Confistulina, the anamorph of *Fistulina hepatica*

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The anamorph of *Fistulina hepatica* is described in detail and a new genus, *Confistulina*, is proposed to accommodate it. A relationship to *Ellula* is suggested, followed by a more general discussion on anamorph–teleomorph homologies.

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L'anamorphe de *Fistulina hepatica* est décrit en détail et un nouveau genre, *Confistulina*, est proposé pour cet anamorphe. Les données suggèrent qu'il existe une affinité entre ce genre et *Ellula*. Les auteurs présentent une discussion générale sur les homologies entre anamorphes et téléomorphes.

[Traduit par le journal]

Introduction

In the course of a revision of the species described in *Ptychogaster* Corda and *Ceriomyces* Corda, *Ceriomyces hepaticus* Sacc. was studied. This is the anamorph of *Fistulina hepatica* (Schaeff.) Sibth., a species widespread in the temperate regions of both the northern and the southern hemispheres. It occurs as a wound parasite or saprophytic on wood of angiosperms (one of the examined cultures is reported from *Pinus* in the U.S.A.), mainly on *Quercus* and *Castanea*, but also for example on *Fagus*, *Platanus*, *Prunus*, and *Tilia*. The early stage of the infection is known as "brown oak," the wood being stained reddish brown by fungal products. The stained wood is highly esteemed in the furniture trade since the quality hardly decreases (Cartwright and Findley 1936; Cartwright 1938).

Cultural characters of *F. hepatica* have been described by various authors (Rothberg 1937; Davidson et al. 1942; Stalpers 1978). A good description of the anamorph is that of de Seynes (1874), based on an in vivo study of conidia in the basidiocarp. The process of conidiogenesis, which has, however, remained unclear, is dealt with in the present paper.

Methods

Isolates were grown in plastic Petri dishes on neutralized 2% malt extract agar (MEA) and cherry decoction agar (ChA) at room temperature (18–20°C) in diffuse daylight. Drop tests on laccase and tyrosinase were performed as described by Käärik (1965) and Stalpers (1978). Preparations for scanning electron microscopy were made according to Samson et al. (1979). Capitalized colour names refer to Ridgway (1912), colour codes to Kornerup and Wanscher (1978).

Descriptions

Confistulina Stalpers anamorph gen. nov.

Coloniae ochraceae vel brunneae. Hyphae septatae, fibulatae vel efibulatae, aliquae oleosae brunneae.

Aliquae hyphae simulum sunt dendrohyphidiis. Blastoconidia ellipsoidea vel ovoidea, normaliter in successione sympodiale formata. Laccase absens, tyrosinase praesens.

HOLOMORPHOSIS: *Fistulina* (Basidiomycetes).

SPECIES TYPICA: *Ceriomyces hepaticus* Sacc.

Colonies ochraceous to brownish. Hyphae septate, at least some septa with clamps. Some hyphae with brownish oily contents. Dendrohyphidium-like hyphae present. Blastoconidia ellipsoid to ovoid, typically formed in sympodial order. Laccase absent, tyrosinase present.

HOLOMORPH: *Fistulina*.

TYPE SPECIES: *Ceriomyces hepaticus* Sacc.

Confistulina hepatica (Sacc.) Stalpers comb. nov.

Figs. 1–19

= *Ceriomyces hepaticus* Sacc. Syll. Fung. 6: 388. 1888 (basionym).

= *Ptychogaster hepaticus* (Sacc.) Lloyd. Mycol. Writ. 3, Polyporoid Issue 2: 32.

