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A METHOD FOR THE MEASUREMENT OF THE INTERNAL EXPOSED SURFACE OF FOLIAGE LEAVES

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While certain authors have pointed out the probable importance of the surface exposed along intercellular spaces of leaves, no published paper seems to have dealt with the measurement of these areas. The obvious significance of this interior surface in relation to the leaf's activities seemed to warrant an attempt at its measurement. Though it was recognized that variations in structure would lessen the value of such findings, a method of procedure has been developed whereby helpful data may be secured.

The leaves of selected Iowa mesophytes were first studied and subsequently certain xeromorphic forms were surveyed for comparison. This introductory paper, however, is offered primarily to present a statement of the method employed in the measurement of a foliage leaf possessing both palisade and spongy mesophyll.

From small rectangles of leaves embedded in paraffin, transverse and tangential* sections were cut, usually ten or twelve microns thick, and stained with Delafield's haematoxylin and safranin. From these sections sample areas (3,600 μ^2) lying between veins were selected for measurement. Camera lucida drawings of the several cell layers were used where direct measurement could not be undertaken. Drawings were generally made with 10x ocular and 90x objective though occasionally the 40x objective was used where the cells were large.

A. MEASUREMENT OF THE MESOMORPHIC LEAF

Computation of the ratio, R, of the internal exposed surface to the external exposed surface of a leaf having both palisade and spongy mesophyll, may be calculated by substituting in the formula,

$$R = \frac{\Sigma(ab \ldots a_n b_n) + 1(cd + 2\frac{ef}{g}) + \frac{hi}{j,}}{2k}$$

the values obtained in each of the measurements designated by letters as outlined below.

*Section parallel to flat surface of leaf (1, 2).

The Palisade

1. The average length of ten cells in the upper palisade layer was determined by direct microscopic measurement from transverse sections and = *a*, (Plate III, fig. 1).

2. Areas were selected* from the tangential section, showing the palisade cells in cross section, and a camera lucida drawing made of each sample. The total length of the palisade cell walls exposed against the intercellular space was found for each sample area by running over the lines representing exposed walls with a Keuffel and Esser No. 1692 chartometer. The average per sample of such lengths = *b*, (Plate III, fig. 2).

3. The measurements as in *a* were repeated for the second palisade layer and = a_1, (Plate III, fig. 1).

4. The measurements as in *b* were repeated on the drawings (tangential) of the second palisade layer and = b_1, (Plate III, fig. 3).

The Spongy Mesophyll

(*Horizontally Exposed Surface*)

1. Camera lucida drawings were made showing the cell walls of selected areas of the spongy mesophyll seen in the transverse section of the leaf. Each drawing was a sample area which included a full zone of spongy mesophyll from palisade to lower epidermis, and had a width of 60 μ. The total length of all completely exposed walls per cell making an angle not greater than 45° with the vertical was found by running over such portions of the lines with the chartometer. The total number of these vertically inclined cell walls thus measured in each sample was counted and the average length of the curved vertically exposed wall per spongy mesophyll cell = *c*, (Plate III, fig. 4).

2. Camera lucida drawings of the spongy mesophyll as seen in the tangential section were made and the total length of the exposed cell walls in each sample was measured with the chartometer and an average of the wall lengths exposed per sample = *d*, (Plate III, fig. 5).

(*Dorsi-ventral Exposed Surface*)

1. The area of the cells outlined in the sample drawing for *d* (tangential section of sponge) was found by planimeter (3),

*Five sample drawings were made wherever sampling was done by drawing.

(Keuffel and Esser planimeter No. 4238) and the average area per sample = *e*, (Plate III, fig. 5).

2. From the drawings for *c* the length of the walls *exposed* along intercellular space and making an angle greater than 45° with the vertical was measured with the chartometer. The total length of these walls for all the samples = *f*, (Plate III, fig. 4).*

3. From the drawings used for *c* the total length of walls (*exposed* and *non-exposed*) which made an angle with the vertical of greater than 45° was measured with the chartometer.* This value = *g*, (Plate III, fig. 4).

From the drawings used for *c* the average number of layers of cells which composed the spongy mesophyll was determined and that number = *l*, (Plate III, fig. 4).

The Inner Epidermis Wall

1. The area of the intercellular space per sample was found by subtracting the area *e* from the sample area (3,600 μ^2). This represented part of the area of the lower epidermis exposed inwardly against the intercellular spaces and = *h*, (Plate III, fig. 5).

2. From the drawings used for *c* the length of inner walls of the lower epidermis cells in each sample area was measured with the chartometer. The average of the total inner wall per sample was found and this number = *i*, (Plate III, fig. 4).

3. The length of the side of the sample area = $j = \sqrt{k}$

The External Surface

A sample area measured on the surface of the leaf = *k*, (k = 3,600 μ^2).

Results

Application of this method to a number of selected leaves gave for R values ranging from 7.8 to 31.3 as recently published (4).

Discussion of Method

Inasmuch as the above method is based upon drawings of leaf material which has been treated with histological reagents, sectioned, and stained, this may have caused alteration of the walls of the living cells. Tests with free hand sections of living leaves were used to determine the effects of commonly used killing reagents upon the cell wall. However, careful measurements before, during,

*When part of the lower wall of a cell was also part of the upper boundary of the cell below it, both walls were included in the total though they appeared as a single line in the drawing. If the same number of samples are not measured in *f* and *g* averages must be used instead of totals.

and after treatment showed neither extension nor shrinkage of the cell wall, though the protoplasts were altered by some treatments (5).

Accuracy is further dependent upon the histological technique used in making the slides. Poorly sectioned material in which the connections between cells are broken will result in great inaccuracies of measurement. Vigorous movements of the slide in liquid reagents after the paraffin has been dissolved tend to wash unanchored cells out of the sections. Also the cell walls must be well stained, otherwise the thin line of wall may be obscured by heavily stained chloroplasts or cell inclusions.

In making the camera lucida drawings, a selected section of the material was placed in focus and the drawing completed without changing the original focus. Only cell walls which lay clearly within the focal depth were drawn. Such a drawing included a section of 2 μ thickness, and overlying or underlying walls were avoided.

Accuracy of the instruments was checked against a Keuffel and Esser full divided Paragon scale, with 500 μ divisions, as a standard. For the measuring instruments used, the error varied between 0.5% and 2.5% depending upon the cell size and magnification. The largest error resulted from use of the chartometer in measuring drawings of cross sections of palisade cells. Under such conditions the error was reduced by taking readings only after the measurement of an entire sample.

Validity of this method as a whole as well as the total error due to instruments, was found by calculating the internal exposed surface of a hypothetical leaf of regular dimensions. By constructing sample sections through the leaf according to specifications and applying the method here described, accuracy of the formula was established. Since this check showed an error less than 2%, we may conclude that the method as outlined is reasonably accurate and may safely be used in leaf measurement. Relatively larger errors occur in the application of the method to the spongy mesophyll than to other leaf parts but such errors in leaves which contain a fair development of palisade are of less significance because the actual surface exposed in the sponge is small compared to that of the palisade, or in the sample volume as a whole.

If the leaves selected are sun leaves and from the upper part of the plant (2), it is probable that the ratio approaches the maximum for that species. Leaves of lower insertion will probably have

lower ratios than sun leaves of higher insertion due to the greater xeromorphy of the latter type of leaf (6). All samples should be selected from a given region of the blade as in certain species there may be progressive variation in thickness from base to apex (7).

Interrupting structures, other than veins, such as oil glands, etc., are deducted automatically when so small that they appear in the drawings. If the intrusions are very large, other methods can be worked out by the investigator to suit the situation.

B. MEASUREMENT OF STRONGLY XEROMORPHIC LEAVES

Leaves which are composed of palisade tissue only may be measured by following the simplified formula:

$$R = \frac{\Sigma(ab \ldots . a_n b_n)^*}{2k}$$

C. MEASUREMENT OF SUCCULENT LEAVES

Where the leaf is composed entirely of sponge tissue the formula is:

$$R = \frac{1(cd + 2\,\frac{ef}{g}) + \frac{2\,hi}{\sqrt{k}}}{2k}$$

If there are no horizontally running intercellular spaces in the latter type of leaf the formula becomes simply:

$$R = \frac{1cd + \frac{2\,hi}{\sqrt{k}}}{2k}$$

D. SHORT METHOD FOR MESOMORPHIC LEAVES

Under certain conditions the investigator may be satisfied with approximate values for *R* and hence may prefer to use a shortened method for making a rough determination. The formula

$$R = \frac{AB + Cd + \frac{k}{1.56}L + \frac{k}{2.4}}{2k}$$

may be followed in making the measurements when *A* = the entire

*The exposed epidermal surfaces are too small to affect greatly the final results where thick leaves are concerned.

depth of the palisade tissue, B = the average of samples taken from the various layers of palisade and measured as in b, C = the total depth of sponge (using ocular micrometer), d = the average of samples taken as in long method, L determined by count as in long method, and k the same as in the long method. Our calculations based on the variations in nine species show that the application of the short formula to mesophytic types of leaves involves an error in the measurement of the horizontal surface of the spongy mesophyll of about 10% of the total internal surface.

E. CONCLUSIONS

Except for veins, application of the internal-external surface ratio to the entire leaf surface will readily give the internal exposed surface of the whole leaf. While deductions for veins in a given leaf would subtract considerably from the calculated total internal surface, it should be recalled that such deductions have not usually been made in the published studies on transpiration, photosynthesis and other leaf activities where external leaf surface has been used as a basis of comparison.

Inasmuch as the oxygen used in respiration, the carbon dioxide used in photosynthesis, and the water lost in transpiration must pass through the cellulose wall of the internal exposed surface and also through the cell membrane against it, it seems that the use of this internal-external surface ratio as a basis of comparing leaf activities would give more valuable results than their expression in terms of external leaf surface.

The writer wishes to thank Professor Robert B. Wylie for suggesting the problem, supplying microscope slides, and in every way assisting in this work.

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PLATE III

The figures are accurate copies of camera lucida drawings of the various tissues of a sun leaf of *Syringa vulgaris*. Sample areas indicated by the bounding lines are reduced one-half. With the exception of figure 1, the drawings are those from an actual series used in the measurement of the internal surface of a Syringa leaf and illustrate the samplings requisite for application of the method. On each drawing is indicated a single measurement of the type to be applied to the sample as a whole.

Fig. 1. Transverse section of leaf through palisade tissue.

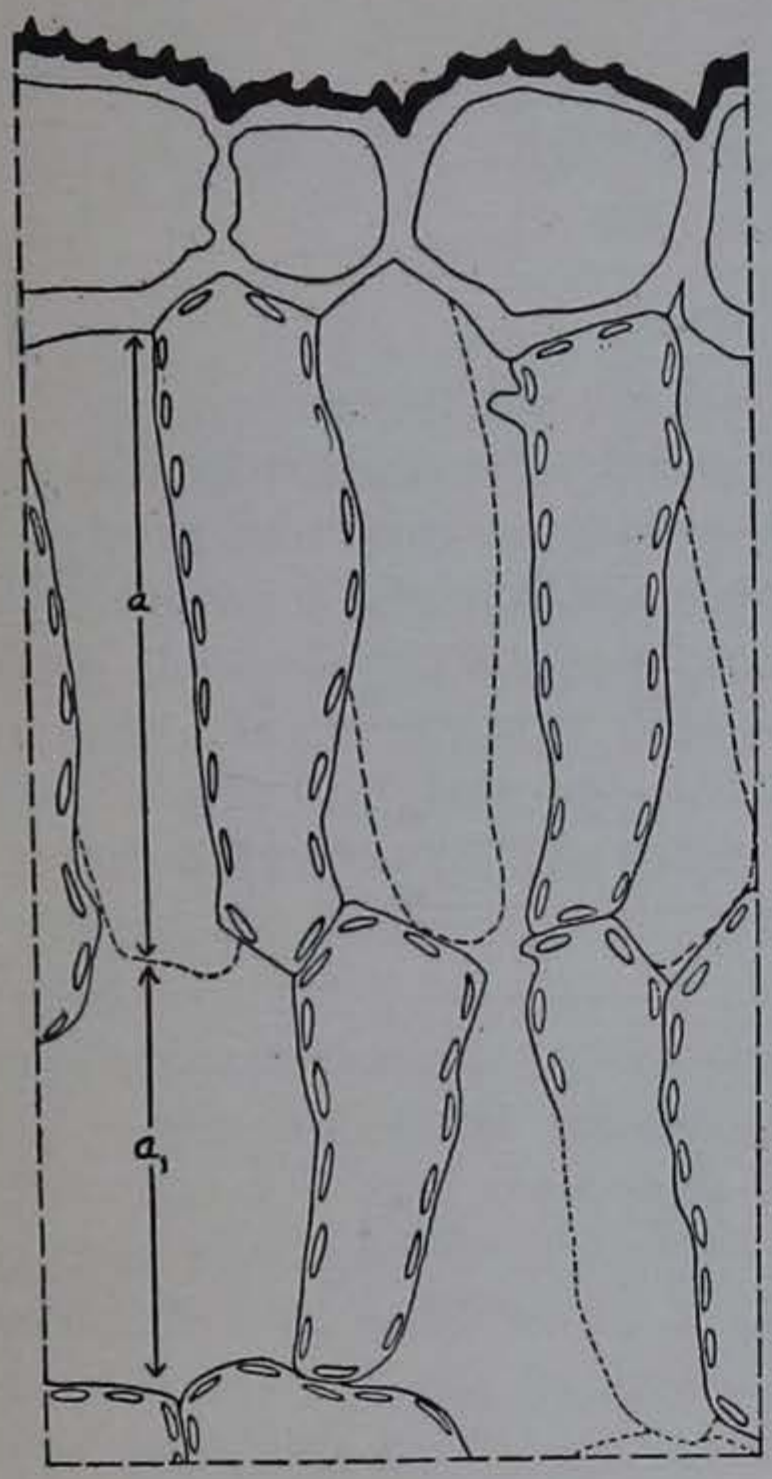
Fig. 2. Tangential section of leaf through upper layer of palisade tissue, used for measurement *b*.

Fig. 3. Tangential section through lower layer of palisade tissue.

Fig. 4. Transverse section of leaf through spongy mesophyll, used for measurements *c*, *f*, *g*, *i*, and *l* (represented by numbers 1, 2, and 3).

Fig. 5. Tangential section of leaf through spongy mesophyll, used for measurements *d*, *e*, and *h*.

PLATE III



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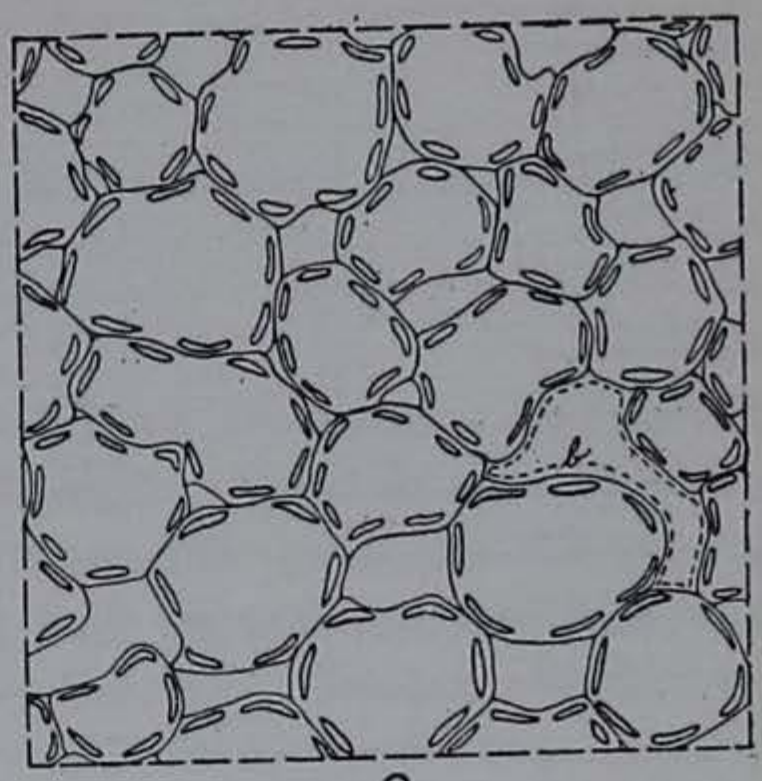

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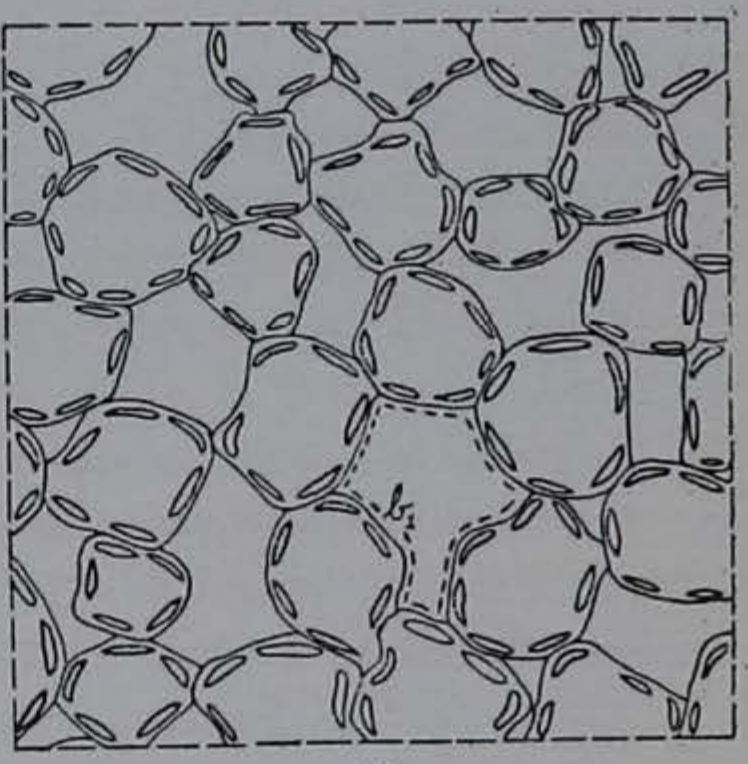

