Studies of conidial anatomy and conidiogenesis in Sporoschisma nigroseptatum using light and electron microscopy

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Abstract: The results of an ultrastructural study of the conidial anatomy and conidiogenesis in Sporoschisma nigroseptatum are presented. The development of the conidial chain involves endogenous conidial ontogeny, apical wall-building, and retrogressive conidial delimitation followed by cessation of apical wall-building, then replacement ring wall-building of additional retrogressively delimited conidia, and extrusion of the true conidial chain through the terminal aperture of the conidiogenous cell. Maturation of conidia involves deposition of two inner wall layers and formation of five distosepta. Conidial chains secede schizolytically. No proliferation of the conidiogenous cell occurs and the conidium is delimited by a cross wall that is discontinuous with the periclinal wall. Each conidium has polar plug-and-socket-like structures that are interlocked between adjacent conidia along the conidial chain. Similar plug-and-socket-like structures are also seen in other Sporoschisma species. The taxonomy of Chalara is also briefly discussed with reference to patterns of conidial wall-building.

Key words: Chalara, conidial chain, conidial ontogeny, ultrastructures.

Résumé : Les auteurs présentent les résultats d’une étude portant sur les ultrastructures de l’anatomie des conides et la conidiogénèse du Sporoschisma nigroseptatum. Le développement de la chaîne conidiale implique l’ontogénie endogène des conides, le développement de la paroi apicale et la délimitation rétrogressive des conides, suivi de l’arrêt de dépôt de la paroi apicale, son remplacement par la construction de parois annulaires de conides rétrogressivement délimitées additionnelles, et l’extrusion de la vraie chaîne de conides à travers l’ouverture terminale de la cellule conidiogène. La maturation des conides implique la dépôt de deux couches pariétales internes et la formation de cinq distoseptas. Les chaînes conidiennes se séparent par schizolyse. Il n’y a pas de prolifération des cellules conidiogènes et la conidie est délimitée par une paroi transverse qui est discontinuée avec la paroi péricline. Chaque conidie possède un bouchon polaire et une cavité d’emboîtement qui unissent les conides adjacentes le long de la chaîne conidienne. On a également observé de telles structures à bouchons et emboîtements chez d’autres espèces de Sporoschisma. Les auteurs discutent également de la taxonomie du Chalara en relation avec la déposition du matériel pariétal.

Mots clés : Chalara, chaîne conidienne, ontogénie conidienne, ultrastructures.

[Traduit par la Rédaction]

Introduction

The genus Sporoschisma Berk. & Broome was recently monographed by Goh et al. (1997) and currently comprises seven species, viz. Sporoschisma mirabile Berk. & Broome (type species), Sporoschisma juvénile Boudier, Sporoschisma nigroseptatum D. Rao & R. Rao, Sporoschisma paricinéatum Goh & K.D. Hyde, Sporoschisma phaeocentri W.H. Ho, K.D. Hyde & Goh, Sporoschisma saccardoi E.W. Mason & S. Hughes, and Sporoschisma unisepatatum Bhat. Species of Sporoschisma are commonly found on decaying wood in terrestrial and freshwater habitats and are either pan-tropical or cosmopolitan in distribution. The genus is characterised by the production of cylindrical, pigmented phragmoconidia, densely pigmented conidiophores, and conidigenous cells that each comprise a swollen venter and a tubular collarette (sensu Minter et al. 1982, 1983a). Successive production of conidia within the swollen venter of conidiogenous cells results in the extrusion of long conidial chains above the tubular collarettes of conidiogenous cells (Goh et al. 1997).

During a study of freshwater fungi on wood submerged in a stream in Hong Kong, we frequently collected S. nigroseptatum. In each conidium, we observed a plug-like protrusion at the distal end and a socket-like depression at the other end. Therefore, we decided to examine the ultrastructure of these conidia and their formation and these results are presented in this paper. In addition to S. nigroseptatum, two other species, S. saccardoi and an undescribed Sporoschisma sp., were examined using scanning electron microscopy. Some taxo-
nomic notes on *Sporoschisma* and the closely related genus *Chalarra* (Corda) Rabenh. are also provided.

