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CONTRIBUTIONS FROM THE CRYPTOGAMIC LABORATORY OF HARVARD UNIVERSITY. XL.

NEW OR PECULIAR ZYGOMYCETES. 2. SYNCEPHALASTRUM AND SYNCEPHALIS.

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(WITH PLATES I AND II)

ALTHOUGH the Mucorineæ, from their great variety and from the ease with which they may be cultivated, offer an attractive field for investigation, they appear to have received but scant attention in this country, if one may judge from the infrequent references to them that are found in our literature, and their meager representation in "Floras" in which they are listed. It must be well known, however, to anyone who has used them for teaching purposes that they are among the most varied and abundant of our moulds. The somewhat limited experience of the writer would indicate that our Mucor flora is a very rich one, since all the described genera, with two or three exceptions, appear to be for the most part well represented in it; and thanks to the admirable papers of A. Fischer and others, but primarily to those of Van Tieghem, whose writings may well serve as models for such work, their systematic study presents comparatively few difficulties except in so far as concerns the more obscure species of the genus Mucor.

The American species included in the group of so-called Cephalideæ seem to be even less well known than those of the mucors proper, and although Professor Farlow has mentioned the occurrence of two species,[1] *Syncephalis sphaerica* and *Piptocephalis Freseniana*, in Massachusetts, with the exception of the present writer's note on Dispira in a former number of the GAZETTE there appears to have been no further mention of the occurrence of any American member of the group; although it comprises many of the more striking fungi that are found in laboratory cultures.

Among the species of Syncephalis which have come under the writer's notice there are several that seem to be distinct from any of the forms previously described, and are of interest not only from the fact that they serve further to illustrate the specific peculiarities of a genus distinguished for its remarkable types, but also for the reason that in two of the forms enumerated the process of spore-formation can be followed with greater accuracy than is possible in any of the commoner species known to the writer. In the same connection a brief account may be given of the sporulation of Syncephalastrum, a genus the characteristics of which appear to be but little known, and prove to possess considerable interest.

It is well known that authorities are by no means agreed as to the homologies of the non-sexual reproductive organs of the Cephalideæ; what we may call for convenience the French school following the opinion of Van Tieghem in considering the "spore-rows" of this group as the homologues of the sporangia in typical mucors, from which they are held to differ merely by reason of the fact that they are cylindrical instead of spherical in shape, and contain a single row of superposed spores endogenously produced instead of a more or less indefinite rounded mass. The walls of these spores having been formed in close union with those of the sporangium, the latter appears finally to break up into a row of spores that present the appearance of having been exogenous in origin. Within the past year the conclusions of Van Tieghem have been further substantiated through the researches of M. Léger, who states that his studies confirm

[1] Bull. Bussey Instit. **2**: 224, 1878; *l. c.* **1**: 431. 1871.

this "sporangial" theory in all respects in so far as concerns the genera Syncephalis and Piptocephalis. The cylindrical sporangia in these cases are said by him to be filled with protoplasm containing many nuclei which becomes simultaneously divided into as many portions as there are spores in each spore row; these masses being separated by an intersporal zone of hyaline protoplasm, as in the sporangia of typical mucors.

Other authors, again, are not inclined to accept this homology, and A. Fischer, for example, in his well-known revision of the Phycomycetes in Rabenhorst's *Kryptogamenflora*, inclines to the opinion that, in the absence of any connecting form between these two supposed types of sporangia, it is as reasonable as well as more simple to assume that these so-called conidia have had an exogenous origin independent of that which has given rise to sporangia of the normal type, and are therefore not homologous. Having been personally inclined to agree with the views expressed by Professor Fischer in this connection, the writer was somewhat surprised to find, in examining the spore rows of a species of Syncephalastrum that has been kept in cultivation for several years in the laboratory, a condition of things which, in so far as this genus is concerned, not only confirms the theories of Van Tieghem just mentioned, but affords at least an approach to the very connecting link between the spherical and the cylindrical form, the absence of which was pointed out by Fischer.

