STUDIES OF COPROPHILOUS ASCOMYCETES

IV. TRIPTEROSPORA, A NEW CLEISTOCARPOUS GENUS IN A NEW FAMILY[1]

By Roy F. Cain[2]

Abstract

A new family Tripterosporaceae is introduced by the description of three species of a new genus, *Tripterospora*, similar in several characteristics to *Podospora* (*Sordaria*) but clearly separable by the production of globose, non-ostiolate ascocarps and of ascospores which lack gelatinous secondary appendages and sheaths. In *T. longicaudata* Cain sp. nov., the type of the genus, and in *T. brevicaudata* Cain sp. nov., early development of the ascocarp is seen to be from a coiled and twisted ascogonium as in many members of the Ascohymeniales, with the ascogonia scattered on the aerial mycelium. Ascospores in all three species are two-celled, the upper cell dark and ellipsoid with a circular germ pore at the apex, the lower cell hyaline. Descriptions and illustrations are given for the known species: *T. longicaudata*, isolated in pure culture from dung and from swiss chard plants in Ontario; *T. brevicaudata*, also observed only in pure culture, obtained from a living branch of yellow birch in Connecticut, and from tomato seed in Ontario; and the third, described by Griffiths as *Pleurage erostrata*, collected on dung in Ontario and Manitoba.

Introduction

A study has been made of three species which resemble the genus *Podospora* in the character of their asci and ascospores. However, these species differ in the evanescence of the ascus wall, the irregular orientation of the asci within the ascocarp peridium, the less extensive development of the paraphyses, and the lack of gelatinous secondary appendages. Furthermore, the ascocarps in all three species are globose, without neck or ostiole. Since there is no genus so characterized, the name *Tripterospora* is proposed.

On account of its inostiolate globose ascocarp, *Tripterospora* should be included in the order Plectascales. Although the writer has pointed out previously (2) that this order is not a natural one, it is, at this time, the only repository for the genus, unless it be included in the ostiolate family Sordariaceae. However, since *Tripterospora* has no close affinity with members of the Aspergillaceae or any other family now known in the Plectascales, it becomes necessary to erect a new family, the Tripterosporaceae.

Descriptions

Tripterosporaceae Cain fam. nov.

Saprophytic; ascocarps globose, superficial, cleistocarpous, developing from ascogonia produced as side branches scattered on mycelium. Asci clavate, in irregular or parallel fascicles, evanescent at maturity. Ascospores dark olivaceous-brown to nearly black, each with a single circular germ pore.

[1]*Manuscript received April 16, 1956.*
Contribution from the Department of Botany, University of Toronto, Toronto, Canada. This study was carried out with the assistance of grants in aid of research from the University of Toronto and the National Research Council of Canada.
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Saprophyticae; ascocarpi globosi, superficiales, sine ostiolo, ab ascogoniis lateralibus dispersis in hyphis orientes. Asci clavati, sive parallele sive in fasciculis irregulariter dispositi, evanescentes. Ascosporae atro-olivaceae, cum foramine germinali singulari orbiculato, praeditae.

Type genus: *Tripterospora* (*tripter*, τριπτήρ, pestle and *spora*, σπορά, seed).

Tripterospora Cain gen. nov.

Saprophytic; ascocarps developing from ascogonia produced as side branches scattered on mycelium, at maturity superficial, globose, without ostiole, covered with flexuous hair-like projections or bare except for surrounding hyphae. Peridium of ascocarp light olivaceous-brown, pseudoparenchymatous, membranaceous to somewhat coriaceous, a few cells in thickness. Asci eight-spored, clavate, stipitate, in irregular fascicles, sometimes with thickened ring in apex, evanescent, leaving spores in mass within ascocarp cavity. Ascospores uniseriate or biseriate, two-celled without gelatinous appendages or sheath; upper cell ellipsoid, smooth, dark olivaceous-brown to nearly black, with circular apical germ pore; lower cell or primary appendage hyaline, without visible contents.

Saprophyticae; ascocarpi superficiales, globosi, pallide olivaceo-brunnei, cum pilis vel hyphis vestiti, ab ascogoniis lateralibus dispersis in hyphis orientes. Peridium ascocarpi pseudoparenchymaticum, membranaceum vel subcoriaceum e cellulis in stratis paucis compositum, non ostiolatum. Asci clavati, octospori, stipitati, evanescentes, ad apicem interdum cum annulo singulo praediti, et in fasciculis irregulariter dispositi. Ascosporae uniseriatae vel biseriatae, biloculares; cellula superior ellipsoidea, levis, atro-olivacea, cum foramine germinali, singulari, orbiculato, apicali praedita; cellula inferior hyalina.

Type species: *Tripterospora longicaudata* Cain.