Minter et al. (1982, 1983a, 1983b) reassessed the processes involved in conidial ontogeny and delimited seven conidiogenous events to describe the development of conidia, viz. conidial ontogeny, conidial wall-building, conidial delimitation, conidial maturation, conidial succession, conidiogenous cell proliferation, and conidiogenous cell regeneration. Their work has been accepted by some workers (e.g., Kirk 1985; Sutton 1986; Nag Raj 1993; Hawksworth et al. 1995, see “mitospore fungi”; Goh and Hyde 1997). Their concepts and definitions of conidial development are adopted in this paper.

Materials and methods

Light microscopy

Freshly collected wood samples were incubated in plastic boxes lined with moistened paper towels and examined with a 10×100 magnification. Fungal fruiting bodies were mounted on slides either in millipore filter water or lactophenol for microscopic examination.

Cryo-scanning electron microscopy (Cryo-SEM)

Freshly collected samples and dried specimens were frozen in Polaron LT-7400 CryoPrep using liquid nitrogen. The specimens were subsequently sublimated at −80 to −90°C for 20 min, frozen down to −150°C, sputter-coated with gold for 40 s, and examined using a Leica Cambridge Stereoscope 440 scanning electron microscope operating at 10 kV and −150°C.

Transmission electron microscopy (TEM)

Freshly collected samples were embedded in 2% (w/v) agar, fixed with 4% (v/v) glutaraldehyde for 4 h, postfixed with 2% (w/v) osmium tetroxide at 4°C overnight, washed with distilled water, dehydrated through a graded alcohol series and acetone series, embedded in Mollenhauer’s resin (Mollenhauer 1964), and polymerized at 60°C for 3 days. Ultrathin sections were stained with lead citrate for 15 min and poststained with uranyl acetate for 40 min and examined using a JEOL 100S transmission electron microscope operating at 80 kV.

Material examined


Results

Light microscopy

Immature conidia of *S. nigriseptatum* were aseptate and hyaline but had the shape of fully matured conidia (Fig. 1). The central septum (S1) was the first-formed septum (Fig. 2) followed by the two outermost septa (S2) delimiting the hyaline end cells (Fig. 3). The penultimate septa (S3) were formed last between the S1 and S2 septa (Fig. 4). Mature conidia were 5-septate with four olivaceous brown inner cells and two hyaline polar cells (rarely 7-septate: two hyaline polar cells and six pigmented inner cells, Fig. 5), with a plug-like protrusion (approximately 1.5 μm wide and 0.5 μm high) at the distal end and a socket-like depression at the proximal end (Figs. 7 and 8). Individual conidia released from the conidial chains possessed a fringe comprising the remains of the conidium-delimiting cross wall at both ends (Fig. 6). Conidia usually became mature before they were extruded from the tubular collarettes of the conidiogenous cells. Mature conidia were usually longer than immature, hyaline conidia.

Cryo-SEM

The distinct plug-like protrusion at one end and the complementary depression at the other end of each conidium of *S. nigriseptatum* were also seen using SEM. The broken edge of the conidium-delimiting cross wall was visible at each end of the conidium (Fig. 9). In *S. saccaroidi*, a minute circular depression was seen at one end of the conidium (Fig. 10). In *Sporoschisma* sp., a faint circular structure within an undulating circular outline was seen at the ends of the conidium (Fig. 11). The conidial walls of these *Sporoschisma* species were covered by a mucous layer of varying thickness (Figs. 9–11).

