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Comparative Studies on the Septal Development in Ascobolaceae and Ciliarieae (Humariaceae)

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ABSTRACT: The septal structure at ascal base in the Ascobolaceae and the tribe of Ciliarieae (Humariaceae *sensu* Rifai) has a very similar hemispherical band as Otideae (Humariaceae). However, the septal structures of these three groups of cup fungi show different types of septal development, namely ascoboloid-, scutellinioid-, and otideoid-type. Species showing the same types of septal structures in the pores of mature asci, but exhibiting transitional differences in their early development stages, indicate a possible monophyletic origin of these groups. The study of septal development may be potentially of great use in the study of phylogeny in the Pezizales.

KEY WORDS: Septal development, Evolution, Pezizales.

INTRODUCTION

Most Ascobolaceae (Pezizales) are found on dung, although some of them may grow on charcoal, soil or rotten wood. This ecological habitat matches very well with members of the tribe *Ciliarieae* (Humariaceae). Most of the Ciliarieae (=Scutellinieae *sensu* Korf, 1973), however, produce hyaline ascospores and hairy apothecia with bright carotenoid pigments. From previous ultrastructural data on ascosporogenesis of *Ascobolus* (Wu and Kimbrough, 1992), *Scutellinia*, *Cheilymenia*, and *Coprobia* (Wu and Kimbrough, 1991), a very close similarity between Ascobolaceae and Ciliarieae (Humariaceae) has been shown.

A similar conclusion was drawn by Kimbrough and Curry (1986) who studied the septal structures in the Scutellinieae and Sowerbyelleae tribes of Pyronemataceae (*sensu* Korf, 1973) (=Humariaceae *sensu* Rifai, 1968) and pointed to a close relationship of Scutellinieae (=Ciliarieae, Rifai 1968) to the Ascobolaceae.

Even though both septal structures and spore ontogeny types are shown to be very similar in the Ciliarieae and Ascobolaceae, the morphological distinction between the two groups is still very prominent. Whether these two groups should belong to the same family, or whether they evolved from the same ancestor, could be confirmed from developmental, cytological and ultrastructural studies. If enough characters correlate, they may be placed in the same family, or they may prove to have only a close phylogenetic linkage.

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The main purpose of this paper is to compare septal development in species of the Ciliarieae (=Scutellinieae, Pyronemataceae *sensu* Korf, 1973) with that of Ascobolaceae and to establish possible relationships among these groups.

MATERIALS AND METHODS

Field collections were processed for transmission electron microscopy following the procedures described by Curry and Kimbrough (1983). The following specimens were observed: *Ascobolus stictoideus* Speg. (FLAS-F53512), on goat dung from Araya, Sucre State, Venezuela; *Cheilymenia coprinaria* (Cke.) Boud. (FLAS-F53436), on cow dung, SW 20th Av., near I-75, Gainesville, Alachua County, Florida; *C. stercorea* (Pers. ex Fr.) Boud. (FLAS-F53435), on cow dung, SW 20th Ave., near I-75, Gainesville, Alachua County, Florida; *Coprobia granulata* (Bull. ex Mérat) Boud. (FLAS-F55143), on cow dung, Sugarfoot Hammock, on south side of SW 20th Ave., ca. 0.5 mi. W. of I-75, Gainesville, Alachua County, Florida; *Scutellinia scutellata* (L. ex Fr.) Lambotte (FLAS-F55434), on wood stump covered with mosses, behind Art Center, Highlands Biological Station, Highland, North Carolina.

