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RECLASSIFICATION OF *PHIALOCEPHALA* BASED ON CONIDIAL DEVELOPMENT

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Leptographium and *Phialocephala* are separated based on conidial development. In *Leptographium*, conidia develop by apical wall building and sympodial or percurrent proliferation of the conidiogenous cells. *Phialocephala* includes species with ring wall building as well as species with apical wall building development. Some species of *Phialocephala* with ring wall building development are distinguished by their phialides with deep set cylindrical collarettes and conidia produced in chains with points of attachment at both ends resembling those in *Chalara* and allied genera. These are placed in *Sporendocladia*. The remaining *Phialocephala* spp. all have apical wall building with proliferation usually resulting in well-defined, and often ornate collarettes. In most cases these are distinct from the poorly-defined collarettes often found in *Leptographium* spp. These *Phialocephala* spp. apparently comprise a heterogeneous group probably affiliated with a number of teleomorph genera. In contrast *Leptographium* spp. are all anamorphs of *Ophiostoma*.

Fungi in the *Leptographium* complex are characterized by robust, erect, dark, mononematous penicillately branched conidiophores with conidia produced in gloeoid masses (Hughes, 1953; Kendrick, 1961, 1962). They are commonly found on wood and are often associated with insects (Goheen & Cobb, 1978; Upadhyay, 1981; Wingfield, 1983; Witcosky & Hansen, 1985). Many are known to cause blue staining of timber (Lagerberg, Lundberg & Melin, 1927; Davidson, 1978) and some species are associated with or cause conifer root disease (Wagener & Mielke, 1961; Smith, 1967). The genus *Leptographium* Lagerberg & Melin in Lagerberg, Lundberg & Melin (1927) was described as associated with stained timber. Grosmann (1932) considered *Hantzschia* Auerswald to be the same as *Leptographium* although she used *Leptographium* due to doubts concerning the validity of the description of *Hantzschia*. Goidanich (1933) used *Scopularia* Preuss in descriptions of members of the *Leptographium* complex. Shaw & Hubert (1952) assigned all members of the complex to the single genus *Leptographium* after indicating difficulties in the use of either *Hantzschia* or *Scopularia*.

In introducing a classification of the Hyphomycetes using conidial development, Hughes (1953) established *Verticicladiella* Hughes for members of the *Leptographium* complex with 'sympodial conidial development'. The genus *Phialocephala* Kendrick (1961) was established to accommodate those species with 'phialidic conidial development'. The three genera, *Leptographium*, *Phialocephala* and *Verticicladiella* therefore comprised the *Leptographium* complex. They are commonly encountered as anamorphs of *Ceratocystiopsis* Upadhyay & Kendrick and *Ophiostoma* H. & P. Sydow (De Hoog & Scheffer, 1984; Griffin, 1968; Hunt, 1956; Olchowecki & Reid, 1974; Upadhyay, 1981).

Recent studies on conidial development in the *Leptographium* complex have shown that conidiogenous cells in species of *Leptographium* and *Verticicladiella* can proliferate both percurrently and sympodially in a single species or even on a single conidiophore (Wingfield & Marasas, 1983; Wingfield, 1985). These observations led to the reduction of *Verticicladiella* to synonymy with *Leptographium* (Wingfield, 1985).

Two *Phialocephala* spp., *P. bactrospora* Kendrick and *P. fusca* Kendrick examined by Wingfield (1985) were found to have distinctly different conidial developments and shapes. On this basis, it was suggested that the genus was not homogeneous.

Phialocephala bactrospora resembled *Chalara* (Corda) Rabenh. in having cylindrical and truncate-ended conidia that are produced within long, cylindrical collarettes apparently typical of ring wall building conidial development (Minter, Kirk & Sutton, 1983). Conidia of *P. fusca* however appeared to develop by apical wall building (Wingfield, 1985). These observations prompted a more detailed examination of conidial development and associated processes in *Leptographium* and *Phialocephala*.

MATERIALS AND METHODS

A light microscopic examination was made of as many *Phialocephala* spp. as possible. The following species were examined: *P. bactrospora* DAOM 28736 (Kendrick, 1961); *P. fusca* DAOM 75852 (Kendrick, 1963); *P. dimorphospora* Kendrick DAOM 71465 (Kendrick, 1961); *P. humicola* Jong & Davis ATCC 22801 (Jong & Davis, 1972); *P. foliicola* P. M. Kirk IMI 285199 (Kirk, 1985); *P. canadensis* Kendrick DAOM 71971 (Kendrick, 1963); *P. fluminis* Shearer, Crane & Miller ILLS 36160 (Shearer, Crane & Miller, 1976); *P. fortinii* Wang & Wilcox Fap-7 (Wang & Wilcox, 1985); *P. fumosa* (Ell. & Ev.) Sutton IMI 176928*b* (Sutton, 1975); *P. ivoriensis* Zucconi & Onofri HBR 126A (Onofri & Zucconi, 1984); *P. mexicana* Onofri & Zucconi HBR 125A (Onofri & Zucconi, 1984); *P. phycomyces* (Auersw.) Kendrick DAP 34098 (Kendrick, 1964); *P. repens* (Cooke & Ell.) Kendrick FH 2900 (Kendrick, 1963); *P. truncata* Sutton IMI 184565 (Sutton, 1975).

A detailed examination of a selected set of *Phialocephala* and *Leptographium* spp. for which cultures were available was made using fluorescence microscopy (FM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The *Phialocephala* spp. included *P. bactrospora* ATCC 44606, and *P. dimorphospora* DAOM 16556. The following *Leptographium* spp. were also examined: *L. procerum* (Kendrick) Wingfield DAOM 33940, *L. pyrinum* Davidson ATCC 34943 (Davidson, 1978), *L. truncatum* (Wingfield & Marasas) Wingfield PREM 45698 = ATCC 58100 (Wingfield & Marasas, 1983; Wingfield, 1985), *L. terebrantis* Barras & Perry CBS 298.85 (Barras & Perry, 1971).

Fungi were grown on 2% malt-extract agar (Difco malt-extract, Difco Bacto agar in 1000 ml water) in Petri dishes and incubated at 20 °C in the dark for two weeks or until onset of sporulation. Specimens for SEM and TEM were cut from agar and fixed in 2·5% glutaraldehyde and osmium tetroxide in 0·1 M phosphate buffer, dehydrated in a graded acetone series. Material for SEM was critical point dried (Cohen, 1970), coated with gold palladium and examined using a Philips or ISI scanning electron microscope.

