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Analysis of conidium ontogeny in anamorphs of *Ophiostoma*: *Pesotum* and *Phialographium* are synonyms of *Graphium*

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Representatives of the anamorph-genera *Pesotum* and *Phialographium*, supposedly characterized by sympodial and phialidic conidiogenesis, respectively, were shown to exhibit more than one kind of conidium formation. Specifically, conidiogenous cells of both genera proliferated percurrently, a process supposedly diagnostic of the related genus *Graphium*. Accordingly, the three genera are merged in *Graphium*, the oldest name.

Over the past several decades, taxonomists have paid considerable attention to the diverse conidial anamorphs of the ophiostomatales. This is at least partly because the characters of these asexual forms have been used as the basis for delimiting sections and even genera among the holomorphs (Münch, 1907; Melin & Nannfeldt, 1934; Goidanich, 1936; Hunt, 1956; De Hoog & Scheffer, 1984). Once the diversity of conidium development had been demonstrated by Hughes (1953), mononematous Ophiostomatalean anamorphs of the *Leptographium* complex were subjected to particularly intensive scrutiny. Their processes of conidium development, though difficult to resolve with the light microscope, were perceived as exclusively blastic–annellidic (percurrent), blastic–sympodial or blastic–phialidic. The anamorphs were accordingly segregated into three anamorph-genera: annellidic analogues in *Leptographium* Lagerberg & Melin (1927), sympodial analogues in *Verticicladiella* Hughes (1953), and phialidic analogues in *Phialocephala* Kendrick (1961) (Kendrick 1961, 1962).

This interpretation of the complex was accepted for twenty years, but was questioned by Kendrick (1980), and has now been superseded by studies based upon scanning and transmission electron microscopy (Tsuneda & Hiratsuka, 1984; Wingfield, 1985; Wingfield, van Wyk & Wingfield, 1987; Van Wyk, Wingfield & Marasas, 1988; Wingfield, Van Wyk & Van Wyk, 1989). These showed that although the conidiogenous cells of species placed in *Verticicladiella* sometimes proliferated sympodially, such conidiogenous cells could at other times proliferate percurrently.

Van Wyk *et al.* (1988) also demonstrated that a false appearance of sympodial development can be produced by the incomplete dehiscence of conidia from percurrently proliferating conidiogenous cells; the outer wall uniting the conidium with the parent cell tears around most, but not all, of the cell circumference, leaving the conidium attached to the side of the cell by a narrow strip of wall material (Fig. 1). When this process happens repeatedly, the laterally attached conidia may deceive an observer using the light microscope into concluding that the cell has undergone repeated sympodial development, rather than the percurrent process that has actually taken place. The same kinds of phenomena have been observed in other, unrelated anamorph-genera, and must now be regarded as among the occupational hazards of studying conidium development (Zhang, Kendrick & Brubacher, 1983; Dykstra, Salkin & McGinnis, 1989).

It became apparent that the genus *Verticicladiella* could not be maintained, and Wingfield (1985) reduced it to synonymy with *Leptographium*, also transferring the 13 species originally described in *Verticicladiella* to *Leptographium*. Subsequently, Wingfield *et al.* (1987) analysed the species of *Phialocephala* and found the genus to be heterogeneous. Some species produce deep cylindrical collarettes, and were disposed in *Sporendocladia* Arnaud: Nag Raj & Kendrick. Other species were retained in *Phialocephala* because their conidiogenous cells produced shallow but distinct collarettes and did not elongate during repeated conidiation.

