Pak.J.Bot., 30(1): 51-68, 1998.

STUDIES ON NUCLEI AND APPENDAGES IN SOME COELOMYCETES

S.Q. ABBAS, B.C. SUTTON* AND A. GHAFFAR**

Department of Botany,
Government Urdu Science College, Karachi, Pakistan.

Abstract

Number of nuclei and the nature, arrangement and number of appendages in 8 genera of Coelomycetes viz., *Kellermania, Alpakesa, Seimatosporiopsis, Parahyalotiopsis, Hyalotiella, Hyalotiopsis, Robillarda and Pseudorobillarda* has been described and illustrated.

Introduction

Although extensive studies have been carried out in which the number of nuclei have been used as a taxonomic criteria for separation of various taxa in fungi (Korf, 1972; Zhang & Minter, 1989; Punithalingam, 1972, 1975, 1989b; Punithalingam & Woodhams, 1986), little seems to have been done in the Coelomycetes except for the reports of Punithalingam (1970, 1974, 1983, 1984a, 1984b; 1989a, 1989b); Punithalingam & Woodhams (1986) and Uecker & Kulick (1986). Similarly Pirozynski & Shoemaker (1971) have described the importance of appendages in the taxonomy of Coelomycetes. The nature of appendages in some taxa of Coelomycetes have been reported (Punithalingam, 1981, 1982, 1983, 1984a, 1989a, 1989b, 1989c; Punithalingam & Woodhams, 1982, 1984, 1986. Recently, Abbas *et al.*, (1998) have proposed a new appendages classification. In the present paper studies on the nuclei and appendages of 8 Coelomycete genera are described and illustrated.

Materials and Methods

Appendage staining: A Modified Leifson's flagellar staining technique was used (Punithalingam & Woodhams, 1984). The stain used was a 1:2 mixture of solution A, containing 1.2% basic fuchsin in 95% industrial methylated spirit and solution B, containing 1.5% tannic acid and 0.75% sodium chloride in distilled water. Phenol (1/1000) was added to solution B to prevent mould growth during storage. The stain was used immediately or the mixture of A and B in a 1:2 ratio was centrifuged @ 2000 r.p.m. for 10 min., and the supernatant used for staining. Conidia obtained by teasing the conidiomata in a drop of water on a microscopic slide were spread to obtain a thin film, dried at room temperature and stained with 1 ml of staining solution. The stain was allowed to stand on the surface of the thin film for 2-5 sec., rinsed with running tap water, dried at room temperature and mounted in Euparol (ASCO Laboratories, England).

*International Myclogical Institute, Bakeham Lane, Egham, Surrey TW20 9TY, U.K.
**Department of Botany, University of Karachi, Karachi-75270, Pakistan.

Nuclear staining: For staining nuclei in hyaline conidia the HCl-Giemsa technique of Hrushovetz (1956), adapted and modified by Punithalingam (1983) was used. Material was either fixed directly in Carnoy's fluid containing absolute alcohol and glacial acetic acid (3:1), or a mature conidioma was transferred to a microscopic slide, teased and spread to produce a thin film of conidia, air dried at room temperature, fixed in Carnoy's fluid for 15 min., rinsed thrice in 95% ethyl alcohol and stored in 70% ethyl alcohol for a minimum period of 24 h or stored for several days until staining. Before staining the slides were immersed in IN HCl for 5 min., at room temperature (23-25°C) followed by hydrolysis in IN HCl at 58.5 - 60°C for 8-9 min. The slides were rinsed thrice in distilled water each for 1 min., rinsed in phosphate (K_2HPO_4) buffer at pH 6.9 and stained in Giemsa stain (Gurrs improved R66 BDH Chemical Ltd, UK), 1:10 diluted in buffer solution for 30 min. The slides were again rinsed with phosphate buffer then in distilled water, air dried at room temperature and mounted in Euparol.

For staining pigmented conidia, a thin film of conidia on a glass slide, air dried at room temperature was fixed in Carnoy's solution for 15 min., and stored for atleast 24 h in 70% ethyl alcohol. A longer period of storage of up to 10 days gave better results. The slides were then immersed in IN HCl for 5 min., at room temperature. Hydrolysis was carried out for 10-20 min., at 60-68°C. Hydrolysis at higher temperatures beyond 60°C and for a longer period of 10-20 min., gave better results compared with the technique suggested by Punithalingam (1983). Slides were rinsed 5 times in distilled water at least for 1 min., each, followed by 4 rinses in phosphate buffer at pH 6.9. The slides were stained in Giemsa stain for 30 min., rinsed in phosphate buffer pH 6.9 followed by rinsing in distilled water. Instead of directly mounting in Euparol, the slides were counter stained with 60% glacial acetic acid (BDH Chem., U.K.) for 10 min., followed by immersion in Citric acid for 5 min., to decolorize the Giemsa stain which improved staining. The slides were then treated with a few drops of Aceto Orcein, gently heated and mounted in Euparol.

For pigmented and thick-walled conidia, a conidial wall cracking technique (Zhang & Minter, 1989) was used. A thin film of conidia on a glass slide was air dried at room temperature. A cover slip was placed on the material and tapped, followed by slight pressure. Routine Giemsa staining method was used since the walls of conidia cracked after tapping and application of pressure, cytoplasm with nuclei came out from cracked conidia which were stained and easily seen.

Microscopy: A Leitz Whetzler microscope fitted with a camera lucida or an Olympus BH_2 microscope equipped with bright field, phase contrast, Nomarski and a camera lucida was used to examine the fungi. Photographs were taken either on an Olympus BH_2 or a Zeiss Photomicroscope, 4720459901 model, fitted with bright field, phase contrast and Nomarski optics, using Pentax 32 black and white film.

Scanning Microscopy: A scanning electron microscope, Hitachi Model S.570, was used. Discs of double sticky tape were fixed on the steel or brass stubs. The material for examination was directly dusted on the stubs or the material was teased in a film of water and a drop of liquid containing conidia was poured on the stub or the stub was touched with a drop of liquid containing conidia. Stubs were gold coated for 4 min., using a Polaron sputter coater. Photographs were taken using Kodak Plus X 125 ASA film.

Results and Discussion

Kellermania Ell. & Everh.
Fig. 1

Conidia of *Kellermania* Ell. & Everh., are characterized by an apical simple or occasionally branched appendage. It is acellular and develops from an outer mucilaginous sheath enclosing the conidia (Sutton, 1968, 1973, 1980). Of the 4 species recognised by Morgan-Jones *et al.*, (1972b), *K. yuccigena* Ell. & Everh., (as *yuccaegena*) has 1-septate conidia and *K. anomala* (Cke) Höhn., has 2-septate conidia. Both have an appendage. The other 2 species with no appendages are *K. attenuata* Morgan-jones, Nag Raj & Kendrick with aseptate conidia and *K. multiseptata* Morgan-jones, Nag Raj & Kendrick with more than 4 septa. Sutton (1980) only recognised 2 species, *K. yuccigena* and *K. anomala* with 1 and 2-septate conidia, respectively. He did not agree with Morgan-Jones *et al.*, (1972b) in placing taxa without appendages in *Kellermania* and considered *Piptarthron* Mont. ex Höhn., as a more suitable genus. Recently Vittal & Dorai (1991) reported *K. intermedia* with aseptate, appendaged conidia.