For TEM, material was embedded according to Spurr (1969) and the epoxy polymerized at 70° for 8 h. Ultrathin sections (60 nm) were cut with glass knives, mounted on copper grids and stained for 20 min in a saturated uranyl acetate solution and 10 min in lead citrate (Reynolds, 1963). Sections were examined with a Philips EM300 transmission electron microscope.

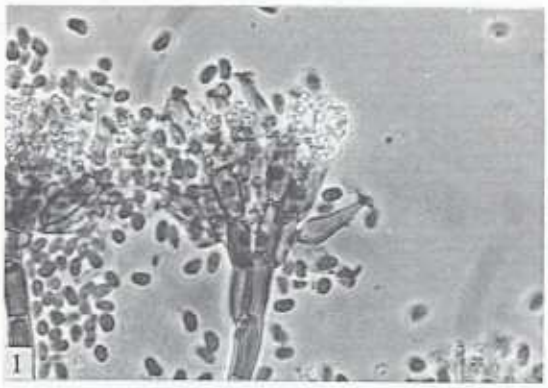
Conidiophores to be examined using fluorescence microscopy were mounted on glass slides in a 0·05% w/v solution of calcaflour white M 2R optical brightener in 0·1 M phosphate buffer. Samples were examined with a Nikon Optiphot Episcopic fluorescence microscope, dark background and ultraviolet light and photographed using Ilford FP4 film.

RESULTS

Most *Phialocephala* spp. had distinct collarettes at the apex of conidiogenous cells (Figs 1–8). These fungi could however be divided into two distinct groups based on collarette morphology and conidium shape. *Phialocephala bactrospora*, *P. truncata*, *P. fumosa*, *P. foliicola* and *P. ivoriensis* had distinctly cylindrical collarettes and elongated catenate conidia with parallel walls. In the second group, the remaining *Phialocephala* spp., had collarettes that varied from being somewhat inconspicuous to cupulate or loosely flaring or irregularly split. In these species, conidia were variably shaped from ovoid to pyriform and in all cases had a single distinct point of attachment.

Collarettes in *P. bactrospora* were cylindrical and gave rise to cylindrical conidia (Figs 9–12). Conidia were produced in chains and had points of attachment at both the base and the apex (Figs 10, 12). Fluorescence micrographs indicated wall building activity at the base of the collarette (Fig. 9) similar to that found in *Chalara* and allied genera (Hawes & Beckett, 1977*a*, *b*). This wall building process would be best be described as ring wall building in the terms of Minter, Kirk & Sutton (1983).