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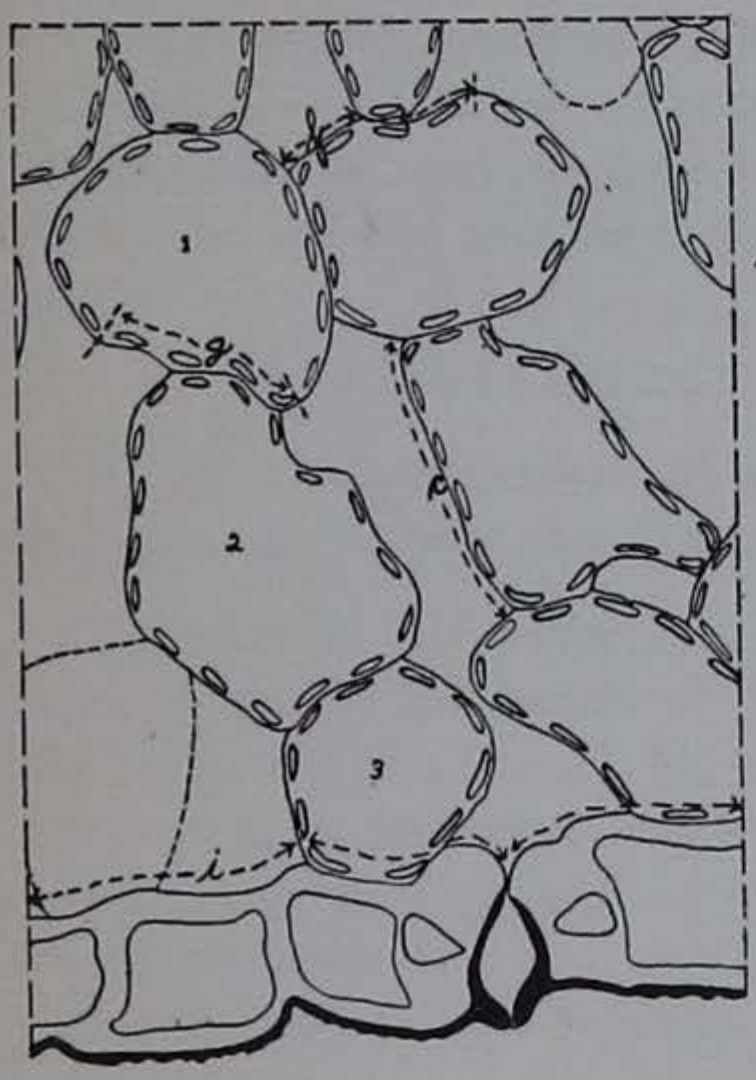
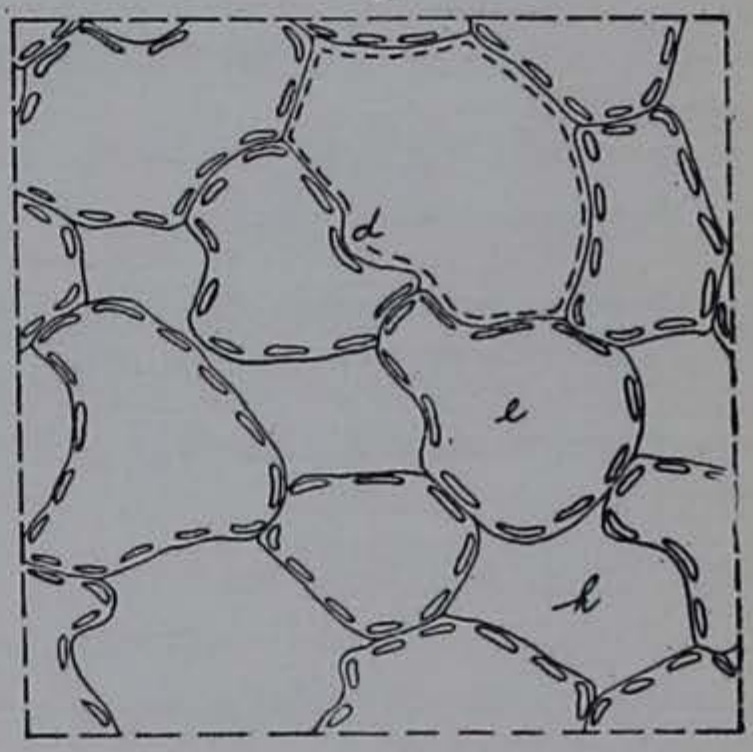


4

5



NOTES ON THE LIFE HISTORY OF APHANIZOMENON FLOS-AQUAE

EARL T. ROSE

With few exceptions, the lakes of Iowa are subject to seasonal growths of objectionable blue-green algae. The two species occurring most commonly are *Aphanizomenon flos-aquae* and *Microcystis aeruginosa*. While the *Microcystis* may be extremely offensive at times, the *Aphanizomenon* is usually the chief offender. With some variation due to seasonal differences, it usually becomes abundant about the first of July, appearing as small green flakes floating on or near the surface of the water. The alga reproduces with extreme rapidity and during the hot, dry periods of later summer and fall accumulates in thick mats on the surface of the water on the leeward sides of the lakes, where decomposition begins. This results in the production of an extremely offensive odor and the death of numerous fish and crustacea, partly through exhaustion of the oxygen, partly by the liberation of toxic substances released by the decomposition, as shown by Prescott. (10) It is known that these offensive growths can be controlled without injury to fish and fish-food organisms by the judicious use of copper-sulfate or chlorine, provided proper consideration is given to temperature, alkalinity of the water and other variable factors. In view of the importance of the algal species named, however, it is surprising that practically no information is available concerning their life histories. In order partially to supply this lack the following observations are reported.

COLLECTION AND CULTURE

Study of a large number of lakes in which *Aphanizomenon* is abundant suggests that the following factors favor its growth:

1. Alkalinity of the water. In all lakes in which the growth is most offensive the waters are strongly alkaline, usually ranging between pH 8 and pH 11, usually 150–300 p.p.m. alkalinity expressed as calcium carbonate.
2. Pollution. Sewage and excreta from farm animals is an important factor favoring excessive growth.
3. Shallow water. The relation here is probably one of tempera-

ture. A shallow lake becomes much warmer than a deep lake. *Aphanizomenon* and other blue-green algae are able to thrive in such warm water. Probably the most important single factor.

4. A deep layer of mud on the bottom. This favors the growth of *Aphanizomenon*, perhaps by providing a favorable place for the overwintering of the species, both as vegetative filaments and in the akinete stage.

All of these conditions occur at Silver Lake in Dickinson County. The material for study was largely secured from this lake.

Aphanizomenon seems never to have been cultured successfully. Repeated attempts to grow it in various culture solutions which have been recommended for blue-green algae, made up with distilled water and nutrient salts or filtered lake water, resulted in failure when the ordinary vegetative flakes, or filaments from these, were introduced into the culture solutions. It was therefore thought advisable to attempt to grow the species from akinetes. Akinetes are formed abundantly, and it has been assumed, although without experimental evidence, that these structures serve to initiate each new season's growth. Such an assumption is, of course, reasonable.

Some akinetes are formed during the summer, but they become abundant in late summer and fall and spore formation occurs until winter.

Silver Lake had supported an extremely abundant growth of *Aphanizomenon* during the season of 1933, and the algae were still abundant in December, when the lake was partially frozen over. In some areas the ice was colored a brilliant green by the included algae. In the open water, vigorous-looking flakes were abundant. These were collected with a plankton net and brought back to the laboratory. Examination showed that most of the filaments in these flakes contained one or more large akinetes. Where more than one akinete was present in a single filament they often occurred in pairs, a situation not heretofore reported. When either solitary or in pairs the individual akinetes were often longer than the dimensions given in the literature, 35–80 μ (Smith (11)), attaining, in some cases, 115 μ. This seems to suggest that the akinetes enlarge substantially before maturation, and that maturation does not occur until cold weather. All of the following notes are based on material collected in December, the akinetes in the summer collec-

tions, although abundant and within the range of the ordinary dimensions cited, failing completely to germinate.

All collections were kept in the cold temperature room at about 5° C in the original collection jars containing the akinetes and lake water, and removed only for obtaining material for study. No results were obtained by using methods described for germination and development of other species of blue-green algae which have been investigated (5, 6, 7, 8, 12). Petri dish and flask cultures using lake water and various recommended culture solutions were not effective; although some germination was obtained, subsequent development failed to occur.

On one occasion a small vial (10 ml. shell vial) containing many akinetes of *Aphanizomenon* was unintentionally left on the laboratory table for two days, consequently being exposed to a little direct sunlight, and room temperature. The vial was nearly full of the spore material in lake water and tightly stoppered. Upon examination it was discovered that many of the akinetes had germinated. The various stages of germination and development to be noted were taken from this and material treated in a similar manner.

SPORE GERMINATION

In view of the fact that flakes of *Aphanizomenon* are seldom, if ever, collected in early spring by the plankton net, it may be assumed that the ordinary vegetative progagation by fragmentation of the flake is not the usual method of reproduction after the winter months. When flakes are obtained in the spring they usually consist of a mass of akinetes in lateral contact with one another having but a few vegetative cells adhering. During mild winters many colonies doubtless continue their vegetative existence until the following summer.

Two methods of akinete germination have been noted and they coincide to some extent with the observations of Fritsch (4) on *Anabaena Azollae*, and with those of Spratt (12) on *Anabaena Cycadeae*.

The first method of germination, which I believe the more usual, involves a combination of two forces, first, that exerted by the swelling of the highly hydrophilic mucilaginous substance which surrounds the endospore, and second, the endospore itself divides, forming a chain of several cells. This results in the rupture of the

exospore and the release of the young sporeling. The cells of these filaments, as well as those formed by the second method, are all of the same general appearance, being broader than they are long. Such filaments would be classed as belonging to the genus *Trichodesmium*. Clusters of similar filaments are common in the early stages of development in *Aphanizomenon* (Plate IV, fig. 17), merging by gradual degrees as the filaments mature into typical vegetative *Aphanizomenon* filaments.

The second method of germination, which, judging from the material and data at hand, seems to be almost as prevalent as the first, consists of the rupturing of the wall of the exospore, and the emergence of the endospore through the rupture. The endospore, or sporeling, is in this case similar to the young sporeling described above except that division of the protoplast is not evident (Plate IV). In this method of germination, one end of the exospore is forced open by pressure exerted upon it by the expansion of the mucilaginous substance which surrounds the endospore. The presence of this mucilaginous substance may be demonstrated by staining with fast-green. The endospore is then forced from the exospore by the rapidly swelling mucilaginous material, the whole process taking approximately twenty-four hours. The protoplast then divides by the formation of septa which grow inward from the lateral walls and thus becomes a young trichome which rapidly increases in length.

There is some evidence that the trichomes of *Aphanizomenon* have two cellular investments or envelopes—an inner investment which completely encloses the protoplasts of the cells, and from which the septations arise during cell division, and an outer investment which is simply a cylindrical sheath which is derived from the inner investment (Plate IV, fig. 8). A somewhat similar account is recorded by Fritsch (4) for *Anabaena*; however, some differences may be noted. In *Anabaena* the outer investment is split into two fresh sheaths on cell division, by the development of an intercellular septum from the inner investment.

FORMATION OF THE FLAKE

One of the characteristics of the Cyanophyceae is the usual presence of a gelatinous or mucilaginous substance surrounding the filaments or cells. This characteristic is not easily observable in *Aphanizomenon*, but its habit of growth is evidence of the presence

of this cohesive material. Mature flakes usually consist of hundreds of trichomes. Flake formation is facilitated by the simultaneous germination of a group of laterally attached akinetes as a unit, the young sporelings becoming attached at the moment of their germination. This has been observed many times (Plate IV, figs. 9, 17; Plate V).

The young filaments described above under akinete germination were mostly from akinetes which had become separated from one another by shaking and handling, consequently the joining of filaments necessitated their union some time after their emergence from the exposure. The fact that many perfectly normal flakes were formed in the cultures further substantiates the existence of a cohesive substance around the filaments, and suggests that its function is to enable the filaments to adhere to each other.

HETEROCYSTS

The young sporeling, soon after having divided to form several cells, often develops a heterocyst, usually toward the middle of the trichome. The writer is not prepared to state that the heterocysts have a function in *Aphanizomenon*. From the experience with this blue-green it would seem that it is practically functionless. Observation of heterocysts from cultures and from material collected in the natural habitat have never given any evidence which might lead one to believe they might function as gonidia, as has been reported in a few isolated instances for *Anabaena*, (2, 4, 12). Heterocysts in a number of blue-greens offer a means of separation of the trichomes in order further to propagate the organisms. In *Anabaena* the breaking point of a trichome is usually between a heterocyst and a vegetative cell. This seldom occurs in *Aphanizomenon*.

The heterocyst is composed of a single cell which in its early state appears as an enlarged, rounded, vegetative cell (Plate IV, fig. 10). Here, however, the inner and outer integuments are in evidence. The protoplasm within the inner investment appears more homogeneous in substance than that of the ordinary vegetative cell. Often there are one or two small granules of cyanophycin present within the cell. A number of young heterocysts were observed which contained a large vacuole (Plate IV, fig. 11). This has not been observed in the later stages of development, and its significance is not known. As the heterocyst matures, the outer investment enlarges separating it into two distinct layers, an outer hya-

line region, and an inner region which is separated from the adjacent vegetative cells by a highly refractive granule (Plate IV, figs. 12, 13, 14, 16). In some cases the heterocysts secrete an enormous amount of mucilage (Plate IV, fig. 16). This is best observed in the living state as the usual preservatives dissolve this material.

AKINETE FORMATION

As is usually the case with organisms which form resistant resting bodies, conditions which are not conducive to continued vegetative existence favor the formation of these structures. As previously noted, akinetes (spores) are formed in abundance early in the winter, at which time collections may easily be made by means of a plankton net. In the summer the organisms often become too abundant, forming the huge mats which later decay on the surface. The conditions resulting from the decay of the algae on the surface cause akinetes to be formed to some extent in those organisms below the surface. Akinetes collected in the summer have, as previously stated, consistently failed to germinate.

The akinetes develop from single cells located near the mid-region of the trichome. This is advantageous due to the fact that the akinetes in the flake will all be united by their lateral walls in a compact bundle. After the decomposition of their adhering vegetative cells, which have previously buoyed up the plant mass, the akinetes sink to the bottom. The simultaneous germination of the akinetes in a bundle facilitates the direct formation of a new flake.

The steps involved in the formation of the akinetes are shown on Plate IV, figs. 2, 3, 4, and 5. Such transformation of a single vegetative cell involves the accumulation of a large amount of reserve food material, thickening of the outer wall, and elongation of the cell. The reserve food materials according to Baumgärtel (1) and Poljansky & Petruchevsky (9) consist of a number of indefinite substances of a glycogen and protein nature. The mature akinete is connected with the vegetative cells at either end by a highly refractive substance which consists apparently of the same material as that which composes the large granules which become so abundant in the akinetes (Plate IV, fig. 5). These granules are the so-called cyanophycin bodies.

DISCUSSION

The foregoing account includes in the main the complete life cycle of *Aphanizomenon flos-aquae*. The cytological details are pre-

sumably similar to those of other spore-forming blue-green algae. Preparations stained with iron alum haemotoxylin show the chromatin condition to be similar to other closely related forms (Plate IV, fig. 15).

A temperature of 8° C. is the minimum at which spore germination may take place. Light is not essential for germination; flake formation, however, did not take place unless the organisms were placed in the light. Temperatures ranging from 15° to 22° C. were found to be best for continued growth and reproduction in culture. The source of light in most studies was a 100 watt lamp placed over the culture chamber. The culture chamber was iced to keep the temperature low, consequently some variation was inevitable.

Species-pure cultures of this organism are easily procured by germinating a mass of spore material in the small vials. The filaments so formed rise to the top of the vial and may readily be removed by means of a pipette to another vial. This method was found to be rapid and effective. The necessity for such a procedure is obvious, since many other organisms are present which rapidly contaminate the culture. Bacteriologically pure cultures were attempted many times, using agar plates and other methods described, but without success.

As noted elsewhere, the various culture solutions recommended for the growth of blue-green algae were of little value in culturing *Aphanizomenon*. Since the use of small shell-vials as culture containers and the obtaining of good growths using redistilled water as a medium, many of the recommended solutions are being tried again.

This study was made possible by a grant from the State Fish and Game Department and State Board of Conservation of Iowa. The laboratory work was done in the Botany Department of the State University of Iowa, under the supervision of Dr. G. W. Martin.