Growth on MEA slow, radius 1–20 mm in 14 days; on ChA 10–25 mm in 14 days. Odour insignificant. Advancing zone raised or appressed in the outermost 2 mm, even in outline, hyphae dense to rather distant. Mycelial mat at first cottony, white, soon with, at least locally, pale buff tinges (Pinkish Buff, Light Buff, 5A4, 4A3), later becoming denser cottony to cottony woolly or locally farinaceous, Ochraceous Buff to Yellow Ocher, Ochraceous Tawny or Tawny (4B8, 5B6, 5C6-8, 5D8, 6D8). In some strains the mycelium behind the first cottony zone collapses and a velutinous mat develops, which is even or forms distinct warts, at first warm buff to Light Ochraceous Buff, becoming Apricot Yellow and finally Tawny (4A4-7, 6D8). The cottony woolly mycelium often produces reddish drops of exudate (near Brazil Red, 7C8, 8C8). Reverse may become darker. Reaction with α-naphthol negative
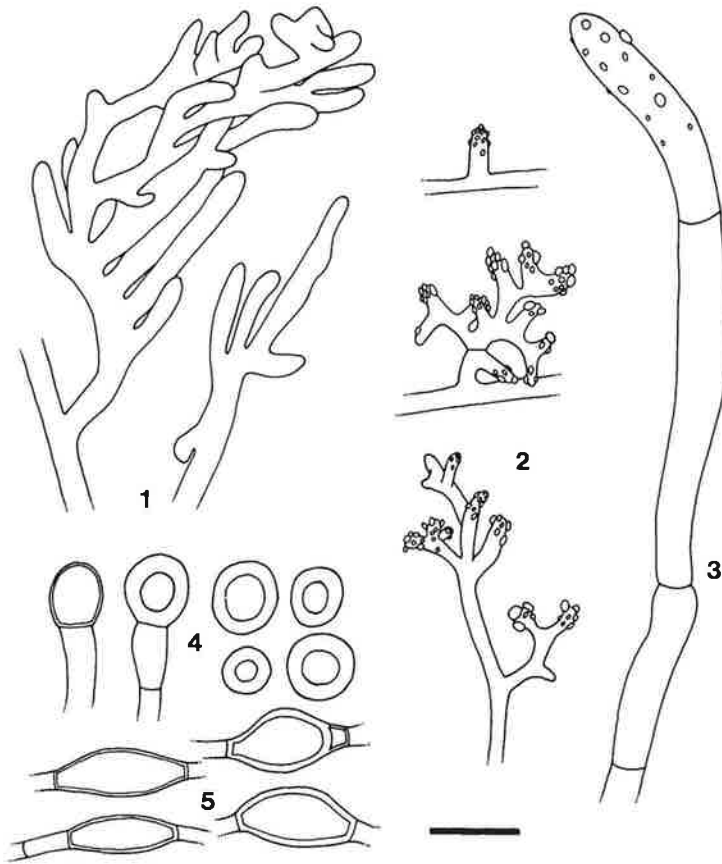
FIG. 1. CBS 513.80, advancing hyphae. FIG. 2. CBS 508.80, dendrohyphidium-like hyphae. FIG. 3. CBS 513.80, hyphae from velutinous part. FIG. 4. CBS 513.80, terminal chlamydospores. FIG. 5. CBS 161.30, intercalary chlamydospores in submerged mycelium. Bar represents 10 μm.

(somewhat ochraceous); reaction with *p*-cresol positive. Cardinal temperatures: minimum 6°C, optimum 24–26°C, maximum 33°C.

Marginal hyphae hyaline, thin walled, 1.5–4(–7) μm wide, with few septa, some of which have clamps with a diameter equal to or narrower than that of the supporting hypha. Clamps may grow out to form a hypha. Branching typically at acute angles; wide hyphae often bearing much narrower hyphae, normally at right angles. Hyphae occasionally with numerous parallel branches which may develop into hyphal knots (Fig. 1). Crystals may be present.

Aerial hyphae hyaline, thin walled, 2.5–4.5 μm wide. Clamps present at most septa, but sometimes lacking, particularly on specialized hyphae. Hyphae sometimes with brownish oily contents, especially in older mycelium, where many hyphae are also covered with small or big exudate drops (Figs. 18, 19), often concentrated at hyphal tips or septa. The mycelium also contains narrow (1.8–2.5 μm) dendrohyphidium-like hyphae with repeated dichotomous branching at the apex (Fig. 2); the apices are covered with small (sub)hyaline granules. Sometimes single short side branches show a similar encrustation. The velutinous parts are composed of a pallisade of 5 to 8 μm wide hyphae, of which especially the apical cell is often covered with exudate drops (Fig. 3). The contents of these hyphae are hyaline. The central hyphae of the warts are hyaline, somewhat irregular in outline, up to 8 μm wide.