In the present studies, the appendage was found to be cellular, enucleate, having protoplasmic connection to the body of the conidium in early stages of conidial development. The inner and outer walls of the appendage were connected with the inner and outer wall of the conidium. In early stages of conidial development the appendage is continuous with the conidial body, later a wall is laid down. Species formerly considered to be aseptate are therefore 1-septate. Similarly 1 and 2 septate species respectively become 2 and 3-septate. *K. yuccigena* (IMI 21864) and *K. anomala* (IMI 182783) showed uninucleate conidial cells. The young aseptate conidium is binucleate and it is presumed that the first nucleus divides mitotically followed by the formation of a median septum thus producing 2 uninucleate cells. No nucleus was seen in the appendage.

Alpakesa Subram. & Ramakr.
Fig. 2 (I,II,III)

Alpakesa Subram. & Ramakr., is another genus bearing 0-3-septate, hyaline conidia with 2-7 simple, hyaline appendages, and a truncate base with a frill. Morgan-Jones *et al.*, (1972a) considered appendages as filamentous, unbranched setulae, surmounting the conidial apex, whereas, Sutton (1980) considered them as extracellular, developing from an outer mucilaginous sheath enclosing conidia. Studies on *A. uniseptata* Morgan-Jones, Nag Raj & Kendrick (IMI 256616) showed that the appendages are unicellular, nucleate, with cytoplasmic connections to the conidial cells in early stages of development and with very clear inner and outer walls. Conidia of *Alpakesa* are characterized by a hyaline region at the base of appendages. This is due to a wide gap between the inner and outer walls observed in *A. uniseptata* and *A. nolinae* (Pollack) Morgan-Jones, Nag Raj & Kendrick (IMI 107246). Modified Leifson's staining technique showed that in conidia with a relatively small gap, the

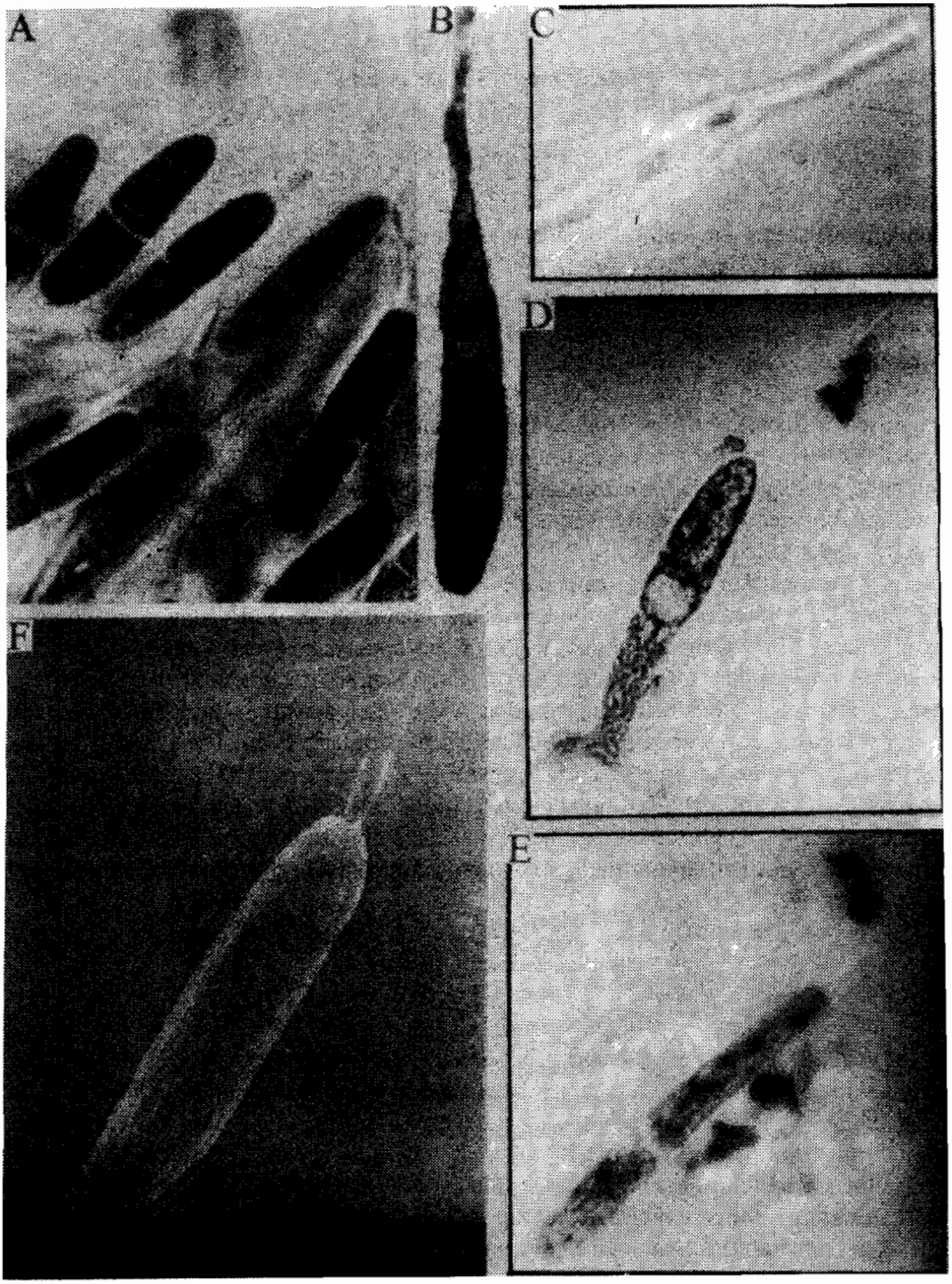

Fig.1. *Kellermania yuccigena*, IMI 121864 (A) Conidia in Lactophenol, 1800X; (B) conidia stained in Leifson's flagella stain, 1800X; (C) conidia in phase contrast, 1000X; (D,E) conidia stained in Geimsa HCl stain (D) young conidium with conidiogenous cells, 1800X; (E) mature conidium, 1800X; (F) conidia in SEM.

