Observations on Gymnoascaceae.

BY

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With Plates XXVII and XXVIII.

INTRODUCTION.

IN May, 1901, Professor Marshall Ward handed to me for investigation three species of *Gymnoascus*, which had been received by him from Mr. Massee, who had collected them on the substrata referred to below. The species were (1) *G. Reessii* (Baranetzky), growing on dung, of what kind could not be determined; (2) *G. setosus* (Eidam), on an old bee's nest; and (3) *G. candidus* (Eidam), *Arachniotus candidus* (Schroeter), on dead grass. Subsequent examination showed that all three species were growing together on the old nest.

The total number of species of *Gymnoascus* actually known is probably about a dozen. Winter[1], in Rabenhorst's Kryptogamen-Flora, describes *G. Reessii*, *G. ruber*, and *G. uncinatus*. Massee[2] mentions *G. Reessii* and *G. ruber* (van Tieghem), but does not notice any other species as found in Britain.

Fischer[3] mentions five species, viz. *G. Reessii*, *G. setosus* (Eidam), *G. durus* (Zukal), *G. umbrinus* (Boudier), and *G. Bourqueloti* (Boudier). Saccardo[4], in 1889, describes six species

[1] Band I. 2. Pilze, p. 15 (1887).
[2] British Fungus Flora, vol. iv, p. 18 (1895).
[3] Engler und Prantl, Pflanzenfamilien, I. 1, p. 294 (1897).
[4] Syll. Fung., vol. viii, p. 823 (1889).

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of *Gymnoascus*, viz. *G. Reessii*, *G. ruber*, *G. aurantiacus* (Peck), Sacc. (*Gymnascella aurantiaca*, Peck), *G. uncinatus* (Eidam), *G. reticulatus* (Zuk.), and *G. setosus* (Eidam).

In three later volumes he adds seven other species, viz. *G. Zuffianus* and *G. Eidami*[1]; *G. Bourquelotii*, *G. umbrinus*, *G. luteus*, and *G. myriosporus*[2]; and *G. ossicola*[3].

During the present year (1902) a new species of *Gymnoascus* has been described, but not figured, by Klöcker[4], under the name of *G. flavus*. Schroeter[5], in treating of the Gymnoascaceae in general, founded two new genera, *Arachniotus* and *Amauroascus*, by breaking up the original genus *Gymnoascus* into three. He did not describe any new forms, but only reclassified those already known. Eidam's *Gymnoascus candidus* belongs to the genus *Arachniotus*, according to Schroeter's classification, which has been generally adopted. It is the one which has been accepted by Matruchot and Dassonville, whose results, as will be seen later (page 590), appear to be confirmed by the work about to be described.

Historical.

The three species may first be considered briefly from the historical point of view.

1. *Gymnoascus Reessii* (Baran.) was first described in 1872 by Baranetzky[6], who founded the genus on this species. He made cultures of the fungus, and worked out its life history in as great detail as was possible with the histological methods then available. His conclusions were afterwards disputed by subsequent workers, who, however, do not seem to have gone into the matter as thoroughly as Baranetzky.

According to Baranetzky the fructifications are formed in the following manner: two swellings arise side by side on a single

[1] vol. x, p. 71 (1892). [2] vol. xi, p. 437 (1895).

[3] vol. xiv, p. 824 (1899).

[4] Bot. Cent., Bd. lxxxix, No. 22, p. 626 (1902), and Hedwigia, Bd. xli, Heft 2, pp. 80–8 (1902).

[5] Cohn's Kryptogamen-Flora von Schlesien, Bd. iii, zweite Lieferung, zweite Hälfte, p. 210 (1893). See also Saccardo, l. c., vol. xi, p. 438 (1895).

[6] Entwickelungsgeschichte des *Gymnoascus Reessii*, Bot. Zeit., p. 145 (1872).

hypha, one on each side of, and quite close to, a transverse wall. These swellings grow out into little branches, which twist spirally round one another and become club-shaped. At this stage Baranetzky observed that the two cells cannot be separated, but he says 'a true copulation does not occur since both cells remain completely closed.' They each become cut off by a wall from the hypha on which they arose. The free end of one cell swells, and becomes cut off by a transverse wall from the part below it. The other cell puts out from its free end a thin cylindrical projection, which is also cut off by a side wall. This cell gives rise to the ascogenous hyphae, and may therefore be called the *ascogenous cell*, while the other may be distinguished as the *sterile cell*. The cylindrical projection lays itself round the swollen end of the sterile cell, and encircles it once by annular growth. It becomes segmented into almost isodiametric cells. Certain of these cells, generally not more than two, grow out into hyphae, which branch copiously without increasing much in length. In consequence there arise thick tufts with many short branches which swell at their ends and form asci. From the base of the sterile cell, meanwhile, grow out thin vegetative hyphae.

The results of the work about to be described, in most of the essential points, confirm those obtained by Baranetzky, but, by the use of modern methods, they have been extended.

In 1877 van Tieghem[1] described under the name *Gymnoascus ruber*, a species which he compared with *G. Reessii*. His account of the development of the reproductive organs is very short, and he gives no figures. This species belongs to Schroeter's genus *Arachniotus*.

In 1883 Eidam[2] described *G. Reessii* as it occurred on a pupa of *Sphinx Galii*. He did not find the reproductive organs described by Baranetzky, but gives the origin of the coiled hyphae as follows: below the dividing wall of a mycelial hypha a lateral branch arises which coils closely round

[1] (1) Sur le développement de quelques Ascomycetes. Bull. de la Soc. Bot. de France, vol. xxiv, p. 159 (1877).

[2] (1) Beitrag zur Kenntniss der Gymnoasceen. Cohn's Beiträge, p. 267 (1883).

the parent hypha or one which is adjacent. After winding round in a close coil for about eight or ten times it becomes loose and septate, and then grows out into branches which are the ascogenous hyphae. Eidam further states that Baranetzky says the method of reproduction described by him only occurs in weak mycelia.

I can find no such statement in Baranetzky's paper; in fact, he distinctly says that his cultures were perfectly normal and strong.

Eidam[1] also cultivated *G. ruber* (*Arachniotus ruber*), and in this species he found the kind of reproductive organs described by Baranetzky in *G. Reessii*, but only the early stages were described. Cell-fusion was not seen, although he sought specially for it, because he had already discovered it in *Eremascus*[2]. Perhaps the stages seen were too young, or the cultures not strong enough, as the ripe fructifications were never formed. In *G. uncinatus*, described by Eidam[1] as a new species, the early stages also agreed with Baranetzky's account of *G. Reessii*. The fungus occurred spontaneously on sparrow-dung, but here again the ripe asci were not obtained in culture.

In 1891 *G. Reessii* was again described by Brefeld[3], who declares that the ascogenous hyphae arise from *solitary* branches, each of which coils itself into a spiral, from which the ascogenous hyphae are produced by branching. Baranetzky figures a few such solitary branches, but regards them as anomalous cases which do not develop farther. Brefeld confirms Baranetzky's account of the formation of the asci on the ascogenous hyphae.

2. *Gymnoascus setosus* (Eidam) was first described by Eidam[4] as a new species at a meeting of the Botanical section of the *Schlesische Gesellschaft für vaterländische Cultur*, in January, 1882. Its habitat was an old wasp's nest. Eidam, in a very

[1] loc. cit. (1), p. 273.

[2] (2) Untersuchungen über die Familie der Gymnoascaceen: Bericht über die Thätigkeit der bot. Section der Schlesischen Gesellschaft, p. 164 (1886).

[3] (1) Ascomyceten, ii, Heft x, p. 158 (1891).

[4] (3) Ueber Entwickelungsgeschichte der Askomyceten: Jahresbericht der Schlesischen Gesell., p. 175 (1883), and Bot. Cent., vol. x, p. 107 (1882).

brief description, says that the mode of origin of the coil which precedes the formation of the asci is the same as in *G. Reessii.* No detailed life-history of this species has yet been given.

3. *Gymnoascus candidus* (Eidam) (*Arachniotus candidus*, Schroeter) was first described in 1886 by Eidam [1], who gives an account of the mature fructifications as found by him growing spontaneously on cooked rice. It was subsequently separated from *Gymnoascus* and placed in a new genus, *Arachniotus*, by Schroeter [2], who at the same time founded the genus *Amauroascus* on other species previously included in the genus *Gymnoascus*. The two new genera both agreed in having a peridium of very thin-walled, similar hyphae; whereas, according to Schroeter's limitations, *Gymnoascus* has a peridium of thick-walled hyphae which branch copiously and form a kind of trellis. *Arachniotus* differs from *Amauroascus* in having colourless, red, or yellow ascospores, while in *Amauroascus* the ascospore-wall is brown or brownish-violet.

