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Conidium development in *Sporothrix* anamorphs of *Ophiostoma*

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Hyalorhinocladiella and *Sporothrix* are two common mycelial anamorphs of *Ophiostoma* that are difficult to distinguish from each other. *Sporothrix* spp. differ visibly from *Hyalorhinocladiella* by the presence of denticles on the conidiogenous cells. *Graphium* and *Sporothrix* are often synanamorphs of the same *Ophiostoma* species and the aim of this study was to determine whether a relationship exists between sympodial conidium development in *Sporothrix* and annellidic development in *Graphium*. Conidium development was examined in *Sporothrix schenckii*, *Ophiostoma nigrocarpum* and *Ophiostoma piceae*. Using fluorescence microscopy, as well as scanning and transmission electron microscopy, distinct denticles were observed on the conidiogenous cells in *Sporothrix* spp. In some cases, these conidiogenous cells were reduced, giving them a *Hyalorhinocladiella*-like appearance. Results of this study suggest that a continuum in patterns of conidium development exists between *Sporothrix*, *Hyalorhinocladiella* and *Graphium*. The linear extent, and the angle of the proliferation stage with reference to the long axis of the conidiogenous cell, appear to determine the form of conidium development.

Ceratocystiopsis H. P. Upadhyay & W. B. Kendr. *Ceratocystis sensu stricto* and *Ophiostoma* Syd. & P. Syd. form part of the *Ceratocystis* Ellis & Halst. *sensu lato* complex (Weijman & De Hoog, 1975; Upadhyay, 1981; De Hoog & Scheffer, 1984). *Ceratocystis s.s.* can be distinguished from *Ophiostoma* by the presence of a *Chalara* (Corda) Rabenh. anamorph (Weijman & De Hoog, 1975; De Hoog & Scheffer, 1984). In *Chalara* species, conidia are formed in phialides (Nag Raj & Kendrick, 1975; Upadhyay, 1981) through a ring wall building process (Minter, Kirk & Sutton, 1983). In contrast, *Ophiostoma* has a diverse group of anamorphs in which conidia develop through apical wall building (Minter *et al.*, 1983). *Ophiostoma* anamorphs include genera such as *Leptographium* Lagerberg & Melin, *Graphium* Corda, *Hyalodendron* Diddens, *Hyalorhinocladiella* H. P. Upadhyay & W. B. Kendr. and *Sporothrix* Hektoen & C. F. Perkins (Upadhyay & Kendrick, 1975; Upadhyay, 1981; Wingfield, 1985; Harrington, 1987).

Sporothrix and *Hyalorhinocladiella* include a number of simple mycelial anamorphs of *Ophiostoma* species (Upadhyay, 1981). Many problems have been experienced in distinguishing between these two genera (De Hoog, 1993). *Sporothrix* species are characterized by elongate conidiogenous cells that arise from undifferentiated hyphae. These cells terminate in clusters of conidium-bearing denticles that extend sympodially and carry hyaline conidia (De Hoog, 1974; Upadhyay, 1981). According to earlier authors, the only difference between *Hyalorhinocladiella* and *Sporothrix* is that the peg-like denticles of the latter genus (De Hoog, 1974) are replaced in the former by low-profile scars on the conidiogenous cells (Upadhyay & Kendrick, 1975; Upadhyay, 1981).

Since it was first introduced as a taxonomic characteristic by Hughes (1953), conidium development has been used by many authors to distinguish between the anamorphs of *Ophiostoma* (Kendrick, 1961, 1962; Crane & Schoknecht, 1973; Upadhyay & Kendrick, 1974). This has unfortunately resulted in an unusual number of genera in the Hyphomycetes as a whole (Kendrick, 1980) and in the Ophiostomatoid fungi in particular (Mouton, Wingfield & Van Wyk, 1994).

Recently many genera in the *Leptographium* and *Graphium* complexes have been reduced to synonymy (Wingfield, 1985; Wingfield, Kendrick & Van Wyk, 1991). The *Graphium* complex initially included *Graphium* with annellidic conidium development (Upadhyay & Kendrick, 1974), *Phialographium* H. P. Upadhyay & Kendr. with phialidic development (Upadhyay & Kendrick, 1974) and *Pesotum* J. L. Crane & Schokn. with sympodial development (Crane & Schoknecht, 1973). These genera were reduced to synonymy with *Graphium* when Wingfield *et al.* (1991) found species of *Phialographium* and *Pesotum* to have conidiogenous cells that proliferate percurrently.

