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# CONIDIUM DEVELOPMENT IN HYALODENDRON AND ALLESCHERIELLA ANAMORPHS OF OPHIOSTOMA AND CERATOCYSTIOPSIS

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Anamorphs of *Ophiostoma* have been separated by their patterns of conidium development. This has resulted in the establishment of an unduly large number of genera. The aim of this study was to consider the validity of including the genera *Hyalodendron* and *Allescheriella* amongst the anamorphs of *Ophiostoma* and *Ceratocystiopsis*. Scanning electron micrographs (SEM) of the so-called *Hyalodendron* anamorphs of *Ophiostoma piliferum* and *O. pluriannulatum* revealed the presence of denticulate conidiogenous cells, typical of *Sporothrix* spp. Secondary conidia found in *Sporothrix* and *Hyalodendron* spp. were also present. SEM and transmission electron micrographs of the purported *Allescheriella* anamorph of *Ceratocystiopsis retusi* revealed conidiogenous cells that proliferate sympodially to form peg-like denticles, characteristic of *Sporothrix* spp. In some cases, however, annellations were also found on these conidiogenous cells. Here they formed an intermediate state between annellidic conidium development in *Hyalorhinocladiella* and sympodial development in *Sporothrix*.

A SEM examination revealed the presence of hyphal swellings in the anamorph of *C. retusi*, previously incorrectly interpreted as conidia, explaining its disposition in *Allescheriella*. We conclude that the anamorphs of *O. piliferum, O. pluriannulatum* and *C. retusi* are *Sporothrix* spp. and that the genera *Hyalodendron* and *Allescheriella* should be removed from the list of Ophiostomatoid anamorphs.

## INTRODUCTION

*Ophiostoma* H.&P. Sydow and *Ceratocystiopsis* H.P. Upadhyay & W.B. Kendr. spp. can be distinguished from *Ceratocystis sensu stricto* based on conidium development. *Ceratocystis s.s.* has *Chalara* (Corda) Rabenh. anamorphs (Weijman & De Hoog, 1975; Upadhyay, 1981; De Hoog & Scheffer, 1984) where conidia develop through ring wall building (Minter, Kirk & Sutton, 1983; De Hoog & Scheffer, 1984). In contrast, *Ophiostoma* spp. have anamorphs characterized by apical wall building (Minter *et al.*, 1983). These include genera such as *Hyalodendron* Diddens, *Sporothrix* Hektoen & C.F. Perkins, *Hyalorhinocladiella* H.P. Upadhyay & W.B. Kendr., *Graphium* Corda and *Leptographium* Lagerb. & Melin (Upadhyay & Kendrick, 1975; Upadhyay, 1981; Wingfield, 1985; Harrington, 1987).

Many anamorphs of *Ophiostoma* have been separated on the basis of conidium development. This trend began with the classic paper by Hughes (1953) introducing this feature as an additional morphological criterion, also separating *Verticicladiella* S. Hughes from *Leptographium* with sympodial and annellidic conidium development, respectively. In recent years, the validity of many of these genera has been questioned and the number of genera has been reduced (Wingfield, 1985; Wingfield, Kendrick & Van Wyk, 1991; Mouton, Wingfield &

Van Wyk, 1993a). Indeed, it has been suggested that anamorphs of *Ophiostoma* and *Ceratocysistiopsis* could be restricted to as few as five genera (Mouton, Wingfield & Van Wyk, 1993b).

The genus *Hyalodendron* was established by Diddens (1934), and is characterized by conidiogenous cells that are indeterminate, producing one to a few holoblastic conidia on short peg-like projections at the tips, or in whorls along the sides of simple or branched conidiophores (Upadhyay, 1981). These primary conidia produce secondary conidia in short, acropetal, lateral or terminal chains (De Hoog, 1979; Upadhyay, 1981). *Hyalodendron* is considered the hyaline analogue of *Cladosporium* Link (Upadhyay, 1981).