Blastic conidia are formed at random or in seemingly retrogressive sequence from a determinate conidiogenous cell apex (Figs. 12–14) or they form dense clusters (Figs. 6, 16, 17). In this last type the second conidium is formed below the first one, but the third and fourth conidia are formed more or less simultaneously below conidium one and two. This results in a dense cluster of up to 8(–10) conidia. One of these conidia, normally the lowest, may in an early state of differentiation grow out into a hypha on which one or more further conidial clusters may be formed (Fig. 10). Secondary clusters generally comprise fewer conidia; maturation may
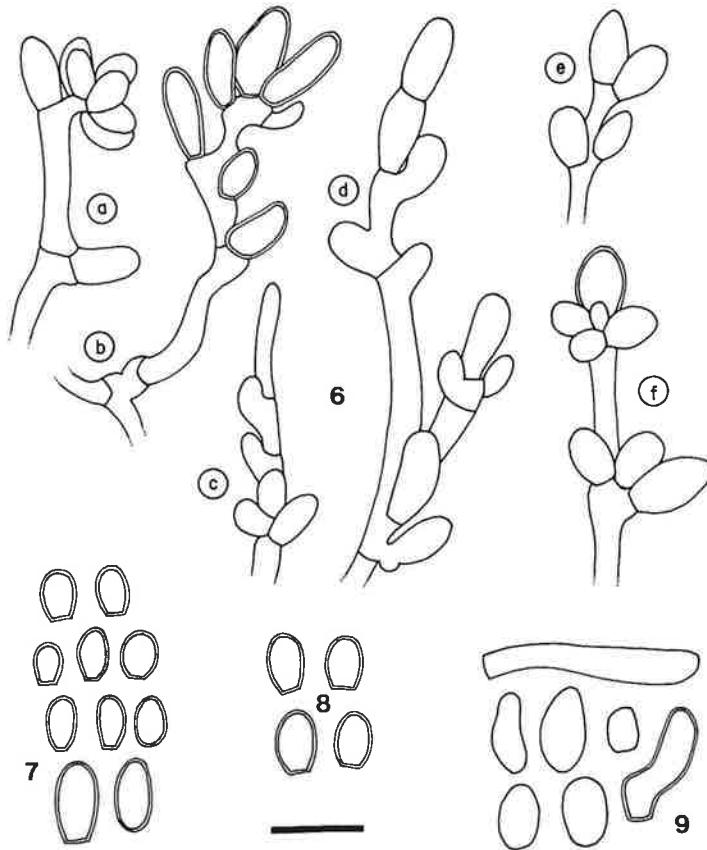
FIG. 6. Conidiogenous structures. Fig. 6a, 6c. CBS 508.80. Fig. 6b, 6d. CBS 161.30. Fig. 6e. CBS 510.80. Fig. 6f. CBS 514.80. FIG. 7. CBS 161.30, blastoconidia. FIG. 8. CBS 514.80, blastoconidia. FIG. 9. CBS 161.30, arthroconidia. Bar represents 10 μm.

cease. The fertile hyphae finally become septate in retrogressive order; the separate cells inflate slightly and may secede as arthroconidia. The walls of both blastoconidia and arthroconidia may become thickened, generally before secession, but the process may continue afterwards. Mature blastoconidia (Figs. 7–8) pale yellowish, ellipsoid to ovoid, (4.5–)5.5–9.5 × (3–)4–5 μm; arthroconidia (Fig. 9) ellipsoid to subcylindrical, (3–)5–10(–22) × 3–4 μm, or knee shaped. Chlamydospores, when present, terminal (Fig. 4), intercalary, or (in CBS 510.80) developing from an inflation of the basal part of a terminal, up to 50 μm long, hyphal cell (Fig. 15); the 4 to 18 μm long apical appendage then disintegrating after delimitation. Chlamydospores hyaline, ellipsoid, 11–15 × 5–7 μm, with walls 0.6–1.5 μm thick. Clamps are absent from septa between conidiogenous loci but may be present immediately below the lowest locus.

Submerged hyphae hyaline, thin walled, 2.5–7(–8) μm wide, sometimes with swellings. Chlamydo-spores (Fig. 5) intercalary, ellipsoid to broadly ellipsoid, 9–15(–25) × 5–8(–10) μm.

SPECIES CODE (STALPERS 1978): 2, (9), (10), 11, (12), (13), (14), 18, 21, (22), (26), 30, 31, 34, 35, (38), (39), (40), 42, (44), (45), 50, 52, 53, 54, (55), (57), 58, 60, 61, (65), (80), (82), 83, 84, 85, 86, 87, 89.