Figs 1–8. Light micrographs of conidiogenous cells of *Phialocephala* species. Fig. 1, *P. canadensis* × 1760. Fig. 2, *P. fluminis* × 1750. Fig. 3, *P. fusca* × 1750. Fig. 4, *P. dimorphospora* × 1750. Fig. 5, *P. illini* × 1750. Fig. 6, *P. mexicana* × 1750. Fig. 7, *P. fumosa* × 1750. Fig. 8, *P. bactrospora* × 1750.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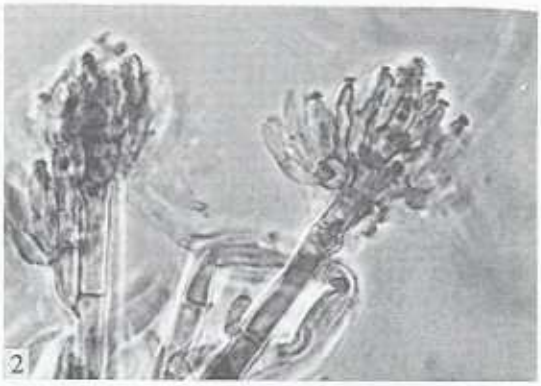
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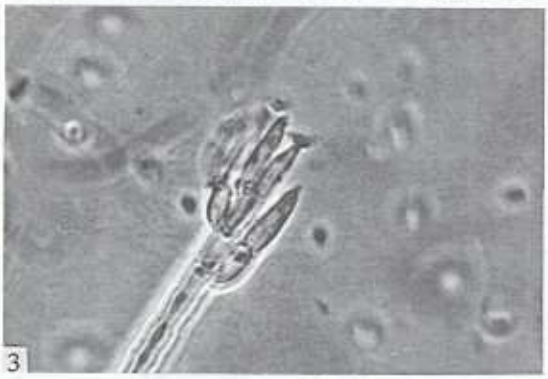
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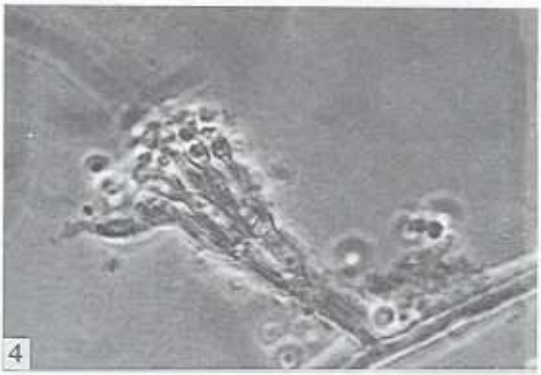
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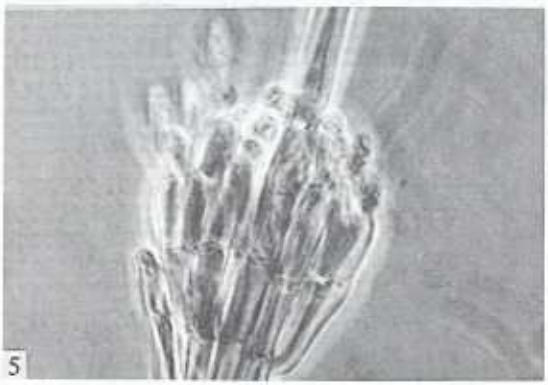
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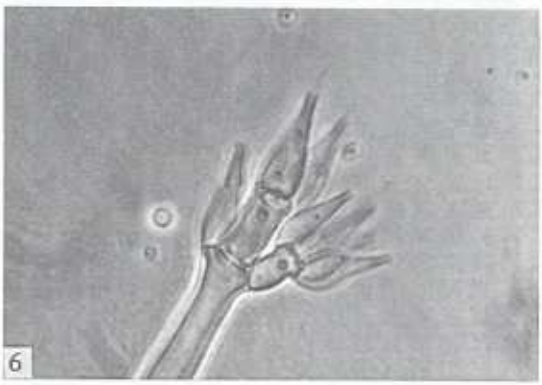
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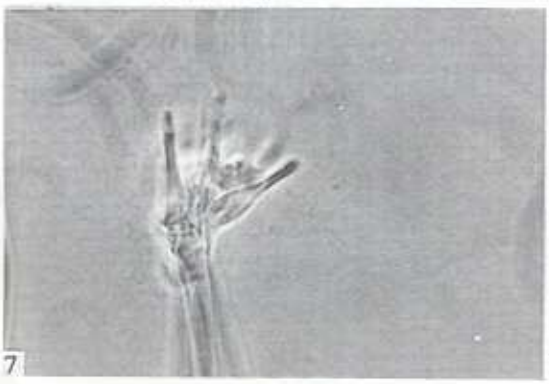
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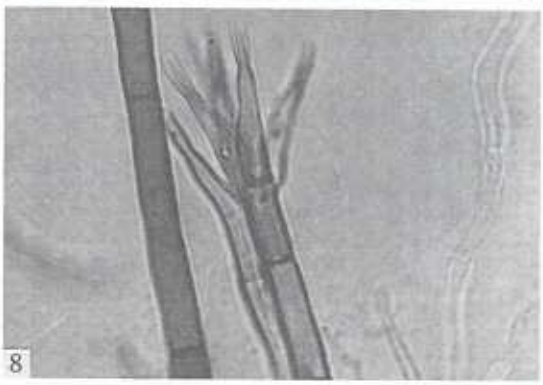
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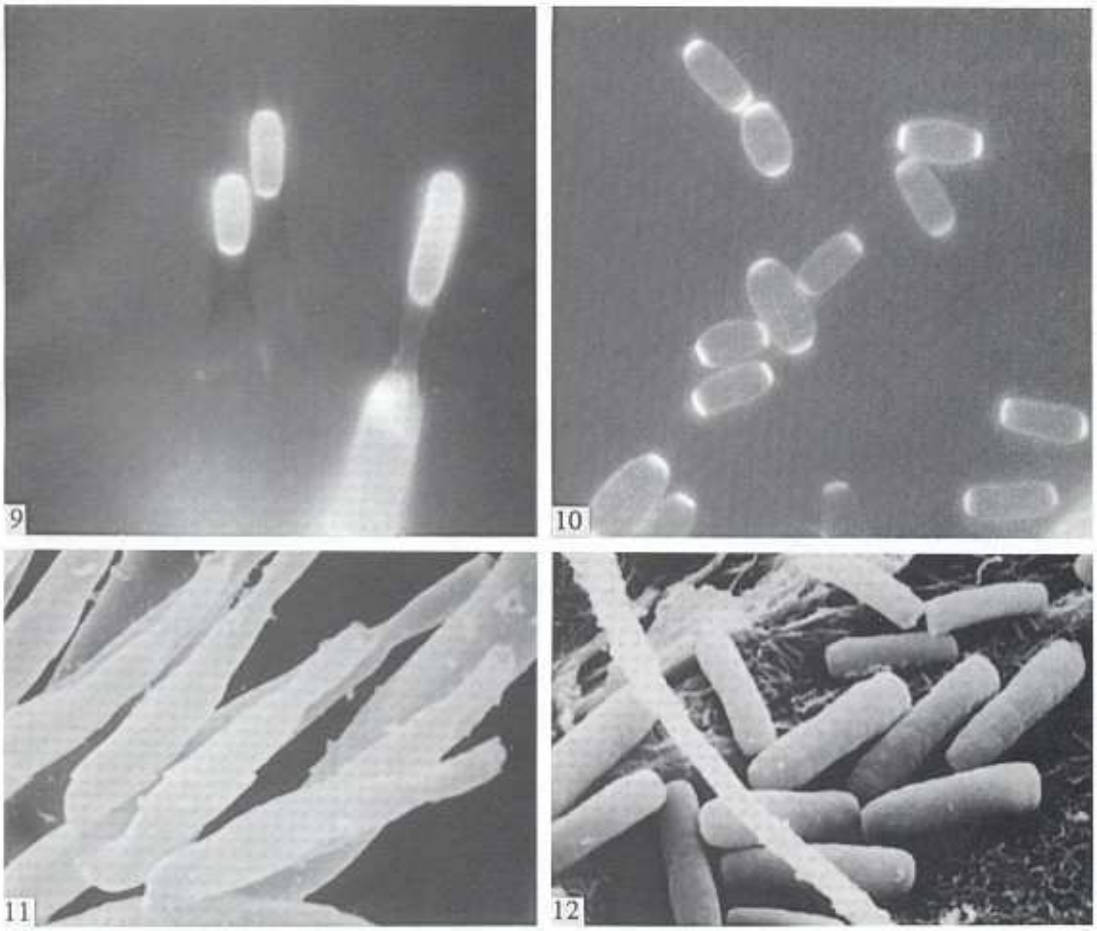
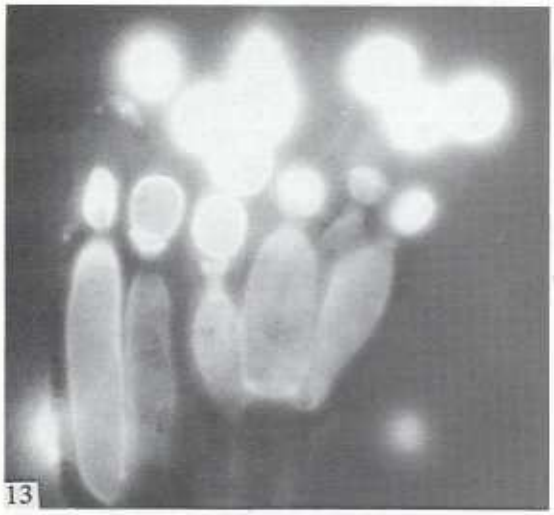


Figs 9–12. Conidia and conidiogenous cells of *P. bactrospora*. Fig. 9, Fluorescence micrograph of conidiogenous cells showing tubular collarettes and wall building ring, × 1750. Fig. 10, Fluorescence micrograph of elongate conidia showing point of attachment at both ends, × 1750. Fig. 11, SEM of conidiogenous cells with tubular collarettes, × 3250. Fig. 12, SEM of elongate conidia with parallel walls that are produced in chains, × 3250.

In *P. dimorphospora*, conidiogenous cells had well-developed collarettes that were approximately cylindrical (Figs 13–18). Conidia, however, had single distinct points of attachment (Figs 16–18) and were not produced in chains. Treatment with calcaflour showed distinct fluorescence at the apex of the conidiogenous cell (Figs 13, 14) indicative of a replacement wall building process of conidial development (Minter, Kirk & Sutton, 1982). Thin sections through conidia showed the single, well-developed point of attachment resulting from conidial secession (Figs 17, 18).

