XXIII. *On the Occurrence of Conidial Fructification in the* Mucorini, *illustrated by* Choanephora. *By* D. D. Cunningham, *M.B., F.L.S., Surgeon H.M.'s Indian Army.*

(Plate XLVII.)

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In the year 1872, Mr. Currey described what he was then justified, by my drawings and descriptions, in regarding as a new genus of Mucedinous Fungi *. The object of the present paper is to show that this fungus, in place of being a member of the Mucedines, belongs to the Mucorini, and that M. de Bary's suggested analogy between the Mucorini and Ascomycetes, in respect of their fructification, is well founded, although the observations which originally suggested it have since been shown to be fallacious.

Ever since first encountering the plant in 1871 I have frequently observed it, especially during the rainy season, on the flowers of *Hibiscus*, and once or twice on those of other plants, such as *Zinnia*, &c. It was, however, only during the present season that I undertook its systematic study and cultivation, in order to determine more accurately its nature and relations.

As encountered under its natural conditions of growth, the plant covers the flowers which it affects with a dense crop of erect fructifying filaments, without any trace of aerial mycelium. It occurs most abundantly and in most luxuriant development at times when there is much moisture in the air, especially when, as is normally the case during the rains, there is an alternation of heavy showers with warm rainless intervals. Failing such conditions, clear weather, with cloudless nights and drenching dews, furnishes the circumstances under which it most commonly occurs. Constant heavy rain or absence of dew are apparently equally repressive to its development. It attacks the flowers immediately after they have matured, and may be found in great perfection covering them whilst still adherent to the flower-stalk and only exhibiting incipient symptoms of decay. Its presence certainly accelerates decay greatly, but it is a cause, not a consequence of advanced putrefaction, as it is not found to occur on flowers in which decomposition has advanced far previous to the access of the conidia.

The affected flowers are readily detected in the early morning by being more or less covered by a white bloom, due to the crowd of immature fructifying heads; but in the course of a few hours they become much less conspicuous, as the conidia, in ripening, pass from white to madder-brown, and finally to a deep purplish black. The entire field is found, on microscopic examination, to be occupied by a series of mulberry-like heads (fig. 1), each composed of numerous dense masses of conidia, varying in colour from snowy white to dark purple or black, and supported on a forest of shining colourless filaments. In addition to such mature forms, other specimens occur bearing a series of

* Journ. Linn. Soc., Bot. (June 20th, 1872) vol. xiii. p. 333, pl. vii.

colourless, hyaline, funnel-shaped processes, replacing the conidial masses (fig. 2), and with only a few conidia or membranous fragments adherent to them. Various immature forms are also represented by filaments, each terminated by a spherical dilatation, which gives origin either to a set of stalked heads covered with sterigmata and young conidia, or to a number of secondary dilatations producing such structures. The stems can be traced downwards to the epidermis of the corolla, from which they emerge without any trace of mycelial filaments appearing on the surface.

On detaching specimens of such fructifying filaments from the basis, and subjecting them to more minute examination, the following points may readily be determined regarding them. The filaments take origin from a more or less defined dilatation, which in its turn is connected with a slender mycelial twig penetrating the tissues of the corolla. The dilatation is generally situated immediately beneath the epidermis; sometimes, however, it appears on the surface. It is occasionally sharply defined from the parent mycelial twig by a septum, and in such circumstances comes to present a considerable resemblance to the basal dilatations in *Pilobolus*. In other cases, however, the transition is very gradual, and the wide base of the fructifying filament passes by insensible degrees into the mycelial twig. The dilatations in many instances, in addition to giving origin to the fertile filaments, produce one or two short blunt tubes (apparently abortive filaments), and in rare cases two perfect filaments may be present.

The stems vary considerably in length, but many measure as much as 8·25 millims. They are generally continuous tubes, but occasionally one or two septa may be present in their course. The superior extremity terminates in a more or less spherical dilatation. This either gives origin to a set of stalked secondary heads, which may be conveniently termed capitella, or, where the plant is luxuriantly developed, to a number of thick processes, each surmounted by a dilatation producing capitella. The capitella, when mature, measure about 0·325 millim. in transverse diameter; they are rounded above, and below pass insensibly into their supporting pedicels. Over the upper half of each a number of projecting spicules is developed. These gradually enlarge above, and the process terminates in the formation of sets of conidia attached to the capitellum by short stalks (fig. 3). As the conidia increase in size they gradually conceal the capitella, and form lobules of the mature mulberry-like fructification. From the comparatively large size of the plant, every step in the development can be readily and accurately followed; and there can be no doubt of the essentially conidial nature of the fructification. When the conidia are mature a septum forms a little beneath the upper extremity of each sterigma, so that the conidia, when detached, carry small portions of the latter with them.