In the genus *Arachniotus* Schroeter places three species, *Gymnoascus candidus* (Eidam), *G. ruber* [3] (van Tieghem), and *G. aureus* (Eidam [4]). Schroeter describes mature asci and conidia, but the life-history has not been worked out until now, as Eidam's cultures were unsuccessful and he saw no conidia.

Methods of Culture and Preparation.

The three species were isolated by means of plate cultures, and the colonies thus obtained were transferred to one of the following culture media :—sterilized horse-dung in tubes, extract of horse-dung in 2 per cent. agar-agar, or beer-wort in 2 per cent. agar-agar. The agar was sterilized in test-tubes. The most convenient method was found to be to grow, fix, and harden the fungus on the agar in the tube, as the species grew equally well on any of the media [5]. The material thus obtained was imbedded in paraffin, and the sections were

[1] (2) loc. cit., p. 5 (1886).
[2] loc. cit., p. 210. See also Saccardo, Syll. Fung., vol. xi, p. 438 (1895).
[3] See p. 573. [4] loc. cit. (2).
[5] As a fixing reagent Flemming's weak solution was used.

stained in various ways. The best results were obtained with Flemming's triple-stain-safranin, gentian violet and orange G, and with toluidine blue and eosin. The latter stain is somewhat uncertain, but when successful the results are very good. The eosin stains the nucleoli red, while the toluidine blue stains the protoplasm blue.

A very useful stain for these Fungi is brazilin[1], which differentiates the nuclei very clearly. Its special advantages are that its effects are very certain, and there is no over-staining. The results seem to be equally good, whether the material is stained before or after cutting.

I. The Life-History of Gymnoascus Reessii.

The original material consisted of little brick-red balls, made up of thick-walled septate hyphae, freely branching and anastomosing, and enclosing a mass of ripe ascospores, spherical in form and of a pale brown colour. These spores were for the most part isolated, but some were still contained in the spherical asci (Pl. XXVII, Fig. 1).

The thick-walled hyphae branch in a peculiar manner, the branches arising almost at right angles to the axis which bears them. Thus anastomosis is facilitated, and also the dense growth which results in the spherical mass of hyphae surrounding the groups of asci. The branches are said by Fischer[2] to be covered with 'short, straight, or slightly bent spines, 10–15 μ long.' Both in the original material and in the cultures subsequently made from it, the ends of the hyphae were blunt (Fig. 1 *a*). The hyphae were either empty or contained a greater or less amount of protoplasm. None of the asci was attached to any hyphae.

The ascospores readily germinated in various nutritive media. Those chiefly used were beer-wort, or horse-dung extract, made up with 10 per cent. or 15 per cent. of gelatine. Colonies were afterwards transferred either to sterilized horse-

[1] Hickson, Q. J. M. S., vol. 44, p. 469 (1901).
[2] Engler und Prantl, loc. cit., p. 294.

dung, or to beer-wort, or horse-dung extract, made up with 2 per cent. agar-agar, placed in test-tubes and sloped. In all these media the fungus grew well, and produced an abundance of ripe ascospores from which other cultures were made.

The ascospore germinates by the bursting of the outer wall and the growing out of the germ-tube (Fig. 2 *a–d*). The germ-tube soon branches close to the spore and becomes septate. Some of the branches grow almost parallel to the main axis in one direction, while adjacent ones grow in a completely opposite direction (Fig. 2 *d*). In some cases the mycelium branches little, and grows straight on ; and in other cases the hyphae branch and curve considerably. In many mycelia, but not in all, the hyphal segments are swollen close to, and on one side of, each septum. This fact has been pointed out by Baranetzky, Brefeld, and others as characteristic of the family. Irregular knots of hyphae appeared in a hanging drop culture, but came to nothing. Apparently these were pathological, and due to the starved condition of the mycelium in the small drop. Similar irregular masses of hyphae have also been observed by Eidam[1] in *Ctenomyces*, a genus closely allied to *Gymnoascus*, and were by him also regarded as pathological.

In cultures on horse-dung the mycelium had completed its first fructifications, and ripe ascospores were obtained, by the first week in July, that is, in about two months from the sowing of the spores.

The vegetative mycelium varies greatly in external appearance according to the nature of the medium on which it is grown. If the fungus is growing on the surface of a dry medium, it forms a very small aërial mycelium, which is soft, flocculent, and perfectly white (Fig. 3). On it the fructifications soon arise as little white bodies which become yellow and then brick-red. But if the medium is wet at the surface, or if the mycelium is sunk in it, e. g. in gelatine or agar, the aërial hyphae cling together in bundles and grow up in strands which stand erect and taper to a point (Figs. 4

[1] loc. cit. (1), pp. 286, 287.

and 6). After a while the hyphae at the ends of the strands separate from one another (Figs. 5, 7, and 8) and grow out into a flocculent mycelium like that grown on a drier medium. On this the fructifications arise. The plants grown under the latter conditions have a much longer period of vegetative growth and are much larger and stronger than the former. In fact the two types would not be taken for the same species they differ so greatly.

So far as could be discovered none of the cultures of *G. Reessii* produced any conidia.

The origin of the coils, which precede the formation of asci, takes place exactly as Baranetzky has described [1] and figured, and, although hundreds of sections were examined, no structures were seen like those described by Eidam [2] and by Brefeld [3]. In every case *two* branches arise from a single hypha, one on each side of a septum. These two branches grow upwards, at right angles to the hypha which bears them, and twist round one another once or twice. Their free ends swell up into club-shaped heads (Fig. 9), each of which now becomes cut off by a transverse wall as a separate cell (Fig. 10). The cells become very closely applied to one another, and soon the wall between them breaks down, and the two cells fuse. The fusion can be seen in specimens stained whole, but much more clearly in microtome sections (Figs. 11, 12, 26–29). At this stage there is usually no differentiation whatever between the two cells. But in some cases a differentiation may be noticed even before conjugation. One cell, that called by Baranetzky the 'sterile cell,' is larger than the other, the 'ascogone' of Baranetzky. The sterile cell is almost straight, whereas the ascogone is longer, smaller in diameter, and is coiled round the sterile cell (Fig. 13). After conjugation the sterile cell grows larger and more spherical, so that the ascogone often comes to lie on its side, some distance from its apex (Fig. 14). The ascogone soon puts out a prolongation, which winds round the sterile cell (Figs. 13, 15, and 16). If the conjugating

[1] loc. cit. [2] loc. cit. (1). [3] loc. cit.

cells are of approximately the same size and shape, so that the apex of the ascogone and of the sterile cell are at the same level, the prolongation winds loosely and irregularly round the two cells (Fig. 15); but if the sterile cell is larger, so that the point of fusion lies some distance from its apex, the prolongation of the carpogone, at least at first, winds closely round the sterile cell (Fig. 14).

After forming a considerable coil round the original conjugating cells the prolongation of the ascogone becomes segmented, as may be seen in solid preparations (Figs. 17 and 19) and also in longitudinal and transverse sections (Figs. 18, 29 *c*). From most of these segments, not merely from one or two, short thick branches grow out, and soon themselves branch (Fig. 18) and form a dense mass of hyphae (Figs. 19 and 20). These are the ascogenous hyphae, and their ends swell up into the rounded asci.

From below the sterile cell, and possibly from below the ascogone also, there eventually grow out a few vegetative hyphae which are longer, thinner, and straighter than the ascogenous hyphae (Fig. 21), but they do not arise till a considerably later stage in the development is reached.

With regard to the behaviour of the nuclei the following facts have been observed. When the two hyphae forming the coil are still quite small each contains a single nucleus of considerable size, in which may usually be seen a nucleolus surrounded by a nuclear zone (Figs. 22 and 23).

At the time of conjugation, however, *both cells contain large numbers of nuclei*, which, at least in certain stages, have each a distinct nucleolus and nuclear zone (Figs. 27 and 28). These nuclei must apparently have arisen by division from the original single nucleus, and cases were noticed, which seem to be intermediate stages, in which there were several, but far fewer, nuclei (Figs. 24, 25, and 26). As the nuclei divide they become smaller in size, because the growth of the divided nuclei does not keep pace with division. When division is completed the nuclei grow until they attain their permanent size. The cells themselves are usually completely

filled with dense protoplasm, but in some stages, apparently the later stages, the protoplasm is vacuolated.

At the time of fusion a considerable portion of the wall between the two cells breaks down, and the nuclei and protoplasm become mingled. Doubtless a nuclear fusion now takes place, but this has not been determined with certainty (Figs. 27 and 28). The nuclei pass over from the sterile cell into the ascogone (Fig. 28), and later into the prolongation of the ascogone (Fig. 16). Evidently they ultimately pass into the ascogonous hyphae, for, within a mass of ripening asci are to be seen ascogenous hyphae containing many nuclei, while the conjugating cells, though retaining their original shape and size, and often showing very distinctly the point of fusion, are completely empty (Fig. 29 *a*, *b*, and *c*). The numbers of nuclei in the ascogenous hyphae are so large that it would seem as if nuclear division occurred in these hyphae, more especially if we consider the enormous numbers of asci produced from one pair of conjugating cells. The small ascogenous hyphae generally show one nucleus, with a nucleolus and nuclear zone, lying in the apex of the hypha, before it has begun to enlarge (Fig. 30 *a*). At a later stage when the apex is beginning to swell (Figs. 30 and 31) we find first two and then four nuclei which are smaller in size than the original nucleus, and apparently have no nuclear zone.