Figs 13–18. Conidia and conidiogenous cells of *P. dimorphospora*. Figs 13, 14, Fluorescence micrograph of conidiogenous cells showing long tubular collarettes with ovoid conidia that appear to be produced in chains × 1750. Fig. 15, SEM of conidiogenous cells with tubular collarettes and conidia, × 6000. Fig. 16, SEM of conidia with distinct truncate points of attachment at one end, × 9000. Fig. 17, TEM of conidiogenous cells with tubular collarette, × 23000. Fig. 18, TEM of conidium with truncate point of attachment at one end, × 18000.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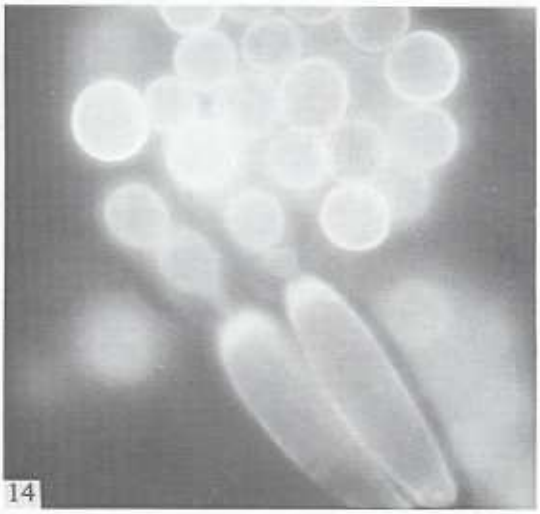
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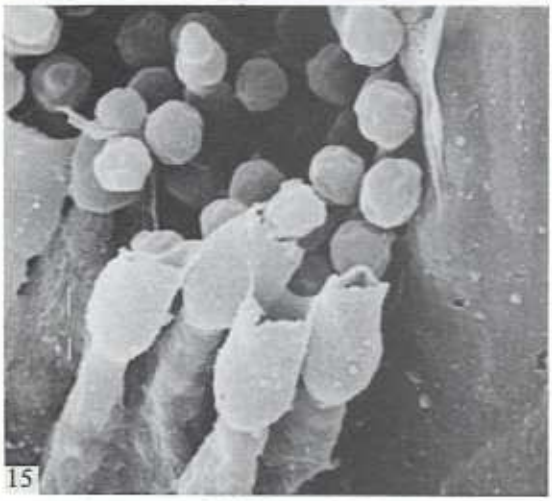
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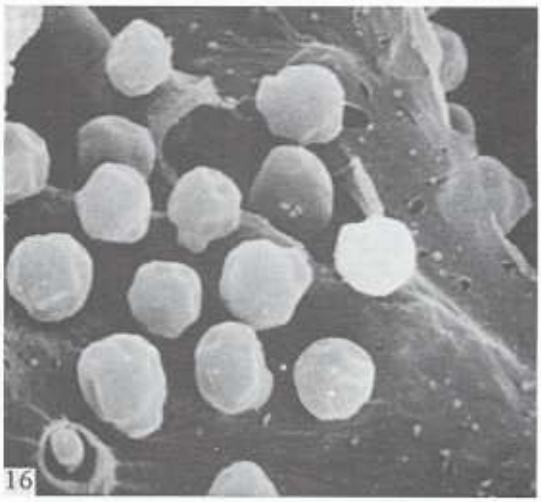
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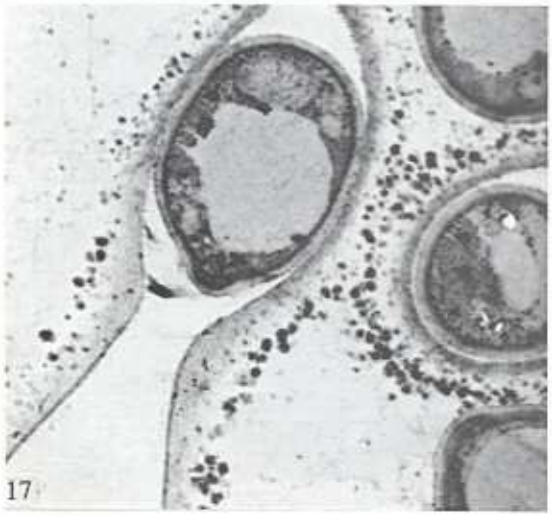
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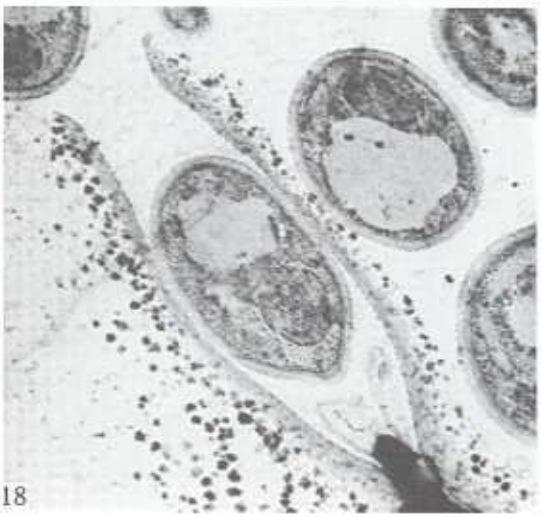
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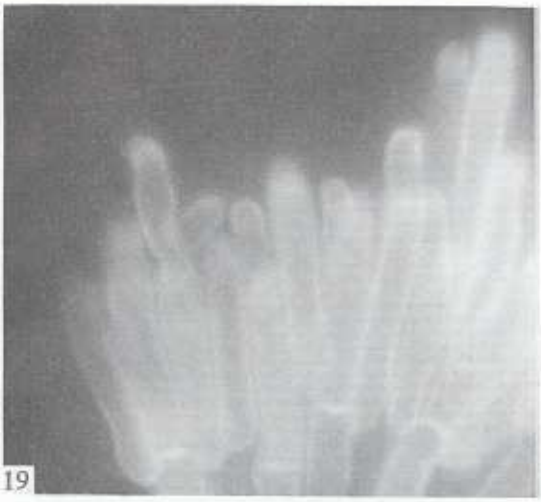
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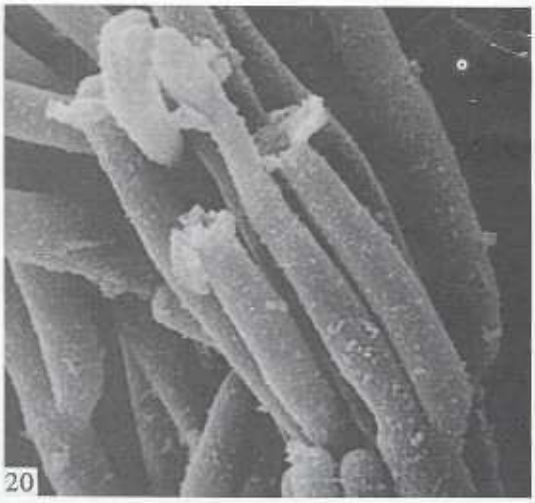
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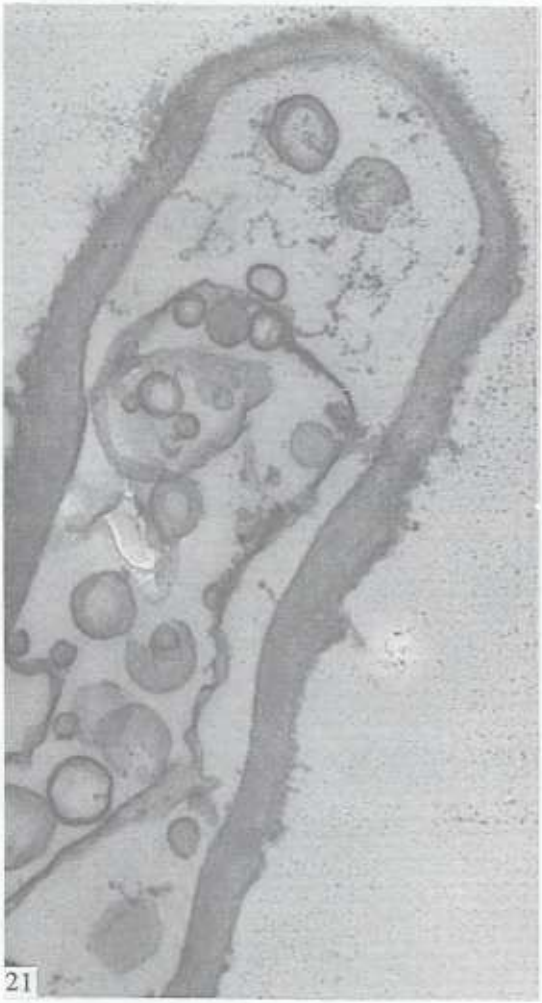
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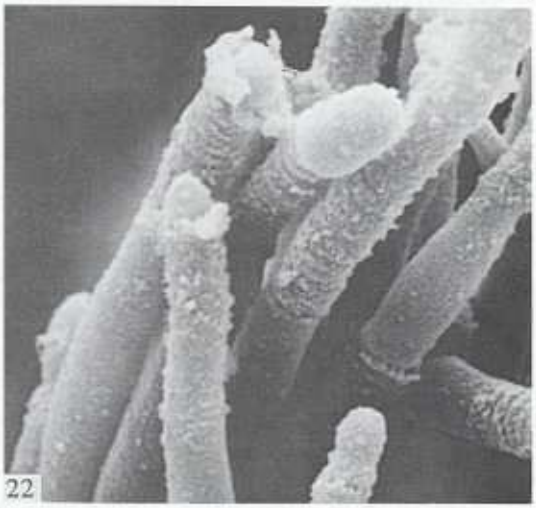
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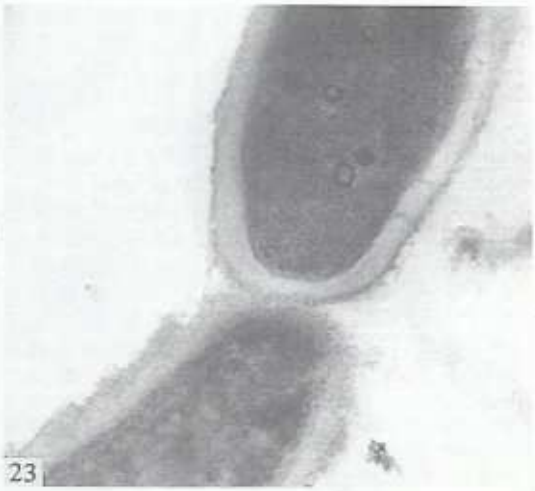
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Conidiogenous cells in *Leptographium terebrantis* (Fig. 19) fluoresced at the apex in a manner suggesting replacement wall building and percurrent proliferation. Micrographs (SEM) of conidiogenous cells (Figs 25–27) showed percurrent proliferation (Figs 20, 22). In addition, collarettes were observed (Figs 20, 22). Remnants of the outer walls resulting from percurrent proliferation of the conidiogenous cells were obvious in thin sections (Figs 21, 23).