A parallel situation to that described above exists among the synnematal anamorphs of the Ophiostomatales, which may be regarded as more complex than, but related to, the *Leptographium* anamorphs. Many of these fungi produce darkly pigmented synnemata with various degrees of apical branching, ultimately forming a dense aggregation of narrow,

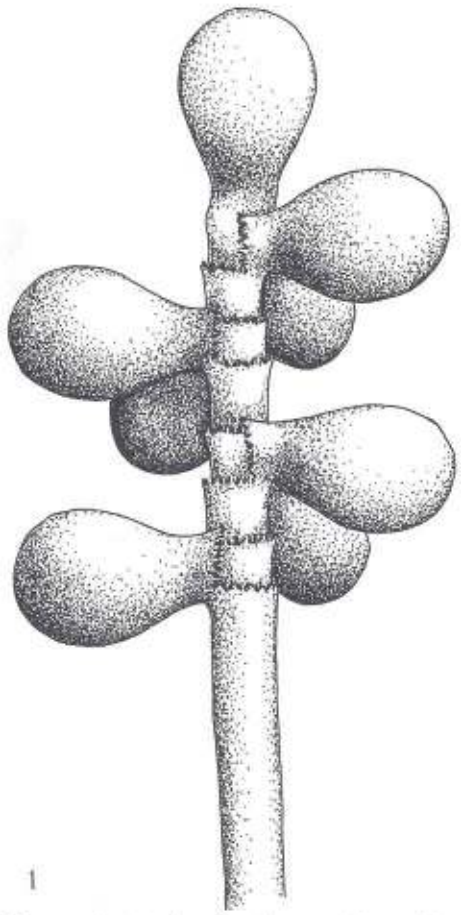


Fig. 1. Diagram showing the effect of repeated incomplete dehiscence of conidia in a percurrently proliferating conidiogenous cell. Under the light microscope, this situation could easily be misinterpreted as arising from sympodial proliferation.

parallel conidiogenous cells which give rise to numerous slimy conidia. Such anamorphs were usually either left unnamed or described in the genus *Graphium* Corda (1837). However, as the analysis of conidiogenesis proceeded, this assemblage was also subdivided into three groupings of generic rank: *Graphium* for those with annellidic conidiogenesis, *Pesotum* Crane & Schoknecht (1973) for the sympodial analogues, and *Phialographium* Upadhyay & Kendrick (1974) for the phialidic analogues. Once the status of *Verticicladiella* and *Phialocephala* had been challenged, it become apparent that conidium development in *Graphium*, *Pesotum* and *Phialographium* would also have to be re-examined. This paper presents the results of such observations.

HISTORICAL BACKGROUND

Corda (1837) established *Graphium* for two species, *G. penicillioides* Corda and *G. tenuissimum* Corda (neither of which appears to be connected with the Ophiostomatales). Hughes (1958) chose *G. penicillioides* as the lectotype species of *Graphium*. No authentic cultures of this species exist. Crane & Schoknecht (1973) took scanning electron micrographs of some of the type material, which convincingly demonstrated the presence of annellations on the conidiogenous cells, but also stated that sympodial proliferation occurred occasionally. The anamorph of *Ophiostoma ulmi* (Buisman) Nannfeldt (*Graphium ulmi* Schwarz 1922), was chosen as the type species of their new genus, *Pesotum*, because they considered its conidiogenesis to be exclusively sympodial.