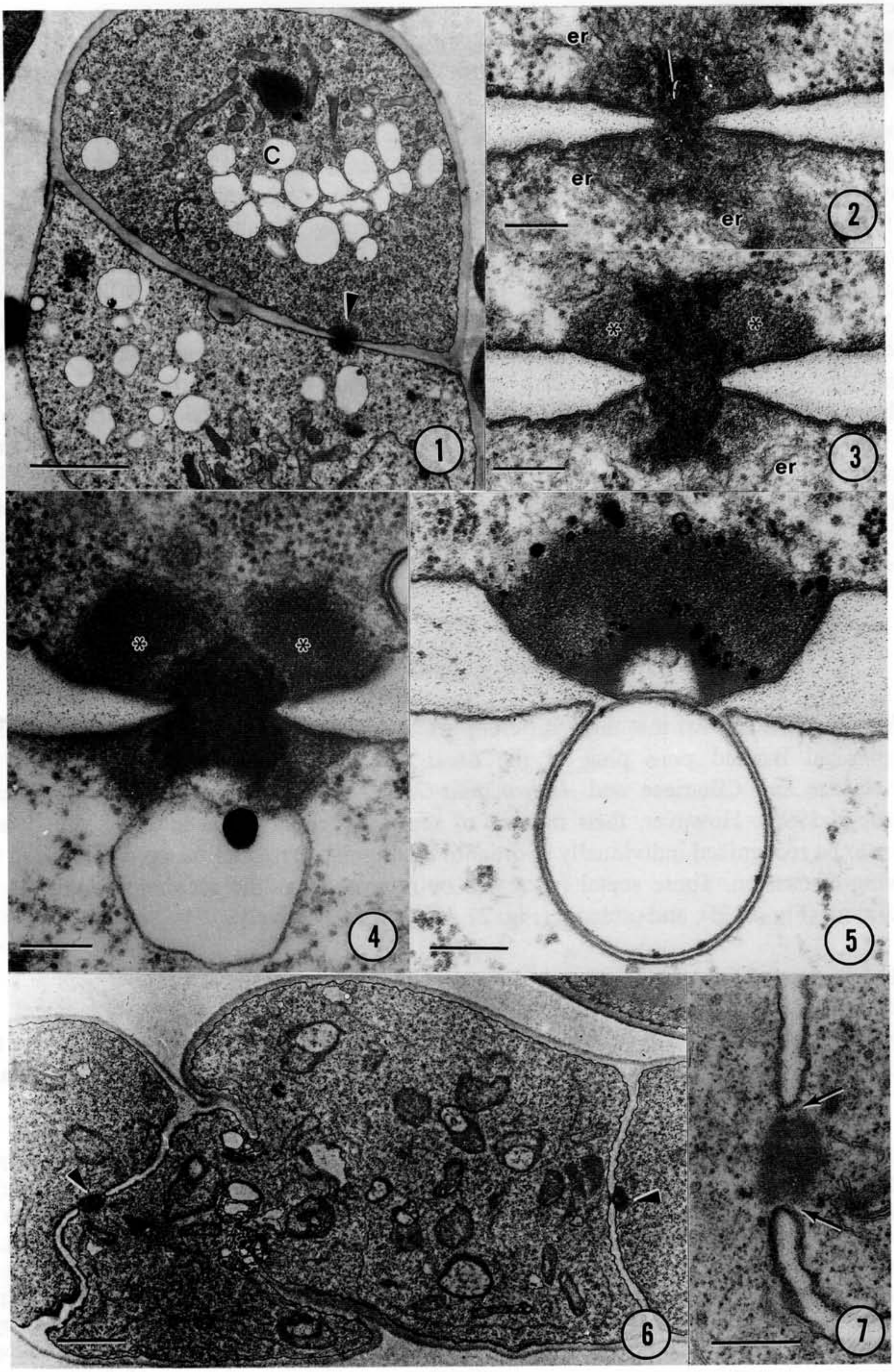
Plastic blocks were sectioned on an LKB Huxley ultramicrotome with a diamond knife. After poststaining with uranyl acetate and lead citrate, the sections were examined at 60 kv, on a JEOL 100-CX electron microscope. In order to observe inner structures inside the septal plugging matrix, some of sections, before examined by electron microscope, were treated by 1% hydrogen peroxide for 1-5 minutes following regular poststaining precedure.

RESULTS

Ascobolus stictoideus

Septal structures in the crozier stage are observed in *A. stictoideus* (Fig. 1). In the central lumen of the pore, there are some delicate vacuoles surrounded by endoplasmic reticulum (Figs. 2-3, arrow). Subsequently, some less electron opaque material is deposited around the vacuoles (Fig. 3, asterisks). More material is accumulated forming arches across the pore (Fig. 4, asterisks). In the mature ascus, an electron opaque hemispherical band is formed (Fig. 5). Usually, the lower part of hemispherical structure is more condensed and electron opaque than the outer dome, and the opening of the pore is sealed by a membranous bulb.