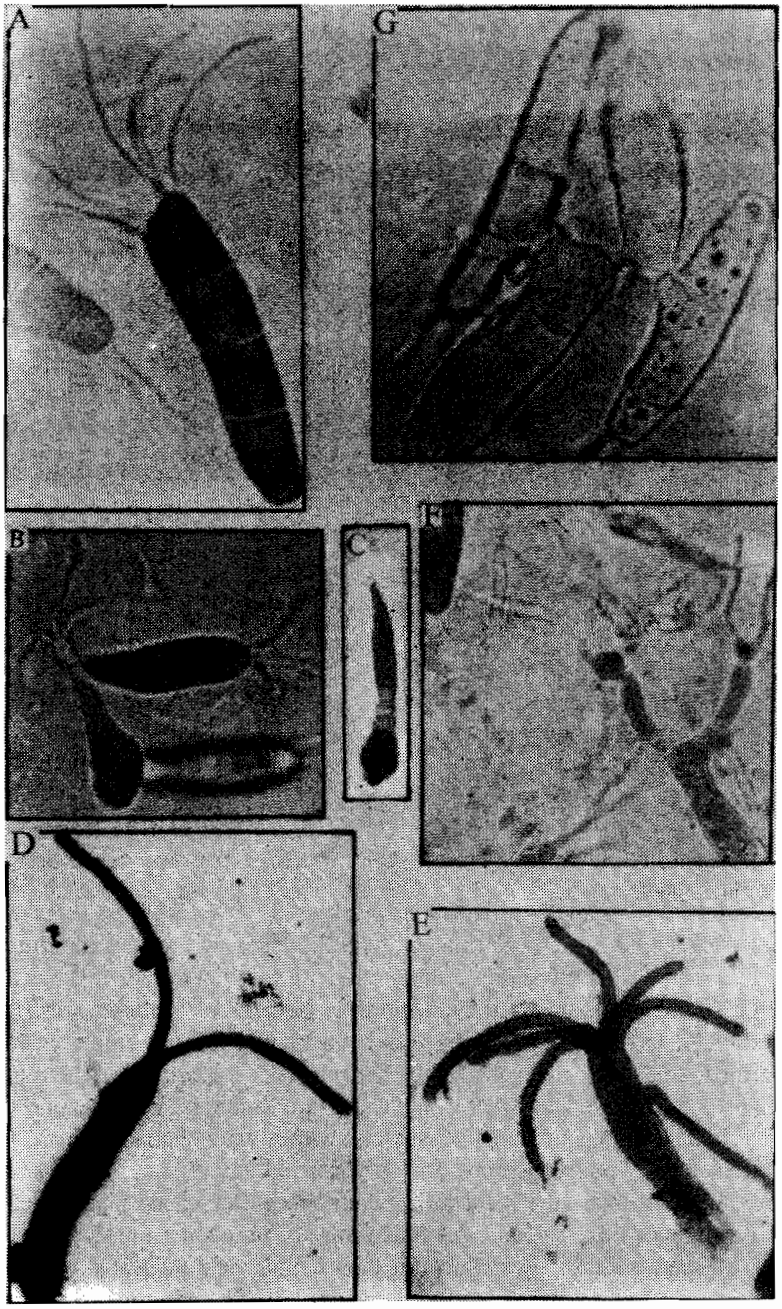

Fig.2.(I). *Alpakesa nolinae*, IMI 107246 (A) Conidia, 3 septate with 7 apical appendages, 1000X; *A. unisep-tata*, IMI 256616 (B) Conidia with 3 apical appendages and gap like structure, 1800X; (C) Conidiogenous cells with percurrent proliferations, 1800X; (D,E) conidia stained in Leifson's flagella stain, 2-many cellular appendages, 1800X; (F) conidium with modification in appendages; (G) conidia showing anastomosis.

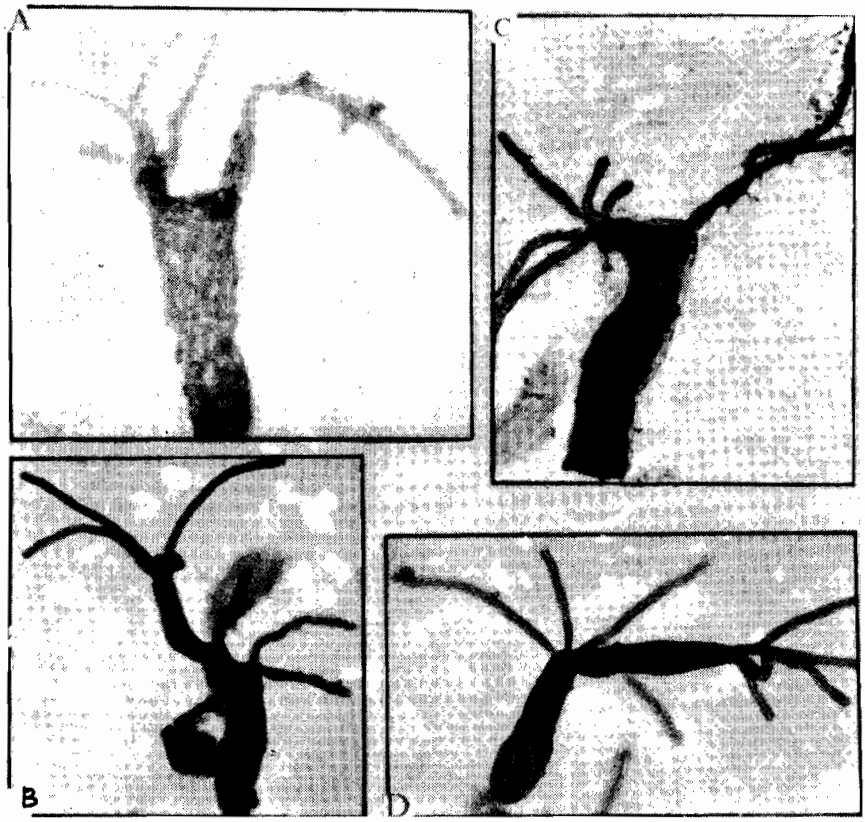

Fig.2.(II). *Alpakesa uniseptata* IMI 256616 (ABCD) conidia stained with leifsons flagella stain, appendages modified variously, 1800X.

outer wall of the appendages is continuous with the outer wall of the conidium, and the very delicate inner wall is similarly continuous with the inner wall of the conidium. In conidia with a wider gap, an additional septum is formed below the gap and in this way an additional cell is formed containing less cytoplasm. From the upper surface of this newly formed cell, the inner wall of appendages is formed. Scanning electron microscopy supports the view that appendages are cellular and developed from the conidial apex. The number of appendages also varies from 1-many which are formed from different loci. Appendages are not only apical, but in some cases arise slightly below the apex or from the next cell. Sometimes branched appendages are also observed. In some conidia, appendages function as conidiogenous cells and bear conidia at apices. This is an additional evidence for their cellular and nucleated nature. Conidial cells are uninucleated. However, Punithalingam (1989b) reported conidial cells as multinucleated. Nuclei are not visible in appendages but the formation of conidia from the tip of appendages indicates the presence of nuclei and supports the work of Punithalingam (1989b). Anastomosis between conidia was also observed.