In *L. truncatum*, both sympodial and percurrent proliferation of conidogenous cells was observed (Figs 24, 25). Collarettes, reminiscent of phialides were also observed (Fig. 25). Thin sections in this species showed similar outer wall remnants to those observed in *L. terebrantis* resulting from secession of conidia and sympodial proliferation (Fig. 26).

Large conidia produced by *L. pyrinum* resulted in distinct scars on conidiogenous cells that indicated either sympodial or percurrent proliferation (Figs 27, 28). In thin sections of these conidiogenous cells, proliferation was clearly sympodial (Fig. 29).

Conidiogenous cells in *L. procerum* appeared to proliferate less between the production of successive conidia than in *L. pyrinum*, *L. terebrantis* and *L. truncatum* although proliferation was apparently sympodial as well as percurrent (Figs 30, 31). Remnants of the outer wall layer of the conidiogenous cells could however be seen at higher magnifications in thin sections (Figs 32, 33).

DISCUSSION

Using SEM and light microscopy Wingfield (1985) showed both percurrent and sympodial proliferation of conidiogenous cells in *Verticicladiella* and *Leptographium*, and on this basis, combined these genera. In this study, TEM and fluorescence microscopy reaffirmed previous observations and added additional examples, particularly of *Phialocephala* spp.

