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The Tissue Types of Stroma in Some Species of *Hypoxylon*

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*Hypoxylon*속 자낭자좌 조직의 형태 연구

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요 약

주사 전자현미경상에서 *Hypoxylon*속 4종(*H. archeri*, *H. bovei*, *H. fragiforme*와 *H. stygium*)의 자낭자좌 조직 형태를 중점적으로 관찰하였다. 본 연구는 *Hypoxylon*속의 자낭자좌의 각 층의 조직상 형태를 가장 심도있게 연구하였으며, 이 자낭자좌들은 동결파쇄법에 의하여 종단 및 횡단 절단되었다. 여러 개의 균일 된 자낭자좌 조직의 형태가 속내의 종간에 각기 다르게 형성되었다. 자낭자좌 조직의 형태는 *Hypoxylon*속의 종 수준에서의 분리동정을 위한 주검색 표로서는 미흡하나 보조 검색 표로는 매우 유용하다.

Key words : *Hypoxylon*, scanning electron microscope, stromata, tissue type

INTRODUCTION

The xylariaceae is an assemblage of sphaeriaceous genera, with obscure but apparently ancient common ancestry, which typically produces dark-colored nonseptate ascospores in smooth perithecia, embedded in stromata (Rogers, 1979; Whalley, 1985). A central core of genera - *Xylaria*, *Hypoxylon*, *Rosellinia*, *Dald-inia*, *Nummularia*, *Kretzschmaria*, *Penzigia Camillea* - are obviously related, regardless of the fact that the generic limits are arguable (Rogers, 1979; Barr, 1990; Eriksson & Hawksworth, 1991; Laessoe, 1994; Lee, 1997). In general, the taxonomical descriptions of species are entirely based on telemorphic (shape, size, color and the kind of surface of the stoma, perithecia, asci, ascospores and its types of ostiolum) and anamorphic (growth form, colonies, location and arrangement of conidiophores, conidiogenous structures) (van der Gucht, 1993). However, there are still many problems (Rogers, 1979).

The anatomical structures of stromata in Xylariaceae have been investigated and have proven to be very useful in taxonomy of these groups (van der Gucht, 1993). However, only a few investigators have emphasized the structure of stroma (Miller, 1928; Martin, 1967; Abe, 1984;

Dargan & Singh, 1986). Miller (1928) observed the structure of stromata and the development and formation of perithecia in *Hypoxylon* and its allied genera. Martin (1967) and Dargan and Singh (1986) also used the types of stromata as one of the keys, having four potential stromal layers. Therefore Abe (1984) tried to observe the stromal tissues of fifteen species of *Hypoxylon* and its allied Genera using SEM. According to their observations, the study of tissue types seems useful, but no enough studies have been considered yet. The object of this study tried to observe the structure of stroma for evaluating this method as a key of taxonomic criterion.

MATERIALS AND METHODS

Four fungi, which are mostly fresh condition, were selected for this experiment: *H. archeri* (collected from Hong Kong), *H. bovei* var. microspora (Malaysia), *H. fragiforme* (England), and *H. stygium* (Philippines). Specimens were collected 4 different countries and deposited at University of Portsmouth.

The experimental procedure was modified with Abe (1984). Small cubes (about 5 mm), containing several perithecia, were cut out from each specimen with razor blade. These cubes were cut vertically or horizontally for observation of tissue types with a freezing fracture methods for SEM (Lee, 1997). For observation of ostiola structure and the surface of stroma, samples were cut vertically. The specimens were dehydrated, critical point dried and coated with gold by ion spattering and observed under SEM T-20.

RESULTS

Hypoxylon archeri Berk., Fl. of Tasmania II, in Hook., Bot. Antarctic Voy. II : 280 (1860) (Fig. 1).