Figs. 1-7. Transmission electron micrographs showing developements of ascoboloid and scutellinioid septal structures. Figs. 1-5. *Ascobolus stictoideus*. 1. A young crozier (C) showing one of the pore plugs (arrow head) (bar = 2 μm). 2. A number of delicate vacuoles (arrow) are present in the central pore lumen of ascogenous hypha which is surrounded by endoplasmic reticulum (bar = 0.2 μm). 3. Some electron opaque material (asterisks) is deposited around the pore (bar = 0.2 μm). 4. Some electron opaque material (asterisks) is deposited around the pore (bar = 0.2 μm). 5. An electron opaque, hemispherical band is laid across the ascal pore (bar = 0.2 μm). Figs. 6-7. *Scutellinia scutellata*. 6. Septal pluggings (arrow heads) in a section of ascogenous hypha. Left septal structure is younger than right side (bar = 1 μm). 7. A translucent zone (arrows) shown in the pore plugging matrix. A higher magnification of septal structure (left) in Fig. 6. (bar = 0.5 μm).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Scutellinia scutellata

Pore plugging structures in the ascogenous hypha and crozier stages are observed (Fig. 6, arrow heads). Electron opaque plugging matrix accumulates in the pore center, but separates from the pore border by a translucent torus (Figs. 7-9). Thereafter, a number of small vacuoles are formed within the plugging matrix (Figs. 9-10, arrow head). Vacuoles push the plugging matrix to both sides forming biconvex arches (Figs. 10-12). The contents of the biconvex arches are removed when they are treated with 1% hydrogen peroxide (Figs. 12, 14). The lower part (opposite to the ascal cell) is usually diminished gradually (Fig. 12, arrow heads). Another layer of less opaque material, very inert to hydrogen peroxide (Fig. 14), is deposited on the main arch (Fig. 11). So, in the mature ascal base, a hemispherical band with two different zones is formed (Figs. 13-14). A membranous veil is usually found on the mature septal arch (Fig. 13, arrow heads). Below the hemispherical band, a poorly formed laminated structure (Fig. 15, arrows) is rarely found within the pore.

This type of septal development is also found in species of *Cheilymenia* and *Coprobia*. In *C. coprinaria* and *C. stercorea*, a similar electron dense arch is formed first at the ascal base and then another less opaque material is deposited, forming a double zonate band (Figs. 19-20). However, there is no additional less opaque layer deposited on the electron dense band in *Coprobia granulata* (Figs. 16-17). In an old ascus, the lower part of the septal structure is occluded by hyphal wall material (Fig. 18).