The name *Hyalodendron* has been assigned to several anamorphs of *Ophiostoma* spp. (Upadhyay, 1981; De Hoog, 1993). For example, Upadhyay (1981) described the anamorphs of *Ophiostoma piliferum* (Fr.) C. Moreau and *Ophiostoma pluriannulatum* (Hedgc.) H.&P. Sydow as having conidiogenous cells that proliferate sympodially forming conidia in acropetal or in branched chains. Cylindrical ramoconidia were also present. The presence of acropetal chains and ramoconidia led him to suggest that these anamorphs of *Ophiostoma* should be placed in *Hyalodendron.*

*Sporothrix* differs from *Hyalodendron* by the occurrence of prominent denticles on the conidiogenous cells (De Hoog, 1974). The formation of secondary and tertiary conidia, characteristic of *Hyalodendron*, can also be found in *Sporothrix* anamorphs such as those of *Ophiostoma perfectum* (R.W. Davidson) de Hoog, *Ophiostoma piceae* (Münch) B.K. Bakshi and *Ophiostoma nigrocarpum* (Davidson) de Hoog (de Hoog, 1974). Given that *Sporothrix* is amongst the most common anamorphs of *Ophiostoma* and *Ceratocystiopsis*, it is also understandable that *Hyalodendron* has been included amongst their anamorphs.

The genus *Allescheriella* Henn. has also been included amongst the anamorphs of *Ceratocystis sensu lato*. T.E. Hinds & R.W. Davidson (1972) first described the species *Ceratocystis retusi* R.W. Davidson & T.E. Hinds and provided an illustrated description (Fig. 1) of its anamorph. The anamorph was described as having hyaline, ovate conidia, borne singly, laterally and apically on hyphae, usually with an annular frill at the base (Hinds & Davidson, 1972). Upadhyay (1981) transferred the fungus to *Ceratocystiopsis* and concluded that the anamorph belongs in *Allescheriella*.

The aim of this study was to consider the validity of applying the generic names *Hyalodendron* and *Allescheriella* to the anamorphs of Ophiostomatoid fungi. Two species, *O. piliferum* and *O. pluriannulatum* with anamorphs that were assigned to *Hyalodendron* by Upadhyay (1981) were considered in the former case. The anamorph of *C. retusi* that has been assigned to *Allescheriella* was examined in the latter case.

## MATERIALS AND METHODS

Isolates of the fungi used in this study originated from the collection of Dr. R.W. Davidson (COLO). They were as follows: *O. piliferum* (COLO-487, COLO-432), *O. pluriannulatum* (C-416), and *C. retusi* (C-494, CO-494). These cultures were obtained from Dr. T. Hinds, U.S. Forest Service, Rocky Mountain Forest and Range Experiment Station, U.S. Department of Agriculture, Fort Collins, Colorado, United States. Isolates were grown on 2 % malt extract agar (20 g Biolab malt extract; 20 g Biolab agar; 1000 ml $H_2O$) in Petri dishes and incubated at 25 °C until the onset of sporulation.

Conidial development was examined using scanning (SEM) and transmission (TEM) electron microscopy. Material for SEM and TEM was cut from the agar and fixed using 3 %

glutaraldehyde and 1 % osmium tetroxide in 0.1 M buffer (pH 7). The material was then dehydrated in a graded acetone series. Specimens for SEM were critical point dried, mounted, coated with gold/palladium and examined with a JSM 6400 scanning electron microscope.

Material for TEM was fixed in a similar manner and embedded according to Spurr (1969) with the following modifications: The material was placed in epoxy resin and acetone (1:1) for 90 min at room temperature. The epoxy resin and acetone was replaced by new epoxy resin for 30 min at room temperature, followed by 30 min at 50 °C. The resin was again replaced and the material was placed in a oven for 60 min at 50 °C. Specimens were embedded in pre-heated gelatin

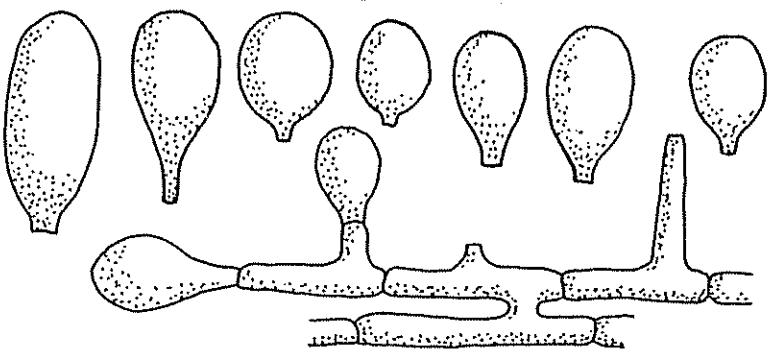

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Fig. 1 Diagrammatic illustration of the hyphal swellings found in *Ceratocystiopsis retusi* (Redrawn from Hinds & Davidson, 1972).

capsules where the epoxy resin was polimerized at 70 °C overnight. Ultrathin sections (60 nm) were made with glass knives, and mounted on copper grids. Sections were stained for 30 min in uranyl acetate followed by 5 min in lead citrate (Reynolds, 1963) and examined with a Phillips 301 transmission electron microscope.