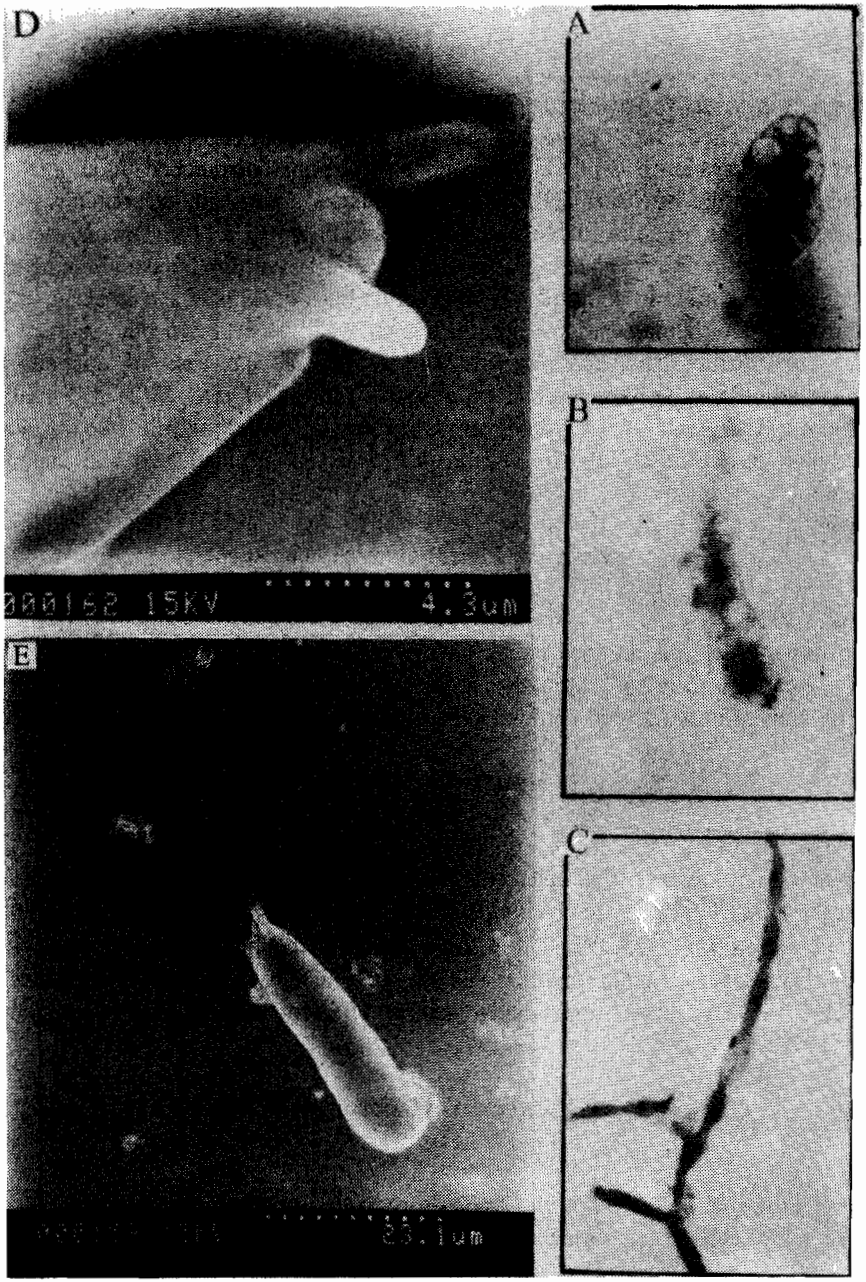

Fig.2.(III). *Alpakesa uniseptata* IMI 256616 (A,B) conidia stained in Geimsa HCl showing nuclei, 1800X; (C) septate mycelium with nuclei, 1800; (D,E) conidia in SEM showing appendages.

Seimatosporiopsis Sutton, Ghaffar & Abbas
Fig. 3. (I,II,III)

Seimatosporiopsis salvadorae Sutton, Ghaffar & Abbas (IMI 148620a) has 3-septate, thick-walled, dark brown conidia with 1-2 apical or subapical, simple, hyaline, cellular appendages and 1-2 lateral, exogenous, occasionally branched, hyaline, filiform appendages inserted on basal cell. These appendages arise from the outer wall with septa at their bases, even in immature conidia (Sutton *et al.*, 1972). During the present studies, it was found that appendages are indeed cellular with cytoplasmic connection to the body of the conidium and with no septa at the base of appendage in early stages of development. The lateral walls of conidia are thin and pale brown but mature conidia become thick-walled and dark brown. The septum at the base of appendages in immature conidia as seen in optical microscopy (Sutton *et al.*, 1972) is actually the lateral dark thick wall of the conidium. Crushing of conidium followed by staining showed that the conidial cells in *Seimatosporiopsis* are uninucleated and appendages enucleated.

Parahyalotiopsis (Thaung) Nag Raj
Fig. 4

Parahyalotiopsis is another genus having 3 septate, pale brown conidia with 2-4 apical appendages arising separately. Studies on *P. borassi* (Thaung) Nag Raj (IMI 180210) showed uninucleated conidial cells with cellular and enucleated appendages.

Hyalotiella Papendrof
Fig. 5

Hyalotiella transvalensis Papendrof (IMI 2101125, 1617741, 137470) has 3-septate hyaline conidia. The apical cell elongates and tapers then divides trifurcately to form branched appendages. Modified Leifson's staining method and scanning electron miscroscopy confirmed the cellular nature of the appendage. The Giemsa HCl technique showed that appendages are enucleated but the conidial cells are uninucleated.

Hyalotiopsis Punithalingam
Fig. 6

In *Hyalotiopsis subramanianii* (Agnihothrudu) Punithalingam (IMI 136542) conidia are 3-septate, each cell multinucleated, apical and basal cells hyaline, median cell pale brown (Punithalingam, 1970; Nag Raj & Kendrick, 1985). In the present study, the use of modified Leifson's flagella staining technique and Scanning electron microscopy confirmed the work of Punithalingam (1970) and Nag Raj & Kendrick (1985). It was observed that from the apex, two appendages arise which divide to form 2-4 branches, each conidial cells have 2-4 nuclei while the middle cell has more nuclei than the end cells.