Sporothrix is commonly found as a synanamorph of *Graphium* (De Hoog, 1974; Upadhyay, 1981; Seifert & Okada, 1993) and conidiogenous cells in the former, proliferate sympodially (De Hoog, 1974; De Hoog, 1993). Given that these two genera commonly occur in the same species of *Ophiostoma*, it would be reasonable to expect a relatedness in themes of conidium development to occur. This could be exemplified by a continuum between sympodial and percurrent proliferation. The aim of this study was, therefore, to determine whether a relationship exists between sympodial and annellidic conidium development in *Sporothrix* and *Graphium* anamorphs of *Ophiostoma*.

MATERIALS AND METHODS

Three species of *Sporothrix*, all connected with *Ophiostoma*, were used in this study. *Sporothrix schenckii* Hektoen & C. F. Perkins was particularly chosen because it is the type species of this genus. This fungus is also believed to be related to *Ophiostoma stenoceras* (Robak) Melin & Nannf. (Berbee & Taylor, 1992; De Hoog, 1993). *Ophiostoma nigrocarpum* (R. W. Davidson) De Hoog was included because it is a typical *Ophiostoma* sp. that has only a *Sporothrix* anamorph. Inclusion of *Ophiostoma piceae* (Münch) Syd. & P. Syd. was justified by the fact that this fungus has a typical *Graphium* anamorph (*Graphium piceae* (J. L. Crane & Schokn.) M. J. Wingf. & W. B. Kendr.), as well as a distinct *Sporothrix* synanamorph. The following cultures were used:

S. schenckii (CBS 937.72, CBS 281.35); *O. nigrocarpum* (CBS 637.66, CBS 638.66); and *O. piceae* (Japan, Collection of Micro-organisms, JCM 6016). Cultures were grown on 2% malt extract agar (20 g Biolab malt extract; 20 g Biolab agar l^{-1} water) and incubated at 25 °C for approximately 3 wk until the onset of sporulation.

Conidium development was examined using scanning and transmission (SEM, TEM) electron microscopy, as well as light and fluorescence microscopy. Material for SEM and TEM was cut from cultures in Petri dishes and fixed using 3% glutaraldehyde and 1% osmium tetroxide (OsO_4) in a 0·1 M phosphate buffer (pH 7). Material was dehydrated in a graded acetone series 50, 70, 95 and 100%. After dehydration, SEM specimens were critical point dried, coated with gold/palladium and viewed with a Jeol 6400 scanning electron microscope. TEM specimens were embedded, after dehydration, 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 and, thereafter, placed in epoxy resin for 30 min at room temperature followed by 30 min at 50°. The resin was replaced with new epoxy resin and the material held at 50° for 60 min. Specimens were then placed in pre-heated gelatin capsules where the epoxy resin was polymerized at 70° overnight. Ultrathin sections (60 nm) were cut with glass knives and mounted on copper grids. Sections were stained for 20 min in uranyl acetate followed by 5 min in lead citrate (Reynolds, 1963) and viewed with a Phillips 301 transmission electron microscope.

Conidia and conidiophores were mounted on glass slides in lactophenol and examined using phase- and interference-contrast microscopy. For fluorescence microscopy, material was mounted on glass slides in buffered calcafluor optical brightener (0·1 M phosphate buffer). Samples were examined with a Zeiss Axioskop fluorescence microscope, using dark field and uv illumination and photographed using Ilford FP4 film.

RESULTS

Scanning electron micrographs of *S. schenckii* revealed conidiogenous cells with clusters of prominent cylindrical conidium-bearing denticles at and near their apices (Fig. 1). Terminal and intercalary conidiogenous cells proliferated sympodially and gave rise to ovoid to elliptical conidia (Figs 1, 2).

In the *Sporothrix* anamorph of *O. piceae*, ellipsoidal to fusiform conidia were formed sympodially at the apex of conidiogenous cells (Fig. 3). In contrast, conidiogenous cells in the *Graphium* anamorph proliferated percurrently to form ellipsoidal conidia (Fig. 4). In many cases, these conidia were arranged at either side of the conidiogenous cells, giving an illusion of sympodial development (Fig. 5).

