus, particularly from the nature of the perithecium with its asci and ascospores, it seems to be most closely allied to *Mycogala*, a genus of the Eurotiaceae. It differs largely, however, in its characteristics and habitat, from the species previously described. It is interesting to note that no other species of *Mycogala* has yet been recorded on *Eucalyptus*.

The main characters of the known species of *Mycogala* are given in the following table:—

Species.	Perithecia. Diameter.	Ascospores.	Habitat and Locality.
M. porietinum (Schrad.) Sacc.	——	Globose, rough, 10–12μ diameter	Decaying wood in Britain, Germany, Lapland, Italy
M. minimum (Fr.) Karst.	1 mm. ..	10–12μ diameter	——
M. firmum Karst. 1896	0·3 mm.	3–4μ diameter	Leaves of living Palms, Leningrad, Russia
M. insigne Pat. 1900	3–6 mm.	Rough, 6μ diameter	Dead trunks of *Daniella* (Leguminosae) West Africa
M. guadalupense Pat. 1902	4–6 mm.	Ovoid or subglobose 6–7 x 4–5μ	Associated with *Lycogala Epidendri* on decaying wood
M. macrospora Jaap. 1910	0·5–1 mm.	18–25μ diameter	——
M. musciola Jaap. 1912	——	7–8μ diameter	—— Linglitz, Germany (Jaap.)
M. fimeti 1912 ..	200–300μ	8–10 x 6μ diameter	—— Hamburg (Jaap.) Dessan (Staritz)
Mycogala from *Eucalyptus*	50–150μ ..	2–4·5μ diameter	*Eucalyptus marginata* Melbourne, Australia

This table shows that *Mycogala firmum* is the only species approximating to the fungus in question, and it is with this form that a further comparison becomes necessary.

The size of the ascospores is approximately similar in both cases, but there is a wider variation in the Australian form, and the spores of the latter are hyaline, while those of *Mycogala firmum* are pale yellow. In addition the host and size of perithecia are different in the two forms. These points are considered sufficient to separate it from *Mycogala firmum,* and the name *Mycogala marginata* is proposed for it.

The asexual spores borne on the aerial branches of *Mycogala marginata* may be compared with the so-called "endo-conidia" of *Thielavia basicola* Zopf. According to Brierley (3), the conidia of *Thielavia basicola* Zopf. are endospores not formed by free cell division within an endo-conidial cell, but are "acrogenously abjointed from the conidiophore." The first conidium is liberated by the differentiation of its walls, into an inner wall and a sheath, and by the rupture of the latter at its apex. The later conidia grow out through the sheath of the first, and are set free by the splitting of their basal walls. The formation of the transverse walls is brought about by the ingrowth of a ring of cell-wall substance, which finally closes in the centre.

In *Mycogala marginata,* the method of spore-formation appears to be different. It was not possible in any instance to detect the sheath through which the conidia push their way out to the exterior—a feature which is readily observed in *Thielavia basicola.* The branches, however, seem themselves to break up into shorter elements—one or more septate—so these free cells appear to be of the nature of "oidia" rather than of conidia.

Thielavia basicola Zopf is a well-known parasite chiefly on tobacco, violet, and many Leguminosae. It was invariably placed in the Ascomycetes on the assumption that the chlamydospores, "endo-conidia," and perithecia belonged to the same fungus. McCormick (20) in 1924, obtained the perithecia in culture, and proved conclusively that all three spore stages belonged to the same form—*Thielavia basicola* Zopf.

It is interesting to note that *Mycogala marginata* also has three types of spores—chlamydospores, oidia—superficially resembling the endo-conidia of *Thielavia basicola* Zopf—and ascospores.

IV. Development of the Perithecium.

The ascospores germinate within twenty-four hours when incubated at 19°C. and soon produce abundant mycelium. After three or four days, the first signs of sexual reproductive organs are visible. The perithecium initial is a coiled septate hypha, the degree of coiling varying widely (Fig. 4).