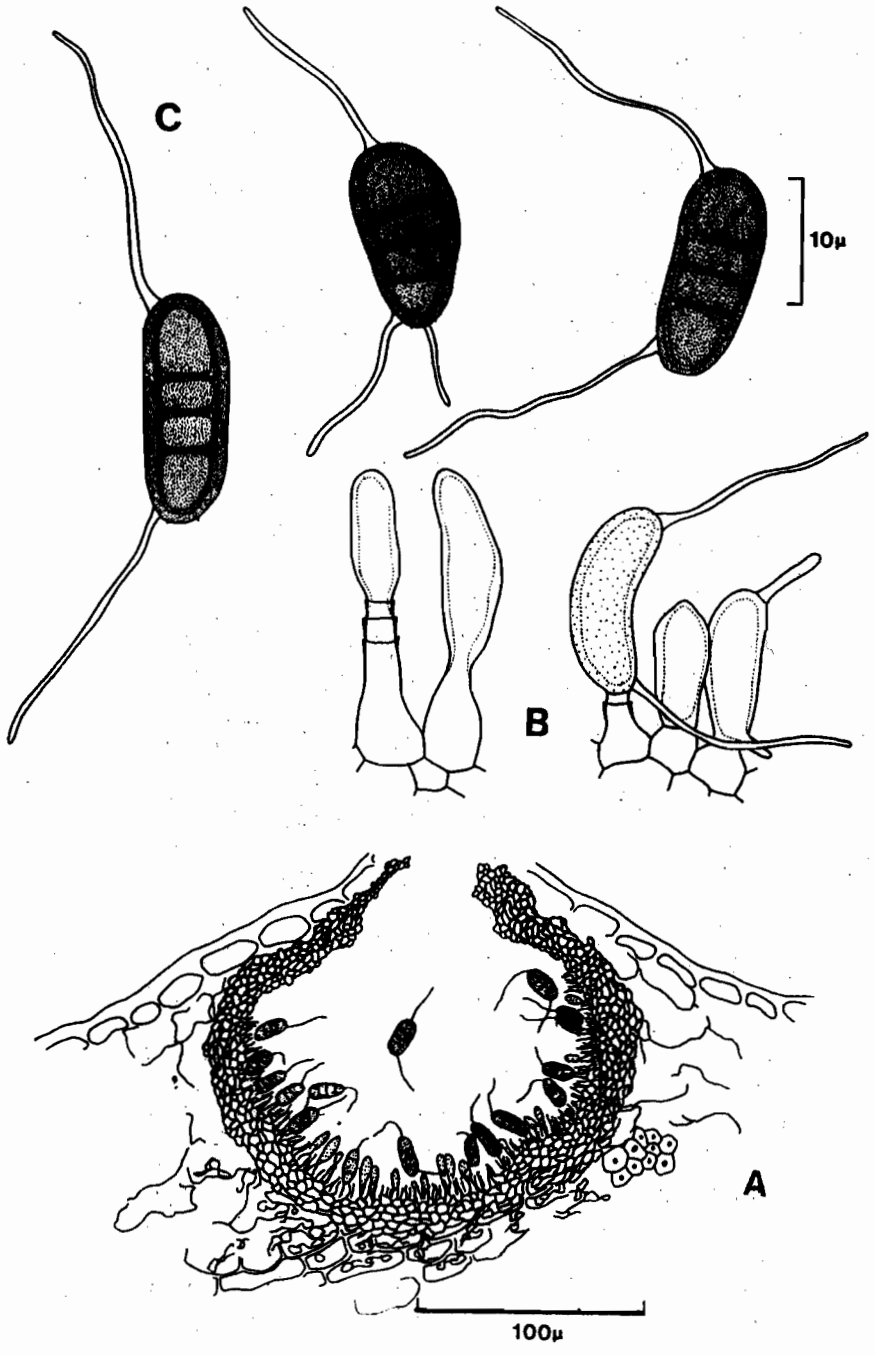

Fig.3.(I). *Seimatosporiopsis salvadorae*, IMI 148620a, type (A) V.S. of conidioma; (B) conidiogenous cells; (C) conidia.

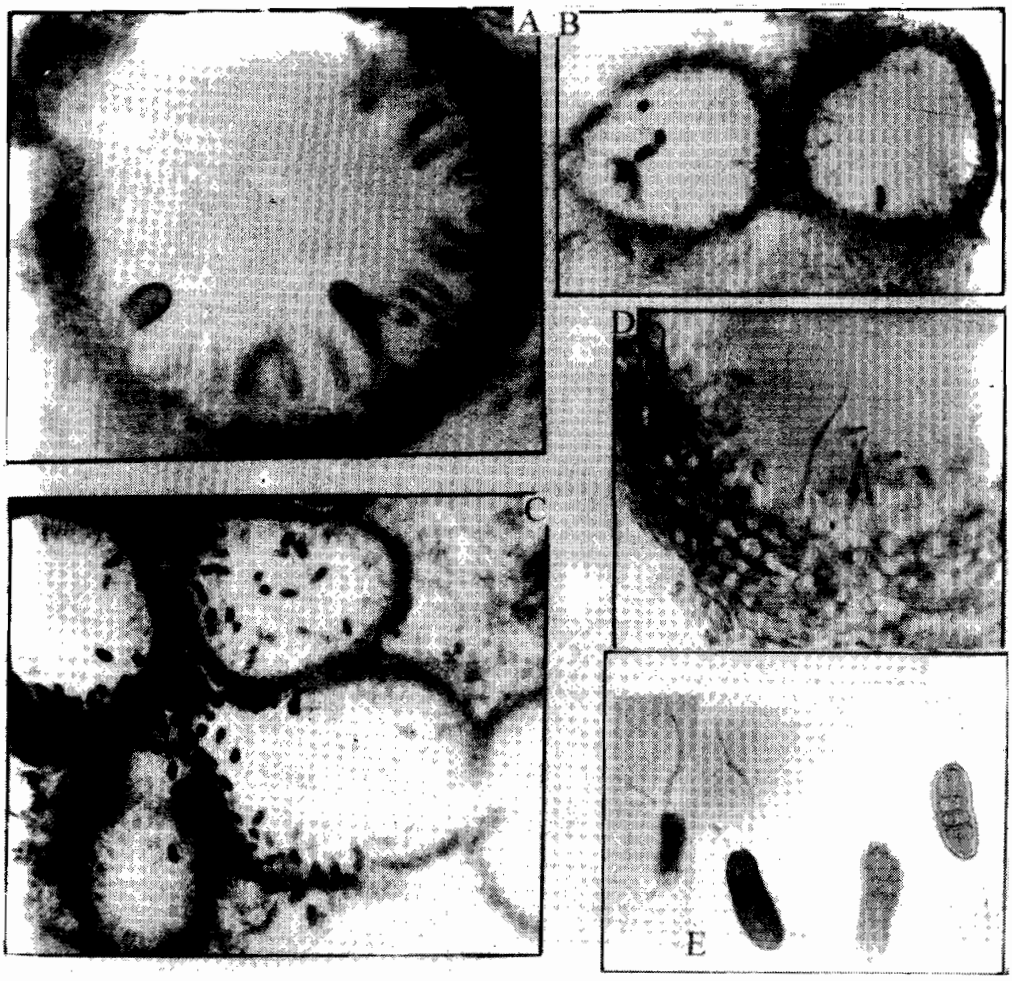

Fig.3.(II). *Seimatosporiopsis salvadorae*, IMI 319159, on *Calotropis procera* (A) V.S. of simple conidioma without paraphyses, 650X; IMI 148620a, type (B) V.S. of conidiomata loosely aggregated, 250X; IMI 319160, on *Capparis decidua* (C) V.S. of conidiomata, more compactly aggregated, 40X; IMI 1486209 (D) conidioma with paraphyses, 1000X; (E) conidia with 1-2 simple or branched apical and basal appendages, 1800X.

Robillarda Cast.
Fig. 7

The present studies on ***Robillarda sessilis*** (Sacc.) Sacc., (IMI 276578, 130854) also confirm the results of Punithalingam (1984a). Appendages are cellular and conidial cells uninucleate. Appendages under scanning electron microscopy were found to be apical and divide to form 3 branches as is found in *Hyalotiella transvaalensis*.