In the stage with two nuclei, the nuclei in some cases lie one above the other (Fig. 30 *b'*), and in other cases side by side (Fig. 30 *b''*), recalling the figures and descriptions given by Harper[1] and others of the development of the asci in the higher Ascomycetes. In *Gymnoascus* also the arrangement of the nuclei in two different planes may indicate that the nucleus has undergone two divisions.

At a still later stage the ascus becomes larger and almost spherical, while, instead of being filled with dense protoplasm, it has a large central vacuole, so that the protoplasm and the eight nuclei it now contains, come to lie on the wall,

[1] Sexual Reproduction in *Pyronema confluens* and the Morphology of the Ascocarp, Annals of Botany, Sept. 1900, vol. xiv, p. 363.

usually, but not always, near the apex (Fig. 31 *b*). The nuclei now increase in size, and the protoplasm also seems to become more abundant, so that the vacuole disappears and the developing spores fill the ascus (Fig. 31 *c*).

At different stages in their development the young spores behave very differently towards stains. At first they are oval in shape and, with the toluidine blue method (cp. p. 576), their nuclei stain a deep pink with the eosin. In some young spores there are two deeply staining bodies (Fig. 32 *a*); in others a single elongated body, which in some cases is thickened at each end (Fig. 32 *b*), and in other cases is thickened in the centre (Fig. 32 *c*). These observations suggest a nuclear fusion in the spores like that in the spores of Uredineae. At a later stage the spores become larger and rounder, and their contents stain more diffusely and not so deeply (Fig. 32 *e*). Finally the spores attain to their full size and become spherical. In this stage they remain colourless with the toluidine blue method (Fig. 32 *f*).

With the triple stain, on the other hand, the ripe spores stain more deeply than those which are still immature. They become strongly coloured by the safranin.

Amongst the ascogenous hyphae are a few thinner, slenderer hyphae, which often contain many small nuclei. These hyphae appear to be vegetative, and may either be those of the ordinary mycelium or those arising from the base of the coil.

Some of the ordinary vegetative hyphae become changed into the thick-walled hyphae described above (p. 576, Fig. 1), which envelop the asci.

II. Gymnoascus setosus.

The original material of this species also consisted of ripe ascospores and vegetative hyphae. The hyphae were so thick-walled, and coloured such a deep brown, that, except at their ends, they were opaque (Fig. 33). Their branching is peculiar, and both the main and the lateral branches end in sharp spines or bristles. They occurred in masses enclosing numbers of spindle-shaped colourless spores, either isolated or still

within the spherical asci. The hyphae do not anastomose, although they branch considerably.

The ascospores germinate by putting out one or two germ-tubes, which soon branch and form conidia by budding (Figs. 34–36). The end of a branch swells into an almost spherical knob, which is a conidium (Fig. 34). Immediately below it other conidia grow out. Branches, usually very short, and either spherical or oblong, arise, chiefly at the septa, but also at other points, and bud out at the top into conidia, which are formed in rapid succession (Figs. 34 and 35). These branches may be thrown off, and then frequently begin a yeast-like budding. The conidial form of this species resembles those of some of the higher Ascomycetes, e. g. *Nummularia*, *Xylaria polymorpha*, &c., as figured by Brefeld [1]. The conidia germinate at once, but their behaviour varies under different conditions. If many conidia are sown in a small hanging drop they begin to bud at once, and the buds fall off as they do in a yeast (Fig. 37). In this connexion it may be noted that Klöcker [2] states that yeast formation does not occur in the Gymnoascaceae, and draws conclusions therefrom in discussing the affinities of the Gymnoascaceae.

If a few conidia are sown in a drop a small mycelium is formed (Fig. 36). Similar differences occur in streak-cultures of conidia. If the spores be grown on 2 per cent. beer-wort agar scarcely any mycelium is formed, and the culture soon consists of nothing but a dense white powdery mass of budding conidia (Fig. 38). But sometimes, apparently if the agar has become drier and more concentrated, a mycelium is first formed (Fig. 39), which, however, soon becomes smothered in the enormous quantities of conidia which it produces. On such a mycelium the conidia-bearing branches somewhat resemble a *Verticillium*, since they are produced, one or more together, chiefly at the 'nodes' of the hyphae, i. e. where the cross-walls occur (Fig. 35). Van Tieghem [3] has described a similar verticillate form in *G. ruber*, but Eidam [4] doubts the

[1] loc. cit. (2), Pl. IX.
[2] loc. cit.
[3] loc. cit. (1), p. 160.
[4] loc. cit. (e), p. 164.

accuracy of this statement, and thinks that van Tieghem may have had a true *Verticillium* in his cultures. The conidial form is always pure white.

This species has now (December, 1902) been kept in culture for a period of eighteen months, but so far it has never produced any other kind of spore but conidia, although it has been grown under various conditions on different media. The cultures are still being continued in the hope of obtaining ascospores. As will be noticed below, other species are known which have only produced conidial forms in artificial cultures.

III. Gymnoascus candidus.

The original material again consisted of a mass of ripe asci and ascospores, and a few slender, colourless, almost unbranched hyphae, which had no connexion with the asci (Fig. 40). Hyphae, asci, and spores were all completely devoid of colour, and, to the naked eye, appeared as small, dense, and perfectly white masses.

The ascospores germinate readily, and ripe fructifications are formed in a few weeks.

On germination the minute ascospores swell considerably, and produce a mycelium of very thin and delicate hyphae. The young coils which precede the asci were first observed about three weeks after the sowing of the spores. Each coil consists of a central club-shaped hypha, the 'sterile cell' (to retain Baranetzky's terminology), surrounded by a thinner hypha, the 'ascogone,' which coils round it in a close, symmetrical spiral (Fig. 41).

The two hyphae may or may not arise from the same hypha; more usually they appear not to do so. Nor do they arise simultaneously, as in *G. Reessii*; for the 'sterile cell' is first formed, and the 'ascogone' afterwards grows round it, as far as the apex, and here, after each has been cut off by a transverse wall (Fig. 43), the two cells fuse with one another (Figs. 44, 45, and 46). The ascogone now segments (Figs. 46–48), and the greater number of the segments thus formed grow out into short thick hyphae (Figs. 46–48), which branch

repeatedly and form round the coil a dense mass of ascogenous hyphae (Fig. 49). Besides the ascogenous hyphae a few vegetative hyphae seem to grow out from the base of the coil, as in *G. Reessii* (Fig. 50).

The development of the asci and ascospores seems to take place exactly as in *G. Reessii*, except that the occurrence of a large vacuole is not so constant. But the exceeding minuteness of the asci and their spores makes the details of their development very difficult to follow, even with the highest available magnification. For the same reason the behaviour of the nuclei is difficult to observe. There is no doubt, however, that the conjugating cells about the time of fusion both contain numbers of small nuclei (Fig. 45), whereas in the youngest stage, as in *G. Reessii*, there seems to be but one large nucleus in each cell (Fig. 43 *a*).

The young asci also appear at first each to have one large nucleus, with a nuclear zone, in the dilating end of the ascogenous hypha (Fig. 51 *a*). This evidently divides into two (Fig. 51 *b*), then into four, and finally into eight (Fig. 51 *c*), which are small after division, but increase in size when the divisions are all completed. Certain slender hyphae, filled at the apex with small nuclei, are apparently vegetative hyphae like those occurring in *G. Reessii* (Fig. 51 *e*). As is the case in *G. Reessii*, the remains of the empty coil may be seen within the mass of ripening asci (Fig. 52). Besides ascospores this species also produces abundant oidia. Each colony produces either oidia or ascospores, or both.

With the naked eye the ascogenous parts of the colonies are of a chalky whiteness and consistency, because the dense masses of minute asci cover up the small cushion of delicate hyphae which is first formed. In cultures grown from single ascospores each colony forms a white circular mass, a centimetre or more in diameter, which usually produces asci at the centre and oidia round the periphery (Figs. 53 and 54). The hyphae forming the oidia are usually erect and branching, and form masses which, to the naked eye, are somewhat flocculent.

As in *G. Reessii* and *G. setosus* the habit of the colonies

differs under different conditions. For example, oidia were sown in plates of beer-wort gelatine. The sowings were made from a pure culture, and yet two different kinds of colonies were formed—a dense kind and a loose kind. This difference was due simply to the fact that the dense colonies were submerged, while the loose form was growing on the surface of the medium.