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11. Smith, G. M. The Fresh-Water Algae of the United States. 1933.
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PLATE IV

Fig. 1. Small flake developed from akinetes in culture. x 362.
Figs. 2, 3, 4, 5. Development of the akinete. Figs. 2, 3, 4 x 850, Fig. 5 x 1260.
Fig. 6. Double akinete. x 650.
Fig. 7. Germination of akinete, showing large cyanophycin granules. x 1260.
Fig. 8. Germination of akinete. Stained in safranin, showing outer investment of protoplast. x 850.
Fig. 9. Young trichomes from 24 hour culture. x 850.
Figs. 10–16. Development of heterocyst. Fig. 15 stained with iron-alum-haemotoxylin showing chromatin in vegetative cells. x 850.
Fig. 17. Portion of flake from a three-day culture, showing the *Trichodesmium*-like cells. x 850.

PLATE IV

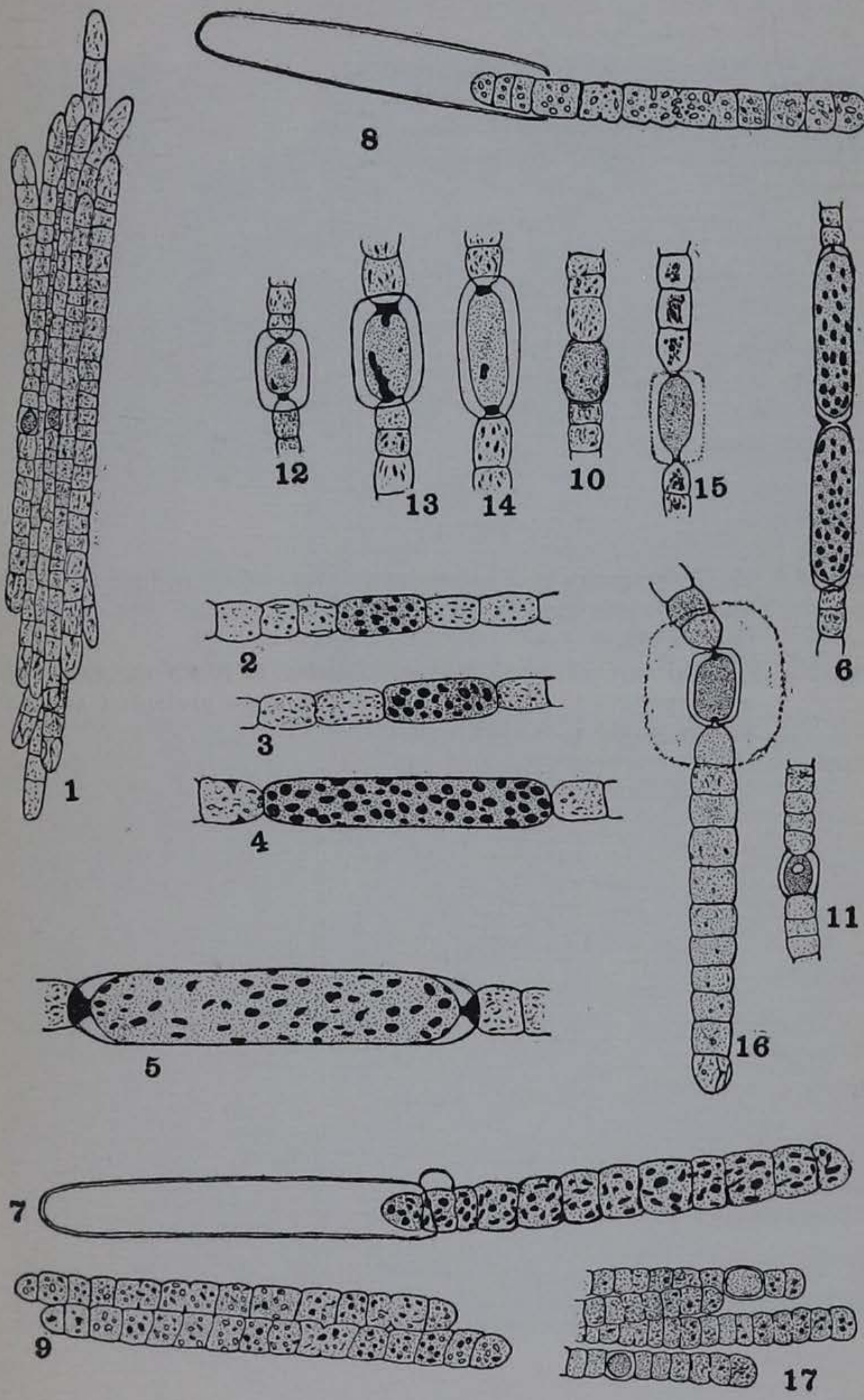
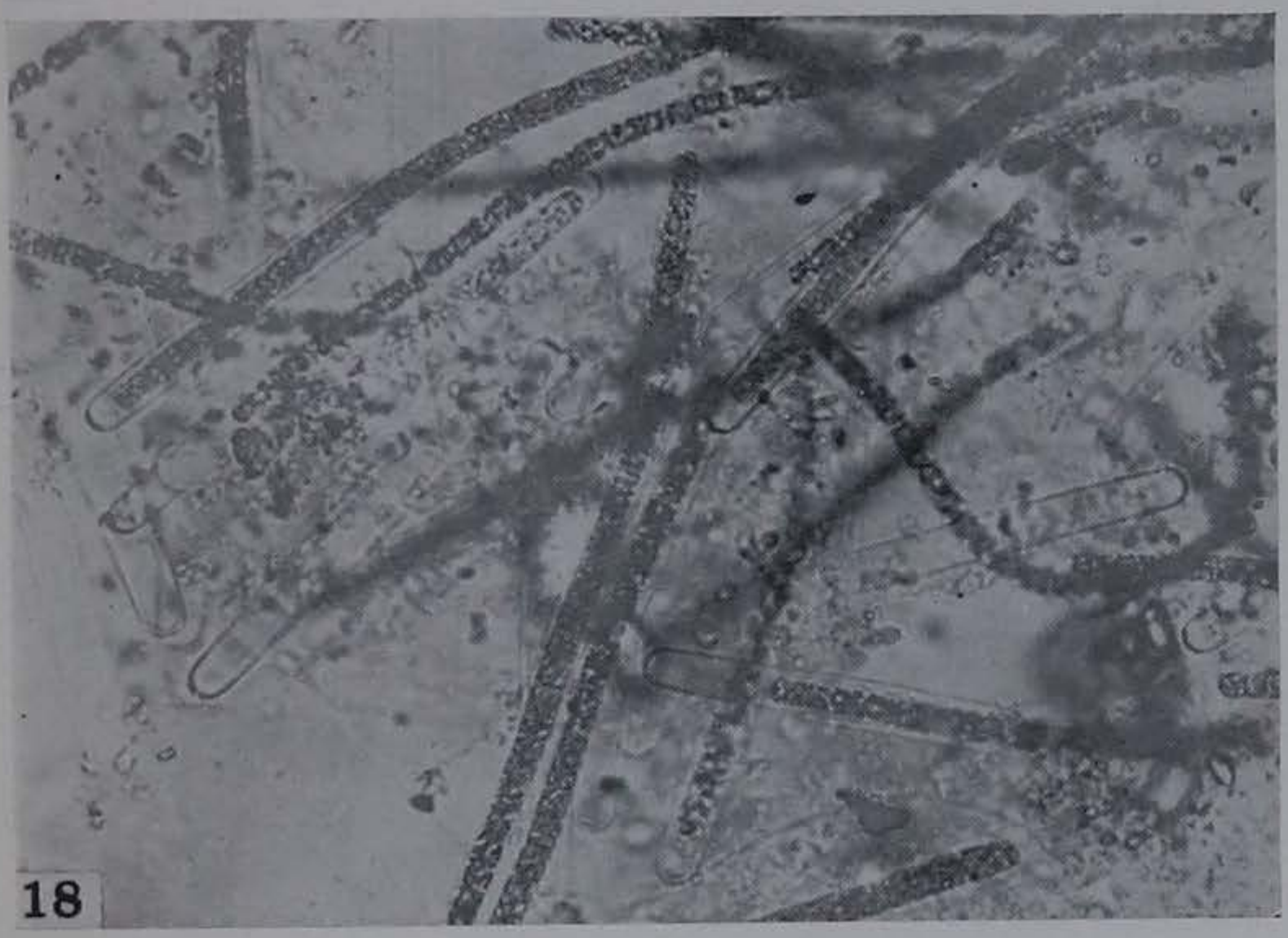


PLATE V

Fig. 18. Photomicrograph of most common method of akinete germination, in which the sporeling is differentiated into cells at time of germination. x 450.

Fig. 19. Photomicrograph of akinete germination in which the sporeling is undifferentiated. The large bodies within the protoplast are cyanophycin granules. x 1350.

PLATE V



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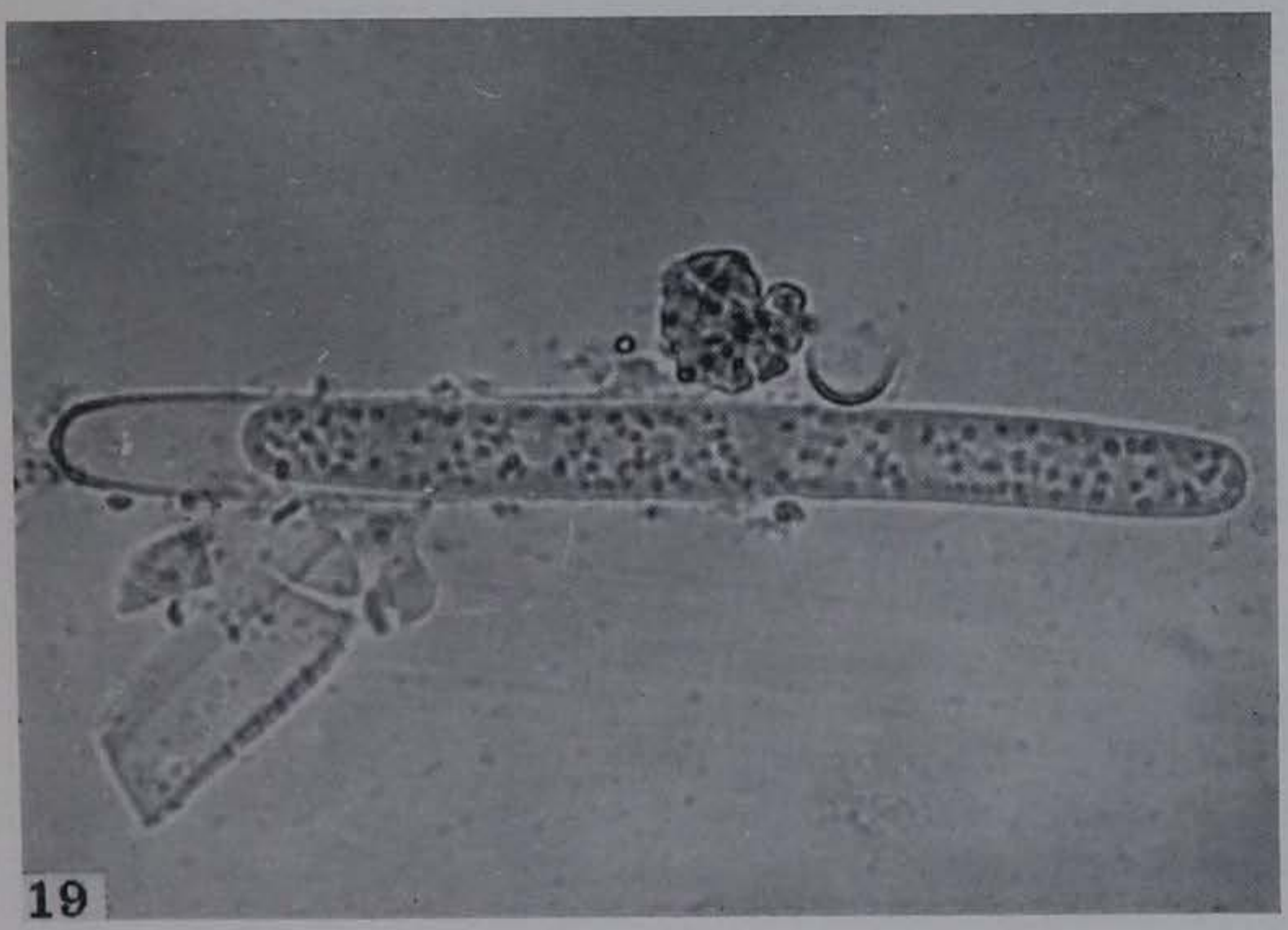


19

THE GENUS STYPELLA

G. W. Martin

Stypella was established in 1895 by A. Möller (Protobasidiomyceten, 75) to accommodate two species of tremellaceous fungi collected on rotten wood in Brazil. Möller regarded the genus as sufficiently distinct to warrant its segregation in a separate subfamily, the Stypelleae. Neither of Möller's species was again reported until 1930, when, believing I had rediscovered one of them, *S. minor*, in Iowa, I published a brief reference to it under that name (Proc. Ia. Ac. 36: 128). More recently Linder has described from Missouri as *Tremella gangliformis* (Mycologia 25: 105. 1933) what is certainly the same fungus as that reported from Iowa. This raises the question as to whether the original reference of the Iowa material was justified, and, if not, just what Möller's genus represents. Whether Möller's types are still in existence, and if so, where they are deposited, I have been unable to learn. The descriptions, however, are ample, and the illustrations clear, and it should be possible, with their aid, to gain a reasonably satisfactory idea of the character of the genus.

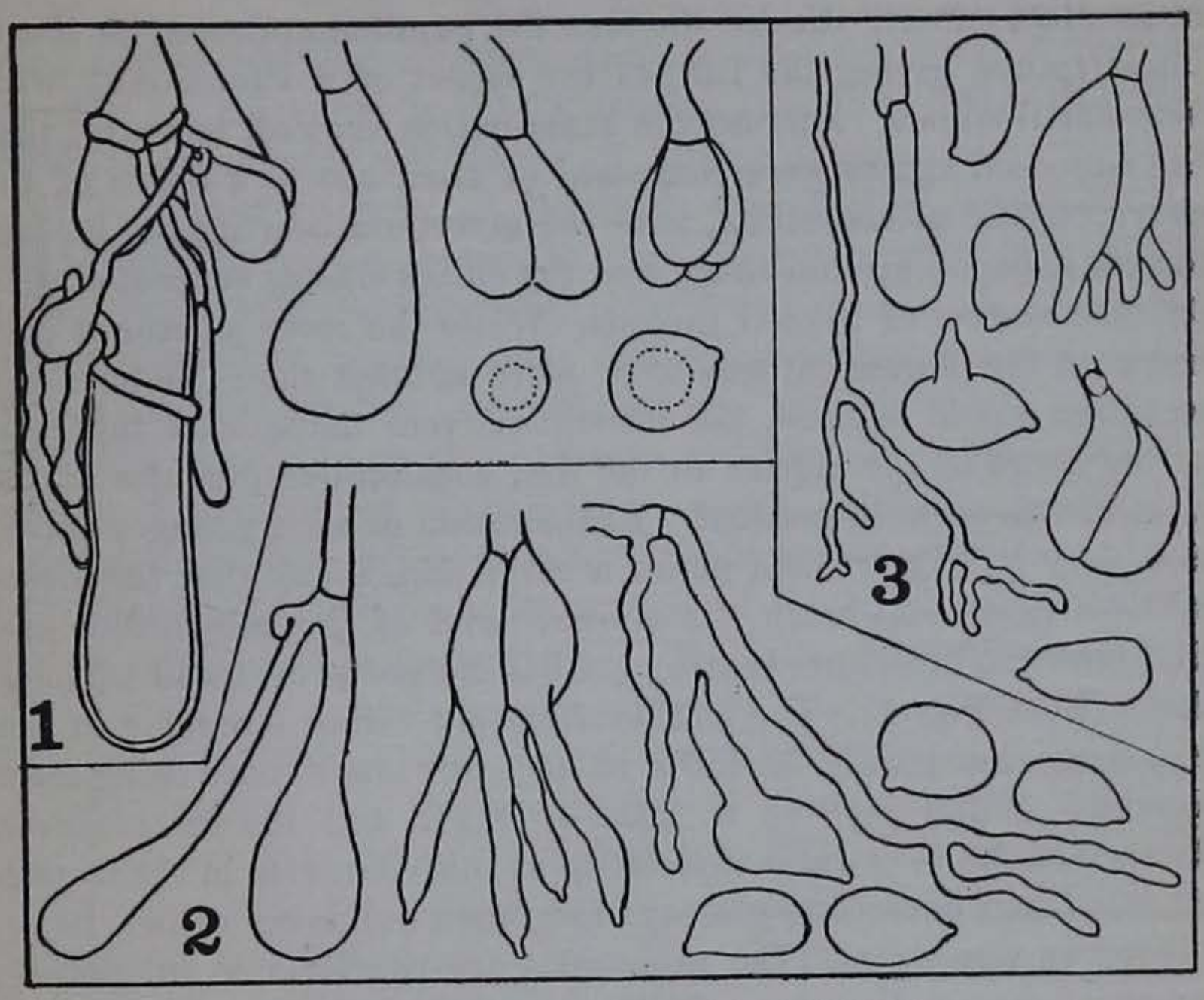
In the last pages of his volume Möller summarizes the less formal discussion of the earlier pages. On p. 166 he says of the Stypelleae: "Entsprechen den Stypinelleen unter den Auriculariaceen. Basidien frei und einzeln an den Myzelfäden, ohne Fruchtkörperbildung." Of the single genus *Stypella* he merely notes: characters of the group. Now *Stypinella* Schroeter, which is a synonym of *Helicobasidium* Patouillard, is, it is generally conceded, characterized by a soft, floccose, non-gelatinous, "tow-like" habit, with the basidia borne singly and at various heights on the hyphae; that is to say, a hymenium is quite definitely lacking. This comparison with *Stypinella* seems to be responsible for the conception of *Stypella* which has been presented in most of the later systematic works which have referred to that genus. Thus Lindau, in the first edition of Engler and Prantl, speaks of it as "wergartig," and this characterization is copied by Killermann in the second edition. Saccardo and Sydow (Syll. fung. 14: 246) merely paraphrase Möller's statement that it is like *Stypinella* but with globose, tremellaceous basidia. Clements, and Clements and Shear say "pileus

byssoid." On the other hand, von Höhnel (Ann. Myc. 2: 749. 1915) suggests that the two species represent early stages of an *Exidiopsis* and a *Heterochaete* respectively, and Burt (Ann. Mo. Bot. Gard. 2: 749. 1915) regards the genus as included in *Sebacina*. Neuhoff, (Bot. Arch. 8: 287. 1924) likewise, regards *Stypella* as a subgenus of *Sebacina* and states that *Exidiopsis* is a synonym of that genus. There is no reason to suppose that any of the last-named authors ever saw Möller's specimens, but they had evidently read his descriptions carefully. In view of the close approach of certain species of *Sebacina* to the fungi under discussion, this disposition of the genus is not unreasonable.