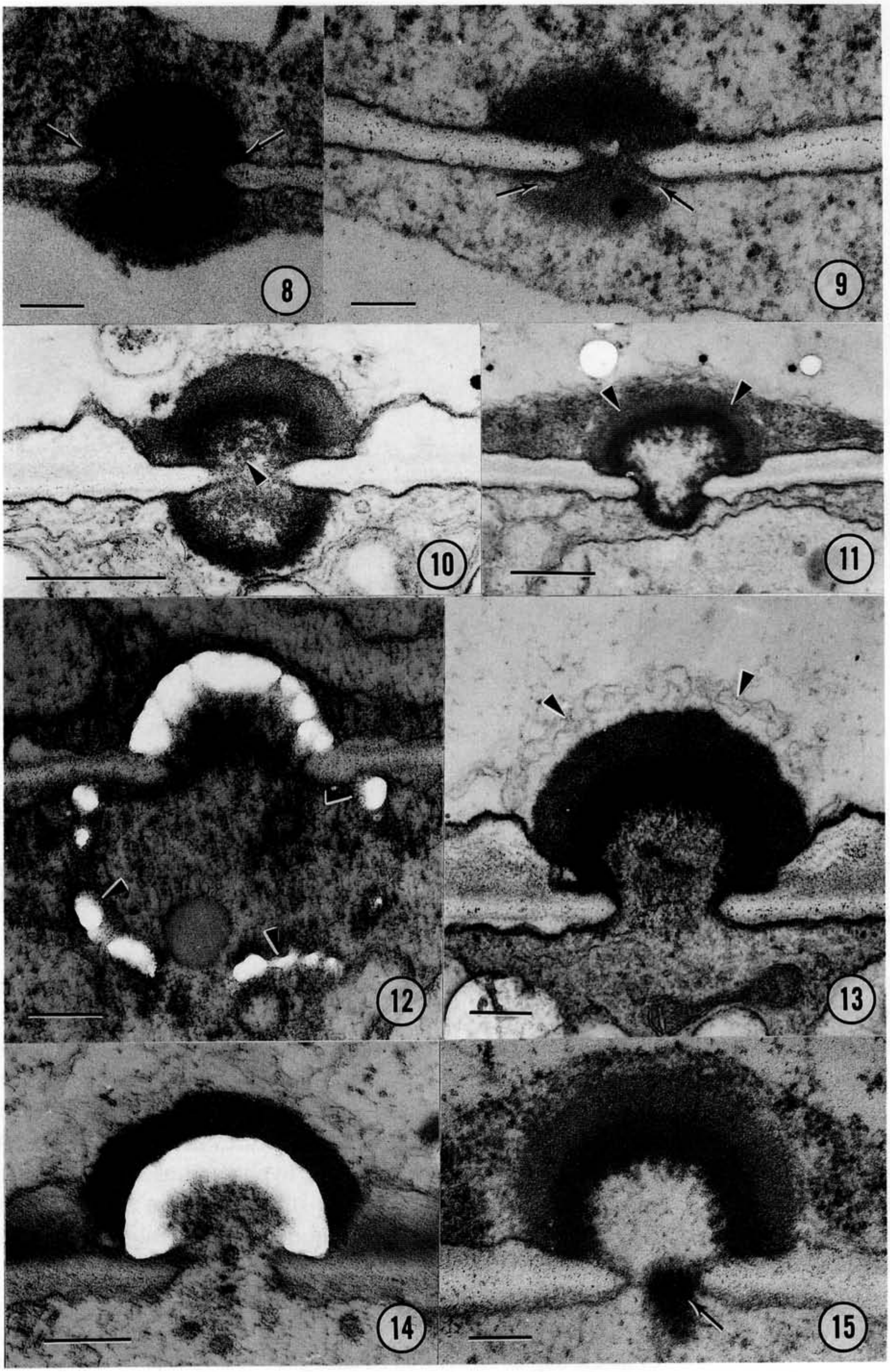
DISCUSSION

This study has shown that three different groups of cup fungi may have a very similar hemispherical banded pore plug at the ascal bases (Fig. 21). They are species of Ascobolaceae and Ciliarieae and *Anthracobia-Otidea* groups of Humariaceae (Wu, and Kimbrough 1995). However, their manner of septal development is totally different and deserve to be recognized individually in order to distinguish them and for convenience in the following discussion. These septal types will be referred to as the ascoboloid (Fig. 21 C), scutellinioid (Fig. 21 B), and otideoid (Fig. 21 A) types respectively.

Ascoboloid type of septal structure

This type is characterized by the presence of delicate vacuoles surrounded by endoplasmic reticulum in the central lumen of the septal pore of ascogenous hypha, and the pore plugging material is secreted around the vacuoles. A hemispherical band is finally