Microscopic examination of the oidium-bearing hyphae shows that they consist of erect hyphae branching dichotomously with great regularity (Figs. 55–57). When more highly magnified the protoplasm in these branches is seen to be collecting into regular squarish masses, each containing a large vacuole (Fig. 55 *c*). Finally, walls appear between the masses of protoplasm, and the walls break up into oidia (Fig. 56 *a*), which are at first flat at the ends, but which later become rounded (Fig. 57). Each oidium (Fig. 57) is larger than an ascus. The oidia readily germinate and form cultures indistinguishable from those grown from ascospores [1].

Amongst the vegetative hyphae of the oidium-bearing mycelium may often be seen thicker hyphae, which, however, bear branches of varying thickness (Fig. 58). Some of the thicker hyphae show the pyriform swellings (Fig. 59), the cyst-like ends to some hyphae, and the beaded appearance of other hyphae (Fig. 60), which are characteristic of the genus *Gymnoascus* and which have also been observed in other genera, e. g. in *Onygena equina* [2].

Besides the erect hyphae oidia may occur on the ascus-bearing mycelium, between the layer of sexual coils and the vegetative hyphae imbedded in the nutritive medium, but lying on the surface between the medium and the ascogenous layer.

[1] The mycelium of another species of *Gymnoascus*, still under culture, behaves in a similar manner.

[2] Marshall Ward, *Onygena equina*, Willd., a horn-destroying fungus, Phil. Trans., series B, 175, vol. cxci, pp. 269–291, Pl. XXI, Figs. 11 and 12, Pl. XXII, Fig. 13 (1899).

The Various Kinds of Reproduction observed in the Gymnoascaceae.

The occurrence of asexual spores has not been observed in all species of *Gymnoascus*. Some species, e. g. *G. Reessii*, seem to reproduce themselves exclusively by means of ascospores. On the other hand, there are species which, at least under certain conditions, produce nothing but asexual spores. As examples may be noted the case of *G. setosus* just described, for, though Eidam[1] succeeded in obtaining the young coil, his cultures did not produce any ascospores. Another case is that of a species cultivated by Matruchot and Dassonville[2], who do not, however, give its name.

The majority of the Gymnoascaceae, however, produce in culture both sexual spores and also various kinds of asexual spores. Frequently these are of the type of chlamydospores, as, for example, in *G. uncinatus*[3]. In *G. setosus* (p. 582), and perhaps in *G. ruber*[3], the conidia arise in a verticillate manner on erect subaërial hyphae. In *G. setosus* conidia may also arise by budding from a germinating conidium (p. 582).

In *G. candidus* (pp. 584, 585) the asexual spores are oidia, resulting from the breaking up into spores of subaërial hyphae, which may either lie horizontally upon the substratum, or, more usually, stand erect and branch copiously.

Conclusions.

The investigations just described leave no doubt as to the occurrence of a sexual process in the Gymnoascaceae, if not in every species, at least in *Gymnoascus Reessii* and in *G. candidus*. Such a process has not before been described, though it was assumed by Baranetzky[4], who, however, expressly states that

[1] loc. cit. (2).

[2] (1) Sur le Champignon de l'Herpès (Trichophyton) et les formes voisines, et sur la classification des Ascomycètes. Bull. Soc. Myc. de France, tom. xv, p. 250 (1899).

[3] Eidam, loc. cit. (1), p. 298.

[4] loc. cit., pp. 148 and 156.

he saw no fusion between the two cells, so that 'fertilization' must take place by means of 'transfusion' through the wall between them.

Eidam[1] also takes a sexual process for granted in the species he cultivated, viz. *G. Reessii*, *G. uncinatus*, and in the closely allied genus *Ctenomyces*.

On the other hand, van Tieghem[2], Zukal[3], and Brefeld[4] emphatically deny the occurrence of any sexual process whatsoever. Van Tieghem, indeed, denies that sexuality occurs in any Ascomycete, on account of what he calls the 'monocarpous Ascomycetes,' i. e. Ascomycetes in which, according to him, the asci arise from a solitary original branch[5]. The cases where actual fusion has been seen he regards as examples of purely vegetative union, comparable to ordinary anastomosis.

Brefeld, according to whose observations the coils in *G. Reessii* are formed from a *single* branch, also, for this reason, considers that any idea of sexuality is quite out of the question. Some cases which he saw of coils made up of two hyphae, like those described by Baranetzky, he regards as pathological.

But undoubted cases of fertilization in which has been seen the union, not only of the conjugating cells, but in some cases of their nuclei also, have now been recorded amongst the Ascomycetes, e. g. in *Sphaerotheca Castagnei*[6] and *Pyronema confluens*[7] by Harper, and also in *Eremascus albus*[8] by Eidam,

[1] loc. cit. (1), p. 300.

[2] loc. cit. (1), p. 96.

[3] Ueber einige neue Pilzformen und über das Verhältniss der Gymnoascen zu den übrigen Ascomyceten: Berichte der Deutschen Bot. Gesellschaft, Bd. viii, p. 295 (1890).

(2) Sur le développement du fruit des *Chaetomium* et la prétendue sexualité des Ascomycètes. Ann. des Sci. Nat., 6e sér., vol. ii, p. 364 (1875).

[4] loc. cit. (1), p. 159.

[5] (3) Sur le développement du fruit des *Ascodesmis*, genre nouveau de l'ordre des Ascomycètes. Bull. de la Soc. Bot. de France, vol. xxiii, p. 271 (1876).

[6] Die Entwickelung des Peritheciums bei *Sphaerotheca Castagnei*: Ber. der Deut. Bot. Ges., Bd. xiii, p. 475 (1895).

[7] Sexual reproduction in *Pyronema confluens*, and the morphology of the Ascocarp. Annals of Botany, vol. xiv, p. 321 (1900).

[8] (3) Zur Kenntniss der Entwickelung bei den Ascomyceten. Cohn's Beiträge, vol. iii, p. 385 (1883).

and in *Monascus* by Barker [1], though not in those forms which are most nearly related to *Gymnoascus*.

The affinities of the Gymnoascaceae have gradually become apparent, as our knowledge of the family has increased by the addition of new genera and species. The investigations which have been recorded above seem to throw some further light on this interesting question.

One of the forms most nearly allied to *Gymnoascus* is *Ctenomyces serratus*. *Ctenomyces serratus* was first described by Eidam [2], and bears a most striking resemblance to *Gymnoascus candidus*; in fact, the description given by Eidam of the development of the coil and of the ascogenous hyphae and asci in *Ctenomyces* would serve equally well for *G. candidus*. Eidam, however, did not see any cell-fusion, or any nuclei in *Ctenomyces*. The only difference between *Ctenomyces* and *G. candidus* is that whereas the former (like most other species of *Gymnoascus* hitherto described) has hard, thick-walled hyphae round the asci, the mycelium of *Gymnoascus candidus* consists exclusively of extremely thin and delicate hyphae.

The resemblance between the two species extends to the asexual spores, but in *Ctenomyces* these are conidia, budded off laterally from the hyphae, while in *G. candidus* they are oidia.

Another closely allied species is *Eidamella spinosa*, a parasite growing on the skin of a dog. Matruchot and Dassonville [3], who founded the genus, made pure cultures which produced asci. The original coil arises exactly as in *Ctenomyces* and in *Gymnoascus candidus* from two branches, which sometimes grow out from one hypha, sometimes from two. But occasionally an anomalous case occurs, in which a single branch coils round the hypha from which it sprang. It is interesting to note that this is what Eidam observed in *G. Reessii*, and what he also records as an occasional occurrence in *Ctenomyces*. *Eidamella* also produces chlamydospores. This species is particularly

[1] Morphology and Development of the Ascocarp in *Monascus*. Ann. of Bot., Jan. 1903.

[2] loc. cit. (1), p. 271.

[3] (2) *Eidamella spinosa*, Dermatophyte produisant des périthèces. Bull. de la Soc. Myc. de France, tom. xvii, p. 123 (1901).

interesting, as the authors point out, because it is the first dermatophyte which has produced asci under artificial culture.

The life-history of *Gymnoascus Reessii* shows affinities in other directions, some of which have already been pointed out by previous investigators. Attention has been drawn to the fact that, though the young coils in this species always consist of two cells which are at first identical, certain variations may occur later which seem to indicate affinities with other genera and species. For example, when the two cells are of the same size and shape at the time of conjugation, they exactly resemble the similar stage which Eidam has described and figured in *Eremascus albus*[1] (cp. Figs. 9–11 with Eidam's figures on his Pl. XIX).

Eremascus was originally placed by Eidam amongst the Gymnoascaceae, and was by him regarded as forming a link between the Mucorineae and the Ascomycetes.