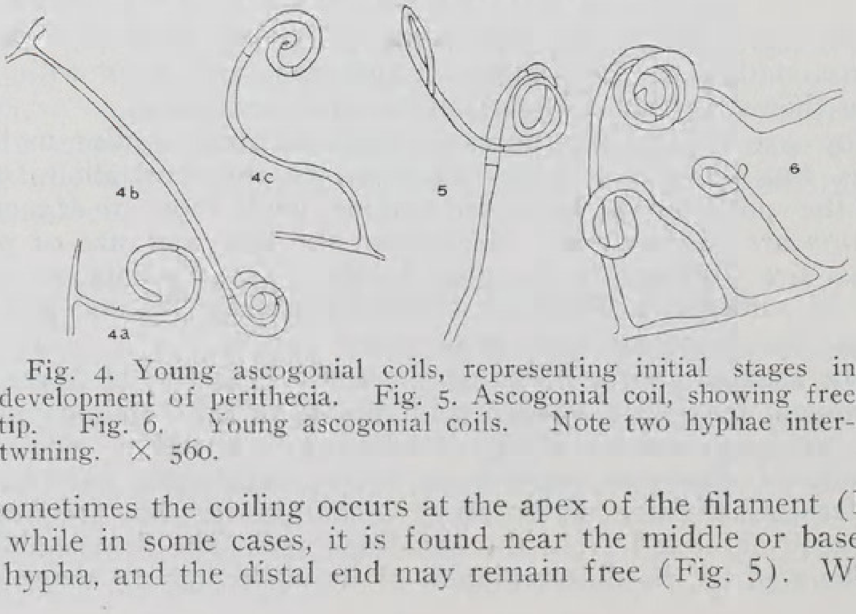


Fig. 4. Young ascogonial coils, representing initial stages in development of perithecia. Fig. 5. Ascogonial coil, showing free tip. Fig. 6. Young ascogonial coils. Note two hyphae intertwining. × 560.

Sometimes the coiling occurs at the apex of the filament (Fig. 4), while in some cases, it is found near the middle or base of the hypha, and the distal end may remain free (Fig. 5). When the distal end of the hypha is free, dichotomous branching frequently occurs (Fig. 8c), a feature also recorded in some members of the Ascobolaceae, where it has been compared to the branching trichogyne of *Lachnea cretea* (12). In *Lachnea cretea,* however, this terminal portion of the archicarp is constantly found protruding from the other coils, and it is so distinct as to be regarded morphologically as a trichogyne, and it is even suggested that the contents of the trichogyne may discharge themselves into the central part of the archicarp, and thus a form of pseudo-pogamy may replace fertilization. In *Mycogala marginata,* this free terminal portion is not such a constant feature. In *Lachnea cretea,* the terminal part consists of about eight or nine cells, but the cross-walls become broken down, so that a free passage occurs from cell to cell.

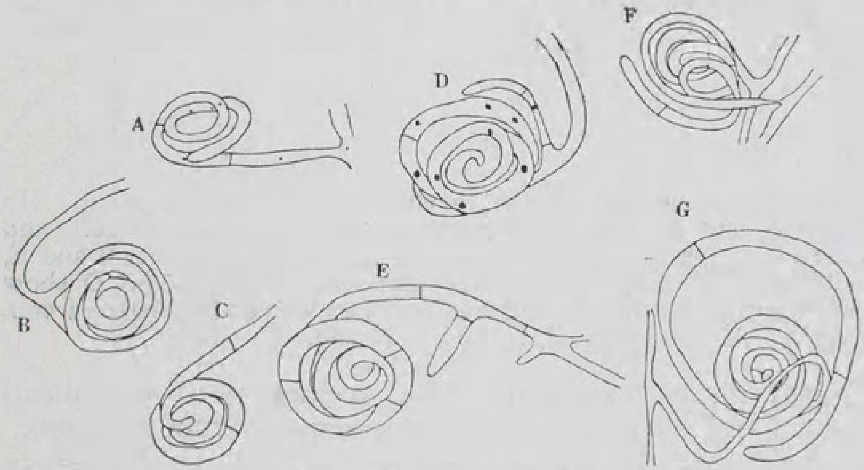


Fig. 7. Later stages in development of coils. B, D, and E show lateral branching at base of ascogonium. G shows long free tip. × 400.