Möller specifically states that there is no fructification, but both by description and drawing he makes it clear that this is merely a matter of definition. He regards the genus as a fulfillment of Brefeld's prophecy (Untersuchungen 7: 24, footnote) that genera would be discovered among the Heterobasidiomycetes corresponding to what he regarded as the Tomentelleae of the Homobasidiomycetes, that is, with free basidia not united into a hymenium. The fact that Brefeld's conception of what constituted a hymenium was inadequate, and that it was adopted by Möller and applied by him to the condition existing in *Stypella,* is important in this connection. The pustular aggregations of basidia-bearing hyphae, massed within a restricted area and connected by a network, more or less developed, of subicular hyphae, constitute a fructification exactly comparable with that found in certain of the more tenuous forms of *Grandinia, Tomentella* and *Corticium.*

The references to the tow-like or byssoid character of the fungus are obviously due, not to Möller's description, but to his comparison with *Stypinella.* True, when the subicular hyphae are well developed (see Plate VI, fig. 4a), these terms are not inapplicable to the dry fructification, but they are much less applicable to it when it is soaked. It is clear that Möller regarded *Stypinella* as the auriculariaceous equivalent of *Tomentella,* and *Stypella* as occupying the same position among the Tremellineae. The emphasis is upon the rather loose and open character of the fructification, and not at all upon a tow-like or byssoid texture.

These contentions are supported by the descriptions of the species. *Stypella papillata,* to be regarded as the type, is made up, according to Möller, of small, irregularly circumscribed areas scarcely ½ mm. in breadth, grouped irregularly into masses up to



EXPLANATION OF TEXT FIGURE

All drawings made with aid of camera lucida, and all reduced in reproduction to x 1500, except upper left hand drawing in Fig. 1, which is reduced to x 683.

Fig. 1. *Stypella papillata.* Cluster of three gloeocystidia, two young and one older, with hyphae bearing probasidia encircling them; also young stages of three basidia, and two spores. Fig. 2. *Stypella minor.* Two probasidia, one nearly mature basidium, paraphysoid and five spores, one germinating. Fig. 3. *Tremella Grilletii.* Paraphysoid, three stages in development of basidia and four spores, one germinating.

1½ cm. in length, the entire group with a smooth, glassy appearance when moist, and papillate under a lens, completely disappearing to the naked eye when dry. Microscopic examination reveals that the papillae are connected by a loose, irregular network of slender hyphae, apparently imbedded in a thin jelly.

Two collections made at Highlands, N. C., during the August, 1933, foray of the Mycological Society of America, are referred to this species. They were both growing on very rotten coniferous wood lying on the ground. The fungus occurred in the form of scattered, irregular patches, the largest of which was about 2½ cm.

in greatest extent. Under the lens the papillae appeared as short, blunt spines, giving the fungus the aspect of a *Protodontia* with very small spines. Microscopic examination showed, however, that the supposed spines were composed of from one to a dozen of the characteristic gloeocystidia, with the mycelium bearing the basidia loosely grouped around them, and the entire cluster connected by a delicate system of hyphal threads. While the more advanced portions of the fructifications were more spinose than Möller's description would suggest, the transition from the spinose tubercles at the heart of the cluster to the flat, cushion-like pustules at the margin was entirely gradual. Examination of such younger tubercles, very lightly crushed under a cover slip, shows that the gloeocystidia first drop below the general level of the substratum and that later the mycelium bearing the basidia grows over and amongst them (Text Fig. 1). The gloeocystidia are rather irregular in size and shape, but mostly 70–150 x 10–16 μ, the longer ones being more slender. Möller says up to 200μ in length and 10μ in thickness. In view of the very great variability of this character in the tremellaceous fungi possessing gloeocystidia, such difference cannot be regarded as significant. The probasidia are pyriform to subglobose, mostly 7–8μ in diameter. Möller says 10μ, but the two basidia shown in his illustration are 6μ and 8μ broad respectively, if checked by the magnification given. The spores are globose, 4.5–6μ in diameter. Möller says "rundlich, 4μ," but illustrates two spores, both still attached to sterigmata, one, apparently about mature, 6 x 4.5μ, the other, obviously immature, 4.5 x 3μ.

It cannot be maintained that the identity of the Highlands collections with *S. papillata* is positively established. Möller's description and illustration give a vivid picture of the fungus he had before him. The differences between his account and the characters of the North Carolina material are such as might readily be explained on the basis of the known wide range of variability in fungi belonging to this group. It seems better, therefore, to use Möller's name for these collections rather than to apply a new one which would in all probability have to be relegated to synonymy.

As stated above, von Höhnel suggested that *S. papillata* (he says *S. minor,* but the context makes it clear that this is merely a slip) might represent an undeveloped *Heterochaete.* But the resemblance between *Heterochaete* and *S. papillata* is confined to the gloeocystidia. In every other respect *S. papillata* is quite distinct

from any known *Heterochaete*, and, as has been pointed out repeatedly by Burt and again by Rogers (see Ann. Myc. 31: 181. 1933), this character alone is not of generic value.

Möller's second species, *S. minor*, is described as outwardly indistinguishable from *S. papillata*, appearing as a thin, gray pubescence, irregularly circumscribed, composed of a loose tangle of delicate mycelium bearing tufts of hyphae which are responsible for the papillate surface. The distinctions between the two species are based mainly upon the presence or absence of gloeocystidia, and the size and shape of the spores. Gloeocystidia are lacking in *S minor*, their place being taken by slender, branched hyphae, about 3μ in diameter, which form the centers of the papillae, and which may be referred to as paraphysoids. The paraphysoids in our specimens are more slender, mostly under 2μ, but thicker, and more irregular and tortuous than those of the forms referred to *Tremella Grilletii*. Möller gives the breadth of the basidia as 4–5μ, but this must be inaccurate, since a subglobose basidium of this diameter could not produce four spores 6 x 3μ. If a calculation is made on the basis of the magnification given for his drawing, the diameter of the basidia is approximately 7–9μ.

The fungus which I have referred to *S. minor* is common in Iowa and Missouri, and is the same as *Tremella gangliformis* Linder (Text Fig. 2; Plate VI, Figs. 1–4). It usually occurs in rather small patches, but occasionally forms moderately conspicuous fructifications several centimeters in extent. It varies all the way from small clusters of almost completely separated papillae connected merely by an extremely tenuous subiculum (Plate VI, Fig. 3) to phases in which the papillae are so densely massed and anastomosed as to form an almost continuous gelatinous network, never, however, losing the papillate character which is the expression of the tufted habit of the fructification. Such fruitings, when dry, are easily visible to the naked eye, and do exhibit, under a lens, a somewhat byssoid appearance (Plate VI, Fig. 4a).

The probasidia are 7–8(–9)μ in width, and after division into two or four, rarely three cells, produce epibasidia of the same thickness as the paraphysoids and mostly 10–15μ long. The longest epibasidium observed, 22μ, was far short of the dimensions given by Linder, 36–40μ, but he figures a basidium producing spores, hence mature, in which the epibasidia are 10μ long, which is in agreement with our material. The spore-size cited in the original description

was 6 x 3μ. Linder describes the spores of *T. gangliformis* as globose to ovoid, 5.5–6 x 4–5μ. My measurements show a considerable range both of size and shape, but in general the shape is oval or short cylindrical and suballantoid, 6–9 x 3–5μ.

A third species, obviously very close to *Stypella minor,* is *Tremella Grilletii* Boud., Bull. Soc. Bot. Fr. 32: 284. 1885. This is beautifully characterized in the original description, of which the following is a translation: Very small, scarcely exceeding 0.3–0.4 mm. in diameter, rounded or sublenticular, pale ashy lilac or subhyaline, in dense clusters, forming cinereus or lilaceous blotches 1–2 cm. in diameter; hymenium appearing very finely papillate under the microscope, and pruinose from the presence of spores; basidia rounded, longitudinally septate, with four flexuous and elongated sterigmata; spores hyaline, oblong, a trifle curved, obtuse and rounded at the tip, obliquely apiculate at the base, which is a trifle narrower, the contents finely granular with a vacuole in the midst, often located near the wall, 8–10 x 3–5 μ. . . . It forms small disks of a pale violaceous gray, and has exactly the appearance of certain discomycetes of the genus Ascophanus.

Bourdot and Galzin list the species as not rare. They give the spore dimensions as 6–10 x 3–5μ, and add measurements of basidia, 8–12 x 6–10μ, and epibasidia, 15–20 x 2μ. They also note the paraphysoidal branches, which are described as simple or forked. In our material these structures are always forked, as shown in text figure 3.

The species is not a *Tremella* as that genus is ordinarily understood at present. Bourdot and Galzin suggest *Exidia,* where its spores would seem to place it. They also note that the pustules may become confluent so as to resemble certain forms of *Sebacina fugacissima.* It is certainly very close to *Stypella minor,* and may not be specifically distinct, in which case the specific name has priority. We have a collection from Iowa which is tentatively referred to Boudier's species, and which differs from *S. minor* as here defined in the slightly larger size and remarkable regularity of the pustules, which are connected by an extremely tenuous subiculum, in the distinctly lilaceous color, in the slightly longer, narrower and more curved spores, 6–8 x 3.5–4μ, and in the more slender paraphysoids, 1–1.5μ in thickness. In the Tremellaceae, none of these distinctions, taken alone, is of specific significance, but taken together, they may justify recognition of the species. Certainly, it must eventually be

included in the same genus as *Stypella minor*, if not merged with that species.

The taxonomic disposition of these species offers considerable difficulty. They do not fit naturally into *Tremella, Exidia* or *Heterochaete*. They are unquestionably closer to the thin, gelatinous, effused species of *Sebacina* (including *Bourdotia*) as that genus is now recognized. But the type species of Sebacina is *S. incrustans* (Pers.) Tul., a familiar, coriaceous, often subpileate fungus which it is difficult to believe is congeneric with these delicate forms. *Sebacina* is in need of thorough revision, a task which will demand long and careful study. But when this has been accomplished, it must include *S. incrustans* and all similar forms, and it is my belief that the delicate, gelatinous forms originating as distinct pustules will have to be excluded. If, however, Möller's characterization of Stypella is studied in the light of the clear species descriptions and the figures, we have a satisfactory genus to receive them. I therefore venture to present the following emendation of Möller's genus:

Stypella Möller emend. Fructifications resupinate, determinate, tubercular, densely gregarious, and united by a delicate subiculum which often disappears upon drying, often anastomosing or confluent into a continuous layer; texture soft, waxy-gelatinous when moist, drying to an almost imperceptible film; probasidia obpyriform to subglobose, becoming longitudinally septate into 2–4 cells, each cell bearing an epibasidium tipped by a sterigma and a spore; gloeocystidia or paraphysoids present, borne in clusters and serving as the centers of the tubercles; spores usually germinating by repetition.

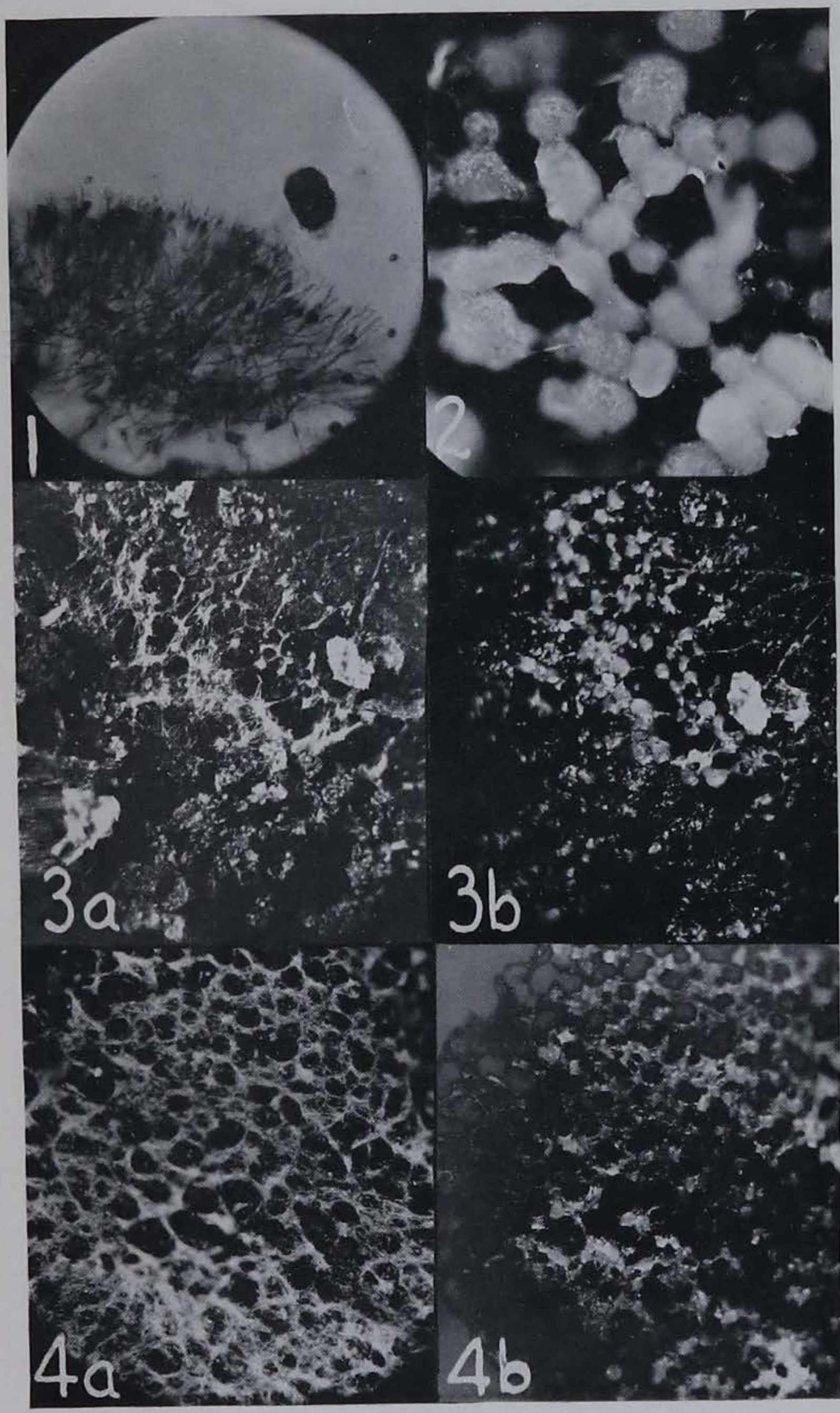
If the genus should find acceptance on this basis, there are several other species now distributed amongst *Exidia, Sebacina* and *Tremella* which should probably be included.

PLATE VI

Photographs by Mr. Donald P. Rogers

Stypella minor Möller. Fig. 1. Mount in Amann's medium, with Nigrosin, showing basidia and paraphysoids, x 233. Fig. 2. Surface view of pustules, soaked, showing anastomoses and subicular hyphae, x 55. Fig. 3. Fructification of scanty growth, x 11; a. dry; b. soaked. Fig. 4. Fructification of denser growth, x 11; a. dry, showing tow-like character; b. soaked, showing anastomosing pustules.

PLATE VI



THREE NEW SPECIES OF MYXOMYCETES

HENRY C. GILBERT

The three species of Myxomycetes presented here were found on the bark of living trees during a study of bark inhabiting forms made in Iowa in 1932 and 1933. A brief report of the known forms found during this study was published in August, 1933.[1] Bark samples collected from various trees were cultured in moist chambers, thus simulating damp or rainy weather for a sufficient length of time to permit maturation.