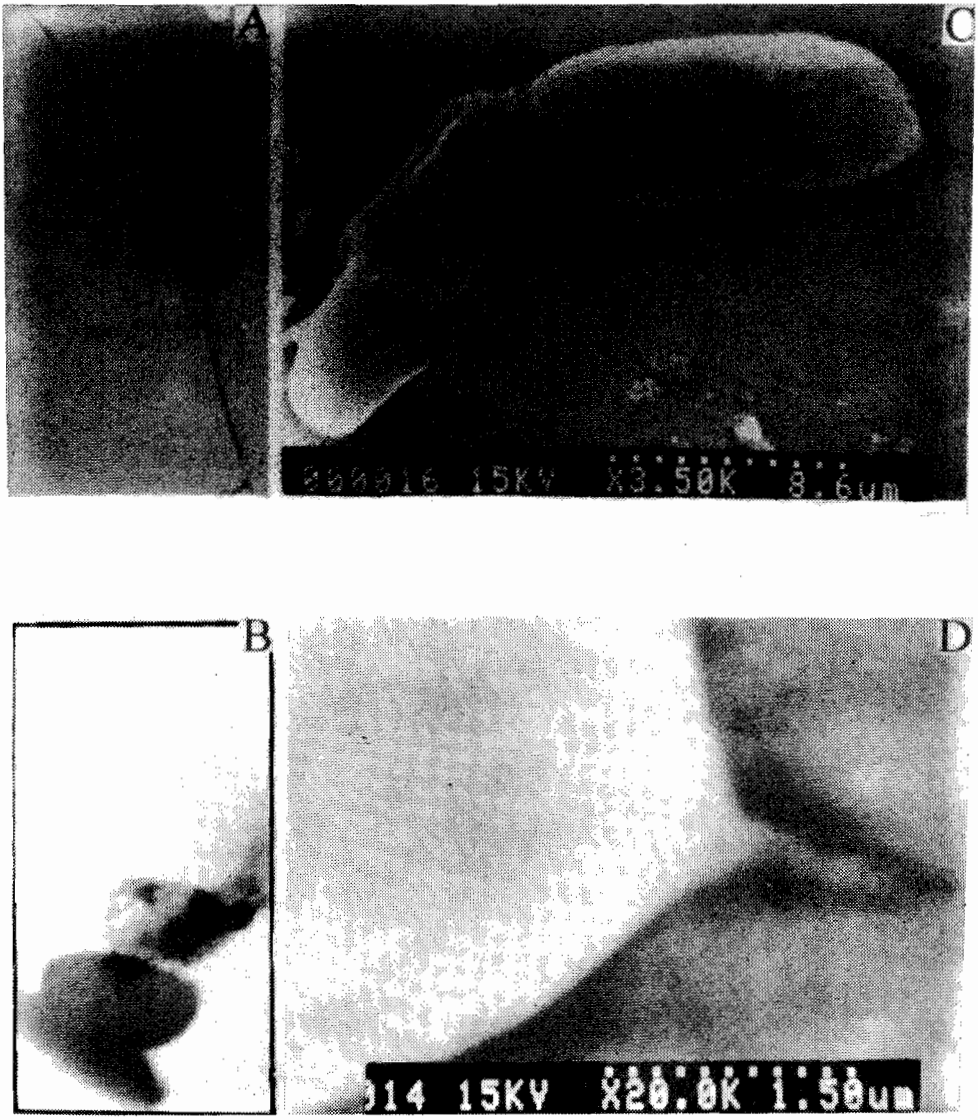

Fig.3. (III). *Seimatosporiopsis salvadorae*, IMI 148620a, type (A) Conidia stained with Giemsa HCl, 1800X; (B) cracked conidia, stained with Geimsa HCl, showing stained nuclei, 1800X; (C) conidiogenous cells in SEM; (D) conidial apical appendage in SEM.

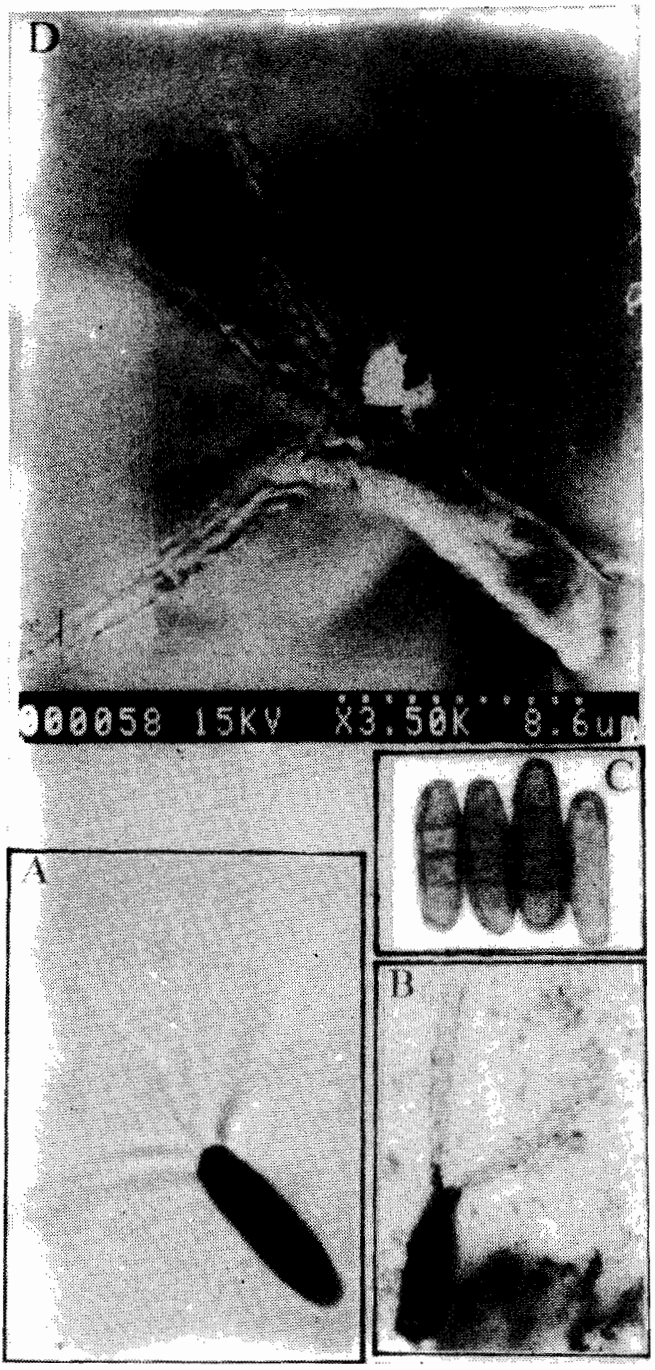

Fig.4. *Parahyalotiopsis borassi*, IMI 180210, type (A) Conidia, 1800X; (B) conidia stained in Leifson's flagella stain, 1800X; (C) conidia stained in Geimsa HCl stain, 1800X; (D) Conidia in SEM;