In connexion with the possibility of a connexion between the Gymnoascaceae and Zygomycetaceae it is interesting to remember that the sexual reproductive organs described and figured by Eidam in *Basidiobolus ranarum*[2] originate exactly in the same way as in *Gymnoascus Reessii*, namely, by the outgrowth of two adjacent cells, close to the septum which divides them from one another, and that these two cells fuse together as in *Eremascus* and *Gymnoascus*.

Schröter, in Engler and Prantl[3], however, places *Eremascus* amongst the Endomycetaceae, which, together with the Saccharomycetaceae, form the group of the Protoascineae.

If, on the other hand, the sterile cell in *Gymnoascus Reessii* grows more rapidly than the ascogone, the latter grows round the former in a manner suggesting *G. candidus*, *Ctenomyces*, and *Eidamella*.

Such a variation which, as it were, unites the type of *G. Reessii* and that of *G. candidus* also very closely agrees with the descrip-

[1] loc. cit. (3).

[2] (4) *Basidiobolus*: eine neue Gattung der Entomophthoraceen. Cohn's Beiträge, Band iv, Heft ii, p. 181 and Taf. xi (1887).

[3] loc. cit., p. 152.

tion and figures drawn by Eidam of the early stages in *Aspergillus* (*Sterigmatocytis*) *nidulans* [1]. The fate of the two hyphae was not determined with certainty, but asci were ultimately formed from the coil.

Another species of *Aspergillus*, e.g. *A. herbariorum* (Wiggers), of which figures are reproduced by Engler and Prantl [2], does not resemble the Gymnoascaceae nearly so closely as *A. nidulans*. In view of recent work on the sexuality of the lower Ascomycetes it would seem worth while reinvestigating, by means of modern histological methods, the life-histories of *Aspergillus* and *Penicillium*.

The obvious resemblances between the early stages of the coil of *Penicillium* and that of *Gymnoascus Reessii* have been noticed by previous investigators, and have led to the families of the Aspergillaceae and the Gymnoascaceae being included, with others, amongst which may be mentioned the Onygenaceae, in the group of the Plectascineae [3].

Previous to their discovery of *Eidamella*, Matruchot and Dassonville had drawn attention to the possibility of a relationship between the Gymnoascaceae and certain dermatophytes [4], especially *Trichophyton* [5], on account of the similarity in the asexual reproduction. The life-history of *Eidamella* confirmed their view, which now seems also to be strengthened by the likeness between *Gymnoascus candidus* and *Eidamella*. These authors place the Gymnoascaceae between the Endomycetaceae, on the one hand, and the Onygenaceae on the other, and give the following classification [5]:—

1. Endomycetées. *Endomyces*.

2. Gymnoascées. *Gymnoascus*, *Ctenomyces*, *Trichophyton*, *Achorion* (?), *Microsporum* (?), &c.

3. Onygénées. *Onygena*.

[1] loc. cit. (3), p. 406 et seq., Pl. XXI, Figs. 8–14.

[2] loc. cit., p. 301, Fig. 214.

[3] Engler und Prantl, loc. cit., p. 293.

[4] loc. cit. (1), p. 240. (3) Sur le *Ctenomyces serratus*, Eidam, comparé aux champignons des Teignes. Bull. Soc. Myc. de France, tom. xv, p. 305 (1899). (4) Sur une forme de reproduction d'ordre élevé chez les Trichophyton. Bull. Soc. Myc. de France, tom. xvi, p. 201 (1900).

[5] (1) p. 251.

Later they place *Eidamella* amongst the Gymnoasceae [1], near to *Myxotrichum*.

With regard to *Endomyces decipiens*, no sexual organs have been found, but the life-history of *G. candidus* tends to confirm the views of Matruchot and Dassonville. In neither *G. candidus* nor *Endomyces decipiens* are there any thickened hyphae, but in both the asci are completely naked and borne on delicate colourless hyphae. In both the mycelium breaks up into oidia [2]. The life-history of *Endomyces* would probably repay reinvestigation, with a view to ascertaining the presence or absence of sexual organs before the production of asci. The chief difference between these two species is that in *Endomyces decipiens* each ascus contains only four ascospores, whereas in *Gymnoascus candidus* there are eight spores in each ascus.

Van Tieghem [3] also compares *Gymnoascus* with *Hypomyces* (*Endomyces*) on the one hand, and on the other with *Penicillium*.

Boudier [4], as well as Matruchot and Dassonville, regards the Gymnoascaceae as having close affinities with *Onygena*. Indeed, he considers that the Gymnoascaceae do not differ essentially from the sessile species of *Onygena*.

Matruchot and Dassonville claim that Marshall Ward's recent work on *Onygena* [5] confirms their views as to the relation between the Gymnoascaceae and the Onygenaceae. Though no definite coil was seen by this author, the resemblances between the two families are very strong. In both the ascus formation is preceded by a coil, and the asci and ascospores develop in the same way. In both families there are chlamydospores ; in both pyriform swellings and cyst-like swellings at the ends of the hyphae occur in the vegetative mycelium. But since definite sexual organs are unknown in *Onygena*, its exact systematic position is uncertain.

[1] loc. cit. (1), p. 128.

[2] loc. cit., p. 155, Fig. 135.

[3] loc. cit. (1), p. 161.

[4] Description de deux nouvelles espèces de *Gymnoascus* de France. Bull. Soc. Myc., tom. viii, p. 43 (1892).

[5] loc. cit.

A comparison of the habitats of the various genera included in the Gymnoascaceae and Onygenaceae is also very suggestive in considering their affinities. For example, many species of *Gymnoascus* live either on the excrements of animals or on various parts of dead or living animals. *G. ossicola* and *G. aurantiaca* have been found growing on old bones. Eidam found *G. Reessii* growing on the dead pupa of *Sphinx Gallii*. *G. umbrinus* has been found on a dead cockchafer, *G. candidus* on the feathers of owls, *G. setosus* on an old bee's nest and on an old wasp's nest, which probably both contained excrements; *G. reticulatus* was found on the decaying horn of a cow, and *G. myriosporus* on the surface of the claws of birds of prey, and also on the excrements of these birds; *Ctenomyces* grows on feathers, *Onygena* on horn; *Eidamella* was obtained from the skin of a live dog, and is, according to Matruchot and Dassonville, related to other dermatophytes, e. g. *Trichophyton*. Moreover, the genera and species included in the Endomycetaceae, the Gymnoascaceae, and the Onygenaceae fall into a series in which there is a gradually increasing complexity in the structure of the fructification.

In *Endomyces decipiens* the asci are naked and solitary, and are produced on the ends of branching hyphae and show a tendency towards aggregation.

In *Gymnoascus candidus* the asci, while still completely without investment, are aggregated together in dense masses, each mass being produced from a single pair of conjugating cells. In other species of *Gymnoascus*, in *Ctenomyces*, and in *Eidamella* the groups of asci are more or less enclosed in a loose investment of thick-walled, branching, and, in most cases, anastomosing hyphae.

In *Aspergillus* and *Penicillium* the still more compact groups of asci are each surrounded by thick-walled hyphae, which form a continuous wall of pseudo-parenchyma—the peridium. In *Onygena* also the asci are enclosed in a complete investment, which in some respects is more differentiated than that of the Aspergillaceae.

In comparing the sexual organs of the forms under con-

sideration *Endomyces* and *Onygena* must be omitted, because in them such organs are unknown. But in all the other species the asci are the product of ascogenous hyphae arising from two cells which in every case are in close contact with one another, and which in two species, *Gymnoascus candidus* and *Gymnoascus Reessii* have been seen to actually fuse. Thus the probability of a sexual process in the allied genera is increased.

Evidently, then, the normal origin of the reproductive organs in this series is by means of two cells arising as branches, either from the same hypha or from two adjacent hyphae. But anomalous cases occur, like those described by Eidam in *G. Reessii* (p. 572) and in *Ctenomyces* (p. 588), in which a single branch coils round the parent hypha. Still more abnormal cases, which are undoubtedly pathological, are the irregular coils like those seen by Eidam in *Ctenomyces* and by the present writer in a starved drop culture of *G. Reessii* (p. 577). Such coils never produce asci, but soon degenerate.

It seems, therefore, as if this series of forms was natural, and based, not upon mere resemblances, but upon real affinities.

DESCRIPTION OF FIGURES IN PLATES XXVII AND XXVIII.

Illustrating Miss Dale's paper on the Gymnoascaceae.

Figs. 1–32. *Gymnoascus Reessii.*

Fig. 1 *a*. Part of the original material, consisting of hard thick-walled hyphae and loose ascospores. (2·F.)

Fig. 1 *b*. The spores more highly magnified. (4·F.)

Fig. 2 *a–d*. Germinating ascospore.

Fig. 3. Photograph of young colonies growing on a dry substratum in a culture plate.

Fig. 4. Photograph of similar colonies on a wet substratum.