KEY TO SPECIES

Ascospores with hyaline basal cell broad (more than 4 μ) and short (less than 5 μ)..... 1. *T. brevicaudata*
Ascospores with hyaline basal cell narrow (less than 4 μ) and 6–8 μ long.....2. *T. erostrata*
Ascospores with hyaline basal cell narrow (less than 4 μ) and 10–17 μ long..3. *T. longicaudata*

1. Tripterospora brevicaudata Cain sp. nov. Figs. 1–10

In artificial culture producing white colonies. Hyphae hyaline, 2–8 μ in diameter, branching infrequently, remotely septate. No conidia or spermatia produced. Ascogonia as side branches scattered on mycelium, becoming irregularly twisted and coiled, surrounded by branches from stalk. Ascocarps abundant (Fig. 1), densely aggregated, mostly superficial or partially immersed, some completely immersed in agar matrix, globose, 300–640 μ in diameter, smooth, very light-brown, but appearing black owing to ascospore mass inside, embedded in a loose mat of light flesh-colored hyphae. Peridium of ascocarp very thin, semitransparent, membranaceous to slightly coriaceous, pseudoparenchymatous. Peridial cells (Fig. 2) small, somewhat angular and irregular in shape, very indistinct, in several layers, light-brown, merging on the inside

into the larger, thin-walled, colorless cells surrounding the asci (Fig. 4); outer cells extending into very long, flexuous, fairly thick-walled, remotely septate, rarely branching hairs measuring 2–4 μ in width, light brownish at base, hyaline at apex, with superficial, scattered, irregularly shaped, very faintly brownish crystals. When first crushed out of fully mature ascocarps, spore mass (with asci completely broken down) held together in a single globose ball by a transparent membrane produced by collapsed, hyaline, swollen cells of the innermost peridial layer. Asci (Figs. 5–7) eight-spored, clavate, 140–170 × 20–28 μ, narrowed somewhat above, with a broadly rounded apex, tapering below to a short stipe with crozier at base, few, forming irregular fascicles, very evanescent, leaving spores in mass in ascocarp cavity. Paraphyses consisting of short chains of swollen cells extending inward from cells of ascocarp peridium. Ascospores (Figs. 5–8) uniseriate or partially biseriate, rapidly becoming biseriate in upper part of ascus when mounted in water, at first hyaline, ellipsoid, with a slightly pointed base, becoming transversely uniseptate; upper cell ellipsoid, 18–26 × 12–17 μ, finally olivaceous-brown to nearly black, but not quite opaque, with irregular granular contents, rarely with one large refractive globule, with an apical, circular germ pore; basal cell or primary appendage remaining hyaline, 3.5–5.0 μ long and 4–7 μ wide at base, papilliform. Without gelatinous appendages or sheath on ascospores.

The ascospores are usually so oriented in the ascus that the hyaline cells are basal, but sometimes a few spores in the upper end of the ascus are inverted. Another occasional anomaly observed is the failure of an ascospore to form a septum, thereby becoming completely colored but with a papilliform base (Fig. 9).

Monoascospore cultures are self-sterile since they fail to develop mature ascocarps. Ascospores have been seen to germinate after 18 hr. in water, producing a small vesicle outside the germ pore with usually one long and one short branch (Fig. 10). Thereafter, the hyphae, hyaline with a few small oil globules, become quite long before branching.

Collections: Isolated in pure culture at Ottawa, April, 1940, by J. W. Groves, from tomato seed, Eastern Ontario (TRTC 31780). Isolated in pure culture at Ottawa, April, 1940, by J. W. Groves from radish seed (registered), British Columbia (**Type** TRTC 31779). Isolated in pure culture by J. R. Hansbrough, from living branch of yellow birch tree infested with bronze birch borer, Conn., July 22, 1945, (TRTC 31781).

Mycelio ex hyphis hyalinis, raro ramosis, remote septatis, 1–2 μ diam. composito. Neque conidia neque spermatia prolata. Ascocarpis ab ascogoniis lateralibus dispersis in hyphis orientibus. Ascocarpis dense aggregatis, superficialibus, vel raro immersis, globosis, abundantibus 300–640 μ, levibus, pallido-brunneis. Peridio ascocarpi tenuissimo, diaphano, membranaceo vel coriaceo, pseudoparenchymatico. Cellulis peridii in stratis externis pallido-brunneis parvis semiangulatis irregularibusque, valde obscuris; cellulis peridii in stratis internis majoribus, hyalinis, tenuiter tunicatis. Pilis ascocarpi longissimis, flexuosis, crasse tunicatis, remote septatis, raro ramosis, 2–4 μ diam., ad basin pallido-brunneis, ad apicem hyalinis, crystallis vestitis.

Ascosporis in globo singulari conglomeratis. Ascis 8-sporis, clavatis, 140–170 × 20–28 μ, basi in stipitem brevem sensim attenuatis, evanescentibus, in fasciculis irregulariter dispositis. Ascosporis uniseriatis vel partim biseriatis, primum hyalinis, ellipsoideis, basi acumenatis, deinde transverse uniseptatis. Cellula superiore ellipsoidea, in maturitate brunneo- vel atro-olivacea haud opaqua, 18–26 × 12–17 μ, cum foramine germinali singulari, orbiculato praedita. Cellula inferiore hyalina, 3.5–5.0 μ longa, ad basin 4–7 μ lata, papilliformi. Ascosporis sine appendicibus gelatinosis et sine strato mucoso.