The fact that these were found and are described from material developed in moist chambers immediately raises a question as to their normal development. In the case of the known species found in this study the sporangia formed in the moist chambers were found to be typical. Furthermore, the sporangia were more uniform and perfect in their development than collections of the same species which had developed in the field. The two species of *Macbrideola,* here described, are very similar. If collected in the field the species *M. decapillata* might be considered a poorly developed form of *M. scintillans.* In the moist chamber, however, the conditions were the same for both, and, as experience has proved, were optimum or nearly so for development. The greater simplicity of *M. decapillata* in this case cannot be ascribed to external conditions.

The possibility of collecting these species mature in the field is probably remote due to their minute size and scattered habit. The number of times that they have developed on bark cultures shows that they are actually common and should be considered rare only in the matter of finding them. The culture method is recommended both for collection and study. It is to be expected that these and perhaps other new Myxomycetes will be found on the bark of living trees wherever climatic conditions favor the growth of algae or lichens on the bark.

Hymenobolina pedicellata sp. nov. Sporangiis late dispersis, globosis, fusce brunneis vel nigris, plerumque corrugatis, stipitatis vel sessilibus, 75–175μ diametro; stipite crasso, sulcato, opaco, scrutis farto, fusco vel cum substrato concolori, 100–250μ longo; peridio

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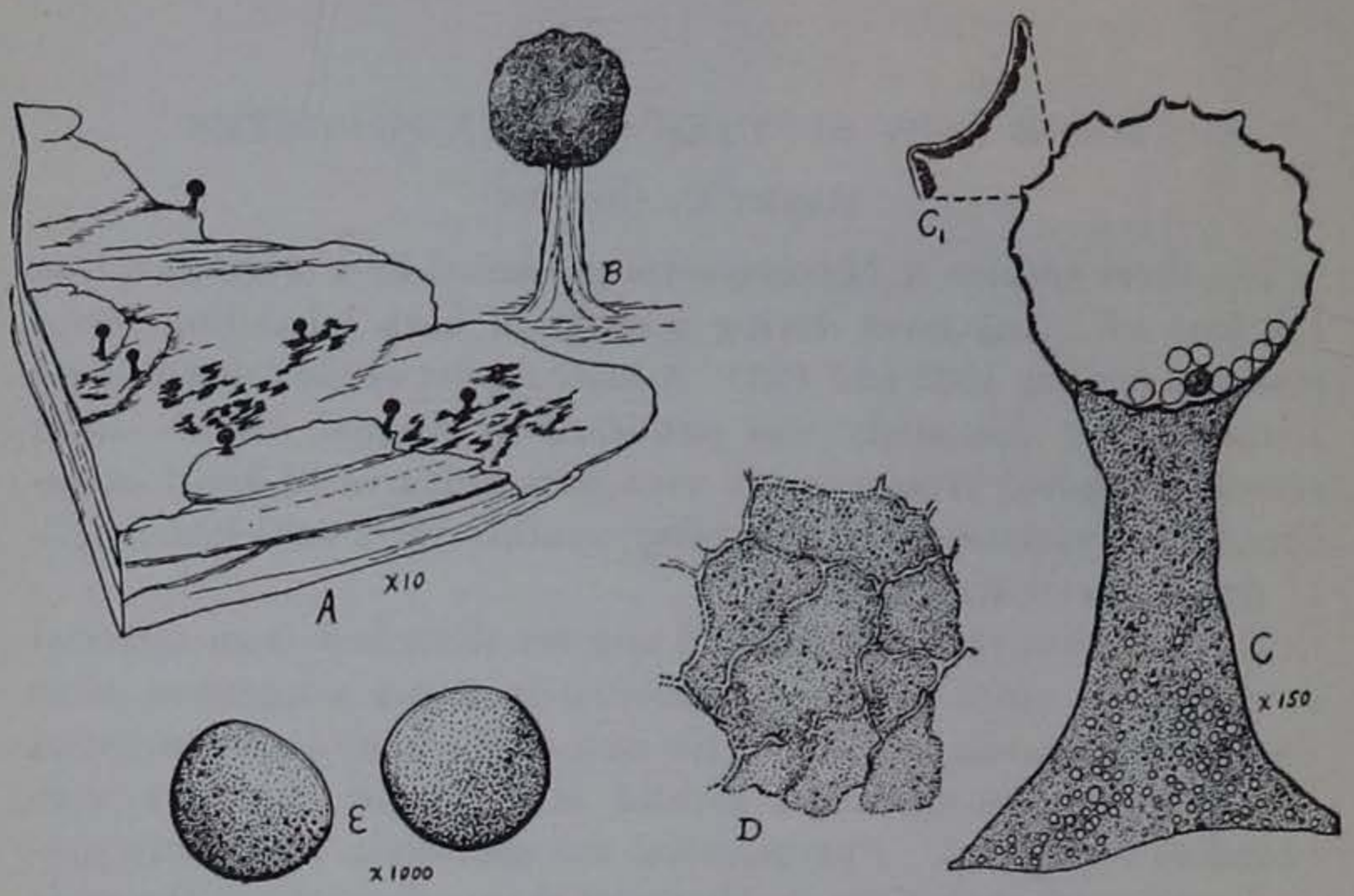
1 Gilbert, H. C., and G. W. Martin, Myxomycetes found on the bark of living trees. Univ. Ia. Studies Nat. Hist. 15(3): 1–8. 1933.



Text figure 1. *Hymenobolina pedicellata.* A, habit sketch; B, a single sporangium enlarged; C, longitudinal section of a sporangium containing a few spores. A detailed cross-section of the peridium is shown at C_1; D, surface view of a piece of peridium; E, spores.

membranoso granula includente, sporis globosis, in cumulo fuscis, sub lente pallido-brunneis, uno latere incoloribus, levibus vel verrucis minutis, 12–14μ diametro.

Sporangia widely scattered, globose, dark brown or black, usually wrinkled, stipitate or sessile, 75–175μ in diameter; stipe thick, furrowed, opaque and stuffed with refuse, often including spores and Protococcus cells, dark or the color of the substratum, 100–250μ long; peridium membranous but containing granular refuse in the inner part, continuous with the surface of the stipe and also forming a wall between the contents of the stipe and the sporangium, on drying forming irregular plates and thin lines of dehiscence; spores globose, dark brown in mass, by transmitted light light brown with a colorless area on one side, contents rose tinted, smooth or faintly and finely warted, 12–14μ in diameter.

Type specimen: Gilbert No. 2117, Milford, Iowa, July 16, 1932.

Habitat: the bark of living trees of Ulmus, in slime flux areas or other wet places, often associated with *Protococcus,* and with other bark inhabiting Myxomycetes.

This minute Myxomycete lives in the thin layer of wet waste matter on the surface of the bark of trees. The small plasmodia seem to develop like those of *Hymenobolina parasitica,* each forming but one sporangium. The sporangia are scattered and often few will be found. They are slow to mature and many dry before the spores are formed. A cross-section of the peridium shows a thin translucent layer on the outside with more or less waste matter on the inside as illustrated in text figure 1, C. The separation of this inner granular waste matter into irregular plates forms thin lines of dehiscence. These are less numerous and less evident when there is little waste matter in the peridium.

This new species undoubtedly belongs to the family *Liceaceae* yet it does not conform to any one of the present genera. Rather than erect a new genus it is placed in *Hymenobolina* for the present. The reasons for this may be briefly stated as follows. The species *Hymenobolina parasitica* does not conform to the original generic limitations set for it. The genus description states that the plasmodium is parasitic on lichens and algae. I have observed that many plasmodia of *H. parasitica* develop and fruit in the absence of either lichens or algae. A membranous lid for the sporangium is also required in the generic description but many sporangia otherwise typically *H. parasitica* do not develop membranous lids. The genus *Hymenobolina* is described as having sessile sporangia. In this new species I consider the stipitate sporangia as typical yet there are some sessile and nearly sessile sporangia found among the stipitate ones in various collections. The spores of this new species are very much like those of *Hymenobolina* and definitely relate it to that genus. It is like this old genus also both in habit and in habitat.

More detailed study of these minute forms is needed. Since such forms as *H. parasitica* develop but one sporangium from one plasmodium it is entirely possible that two sporangia very close together may be two species. The idea of intergrading forms and variation in species of this kind cannot be considered in the same way as it is in the case of colonial forms. Differences which have been observed such as presence or absence of a stipe, or possession or lack of a definite lid may actually be species differences in these uni-sporangiate forms.

MACBRIDEOLA gen. nov. Sporangiis stipitatis; stipite pellucido, cavo, sicut columella in sporangium inserto; plerumque sine capil-

litio; peridio membranoso, pellucido, evanido vel persistente; sporis brunneis.

Sporangia stipitate; stipe translucent, appearing hollow, extending into the sporangium as a columella; capillitium typically lacking although divisions of the columella may be found in some sporangia; peridium membranous, translucent, evanescent or persistent; spores brown. Type species *M. scintillans.*

This new genus is named in honor of Dr. Thomas H. Macbride, late President Emeritus of the University of Iowa. For more than forty years Dr. Macbride collected and studied the Myxomycetes. His contributions to our knowledge of the North American forms of this group are the greatest ever made by any one person. This new genus is particularly appropriate to commemorate Dr. Macbride's work because the type species, *M. scintillans,* finds its ideal habitat in the beautiful woodlands of the Iowa country in which Dr. Macbride labored. It is hoped that this beautiful though minute Myxomycete may bear this name and ever remind us of the work of a great and good scholar.

Included in this new genus are two forms not referable to any of the present genera but showing relationship to several in various characteristics. In the type species the columella is usually undivided and like that of *Enerthenema.* The peridium is persistent and even more firmly attached to the columella than the similar attachments in *Barbeyella.* The stipe seems more like that of a *Comatricha* or *Lamproderma.* The rudiments of capillitium found in some sporangia suggest that it may be related to *Echinostelium.* In the classification given by Lister[2] it would come under the *Stemonitaceae* while in that of Macbride[3] it would be in the *Lamprodermaceae.*

Macbrideola scintillans sp. nov. Sporangiis dispersis, globosis, fusce brunneis vel colore aeneo, 75–125μ diametro; stipite subulato, pellucido, cavo, ad basim flavo, supra brunneo, in sporangium inserto, 50–100μ longo; peridio tenui, nitido, pellucido, tenaci, ad collumellam haerente; columella deminuente, percurrente, in peridio merso; capillitio nullo; sporis globosis, verrucis magnis, magnitudine et distributione irregularibus, ornatis, 8–9μ diametro.

Sporangia scattered, globose, dark brown or metallic bronze, 75–

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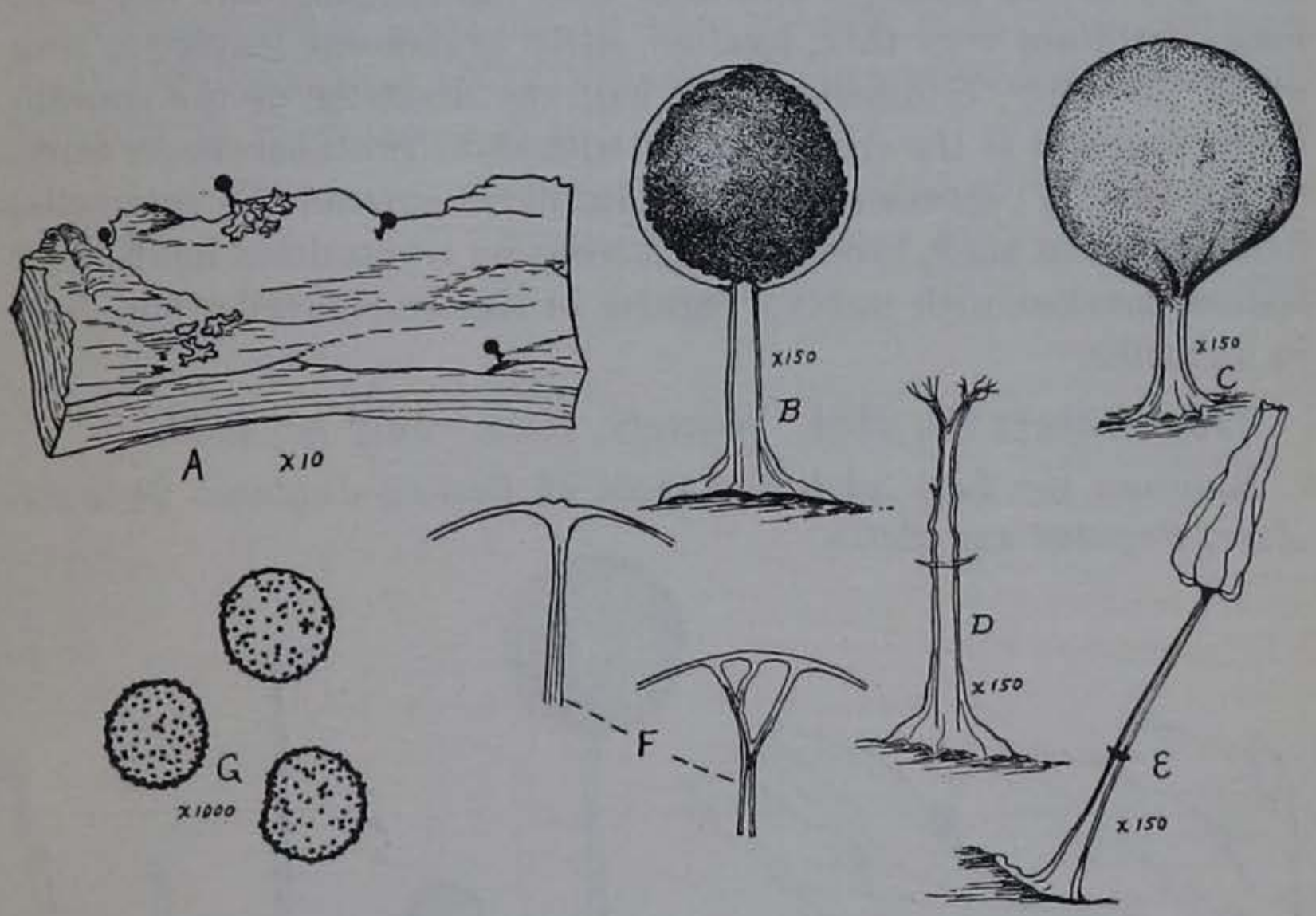
[2] Lister, G., Mycetozoa, London, 1925.
[3] Macbride, T. H., North American Slime-Moulds, New York, 1922.

125μ diameter; stipe tapering, translucent, appearing hollow, yellow at the base, brown above, continuing into the sporangium, 50–100μ long; peridium thin, shining, translucent, tough, strongly attached to the columella; columella tapering, reaching the apex where it fuses with the peridium; capillitium lacking; spores globose, thick walled, brown, marked with large warts irregular in shape and distribution, 8–9μ diameter.

Type: Gilbert No. 1741. Iowa City, Iowa. May 14, 1932.

Habitat: the bark of living trees of *Ulmus, Quercus, Tilia* and *Juglans.*



Text figure 2. *Macbrideola scintallans.* A, habit sketch; B, a mature sporangium by transmitted light showing the spore mass shrunk away from the translucent peridium; C, a large sporangium; D, showing unusual divisions of the tip of the columella; E, stipe and typical columella with the tough peridium turned inside out during spore dispersal; F, showing attachment of columella and peridium; G, spores.

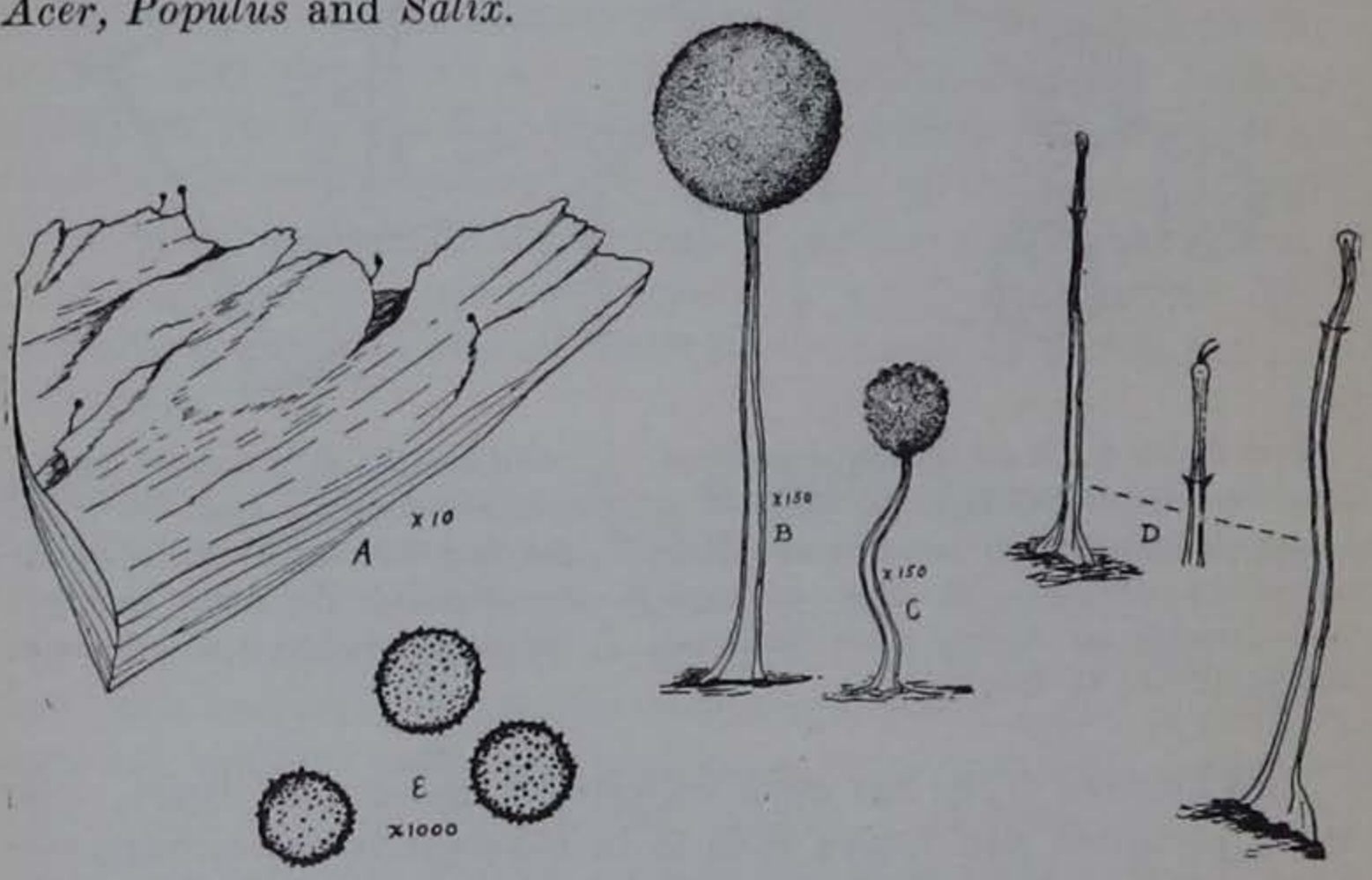
This minute form has been collected a number of times. The stipe, peridium and spores seem to be constant in their characteristics. The variations found in the columella are not sufficient to mislead one in identification. It is often associated with other bark inhabiting Myxomycetes.