The genus *Phialocephala* is heterogeneous and two distinct groups of species can easily be separated based on conidial development. One group has conidia that are clearly formed by ring wall building (Minter *et al.*, 1983). In the second group, conidia apparently develop by replacement wall building (Minter *et al.*, 1982). The *Phialocephala* spp. with ring wall building conidial development are easily distinguished by the cylindrical conidia that often have obtuse ends. Conidia are always produced in chains from a wall building ring in the conidiogenous cell (Minter *et al.*, 1983) and therefore, have attachment points at both ends. These *Phialocephala* spp. most closely resemble *Chalara* and allied genera (Nag Raj & Kendrick, 1975).

Observations in this study suggest that the ring wall building species of *Phialocephala* form a homogeneous group that would be well distinguished in their own genus. The genus *Sporendocladia* would appropriately accommodate these species. *Sporendocladia* originated in the description of *S. castaneae* from *Castanea* cupules in France (Arnaud, 1954). Sutton (1975) showed that this fungus was identical to *Spicaria fumosa* Ell. & Ev. collected on *Castanea* cupules in the United States (Ellis & Everhart, 1883) and transferred the name of this species to *Phialocephala* as *P. fumosa*. Sutton (1975) noted that the description of *S. castaneae* was invalid and that *Spicaria* could not be used due to its *nomen confusum* status (Brown & Smith, 1957).

We propose the following synonymies.

Sporendocladia fumosa (Ell. & Ev.) M. Wingfield, comb. nov.

Spicaria fumosa Ellis & Ev., *Bull. Torrey bot. Cl.* **10**: 97 (1883).

Phialocephala fumosa (Ell. & Ev.) Sutton, *Trans. Br. Mycol. Soc.* **64**: 411 (1975).

Sporendocladia castaneae Arnaud, *Bull. Soc. mycol. Fr.* **69**: 279 (1954), nom. inval., Art. 36.

Sporendocladia castaneae Arnaud ex Nag Raj & Kendrick, *A monograph of Chalara and Allied Genera*: 162 (1975).

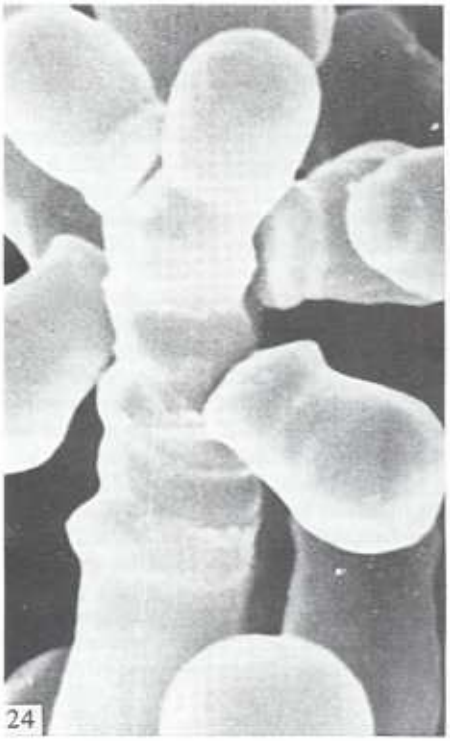
Sporendocladia bactrospora (Kendrick) M. Wingfield, comb. nov.

Phialocephala bactrospora Kendrick, *Can. J. Bot.* **39**: 1083 (1961).

Sporendocladia foliicola (P. M. Kirk) M. Wingfield, comb. nov.

Phialocephala foliicola P. M. Kirk, *Mycotaxon* **23**: 337 (1985).

Figs 19–23. Conidiogenous cells of *Leptographium terebrantis*. Fig. 19, Fluorescence micrograph showing apical wall building, and apparently also collarettes and annellations, × 1750. Fig. 20, SEM of conidiogenous cells showing collarettes, × 5600. Fig. 21, TEM of conidiogenous cells with remnants of outer wall layer after secession of conidia, × 40000. Fig. 22, SEM of conidiogenous cells showing percurrent proliferation and the presence of collarettes × 5600. Fig. 23, TEM of conidiogenous cell and conidium showing remnants of outer wall layer after secession, × 40000.



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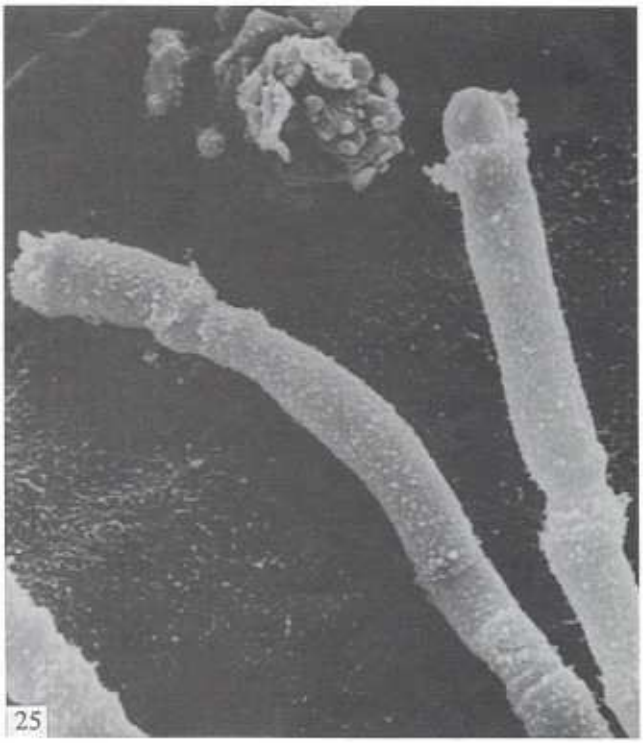