## RESULTS

Scanning electron micrographs of the anamorph of *O. piliferum* revealed conidiogenous cells typical of *Sporothrix* spp. Here distinct, peg-like denticles could be distinguished at the conidiogenous loci (Fig. 2). Conidia were typically cylindrical to clavate and commonly exhibited secondary conidiation (Figs 3, 4).

Conidium development in *O. pluriannulatum* was similar to *O. piliferum* revealing prominent peg-like denticles on the conidiogenous cells. These denticulate conidiogenous cells are characteristic of those found in *Sporothrix* spp. (Figs 5, 6). An abundance of clavate ramoconidia characterized by more than one attachment point were found (Fig. 7).

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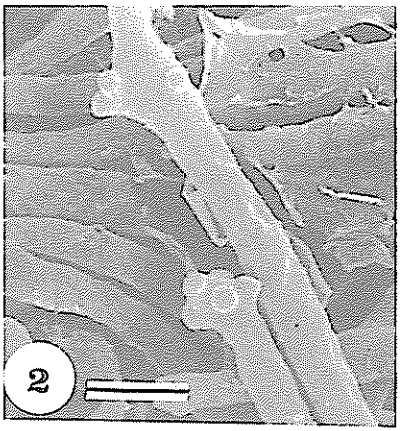
Figs 2-7 Conidiophores, conidiogenous cells and conidia of *Ophiostoma piliferum* and *Ophiostoma pluriannulatum*. Fig. 2 SEM of *O. piliferum* showing distinct peg-like denticles at the apex of the conidiogenous cell (Bar = 2 μm). Figs 3-4 Cylindrical to clavate conidia of *O. piliferum* exhibiting secondary conidiation (Bar = 2 μm). Figs 5-6 SEM of denticulate conidiogenous cells of *O. pluriannulatum* forming *Sporothrix*-like structures (Bar = 2 μm). Fig. 7 Fusiform to clavate ramoconidia of *O. pluriannulatum* have more than one basal delimiting septum (Bar = 2 μm).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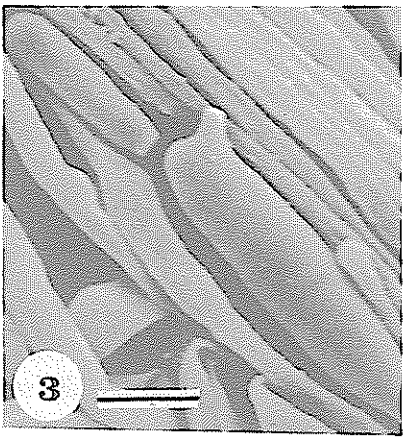
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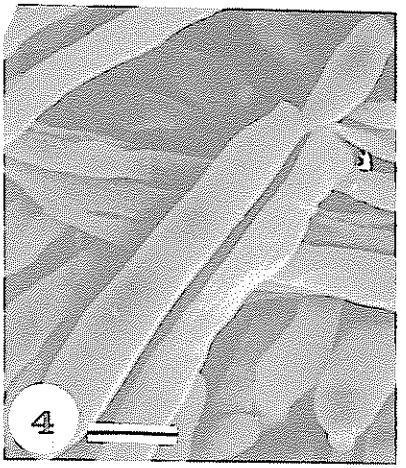
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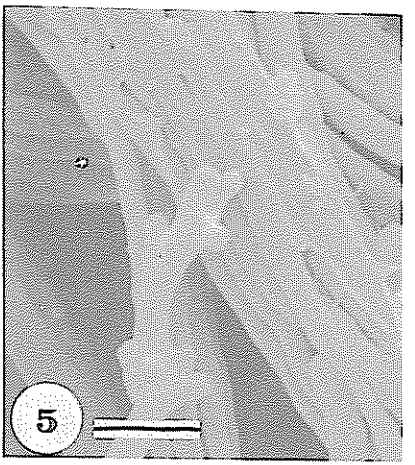
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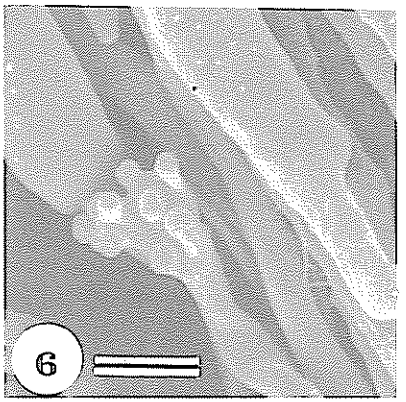
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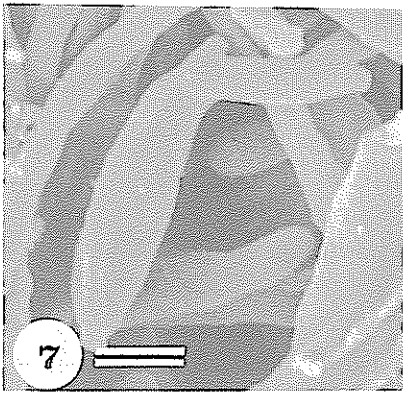
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In *C. retusi*, conidia most commonly developed from conidiogenous cells bearing *Sporothrix*-like denticles (Fig. 8). Occasionally, however, conidiogenous cells had distinct annellations typical of percurrent proliferation (Fig. 9). Such percurrent proliferation could also be observed in the formation of secondary conidia.