Figs. 8-15. Transmission electron micrographs showing the development of scutellinioid septal structure. Figs. 8-15. *Scutellinia scutellata*. 8. Pore plugging matrix in ascogenous hypha with a translucent zone (arrows) (bar = 0.2 μm). 9. Some small vacuoles are present in the central lumen of pore plugging matrix (bar = 0.2 μm). 10. More vacuoles (arrow head) are present in the pore lumen and biconvex bands are formed (bar = 0.5 μm). 11. Another less electron opaque layer (arrow heads) is laid over one of the biconvex bands in ascal cell (bar = 0.5 μm). 12. After bleached by 1 % hydrogen peroxide, the lower part of biconvex bands degenerating (arrow heads) (bar = 0.2 μm). 13. A hemispherical band in a mature ascal base and the upper part covered by a membranous structure (arrow heads) (bar = 0.2 μm). 14. A hemispherical band was treated by 1 % hydrogen peroxide. The lower part is bleached and formed earlier than upper part (bar = 0.3 μm). 15. In certain cases, another laminated structure (arrow) is found under the dome-shape plugging band (bar = 0.2 μm).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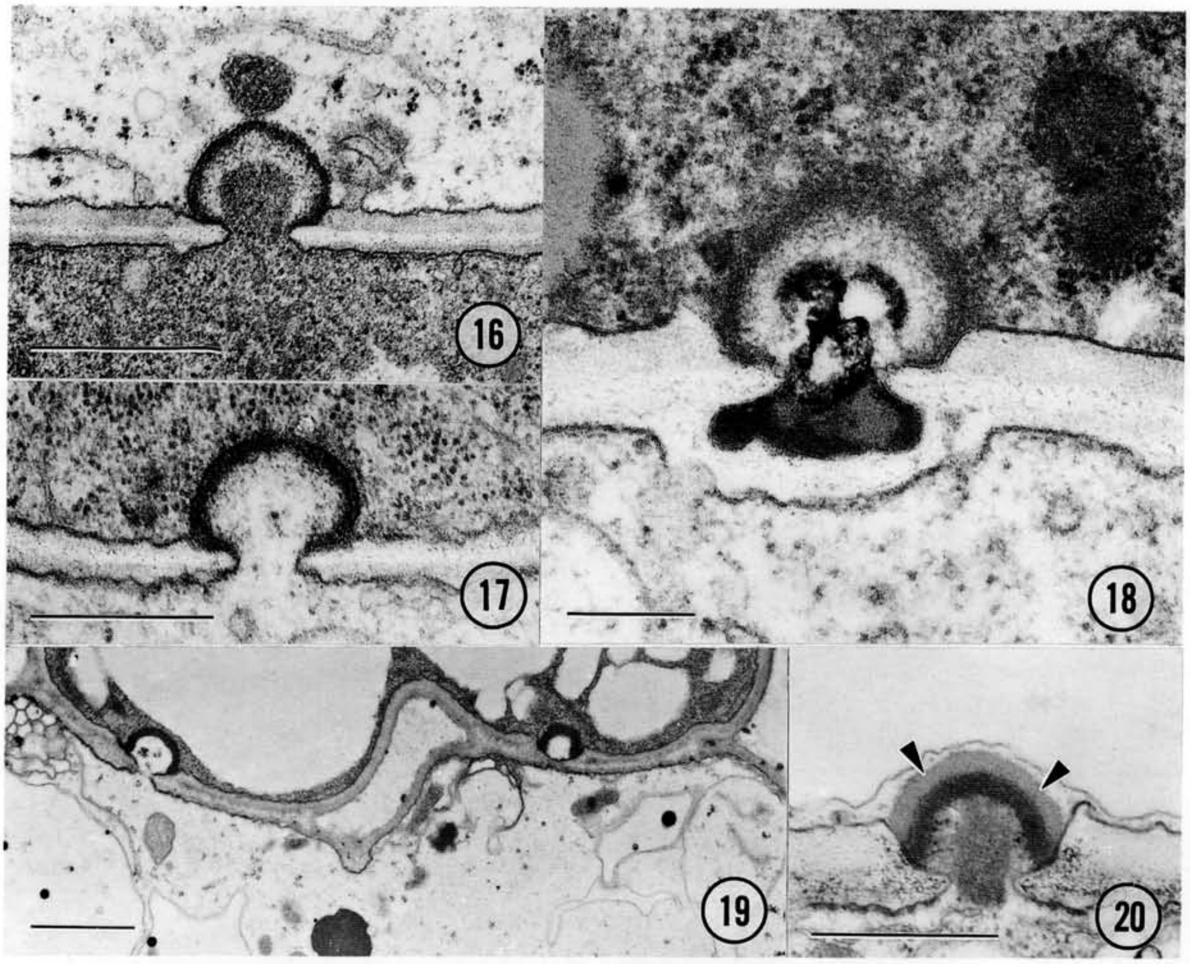

Figs. 16-20. Transmission electron micrographs of septal structures in *Coprobia* and *Cheilymenia*. Figs. 16-18. *Coprobia granulata*. 16. An electron dense hemispherical band at the ascal base (bar = 0.5 μm). 17. An electron dense hemispherical band at the ascal base (bar = 0.5 μm). 18. A mature septal plugging at the ascal base and lower part is occluded by hyphal wall material (bar = 0.2 μm). Figs. 19-20. *Cheilymenia stercorea*. 19. Two early ascal plugging structures at the ascal bases, showing only electron dense hemispherical bands (bar = 1 μm). 20. A mature ascal plugging structure, showing another less opaque material (arrow heads) laid over electron dense hemispherical band (bar = 0.5 μm).

formed in the mature ascal base. The pore diameter for ascogenous hypha is 0.14-0.27 μm and for the ascal base is a little smaller, 0.2 μm.