The lower portion of the capitellum produces no sterigmata; but whilst the formation of conidia is occurring over the upper portion, its walls gradually thicken, and ultimately the line of demarcation between the two portions comes to be sharply defined by the projecting margin of the thickened membrane of the lower one. The fertile portion now comes to appear as though it were fitted into the other, as an acorn is into its cup. As the conidia are developed, the protoplasmic contents of the filaments pass successively upwards into the terminal dilatations, into the capitella, and finally into the conidia, leaving the former structures full of watery fluid. When the ripe conidia become detached, the thin membrane of the upper portion of the capitellum usually breaks up, leaving only delicate threads attached to the rim of the lower portion, or collapses and disappears entirely. Occasionally, however, it persists in the form of a filmy sac projecting from the cup of the lower portion (fig. 4). The persistent portions of the capitella now come to appear as a series of pedicillate funnels; and as conidia not unfrequently fall into them, or are carried down attached to the collapsing upper portion, it might readily be supposed that they had been developed within the funnels. Taking the development and structure of this form of fructification alone there would seem to be very conclusive grounds for regarding the plant as of a mucedinous nature; but the facts in regard to its anatomy and life-history, still to be described, show what false conclusions may be arrived at where one form alone of asexual fructification is employed as a basis for the classification of fungal organisms.

On proceeding to examine those portions of the plant which remain concealed within the tissues of its host, the following results are arrived at. The mycelial system is composed of a series of main tubes, which are branched, devoid of septa, and full of a granular yellowish protoplasm, in which a continuous streaming motion may be detected, and of a set of branched radicles or haustoria, taking origin from the branches of the main tubes. These radicles are at first full of contents similar to those of the main tubes; but they soon become emptied of these, and are, when mature, full of watery fluid and provided with a basal septum (fig. 20). The mycelium is therefore in every respect characteristic, not of the Mucedines, but of the Mucorini. The cells of the corollar tissue of the host do not appear to be penetrated either by the main tubes or by the radicles; but the latter are closely applied to them, and in some cases appear to adhere to them by special dilatations.

At certain points, where they approach the surface, the mycelial filaments give origin to slender twigs, which generally follow a course more or less parallel to the parent tubes. Such twigs are destined to produce the aerial conidiiferous filaments. After growing for some distance, they either terminate in a distinct dilatation or, more rarely, pass on directly into the aerial filaments. Sometimes the tips of the twigs force their way to the surface previous to forming their terminal dilatations; but the latter are generally produced immediately beneath the epidermis. In such cases the resistance which they encounter causes the filaments in many instances to follow a more or less contorted course. The dilatations are frequently directly continuous with their parent tubes; sometimes, however, they are eventually separated from them by transverse septa.

Such are the features commonly presented by the plant; but in certain cases the mycelium is capable of producing the apparatus of sexual production. This happens comparatively rarely, and I have as yet been unable to determine what the precise conditions are under which it occurs. In these cases the mycelial tubes, after having given origin to the common conidial fructification, produce a number of short branches, which, in place of growing in length, swell out into thick club-shaped processes (fig. 11). In most cases these arise directly from the mycelial tubes, and remain continuous with them; but in some instances they are borne on short stalks, and are separated from the parent tube by a septum. Sometimes the clubs are isolated, but in general several, and frequently a considerable number are produced, in close proximity to one another. When several are present, only one or two of them undergo further development, and the rest soon lose their contents and remain as a series of empty saccular protrusions around those which continue to grow (fig. 12). The latter enlarge considerably, and a great accumulation of granular protoplasm takes place within them. As this accumulation increases, the clubs gradually assume an arcuate outline, one side projecting outwards, whilst the other becomes deeply concave (fig. 14). The contents now accumulate towards the apex, and a septum forms beneath them, separating the process into two unequal portions. The upper portion is filled by a dense mass of granular protoplasm; the lower is hyaline, and distended with a clear watery fluid. Where two of the arcuate bodies are situated sufficiently close, their superior extremities approach one another like the blades of a curved pair of forceps, and the apices of the terminal cells are thus brought into close contact (fig. 13). In all cases in which I have been able to determine the point accurately, the opposed organs have been derived from two distinct mycelial filaments. In many cases, however, the relations of the processes and filaments are very much obscured by the presence of the abortive dilatations previously alluded to; and in some the appearances seemed to indicate that contact occasionally occurred between processes arising from the same filament. After the terminal cells have come into contact, the mutual pressure which they exert causes their opposed surfaces to become flattened out, so as eventually to appear as a transverse partition separating the two masses of protoplasmic contents. This partition is next absorbed, and the two masses become fused together. The result is the formation of an oblong body, which at first presents more or less distinct lobes, corresponding to its separate parent cells, but which soon becomes smoothly convex. The entire sexual apparatus at this stage has the form of two curved empty cells or suspensors, connected above by a convex keystone-like cell full of granular contents (fig. 15, *a*). A great increase in size ensues in this latter cell, but the growth does not take place uniformly through its entire mass. On the contrary, it is confined to the convex surface, which, in consequence, comes to project more and more prominently beyond the suspensors (fig. 15, *b*). The terminal surfaces of the latter, which were originally directed obliquely towards one another, alter their relative directions as the growth progresses. Their upper edges are more and more widely separated by the unequal increase in the intervening mass; and the surfaces eventually come to lie almost in the same plane, in place of diverging, as at first (fig. 15, *c*). The young zygospore now appears as an almost hemispherical body, bounded on one side by a flat face, corresponding with the terminal surfaces of the suspensors. This appearance is, however, transitory; for, as its growth continues, the zygospore gradually assumes a rounded outline, and eventually appears as a sphere supported on one side by two dilated suspensors (fig. 15, *d*). This sphere is filled with coarsely granular yellowish contents. These next become separated from the cell-wall and are invested by a distinct membrane (the future exospore), which rapidly increases in thickness and acquires a deep-brown colour, and a second delicate membrane forms within it. Whilst these processes of growth and development have been taking place in the membranes, another series of changes has been advancing in the contents. The contents are at first evenly granular, but, as maturation proceeds, a separation of oily globules takes place. These globules increase in number and size, and, gradually running together, are represented in the ripe zygospore by a single large drop of oil. The mature zygospores measure from 0·07 to 0·05 millim. in diameter. They are covered externally by a thin membrane belonging to the parent conjugating cells (fig. 16). Their intrinsic membranes consist of a thick dark-brown exospore and a delicate colourless endospore (fig. 16). None of those tubercles or irregular thickenings of the spore-membranes are present which are so conspicuous in the zygospores of other Mucorini; and the ripe structures resemble those of some of the Peronosporeæ rather than those of the nearest allies of the plant.