2. *Tripterospora erostrata* (Griff.) Cain comb. nov. Figs. 11–16

≡ *Pleurage erostrata* Griff. Mem. Torrey Botan. Club 11 : 71. 1901.

Ascocarps (Fig. 11) scattered, entirely superficial, globose, non-ostiolate, 200–250 μ in diameter, black, opaque, completely covered with very long, flexuous, septate, brown hairs (Fig. 13) measuring about 1 millimeter in length and 4–5 μ in diameter; wall of hair thickened at base, thinner toward apex. Peridium of ascocarp lacking suture marks and special lines of dehiscence, membranaceous, pseudoparenchymatous, composed of dark-brown angular cells in several layers and mostly about 10 μ in diameter (occasionally up to 15 μ) (Fig. 12). Asci eight-spored, clavate, 50–70 × 16–20 μ, forming irregular fascicles, somewhat narrowed in upper part, broadly rounded at apex, with a stipe measuring about 20 μ in length, very evanescent. No paraphyses seen. Ascospores (Figs. 15, 16) mostly biseriate, at first hyaline, one-celled, somewhat pestle-shaped, becoming transversely uniseptate; upper cell finally dark-brown and opaque, ellipsoid, acutely rounded above, with a circular germ pore at apex, broadly rounded to truncate below, 10–12 × 6.5–7.5 μ; basal cell or primary appendage remaining hyaline, cylindrical, straight, 6–8 × 3 μ. Without secondary gelatinous appendages or sheath on ascospores.

Collections: Developed in laboratory, Toronto, Feb. 1940, on rabbit dung collected northwest of Burford, Brant Co., Ont., April 29, 1939, (TRTC 31777). Developed in laboratory, Winnipeg, Man., Jan. 25, 1933, on horse dung collected at Manitoba Agricultural College, G. R. Bisby, 4607 (TRTC 31778).

3. *Tripterospora longicaudata* Cain sp. nov. Figs. 17–33

Colonies on modified Leonian's agar + 0.3% yeast extract, and on "V-8" vegetable juice agar, very similar, at seven days in test tube culture, expanded to a diameter of about 2 in., margin very indefinite, surface irregularly pruinose, with scanty aerial growth, gray in color or with a pinkish cast. Under a lens, aerial mycelium appearing cottony with minute irregular areas. Central half of colony somewhat darkened with ascocarp production and black in reverse. Hyphae hyaline, 1.5–4.0 μ in diameter, frequently anastomosing. No conidia produced. Ascogonia initiated when mycelium is only a few days old, developing rapidly thereafter as side branches scattered on mycelium, becoming irregularly twisted and coiled (Fig. 21), surrounded by branches from stalk which proceed to form peridium of ascocarp, adjoining hyphae not

involved in peridial production, few hyphae extending out from young ascocarp. Ascocarps (Figs. 17–18) densely aggregated, forming a continuous layer over surface of agar, mostly superficial, with a few embedded in agar, globose, 150–250 μ in diameter, covered with a thin, arachnoid weft of mycelium, appearing black and shining by reflected light because of colored ascospores within. Peridium light-brown, pseudoparenchymatous, semi-transparent, coriaceous, about 15–25 μ in thickness. Peridial cells very irregular and interlocking, in three to five layers, the outer (Fig. 19) 4–12 μ in diameter, the inner (Fig. 20) somewhat larger (8–16 μ in diameter). Wall of peridial cells very thin and light yellowish-brown. When first crushed out of fully mature ascocarps, spore mass (with asci completely broken down) held together in a single globose ball by means of a transparent membrane not readily dissolving in water and without cellular structure (Fig. 18) but formed from collapsed hyaline cells of the nutritive layer within the peridium. Asci eight-spored (Figs. 24–27), clavate, 75–85 $\times$ 15–18 μ, narrowed somewhat above middle, at maturity showing a distinct thickened ring in broadly rounded apex, narrowed to a short stipe with crozier at base, forming irregular fascicles, not in a single parallel layer or oriented in any single direction, very evanescent. Paraphyses (Figs. 24, 25) consisting of short chains of swollen cells measuring 10–18 $\times$ 4–9 μ each containing two or three oil globules, separating freely from asci in crushed mount. Ascospores (Figs. 28–33) biseriate, at first elongate with a swollen upper end, becoming transversely uniseptate; upper cell darkening through olivaceous-brown, with a single refractive oil globule, to nearly black and opaque at maturity, ellipsoid, 13–15 $\times$ 8.0–9.5 μ, with an apical, circular germ pore measuring 1.5 μ in diameter; basal cell or primary appendage remaining hyaline, 10–17 $\times$ 3 μ, cylindrical, straight or slightly curved. Without gelatinous appendages or sheath on ascospores.