Fig. 5. Photograph of older colonies in which the upper part of the mycelium grown on a wet substratum is becoming flocculent.

Fig. 6. Drawing of a mycelium on a wet substratum.

Fig. 7. An older stage of the same, in which the aërial hyphae are separating from one another.

Fig. 8. Still older stage of the same.

Fig. 9. Early stage in the formation of the sexual organs.

Fig. 10. The sexual organs more twisted round one another.

Figs. 11 and 12. Surface views of conjugating sexual cells. In 11 the two cells are of the same shape and size, in 12 one is larger; but both are coiled.

Fig. 13. A similar stage where one cell is much straighter than the other.

Fig. 14. A later stage of a form like that in Fig. 13. *a*, the outgrowth of the 'ascogone.'

Fig. 15. Two coiled cells after conjugation, showing the outgrowth *a*.

Fig. 16. Section of a similar stage, showing nuclei.

Fig. 17. Section of the segmented outgrowth round the end of the 'sterile cell.'

Fig. 18. The segments of the outgrowth forming branches which are the ascogenous hyphae.

Fig. 19. Surface view of segmented and branching outgrowth, *a*, vegetative hyphae.

Fig. 20. Group of ascogenous hyphae produced from a pair of sexual cells.

Fig. 21. Section showing vegetative hyphae springing from the base of the sexual organs.

Fig. 22. Section of young sexual cells, each containing a single nucleus.

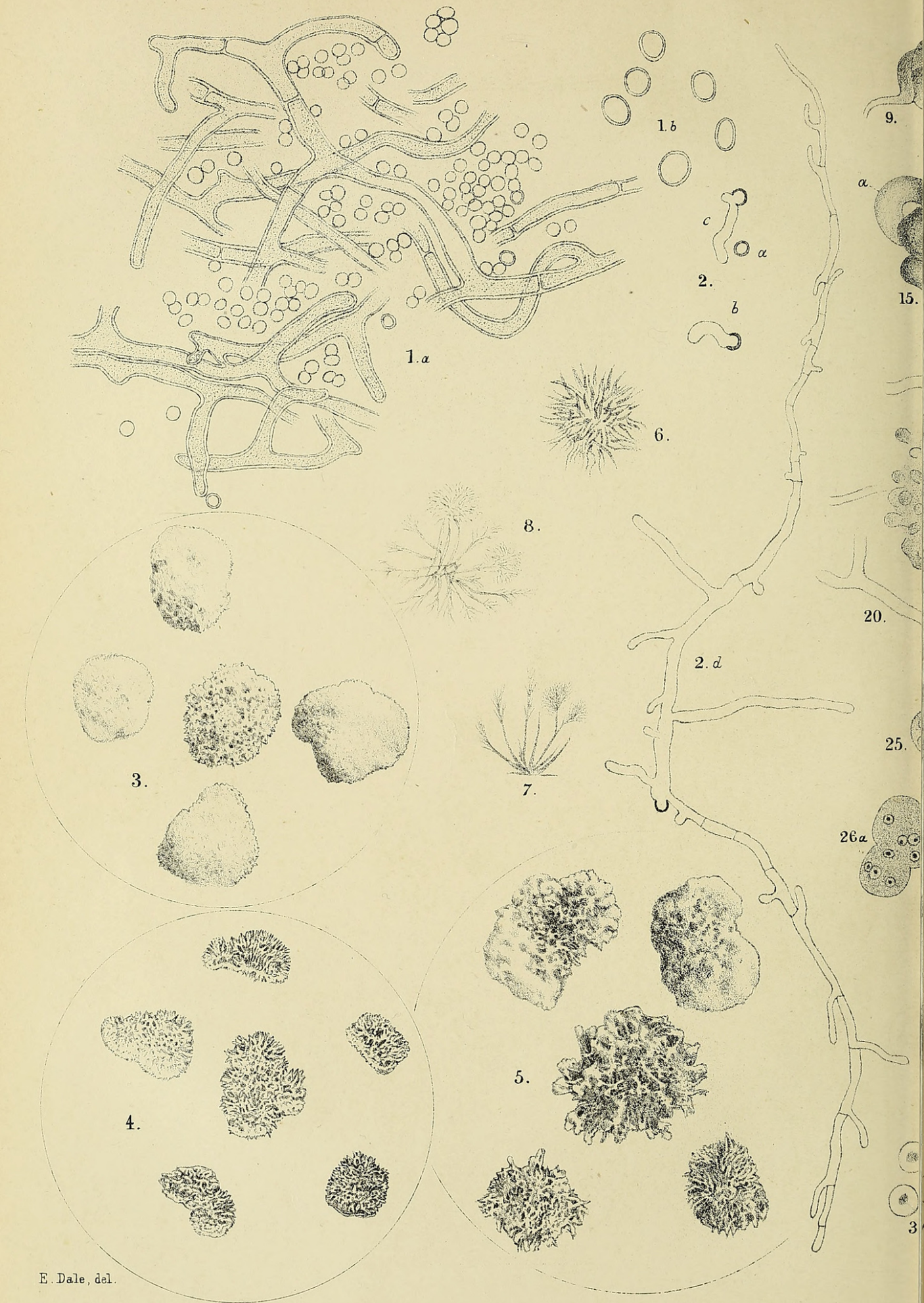
Fig. 23. Later stage, after nuclear division and the formation of a dividing wall below the 'sterile cell.' The nuclei have increased in size, and show a distinct nucleolus and nuclear zone.

Fig. 24. A later stage in which the nuclei have undergone division.

Fig. 25. A still later stage in which the nuclei are more numerous and smaller.

Fig. 26 *a, b, c*. Conjugating sexual cells in transverse section.

Fig. 27. The same in longitudinal section, showing many small nuclei.



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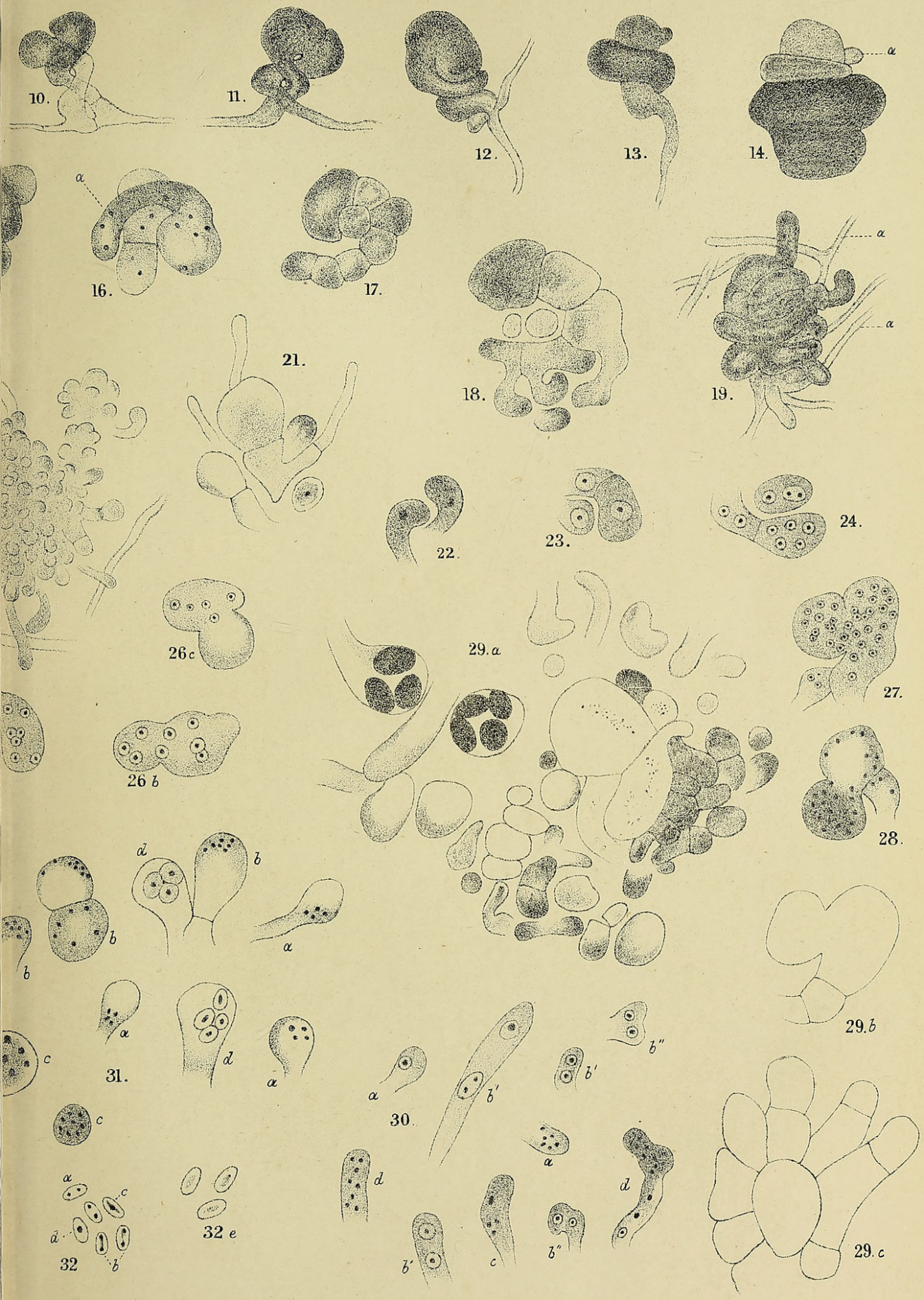