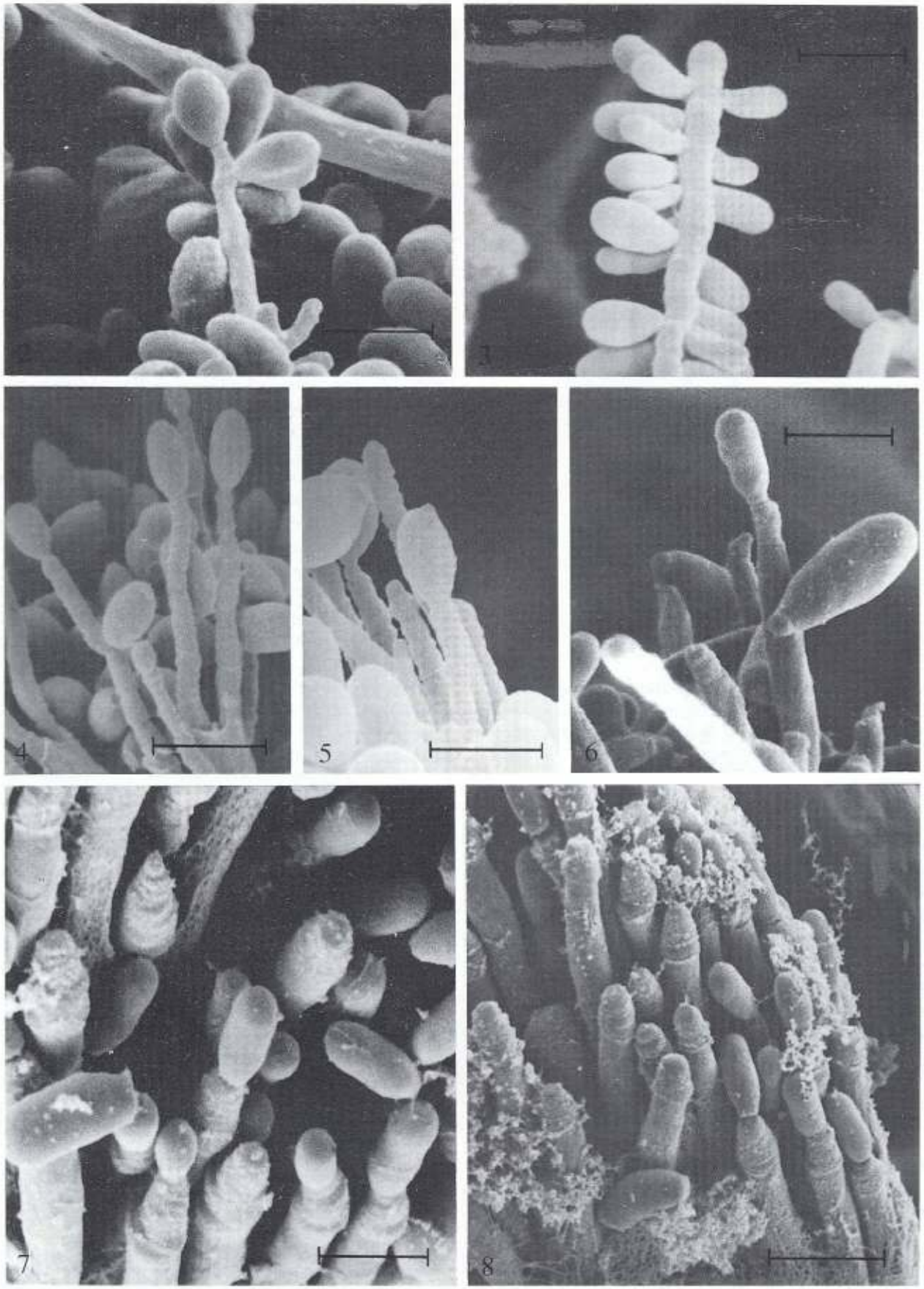
However, their conclusion was not as straightforward as it might seem. They gave two groups of illustrations. The first were of the synnematous anamorph, and their figures 2–6 do suggest that sympodial conidiogenesis was in progress, although the general outline of the conidiogenous cell remained uncharacteristically smooth after repeated conidiogenesis. Their second group of illustrations were of an associated mononematous anamorph. Here, conidiogenesis was indubitably sympodial, but the conidia were borne on conspicuous pegs, which remained after the secession, and gave the fertile region of the conidiogenous cell a distinctly denticulate appearance. Perhaps because there had been some confusion concerning the appropriate generic disposition of this mononematous anamorph, Crane & Schoknecht decided to pool the evidence of sympodial conidiogenesis, and they described both mononematous and synnematous anamorphs as parts of *Pesotum ulmi* (Schwarz) Crane & Schoknecht.

We consider that this lumping of synnematous and mononematous anamorphs is unjustified, especially because we have demonstrated that the kinds of conidiogenesis they exhibit are, in fact, completely distinct. The mononematous anamorph should clearly be placed in the anamorph-genus *Sporothrix* Hektoen & Perkins. The synnematous anamorph is discussed below.