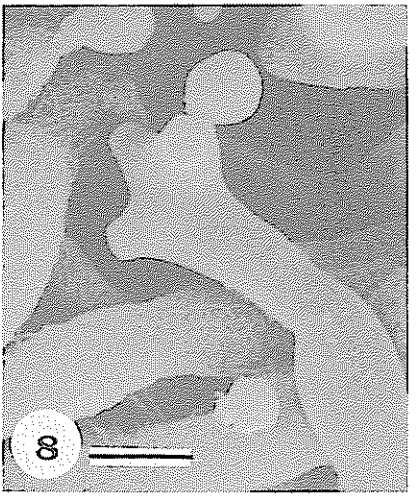
Despite repeated attempts, it was not possible to obtain TEM micrographs of *C. retusi* conidiogenous cells exhibiting percurrent proliferation. This was undoubtedly due to their infrequent occurrence. TEM sections through typical *Sporothrix* conidiogenous cells showed that conidiogenous loci become plugged with wall material after conidial secession (Fig. 10).

Although Hinds & Davidson (1972) reported single apically and laterally borne conidia in *C. retusi,* none could be found in this study. Several apical and intercalary hyphal swellings resembling chlamydospores were, however, observed (Fig. 11).

## DISCUSSION

Results of this study show conclusively that assignment

Figs 8-11 Conidia and conidiogenous cells of the anamorph of *Ceratocystiopsis retusi*. Fig. 8 Conidiogenous cells of *C. retusi* have short, cicatrized, peg-like denticles typical of *Sporothrix* spp. (Bar = 2 μm). Fig. 9 SEM of conidiogenous cells with distinct annellations (Arrows) (Bar = 2 μm). Fig. 10 TEM of a section through a conidiogenous cell revealing two conidiogenous loci. The one conidiogenous locus (Arrow◊) is plugged with wall material while a new conidium is being formed at the other (Arrow ←——) (Bar = 1 μm). Fig. 11 SEM of apical and intercalary hyphal swellings (Bar = 2 μm).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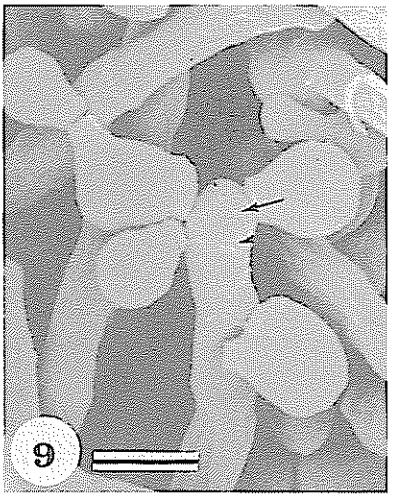
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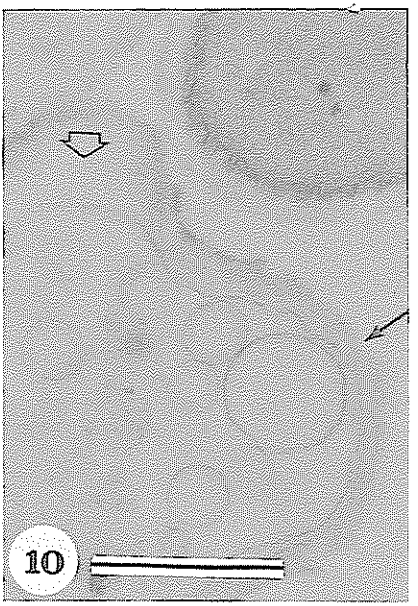
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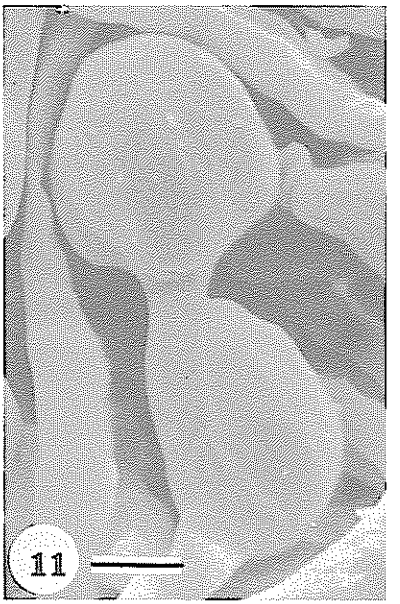
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of the anamorphs of *O. piliferum* and *O. pluriannulatum* to *Hyalodendron* cannot be justified. Anamorphs of these two species are typical of *Sporothrix* with well developed, denticulate conidiogenous cells. The type species of *Hyalodendron,* i.e. *H. lignicola* Diddens is a Basidiomycete and the name should not, therefore, be applied to anamorphs of *Ophiostoma* (De Hoog, 1993).