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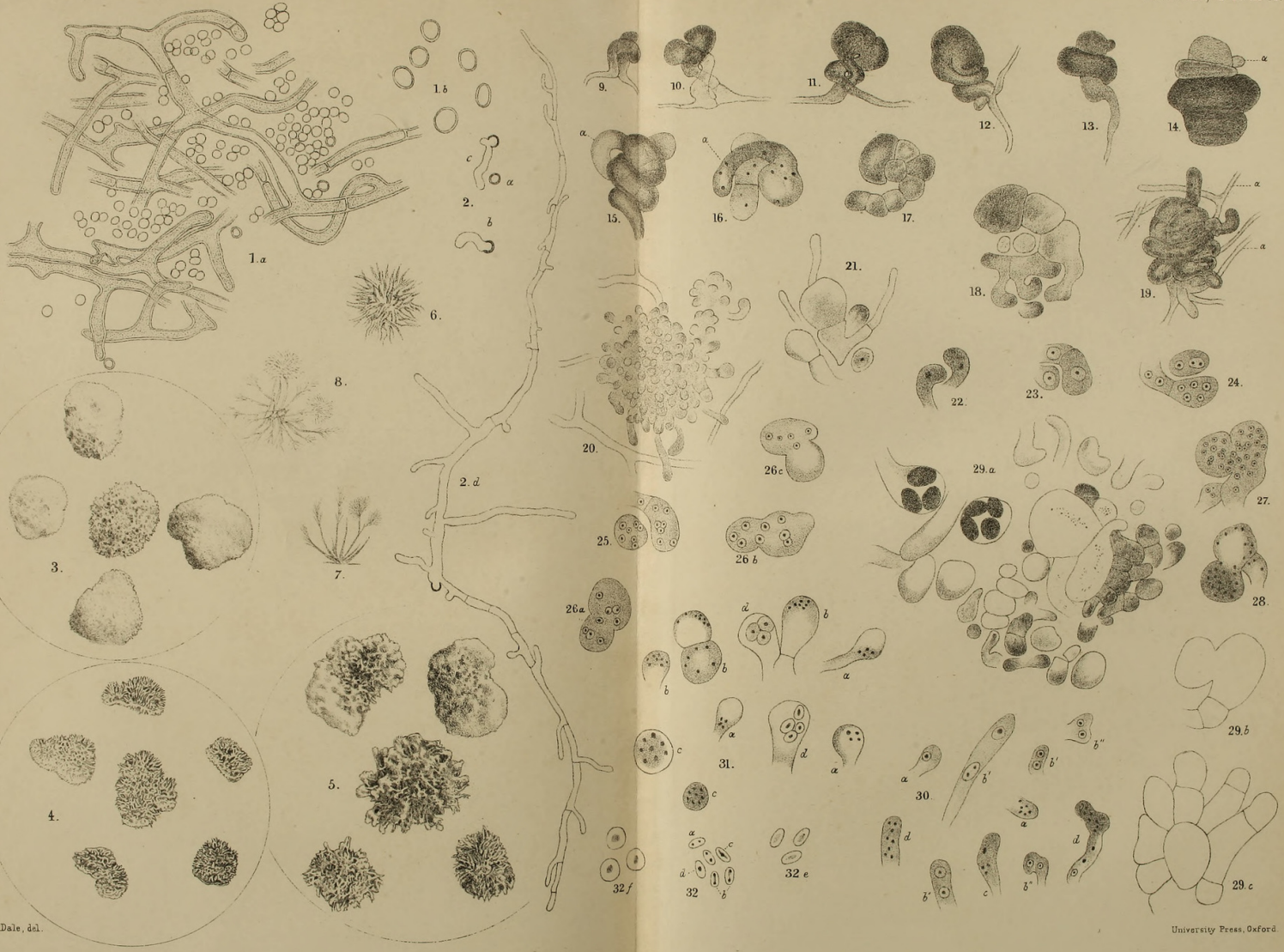

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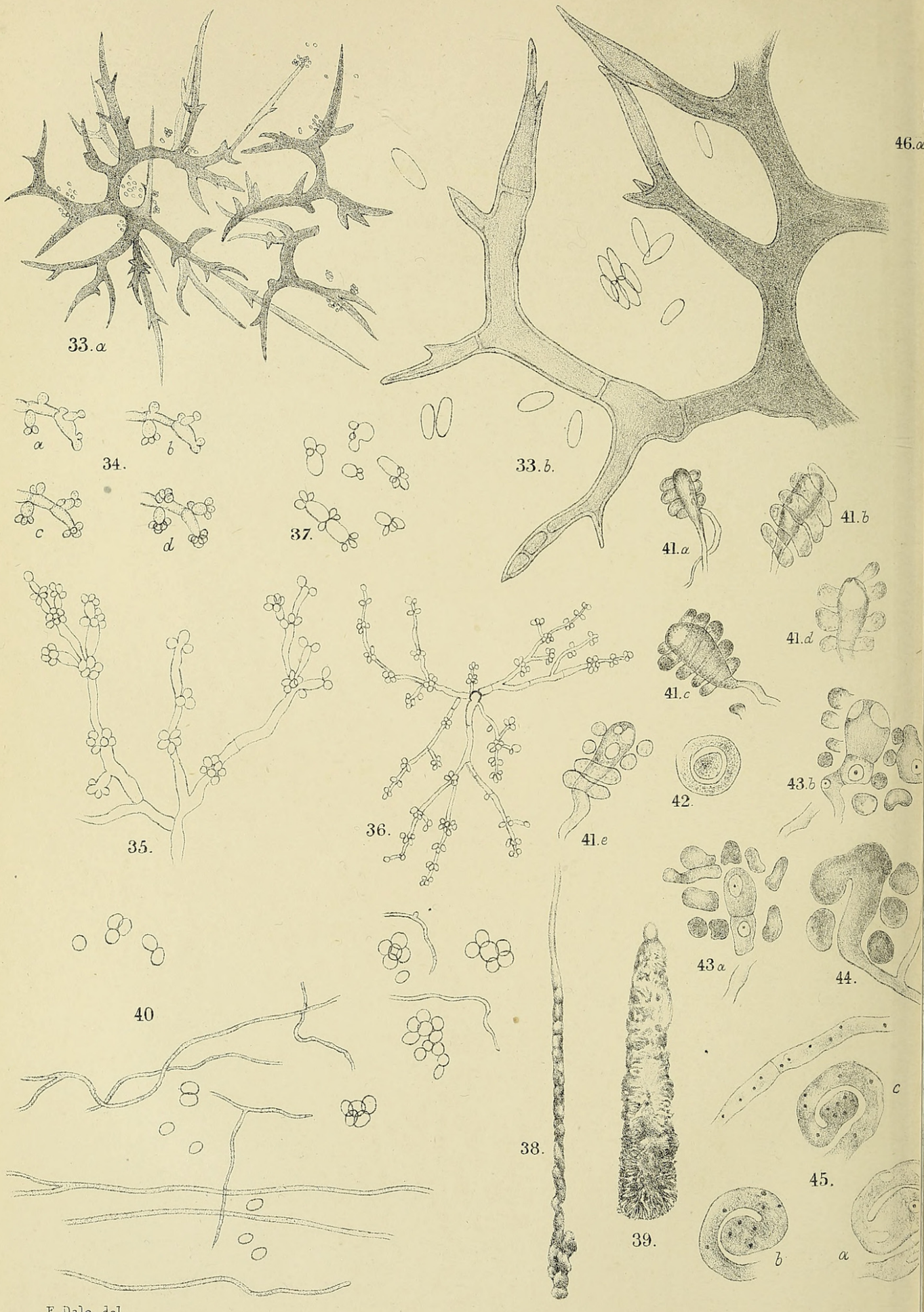
33.a
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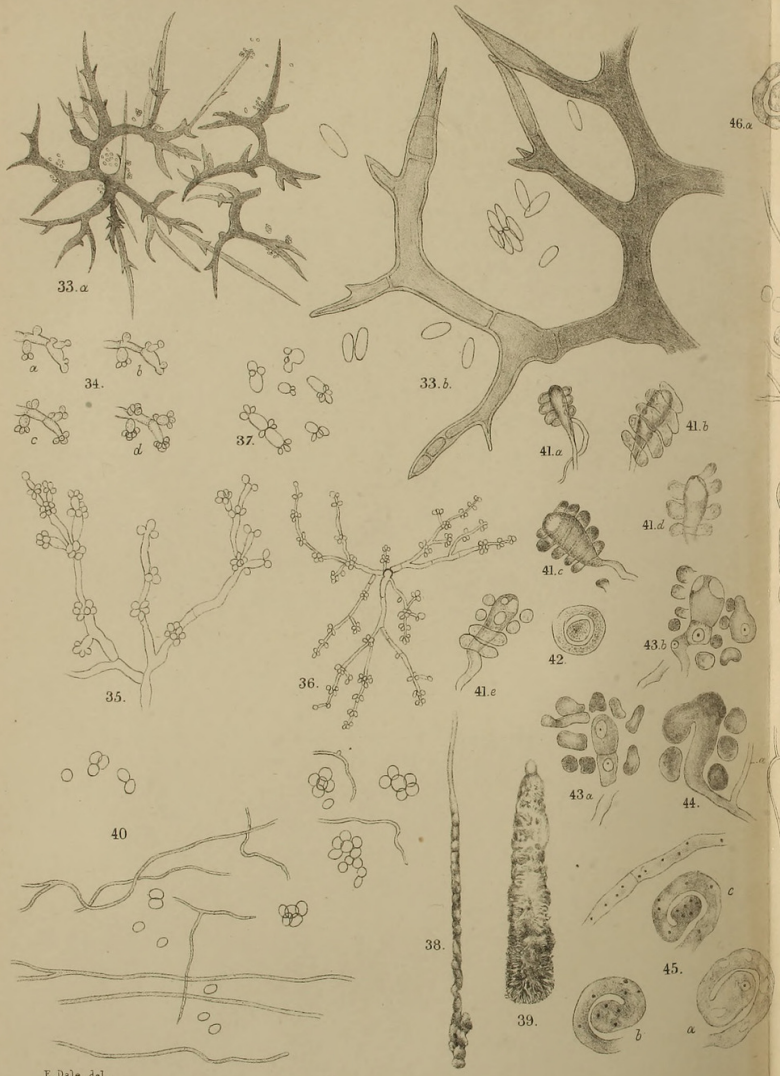