Crane & Schoknecht also aggregated the unnamed synnematous and mononematous anamorphs of *Ophiostoma piceae* (Münch) H. & P. Sydow, finding it difficult to demonstrate sympodial proliferation in the synnematous anamorph, but easy in the mononematous synanamorph, and ultimately describing the two together as the second species of *Pesotum*. Again, the denticulate conidiogenous cell of the mononematous anamorph places it in *Sporothrix*.

Upadhyay (1981) disposed the synnematous anamorphs of *Ceratocystis torticiliata* Olchowecki & Reid, and *Ophiostoma brunneo-ciliatum* Mathiesen-Kaarik, in *Pesotum*. Hutchison & Reid (1988*a*) also placed the synnematous anamorph of *Ceratocystis novae-zelandiae* Hutchison & Reid in *Pesotum*. Upadhyay & Kendrick (1975) erected *Hyalopesotum* Upadhyay & Kendrick, distinguished from *Pesotum* only by its reduced pigmentation, for the synnematous anamorph of *Ceratocystis introcitrina* Olchowecki & Reid. Upadhyay (1981) placed the synnematous anamorphs of *Ceratocystis arborea* Olchowecki & Reid, and *Ophiostoma araucariae* (Butin) De Hoog & Scheffer in this segregate genus. Hutchison & Reid (1988*b*) described *Hyalopesotum pini* Hutchison & Reid, for which no teleomorph is known. Thus five taxa have been referred to *Pesotum* and four to *Hyalopesotum*.

Wright & Cain (1961) described *Ceratocystis sagmatospora* Wright & Cain [= *Ophiostoma sagmatospora* (Wright & Cain) Solheim] with a synnematous anamorph which they referred to *Graphium*. Their drawing of the conidiogenous cells showed distinct, slightly flaring collarettes. Upadhyay & Kendrick (1974) confirmed this observation, assumed that conidiogenesis was exclusively phialidic, and made this



Figs 2, 3. *Pesotum ulmi*. Scanning electronmicrographs of conidiogenous cells. **Fig. 2.** Sympodial proliferation. Scale bar = 2 μm. **Fig. 3.** Repeated percurrent proliferations, accompanied by incomplete dehiscence of the conidia. Scale bar = 2 μm.

anamorph the type species of the anamorph-genus *Phialographium* Upadhyay & Kendrick. Collarettes were illustrated, most of those of the conidiogenous cells of the synnematous anamorph of *Ophiostoma davidsonii* (Olchowecki & Reid) Solheim being slightly flared (Upadhyay, 1981). *Ophiostoma olivaceum* Mathiesen, *O. davidsonii* and *Ceratocystis columnaris* Olchowecki & Reid were placed in *Phialographium* though binomials were not provided. Solheim (1986) placed the synnemtous anamorph of *Ophiostoma cucullatum* Solheim in *Phialographium*.

Upadhyay & Kendrick (1975) suggested that conidiogenesis in the synnematous anamorph of *Ophiostoma sparsum* (Davidson) De Hoog & Scheffer was phialidic, and established the segregate anamorph-genus *Graphilbum* Upadhyay & Kendrick as the hyaline analogue of *Phialographium*. Upadhyay (1981) also referred the synnematous anamorph of *Ophiostoma ips* (Rumbold) Nannfeldt to *Graphilbum*, noting that conidiogenesis could be either phialidic or annellidic. Thus five anamorph-taxa have been referred to *Phialographium* and two to the closely related *Graphilbum*. It is beyond the scope of the present paper to reassess *Hyalopesotum* and *Graphilbum*, but these genera should be re-examined.

In addition, although we are not addressing the question of the circumscription of *Ophiostoma* and *Ceratocystis*, it is our opinion that all species of *Ceratocystis* mentioned in this paper should be transferred to *Ophiostoma*, because we concur with De Hoog & Scheffer (1984) that all members of *Ceratocystis sensu stricto* have *Chalara* anamorphs.