SPECIES CODE (NOBLES 1965): 1, 5, 21, (26), 33, 34, 35, 37, (38), (39), 46–47, 54.

MATERIAL EXAMINED: Living strains: CBS 161.30, GREAT BRITAIN, K. S. G. Cartwright; CBS 339.69, from Quercus robur, Horst, NETHERLANDS, P. J. Bels; CBS 508.80 = FP-94393-Sp, from Castanea sp., Bell, Madison, U.S.A., F. F. Lombard; CBS 509.80 = FP-103444-S, from Quercus velutina, Spalding County, GEORGIA, U.S.A., F. F. Lombard; CBS 510.80 = FP-71334-S, from Quercus coccinea, Arlington Cemetery, VIRGINIA, U.S.A., F. F. Lombard; CBS 511.80 = FP-24041-S, from Castanea sp., Mt. Pisgah, NORTH CAROLINA, U.S.A., F. F. Lombard; CBS 512.80 = LOO-14582-S, from Castanea dentata,
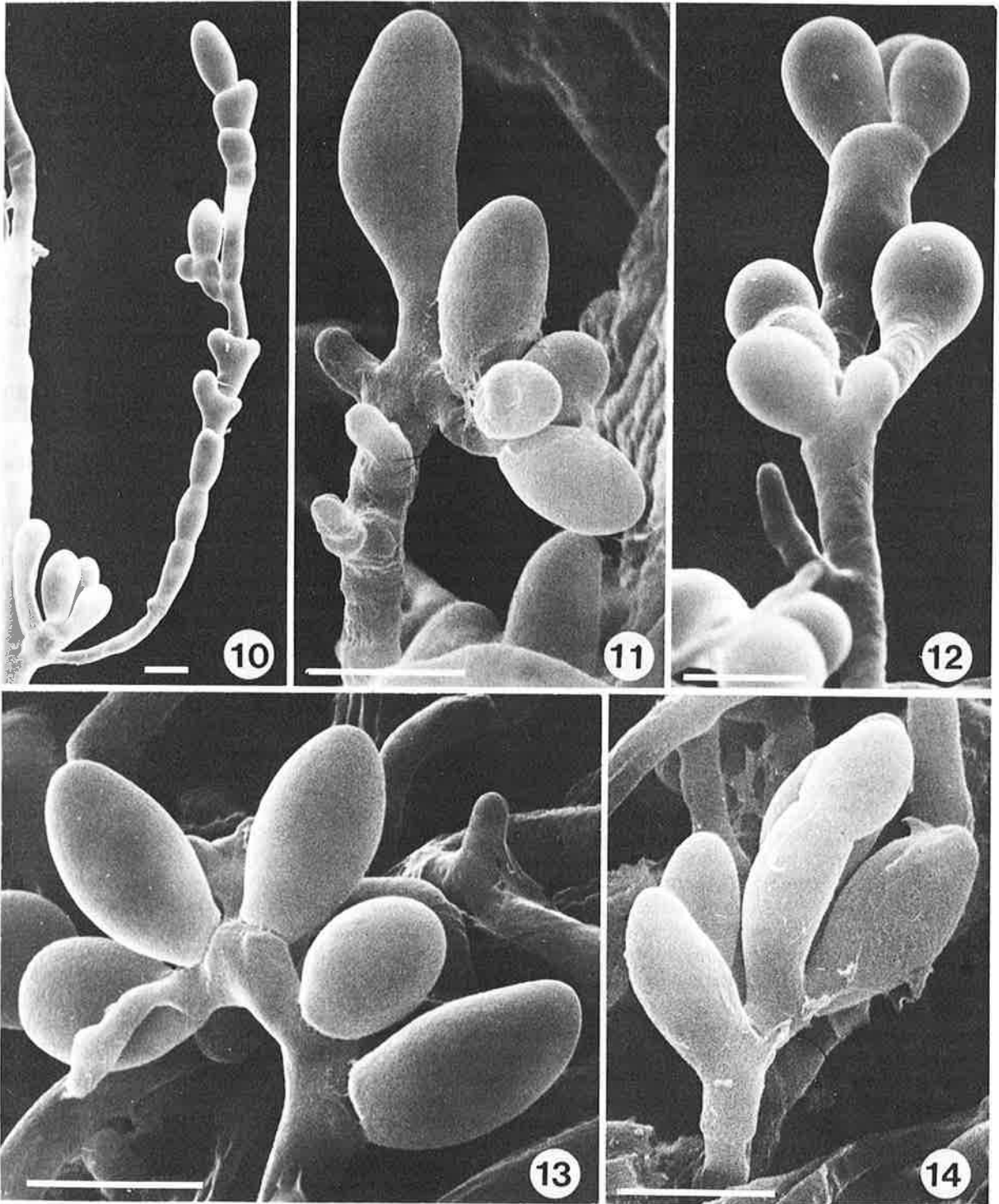
Figs. 10–14. Conidiogenous structures. Figs. 10, 12. CBS 339.69. Figs. 11, 14. CBS 316.75. Fig. 13. CBS 305.80. Bar represents 5 μm.
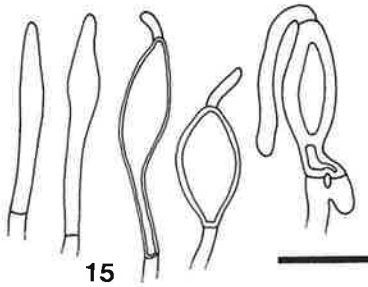
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FIG. 15. Developmental stages of terminal appendiculate chlamydospores. Bar represents 10 μm.