Septa have not been observed in the free portion of the archicarp of *Mycogala marginata.* An antheridium is not developed, but hyphae grow out laterally from the stalk region of the ascogonium, and these often have the appearance of antheridial branches (Figs. 7B, D, E, 8E). Similar branching has been recorded by Gwynne-Vaughan and Williamson (16) in some members of the Ascobolaceae, and is regarded by them as contributing to the formation of the sheath.

Another feature showed by some members of the Ascobolaceae, e.g., *Saccobolus depauperatus* (16), is the fact that two coils are often found together (Fig. 6), suggesting that more than one may be concerned in the formation of an ascocarp.

Gwynne-Vaughan in discussing the Ascobolaceae describes the cells of the archicarp as being multi-nucleate. Fraser (12) describes the archicarp of *Lachnea cretea* as a multi-nucleate structure, and there is no evidence either when the ascogenous hyphae are first formed, or, at a later stage, of an arrangement in regular pairs. In *Mycogala marginata* the segments of the ascogonial coil are at first multi-nucleate, but later uninucleate segments are found (Fig. 8). In many cases, binucleate segments are observed, and these would represent a stage before the uninucleate condition is reached.

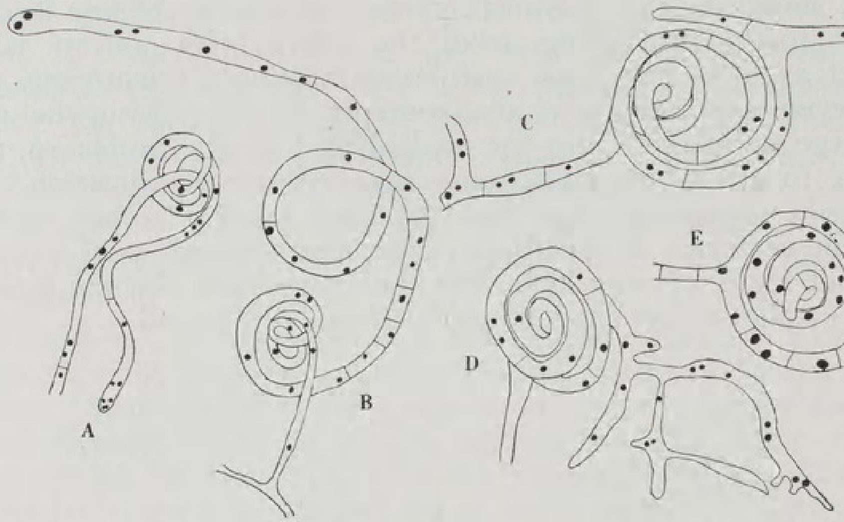


Fig. 8. Ascogonial coils in varying stages of development. In Figs. A and B, note multi-nucleate segment at tip of coil, and nuclei showing a tendency to associate in pairs. In Figs. B and E, note bi-nucleate and uninucleate segments. Figs. C and D show branching at tip of coil, and Fig. E shows a branch at base of coil. Fig. A. × 200; Figs. B, C, D, E. × 480.

As previously mentioned, the formation of the sheath is brought about by lateral branches given off from the stalk, but it is likely that the surrounding hyphae also contribute to its formation, as branches from the mycelium have been observed to pass up into the fruiting-body. The original ascogonium is visible in the young ascocarp, when cut in section, as large usually uninucleate segments only faintly stained and sometimes branched (Figs. 9, 10).