The species of Syncephalastrum, three of which have been described, appear to be in general of tropical origin, since in all cases in which they have been observed, with perhaps one exception, the material on which they have been cultivated has been brought from the warmer regions of the earth. In the writer's laboratory substances from Africa, China, Ceylon, and Java have repeatedly yielded the same species, which, although very variable in cultivation in regard to its branching and to the number of spores in each spore-row, cannot be separated from *S. racemosum* Cohn. It grows and fruits luxuriantly on agar, and is sharply distinguished from other members of the Cephalideæ in that its fertile and vegetative hyphæ are uniform, as well as from the fact that it is never even partially parasitic in its habit. The undifferentiated fertile branches end in a spherical head, from which, as in some species of Syncephalis, the spore-rows radiate in all directions, forming an Aspergillus-like fructification. The spore-rows arise as cylindrical cells, formed by budding directly from this head, which normally contain at maturity a single row of superposed spores (*fig. 3*) resulting from the separation of the protoplasmic contents of each cell into a number of distinct portions corresponding to that of the mature spores. As far as has been seen, this separation appears to occur simultaneously, and not by gradual constriction, the successive masses becoming separated by a hyaline intersporal substance exactly similar in appearance to that which occurs in ordinary sporangia (*fig. 1*). After this separation has been effected, each mass surrounds itself with a wall visibly distinct from that of the cylindrical mother-cell (*figs. 2–3*), within which the spores thus formed are practically free. That this is the case is readily demonstrated by crushing the spore-rows under a cover glass, and in such preparations abundant instances may be observed in which the spores have been forced out of the sporangia, as the cylindrical mother cells must undoubtedly be called, which may thus be left wholly empty or but partly filled with spores that may lie more or less irregularly in its interior (*figs. 2* and *4*). By selecting a head not fully mature it is often possible by careful crushing to force all the spores out of the sporangia through their ruptured tips, leaving them empty but still intact and adherent to the fertile head. In nature the spores are freed by the eventual disappearance of the sporangium wall, which shrivels and breaks up without undergoing the deliquescence characteristic of all other Cephalideæ at this stage; so that the spores are dispersed in a dry condition instead of cohering in a viscous drop. The sporangial nature of the spore-rows is further shown by the fact that it is by no means unusual to find instances in which the spores are formed not in single rows, as in *fig. 3*, but more irregularly through the occurrence of longitudinal or oblique planes of separation, as in *figs. 1* and *2*. In such cases the sporangium is more or less distinctly swollen terminally, as in *fig. 2*, and presents a condition which may well be regarded as intermediate between an ordinary sporangium and the more typical uniseriate type represented in *fig. 3*.

Although the species of Syncephalastrum are not, as has been mentioned above, in any degree parasitic, and although there are certain important structural differences which distinguish them from other Cephalideæ, their close relationship to the latter can hardly be doubted. It would therefore seem quite safe to assume that the corresponding spore-rows in Syncephalis would prove to have an exactly similar mode of development. An examination of two undescribed species of this genus, however, shows conclusively that the processes in the two cases are by no means identical, and that in this instance we have a far more definite approach to the ordinary exogenous type of spore formation than is found in Syncephalastrum.