Macbrideola decapillata sp. nov. Sporangiis late dispersis, globosis, fusce brunneis, 50–100μ diametro; stipite gracili, pellucido, cavo ad basim luteo, supra brunneo, in sporangium insertum, 125–250μ altitudine; peridio tenuissimo, hyalino, evanido, circum stipitem annulum relinquente; columella ad medium sporangium pertinens; capillitio nullo; sporis globosis, circum columellam agglutinatis, cumulo fusce brunneis, sub lente spadiceis vel violaceis, verrucosis, 7–9μ diametro.

Sporangia widely scattered, globose, dark brown, 50–100μ in diameter; stipe slender, translucent, appearing hollow, yellow at the base and brown above, continuing into the sporangium, 125–250μ long; peridium very thin, hyaline, early evanescent leaving a ring about the stipe; columella about half the diameter of the sporangium, rounded at the end or rarely with short protuberances; capillitium lacking; spores globose, agglutinated around the columella, dark brown in mass, brown or violaceous by transmitted light, thick walled, marked with warts irregular in size and distribution, 7–9μ in diameter.

Type: Gilbert No. 2184. Waverly, Iowa. July 30, 1932.

Habitat: the bark of living trees of *Ulmus, Juglans, Quercus, Acer, Populus* and *Salix*.



Text figure 3. *Macbrideola decapillata.* A, habit sketch; B, a very large, young sporangium with peridium yet intact; C, a small sporangium dry and with peridium gone; D, types of stipe and columella; E, spores.

This minute species is but little larger than *Echinostelium* and much like it in structure. Collection and handling are difficult. The peridium drops away in drying. The spores are but slightly agglutinated if well matured and usually drop off if the mount is handled much after drying. The result is that from numerous collections I have left but little herbarium material. It is advisable with species as delicate as this to make a few permanent slide mounts before the collection is dried. The small size, long stipe and evanescent peridium set this form apart from *M. scintillans*.

All of my collections of Myxomycetes have been placed in the Department of Botany, Oregon State College, Corvallis, Oregon. Type specimens of these new species will also be found at the University of Iowa, Iowa City, Iowa, and in the United States National Museum at Washington. D. C.

THE BASIDIUM

DONALD P. ROGERS

It is a truism that during the development of mycology increasing dependence has come to be placed upon microscopic criteria in the classification of the fungi. Character of spore and minute structure of sporocarp have been found to furnish the most reliable basis attainable for the delimitation and identification of species. Arguing from this, a number of workers have erected genera or even larger categories on characters which have been proved valid only in the distinguishing of species—on spore ornamentation, it may be, or on various accessory hymenial organs. Saccardo's sporologic arrangement of the imperfecti and pyrenomycetes is the most striking example of such inappropriate consistency; only slightly less thoroughly scholastic are some of the modern revisions of the hymenomycetes.

Since the time of Tulasne the morphology of the basidium has been an important factor in the classification of the basidiomycetes. That great mycologist left lucid and accurate descriptions of the principal basidial types known today. Present understanding of the basidium may be said to begin with his work; present taxonomic arrangements of the basidiomycetes, insofar as they are not Friesian, are founded upon relationships which he described on grounds of basidial morphology. But his work ended before he had expressed his conclusions in a system; and it was left to later mycologists, and above all to Patouillard, to embody his method and results in classification. Patouillard based his arrangement, (20) the nearest approach to a natural system that has yet been proposed, on other microscopic criteria in addition to basidial form—on texture of basidiocarp, accessory hymenial organs, spore germination—and, to an extent, on the macroscopic character of hymenial configuration; his principal groups, however, are based on the basidium. Other workers have gone much farther than he in microscopic subdivision; still others have not placed a high value on minute as opposed to gross characters; perhaps the majority have assented to the removal of phragmobasidiate forms from holobasidiate groups, but among the latter have not seen fit to depart from Friesian orthodoxy. This is essentially an acceptance of the evi-

dence of the basidium, as far as that has been made clear, and a rejection of other microscopic criteria as applied to the higher taxonomic categories.

It may be asserted that the greater part of whatever truth and permanence inhere in the present conceptions of basidiomycete relations has been attained through study of the basidium. Both the kinship of the rusts, smuts, gasteromycetes with the hymenomycetes and the distribution among its natural subdivisions of the fungi included in this last vaguely defined group have been deduced from basidial characters. The sharp separation of the gasteromycetes from the hymenomycetes, a taxonomic convention comparable with the division between the pteridophytes and spermatophytes, is unnatural in exactly the degree in which it takes no cognizance of the basidial evidence. It is not improbable that any future approximation of ultimate truth concerning basidiomycete relations will be based on observation and interpretation of the basidium. Meanwhile it appears desirable and expedient to apply such knowledge as is available to the working out of a tentative series of relations among these fungi and to the criticism of some of the accepted taxonomic arrangements.

The antecedents of the basidium and the ancestors of the basodiomycetes are in all probability to be found somewhere among the less highly specialized ascomycetes. The evidence for this statement has been treated in detail by several authors, notably by Gäumann (16, pp. 398–404; 17, pp. 420–426). Briefly, it has been shown, largely on cytological grounds, that the basidium is an organ completely homologous with the ascus, differing fundamentally only in the exogenous formation of the definitive spores. Furthermore, only among the ascomycetes is there to be found a structure even remotely assimilable to the dikaryophase of the basidiomycetes, already highly developed even in the simplest forms; and the homologies between the ascogenous thallus and the basidiomycete diplont are many and striking. The relation between the cytologically antithetic generations in the two classes is roughly comparable to that between those of the bryophytes and the tracheates: in the ascomycetes the haplont is well developed and the diplont present only as a parasitic fruiting thallus; while in the basidiomycetes the haplont becomes progressively more insignificant and the diplont, an assimilative as well as a fruiting phase, occupies a larger and larger part of the cycle. It appears proper to regard this shift

in the development of the two phases, which, as Gäumann (16, pp. 371–373; 17, pp. 393–395) has pointed out, commences among the simplest of the ascomycetes, as a continuous process and not two distinct ones, to see in the increasing emphasis upon the diplont among the basidiomycetes a phyletic continuation of a line of development that has not been completed anywhere among the ascomycetes. There appears no good reason to doubt the complete homology of the crozier of the ascogenous hypha and the clamp of the basidiomycete dikarophase. The presence of double fusion and reduction in certain of the higher ascomycetes, even if it can be said to be established, offers no very serious obstacle to the homologies here described. It may be that the basidiomycetes represent one general type of evolution and the more highly developed ascomycetes another from a primitive ascus type lacking any special mechanism for spore discharge. There is no serious objection, however, to the derivation of the basidium from some more highly developed, explosive ascus.

No such high degree of probability can be predicated of any hypothesis which seeks to establish which particular basidial type is nearest to the ascomycetous ancestor. Perhaps the greatest certainty belongs to the negative assertion that the "primitive basidium" is not to be found among the biologically highly specialized groups of the rusts and smuts. The primitiveness of the smuts was formerly asserted on grounds of variability in spore number and in the organization of the promycelium. The development of the promycelium from a thick-walled resting spore and the cytological phenomena in the smuts, to name but two characters, oppose this theory, and it is no longer of any importance. Phyletic transition through the rusts is argued chiefly on the asserted presence among them of "sex organs"—that is to say, female gametangia and receptive organs and male gametes. There is nothing in the recent careful accounts of Craigie (11) and R. Allen (1) to indicate a fundamental dissimilarity between the manner of initiation of the dikaryophase in the rusts and the hypha-conidium fusions of the hymenomycetes, and it seems preferable to follow Gäumann (16, p. 454; 17, p. 591) in interpreting copulation in the rusts as essentially somatogamous. The rust basidium, with its specialized organ of conservation (teliospore), is no nearer than that of the smuts to the ascus. For the rest, "Es ist doch unmöglich, Pilze, deren ganze Organization durch ihren extremen Parasitismus bedingt ist, als

Vorläufer solcher Pilzgruppen anzusehen, die ausschliesslich oder vorwiegend Saprophyten enthalten'' (Janchen, quoted by Neuhoff (19, p. 278)). No more credible is the derivation advanced chiefly by the Besseys (3, 4, 5) of the holobasidiate series from hypogaeous ascomycetes, through similarly hypogaeous gasteromycetes, by progressive reduction of the fruiting body, or that suggested by C. W. Dodge, (12) which similarly would have the higher hymenomycetes consist essentially of angiocarps become gymnocarpous. Either would, in general, constitute an utterly unparalleled example of evolutionary back-tracking, and of a large and extremely varied group arising from forms highly advanced, highly specialized, even to the extent of being degenerate in some of their most characteristic aspects. Specifically, the transition in spore production *among angiocarpous fungi* from endogenous to exogenous-sessile to exogenous-sterigmate and ultimately to exogenous-expulsive—the violent discharge of the spore from the sterigma is surely a fundamental basidiomycete character—is difficult of defense; what is true of the first of the schemes under consideration is true, to a slighter extent, of the second. Furthermore, sterigmate and sessile spores frequently occur within a single genus, in numerous widely separated genera of the gasteromycetes; the transition from asterigmate to sterigmate would under the theory in question have occurred many times over without having anywhere the slightest biological significance. The reading of the series in the reverse order—propulsive, sterigmate-deciduous, sessile—is biologically defensible; the sterigma is incomprehensible except as an organ contributing to spore dispersal, and consequently cannot have arisen, but only have degenerated, in forms where it disappears long before the spores are freed from the spore cavity. The evolution of a fairly extensive haplont from the reduced condition typical of the gasteromycetes, in which it is represented by only a brief early stage in basidiospore development, is equally improbable, and an equally conclusive objection to the derivation of gymnocarpous from angiocarpous basidiomycetes.

The most primitive basidium is therefore to be sought somewhere among the saprobic gymnocarps—more specifically, among those *Tremellales* and *Thelephoraceae* which do not show marked somatic development. There is not the absurdity that Janchen and Neuhoff see in positing some phragmobasidium as the primitive type. Once granted the proposition that the ascus, of a sort either lacking or

secondarily deprived of the means of discharging its spores outright, has developed exogenous spore production, some sort of case can be made out for almost any basidial type; witness Buller's argument (8) for an auriculariaceous basidium and Neuhoff's (19) for the holobasidium. Although there is no incontrovertible evidence that the evolution of the basidium has at any level been monophyletic, yet the numerous fundamental similarities among the various basidial types speak for their origin from an at most greatly restricted ancestral group. The complete series of basidial types being represented in resupinate forms only, it is proper to reason that the greater part of the fundamental morphological evolution has occurred at this level of development, and also to postulate the rise of the basidiomycetes from some such resupinate form as *Ascocorticium*. Further, the sterigma-spore relation, as has been shown by Buller, is morphologically and physiologically a fixed one throughout the gymnocarpous basidiomycetes; this being the case, it has either developed *de novo*—and scarcely more than once—in the course of basidial evolution, or else it represents, likely in a modified form, a biological adaptation already present in the ancestral ascomycete. The first implies development of all basidiomycetes from a single basidiomycetous ancestor; polyphyletic evolution of the group has any considerable probability only under the second hypothesis. Further consideration of the question must be founded upon a more minute examination of the various basidial types, to be undertaken in the following pages.

The concept of basidial morphology which offers the most satisfactory basis for comparative study is that proposed by Neuhoff (19) as the fruit of his studies of the *Auriculariaceae* and *Tremellaceae*. The structurally complex basidium of these groups typically arises as a vesiculose body, containing at first the basidial dikaryon and later the zygote. This primary vesicle may constitute a more or less protracted resting stage and usually remains morphologically distinguishable throughout the development of the organ; it is the probasidium. Neuhoff chooses to interpret it ecologically, as essentially a resting organ; but while it assuredly reaches its highest development as a sclerotized resting cell, there are everywhere among the basidiomycetes resting stages in basidial ontogeny which cannot properly be assimilated to the probasidium. It therefore appears preferable to interpret it morphologically, to see in it the actual vestigial ascus vesicle, which in most holobasidia is no longer

differentiated. An obvious corollary of this assumption is that the primitive basidium must be one having a probasidium. The mature basidium in the heterobasidiomycetes under consideration typically consists of a probasidium together with one or several more or less tubular appendages borne upon it, serving to separate the place of spore formation from the place of origin of the basidium and of karyogamy, to raise the spores to and beyond the surface of the basidiocarp, and serving also in some degree in the stead of the basidium as a whole in actual spore formation. These appendages are called by Neuhoff the epibasidia, and in the mature basidium possessing them the original vesicle is called the hypobasidium. Their number is most frequently four if meiosis occurs in the hypobasidium; if the diploid nucleus remains undivided long after the "germination" of the probasidium, only one epibasidium is formed, and the nucleus undergoes reduction some time after migrating into it from the hypobasidium. Neuhoff advances the profitable hypothesis that the morphological development was originally, in the course of phylogeny, the result of the cytologic, that the formation of the four epibasidia was originally occasioned by the thrusting out of the wall of the primary vesicle by the four haploid nuclei independently, and the single epibasidium by the activity of the entire protoplast acting as a unit with its single nucleus. Following him in this, it seems proper to consider that the basidiomycetes present fundamentally two phyletic tendencies, to divide them for purposes of comparative morphology between forms having epibasidial and those having hypobasidial meiosis. This is not to accept in the slightest degree the two basidiomycete series, the stichobasidial and the chiastobasidial, recognized by Juel; the folly of that arrangement has been demonstrated many times over, notably by Neuhoff. Nor is it intended to suggest by the inclusion of all basidiomycetes in one group or the other that there are in simplified types ontogenetic stages which correspond to the morphological differentiations of the more complex, as implied by Donk (13, pp. 78–81). His distinction between probasidium, "that part or stage in which karyogamy occurs," and metabasidium, "that part or stage in which the diploid nucleus divides," introduces a false complexity into the interpretation of the phylogenetically simplified basidium of the *Hymeniales*; in this group there is no epibasidium, and no need for such a distinction as Donk's. The two series here noted justify themselves by furnishing a theoretical basis for observed morphological differences.