The surface of stromata is shiny and smooth, but sometime perithecia produced. The discs of this species are very small and not so distinct compared with other species in sect. of Anulata. The disc of around open papillate ostiola covered with cubical or angularical minutes. Ascomata - bearing part are distinctively divided into three layers: inner layer very narrow with thick - walled to matrix; middle layer thin - walled textura angularis; outer layer thick - walled textura angularis. The width of asci - bearing layer in cross section perithecium is less than 50 um thick.

Hypoxylon bovei Speg. var. ***microspora*** Mill., Monograph: 95 (1961) (Fig. 2).

The surface of stroma is not smooth with warty hypha. Small and very distinct annulate discs found but ostiola are mainly not open. Ascomata - bearing part seems to be divided into three layers: inner layer very narrow, thin - walled textura prismatica; middle layer very thick - walled textura angularis with a very small pore in the cell, and decreasing thickness along with the distance outside the ascomata. The width of asci - bearing layer in cross section perithecium is so wide, having thick-walled textura angularis to thick-walled texture prismatica.

Hypoxylon fragiforme (Pers. ex Fr.) Kickx, Flore crypt. des Environs de Louvain: 116 (1835) (Fig. 2).

Surface of stroma is heavily covered with warted hypha, having a rough surface. Ostiola are mainly open but sometime matrix hypha close up ostiola, without annulate disc. The

types of texture stroma are similar with *H. howeianum*. Ascomata - bearing part can be divided into three layers and consisted of fine walled textura angularis, increasing diameter of cells from inner layer to outer layer. The tissue of most inner layers is composed of thick - walled prismatica but very thin layer.

Hypoxylon stygium (Lev.) Sacc., Syll. F. 1: 379 (1882) (Fig. 3).

Stromata are very shiny, hardy and black carbonaceous. The surface is smooth but sometime matrix hyphae observed on surface of stroma. Ostioles are distinct but very small compared with *H. truncatum*. Pore of annulate discs are globose to pentagonal angular and 2-3 um in diameter. Ascomata - bearing part is either thick-walled texture globulosa or thick-walled texture angularis.

DISCUSSION

For observation of tissue types of each layer in stroma, cutting stroma with razor blades and sectioning of tissue layers vertically or horizontally with a freezing fracture methods for SEM are suitable method in hard and brittle stromata in the species of *Hypoxylon* (Abe, 1984; Lee, 1997). Even though tissue types could be observed without any pre-treatment, fixation and dehydration were clearly need. Without proper fixation and dehydration of specimens, the collapse of tissue structure clearly occurred. Deformation by pressure of the razor blade also was not occurred with a freezing fracture method. This method is suitable for observation of soft, easily deformable tissues, thin-walled hyphal elements of the protoplasm of hyphae.

A stroma is divided into two or three parts. "Epistroma" and "hypostroma" termed by Fuisting (1867), and "ectostroma" and "entostroma" designated by Ruhland (1900) for the surface layer and inner layer respectively. Wehemeyer (1926) divided into three parts for ectostroma and Miller (1928) defined the terms for layers of a stroma. Later Martin (1967) divided a stroma into four parts for the taxonomical cri-teria for the Xylariaceae. Recently, Abe (1984) divided into three parts, S.L., P.S. and B.S., temporarily for description of the tissue types. He observed fifteen species of *Hypoxylon* and its allies and defined schematic types of stromal tissues, having four to five different tissue typ-

Table 1. Tissue types in each part of stroma in the genus *Hypoxylon*.

Species	Tissue of disk	Tissue of S.L.	Tissue of A.S.		
			inner layer	middle layer	outer layer
H. archeri	minute disc, TC-TA		NT-M,	tTA	TTA
H. bovei	Small, distinct annulate		NtTP	TTA	decreasing thickness of TTA
H. fragiforme	not present		TP	TC-TA	increasing thickness of TC-TA
H. stygium	annulate disc	PA	TTA TTG	TTA, TTG	TTA, TTG

S.L.: Surface layer of a stroma; A.S.: Ascomata-bearing part of stroma; TC-TA: thick cubical to thick angularis; NT-M: narrow with thick-walled to matrix; tTA: thin-walled textura angularis; PA: prismatica to angularis; TTA: thick-walled textura angularis; NtTP: narrow with thin-walled textura prismatica; TP thick-walled prismatica; TTG: thick-walled texture globulosa; -: not observed.

es. Even though Martin (1976) found common characters of stroma, which enables one to divide stromata, Abe (1984) did not find in his anatomical structure.