This septal development type is found in all members of Ascobolaceae (Wells, 1972; Kimbrough and Curry, 1985). The term "ascoboloid" was first coined by Kimbrough and Curry (1985) to describe a hemispherical structure in the ascal septa of taxa of the Ascobolaceae. Even though they did also observe the septal structures in ascogenous hyphae, however, the developmental connection between ascogenous hypha and ascal base was not emphasized. In this study, we examined carefully the septal structures from the ascogenous hyphae, croziers and ascal bases, and suggested a unique developmental type in the Ascobolaceae.

Scutellinioid type of septal structure

This type is characterized, in the septal structures of ascogenous hyphae, by the presence of a translucent torus by the pore border in the plugging matrix within which delicate

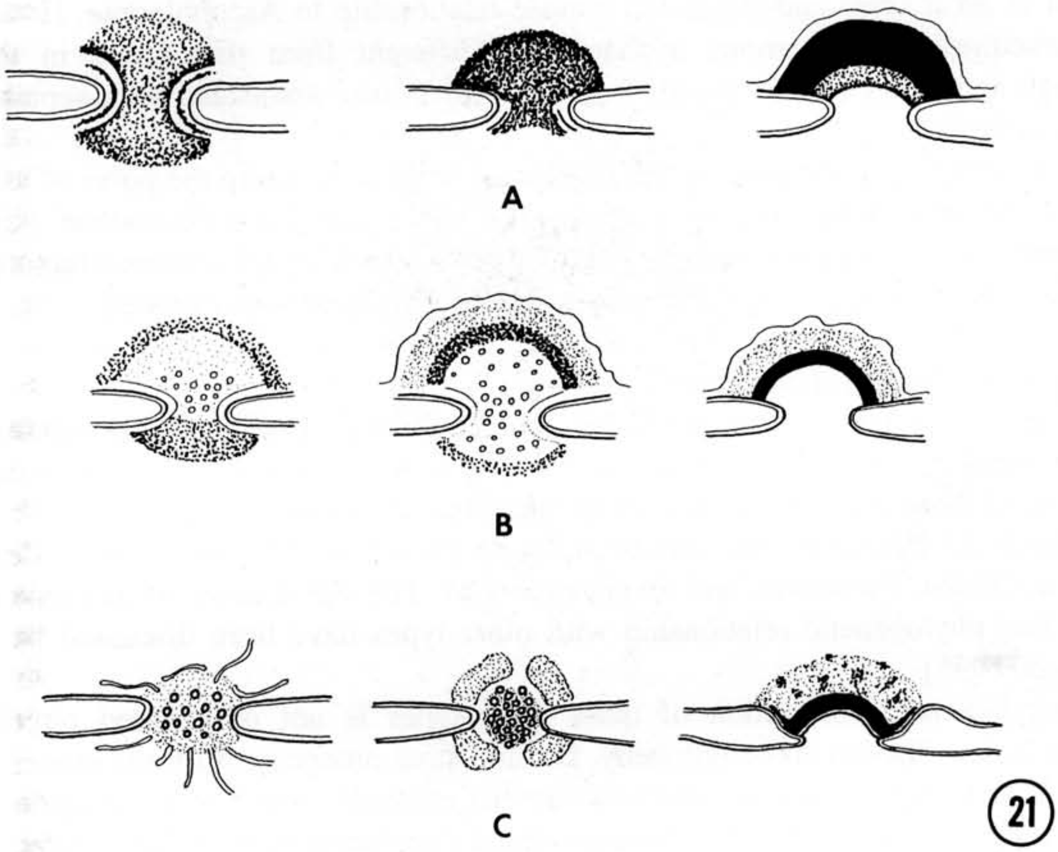

Fig. 21. A comparision of septal developments between anthracobioid (A), scutellinoid (B) and ascoboloid (C) types.

vacuoles are visible. When the ascogenous hyphae mature and become young asci, a plugging matrix is pushed toward both sides forming two hemispherical archs. In the base of mature asci, only one hemispherical band is left in the ascal cell on which later another layer of wall material will be deposited. The pore diameter of ascogenous hypha is 0.3-0.36 μm and of ascal base is 0.2-0.3 μm.