The species of Syncephalis appear to vary very greatly in so far as concerns the ease with which the changes connected with spore formation may be observed, and the distinctness with which it may be followed depends, not on the size of the spores themselves, but on the width of the interval which separates successive spores in given species. In the few common forms which the writer has recently had an opportunity of examining in a fresh condition, in connection with the preparation of the present note, namely *S. cordata*, *S. depressa*, *S. cornu*, and *S. nodosa*, phenomena, which in the two species just mentioned are readily seen, can be made out only with great difficulty. The form in which the true condition of things is most strikingly shown is an undescribed species from Liberia, which, though so closely allied to *S. cordata* that it was at first mistaken for that species, presents well-marked specific differences. This species, a description of which is reserved for a subsequent paper, is characterized by producing rather small oblong spores in somewhat elongate spore-rows, the former during their formation being separated by so wide an interval that the various accompanying changes may be readily seen even without the use of high magnifications. The successive steps in this process may be summarized as follows: Beginning with the immature "sporangial filament," if we may use this term to indicate the structures from which or within which the spores are eventually produced, we find them filled as usual with undifferentiated granular protoplasm. The first indication of spore-formation is seen in the appearance of successive indentations of the protoplasm which correspond to the future lines of separation between the successive spores. These indentations extending completely around the sporangial filament thus divide it by a series of successive rings into a number of segments corresponding to the number of spores to be produced. As this indentation gradually increases the protoplasmic mass within the sporangial filament becomes correspondingly constricted, and by treating the specimen with eosin or other stains the indented area may be seen to be made up of two parts (*figs. 39–41*), the one hyaline (*a*) unable to absorb stain and resembling in appearance the intersporal substance of ordinary sporangia, the other (*b*) acting toward stains like granular protoplasm. As the development proceeds the indentation just described and the corresponding constriction of the protoplasmic contents become more and more pronounced, while at the same time the stainable portion (*fig. 40 b*) of what may conveniently be called the "intermediary zone" increases in volume. At this stage the protoplasmic contents of the sporangial filament has become separated into distinctly formed oblong portions connected by gradually narrowing protoplasmic isthmuses (*fig. 41*). These oblong portions become eventually completely separated and are surrounded by a distinct wall which, however, on either side is hardly distinguishable from the wall of the sporangial filament (*fig. 42*). In this mature condition the intermediary zones may be seen as distinct rings (indicated by dotted lines in *fig. 42*), often distinctly elevated above the adjacent surface of the spores, probably by reason of the fact that they begin to become deliquescent almost as soon as the spore wall is formed. While these changes are taking place the tip of the sporangial hypha undergoes a somewhat similar modification. A cap appears within it (*fig. 39*) which is at first composed of non-stainable material like that of the intermediary zones, but which, like the latter, soon shows a distinction between a stainable and non-stainable portion (*fig. 40*). This terminal cap eventually shares a fate similar to that of the intermediary zones, becoming deliquescent and leaving the terminal spore of the row evenly oblong like the rest.

Having had abundant material of this species growing in a fresh condition it was possible to verify many times the course of development just described, the correctness of which was further substantiated by the examination of a second species subsequently described as *S. pycnosperma*. The sporangial hyphæ of this species are far larger than those of the form last described, although the intermediary zones are relatively narrower. The process by which the latter arise is exactly similar to that of the African form, except that the stainable portion of the zone is proportionately less well developed, forming finally a thin "separation disk" (*fig. 36 b*) which, as the spore matures, loses its power of absorbing stain and is converted into a refractive oily substance. That portion of the zone, moreover, which was at the outset unstainable persists as the spore matures, being converted into a thick wall firmly united to that of the spore and barely distinguishable from it (*fig. 38 a*, in which this distinction is much exaggerated), and it is the persistence of this area (*a*) which gives to the ripe spores their peculiar form (*figs. 34, 37, 38*). The same differences may be noted in the phenomena which occur at the apex of the sporangial filaments, the process being even more clearly marked than in the species last described. The portion of the filament separated above the terminal spore is here so large that it has the appearance of a definite small cell; and as the spore matures it passes through the same changes which have just been described as characteristic of the separation zones. The portion which is at first stainable becomes converted into oily material, and disappears together with the portion of the wall of the sporangial filament that immediately surrounds it; while the non-stainable part, as in the case of the zones, is transformed into a permanent wall. The terminal spore thus ends in a cup-like depression (*fig. 38*, lower end), by which it is at once distinguished from the two other spores which compose the spore-chain at maturity.

It is thus apparent, in these two instances at least, that the process of spore formation is distinctly different from that which has been described in Syncephalastrum, from the fact that the contents of the sporangial filament is converted into spores, not through its simultaneous separation into successive masses, but as the result of a more or less gradual intrusion of "intermediary zones" which develop from the periphery inward till the protoplasmic content is cut into segments. In the species last described it is evident that the intermediary zones consist of two parts; one of which is, or at least becomes, a permanent structure which, though formed earlier than and independently of the spore wall proper, is ultimately closely united with it; while the other constitutes an intermediate portion ultimately converted into an oily substance, although at first it seems to be protoplasmic, and corresponding to the plane of separation between adjacent spores, their function in this process being evident.