MATERIAL AND METHODS

Cultures examined were (1) *Pesotum ulmi* Crane & Schoknecht, the type species of *Pesotum* obtained from the forest pathology culture collection of the Department of Plant Pathology, University of Minnesota; (2) *Pesotum piceae* Crane & Schoknecht from the culture collection of Dr R. W. Davidson; and the unnamed anamorph of (3) *Ophiostoma davidsonii* (Olchowecki & Reid) Solheim (IMI 176524) which is thought to be phialidic fide Upadhyay (1981). In addition, we refer to illustrations (Wingfield *et al.*, 1989) of the unnamed anamorph of *Ophiostoma cucullatum* Solheim (reported by Solheim [1986] to be phialidic). Unfortunately, no culture or other material of *Phialographium sagmatosporae* Upadhyay & Kendrick, the type species of *Phialographium*, could be obtained for electron microscopic examination.

The available cultures were examined by SEM and in *O. davidsonii*, by TEM, according to Wingfield *et al.* (1987).

RESULTS AND DISCUSSION

Conidiogenous cells of the anamorph of *Ophiostoma cucullatum* Solheim produced collarettes, indicative of phialidic conidiogenesis; annellations, diagnostic of percurrent conidiogenesis and sympodial proliferations (Wingfield *et al.*, 1989). The annellations, and apparent sympodial proliferation of the conidiogenous cell, or the misleading appearance of this as a result of incomplete conidium dehiscence, suggested that the synnematous anamorphs of *Ophiostoma* almost certainly exhibit more than one kind of conidiogenesis, and that the rationale for maintaining them in three separate anamorph-genera was inadequate.

While conidiogenesis in *Pesotum ulmi* (anamorph of *Ophiostoma ulmi*) may sometimes either be, or appear, sympodial (Fig. 2), many of the cells show evidence of repeated percurrent conidiogenesis with incomplete dehiscence (Figs 1, 3). *Pesotum piceae* (anamorph of *Ophiostoma piceae*) similarly shows regular percurrent proliferations (Figs 4, 5), which strongly suggests that the original appearance of sympodial conidiogenesis (Fig. 6) is also probably the result of incomplete secession of annellidic conidia. Conidiogenous cells of the synnematous anamorph of *Ophiostoma davidsonii* are annelated (Figs 7–10), which suggests that its placement in *Phialographium* is incorrect.

The segregation of the synnematous anamorphs of *Ophiostoma* spp. among three (or more) anamorph-genera is inappropriate. A list of the taxa placed in these genera is provided, but new combinations are made only where this is necessitated by the existence of binomials in *Pesotum* or *Phialographium*.

Graphium Corda, *Icones Fungorum* **1**: 18 (1837).

= *Pesotum* Crane & Schoknecht, *Amer. J. Bot.* **60**: 347 (1973).

= *Phialographium* Upadhyay & Kendrick, *Mycologia* **66**: 183 (1975).

Graphium piceae (Crane & Schoknecht) Wingfield & Kendrick comb. nov.

= *Pesotum piceae* Crane & Schoknecht, *Amer. J. Bot.* **60**: 348 (1973).

[anamorph of *Ophiostoma piceae* (Münch) H. & P. Sydow]

An earlier binomial, *Graphium pirinum* Goidanich, exists for the synnematous anamorph of *Ophiostoma catenianum* Goidanich (1935), but although this species was synonymized with *O. piceae* (Upadhyay, 1981), we have been unable to examine the type material. Should it be the same as *O. piceae*, the earlier name for the anamorph would take precedence. A binomial is provided for *Graphium* because we believe that this is appropriate and necessary.

Since a binomial in *Graphium* already exists for the anamorph of *Ophiostoma ulmi*, only the appropriate nomenclature is given.