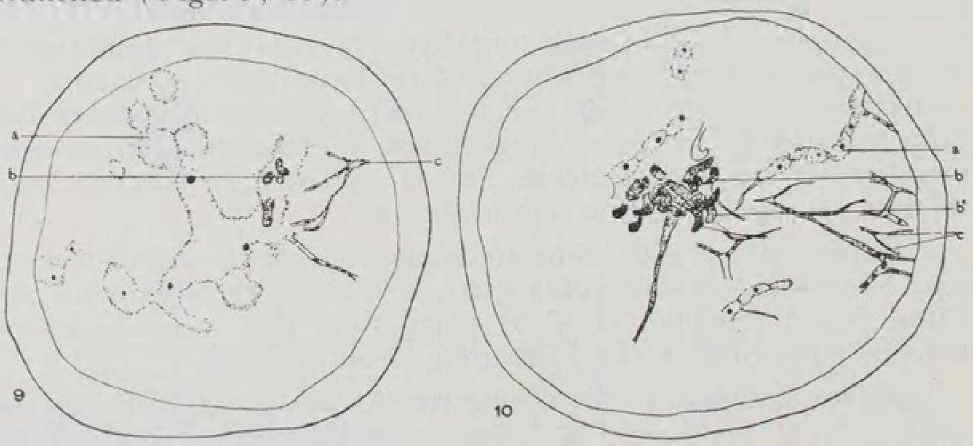
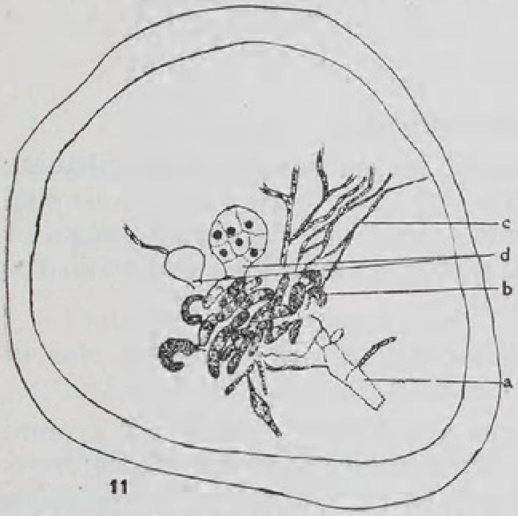


Fig. 9. Section of very young ascocarp. × 420. (a) original ascogonial coil, (b) ascogenous hyphae, deeply stained, (c) "nurse hyphae." Fig. 10. Section of ascocarp, at a slightly later stage. × 420. (a) segments of original ascogonial coil, (b) ascogenous hyphae, deeply stained—note re-curved branches at b^1. (c) "nurse hyphae."

From the ascogonium, ascogenous hyphae arise and are visible as a mass of deeply stained hyphae, situated generally at about the centre of the ascocarp. These hyphae are very irregular in form and the branches are rather short. In many cases they are sharply recurved, resembling the crooks of a crosier system (Figs. 10, 11, 13). Asci arise from the ascogenous hyphae and appear to come from the penultimate cell of a crosier (Fig. 13), thus resembling *Thielavia terricola* (11). It was not possible to observe the nuclear detail of the ascogenous hyphae.

In the young ascocarp, one finds also a system of fine hyphae much branched and intertwined; nuclei are visible in these hyphae, but septa cannot be distinguished. They develop from the ascogenous hyphae and often seem to come from the cells



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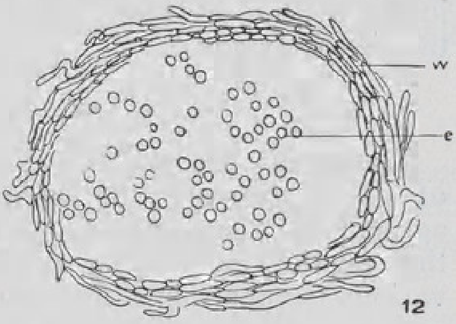

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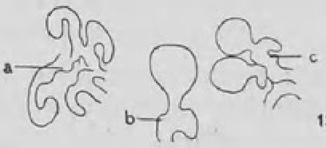

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Fig. 11. Section of young perithecium, showing beginning of ascus formation. × 485. (a) segments of original coil, (b) ascogenous hyphae, (c) "nurse hyphae," (d) asci.