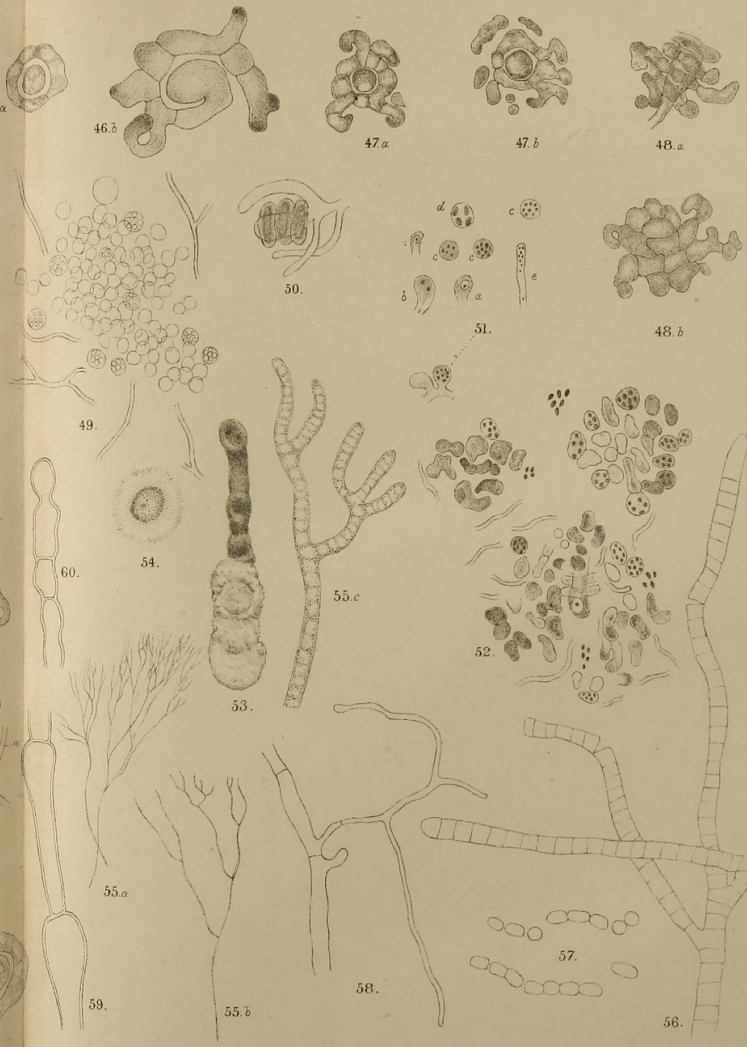

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Fig. 28. Conjugating cells, showing the passage of nuclei from the 'sterile cell' into the 'ascogone.'

Fig. 29 *a*, *b*, *c*. Sections of the old sexual cells as they occur in the centre of the ascocarps after their contents have passed into the ascogenous hyphae. 29 *a* shows ascogenous hyphae, vegetative hyphae, and developing asci. 29 *c* shows the segmented outgrowth of the ascogone with some of its branches.

Fig. 30 *a–d*. *Development of asci*. *a*. Young ascus with a single large nucleus. *b*. Older ascus with the nucleus divided into two. The nuclei sometimes lie in one plane, *b'*, sometimes in another, *b''*. *c*. Stage with four nuclei. *d*. Stage with eight or more nuclei.

Fig. 31 *a–d*. *Development of ascospores*. *a*. Stage with four nuclei and a large vacuole. *b*. Stage with eight nuclei and a large vacuole. *c*. The eight nuclei enlarged in size, and surrounded by so much protoplasm that the vacuole has almost disappeared. *d*. The young spores surrounded by their walls.

Fig. 32 *a–f*. *Ascospores*. *a*. Ascospore with two deeply staining bodies, *b*, the two bodies united by a stained protoplasmic strand. *c*. Spore with a densely stained central body with stained protoplasmic strands at each end of it. *d*. Spore with deeply stained central body. *e*. Larger spores diffusely stained. *f*. Mature spores.

Figs. 33–39. *Gymnoascus setosus*.

Fig. 33 *a*. Part of the original material showing thickened spiny hyphae and loose ascospores (2·D). 33 *b*. Part of the same more highly magnified. (4·F.)

Fig. 34. Formation of conidia. (4·F.)

Fig. 35. Conidial branches. (4·F.)

Fig. 36. Conidium producing a small mycelium bearing other conidia.

Fig. 37. Conidia budding. (4·F.)

Fig. 38. Streak culture consisting almost exclusively of masses of conidia.

Fig. 39. Streak culture consisting of a mycelium bearing conidia.

Figs. 40–60. *Gymnoascus candidus*.

Fig. 40. Part of the original material showing conidia and vegetative hyphae.

Fig. 41 *a–c*. Young stages of the young coil, consisting of a thick straight cell surrounded by a thin coiled hypha. Longitudinal sections or surface view.

Fig. 42. The same in transverse section.

Fig. 43 *a* and *b*. Longitudinal section of an older stage showing the central cell cut off by a transverse wall.

Fig. 44. Conjugating cells in longitudinal section. *a*. Vegetative hyphae.

Fig. 45. Conjugating cells in transverse section.

Fig. 46 *a*. Central cell surrounded by the ascogone divided into segments.

Fig. 46 *b*. Conjugating cells with the ascogone segmented and branching.

Fig. 47 *a*, *b*. Transverse section of central cell surrounded by segmented and branching ascogone.

Fig. 48 *a*. Longitudinal section of central cell surrounded by the segmented and branching ascogone.

Fig. 48 *b*. The same in surface view.

Fig. 49. Group of young asci developed from a pair of conjugating cells.

Fig. 50. Young sexual coil showing origin of vegetative hyphae.

Fig. 51 *a-e*. Development of asci.

Fig. 52. Group of developing asci.

Fig. 53. Photograph of a streak culture on beer-wort agar.

Fig. 54. Sketch of a colony bearing asexual spores round the circumference, and ascospores in the centre.

Fig. 55. A branch in which the protoplasm is dividing into masses.

Fig. 55 *a*. Part of a mycelium about to break up into oidia. 55 *b*. Part of the same more highly magnified.

Fig. 56. A branch breaking up into oidia.

Fig. 57. Mature oidia.

Figs. 58, 59, 60. Parts of the old vegetative mycelium.

(Figs. 9–32 and 41–52 were drawn with the camera lucida, the lenses used being Zeiss 1·5 oil immersion objective and no. 4 eye-piece.)