Collections: Isolated in pure culture Jan. 1956, from horse dung collected North of Palgrave, Peel Co., Ontario, Oct. 10, 1955, (**Type** TRTC 31528). Isolated in pure culture by Mary E. Elliott, from plant of swiss chard grown at Ottawa, Ontario, summer 1949 (TRTC 31725).

Mycelio ex hyphis hyalinis, 1.5–4.0 μ diam. composito. Neque conidia neque spermatia prolata. Ascocarpis ab ascogoniis lateralibus, irregularibus, dispersis in hyphis orientibus. Ascocarpis dense aggregatis, superficialibus, vel raro immersis, atris, globosis, abundantibus, 150–250 μ, cum hyphis arachnoideis vestitis. Peridio ascocarpi pallido-brunneo, pseudoparenchymatico, diaphano coriaceo, circa 15–25 μ crasso. Cellulis externis peridii irregularibus, 4–12 μ, pallido-brunneis, tenuiter tunicatis; cellulis internis peridii majoribus, 8–16 μ. Ascosporis in globo singulari conglomeratis. Ascis 8-sporis, clavatis, 75–85 $\times$ 15–18 μ, superne late rotundatis, ad apicem cum annulo singulo praeditis, basi in stipitem brevem attenuatis, evanescentibus, in fasciculis irregulariter dispositis. Paraphysibus brevibus cellulis vesiculiformibus, hyalinis, 10–18 $\times$ 4–9 μ constitutis. Ascosporis biseriatis, primum pistilliformibus, hyalinis, deinde transverse uniseptatis. Cellula superiore ellipsoidea, in maturitate brunneo- vel atro-olivacea opacoque, 13–15 $\times$

8.0–9.5 μ, cum foramine germinali, 1.5 μ, singulari, orbiculato praedita. Cellula inferiore hyalina, 10–17 × 3 μ, cylindracea, recta vel subrecta. Ascosporis sine appendicibus gelatinosis et sine strato mucoso.

Relationship

The three species included here in *Tripterospora* appear to be fairly closely related. The superficial ascocarps are much alike although larger in *T. brevicaudata* than in the other two species. The hairs on the ascocarp of *T. erostrata* are longer, broader, and with a thicker wall than those of *T. brevicaudata*, while in *T. longicaudata*, there are no hairs distinct from the hyphae. The ascospores provide the most distinct means of separation. *T. brevicaudata* has the largest ascospores, the dark cell being 18–26 × 12–17 μ; *T. longicaudata* is intermediate with a measurement of 13–15 × 8.0–9.5 μ; and in *T. erostrata*, the dark cell is 10–12 × 6.5–7.5 μ. The respective measurements of the hyaline basal cell of the ascospores for the above three species are as follows: 3.5–5 × 4–7 μ, 10–17 × 3 μ, and 6–8 × 3 μ, the first measurement given indicating the length, the second the width taken at the septum. *T. longicaudata* is distinguished by having the longest basal cell; *T. brevicaudata*, the broadest. The paraphyses are of no particular diagnostic significance, being either absent or short and inconspicuous.

Here, then, we have three species whose inostiolate ascocarps would place them, as mentioned above, in the present-day order Plectascales. But the "cleistothecium" of *Tripterospora* develops from an ascohymenial type of ascogonium, coiled and twisted, and at approaching maturity encloses clavate asci with a thickened apical ring such as are characteristic of *Podospora* (*Sordaria*) (1). The arrangement of the asci within the ascocarps of *Tripterospora* is intermediate between that of *Podospora* and typical members of the Plectascales. The same can be said for the paraphyses: prominent in *Podospora*, reduced to a few short chains of cells in *Tripterospora*, and obsolete in most of the Plectascales. It appears that the cleistothecial habit of this new genus has been of fairly recent origin from an ostiolate member of the Ascohymeniales, quite possibly a taxon very similar to, if not identical with, the present-day species of the genus *Podospora*.

The evolution in ascospore structure, too, seems clear. The *Tripterospora* spore is a *Podospora* type which has lost its gelatinous secondary appendages.

The species of the genus *Zopfiella* Winter (4) may represent a further evolution by the addition of a septum in the dark, ellipsoid cell, with retention of the hyaline appendage as a single cell or its division also into a bicellular structure. Further treatment of this genus will be left to a future publication.

Meanwhile it is important to point out once again that a family like the Tripterosporaceae, which would be incorporated by today's schemes of classification in the Plectascales, is almost certainly in the evolutionary line of certain Ascohymeniales, since so many of the characteristics considered to be indicative of homology are actually examples of parallel evolution between more or less distinct groups.