TEM

In *S. nigriseptatum*, two septa were visible in the conidiophores (Fig. 13). The walls of conidiophores and conidiogenous cells comprised two layers: a thick, electron-dense outer wall layer (1.3–1.8 μm) and a thin, less electron-dense inner wall layer (0.3–0.5 μm) (Figs. 14–17). The septa in the conidiophores contained an outer ring of electron-dense material (Fig. 17). The outer wall layers of the conidiophores and conidiogenous cells comprised fibrillar electron-dense deposits (Fig. 19). A thin discontinuous layer comprising granular electron-dense deposits (approximately 40 nm thick) covered the outer surfaces of the conidiophores and conidiogenous cells (Fig. 19).

Formation of conidia occurred in the swollen venters of conidiogenous cells as shown in Fig. 14. The cytoplasm of the venter comprised lipid globules, membrane profiles, and presumably membrane-bound glycogen rosettes. Strands of peripheral endoplasmic reticulum were seen near the inner wall of the venter. Lipid globules accumulated and appeared to coalesce near the conidial primordia. New wall material was incorporated and the cytoplasm extended towards the tip of the venter and the conidial primordia extended distally (Fig. 14). The conidium-delimiting cross wall, measuring up to 900 nm thick, was deposited centripetally in a layer, perpendicular to the axis of the conidiogenous cell. The formation of the conidium-delimiting cross wall appeared to be independent of the formation of the primary conidial wall (Figs. 14 and 15). The resulting conidium-delimiting septum was less electron dense than the peripheral conidial wall (Figs. 14, 15, 23, 25, 26, and 28). Conidia within the tubular collarette of the conidiogenous cell were separated from the wall of the collarette by an electron-translucent layer 1–1.5 μm thick (Figs. 14 and 15).

During maturation of conidia, an additional wall layer was deposited onto the inner surface of the primary conidial wall (Figs. 20 and 21). The conidial wall was transformed from the 120- to 200-nm-thick bilamellate wall (Fig. 21) into a 0.7- to 1.4-μm-thick trilamellate wall (Fig. 20). The mature trilamellate wall comprised one outer, first-formed primary wall layer and two inner, subsequently formed secondary wall layers (Fig. 20). In maturing conidia, the middle wall layer of the inner brown cells was densely laden with electron-dense

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Figs. 1–8. Light micrographs of *S. nigroseptatum*. Fig. 1. Hyaline, one-celled immature conidium. Fig. 2. Immature conidium with a developing central septum (S1). Note that the conidium is lightly pigmented, except at the polar regions. Fig. 3. Pale brown immature conidium with a central septum and two outer septa (S2). Fig. 4. Maturing conidium with one penultimate septum formed (S3). Fig. 5. Unusual 7-septate conidium. Fig. 6. Schizolytically detached conidium with a fringe of residual wall material shown at one end (arrowheads). Fig. 7. Mature conidia with the plug-and-socket-like structures (PG, SC). IC, inner brown cell; OC, outer hyaline cell; PC, penultimate brown cell. Fig. 8. Conidia extruding from the tubular collar of conidiogenous cell with distinct plug-like protrusion formed at the distal end. Figs. 1–6 and 8 in lactophenol; Fig. 7 in filtered water. Scale bars = 10 μm.

Fibrillar material, which was very sparse in the hyaline polar cells (Figs. 23, 24, 26, and 27). The periclinal wall of the four central brown cells was thick (1–1.4 μm), comprising a 120- to 150-nm-thick outer layer, a 400- to 700-nm-thick middle layer, and a 400- to 620-nm-thick inner layer. The periclinal wall of the polar hyaline cells was much thinner (700–750 nm), with an approximately 100-nm-thick outer layer, a 250- to 400-nm-thick middle layer, and a 200- to 400-nm-thick inner layer (Figs. 12, 26, and 27). The outer wall layer was continuous along the conidial chain, linking the conidia together (Figs. 12, 23 and 26). At this stage, the conidium-delimiting cross wall sometimes lysed, more or less medially, to release individual conidia (Figs. 12 and 28).