Fig. 12. Section of mature perithecium, showing cavity filled with spores. × 300. w—perithecium wall, e—ascospores.

Fig. 13. (a) Ascogenous hyphae, showing recurved tips, (b), (c) recurved branches of ascogenous hyphae, showing asci arising from penultimate cell of crosier. × 485.

of the ascogonial coil itself. They appear to correspond to Brefeld's digestive hyphae, although Dodge has shown that this is not their sole function, and he states that "the fine hyphae probably take no greater part in the digestion of the stromatic tissue than do the other elements occupying the cavity." Dodge found that these asci themselves frequently bear asci directly, and it was noted in one or two cases in *Mycogala marginata*.

As the ascocarp matures, the contents of the interior disorganize and eventually the centre of the cavity is occupied by the darkly-stained mass of ascogenous hyphae, accompanied by the finer hyphae. In a ripe perithecium, the entire cavity is filled with ascospores (Fig. 12).

It is interesting to note the similarity that exists between *Penicillium Brefeldianum* and *Mycogala marginata* in the nature of the hyphae occupying the central region of the young ascocarp. In the latter, asci arise from a crosier system, but in *Penicillium Brefeldianum* Dodge (7) states that the asci arise as " short-side or terminal swellings from the thick primary ascogenous hyphae, and are formed in the same position as a branch "; and he believes that the formation of a crosier system may not be an impossibility in this case.

A striking resemblance also occurs between the young coils of *Mycogala marginata* and those of some members of the Ascobolaceae, which is rather surprising between such widely separated members of the Ascomycetes. Similarities also occur in the method of formation of the sheath in our species and in the Ascobolaceae.

V. Discussion.

The genus *Mycogala* is grouped in the family Aspergillaceae of the Plectascales on account of the nature of its sexual apparatus. Gäumann Dodge has traced out the development of the perithecium in the Aspergillaceae, and has classified the various members into five groups:—

(1) *Penicillium " crustaceum " Group, and occasionally also in Aspergillus nidulans.*

Two equal copulation branches are formed which coil around one another helically, come into communication, and the contents of one migrate into the other, after which the copulation branches are surrounded by a dense hyphal knot. Thus here, there is no distinction between the antheridium and ascogonium, a feature also found in the lowest family of the Plectascales—Gymnoascaceae.

(2) *Penicillium vermiculatum Group.*

This includes as yet only one species. This group differs from the previous one in the fact that both copulation branches are differentiated morphologically into antheridium and ascogonium, but the antheridium does not appear until a long time after the formation of the ascogonium, after the latter has become uninucleate. Although the antheridium comes into communication with the ascogonium, according to Dangeard's observation, its single nucleus is functionless. The ascogonium divides into numerous binucleate cells, each of which may develop ascogenous hyphae.

(3) *Aspergillus herbariorum-repens Group.*

There is developed a coiled, one or more septate ascogonium. The cells are multi-nucleate, the terminal one usually the longest. Before or after septation of the ascogonium, the antheridium arises and climbs along the ascogonial helix from either a different hypha, or a coil—usually the basal one of the ascogonium. The antheridium appears to be functionless, as it often does not grow to its full length, or may be entirely absent. In all cases, with or without antheridium, the ascogonium develops parthenogenetically, and divides into binucleate cells, some of which grown into ascogenous hyphae.

Thus here we get functional degeneration of the antheridium which forms often when the ascogonium has reached the stage when fertilization would occur.

(4) *Aspergillus flavus, A. fumigatus, and A. Fischeri.*

A coiled ascogonium is developed which divides into binucleate cells and develops ascogenous hyphae in the usual way, without any formation of an antheridium.