Acknowledgments

For their contribution of cultures used in this study the writer is deeply indebted to the following: Dr. G. R. Bisby, Miss Mary E. Elliott, Dr. J. W. Groves, and Dr. J. R. Hansbrough. For their assistance in preparing stained sections and revision of the manuscript I am very grateful to Miss Luella K. Weresub and Prof. J. F. Morgan-Jones.

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EXPLANATION OF FIGURES

FIGS. 1–10. *Tripterospora brevicaudata.*

FIG. 1. Ascocarp × 30. FIG. 2. Cells of ascocarp peridium. × 1300. FIG. 3. Irregularly coiled ascogonium with branches beginning to form the ascocarp peridium. × 900. FIG. 4. Nutritive cells from inner part of young ascocarp, before ascus formation. × 800. FIG. 5. Young ascus with hyaline ascospores. × 1300. FIGS. 6, 7. Asci with ascospores nearly mature. × 1300. FIG. 8. Five mature and two immature ascospores. × 1300. FIG. 9. One mature ascospore in which the septum has not been developed. × 1300. FIG. 10. Mature ascospore germinating after 18 hr. in water. × 600.

FIGS. 11–16. *Tripterospora erostrata.*

FIG. 11. Ascocarp. × 60. FIG. 12. Cells of ascocarp peridium. × 900. FIG. 13. Mid, apical, and basal portion of hair from ascocarp peridium. × 900. FIG. 14. Ascus with nearly mature ascospores. × 900. FIG. 15. Mature ascospores. × 900. FIG. 16. Mature ascospores. × 1300.

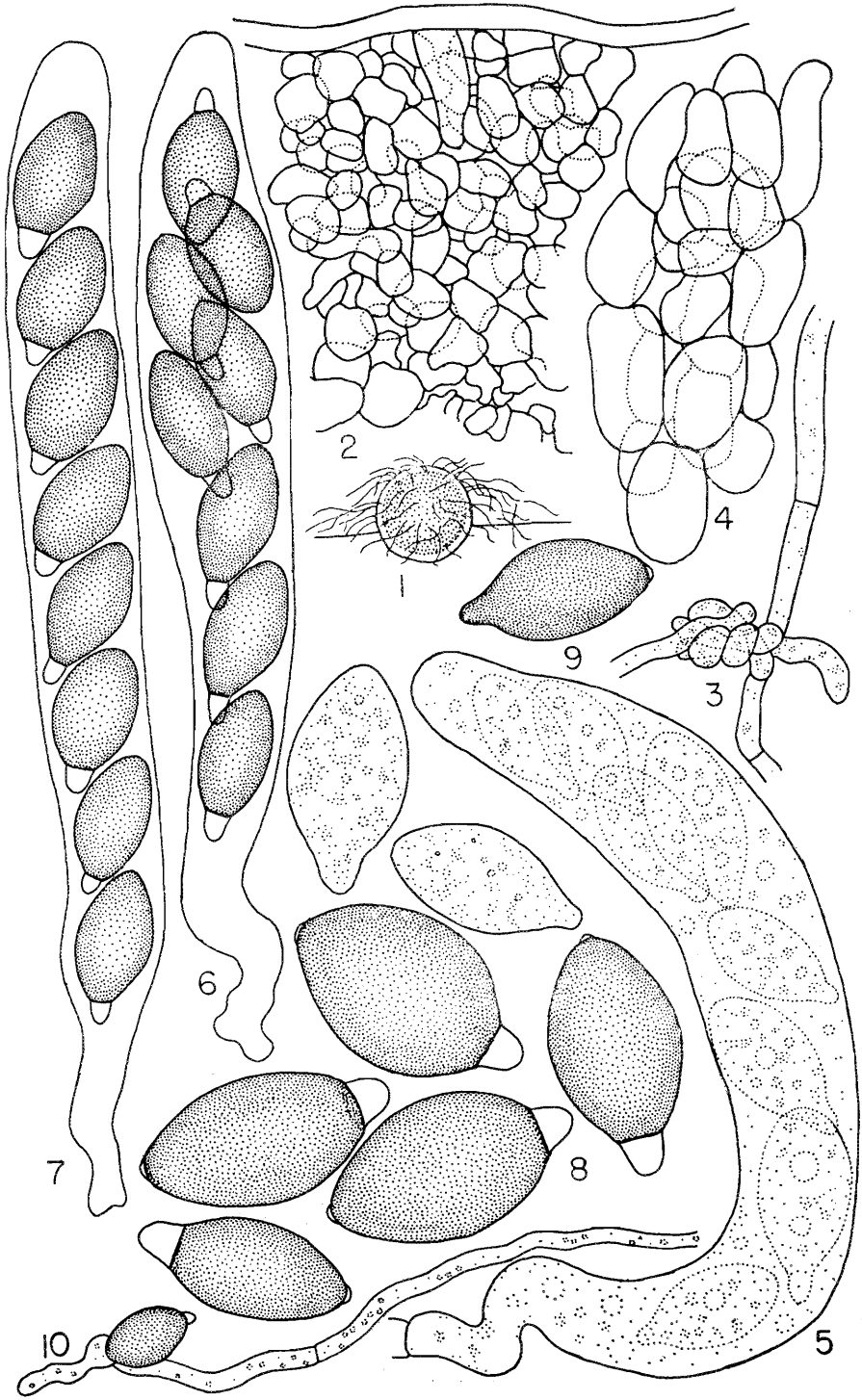
FIGS. 17–33. *Tripterospora longicaudata.*