The *Sporothrix* anamorph of *O. nigrocarpum*, like other *Sporothrix* species, has conidiogenous cells with distinct peg-like denticles (Fig. 6). In some cases, however, these denticles were absent or reduced, making such conidiogenous cells almost indistinguishable from those of *Hyalorhinocladiella* (Fig. 7). They were, however, characterized by having a swollen and distorted appearance at the loci of conidial secession (Fig. 7). Terminal conidiogenous cells in *O. nigrocarpum* proliferated sympodially to form numerous conidia (Fig. 6). These obovoid to broadly ellipsoidal conidia were formed singly but occurred in aggregated slimy masses (Fig. 8). Fluorescence micrographs of the conidiogenous cells of *O. nigrocarpum* revealed brightly fluorescing areas at the apices of the denticles indicative of wall building activity in this region (Fig. 9).

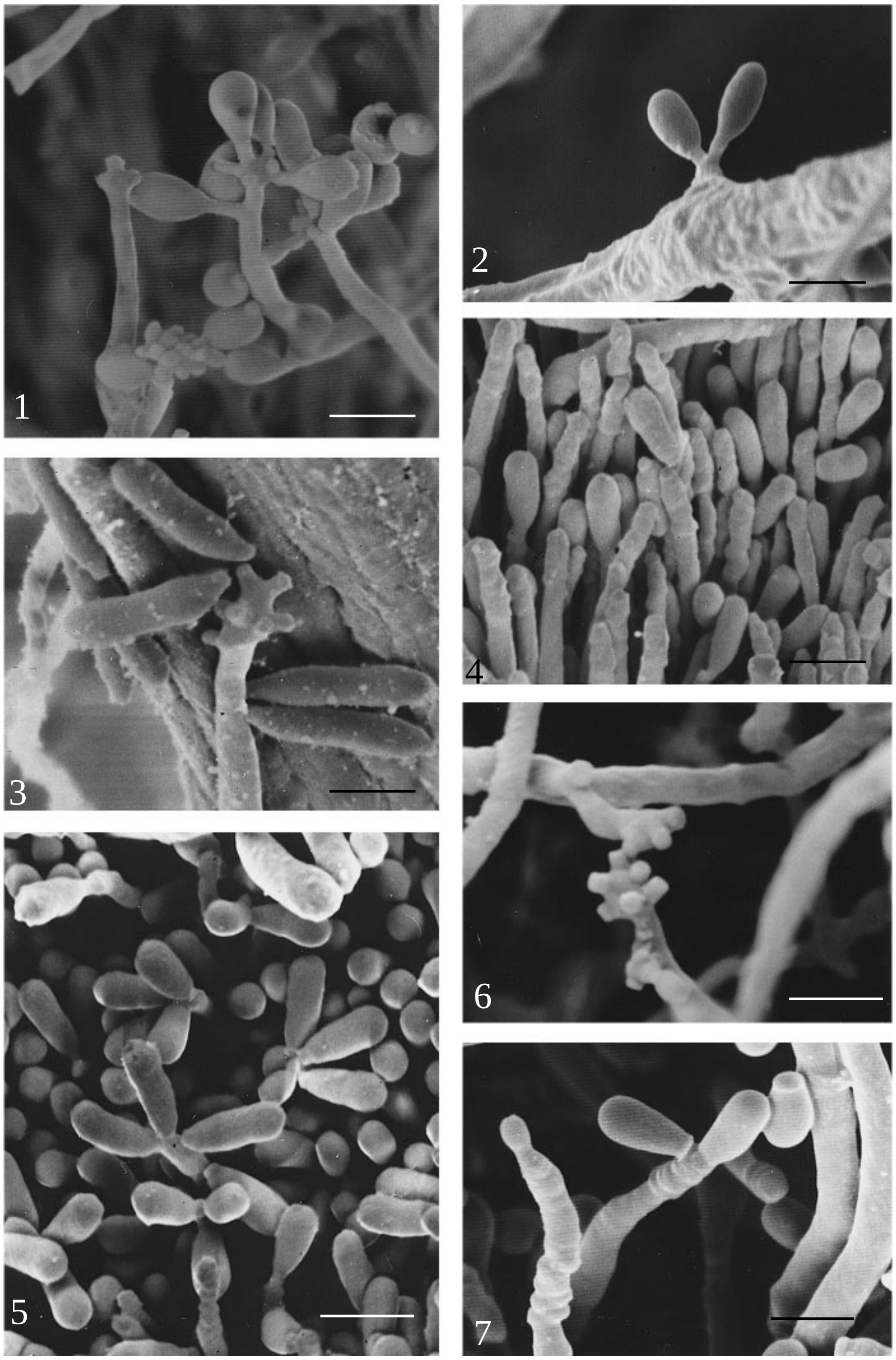
Despite concerted efforts, TEM sections through the *Hyalorhinocladiella*-like conidiogenous cells (shown in Fig. 7) could not be obtained, presumably because these are uncommon. Transmission electron micrographs of sections through the conidiogenous cells of the *Sporothrix* anamorph of *O. nigrocarpum* revealed multiple conidiogenous loci or denticles on the conidiogenous cells (Figs 10, 11). These were typical of the *Sporothrix*-like conidiogenous cells.