The tissue types of each layer in four species of the genus *Hypoxylon* are summarised in Table 1. The types in tissue layer studied by Abe (1984), but not enough description as a key of taxonomical criterion. Therefore this study have focused on the description of ascomata-bearing part and annulate disc. *Hypoxylon* fragiforme in the section of *Hypoxylon* quite differ from others in the section of Annulata with tissue types. From these observations, the study of tissue types seems useful, but still no enough studies considered in basal part of the stromata yet. The conclusion of the present study clearly requires further intensive investigation.

ABSTRACT

The tissue types of stromata were observed intensively in four species of *Hypoxylon* under a scanning electron microscope (SEM). These stromata were sectioned with a freezing fracture method for observation. Several tissue types were recognized and stable in each species. This study presents the most intensive observation of tissue types of each layer of stromata. It will be useful for taxonomic criteria for the species level. However, the tissue types can not be major taxonomical criteria for the genus *Hypoxylon*.

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FIGURE LEGENDS

Fig. 1. Scanning electronic microscope micrographs of *Hypoxylon archeri*.

A. Small disc with open papillate ostiole (arrow).

B. Cubical to angularical pores of annula disc around ostiole.

C. Structure of wall layers of perithecium, having three different layers (black and white arrows) and asci and ascospores layer (arrowhead).

Bars: A: 100 um; B: 3 um; C: 2 um

Fig. 2. A and B: Scanning electronic microscope micrographs of *Hypoxylon bovei* var. *microspora*; C: Scanning electronic microscope micrographs of *H. fragiforme*.