Stony Creek, PENNSYLVANIA, U.S.A., L. O. Overholts; CBS 513.80 = OKM-6138-Sp., Rock Creek Park, U.S.A., O. K. Miller; CBS 514.80 = HHB-133-Sp., from *Pinus virginiana*, Laurel, MARYLAND, U.S.A., H. H. Burdsall; CBS 580.81, from *Quercus robur*, Baarn, NETHERLANDS, J. A. Stalpers.

Discussion

The anamorph has formerly been placed in *Ceriomyces* Corda and in *Ptychogaster* Corda. *Ceriomyces* is a nomen dubium (Donk 1960). *Ptychogaster* is based on the anamorph of *Tyromyces ptychogaster* (Ludw.) Donk, a species with chains of swollen arthroconidia; moreover, *Tyromyces* is not related to *Fistulina*. Neither *Ptychogaster* nor *Ceriomyces* are thus applicable for the accommodation of the anamorph of *Fistulina*. Since no other suitable genus is available, a new genus, *Confistulina*, is introduced. The anamorphs found in species of *Squamanita* Imbach (incl. *Dissoderma* Sing.) seem to be close to *Confistulina*. However, contrary to *Confistulina* they are modified clamp connections (Bas 1965; Watling 1979) and thus cannot be considered as congeneric.

The relatives of *Fistulina* are generally sought in the cyphellaceous fungi, but comparative studies have never revealed a closely related genus (e.g., Agerer 1978). The appendaged chlamydospores in CBS 510.80, however, may indicate a relationship on the anamorph level. There are only three basidiomycetous anamorph genera with this peculiar character, viz. *Michenera* Berk. & Curt., *Ellula* Nag Raj, and *Fibulocoela* Nag Raj. The last genus is left out of the following discussion because it has nonostiolate immersed conidiomata. The possibility that *Ellula* is not a holobasidiomycetous anamorph but an immature heterobasidious teleomorph cannot be excluded, but the structure of the fruit body and the principally sympodial branching of the conidiophores support Nag Raj's thesis that the relationship is with the Holobasidiomycetes. Detailed descriptions of *Michenera* and *Ellula* are published by Lyman (1907) and Nag Raj (1980), respectively.