According to these observations, the process by which the zygospore of *Choanephora* is formed is intermediate in characters between those occurring in *Phycomyces* and *Piptocephalis*. The process in common with those of both these genera is carried out by the conjugation of two curved cells. It differs from that in *Piptocephalis* and agrees with that in *Phycomyces* in the simple nature of the union of the conjugating cells. On the other hand, it differs from that in *Phycomyces* and agrees with that in *Piptocephalis* in the nature of the phenomena occurring after conjugation, in the unequal growth of the combined mass, and the consequent development of a zygospore, which is not situated between two lateral suspensors, as in *Phycomyces*, but is attached by its basal aspect to the summit of these structures.

I have hitherto failed in successfully cultivating the zygospores. The difficulties in preserving such bodies in a tropical climate in conditions favourable to germination and, at the same time, secure from the attacks of fungi and of animal enemies of various kinds, are very great, and frequently almost insuperable.

The results of the study of the plant under its normal conditions are, then, to show that it possesses a mycelium and sexual reproductive apparatus of the recognized Mucorine type, but that the asexual fructification, in place of being sporangial, is of a truly conidial nature.

After having studied the features presented by the plant in its natural condition, I proceeded to undertake a prolonged series of artificial cultivations, with a view of, as far as possible, obtaining a complete knowledge of its life-history. Some of these cultivations were conducted on a large scale; but the results of these were corrected and checked by those of a much more extended series, in which the spores were isolated in such small numbers as to allow of ready individual identifications. Some of the latter cultivations were conducted on the cellular system, as recommended by MM. Van Tieghem and Le Monnier *; but the greater number were carried out on the plan originally introduced by M. de Bary, and so successfully practised by Brefeld †. There can be no question of the theoretical superiority of the cellular cultivations; and the procedure

* "Recherches sur les Mucorinées." Par MM. Ph. Van Tieghem et G. Le Monnier. Ann. des Sc. Nat. 5e série, t. xvii. p. 261.

† Botanische Untersuchungen über Schimmelpilze von Dr. Oscar Brefeld.

is, judging from MM. Van Tieghem and Le Monnier's experience, capable of giving excellent practical results. Working here, however, I have found that with many fungi it is impossible to provide sufficient nourishment in this way or, at all events, to furnish all the conditions necessary for complete development. A luxuriant mycelium is often produced, but fructification is seldom freely developed unless extra nutritive material be added; and the process of introducing this into the cell practically reduces the cultivation very much to one carried out on M. de Bary's method, where the spores are kept under continuous observation in a moist chamber containing both the microscope and cultivation. The cellular method, where successful, may allow of conclusions being more rapidly arrived at; but the other, when carried out with sufficient care, and repeated often enough, gives results which are very nearly, if not quite, as good.

The medium employed in the cultivations in the present case consisted of fresh decoctions of the corollæ of *Hibiscus*, and, in one or two instances, of boiled water alone.

The ripe conidia are obovate in form, and, as previously mentioned, generally have a small projection at the narrower extremity, formed by the tip of the parent sterigma (fig. 6). They vary considerably in size, but average specimens measure about 0·02 millim. by 0·011 millim. They consist of a purplish-brown exospore, containing a mass of granular protoplasm invested by a delicate endosporic layer. They present no distinct indications of a nucleus, either when first detached from the sterigmata or during the course of germination; but a clear space or vacuole may be present in the protoplasmic contents. When sown in water no change takes place in them, and germination does not occur. On their introduction into *Hibiscus*-decoction, the first phenomenon observed is the commencement of movement among the granules of the protoplasm. This begins almost immediately, and very soon attains considerable activity; the streaming of the protoplasm is in fact established so far as the limited area of the interior of the conidium will allow of such a phenomenon. A certain amount of enlargement of the cell now occurs; but this is slight as compared with that taking place in many Mucorini. In two cases in which the increase was measured the conidia, when first sown, were 0·018 by 0·012, and 0·02 by 0·012 millim.; and on germination were 0·02 by 0·02, and 0·02 by 0·015 millim. As a rule there is little or no increase in the length of the cell, but the transverse diameter increases until it nearly or quite equals the longitudinal one and the entire body assumes a spherical form.