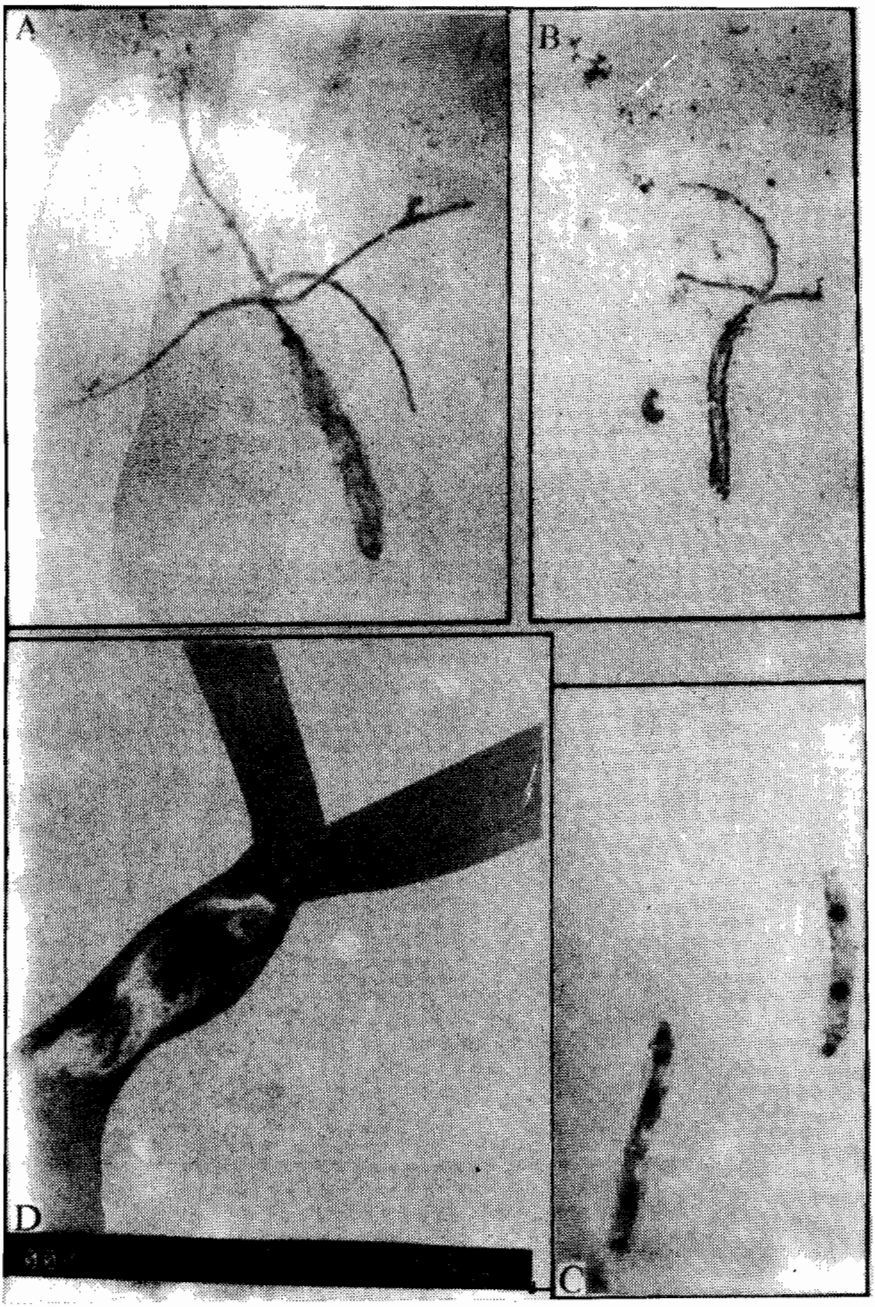

Fig.5. *Hyalotiella transvalensis*, IMI 2101125 (A) conidium stained in Lactophenol, 1800X; (B) conidium stained in Leifson's flagella stain, 1000X; (C) conidia stained in Geimsa HCl stain, 1800X; (D) conidium in SEM.

Fig.6. *Hyalotiopsis subramanianii*, IMI 136542 (A,B) conidia in SEM showing dichotomously branched appendages; conidia stained in Geimsa HCl stain showing multinucleate conidial cells, (C) conidium with conidiogenous cell; (D) conidia without conidiogenous cells.

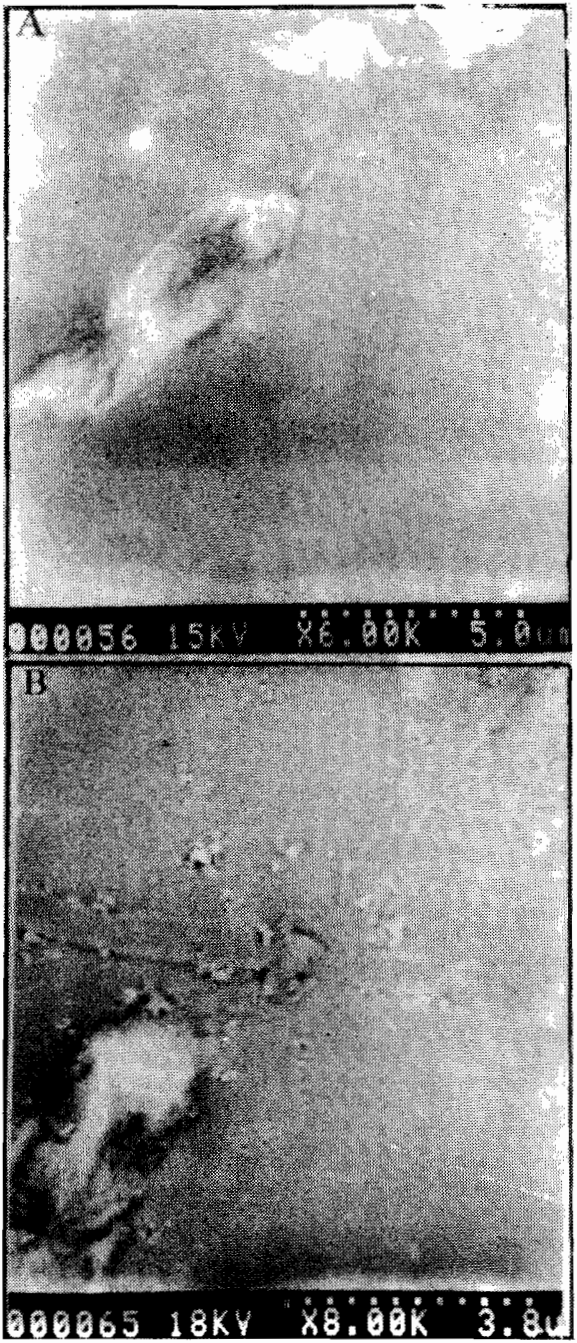

Fig.7. *Robillarda sessilis*, IMI 276578 (A.B) Conidia in SEM, showing appendages.