Conidiogenesis in these genera is similar. The conidia arise by conversion of preexisting elements and can be considered as solitary and terminal chlamydospores (Kendrick and Watling 1979). They are formed in cupulate to tubular conidiomata, while those of *Fistulina* are only found in the mycelium. Nevertheless, there are similarities. In a young stage basidiocarps of *Fistulina* are entirely covered with cupulate structures. The downwards-oriented cupulae are converted into fertile tubes, while those at the upper surface disintegrate. This last type of cyphellaceous structure is very close to that of the conidiomata of *Ellula guaduae* (Viégas) Nag Raj. The cupulae are initially closed or have a narrow pore, which opens like a sea anemone, the wide occluding hyphae bending outward (Fig. 20). These hyphae form the sterile part of the then cupulate to tubular body. The bottom of the cupulae generally remains sterile, but sometimes a hymenial layer is formed, of which only few basidia mature before the whole structure withers away. Up to the formation of the basidioles each single cupula or tube is comparable with a conidioma of *Ellula*. In *Ellula*, however, basidiole-like structures develop into chlamydospores, while in *Fistulina* they either remain undifferentiated or become basidia. In conclusion, although cyphelloid conidiomata have not (yet?) been found in *Fistulina*, there appears to be a taxonomic relationship with *Ellula*.

Michenera differs in several respects from both *Ellula* and *Fistulina*: it has strongly branched, thick-walled "binding hyphae," it lacks clamps, the conidioma is cup shaped rather than tubular, and the chlamydospores are coloured.

Anamorph–teleomorph homologies

In the above discussion arguments are given for the hypothesis that the thallic chlamydospores of *Ellula* and *Michenera* are homologous with basidioles casu quo basidia. However, there are also two types of blastic conidiogenesis in basidiomycetous anamorphs which are comparable with teleomorphic structures. (*1*) Blastoconidia which are simultaneously formed on denticles at the apical part of a conidiogenous cell are homologous with basidiospores. This is supported by the following arguments. (*i*) The shape of the conidia and the basidiospores is nearly identical (examples: *Bondarzewia berkeleyi* (Fr.) Bond. & Sing., *Heterobasidion annosum* (Fr.) Bref., *Hyphoderma mutatum* (Peck) Donk, all species of *Dichostereum* Pilat). (*ii*) The ornamentation of the conidia is comparable with that of the basidiospores, though less pronounced. The basidiospores of *Bondarzewia berkeleyi* have distinct ridges, while the conidia are warted or have very low ridges; the basidiospores of *Laurilia sulcata* are echinulate, the conidia minutely warted to roughened; the basidiospores of *Heterobasidion annosum* are
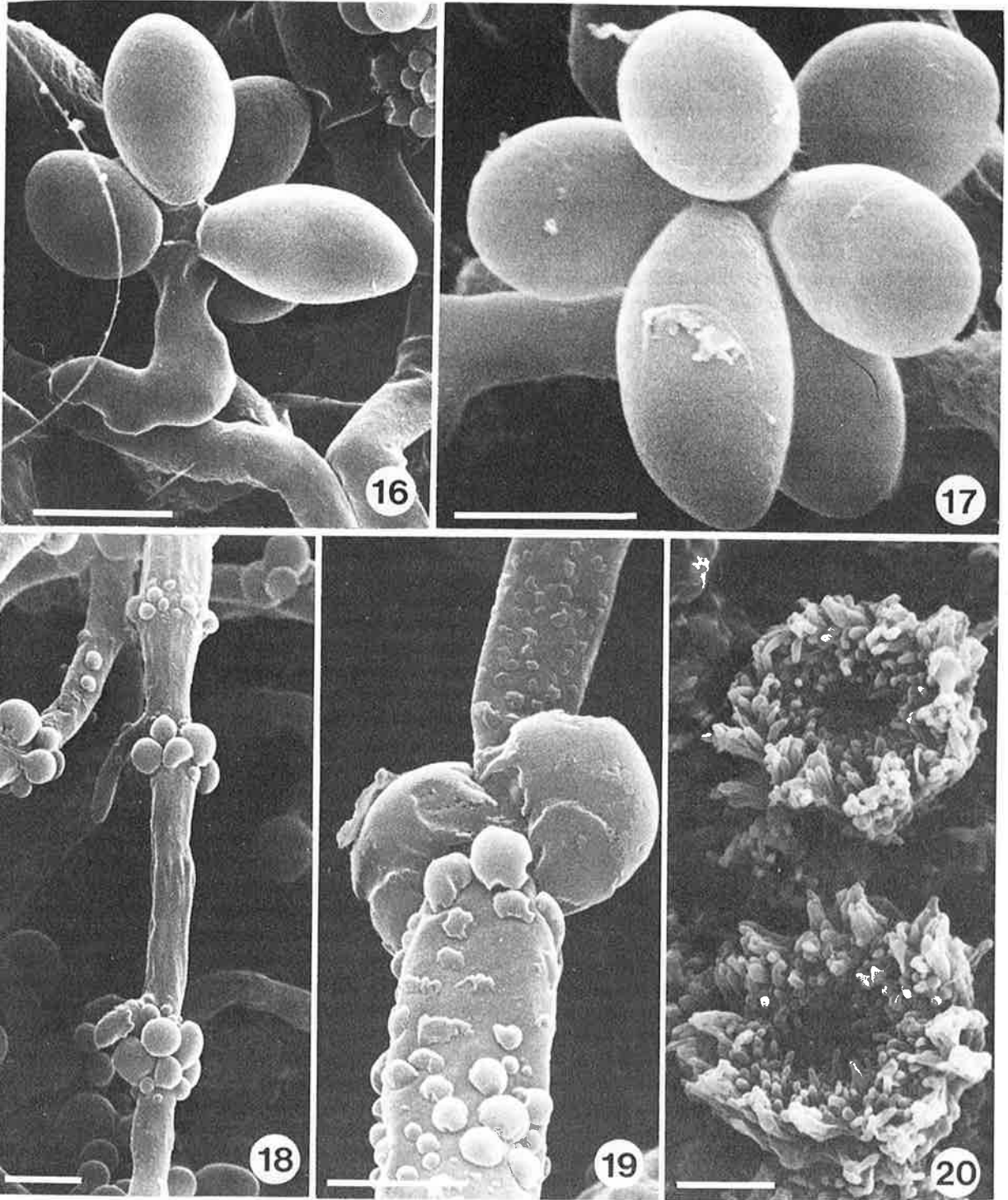
FIGS. 16–17. CBS 316.75, conidiogenous structures. FIGS. 18–19. CBS 161.30, hyphae with exudate drops. FIG. 20. Young basidiome. Bar represents 5 μm in Figs. 16–19, 50 μm in Fig. 20.