The germinal tubes next make their exit. This process takes place very soon; in one case, where special observations were taken in regard to this point, some of the tubes had already attained a length of 0·025 millim. half an hour after the conidia were sown. The precise time of emission, however, varies, being considerably delayed in the case of conidia which have been kept for any length of time previous to being sowed; but germination usually occurs, at latest, within five hours, and, as a rule, takes place much sooner. The majority of conidia give origin to one or two tubes only; but in rare instances three are produced. The phenomena attending germination vary somewhat with the ripeness of the conidia. In immature specimens the exospore shows no distinct evidences of rupture, but appears rather to be pushed in front of the protruding tube and to blend insensibly with it. Where, however, the conidia are thoroughly ripe, a distinct rupture of the exospore occurs, the line of fracture being distinctly evident against the budding-tube (figs. 7, 8). As the latter increases in age, and as its walls become distinctly differentiated from its contents in the form of a definite membrane, this line of demarcation is gradually obscured, and finally disappears, the two membranes apparently being fused together.

The germinal tubes generally arise from the sides of the conidia, so that the axis of growth of the new plant is at right angles to that of the parent; occasionally, however, the tubes are emitted from one or other extremity (fig. 7). They are thick, and are usually undivided for some distance; but in some cases, as they escape from the conidia, they form a more or less marked dilatation, from which two filaments of equal size arise. The streaming of the protoplasm is very active in the young filaments, and, as the latter grow, more and more of the contents of the conidia pass into them. As the process continues, the conidia are gradually emptied, for some time, however, retaining a narrow peripheral layer of protoplasm, and ultimately being left as brown sacs full of watery fluid (fig. 8). The filaments continue to grow and ramify rapidly; and from the main system of tubes thus produced short lateral filaments are developed. These give off several branches, and, soon becoming emptied of their protoplasmic contents, are shut off at their origins by transverse septa. The main tubes are at first entirely devoid of septa; but, as they increase in length, the protoplasm advances within them, so as to leave large tracts occupied by watery fluid only; and in such tracts septa are occasionally formed.

After the mycelium has continued to grow for some time, fructification, in the form of aerial conidiiferous filaments, begins to be produced. The filaments, in the artificial cultivations, may be traced directly to the parent tubes, no intermediate dilatations, such as occur in the natural plant, being formed. Where the conditions of nutrition are very highly provided, the formation of reproductive bodies is delayed, and the mycelium continues to grow luxuriantly for a considerable time. Under fair nutritive conditions—where the decoction is a strong one—a crop of fructification is usually produced within twenty-four hours from the commencement of the cultivation; but this is more or less determined by the time of day at which the sowing of the conidia is effected, as it is only during the hours of darkness that the aerial filaments are produced. Supposing the sowing to have taken place at 10 A.M., the germinal tubes will generally have been emitted within an hour; and by sunset an abundant mycelium will have been developed. On the following morning at dawn (say at 5 A.M.) a crop of young conidial heads will be present; and this will mature during the course of the next few hours. The rapidity with which the conidia are formed and matured is very remarkable, and is rendered very manifest by the great change in colour which the heads undergo during ripening.

The conidial heads, when the plant is well nourished, are precisely similar to those of the uncultivated specimens; but the capitella are generally fewer in number, and the conidia of comparatively smaller size than in the latter. Every step in the development of the heads can here be accurately followed, and the nature of the process is seen to be as follows:—The aerial filaments swell out at the apex, and the protoplasm, which is continually streaming into them, accumulates in the dilated extremities. A series of prominences now appears on the dilatations, and each of its members, after growing outwards for a short distance, in its turn becomes dilated apically, accumulates protoplasm, and is ultimately converted into one of the capitella previously described. Over the upper hemisphere of each capitellum sterigmata are now developed, and the formation of conidia follows in due course (fig. 3). The only feature distinguishing the capitella of cultivated from those of natural specimens of the plant lies in the fact that the lower barren hemispheres of the former do not become so thickened as to form the distinct funnel-shaped structures so characteristic of the natural specimens subsequent to the fall of the conidia.

Such are the characters of the conidial fructification developed on a mycelium derived from the conidia of the natural plant cultivated in a medium affording abundant nutritive material. Where, however, a weak medium is employed, or where too many conidia are sown in a strong medium, or where the mycelium continues to produce fructification after the nutritive properties of the medium have been exhausted during the course of the cultivation, various abortive forms are developed deviating considerably from the normal type. In the natural plant the heads, as previously mentioned, frequently produce thirty or even more sterigmatous capitella, and those arising from a well-nourished mycelium in artificial cultivations may produce as many as fifteen or twenty. In those cases, however, in which nutrition is imperfect, only a small number of capitella are produced, and filaments are encountered with numbers diminishing through various degrees until we find specimens with only two capitella. The process of abortion does not, however, reach its climax here; for a further stage occurs in which no capitella are produced, and in which the dilated extremity of the filament gives direct origin to the sterigmata (fig. 5).

Numerous cultivations of conidia obtained in primary cultivations always gave a like result, however strong a nutritive medium was employed. Abundant fructification was frequently produced; but the heads were invariably of a poor type and the conidia of small size. Only a few capitella were produced in any case, and the proportion of heads failing to produce capitella at all was very large. Tertiary cultivations of conidia obtained from the previous series usually failed entirely to produce fructification. The conidia in many cases germinated, but the mycelium was, as a rule, very feeble and soon ceased to grow. Cultivated under the artificial conditions which were employed, the plant appears to undergo a progressive loss of vigour with each generation, leading to the dying out of the members of the third generation without any provision for reproduction.