A similar series of changes may be made out with sufficient distinctness in *S. Wynneæ* described below; but although in some of the common species, like *S. nodosa* and *S. cordata*, it is possible to observe the progressive constriction of the contents of the sporangial filament into portions corresponding to the spores, the extreme narrowness of the intermediary zones in these species renders it almost impossible to follow out the process in detail, yet it may be fairly assumed that these details are not essentially different from those above described.

In the species subsequently described as *S. tenuis* a somewhat scanty supply of mounted material only has been available for study in this connection, so that it has been impossible to determine the character of its intermediary zones. It will be noticed, however, that the species is peculiar in one respect, in that the development of its sporangial hypha recalls that of Dimargaris, from the fact that the portion corresponding to the terminal spore appears to bud, as it were, from that corresponding to the basal spore after the latter has become almost fully formed and has assumed its more or less characteristic shape (*figs. 23, 24, 27, 28*).

Syncephalis Wynneæ and *S. pycnosperma* possess a further interest from a structural standpoint, in that they illustrate an extreme development of the type hitherto represented only by *S. fusiger*. Bainier, in his description of the latter species, distinguishes it as the type of a new genus which he calls Microcephalis, for the reason that the sporangial filaments arise in pairs from a common basal piece, corresponding to the basal spore or spores which bear similar relation to the erect spore-rows in species like *S. cordata* or *S. nodosa*. In *S. fusiger* this piece, instead of becoming converted into one or more spores, remains sterile and constitutes a specially developed organ, or secondary sporophore, which this author compares to the separable sterile piece on which the spore-rows are inserted in the species of Piptocephalis, although in the last instance this piece would seem to be more properly comparable with the swollen extremity of the fertile hypha in Syncephalis. So marked a differentiation of this secondary sporophore as is found in *S. Wynneæ* might seem to call for generic recognition were it not for such connecting links between this and the ordinary forms as are furnished by *S. pycnosperma* and *S. fusiger*, in view of which the character can hardly be considered of more than sub-generic value.

The zygospores of species of Syncephalis were first discovered by Van Tieghem in the common *S. cornu*, but, as far as the writer isaware, have been observed in only one other instance, those of *S. nodosa* having been described and figured by Bainier in his well-known "Étude sur les Mucorinées." The last mentioned species is very common in this country, and one seldom fails to obtain its zygospores in abundance whenever it grows on a copious substratum of other mucors. From the peculiarities presented in the formation of these zygospores Bainier, in the paper above cited, separates it from other species of Syncephalis as the type of a distinct genus which he calls Calvocephalis; but since there are certain errors both in his figures and descriptions it may be of interest briefly to review the process in connection with the figures given in *Plate I.*

The zygospores of this species are always found in groups of from four or five to twenty or more, which are readily visible as white flecks scattered over the infested mass of mucors. The formation of the gametes is always preceded by the twisting together of two hyphæ, one of which forms a rather close spiral around the other, which is itself but slightly twisted. The latter ends in a swollen extremity (*figs. 18–20 y*) which becomes separated by a septum from the filament that bears it. The tip of the enveloping hypha winds about this swollen extremity, taking a last turn almost completely around it (as is shown, seen from above, in *fig. 18*). The helix thus formed is then separated from the hypha below by a septum (*figs. 18–19 z*), while its apex conjugates laterally or subterminally with the extremity of the inner hypha (*y*). As a result of this conjugation the spore arises, not between the two conjugating tips, but by budding from the helix just mentioned at a considerable distance from the point of conjugation and always close beside the septum (*z*). The mature zygospore is thus borne on a single short stalk which connects it with the helicoid gamete, while the filament below the septum (*z*) buds out at various points to form the curious bladder-like outgrowths which are apparently always associated with the zygospores of members of this genus (*figs. 20–21*). These outgrowths are even more copiously developed in the zygospores of *S. cornu* (*fig. 17*), but in *S. reflexa*, the zygospores of which do not seem to have been previously observed, they attain an even greater luxuriance (*figs. 15–16*). The zygospores themselves are irregularly bullate, about 21μ in diameter, and of a pale yellowish color. The material figured was found in a culture of mouse dung made some years since at New Haven, Conn.