minutely warted, the conidia seem to be smooth, even when examined with the SEM. Ultrathin sections, however, revealed some local wall thickening (Hanlin 1982). (*iii*) The reaction of the basidiospores with Melzer's reagent is comparable with that of the conidia, but stronger. The intensity of the amyloid reaction seems to be correlated with the degree of ornamentation. The wall layer responsible for the ornamentation is the perisporium, which is very thin in smooth spores or conidia (Capellano and Keller 1978). The basidiospores of *Bondarzewia berkeleyi* and *Laurilia sulcata* are strongly amyloid, as are the conidia of *B. berkeleyi*, but the conidia of *L. sulcata* are weakly amyloid. The basidiospores of *Heterobasidion annosum* are weakly amyloid, the conidia are not. (*iv*) The ultrastructural development of the conidial denticles and the sterigmata is identical. Hanlin (1982) described the development of conidial denticles in *Spiniger meineckellus* (A. J. Olson) Stalpers, the anamorph of *Heterobasidion annosum*. They are formed by an extension of the two innermost wall layers, which break through the two outer layers. Tu et al. (1977) demonstrated the same phenomenon for the sterigmata of *Thanatephorus cucumeris* (Frank) Donk. Examples of anamorphic basidiomycetous genera belonging to this group are *Dexhowardia* J. J. Taylor and *Spiniger* Stalpers. (2) Blastoconidia which are broadly attached and are formed in more or less sympodial order seem to be homologous with basidioles casu quo basidia. The following facts support this hypothesis. (*i*) The shape of the conidia and immature basidia is similar. The conidia of *Allescheriella crocea* (Mont.) Hughes and the young basidia of its teleomorph *Botryobasidium croceum* Lentz are globose to subglobose; the conidia of *Sporotrichum aureum* Link and the young basidia of its teleomorph *Poria (Pycnoporellus) metamorphosa* Fuckel are ellipsoid. The same applies to *Confistulina* and *Fistulina*. (*ii*) The branching pattern of the conidiogenous and basidiogenous hyphae is identical. (*iii*) The conidia of *Sporotrichum aureum* have two, four, or finally eight nuclei, the same number found in the basidia. Examples of anamorphic basidiomycetous genera belonging here are *Allescheriella* P. Henn., *Confistulina* Stalpers, *Glutinoagger* Sivanesan & Watling, and *Sporotrichum* Link.

Acknowledgment

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