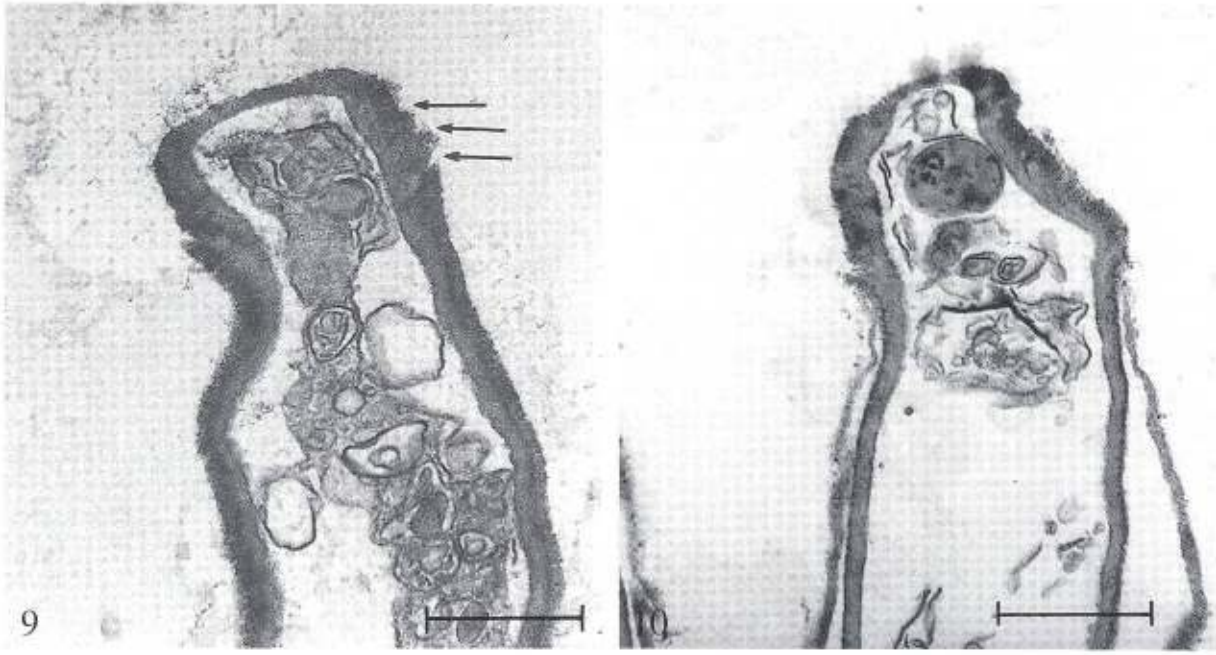
Graphium ulmi Schwarz, *Meded. Phytopathol. Lab. 'Willie Commelin Scholten'* **5**: 13 (1922).

= *Pesotum ulmi* (Schwarz) Crane & Schoknecht, *Amer. J. Bot.* **60**: 348 (1973).

[anamorph of *Ophiostoma ulmi* (Buisman) Nannfeldt]

Figs 4–6. *Pesotum piceae*. Scanning electronmicrographs of conidiogenous cells. **Figs 4, 5.** Percurrently proliferating cells. Scale bar = 2 μm. **Fig. 6.** Cells which appear to be proliferating sympodially. Scale bar = 3 μm.

Figs 7, 8. Synnematous anamorph of *Ophiostoma davidsonii*. Scanning electronmicrographs of conidiogenous cells, both showing multiple annellations. Scale bars = 2 μm (Fig. 7) and 3 μm (Fig. 8).



Figs 9, 10. Transmission electronmicrographs of sagittal sections through conidiogenous cells of *Ophiostoma davidsonii*. **Fig. 9.** Annellations are clearly visible (arrow). **Fig. 10.** Sympodial or angled percurrent proliferation. Scale bars = 7 μm.

Graphium sagmatosporae (Upadhyay & Kendrick) Wingfield & Kendrick comb. nov.

= *Phialographium sagmatosporae* Upadhyay & Kendrick, *Mycologia* **66**: 183 (1975).