Of the new species of Syncephalis previously referred to, three may be characterized as follows:

Syncephalis Wynneæ, nov. sp. *Plate I, figs. 5–12.*

Color white turning to pale straw color. Fertile hyphæ erect, straight, usually septate at the base, with well-marked rhizoids, tapering slightly towards the tip, distally enlarged, not abruptly, into a comparatively small head from all portions of the surface of which are produced secondary sporophores, the latter clavate, swollen at the tip, whence each gives rise to about a dozen sporangial filaments from each of which are produced two spores. Spores irregularly long-oval, usually slightly asymmetrical, involved at maturity in a mucus drop, 16–19 by 6μ. Secondary sporophore about 25μ long. Fertile hypha 400–475μ long, the head including spore *in situ* about 100–120μ in diameter.

On *Wynnea macrotis* Berk., Cranberry, North Carolina.

This species was found in a single instance, growing out of doors on a large clump of its remarkable host, which appears to be not uncommon in the Carolina mountains, and on which it seemed to be truly parasitic, growing not very densely and inducing a rapid decay in the large spoon-shaped apothecia. Attempts to cultivate it on potato-agar were unsuccessful, and no zygospores were found in the material examined. The species is especially noteworthy from the marked differentiation of its secondary sporophores, to which reference has been made above.

Syncephalis pycnosperma, nov. sp. *Plate II, figs. 32–38.*

Vegetative hyphæ slender with nodular anastomoses. Fertile hyphæ rather short and stout, commonly constricted at the basal septum, distally not abruptly enlarged to form a small head from all parts of which are produced numerous clavate secondary sporophores, the latter rarely furcate, distally gradually expanded and two to four-lobed, each lobe giving rise to a sporangial filament producing invariably three spores. Spores subrectangular or angular in section, thick-walled, involved in mucus at maturity, 13–16 by 7–8μ. Fertile hyphæ 300–350 by 25μ (towards the base) to 17μ (towards the apex). Secondary sporophores about 24μ long.

On dung of mice (New Haven, Conn.) and of sheep (Cambridge, Mass.).

This striking species has been met with but twice, growing not very abundantly on rather old cultures. Its peculiar spores and secondary sporophores, which have already been described in detail, serve to distinguish it at once from all other known species.

Syncephalis tenuis, nov. sp. *Plate II, figs. 22–31.*

Fertile hyphæ septate at the base, very elongate, tapering to a slender extremity which expands abruptly to form the fertile head, the latter somewhat flattened and bearing from six to many sporangial filaments arising from all parts of its upper surface or arranged in a more or less definite circle each producing two spores. Spores sub-cylindrical to asymmetrically oval, truncate or bluntly rounded, the cylindrical form 20–25 by 7μ, the oval form 25–27 by 10–11μ. Fertile hyphæ 500–700 by 7μ (at the base) to 4–5μ (at the apex). Sporiferous head (without spores) 10–20μ in diameter.

On Sphagnum in laboratory cultures, Kittery Point, Me.

This species is remarkable for its very slender habit and relatively large spores. It has made its appearance twice in cultures of Sphagnum on which were zygospores of an unknown zygomycete,[2] the orange yellow coherent waxy masses of which are not infrequently found in swampy places on this host, usually at the tip of its axis, occurring more rarely on other substances like decaying wood, etc. These zygospores, which are oblong and orange and are produced by budding upward from the point of union of the two gametes as in species of Syncephalis, although they are widely different in their color, form, and condition of aggregation from any of the known zygospores of this genus, may possibly be connected with the present species; but as all attempts to cultivate them under test conditions have thus far proved fruitless, and as the same cultures of Sphagnum on which they were growing have also yielded a new Martensella (in my opinion a zygomycete), two species of Mortierella, and a peculiar orange-colored Mucor, it is doubtful which, if any, of all these forms should be connected with them. The species is very inconspicuous, extremely delicate, and does not grow luxuriantly. The two varieties, the one with nearly cylindrical and the other with sub-oval spores, might be mistaken for distinct species, the latter variety producing fewer and larger spores borne on a smaller head terminating a more slender stalk; but the material examined appears to show much variability in these respects.

CAMBRIDGE, MASS.