It is a necessary part of any reasoning based on Neuhoff's concept that in no case can the epibasidium be equated to the sterigma. The sterigma of the heterobasidiomycetes is exactly what it is in the homobasidiomycetes, a subulate organ—Tulasne's *spicule* describes it—on which the spore is borne and which serves in its discharge after the manner described by Buller; sterigmata are to be found borne upon the tips or sides of epibasidia. The epibasidia differ not only in function and morphology, but also in their homologies, from the sterigmata; whereas they represent in a greater or less degree either the ascospores themselves (when several epibasidia are present) or else the germinating ascus protoplast (when there is but one), the sterigma and basidiospore are better homologized respectively with the sterigma and conidium or secondary spore by which certain ascospores are known to germinate. This conception, suggested by the author (21) on the basis of the *Tulasnella* basidium, finds its confirmation in its applicability to the interpretation of the various hypha-like complexities of which the epibasidia in their development are capable. Until the virtual completion of the present discussion it was not known to him that the same conception—that of the germinating ascus—had long ago been carefully developed by Vuillemin. "Une baside est un asque dont chaque cellule-fille, avant de passer à l'état de spore, fait saillie au dehors et se transforme en une sorte de conidie pour mieux s'adapter au transport par le vent." (22)

The three major divisions of the class *Basidiomycetes—Heterobasidiomycetes, Hymeniales* (hymenomycetes *sensu stricto*), *Gasteromycetes*—can be accurately delimited and established as reasonably homogeneous groups by the general properties of the basidium. Among the *Heterobasidiomycetes* the basidium (Plate VII, fig. 1–20) generally shows morphological differentiation into hypo- and epibasidium; it frequently shows regular septation; and most notable of all, not only is there great variation in basidial morphology from group to group and from species to species, but under suboptimum conditions the heterobasidiomycete basidium is capable of any of the modifications possible to ordinary mycelium—indefinite elongation, repeated irregular septation, branching, oidium formation—and of direct germination, by hyphae instead of by basidiospores. This variability is reflected in the basidiospores, in their occasional or regular septation and their germination by repetition or conidia as well as directly. So universal is this capac-

ity for variation, phyletic and ontogenetic, that it may well be taken to be the surest criterion of the group; and the types of response possible to the more typical forms must be taken into account in any attempt at interpretation of the more aberrant. It is worthy of note here that the suboptimum condition most frequently responsible for such variations in heterobasidia is the presence of excessive moisture—either saturated atmosphere or a highly deliquescent hymenial matrix—and that the biological significance of the variability is to be seen in the necessity for the gonotocont to reach above a water film if the products of meiosis are to be discharged as air-borne spores; either an appendage (epibasidium) elongates until it reaches the surface, or else the potential spore protoplast develops in hyphal fashion. Excessive moisture has likewise been shown to be responsible in some cases for ascospore germination by conidia, and for germination of ascospores while in the ascus. Neuhoff attempts to elucidate basidial evolution as the varying response to desiccation of an already established homobasidial type. Insofar as ecological factors can be held to be operative, it would appear better to concede at least an equal potency to the factor of humectation, as an unfavorable influence to be overcome, and *pari passu*, as an influence permitting precocious germination, as suggested by Vuillemin, and to attempt, as here, to recognize its possible influence in the original evolution of an exosporous tetrasporocyte.

The remaining basidiomycetes, united in the subclass *Homobasidiomycetes*, are uniformly characterized by the lack of differentiated hypo- and epibasidia and by the absence of septation or of any marked potentiality for ontogenetic variation in the basidium. The subclass is commonly divided into the *Hymeniales* (or *Hymenomycetes*, in a restricted sense), characterized by being at maturity gymnocarpous, and the *Gasteromycetes*, all angiocarpous. The groups are in an equal degree separable, and their relations much better indicated, by basidial characters. In striking contrast to the *Heterobasidiomycetes*, which are marked by variability, the *Hymeniales* show in basidial morphology a high degree of fundamental fixity. The basidium of the *Hymeniales* (Plate VII, fig. 21–26), the homobasidium *par excellence*, throughout the group shows no variation, with comparatively minor exceptions, other than in the proportions of its various parts; it consists of an ellipsoid to cylindric or clavate vesicle which developes only in size from the

time it is separated from the parent hypha to the beginnings of the sterigmata. It constitutes a simplified type, with no fundamental morphological elaboration except the sterigma by which violent spore ejection is accomplished, a simplification which once having been achieved in phyletic development persists throughout the range of basidiocarp evolution. The basidium still serves as a valid indication of taxonomic relations, but it is in this group of less use than elsewhere in phylogenetic elucidation.

Just as the heterobasidial types are comprehensible in the generalization of primitive variability and the hymenomycete basidium in that of simplification and fixity, so the basidia of the *Gasteromycetes* (Plate VII, fig. 27–29) are universally characterized by the degenerative variability that is occasioned by loss of function. The simple vesicle is retained; but the sterigma, no longer serving as a projector for the spore, in some forms becomes grotesquely long and tenuous and in others progressively shortened and obsolescent, until the spore is borne directly upon the basal cell. Where the sterigma remains the spore is borne not obliquely, as in all forms where forcible discharge occurs, but directly along its axis. (7) Such degenerative variations, however marked, are far from fundamental; they are to be interpreted only as secondary responses to an originally teratological or ecological modification of gymnocarpous fungi, and indicate the closest kinship of some of the gasteromycetous groups in which they are least advanced with comparable forms among the *Hymeniales*.

"As the founder of the Naples Biological Station has caustically remarked, it is a curious fact that every investigator is convinced that the type which he is studying has a monopoly of most of the primitive features, and that other types are secondarily modified." (18) It seems no more than fair to preface with such a caveat the suggestion that the *Tulasnella* basidium (Plate VII, fig. 1–5), as highly organized as any existent type, is phylogenetically closest of existent types to the antecedent ascus. It consists invariably of a more or less pyriform hypobasidium, in which occur karyogamy and meiosis, and four to eight epibasidia borne upon it; these receive the nuclei and cytoplasm from the hypobasidium, are separated from it by basal septa, and either at once or following a division of the single nucleus each contains elongate apically to produce above the surface of the fructification sterigmata and spores. There appears no more satisfactory hypothesis for the formation of the

sharply delimited mature epibasidia, which may be capable of producing basidiospores while the hypobasidium beneath them is collapsed and disintegrating, than that of their essential homology with ascospores—that is, the interpretation of them as an alternative mode of development of the protoplasts otherwise destined to mature as ascospores. The *Tulasnella* basidium, then, the one in which the ascospore stage is most completely retained in basidial ontogeny, is held to be most primitive. It may be noted here that the subdivision of the family *Tulasnellaceae* was earlier based, as in other groups, on presence or absence of gloeocystidia, but that basidial morphology, here as elsewhere, has been urged as a much more reliable indication of such lesser, as of more general, relationships.

The *Tremella* type (Plate VII, fig. 6) represents another development of the basidium with hypobasidial meiosis: here the walls separating the tetracyte protoplasts typically are laid down immediately after the second division, before the initiation of the epibasidial outgrowths; the hypobasidium having become "cruciately" septate by the more or less regular formation of three longitudinal walls, each segment, like the epibasidium in *Tulasnella,* proceeds independently to the formation of an apical filament bearing a sterigma and basidiospore. It may well be that the tremellaceous basidium has been derived directly from the tulasnellaceous type, by the transfer of basidial septation to an earlier point in growth. The possibility of independent derivation from the ascus is not, however, to be lost sight of completely. It is perhaps noteworthy also that there occur in certain resupinate species of the *Tremellaceae* (*Sebacina,* subgenus *Bourdotia*), along with longitudinally septate basidia, occasional others in which no septa are to be found even when the epibasidia have attained a considerable length; it has not yet been shown whether the hypobasidium remains undivided through the time of spore formation. At any event, there is here a suggestion of a possible derivation of the holobasidium; but the relations among the resupinate basidiomycetes are too complex, and variability too great, for this suggestion to be embodied in a definite hypothesis. It is to be borne in mind that such tardy septation is no more than a rare anomaly, perhaps only temporary even in the basidia in which it occurs; it is nevertheless valid as a suggestion of what may have occurred in phylogenetic history.

The basidial form characteristic of the *Dacrymycetaceae* offers an anomaly which has hitherto received no acceptable elucidation. The basidium (Plate VII, fig. 7) here arises as a considerably elongate clavate organ, in which karyogamy and meiosis occur; two very thick, long, horn-like appendages arise from the periphery of its truncate summit, each bearing at its tip a sterigma and spore. There is nowhere in the basidium any regular septation; but adventitious septa are occasionally formed both in the clavate portion and in the appendages; this, and the invariable germination of the spores by conidia (Plate VII, fig. 8), serve to confirm the assignment of the family to the heterobasidiomycetes. Their great size and their ability to become septate mark the appendages as epibasidia. The clavate portion is the highly modified hypobasidium. As ancestors for the dacrymycetaceous basidium have been suggested the smuts and the equally specialized and aberrant parasitic genus *Kordyanella*; such a derivation, roundabout and tenuous as it is, can scarcely be accepted for forms which somatically and biologically—not to enumerate basidial similarities—show many characteristics of other heterobasidiomycetes among the *Tremellaceae* and *Auriculariaceae*. On the other hand, of a group of the forms included in *Corticium* section *Botryodea* by Bourdot & Galzin, (6) forms showing their essential heterobasidiomycetous nature by possession of long, stout epibasidia and spore germination by repetition, one form, *C. sterigmaticum,* with the reduced number of two epibasidia (Plate VII, fig. 20), shows the most striking resemblance to the *Dacrymyces* type. The epibasidia arise from the cylindric basal cell exactly as in the latter form; except in length of the hypobasidium, there is no difference in form in the two basidia; it appears highly probable that the dacrymycetaceous type has been derived from a form with just such a basidium. The two forms in question show alike a secondary reduction in the number of epibasidia, correlated with larger spores; and the clavate hypobasidium of *Dacrymyces,* as in other groups, represents a response to the development of a compact hymenium. The most primitive member of the *Dacrymycetaceae* is probably, like the corresponding form in other families, a resupinate, *Ceracea*; nodulose, cerebriform, and spathulate fructifications represent an advance in somatic development.

The basidial types characterized by a single epibasidium may have arisen directly from the ascus or with about equal possibility

from the *Tulasnella* basidium, with its undivided basal cell; there seem to be no important grounds for preferring the one hypothesis to the other, the first being here accepted from considerations of logical economy; in either event the transversely septate basidium marks a distinct line of phyletic development. If the primitive auriculariaceous basidium be assumed to be one with a persistent hypobasidium, clearly distinguishable in all stages, an obvious example of it has so far been found only in such *Saccoblastia* material as shows distal germination of the probasidial sac (Plate VII, fig. 9–12). A hypobasidium is perceptible in a lesser degree in other forms, such as *Platygloea* (*Achroomyces*) (19); but in the very nature of the case there cannot be such sharp demarcation between the hypobasidium and a single thick epibasidium as when the appendages are several. The probasidium here passes repeatedly from a persistent, morphologically apparent organ to a stage; this is true to an extent in the atypical *Saccoblastia* basidia, even in that form where, if ever, the probasidium is a sharply delimited organ. The presence of division into hypo- and epibasidium, although not striking, characterizes many of the simple resupinate *Auriculariaceae*. It is, however, no great step from this to the situation in which there is no interruption in development from initial cell to mature basidium and no marked delimitation of basidial parts; and the two are to be found side by side. In *Iola* and *Eocronartium*, forms entirely comparable biologically and doubtless very closely related phylogenetically, the hypobasidium is respectively present and absent; in the somatically highly developed genus *Septobasidium* (Plate VII, fig. 15) both types occur, without showing correlation with other characters. In *Auricularia* (Plate VII, fig. 14) the hypobasidium is entirely lacking; in *Helicobasidium purpureum* (Plate VII, fig. 13) there is an evident probasidial stage but no delimitation of two parts in the mature basidium. The epibasidium likewise is capable of varying emphasis; in *Saccoblastia* among the more primitive forms and *Septobasidium* among the more advanced the portion in which meiosis and spore formation occur is often abstricted (Plate VII, fig. 12) and, like the *Tulasnella* epibasidium, capable of proceeding with its functions while the remainder of the basidium collapses beneath it or is separated completely from it. The manner of bearing the spores also varies; in *Auricularia* and *Platygloea*, where the mature basidia form a palisade, the epibasidial segments put out each a filament which reaches to the sur-

face before producing a sterigma and spore; this is the equivalent of the filament which carries the spore to the surface in those tulasnellaceous forms (*Gloeotulasnella*) in which the basidia are embedded in a gelatinous matrix. In other *Auriculariaceae* with less compact hymenia the basidia are curved so as to expose the whole convex side at the surface, and the sterigmata arise directly from the epibasidial segments (Plate VII, fig. 13), as in *Tulasnella violea.*

If considerations already advanced here are given adequate weight it would appear impossible to doubt seriously that the *Uredinales* have arisen not from non-basidiomycetous but rather from auriculariaceous ancestors; the morphology of the basidium alone is incontrovertible evidence for this. The teliospores are sclerotized probasidia such as are already present among the *Auriculariaceae,* and the so-called rust promycelium is an epibasidium (note Fig. 15, of *Septobasidium,* which would serve well as a diagram of a germinating teliospore). In the course of the evolution of the group as parasites there has been infinite variation in the arrangement of the basidia, in biological relations, in accessory spore forms, and, most obviously, in reduction of the basidiocarp; and along with all this the basidium has remained constant to so great an extent that the phylogenetic relations within the order must be deduced almost entirely from what may be regarded as somatic characters. What basidial variation there has been is probably to be accounted for largely by variation in the fruiting layer, and consequently may be regarded as of only secondary significance. It appears logical to follow Neuhoff in considering the uredospores abortive probasidia, and to go beyond him in interpreting the aeciospores as diploid conidia likewise essentially probasidial in nature; such structures are not unknown among the saprobic basidiomycetes, and there is ample evidence for the homology in germination phenomena. The pycniospores, whatever properties may be ascribed to them, are essentially haploid conidia; they may, like conidia in numerous other groups, serve to mix the strains in heterothallic species.

The rusts being essentially *Auriculariaceae* highly evolved as plant parasites obligate throughout their life cycle, the smuts are equally to be characterized as derivatives of the *Auriculariaceae* whose diploid phase has become specialized as a parasite, but whose haplont remains saprobic. In many *Ustilaginales* the basidium

(Plate VII, fig. 16, 18) constitutes the entire haploid portion of the cycle; the numerous conidia or "sprout-cells," which in the forms with septate epibasidium are cut off in considerable numbers from the sides of the segments, conjugate in pairs (Plate VII, fig. 17) and the diplont, which alone is capable of infecting the host, is thus almost at once reinitiated. In other forms the typical basidiospores by which in the *Auriculariaceae* the tetraspore protoplasts germinate are replaced not by conidia but by short hyphal outgrowths—a phenomenon frequently to be observed in more typical members of the heterobasidiomycetes—which conjugate directly (Plate VII, fig. 18), either with those produced by other segments of the same basidium or with those arising from other chlamydospores. In the *Tilletiaceae* transverse septation of the epibasidium becomes completely obsolete, occurring only behind the entire protoplast as it migrates out into the elongate promycelial tube; germination occurs at the apex of the basidium, by sprout-cells or hyphae. In this order, the logical end of the phyletic development commenced with the primitive *Auriculariaceae*, the haplont has almost completely dropped out; since it is incapable of disseminating the fungus to any degree (the basidiospores being unable to withstand even brief desiccation and, with a few exceptions, being incapable of infecting the host), the basidium has degenerated from the form adapted to violent spore discharge and has undergone all sorts of modifications by which the certainty and rapidity of initiation of the diplont are increased: has in a sense passed to partial cleistogamy. These modifications being without exception such as are to be found in any of the heterobasidial types subjected to unfavorable conditions, the terminology and homologies by which other members of the group are best understood are to be applied without qualification to the smuts.

Basidia which not only suggest, but which may be held actually to constitute, transitional forms between the hetero- and homobasidium are, as implied, by no means lacking. A number of the *Botryodea* forms of *Corticium* have basidiocarps exactly like those of species of *Tulasnella* and *Sebacina*; the basidia (Plate VII, fig. 19) arise as inflated subglobose bodies whose resemblance to the phragmobasidiomycete probasidium is very striking; from the apex of such a cell arise four—or in some species only two, or up to eight—thick, greatly elongate appendages, sterigmate at the tips, capable of such irregularities as occasional branching or the formation of septa be-

hind the migrating protoplast; the spores may regularly germinate by repetition. It has already been submitted by the author (21) that these fungi may have arisen from *Tulasnella*-like forms by the dropping out of regular septation in the basidium. The alternative hypothesis that the ancestral form may have been tremellaceous cannot logically be excluded; the *Tremella* basidium and the transitional holobasidium under discussion have this in common, that to a much higher degree than in *Tulasnella* it is in them the hypobasidium, or a segment of it, that is concerned in the production of the spores; in *Tulasnella* it is the almost disarticulate epibasidia. It may even be held, as in the system of Neuhoff, that the holobasidium arose directly from the ascus. To this last, however, it is to be objected that three lines so closely related as the tulasnellaceous, the tremellaceous, and that represented by the transitional holobasidium in question can scarcely have arisen independently of each other; and the structural complexities of the phragmobasidia —notably, that of *Tulasnella*—are much better explained by the hypothesis of ascus germination here supported than as secondary complications, in the sense held by Neuhoff. There is little to choose between derivation from *Tulasnella* and from *Sebacina*; the author, considering the tulasnellaceous basidium the more primitive of the latter two, prefers to see in it the ancestor of the holobasidium. Whatever their origin, according to the criteria here recognized the holobasidiomycetes under discussion are heterobasidial. They are so strikingly intermediate in all their characters and affinities, however, that their assignment to any existent group must bring with it objections. Donk has segregated one portion of the section *Botryodea*, in which these forms are included, as the genus *Botryobasidium*; this he has included in the *Tulasnellaceae*. The affinities of the particular species published for *Botryobasidium* are by no means with *Tulasnella*; they possess short, stout, cylindric basidia (Plate VII, fig. 21) upon which the sterigmata are borne directly, and so far as can be ascertained, never have germination by repetition. The case is rather different for other *Botryodea* species, notably *Corticium sterigmaticum, C. cornigerum,* and an undescribed species which in everything except basidial septation is much like *Gloeotulasnella calospora* and *Sebacina calospora*; these are the forms referred to earlier as heterobasidiomycetous. But in actual morphology these forms are no closer to *Tulasnella* or *Sebacina* than the latter are to each other, and cannot properly be

placed in a family with either; the one, as noted, might even be considered one of the *Dacrymycetaceae*; and the only logical disposition of them would be to place them in a family of their own in the heterobasidiomycetes. But it is questionable, in view of the abundant and close transitions toward purely homobasidial types, whether such segregation would result in a clearer expression of relationship; and it appears preferable to accept only generic segregation, treating the group taxonomically as related to *Corticium*, with the express reservation that it *is* transitional, with affinities and characters on the other side of the line. The fungi under discussion, like Vuillemin's protobasidiomycetes, constitute "un group dont il est plus aisé de suivre l'enchaînement que de tracer les limites." (22)