In immature conidia, the developing central septum (S1) was thin (40–70 nm) and less electron dense (Fig. 24). At maturity, five septa were formed and they became thickened (S1 and S3 septa: 1.1–1.3 μm thick; S2 septum: approximately 0.9 μm thick) (Figs. 12, 18, 26, and 27). The septa were bilamellate with an inner electron-dense wall layer and an outer, less electron-dense wall layer (Figs. 12, 18, 26, and 27). The outer, less electron-dense septal wall layer (320–400 nm thick in brown cells, 90–100 nm thick in hyaline cells) was continuous with the inner conidial wall layer, while the inner, electron-dense septal wall layer (300–450 nm thick) was continuous with the middle conidial wall layer (Figs. 26 and 27). The conidial septum was of the simple pore type (Fig. 22).

Each conidium had a plug-like protrusion at the distal end and a socket-like depression at the proximal end (Fig. 18). These protrusions and depressions were formed at the central pore of the conidium-delimiting septum (Figs. 12, 25, and 28). The plug-like protrusion at the apex of each conidium was plugged into the socket-like depression at the base of each adjacent conidium along the conidial chains (Fig. 12). In immature conidia, where only the central septum had been formed, the plug-like structure was up to 1.5 μm in diameter, 0.4 μm high, with a thin pigmented layer (approximately 150 nm thick) (Figs. 25 and 29). At maturity, the plug-like structure was up to 2.3 μm in diameter, 0.7 μm high, with a thicker electron-dense layer (approximately 500 nm thick) (Figs. 28 and 30). The plug-and-socket-like structures comprised two wall layers, which were in continuity with the
Figs. 9–12. Cryo-SEM and TEM of Sporoschisma species. Fig. 9. Conidia of *S. nigroseptatum* with plug-and-socket-like structures at the ends and fringes of residual conidium-delimiting cross wall (arrowheads). Fig. 10. Conidium of *S. saccharoi* with a minute plug-like structure (arrowhead) and a fringe of residual conidium-delimiting cross wall (double arrowhead). Fig. 11. Conidia of *Sporoschisma* sp. Note that the conidia are rough walled and possess a faint circular structure (arrowhead) within an undulating circular outline. Fig. 12. TEM of a mature 5-septate conidium of *S. nigroseptatum*. Note the conidium-delimiting cross wall (DS), plug-like structure (PG), socket-like structure (SC), and large coalesced lipid globules (G) in each cell. IC, inner cell; OC, outer cell; PC, penultimate cell; S1, central septum; S2, outer septum; S3, penultimate septum. Scale bars = 5 μm.

Discussion

Conidiogenesis

*Conidiogenesis in S. nigroseptatum*

Conidiogenesis in *Chalara*, a genus similar to *Sporoschisma* with species that produce conidial chains through long tubular collarettes of conidiophores, has been discussed by Nag Raj and Kendrick (1975), Hawes and Beckett (1977a, 1977b, 1977c), and Ingold (1981). Sutton and Hennebert (1994, in Hawksworth et al., 1995), who illustrated the 43 known combinations of conidiogenous (or mitosporogenous) events in the dermatocystes, described *Chalara* as follows: “conidial ontogeny holoblastic with new inner walls constituting the conidia laid down retrogressively by diffuse wall-building, delimitation retrogressive, loss of apical wall-building followed by replacement ring wall-building at the base of the conidiogenous cell adding more retrogressively delimited...
conidia, the outer (original) conidiogenous cell wall breaks as a connected chain of conidia is formed, collarette variable, one locus per conidiogenous cell, secession (schizolytic). The conidiogenous events in *S. nigrospatum* are best described under this category. Regeneration of conidiogenous cells, omitted in Sutton and Hennebert's (1994, in Hawksworth et al. 1995) description, has not been observed in species of *Sporoschisma*, although we have observed capitulate setae produced from torn tubular collarettes of aged conidiogenous cells in culture.