[2] This fungus corresponds closely to the description of *Endogone xylogena* Schröter.

EXPLANATION OF PLATES I AND II.

The figures are reproduced by photolithography from camera drawings made with the combinations of Zeiss and Leitz objectives noted, and reduced about one-fourth in the reproduction.

Syncephalastrum racemosum Cohn.

FIG. 1. Young sporangium in which the contents has separated into seven masses separated by intersporal substance. The spore walls have not yet formed. One of the spores is lateral in position. $\frac{1}{16}$. oc. 4.

FIG. 2. Sporangium containing mature spores, some of which have escaped from the base. Two lie free within the sporangium ; those near the extremity remaining side by side in the same position in which they were formed. $\frac{1}{16}$. oc. 4.

FIG. 3. Normal sporangium containing single row of superposed mature spores. $\frac{1}{16}$. oc. 4.

FIG. 4. Mature sporangium crushed, from which the spores are escaping. $\frac{1}{16}$. oc. 4.

Syncephalis (*Microcephalis*) *Wynneæ* Thaxter.

FIG. 5. General habit of fertile hypha showing head on which the spores are still *in situ*. C. oc. 2.

FIG. 6. A similar fertile hypha in which the spores have separated and adhere in a viscous mass. C. oc. 2.

FIG. 7. Tip of fertile hypha showing secondary sporophores from which numerous sporangial filaments are in process of development. J. oc. 2.

FIG. 8. Tip of mature fertile hypha showing secondary sporophores from which the spores have fallen. Seen in optical section. D. oc. 4.

FIG. 9. Secondary sphorophore bearing immature sporangial filaments. J. oc. 2.

FIG. 10. A similar sporophore in which the spores are nearly mature. D. oc. 4.

FIG. 11. Sporangial filament with its two spores nearly mature. J. oc. 4.

FIG. 12. Two mature spores. J. oc. 4.

Syncephalis reflexa Van Tieghem.

FIG. 13. Fertile hypha with the spores mature. C. oc. 4.

FIG. 14. The same, showing young sporangial filaments. C. oc. 4.

FIG. 15. Zygospore with sterile outgrowths from conjugating hyphæ J. oc. 2.

FIG. 16. The same. A zygospore seen in optical section. J. oc. 2.

Syncephalis cornu Van Tieghem.

FIG. 17. Zygospore with sterile outgrowths. J. oc. 2.

Syncephalis nodosa Van Tieghem.

FIG. 18. Gametes (x, y) seen from above in process of conjugation. The gamete x separated from the hypha which bears it by a septum (z). J. oc. 2.

FIG. 19. The same. A zygospore has begun to bud from the gamete (x) just above the septum (z). J. oc. 2.

FIG. 20. Conjugating filaments viewed laterally, the inner shown by dotted lines through the outer. Letters as in previous figures. The spiral filament which bears the gamete (x) producing sterile outgrowths. J. oc. 2.

FIG. 21. Two similar conjugating hyphæ, the inner not indicated by dotted lines.

FIG. 21*a*. Mature zygospore seen in optical section, its connection with the gamete (x) still attached. J. oc. 2.

Syncephalis tenuis Thaxter.

FIG. 22. General habit of mature fertile hypha, about one-sixth of its length indicated by dotted lines. D. oc. 4.

FIG. 23. Terminal portion of fertile hypha showing immature sporangial filaments, the terminal half of which is just beginning to form. D. oc. 4.

FIG. 24. Fertile head from which the basal halves of the sporangial filaments have been produced. D. oc. 4.

FIG. 25. Mature head of variety with larger spores. D. oc. 4.

FIGS. 26–29. Successive stages in development of sporangial filament. J. oc. 2.

FIG. 30. Separated spores of elongate type. J. oc. 2.

FIG. 31. Spore of large spored variety. J. oc. 2.