The conidial form of fructification was, however, not the only one developed in these cultivations. On the contrary, two other forms, one sporangial the other chlamydosporous, were obtained.

The former of these occurs very frequently, and, in fact, under conditions to be presently described, it appears almost invariably. That this fructification really belongs to the same plant as the zygosporic and conidial forms previously described, there can, I believe, be no doubt. The grounds for this belief are as follow :—1. Numerous careful cultivations of limited numbers of conidia have given a mycelium producing such sporangia. 2. The cultivation of spores from a sporangium has resulted in the development of a mycelium bearing the conidial form of fructification. 3. Sporangial filaments have been traced to the same mycelial tubes as conidiiferous ones. 4. The conditions securing the development of sporangial filaments on a mycelium produced from conidia have been in a great degree determined. 5. Specimens of *Hibiscus*-decoction when exposed to the air, although they become the site of the development of various moulds, do not show any forms producing such sporangia save when the conidia of *Choanephora* have been introduced into them.

The first thing to be noted in reference to the sporangial fructification is, that it does not appear to occur on the plant in its natural state. The conditions under which it occurs are those in which the poorer types of conidial fructification are produced. It occurs along with these:—1. On mycelia growing on an exhausted medium; 2. On mycelia developed from the conidia produced in artificial cultivations. The sporangial fructification has never been obtained apart from the conidial form; but the proportions of the two forms on the same mycelium varies greatly in different instances. As a rule, however, the conidial heads on any mycelium considerably exceed the sporangial ones in number. Any cultivation in which conidial heads with very few or no capitella abound may be expected to produce sporangia. The two forms of fructification occur simultaneously; but in those cases where sporangia are produced on a mycelium derived from the natural plant, cultivated in a medium which was originally rich enough to secure the formation of well-developed conidial heads, the sporangia do not occur simultaneously with such heads, but along with the poorer types so frequently produced by those portions of mycelium which continue to grow and produce fructification on the partially exhausted medium.

The sporangial filaments do not, as a rule, attain a length equalling that of the conidial ones with which they are associated, and are frequently somewhat curved at the apex. The superior extremity of each filament dilates into a small rounded sporangium, which, from the presence of numerous calcareous particles, is conspicuously tuberculated (figs. 9 & 10). The sporangia are separated from the filaments by a transverse partition, which is sometimes flat, at others arched upwards into a small prominent columella (fig. 10). They vary somewhat in size, but, on an average, measure about 0·027 millim. in diameter. When first formed they are pale yellow, but as the spores mature they assume a fine deep Vandyke-brown colour. This colour is, in any case, due to that of the contained protoplasm or spores; for on the evacuation of these the sporangial membrane is seen to be colourless. The sporangia at first present an even rounded outline; but as the spores enlarge and approach maturity, they come to fill the cavity completely, and pressing upon the walls of the sporangium stretch them tightly, and cause them to assume a nodulated contour, corresponding with that of the contained mass of spores (fig. 9). When mature the sporangia rupture irregularly, the line of fracture being in general more or less vertical; and subsequently they disintegrate and disappear, leaving the spores free. The number of spores produced within any sporangium is very limited, seven or eight being a common number, although in rare instances there may be various numbers up to seventeen or eighteen. The spores vary greatly in size, even within the same sporangium, ranging from 0·02 × 0·013 millim. to 0·013 × 0·009 millim. When mature they generally present a more or less marked ovate form, one extremity being narrow and pointed, or slightly truncate, the other evenly rounded (fig. 10). They are of a rich brown colour, which is mainly due to the exospore, but which is intensified by the yellowish colour of the protoplasm. It is worthy of notice that the protoplasm is sometimes evenly diffused, while at others it is more or less aggregated into two distinct masses, one towards either extremity of the cell.

When sown in decoction of *Hibiscus*, the first change observed in the spores is a general diffusion of the protoplasm where this has been aggregated and a commencement of protoplasmic streaming; at the same time the cell increases somewhat in size, swelling out so as to assume a more or less rounded outline, and, finally, one or two germinal tubes are emitted, which soon begin to ramify in the fluid. This is all that I have been able to observe regarding their development save in one instance; in all the others the growth of the mycelium was rapidly arrested. As in the case of that developed from the poorer types of the conidial fructification, the mycelial filaments soon ceased to grow, and rapidly passed on to decomposition. In the exceptional case where this did not occur, the decoction in which the spores were sown was a very strong one. The spores germinated freely, producing a vigorous mycelium, which at the close of twenty-four hours was covered with a crop of well-developed polycapitellar conidial heads.

The fourth form of reproductive bodies belonging to *Choanephora* consists of true chlamydospores. These appear to occur much less frequently than any of the other forms of fructification. The only occasion on which they were observed was in a cultivation of the conidia of the naturally developed plant. In this case the decoction employed was a very weak one. The mycelium was very rapidly arrested in its growth; in fact in some cases no mycelium was formed, the germinal tube immediately on its exit from the conidium proceeding to form a chlamydospore. Whether formed in the course of mycelial filaments, or arising directly from conidia, the chlamydospores had the same characters. They were broadly fusiform in outline, and varied considerably in size (fig. 18). On an average they measured about 0·03 × 0·016 millim. They consisted of a mass of highly refractive finely molecular or clouded protoplasm, and stood out very conspicuously among the empty and collapsed filaments within which they were contained (figs. 17, 18). Their further development was not observed.