DISCUSSION

This study has given rise to some interesting observations concerning the relatedness between modes of conidium development in *Sporothrix* on the one hand, and *Hyalorhinocladiella* (including its macronematous analogues *Graphium* and *Leptographium*) on the other. Conidiogenous cells in typical species of *Sporothrix* proliferate sympodially with each successive apex giving rise to a single conidium (De Hoog, 1974; Upadhyay, 1981). In most cases, a distinct denticle remains after conidial secession. In some species of *Sporothrix* such as the anamorph of *O. nigrocarpum*, the extent of proliferation is reduced. This can give rise to apparently annellated conidiogenous cells more typical of those in *Hyalorhinocladiella*.

Results of this study confirmed the notion that a continuum most probably exists between the fundamental process of conidium development in *Sporothrix* as opposed to *Hyalorhinocladiella*, *Graphium* and *Leptographium*. The most important issue here appears to concern the proliferation stage in conidial development. Where proliferation is extensive and displaced from the main axis of the conidiogenous cell, conidia secede leaving denticles typical of *Sporothrix* (Fig. 12*a*). Here, the point of secession might be viewed as a single annellation, separated from others by a region of proliferation.

In cases where the proliferation stage is reduced but still displaced from the main axis of the conidiogenous cell, this

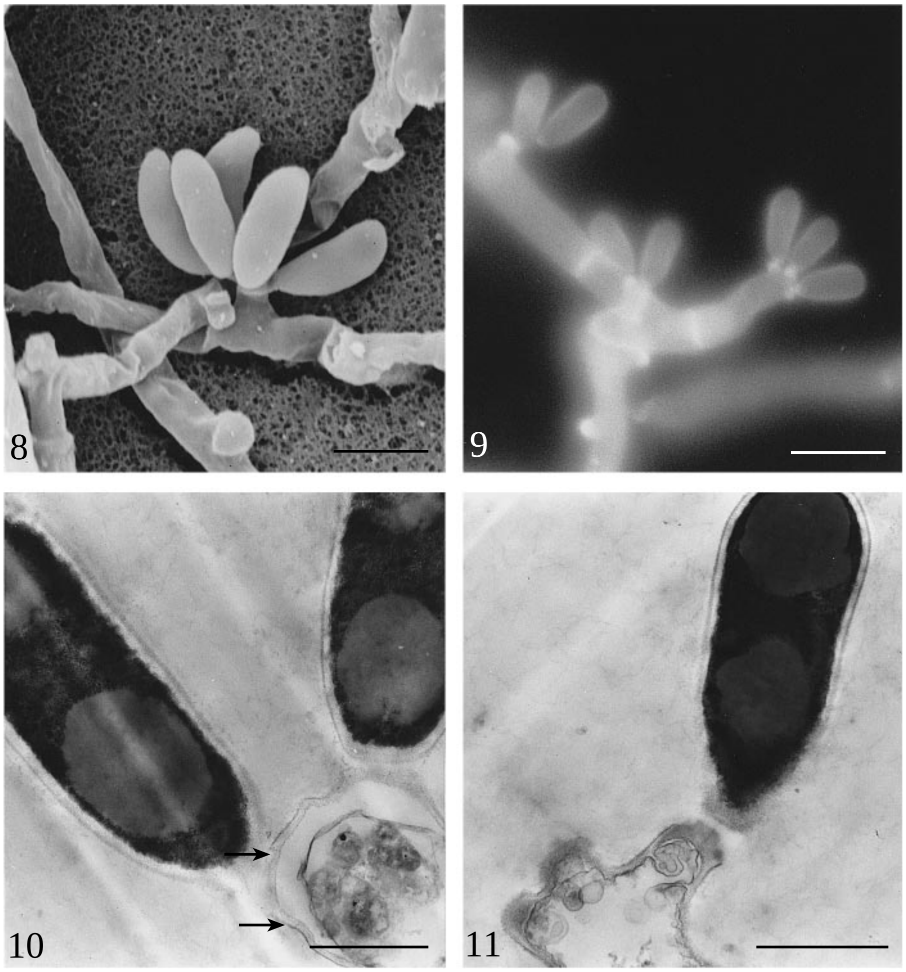


Figs 1–7. Conidia and conidiogenous cells of *Sporothrix schenckii*, *Ophiostoma piceae* and *Ophiostoma nigrocarpum*. **Figs 1–2.** Scanning electron micrographs of *S. schenckii* revealing terminal and integrated denticulated conidiogenous cells with oval to ellipsoidal conidia (bar, 2 µm). **Fig. 3.** SEM of the *Sporothrix* anamorph of *O. piceae* showing a cluster of conidium-bearing denticles at the apex of the conidiogenous cell (bar, 2 µm). **Fig. 4.** SEM of the conidiogenous apparatus of the *Graphium* anamorph of *O. piceae* bearing annellated conidiogenous cells (bar, 2 µm). **Fig. 5.