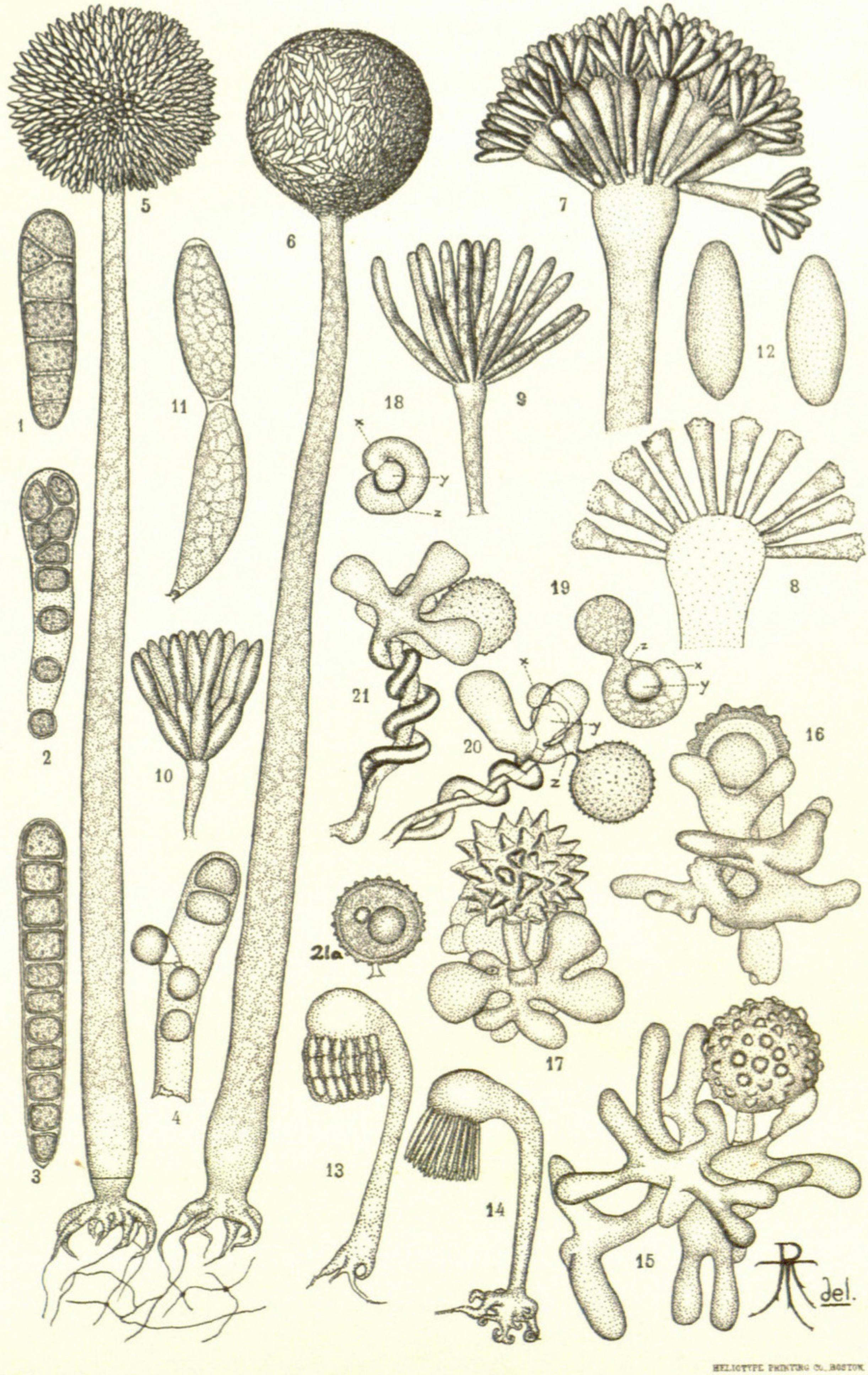
Syncephalis (*Microcephalis*) *pycnosperma* Thaxter.

FIG. 32. General habit of nearly mature fertile hypha. D. oc. 4.

FIG. 33. Terminal portion of fertile hypha showing numerous secondary sporophores from which the sporangial filaments are in process of formation. J. oc. 2.

FIG. 34. Mature head seen in optical section showing secondary sporophores, one of them furcate, from most of which the spores have fallen. J. oc. 2.

FIG. 35. Young sporangial filament showing two intermediary zones, the lower not completed, the upper nearly complete; *b*, the separation disk; *a*,



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