[anamorph of *Ophiostoma sagmatospora* (Wright & Cain) Solheim]

A number of *Ophiostoma* spp. or *Ceratocystis* spp. that require transfer to *Ophiostoma* possess anamorphs which have been assigned to *Pesotum* or *Phialographium*, though no binomials were provided for them. Binomials are not provided for these anamorphs, since this would be appropriate only after detailed studies of the types. Similarly, anamorphs assigned to *Hyalopesotum* and *Graphilbum* require further study and probably disposition in *Graphium*. These purported species of *Pesotum*, *Phialographium*, *Hyalopesotum* and *Graphilbum* are listed below to assist future investigators.

***Holomorphs with anamorphs formerly assigned to* Pesotum:**

Ophiostoma brunneo-ciliatum Mathiesen-Kaarik, *Meddel. Statens Skogsforskningsinst.* Sweden **43**: 44 (1953).

Ceratocystis columnaris Olchowecki & Reid, *Can. J. Botany* **52**: 1689 (1974).

Ceratocystis torticiliata Olchowecki & Reid, *Can. J. Botany* **52**: 1701 (1974).

Ceratocystis novae-zelandiae Hutchison & Reid, *N.Z. J. Botany* **26**: 70 (1988).

***Holomorphs with anamorphs that were formerly assigned to* Phialographium:**

Ophiostoma cucullatum Solheim, *Nord. J. Bot.* **6**: 202 (1986).

Ophiostoma davidsonii Olchowecki & Reid, *Can. J. Bot.* **52**: 1698 (1974).

Ophiostoma olivaceum Mathiesen, *Sv. Bot. Tidskr.* **45**: 212 (1951).

***Anamorphs assigned to* Hyalopesotum *or* Graphilbum.**

Hyalopesotum introcitrina Upadhyay & Kendrick, anamorph of *Ceratocystis introcitrina* Olchowecki & Reid.

Hyalopesotum anamorph of *Ceratocystis arborea* Olchowecki & Reid

Hyalopesotum anamorph of *Ophiostoma araucariae* (Butin) de Hoog & Scheffer

Hyalopesotum pini Hutchison & Reid

Graphilbum sparsum Upadhyay & Kendrick

anamorph of *Ophiostoma sparsum* (Davidson) de Hoog & Scheffer

Graphilbum anamorph of *Ophiostoma ips* (Rumbold) Nannfeldt, *Sv. Skogsv. Tidskr.* **32**: 408 (1934).

Considerable confusion has evidently arisen from the assignment of generic status to many anamorphic fungi based solely on their modes of conidium ontogeny. Anamorphs of *Ophiostoma* provide some of the best examples of this problem. This study has added a sequel to previous investigations aimed at reconsidering generic subdivisions based on condium ontogeny for anamorphs of *Ophiostoma*. Detailed studies of particular species based on type material should, however, follow and, where it is considered appropriate, binomials provided for these anamorphs. In addition, studies on conidium development in anamorphs of *Ophiostoma* such as *Graphilbum*, *Hyalopesotum*, *Hyalorhinocladiella* and *Pachnodium* should be undertaken.

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REFERENCES

Corda, A. K. J. (1837). *Icones Fungorum* **1**, 1–32. Prague.

Crane, J. L. & Schoknecht, J. D. (1973). Conidiogenesis in *Ceratocystis ulmi*, *Ceratocystis piceae*, and *Graphium penicillioides*. *American Journal of Botany* **60**, 346–354.

De Hoog, G. S. & Scheffer, R. J. (1984). *Ceratocystis* versus *Ophiostoma*: a reappraisal. *Mycologia* **76**, 292–299.

Dykstra, M. J., Salkin, I. F. & McGinnis, M. R. (1989). An ultrastructural comparison of conidiogenesis in *Scedosporium apiospermum*, *Scedosporium inflatum* and *Scopulariopsis brumptii*. *Mycologia* **81**, 896–904.