** Ellipsoidal conidia of the *Graphium* anamorph of *O. piceae* carried in an apparently false sympodial manner on the conidiogenous cells (bar, 2 µm). **Fig. 6.** Terminal conidiogenous cells of *O. nigrocarpum* showing prominent denticles (bar, 2 µm). **Fig. 7.** Conidiogenous cells of the *Sporothrix* anamorph of *O. nigrocarpum* with reduced denticles resulting in a *Hyalorhinocladiella*-like structure (bar, 2 µm).

cell has a distorted appearance. It might thus be considered as an intermediate stage between exclusively sympodial and exclusively percurrent proliferation (Fig. 12*b*). The latter pattern was seen in some conidiogenous cells in *O. nigrocarpum* as well as in a previous study of the apparent *Hyalorhinocladiella* anamorph of *Ophiostoma minus* (Hedgc.) Syd. & P. Syd. (Benade, Wingfield & Van Wyk, 1996).

In typical species of *Hyalorhinocladiella*, *Graphium* and *Leptographium*, proliferation occurs along the main axis of the conidiogenous cell. It must, therefore, penetrate the scar left after secession of the previously formed conidium (Fig. 12*c*). Here proliferation is typically percurrent and might be seen at the opposite end of a continuum with *Sporothrix*.

Sections through conidiogenous cells of the *Sporothrix* state



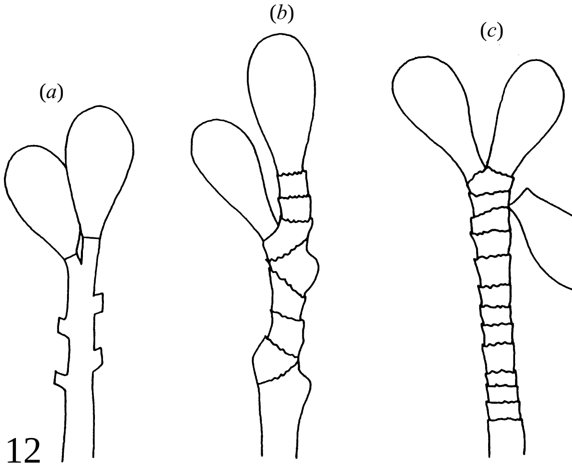
Figs 8–11. Conidiophores, conidiogenous cells and conidia of *Ophiostoma nigrocarpum*. **Fig. 8.** Ellipsoidal to clavate conidia of *O. nigrocarpum* carried sympodially on the conidiogenous cell (bar, 2 µm). **Fig. 9.** Fluorescence micrograph of the *Sporothrix* anamorph of *O. nigrocarpum* showing brightly fluorescing areas at the apices of the peg-like denticles (bar, 3 µm). **Fig. 10.** Transmission electron micrographs of *O. nigrocarpum* showing conidia holoblastically formed, leaving wall remnants (arrows) on the conidiogenous cells at the conidiogenous loci (bar, 1 µm). **Fig. 11.** Conidiogenous loci plugged by wall material after delimitation and secession of the conidia (bar, 1 µm).