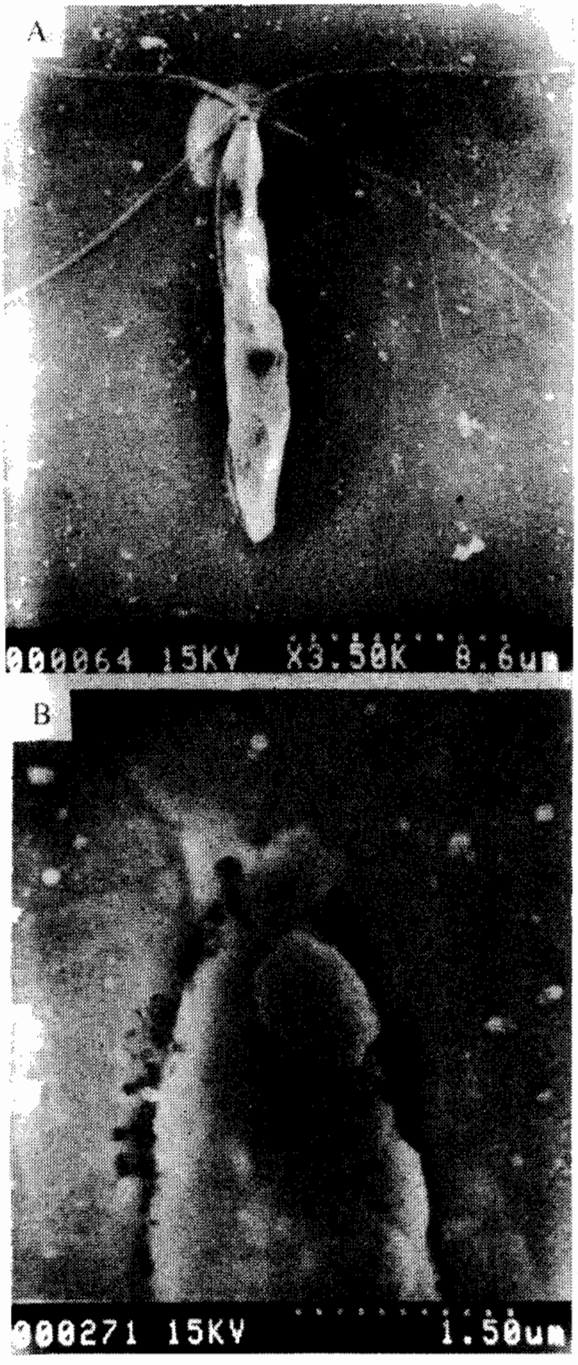

Fig.8. *Pseudorobillarda phragmitis*, IMI 60678 type (A,B) conidia in SEM showing appendages.

Pseudorobillarda Morelet
Fig. 8

In *Pseudorobillarda phragmitis* (Cunnell) Morelet (IMI 70678) appendages under scanning electron microscopy were found to be 3-4 tube like apical structures which arise from a larger tube like structure. Punithalingam & Woodhams (1986) described them like the arms of an umbrella attached to the body, surrounded by a mucilaginous sheath which becomes everted by dissolving these sheath from base to the apex.

References

Abbas, S.Q., B.C. Sutton and A. Ghaffar. 1998. Use of conidial appendages as taxonomic criteria. *Pak. J. Bot.*, 29: 199-205.

Hrushovetz, S.B. 1956. Cytological studies of *Helminthosporium sativum*. *Canad. J. Bot.*, 34: 321-327.

Korf, R.P. 1972. Synoptic key to the genera of Pezizales. *Mycologia*, 64: 937-955.

Morgan-Jones, G., T.R. Nag Raj and B. Kendrick. 1972a. Genera Coelomycetarum. *V. Alpakesa* and *Bartalinia*. *Can. J. Bot.*, 50: 877-882.

Morgan-Jones, G., T.R. Nag Raj and B. Kendrick. 1972b. Genera Coelomycetarum. V.: *Kellermania*. *Can. J. Bot.*, 50: 1641-1648.

Nag Raj, T.R. and B. Kendrick. 1985. *Ellurema* gen. nov., with notes on *Lepteutypa cisticola* and *Seiridium canariensa*. *Sydowia*, 38: 178-193.

Pirozynski, K.A. and R.A. Shoemaker. 1971. Some Coelomycetes with appendaged conidia. *Can. J. Bot.*, 49: 529-541.

Punithalingam, E. 1970. Studies on Sphaeropsidales in culture. *Mycol. Pap.* (CAB, IMI) Kew, 119: 1-24.

Punithalingam, E. 1972. Cytology of *Fusarium culmorum*. *Trans Br. mycol. Soc.*, 58: 225-230.

Punithalingam, E. 1974. Cytology of *Septoria leucanthemi*. *Trans. Br. mycol. Soc.*, 63: 255-260.

Punithalingam, E. 1975. Cytology of some *Fusarium* spp. *Nova Hedwigia*, 26: 275-302.

Punithalingam, E. 1981. Conidiation and appendage formation in *Tiarosporella paludosa* (Sacc. & Fiori) Höhnel. *Nova Hedwigia*, 34: 539-566.

Punithalingam, E. 1982. Conidiation and appendage formation in *Macrophomina phaseolina* (Tassi) Goid. *Nova Hedwigia*, 36: 249-290.

Punithalingam, E. 1983. The nuclei of *Macrophomina phaseolina* (Tassi) Goid. *Nova Hedwigia*, 38: 339-367.

Punithalingam, E. 1984a. The nuclei and conidial appendages of *Robillarda sessilis* (Sacc.) Sacc. *Nova Hedwigia*, 40: 45-58.

Punithalingam, E. 1984b. The nuclei and 'conidial appendages' of *Chaetoconis polygoni* (Ell. & Ever.) Clem. *Nova Hedwigia*, 40: 99-112.

Punithalingam, E. 1989a. Techniques for staining fungal nuclei and appendages. *Bot.J. Linn. Soc.*, 99: 19-32.

Punithalingam, E. 1989b. The significance of nucleate conidial appendages in Coelomycetes. *Studies in Mycology*, 31: 113-121.

Punithalingam, E. 1989c. The nuclei and conidial arms in *Crucellisporium species*. *Nova Hedwigia*, 48: 290-307+8 plates.

Punithalingam, E. and J.E. Woodhams. 1982. The conidial appendage in *Phyllosticta* spp. *Nova Hedwigia*, 36: 151-198.

Punithalingam, E. and J.E. Woodhams. 1984. A modified Leifson's flagella staining technique for revealing appendages in fungi. *Nova Hedwigia*, 40:31-43.

Punithalingam, E. and J.E. Woodhams. 1986. The conidial appendages and nuclei in *Pseudorobillarda* spp. *Nova Hedwigia*, 43: 485-498+8 plates.

Sutton, B.C. 1968. *Kellermania* and its generic segregates. *Can. J. Bot.*, 46: 181-196.

Sutton, B.C. 1973. Ch.11. *Coelomycetes*, PP. 513-582. In: The *Fungi* IVA (Eds) G.C. Ainsworth, F.K. Sparrow & A.S. Sussman. Academic Press, New York and London, pp. 621.

Sutton, B.C. 1980. *The Coelomycetes* (CAB, IMI) Kew, Surrey, U.K. pp. 696.

Sutton, B.C., A. Ghaffar and S.Q. Abbas. 1972. *Seimatosporiopsis* gen. nov. (Sphaeropsidales). *Trans. Br. mycol. Soc.*, 59: 295-300.

Uecker, F.A. and M.M. Kulik. 1986. *Pseudorobillarda sojae*, a new pycnidial Coelomycete from Soybean stem. *Mycologia*, 78: 449-453.

Vittal, B.P.R and M. Dorai. 1991. Three new Deuteromycetes from *Eucalyptus* leaf litter. *Mycol. Res.*, 95: 501-503.

Zhang, B.C. and D.W. Minter. 1989. Morphology, cytology and taxonomy of *Choiromyces gangliiformis* (Ascomycotina, Pezizales). *Mycol. Res.*, 92: 91-94.

(Received for publication 27 July, 1997)