A. Papillate ostiole in the center of annula disc and warted hypha on the surface of annula disc.

B. Cross section of perithecium, showing degreasing thickness along with the distance outside ascomata.

C. Warted hyphae on surface of stromata and open umblicate (white arrow) and close umblicate ostiole (black arrow).

D. Very narrow thick-cell wall layer and increasing of thickness of the cell wall.

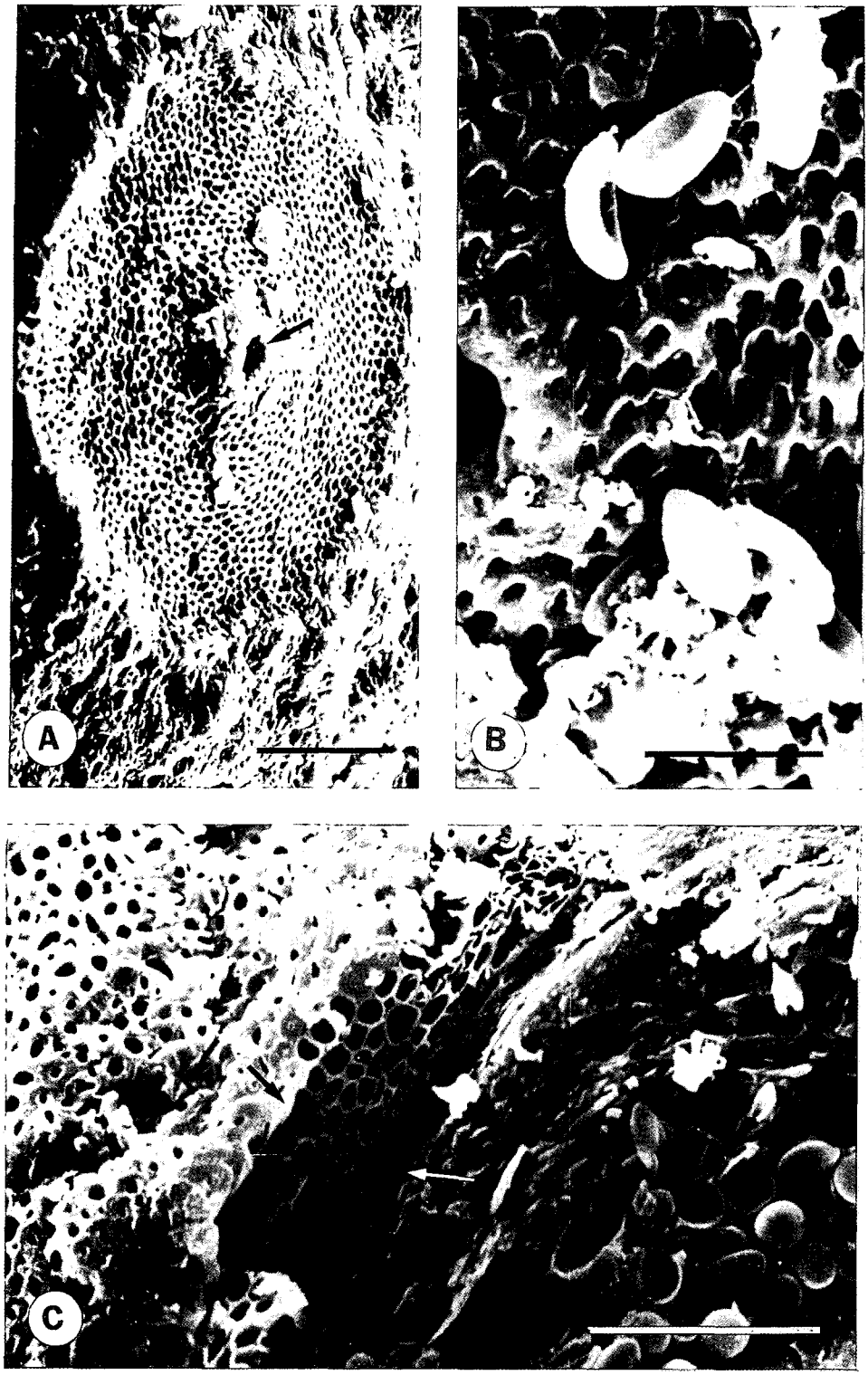
Bars: A: 1,000 um; B: 20 um; C: 5 um; D: 10 um