of *O. nigrocarpum* in this study were reminiscent of those in polyphialides of the microconidial state of *Fusarium chlamydosporum* Wollenw. & Reinking (Van Wyk *et al*., 1991). In both cases, the single conidiogenous loci are plugged after conidial secession (Fig. 13). A distinct frill or annellation can be distinguished at the point of secession (Fig. 13*b*). Previously, Van Wyk *et al*. (1991) suggested that the conidiogenous locus in polyphialides of *F. chlamydosporum* is merely a phialide with a single conidium (Fig. 13*a*). Likewise, the conidiogenous locus in *Sporothrix* might be considered as an annellide with a single conidium. If proliferation were to occur through this point of secession, as has been observed in the anamorph of *O. minus*, typically annellated conidiogenous cells would result (Fig. 13*b*).

Conidia of *Sporothrix* are physically separated from each other, tend to be larger than those of *Hyalorhinocladiella*, *Graphium* and *Leptographium* and appear to be relatively dry. In contrast, conidia of the latter genera are aggregated, small and produced in gloeoid masses (De Hoog, 1974; Upadhyay, 1981). Where both forms occur in a single species, it must be assumed that they are adapted to different modes of dispersal. These fungi occur in association with insects (Upadhyay, 1981; Whitney, 1982; Perry, 1991). Their different morphological manifestations are presumably associated with their dispersal by different forms of insects or other related microscopic animals.

Results of this study suggest that patterns of conidial development in *Sporothrix* and *Graphium* anamorphs of *Ophiostoma* are fundamentally similar. This fact must, however, not be confused with the overall manifestation of each of these genera which is obviously very different. If extent of proliferation is the major factor separating these two patterns, this remains genetically controlled. The various states are probably formed in response to different environmental conditions.

This study provides further evidence of pleomorphism in patterns of conidium development amongst anamorphs of *Ophiostoma* (Wingfield, 1985; Wingfield, Van Wyk & Van Wyk, 1989; Wingfield *et al*., 1991). Furthermore, it emphasizes the danger of placing undue emphasis on conidium development in the taxonomy of conidial fungi. Amongst anamorphs of *Ophiostoma*, conidium development can be a most misleading taxonomic character. It should, therefore, only be used in conjunction with other morphological



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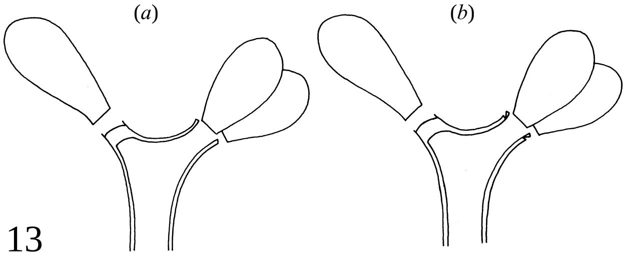

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Figs 12, 13. Fig. 12. Schematic illustration of a continuum in patterns of conidium development between *Sporothrix* and *Graphium*. (*a*) Conidia in *Sporothrix* spp. are formed by extensive and displaced proliferation of the conidiogenous cell. After secession prominent denticles are left on the conidiogenous cell. (*b*). In cases where proliferation is reduced, but still displaced, an intermediate stage is formed between exclusively sympodial (*Sporothrix*) and exclusively annellidic (*Graphium*). (*c*) In *Graphium* spp., proliferation is not displaced laterally and occurs percurrently along the main axis of the conidiogenous cell resulting in annellations. **Fig. 13.** Schematic representation of conidium development in *Fusarium chlamydosporum* and *Sporothrix* sp. (*a*) Microconidia of *F. chlamydosporum* are formed singly on conidiogenous loci. After secession of the solitary conidium, the conidiogenous locus is plugged and a new locus develops sympodially. (*b*) Conidia in *Sporothrix* spp. are formed on denticulate conidiogenous cells. After secession of a single conidium, the conidiogenous locus is plugged and a new locus is formed sympodially. If proliferation occurs through this point of secession, an annellation will be formed on the conidiogenous cell.

characteristics. Investigation into the factors associated with the onset of the various kinds of conidiogenesis promises to be a most rewarding study of value to taxonomists as well as ecologists.

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