According to the observations detailed above, *Choanephora* is a genus of Mucorine fungi capable of producing four distinct forms of fructification. Of these, one is the result of an undoubted sexual process, while the remaining three are produced by the differentiation of portions of the general protoplasmic constituents filling the filaments of the plant. The following is a tabular statement of the forms of reproductive bodies :—

I. Sexual fructification	Zygospores.
II. Asexual fructification	Conidia, Sporangial spores, Chlamydospores.

The sporangial and chlamydosporic reproductive bodies appear to be more closely allied to one another than to the conidia. They are new and independent cells formed within the tubular system of the parent plant; but the conidia are merely isolated portions of that system, being, in fact, merely the tips of the terminal filaments of the aerial portion of the plant. The distinction, moreover, is not merely an anatomical one; the difference in the nature of the conditions favouring the development of the various forms of fructification indicates a physiological distinction also. The conidial fructification is the form characteristic of active nutrition and vegetative growth. Given a very rich nutritive medium and fully developed normal conidia, a luxuriant development of mycelium occurs, followed, sooner or later, by an abundance of conidial fructification. With diminishing nutrition there is progressively poorer mycelial development and less-developed conidial fructification. When this degeneration has reached its utmost limit, when the conidial fructification is reduced to its poorest and simplest type, the sporangia begin to make their appearance; and when conditions of nutrition are too greatly lowered even to allow of this, we find the chlamydosporic fructification providing for the preservation and diffusion of the plant.

The fact that the occurrence of various forms of fructification may be determined by conditions of nutrition should be constantly borne in mind in the study of organisms with polymorphic reproductive apparatus. The observation of the fact is not a new one; but the present instance appears to be one of the clearest demonstrations of it which has yet been afforded. It is not, however, merely in respect of this, although no doubt closely associated with it, that the phenomena exhibited by *Choanephora* deserve attention. They appear, in addition, to be capable of affording a possible explanation of certain conflicting conclusions which have been arrived at as the result of recent observations on the Mucorini conducted by highly competent authorities. I allude especially to those of MM. Brefeld, Van Tieghem, and Le Monnier. The former author has been led by the result of his observations to divide the Mucorini, or Zygomycetes as he prefers to style them, into two subfamilies, one of which he distinguishes as characterized by sporangial, the other by conidial asexual fructification. MM. Van Tieghem and Le Monnier, on the other hand, deny that true conidial fructification ever occurs in the Mucorini, and affirm that in those cases where it has been stated to occur, the supposed conidia were either monosporous sporangia or aerial chlamydospores.

The phenomena presented by *Choanephora* show both these conclusions to be incorrect; for, on the one hand, they demonstrate that true conidia really do occur in the Mucorini, and, on the other, that true conidia may occur in the same species with sporangia. Whilst thus proving the incorrectness of the conclusions of the distinguished observers just named, the present observations afford a means of reconciling the apparently conflicting results upon which these conclusions are founded. They do so by showing that although the observations of MM. Brefeld, Van Tieghem, and Le Monnier may have been correct, their conclusions are not so, merely because of the assumption that all the phenomena presented by the species under observation had presented themselves to each observer. M. Brefeld, because he never encountered any but a conidial form of asexual fructification in *Chætocladium* and *Piptocephalis,* assumes that sporangia never are produced in these genera; whilst MM. Van Tieghem and Le Monnier, believing that the forms of asexual fructification observed by them were either sporangial or chlamydosporic, at once conclude that conidial forms never can occur.

In reference to Brefeld's observations, it is of importance to note that he knew *Chætocladium* and *Piptocephalis*, his conidial genera, only as parasitic organisms, that is, only under conditions in which their nutrition was at its highest,—conditions which the phenomena of *Choanephora* justify us in regarding as those securing the production of a luxuriant mycelium, occasionally of the sexual fructification, and constantly of the conidial form of asexual fructification.

MM. Van Tieghem and Le Monnier, however, studied one of these genera, *Chætocladium*, under different circumstances. They observed it not only as a parasite on other Mucorini, but as growing independently and deriving its nourishment directly from the surrounding media. In reference to the conclusions of these observers regarding the nature of the fructification in this genus and in *Piptocephalis*, three possibilities suggest themselves in endeavouring to reconcile their statements with those of Brefeld. It may be (1) that in both genera they only met with true sporangial fructification; or that (2) they encountered both sporangial and conidial forms; or possibly even (3) that in certain cases they met with conidial forms alone. It may appear to be presumptuous to suggest the latter two possibilities; but when carefully studied in connexion with the present series of observations, their interpretations of many of the phenomena actually observed appear to have been in some degree influenced by a foregone conclusion of the impossibility of the occurrence of conidia. In reference to the possibility of the form of fructification observed by MM. Van Tieghem and Le Monnier having been of a different nature from that obtained by Brefeld in his cultivations, it may be noted that the former authors give an account of the phenomena attending germination in *Chætocladium* differing materially from that furnished by Brefeld. They affirm that germination occurs with hardly any antecedent enlargement of the spores; Brefeld describes the conidia as enlarging considerably prior to germinating.