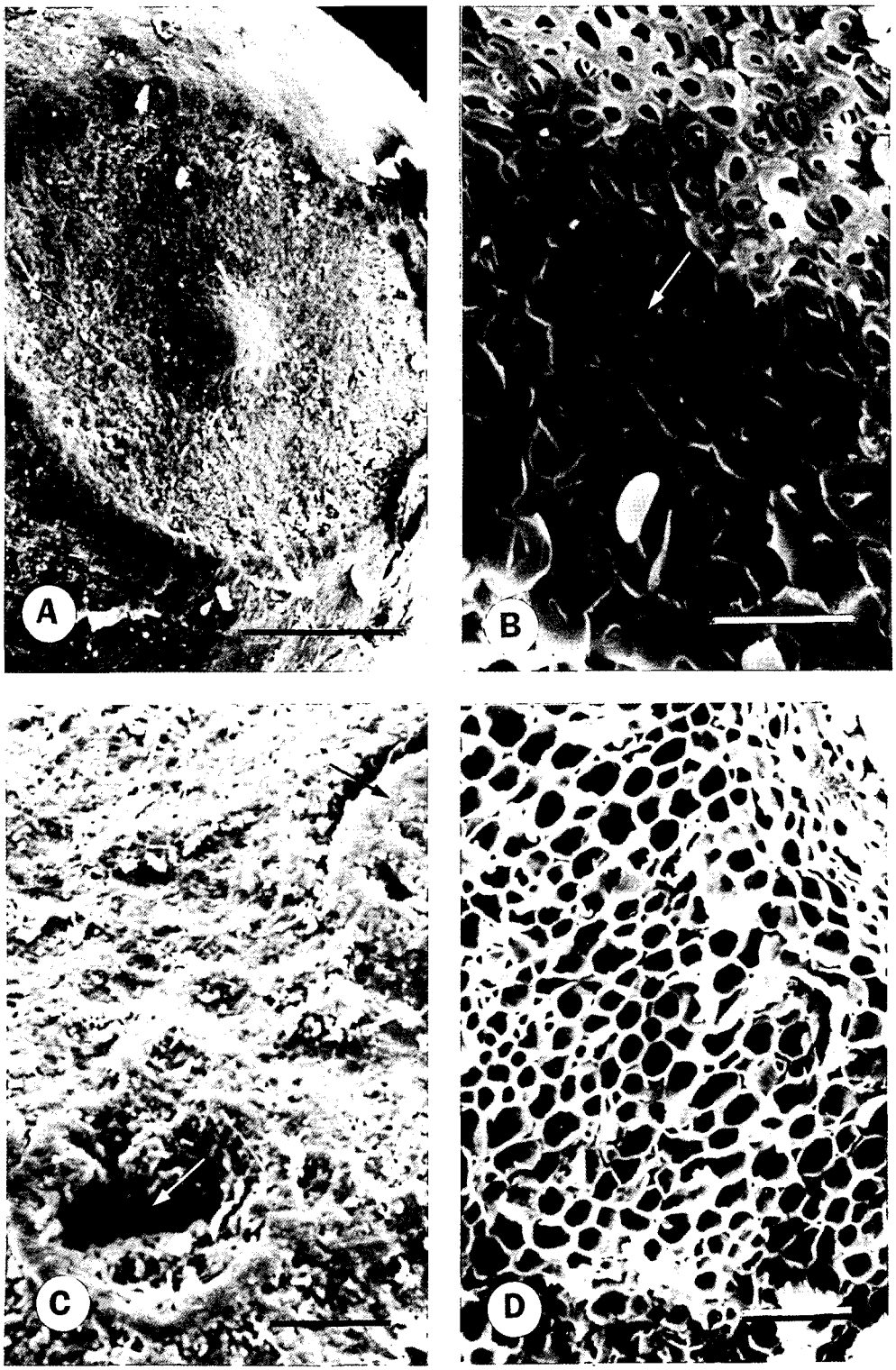
Fig. 3. Scanning electronic microscope micrographs of *Hypoxylon stygium*.