We assume that the assignment of anamorphs of *O. piliferum* and *O. pluriannulatum* to *Hyalodendron* was based on the production of secondary and tertiary conidia. Indeed, secondary conidia can lead to the illusion of ramoconidia typical of *Hyalodendron* and *Cladosporium* and thus be misleading. Production of secondary and subsequent conidia from large primary conidia is a common characteristic in *Sporothrix* anamorphs of *Ophiostoma* (De Hoog, 1974).

The presence of typical *Sporothrix* conidiogenous cells as found in the anamorph of *C. retusi*, calls into question the assignment of this fungus to *Allescheriella.* These structures are not common and they were clearly not observed by previous authors. Here we assume that Hinds & Davidson (1972) mistook the commonly occurring intercalary and apical hyphal swellings for conidia. The same error was probably made by Upadhyay (1981) which would explain why he assigned the fungus to *Allescheriella.*

The observation of percurrently proliferating conidiogenous cells in the anamorph of *C. retusi* was of particular interest. This form of development is typical of *Hyalorhinocladiella* anamorphs of *Ophiostoma* (Benade, Wingfield & Van Wyk, 1996). In fact, it has previously been suggested (Benade, Wingfield & Van Wyk, 1997) that a continuum probably exists between the patterns of conidium development in *Hyalorhinocladiella* and *Sporothrix*. The issue

at hand appears to concern the extent of the proliferation phase and the angle of displacement of this growth. It would be of interest to examine other species of *Sporothrix* to determine how commonly this overlapping of patterns of conidium development occurs.

The results of this study add further support to the contention of Mouton *et al.* (1993b) that there is an excessive number of names for anamorphs of *Ophiostoma* and *Ceratocystiopsis*. Certainly, in this case, the genera *Hyalodendron* and *Allescheriella* should be removed from the list of Ophiostomatoid anamorphs. It might also be expected that the various patterns of conidium development in *Ophiostoma* are fundamentally related. These fungi, therefore, include outstanding examples for further fundamental study of patterns of conidium development in anamorphic fungi.

## ACKNOWLEDGEMENTS

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