The grounds on which there is reason to suspect that the interpretation of phenomena given by MM. Van Tieghem and Le Monnier has occasionally been somewhat influenced by a foregone conclusion, and by a desire to avoid the complication incident on the recognition of two subfamilies of Mucorini, as proposed by Brefeld, must be considered somewhat in detail in order to be appreciated.

In reference to *Chætocladium*, these writers allow that the bodies which they regard as sporangia invariably contain only a single spore. Here the question at once arises, How are monosporous sporangia to be distinguished from conidia? MM. Van Tieghem and Le Monnier apparently distinguish them by the fact that in germinating, or when exposed to pressure, the contents of the cell, if of a sporangic nature, escape, partially or completely, as a homogeneous spore-like body. But such a phenomenon may surely occur in conidia or spores in which there has been a distinct formation of a firm exospore including an endosporic sac. If one look for examples of such an occurrence among Mucorini, although cases of complete escape of the endospore are perhaps unknown, there is no great difficulty in obtaining examples of partial escape. Turning to MM. Van Tieghem and Le Monnier's own account of the germination of the sporangial spores of

Phycomyces, we find it stated :—" Si la spore jeune n'avait pas acquis de double contour, il n'y a pas d'exospore percée par le tube, et le contour externe de la spore est seulement plus noir que celui du filament qui en procède ; mais si la membrane s'était déjà séparée du protoplasme par un contour interne, la spore, en se dilatant, brise un exospore, qui se décolle souvent sur tout le pourtour et continue à l'envelopper en partie." If we compare this description and the illustration accompanying it with those referring to the phenomena occurring in the case of the so-called sporangia of *Chætocladium*, it is evident that the difference in the two cases is one of degree and not of kind.

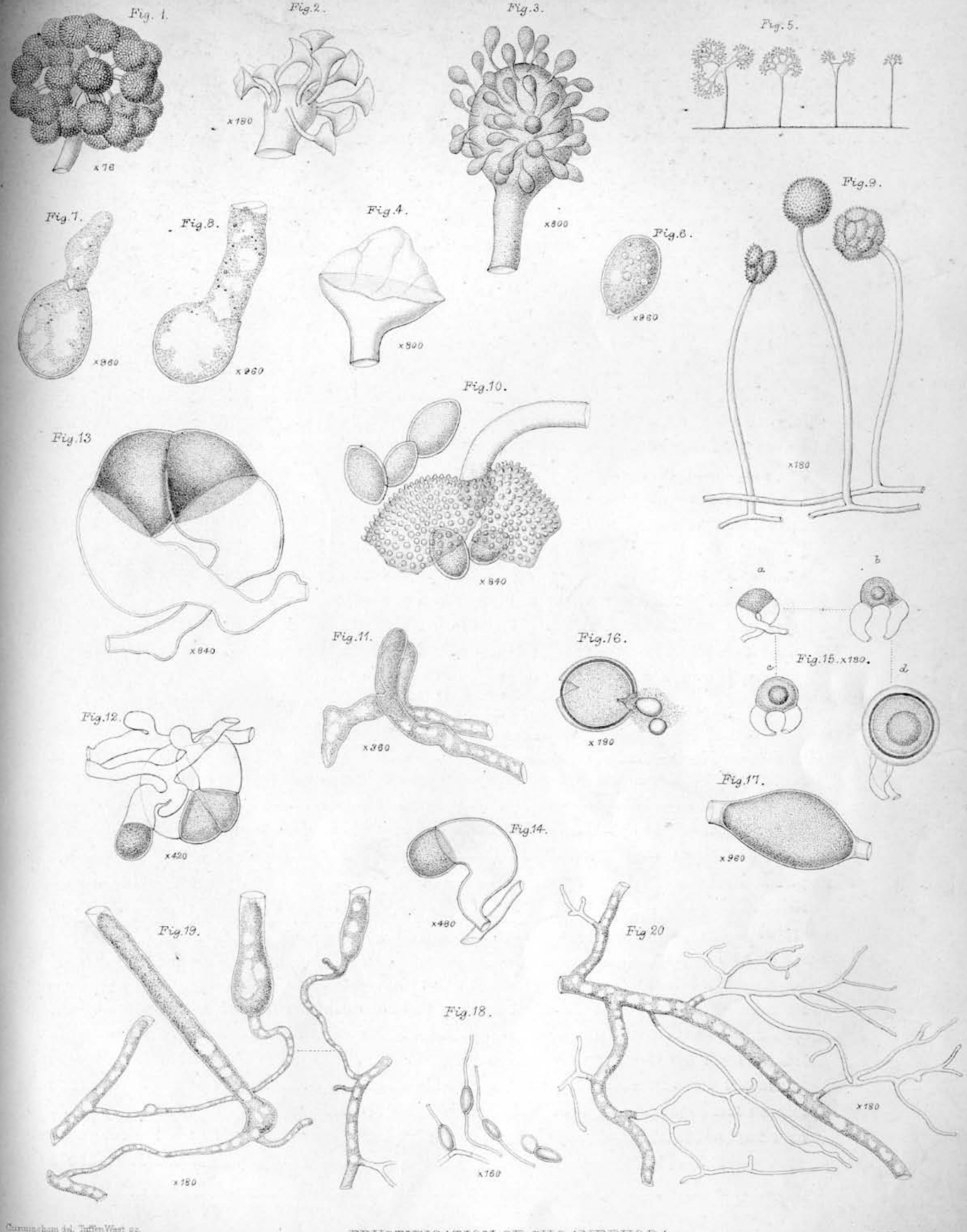
In regard to the phenomena presented by *Piptocephalis*, it is evident that further observation is yet wanting. To those not already convinced of the necessary absence of conidia, neither the descriptions nor illustrations furnished by MM. Van Tieghem and Le Monnier afford satisfactory evidence of the sporangial nature of the fructification. That the sporoid cells are not formed successively by continuous budding, like the conidia of *Penicillium* and *Aspergillus*, but by the segmentation of previously continuous rod-like bodies, all observers are agreed ; but that this affords definite proof of the sporangial nature of the fructification is certainly not a fair conclusion. A very similar process occurs in the filaments of *Oidium lactis* and in the ascal spores of certain *Sphæriæ*, where the filaments or cells breaking up into a series of conidia or secondary spores, as the case may be, can in no sense be regarded as sporangia. There is another difficulty, moreover, in the way of accepting the interpretation proposed by MM. Van Tieghem and Le Monnier. This lies in the fact that they affirm that the spores are held together by a substance similar to that occupying the interspaces between the spores in the sporangia of other Mucorini. It is somewhat difficult to realize in what the similarity lies, seeing that in other Mucorini the special function of the material appears to be to ensure the separation and diffusion of the spores due to its property of swelling up in contact with water, whereas here it is supposed to hold them together although immersed in a globule of water.