The typical homobasidiomycetes have in all probability arisen from such forms in more than one series. One line of development, whose members are marked by very short, thick-celled hyphae, loose and branching at right angles and often colored, and by stout, short-cylindric basidia borne in botryose fashion (*Botryobasidium*) leads through forms with somewhat spinulose spores (*Tomentella isabellina*, identical in all respects save spore surface with typical species of *Botryobasidium*, and by no means to be separated on this single character) to typical species of *Tomentella*, whose more hypochnoid species show considerable structural similarity, basidial and somatic, with *Botryobasidium*. *Tomentella*, in turn, is by no means to be separated widely from *Caldesiella*; here again arises a question of practical taxonomy. *Caldesiella*, like *Tomentella* in all respects save one, has been separated not only as a genus but even as a member of a separate family, the *Hydnaceae*, because its hymenium is somewhat nodulose or warted instead of only slightly nodulose or warted, as in *Botryobasidium* and *Tomentella*. It has been well said that the Friesian subdivisions of the hymenomycetes have been defined by arguing backwards from the hymenophore configuration of end forms; to what extent this arrangement must be retained will not be settled until a greater amount of critical work on relationships has been done; but not by the wildest exercise of the imagination can *Caldesiella* be held to be anything other than a *Tomentella* with hymenophore sculpturing in somewhat higher relief; and to separate it by a family line, whatever may be said of the convenience of Friesian characters for architects of keys, is a detrimental and irrational exaggeration. The present

case is cited as only one of numerous possible examples. Either a taxonomic system is to be worked over and modified, endlessly, to express relationship and degrees of relationship, or else it ought to be frankly considered an indexing arrangement and simplified as such, with splittings and bracketings wherever they add to the symmetry of the key. One may then fit fungi as he finds them into named spaces or numbered blanks in already prepared columns, as has been done with imperfects. Patouillardian categories are unquestionably more difficult to follow in keys than Friesian, for one who knows nothing of mycology, but are surely no more disconcerting than for one who recognizes a *Tomentella* under his lens or a *Coniophora* under his microscope to discover that he must find it in the *Hydnaceae* or in *Merulius* of the *Polyporaceae*. It appears not inappropriate, in connection with the difficulties of the more natural classification, to remember the words of an American phanerogam taxonomist, who has said, "I'm not writing keys for boy scouts."

A second homobasidiomycete group set sharply apart by the morphology of its basidium is that made up of the *Urnigera* sections of *Corticium, Gloeocystidium, Grandinia* and *Poria*. The arid, more or less chalky fungi here included bear on a scanty subiculum basidia (Fig. 23–25) which, arising as ovoid or short-clavate bodies, just before spore production elongate considerably, bearing on the summit of a more or less distinct apical portion a crown of slender sterigmata, regularly more than four, and typically eight in number. The urn-shaped basidium and the delicate crown of sterigmata are highly distinctive; it is unthinkable that the group should represent more than a single line of development. This being the case, the forms with smooth hymenia, having or lacking gloeocystidia, those with tuberculose hymenium, even here often partly smooth, and those whose hymenium is thrown into ridges and pits, are assuredly not to be distributed among three families, as though the warted portion of a fructification were more closely related to *Hydnum* and the smooth part to *Thelephora* than either is to the other. This *Urnigera* series demonstrates more clearly than any case yet unsettled the fallacy of subdivision according to hymenial configuration. It is assuredly only a question of time until other series such as this, as the *Tomentella* group, as *Coniophora* and its relatives will be traced out and given adequate taxonomic recognition; as well retain *Tremellodendron* in *Clavaria, Lentzites*

in the *Agaricaceae*, *Tremellodon* in *Hydnum*, *Auricularia* in *Exidia*, as attempt to retain the old groupings as they are now generally allowed to stand. The *Urnigera* basidium itself stands in need of phylogenetic elucidation. There are in *Corticium* in the present all-inclusive sense several minute, arid-crustose forms assigned by Bourdot & Galzin to the section *Athele* which possess the same scanty, fine mycelium, a similar ovoid basidium, and sterigmata ranging in number from four to eight, that mark the *Urnigera* types. There is not the intervening superficially epibasidium-like prolongation before the sterigmata are put out; even so, it seems not unlikely that among them the ancestral type is to be found for the highly characteristic *Urnigera* group. This is no more than a suggestion; whatever may be the affinities of the *Urnigera* basidium, they are not immediately with any of the heterobasidiomycetes; it must be taken to be a secondary development.

There is still the question of the extent to which information can be drawn from the basidium as to the actual relations among the remaining *Hymeniales*. The stout, thin-walled, cornute basidium of *Coniophora* and related species of *Merulius* is quite possibly as constant an indication of a member of this series as are texture of fructification and spore morphology and color. The flexuous, irregular, greatly elongate basidium of *Aleurodiscus* (Plate VII, fig. 26) and the scarcely distinct genera *Dendrothele* and *Vuilleminia* is a better criterion of the group than other structural characters, and marks it as a phyletic unit. But in the majority of the hymenomycetes the basidium does not furnish sufficient indication of kinship to permit the elaboration from its characters alone of a natural system of classification. There apparently arose from the most primitive holobasidiomycetes a number of lines of development; these may be taken to have persisted as the more strikingly distinct series met with among the somatically little developed *Thelephoraceae*. But higher development involved hymenophore elaboration and the rise of a compact hymenium; a number of lines never progressed so far, and those that did approximated a common clavate, slender-sterigmate basidial type (Plate VII, fig. 22), so closely that differentiation upon basidial characters becomes in the better developed forms scarcely possible. It may well be that differences persist; but these must be studied in extensive series of forms, and in most of the basidiomycetes, particularly those above the *Thelephoraceae* in the present system, probably will never be

of any use except in conjunction with other details of microscopic structure, with texture, color in many cases, and spore morphology. The abundant and varied details of minute morphology to be taken into account in such a study, furthermore, contrary to what is still the almost universal practice, must be treated in combination, as mutually supplementary. Refusals to acquiesce in the arbitrary multiplication of "genera" on the basis of possession of gloeocystidia, of cystidia, of the two together, have been all too few. Among the heterobasidiomycetes there is no such correlation between occurrence of these bodies and basidial as well as other fundamental structural differentiation as would be implied by their extensive use as the sole criteria of new taxonomic segregates. Preliminary studies by the writer would indicate that in the *Thelephoraceae* there is no more connection between the presence of differentiated sterile hymenial organs and such grouping as is clearly indicated by general structure and by such characteristic basidial peculiarities as are present than was found to exist in *Sebacina.* It is significant in this connection to note that most of the sections based on structure that have been erected within *Peniophora* (6) essentially duplicate similar sections in *Corticium,* and that in *Gloeocystidium* have been assembled forms with typical homobasidia, *Urnigera* forms, and even one species (6, p. 261) that failed of being assigned to *Sebacina* (*Bourdotia*) only because the workers who described it failed to observe the basidial septa which (teste Donk) were actually present, this heterogeneous assemblage having no common character but gloeocystidia. Spore ornamentation is a criterion equally delusive when applied uncritically. The spiny *Tomentella* spore is a consistent recognition character for that group; but to put *Corticium tulasnelloideum* and *C. fumosum* (*C. sulphureum* Pers. *non* Fr.) in *Hypochnus* (i. e., *Tomentella*) because their spore wall is roughened is to set a purely verbal consistency against the indications of every possible gross and microscopic character. Relations are to be argued not from verbal but from actually visible characters. The result ultimately to be achieved is a system of classification whose lines will run in many instances perpendicular to those now most commonly recognized, whose series will be phyletic units including, it may be, forms from the ancestral *Thelephoraceae* and from the more highly evolved *Hydnaceae, Polyporaceae, Clavariaceae,* and *Agaricaceae.* The outlines have already been drawn by Patouillard; numbers of minute

but extremely significant forms have been added to the list of known species by Bourdot & Galzin, to the great improvement of the understanding of the whole; they have also applied to a higher and very useful degree the concepts of Patouillard in further critical rearrangement of old as well as new fungi; a highly significant contribution here is being made at this time in the radically revisionary work of Donk. (13, 14)

The arrangement of the *Gasteromycetes* is based even less than that of the *Hymeniales* on knowledge of basidial morphology, or on any minute structural criteria other than disposition of fertile areas. The transition from gymnocarpous to angiocarpous homobasidiomycetes is if anything less abrupt than from hetero- to homobasidial forms. *Secotium agaricoides* and a number of other fungi often included in a family with it can represent, as shown by Conard, (9) nothing but stipitate *Hymeniales*—agarics or boletes—which, perhaps as an ecological response, having failed to open, have set free their spores in the cavity between pileus and stipe. According to Fischer, (15) "the chief characteristic of the gasteromycetes in contradistinction to the hymenomycetes consists in this, that the basidia at the time of spore formation lie within the fruiting body;" this obvious chief taxonomic criterion can by itself explain the other criterion, equally valid but generally disregarded, of basidial morphology, and can account quite satisfactorily for the rise of the whole group. It is not intended to suggest that all gasteromycetes arose from a single form; basidial and especially spore characters as well as general morphology, carried over from the ancestral fungi, show them in all likelihood to be made up of the derivatives of several hymenomycetous groups; but it is a quite satisfactory hypothesis, with no acceptable competitor, that all gasteromycetes have arisen, directly or remotely, from stipitate hymenomycete groups; and relations within the angiocarpous group may to a considerable degree be elucidated by tracing their descent from the various types of more hymenomycete-like members. As earlier noted, the variations in the gasteromycete basidia are comprehensible as representing various degenerative tendencies in a spore-discharge mechanism which is no longer free to discharge its spores, some spores, produced on long, fragile sterigmata (Plate VII, fig. 29), being freed by fracture, and others, borne directly on the basal cell (Plate VII, fig. 27, 28), by disintegration. The arrangement, as well as the form of the sterigmata, shows degener-

ative tendencies: often they are scattered irregularly upon the summit of the basidium; and often they are borne as well upon the sides; the pleurosporous basidium of *Tulostoma* is no more than one striking example of this type. The basidium cannot be here, any more than in the *Hymeniales,* the sole and sufficient criterion of phyletic affinity within the great group; carpophore morphology, among fungi so highly elaborated as these, must be the chief object of study; but, as suggested by Corner, (10) the basidium must be taken into account. If inadequate consideration be given the indications of basidial morphology, phylogenetic absurdities can scarcely fail to result.

In general, it may be asserted that only by study of the basidium can the larger phylogenetic tendencies of the basidiomycetes be understood; that although in many cases basidial morphology cannot alone furnish the key to relationships, in no case is it possible for relations to exist which are denied by basidial characters. The present discussion represents an attempt to evaluate and interpret what information is at hand concerning the basidium. It is intended to set forth the results of the examination of such evidence from the point of view of one phylogenetic concept. There have been, and will yet be, many other points of view, some of them, surely, adapted to the settling of questions which have here only the most unsatisfactory of answers—the question, for example, of which there is here only the beginning of a treatment, of the exact relation existing among the primitive examples of the various types of heterobasidia. "Scientific hypotheses have in their nature no pretension to permanence, and . . . should be judged by their capacity for bringing to light further generalizations, to which, in turn, they yield place." (2)

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The indebtedness of the author to Neuhoff and Gäumann must have been evident throughout the present discussion to anyone acquainted with their published work; he desires to make general acknowledgment of such indebtedness at this time. To those whose privilege it has been to be present at any discussion of the basidiomycetes by Professor G. W. Martin, his authorship and inspiration of much that has been said here must be even more apparent. To his instructor, Dr. Martin, for constant encouragement and direct assistance in this and throughout his mycological studies, the author wishes here to record his gratitude.

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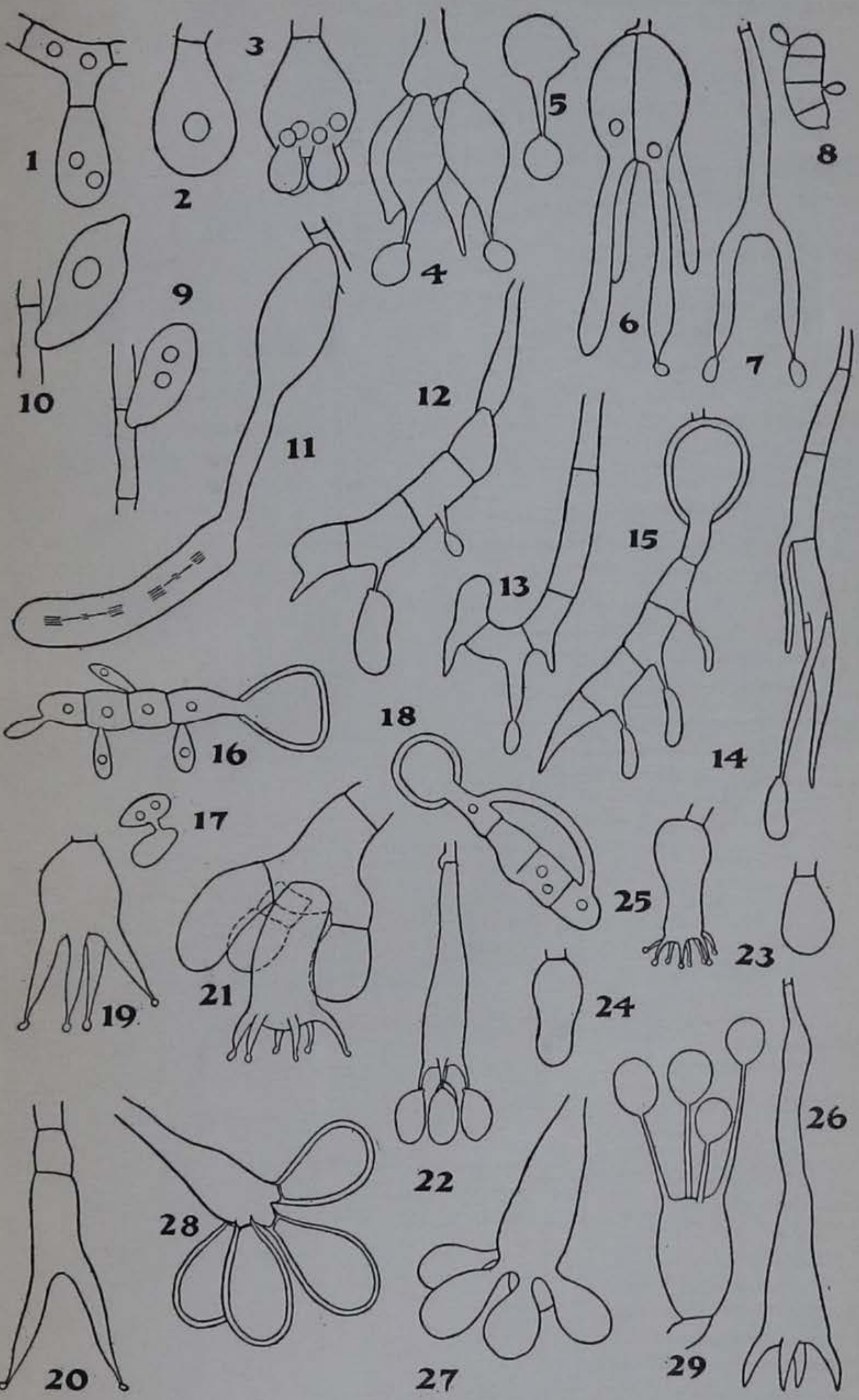
PLATE VII

1–5. *Tulasnella violea.* Stages in basidial development; spore germination by repetition.
6. *Tremella aurantia.*
7, 8. *Dacrymyces.* Basidium; spore germination by conidia.
9–12. *Saccoblastia sebacea.* Probasidia; complete basidium (atypical), with distal origin of epibasidium (reconstructed: diagrammatic); epibasidium with spores.
13. *Helicobasidium purpureum.*
14. *Auricularia* (reconstructed: diagrammatic).
15. *Septobasidium.*
16–18. *Ustilago* (reconstructed: diagrammatic).
19. *Corticium (Botryodea) calospora* sp. ined.
20. *Corticium sterigmaticum.*
21. *Botryobasidium coronatum.*
22. *Hymeniales.* "Typical" homobasidium (diagrammatic).
23–25. *Corticium (Urnigera) subtrigonospermum* sp. ined.
26. *Aleurodiscus* (diagrammatic).
27, 28. *Melanogaster variegatus.* Immature and mature basidia.
29. *Lycoperdon.*

Figs. 1–6, 9, 10, 12, 13, 19–21, 23–25, 27, 28 are redrawn from camera lucida drawings by the author; fig. 7 is redrawn from a drawing by Martin, 8 after Fisher, 15 from a figure by Coker, 16, 17 after Harper, 29 after Coker & Couch. Most of the figures have been altered in a greater or lesser degree, either after examination of a greater range of objects or, with the borrowed figures, according to other figures. It is not intended that any figure should be a representation of a particular object, but of the structure typical of the taxonomic unit—genus or species—in question.

PLATE VII



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