A. Very papillate and distinctable close ostiole and small annula disc.

B. Globose to pentagonal angular pores of annula disc, ranging 2~3 um in diameter.

Bars: A: 40 um; B: 2 um


A
B
C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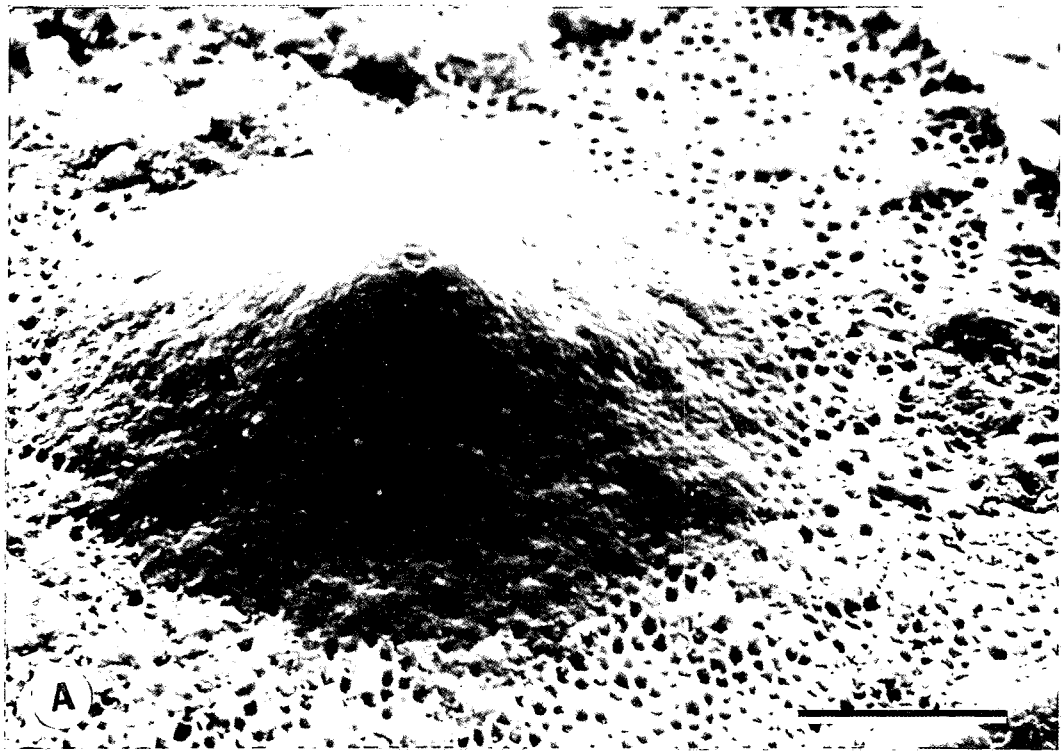
B
C
D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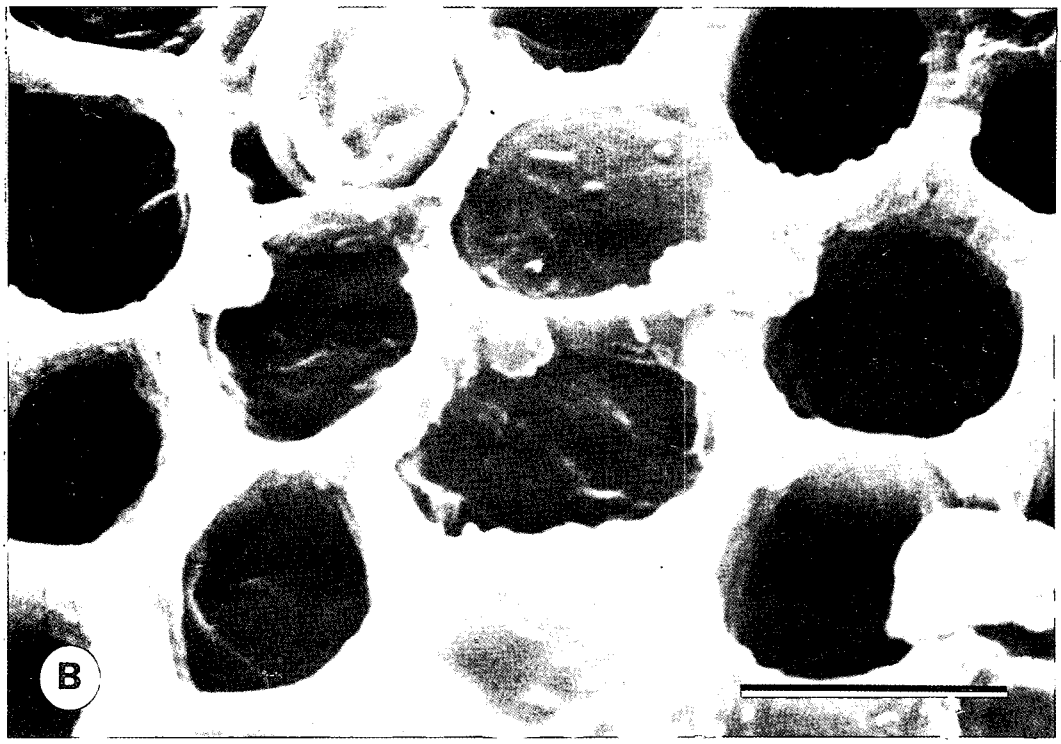
B