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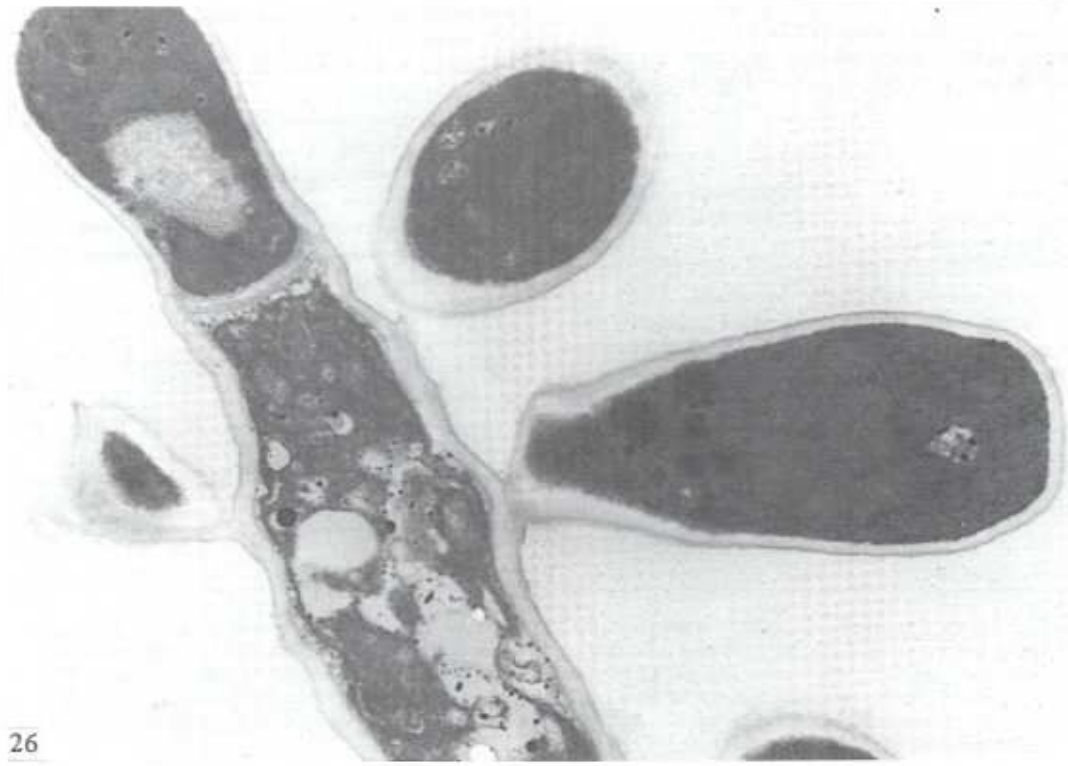

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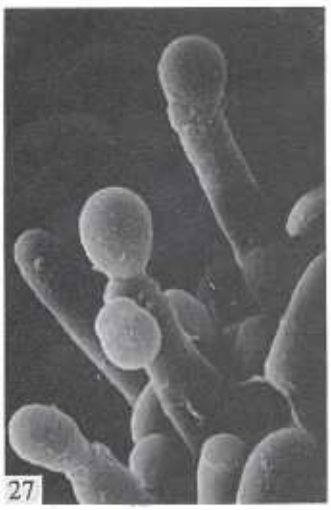
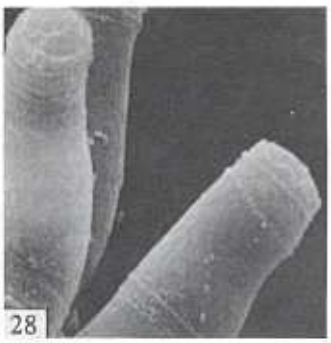
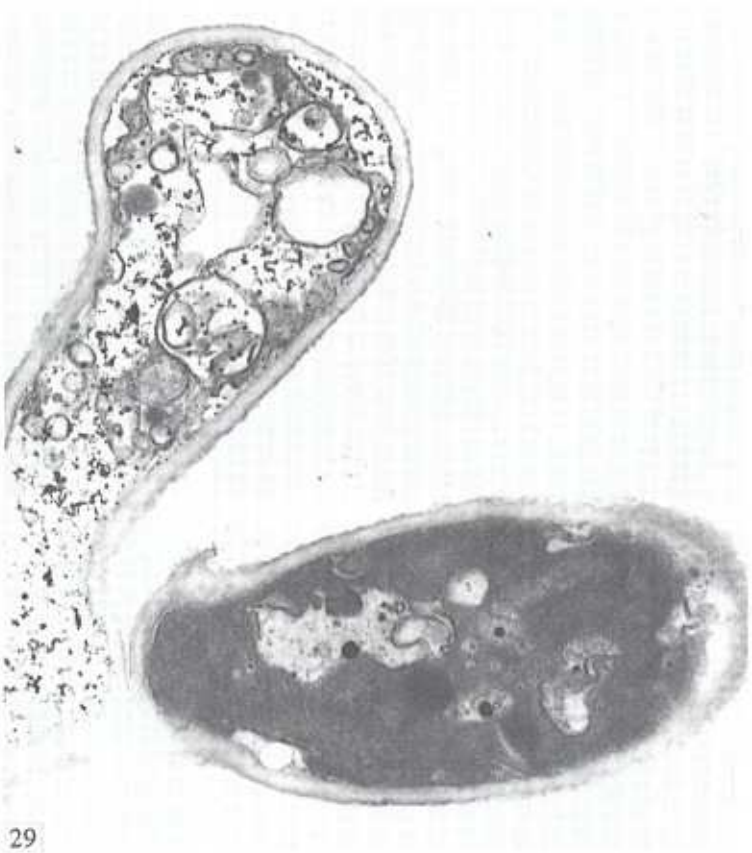






Figs 27–29. Conidiogenous cells and conidia of *Leptographium pyrinum*. Fig. 27, SEM of conidiogenous cells showing sympodial proliferation, ×2500. Fig. 28, SEM of condiogenous cells with annellations indicative of percurrent proliferation, ×4500. Fig. 29, TEM of conidiogenous cell and conidium with sympodial proliferation, ×24000.

Sporendocladia ivoriensis (Zucconi & Onofri) M. Wingfield, comb. nov.

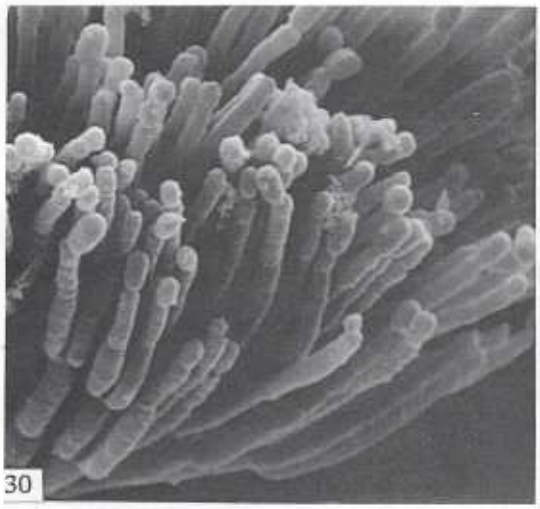
Phialocephala ivoriensis Zucconi & Onofri, *Mycotaxon* **20**: 189 (1984).

Sporendocladia truncata (Sutton) M. Wingfield, comb. nov.