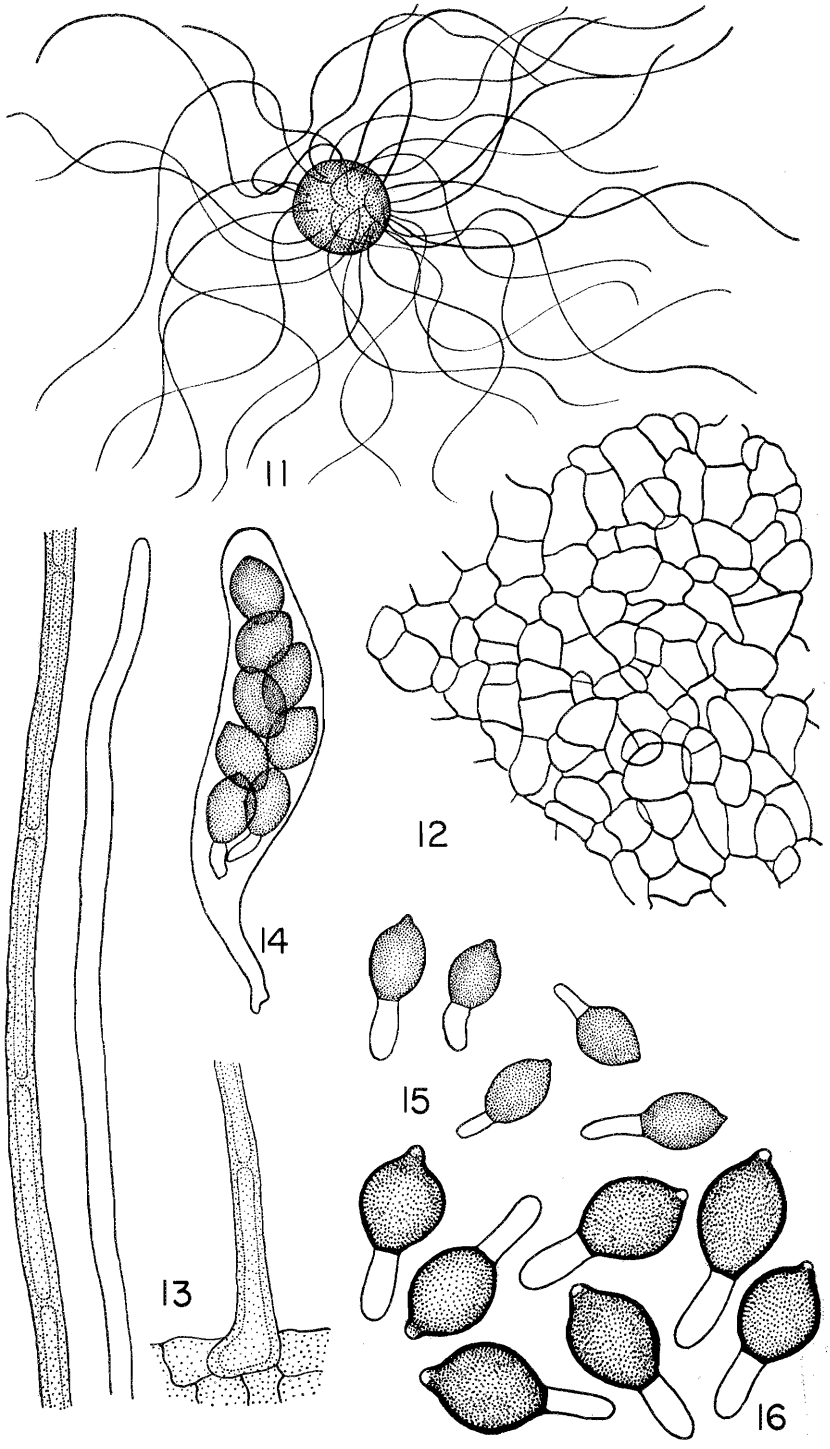
FIG. 17. Ascocarp. × 100. FIG. 18. Ascocarp crushed open with globose mass of ascospores extruded but held together by membrane. Asci have broken down. × 100. FIG. 19. Outer layer of cells from ascocarp peridium. × 1140. FIG. 20. Inner layer cells from ascocarp peridium. × 1140. FIG. 21. Hyphae with coiled and twisted ascogonia, four of which have branches from stalk beginning to form peridium. × 1140. FIG. 22. Ascogonial cells with croziers and young asci in various stages of development. × 1140. FIG. 23. Crozier on ascogonial cell with four nuclei stained with aceto-orcein. × 1140.