(5) *Penicilliopsis clavariaeformis.*

The formation of the fructification is no longer introduced by the formation of an ascogonium. Conidiophoric coremia radiate laterally or terminally without apparent reason, and change into perithecia in a still unknown manner.

Thus in these five groups, according to Gäumann Dodge, there is a gradual degeneration of sexuality, because of the retardation in the formation of the antheridium.

Mycogala marginata falls into the fourth group of this series, since an antheridium is not developed, but the ascogonium produce ascogenous hyphae from which asci are developed. A striking resemblance to *Thielavia* has already been noted both in the sexual apparatus, the development of the perithecium, which is of the same type as in *Thielavia terricola*, and also in the well-developed conidial apparatus.

Although *Mycogala* is a member of the Plectomycetes, it is interesting to note the resemblances found between this genus and some of the higher Discomycetous forms—namely, the Ascobolaceae—indicating that in spite of the different nature of the fructifications, the sexual apparatus is very similar in these widely separated groups.

VI. Latin Description.

Mycogala marginata.—Perithecia solitaria, vel bina, vel terna, in superficie sita, atro-rufa aut nigra quando matura sunt, non ostiolata, scissuris irregularibus dehiscentia; diametro a 50 ad 150μ. Asci subsphaerici, hyalini, tenerrimi, sporas octonas continentes. Ascosporae sphaericae, lenes, hyalinae, diametro a 2 ad 4.5μ, saepe conglomeratae.

Duo typi sporarum sine sexu:—

(*a*) Hyphae praebent chlamydosporas aut solitarias aut catenatas, atrorufas, parietibus crassis, situ aut intercalario aut terminali, forma prope sphaerica, diametro a 6 ad 8μ.

(*b*) Rami aerii producunt elementa spisse saeptata, cylindrica, hyalina, quae in elementa minora separantur, quorum unum aut plura saeptata sunt. Haec naturam oidiorum habere videntur.

VII. Summary.

1. A new species of *Mycogala* was obtained from Jarrah (*Eucalyptus marginata*) timber, and is described as *Mycogala marginata*.

2. The fungus was grown on various culture media, and the cultural characteristics are outlined.

3. There are two types of asexual spores:—

(*a*) Chlamydospores, occurring either singly or in chains, may be formed in either a terminal or intercalary position in the hyphae. They are approximately spherical in shape, 6–18μ diameter, and dark brown in colour with a thick wall when mature.

(*b*) Spores of the nature of "oidia" are formed by the transformation of aerial branches into cylindrical, hyaline elements, which again break up into smaller segments one or more septate.

4. Comparison is made between these oidia and the so-called endo-conidia of *Thielavia basicola* Zopf, and the method of formation of the spores in the two cases is found to be different.

5. The perithecium initial is a coiled septate ascogonium, composed of segments at first multi-nucleate, but later becoming uninucleate. An antheridium is not developed.

6. The wall of the perithecium is formed from lateral branches arising from the basal port of the ascogonium. The surrounding hyphae are also considered to contribute towards the formation of the sheath.

7. The young ascocarp contains three kinds of hyphae:—

(*a*) Large, faintly staining hyphae, composed of uninucleate cells, which represent the ascogonial coil, and disappear as the ascocarp matures.

(*b*) Darkly-stained ascogenous hyphae, which arise from the ascogonium and branch irregularly, the branches being short and often recurved at the tips.

(*c*) Finer hyphae much branched, which correspond to the digestive hyphae of Brefeld, and arise from the ascogenous hyphae and also from the ascogonium itself. Occasionally they have been found to bear asci directly.

8. Asci arise from the ascogenous hyphae, and appear to come from the penultimate cell of a crosier, thus resembling *Thielavia terricola* (11). Ripe asci contain eight spores.

9. A mature fruiting body contains only ascospores, freed by rupture of the walls of the asci.

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