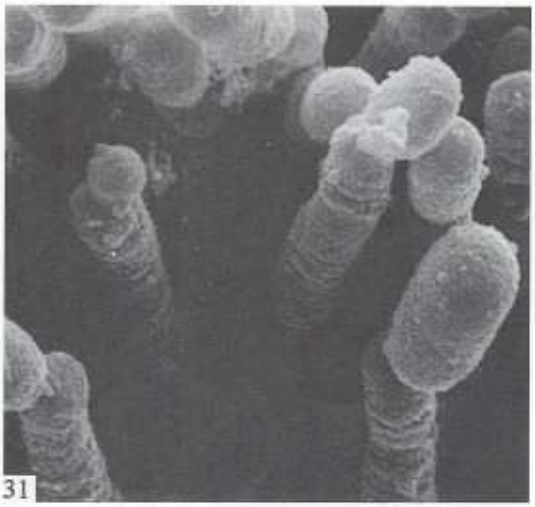
Phialocephala truncata Sutton, *Trans. Br. Mycol. Soc.* **64**: 414 (1975).

The remaining *Phialocephala* spp. with replacement wall building are most clearly distinguished by conidia with a single attachment point. In general conidia of these species are not produced in chains. This characteristic might, however, be misleading as certain species such as *P. dimorphospora* can appear to produce chains of conidia. This species has often been illustrated with conidia in distinct chains (Kendrick, 1961; Nag Raj & Kendrick, 1975; Carmichael *et al.*, 1980). The apparent chains of conidia are unlike those in ring wall building species. These conidia apparently remain in loose chains due to the existence of long

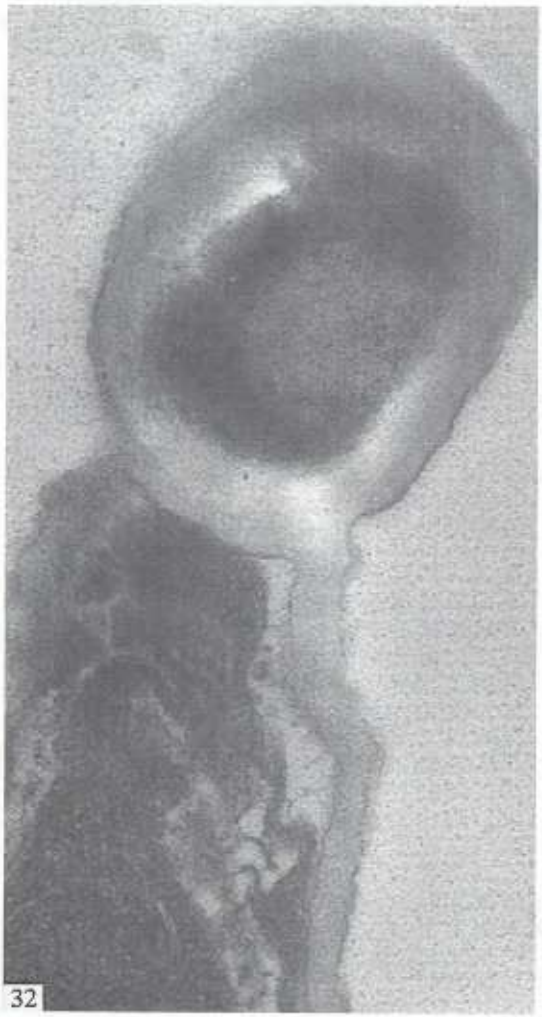
Figs 24–26. Conidiogenous cells and conidia of *Leptographium truncatum*. Fig. 24, SEM of conidiogenous cells showing sympodial proliferation and conidia with truncate bases, ×12000. Fig. 25, SEM of conidiogenous cells with annellations and collarettes, ×5600. Fig. 26, TEM of conidiogenous cell and conidia with sympodial proliferation, remants of outer wall layers and conidia with truncate bases, ×20000.



30



31



32

33

cylindrical collarettes in this species. They are however, clearly, produced by replacement wall building and have a single point of attachment. The observations here are supported by those of Carroll & Carroll (1974) in their detailed study of conidium development in *P. dimorphospora*.

Species of *Phialocephala* are easily separated from *Sporendocladia*. Their separation from *Leptographium* is, however, less easily resolved. Although *Phialocephala* species have apical wall building conidium development in common, they are apparently distinct from *Leptographium* in which conidiogenous cells lengthen in the process of conidiogenesis. In *Phialocephala* spp., conidiogenous cells do not lengthen beyond the apex of the collarettes and collarettes are usually well-developed and often ornate.

Proliferation of conidiogenous cells in all *Leptographium* spp. examined here and previously (Wingfield, 1985; Wingfield & Marasas, 1983) appears to be either sympodial, percurrent or both sympodial and percurrent. Proliferation sometimes results in the formation of collarettes but these collarettes are usually seen in addition to obvious elongation of the conidiogenous cells. These collarettes apparently arise when an unusually large conidium is produced. In thin sections of *Leptographium* conidiogenous cells presented here, the remains of the outer cell walls after conidial secession can easily be distinguished and the origin of the collarettes in *Leptographium* spp. can be deduced. These collarettes are quite distinct from those in *Phialocephala* spp. Teleomorphs of *Sporendocladia* spp. are likely to be species of *Ceratocystis* sensu stricto. Separation of the ring wall building anamorphs from those with apical wall building is consistent with the division of *Ceratocystis* into *Ceratocystis* sensu stricto and *Ophiostoma* (De Hoog, 1974; De Hoog & Scheffer, 1984; Weijman & De Hoog, 1975). *Ceratocystis* sensu stricto has anamorphs in which conidia develop by ring wall building and are placed in the genus *Chalara*, species of which are similar to *Sporendocladia*. Teleomorphs of *Phialocephala* are likely to be found in numerous and possibly unrelated genera. *Ophiostoma* anamorphs are most appropriately accommodated in *Leptographium*.

This study has attempted to consolidate *Phialocephala* species, and improve our understanding of their generic circumscription. The species remaining in *Phialocephala* unfortunately still represent a heterogeneous group that remains inadequately defined. Further studies including genetic and chemical tests as well as attempts to establish connexions between anamorphs and teleomorphs should be attempted and encouraged.

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Figs 30–33. Conidiogenous cells and conidia of *Leptographium procerum*. Fig. 30, SEM of conidiogenous cells showing sympodial and percurrent proliferation, × 2500. Fig. 31, SEM of conidiogenous cells proliferating sympodially and percurrently, × 12500. Fig. 32, TEM of conidiogenous cell and conidium with sympodial proliferation, × 80000. Fig. 33, TEM of conidiogenous cell showing remnants of outer cell wall after secession, × 80000.

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