Goidanich, G. (1936). Il generi di Ascomiceti *Grosmannia* G. Goid. *Bolletino della Stazione di Patologia Vegetale* **16**, 26–60.

Hughes, S. J. (1953). Conidiophores, conidia and classification. *Canadian Journal of Botany* **31**, 577–659.

Hughes, S. J. (1958). Revisiones hyphomycetum aliquot. *Canadian Journal of Botany* **31**, 577–659.

Hunt, J. (1956). Taxonomy of the genus *Ceratocystis*. *Lloydia* **19**, 1–58.

Hutchison, L. J. & Reid, J. (1988*a*). Taxonomy of some potential wood-staining fungi from New Zealand. 1. Ophiostomataceae. *New Zealand Journal of Botany* **26**, 63–81.

Hutchison, L. J. & Reid, J. (1988*b*). Taxonomy of some potential wood-staining fungi from New Zealand. 2. Pyrenomycetes, Coelomycetes and Hyphomycetes. *New Zealand Journal of Botany* **26**, 83–98.

Kendrick, W. B. (1961). The *Leptographium* complex. *Phialocephala* gen. nov. *Canadian Journal of Botany* **39**, 1079–1085.

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Kendrick, W. B. (1962). The *Leptographium* complex. *Verticladiella* Hughes. *Canadian Journal of Botany* **40**, 771–797.

Kendrick, W. B. (1980). The generic concept in hyphomycetes – a reappraisal. *Mycotaxon* **11**, 339–364.

Melin, E. & Nannfeldt, J. A. (1934). Researches into the blueing of ground woodpulp. *Svenska Skogsvardsforeningens Tidskrift* **32**, 397–616.

Münch, E. (1907). Die Blaufaule des Nadelholzes. *Naturwisssenschaftliche Zeitschrift für Forst- und Landwirtschaft* **5**, 531–573.

Solheim, H. (1986). Species of Ophiostomataceae isolated from *Picea abies* infested by the bark beetle *Ips typographus*. *Nordic Journal of Botany* **6**, 199–207.

Tsuneda, A. & Hiratsuka, Y. (1984). Sympodial and anellidic conidiation in *Ceratocystis clavigera*. *Canadian Journal of Botany* **62**, 2618–2624.

Upadhyay, H. P. (1981). A monograph of *Ceratocystis* and *Ceratocystiopsis*. University of Georgia Press: Athens, GA.

Upadhyay, H. P. & Kendrick, W. B. (1974). A new *Graphium*-like genus (conidial state of *Ceratocystis*). *Mycologia* **66**, 181–183.

Upadhyay, H. P. & Kendrick, W. B. (1975). Prodromus for a revision of *Ceratocystis* (Microascales, Ascomycetes) and its conidial states. *Mycologia* **67**, 798–805.

Van Wyk, P. S., Wingfield, M. J. & Marasas, W. F. O. (1988). Differences in synchronization of stages of conidial development in *Leptographium* species. *Transactions of the British Mycological Society* **90**, 451–456.

Wingfield, M. J. (1985). Reclassification of *Verticicladiella* based on conidial development. *Transactions of the British Mycological Society* **85**, 81–93.

Wingfield, M. J., Van Wyk, P. S. & Van Wyk, P. W. J. (1989). Conidial development in the anamorph of *Ophiostoma cucullatum*. *Mycological Research* **93**, 91–95.

Wingfield, M. J., Van Wyk, P. S. & Wingfield, B. D. (1987). Reclassification of *Phialocephala* based on conidial development. *Transactions of the British Mycological Society* **89**, 509–520.

Wright , E. F. & Cain, R. F. (1961). New species of *Ceratocystis*. *Canadian Journal of Botany* **39**, 1215–1230.

Zhang, T.-Y., Kendrick, W. B. & Brubacher, D. (1983). Annellidic (percurrent) and sympodial proliferation in congeneric hyphomycetes, and a new species of *Sporoidesmiella*. *Mycotaxon* **18**, 243–257.