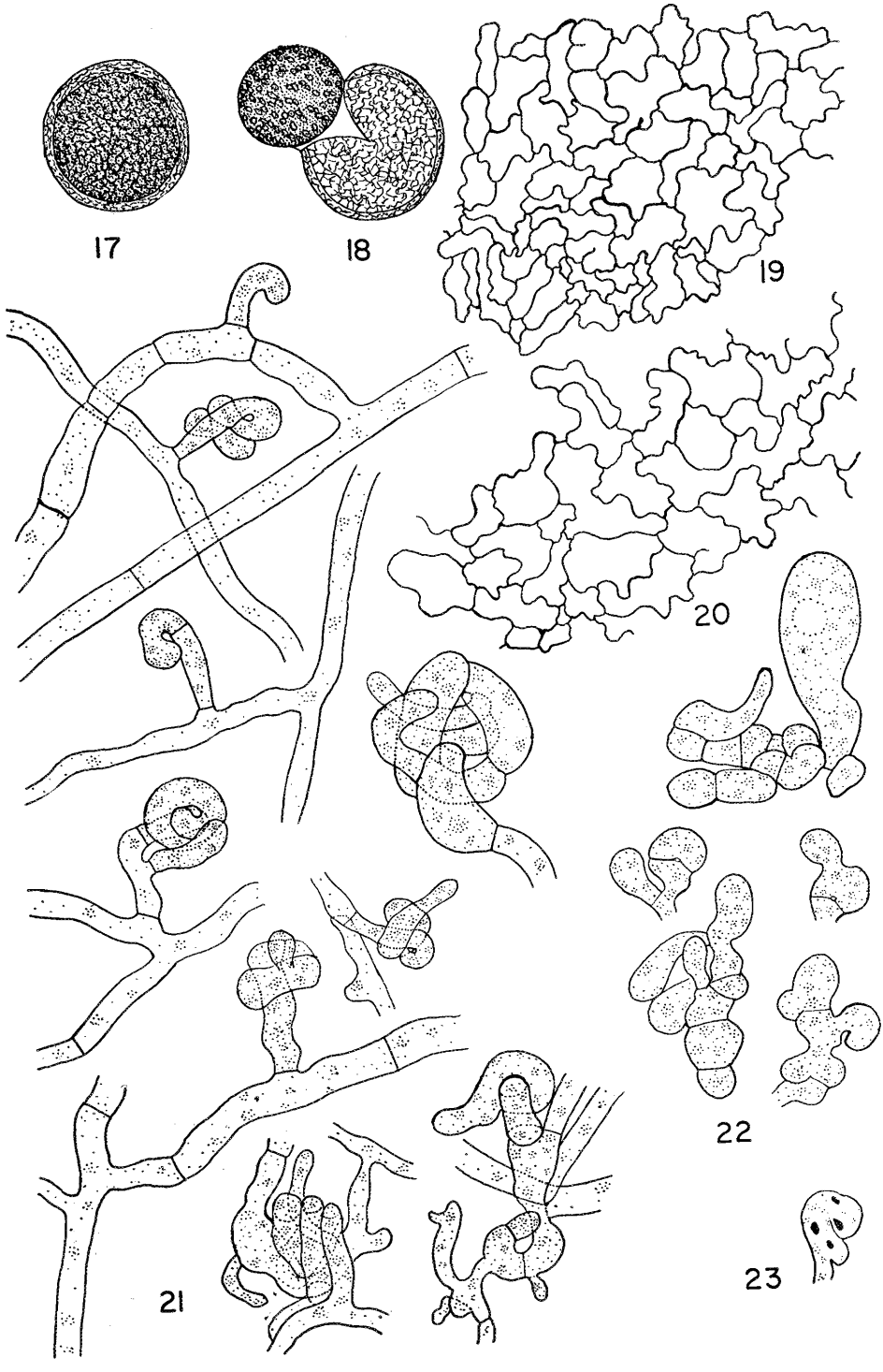
FIG. 24. Paraphyses and asci, one very small and immature, four just after ascospore delimitation. × 1140. FIG. 25. Paraphyses and three asci with immature ascospores. × 1140. FIG. 26. Two asci with ascospores just prior to septum formation. × 1140.