This septal development type has so far been found only in members of Ciliarieae (Humariaceae). There are two major differences separating scutellinioid type from ascoboloid type, (i) the presence of a translucent torus by the pore border of ascogenous hyphae, (ii) biconvex arches are formed due to an assumptive pressure created inside the plugging matrix. However, the components of the hemispherical structure of the ascoboloid type are secreted by endoplasmic reticulum and deposited around the vacuoles located within the pore lumen. The hemispherical structure of the scutellinioid type is obviously composed of two different layers. The inner layer is very osmiophilic and perhaps constituted of lipid or lipoprotein which provides very good occlusion capability. These components are removed with the hydrogen peroxide treatment. In contrast to the inner layer, the outer dome is inert to hydrogen peroxide and may be composed of different materials such as polysaccharides which have been suggested to be in the plugging matrix of *Helvella* and *Gyromitra* (Kimbrough and Gibson, 1989). Information about the chemical composition of septal structures is very limited thus far.

Kimbrough and Curry (1986) also studied the septal structures of ascogenous hyphae and ascal bases separately in members of Ciliarieae. A similar hemispherical structure was described in ascal septa and suggested a close relationship to Ascobolaceae. However, the septal structures of ascogenous hyphae were different from that found in this study. Kimbrough and Curry (1986) found Woronin bodies were associated with septal plugs of ascogenous hyphae.

It is an unusual phenomenon to find laminated structures within the pores of ascal bases (Fig. 15), because these structures were only described from Pezizaceae (Curry and Kimbrough, 1983). This phenomenon and the potential to form a biconvex structure suggest a possibility that Ciliarieae and Pezizaceae might be phylogenetically related.

Otideoid type of septal structure

This septal type has been described (Wu and Kimbrough, 1995) and is different from the two other types by the presence of two translucent zones adjacent to the pore borders, and the absence of those delicate vacuoles in the plugging matrix (Fig. 21).

This septal development type has been found in species of *Anthracobia*, *Caloscypha*, *Octospora*, *Otidea*, *Pyronema*, and *Sphaerosporella*. The significance of this type of septal structure and phylogenetic relationship with other types have been discussed by Wu and Kimbrough (1995).

The phylogenetic connection of these three types is not only based on the septal development, but also on spore ontogeny. During spore ontogeny, all three groups of fungi form condensed granules in the perisporic sac that gradually attach to the epispore forming ornamentations or secondary wall. Additionally, a translucent zone in the perisporic sac is found consistently in some pyrophilic (*i.e.*, *Anthracobia*, *Octospora*, *Sphaerosporella* etc.) (Merkus, 1974; Wu and Kimbrough, 1993; 1994) and soil-inhabiting species (*i.e.*, *Scutellinia* etc.) (Wu and Kimbrough, 1991) which suggests a strong connection with Ascobolaceae, which have a unique spore ultrastructure (Wu and Kimbrough, 1992).

Species showing the same type of septal structures in the pores of mature asci, but exhibiting transitional differences in their early development stages, point to a possible monophyletic origin of these groups. The study of septal development may be potentially of great use in the study of phylogeny in the Pezizales.

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盤菌目 Humariaceae 科 Ciliarieae 亞科與 Ascobolaceae 科，其子囊底部及子囊再生菌絲隔板構造之比較研究

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摘　　要

在 Ascobolaceae 科及 Humariaceae 科 Ciliarieae 亞科的子囊底部的隔板構造與 Humariaceae 科 Otideae 亞科的拱型隔板構造十分類似。但其形成過程並不相同，可分為 ascoboloid, scutellinioid, 和 otideoid 三型。子囊菌類在成熟的子囊底部具有相同的隔板構造，但在初期的分化形成過程卻顯現出差異性，此種現象顯示這些子囊菌類可能由單一族群演化而來。有關子囊底部及再生菌絲隔板形成的過程可能有助於盤菌目演化學上的研究。

關鍵詞：隔板形成過程，演化，盤菌目。

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