The same tendency to insist on the impossibility of the occurrence of conidial fructification appears more or less in the account given by the same authors of the phenomena observed by them in *Mortierella* and *Syncephalis*. The difficulties in the way of accepting their conclusions regarding the nature of the fructification in the latter genus are precisely those alluded to in connexion with *Piptocephalis*. With regard to both genera it may, moreover, be remarked, that even if the observers be correct in denying the conidial nature of the fructification, it is not clear on what grounds they distinguish it as sporangial rather than chlamydosporous. As to *Mortierella*, the doubtful interpretation does not refer to sporangia, but to certain bodies which they regard as aerial chlamydospores. No doubt their observations regarding the occasional formation of such bodies beneath the termination of the parent filament would, if confirmed, be practically conclusive. Confirmation is, however, certainly required, as the whole character and history of the development is otherwise strongly suggestive of the conidial nature of the bodies in question. It is in this respect very significant that the aerial chlamydospores are described as occurring under conditions of high nutrition, not on the same mycelium with the other recognized form of chlamydospores, and even, to a certain extent, in an inverse relation to the sporangia. The description, too, of the different degrees of development of the filaments and dilatations bearing the aerial chlamydospores recalls very forcibly the phenomena occurring in the case of the unequivocally conidial fructification of *Choanephora*.

The above observations have not been made with any desire to detract from the value of the brilliant series of observations recorded by MM. Van Tieghem and Le Monnier, but simply with a view to pointing out that the subject cannot yet be regarded as satisfactorily decided. It is not, of course, to be assumed that because one genus of Mucorini has been shown to possess both sporangial and conidial forms of fructification, all other genera must necessarily do so also. Still as one has been shown to do so, and as the production of the various forms of fructification has been shown to be closely connected with variations in the conditions of the nutrition of the plant, there are some grounds for suspecting that the phenomenon may not be an exceptional one, and there is certainly reason for renewed investigation in order to determine the point.

DESCRIPTION OF PLATE XLVII.

Fig. 1. Mature conidial head of *Choanephora*. × 76.
Fig. 2. Head after the fall of the conidia. × 180.
Fig. 3. Immature capitellum with young conidia. × 800.
Fig. 4. Capitellum after fall of conidia. × 800.
Fig. 5. Diagram of the various types of conidial heads.
Fig. 6. Mature conidium. × 960.
Fig. 7. Conidium and young germinal tube. × 960.
Fig. 8. Conidium and portion of young mycelium. × 960.
Fig. 9. Sporangial fructification. × 180.
Fig. 10. Ruptured sporangium and mature spores. × 840.
Fig. 11. Commencement of sexual process; formation of club-shaped dilatations on the mycelium. × 360.
Fig. 12. Conjugation of sexual cells; isolated sexual cell and empty dilatations. × 420.
Fig. 13. Sexual cells in contact. × 840.
Fig. 14. Mature sexual cell, showing arcuate form. × 480.
Fig. 15. *a*, Conjugated cells; *b*, commencing growth of the zygospore; *c*, continued development of the zygospore; *d*, mature zygospore. × 180.
Fig. 16. Ruptured zygospore, with escape of oil and granular protoplasm. × 180.
Fig. 17. Chlamydospore. × 960.
Fig. 18. Chlamydospores and mycelial filaments. × 180.
Fig. 19. Origin of conidiiferous filaments. × 180.
Fig. 20. Mycelial filaments. × 180.



Cunningham del. Tuffen West sc.

FRUCTIFICATION OF CHOANEPHORA.

West Newman & Co imp.