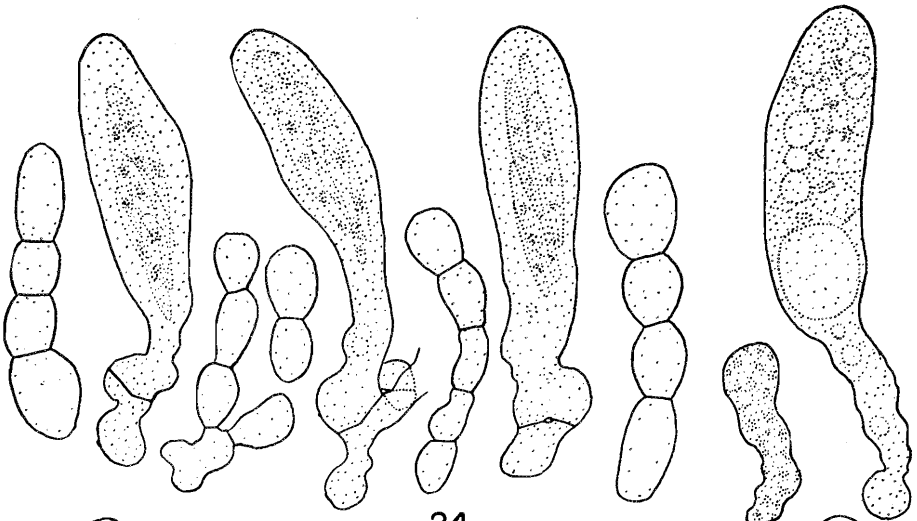
FIG. 27. Asci, one with nearly mature ascospores and one after ascospore discharge, each showing thickened ring in apex. × 1140. FIG. 28. Seven young hyaline ascospores at various stages of development before septum formation. × 1140. FIG. 29. Eleven ascospores not fully mature but after septum formation. Upper cell is light olivaceous-brown with refractive oil globule. × 1140. FIG. 30. Twenty-one ascospores, fully mature, many with basal cell collapsed. Apical germ pore visible in some. × 1140. FIG. 31. Giant ascospore, produced singly in an ascus, upper cell is black and opaque, lower hyaline. Note the short, broad, triangular basal cell. × 1140. FIG. 32. Mature ascospore in longitudinal optical section showing thickness of wall and apical germ pore. × 1140. FIG. 33. Two mature ascospores, optical section, end view showing thickness of wall and germ pore in one. × 1140.

NOTE: FIGS. 1–33 follow.









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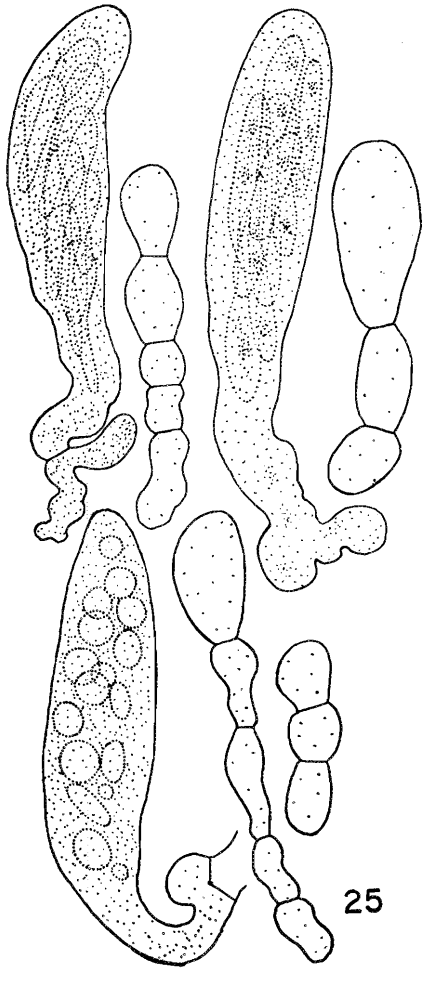

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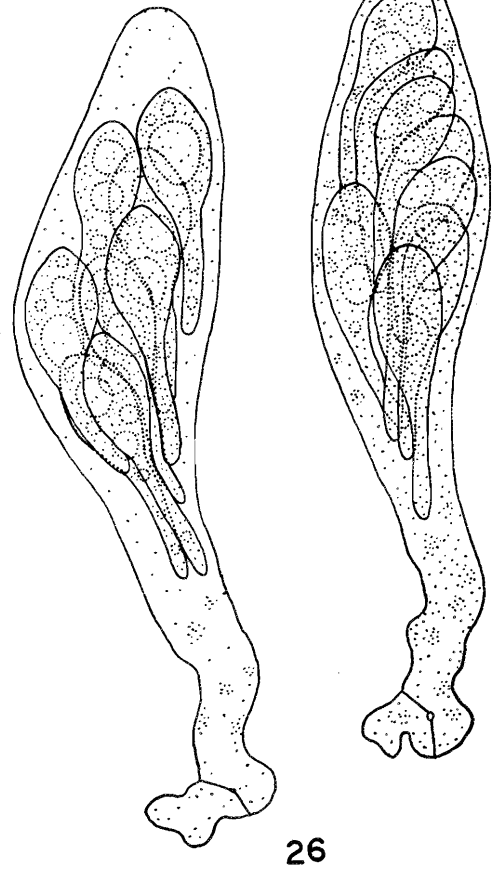

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