**Conidiogenesis in Chalara and Sporoschisma and types of septation**

Minter et al. (1982, 1983a) pointed out that in *Chalara* species, conidiogenous cells do not proliferate (as in other fungi with true-chain phialides, sensu Minter et al. 1982, 1983a, 1983b) and a discrete event of conidial maturation is usually absent. Furthermore, the conidiophore-delimiting septic in *Chalara* species is a distoseptum (sensu Hawksworth et al. 1995), and both the inner and outer conidial wall layers are laid down within the hypophial wall-building ring (Minter et al. 1982, 1983a). *Sporoschisma nigrospatum* is similar to *Chalara* species in that no proliferation in the conidiogenous cell occurs. It differs from *Chalara* species because (i) only the outer primary conidial wall layer is formed within the hypophial wall-building ring (Fig. 14), (ii) two additional wall layers are deposited onto the inner surface of the primary conidial wall, after the conidium is delimited (Figs. 12, 18, 20, and 21), i.e., conidial maturation occurs, and (iii) the formation of the conidiophore-delimiting septum appears to be independent of the formation of the periclinal conidial wall (Figs. 14 and 15). The conidiophore-delimiting septum in *S. nigrospatum* not only differs from those of *Chalara* species, it also differs from the conventional concepts of euseptum (i.e., a cell-separating structure comprising multilayered walls that are structurally similar to the lateral wall; Hawksworth et al. 1995) and distoseptum (i.e., a cell-separating structure with the individual cells each surrounded by a sac-like wall layer distinct from the outer wall; Hawksworth et al. 1995). This conidiophore-delimiting septum is neither structurally similar to the lateral wall nor surrounded by a sac-like wall layer. It is a cross wall created by de novo synthesis within the cell. Ultrastructural studies are needed to examine the delimiting septa, cross walls, and similar structures in other mitosporic fungi before it is possible to conclude whether the conidiophore-delimiting septum in *Sporoschisma* represents a unique type of septum.

**Origin of the conidial wall**

There are three major modes of conidium development in deuteromycetes, viz. holoblastic, enteroblastic, and thallic (Kendrick 1971). In describing the origin of conidial wall, Hennebert and Sutton (1994) proposed three additional terms, viz. "endogenous," "holoblastic" (cf. holoblastic), and "enterogenous" (cf. enteroblastic). The conidium is holoblastic when outer and inner wall layers of conidigenous cell and conidium are continuous, enterogenous when the inner wall layer of the conidiogenous cell forms the outer wall of the conidium, and endogenous when no wall layers are continuous between the conidium and the conidiogenous cell (Hennebert and Sutton 1994). Coincidentally, Tiedt (1993) introduced "endoblastic" to describe conidium development in *Aspergillus niger* Tiegh. Where none of the phialide wall layers are involved in the formation of new conidial wall layers during conidial maturation. In *Chalara* (Hawes and Beckett 1977b) and *S. nigrospatum*, the primary conidial wall is formed by de novo synthesis, in which wall material is deposited within the inner surface of conidiphore wall. In other words, no conidiophore wall layers are involved in the formation of the primary conidial wall. In this sense, the conidial ontogeny in *Chalara* and *S. nigrospatum* is of the endoblastic type (sensu Tiedt 1993), i.e., "endogenous wall formation" (sensu Hennebert and Sutton 1994), rather than "holoblastic" conidial ontogeny, or holoblastic wall formation as described by Sutton and Hennebert (1994, in Hawksworth et al. 1995).

**Conidial chains**

For describing conidial chains, Minter et al. (1982, 1983a, 1983b) adopted the terms "true conidial chains" (in which there is a continuity of wall layer(s) along the conidial chains) and "false conidial chains" (in which there is no continuity of wall layers in the chains), as introduced by Subramanian (1972). Minter et al. (1983a) also proposed two additional terms, "with connectives" and "without connectives," to replace the terms "connected conidial chains" and "disconnected conidial chains" (Gams 1978), respectively. In *S. nigrospatum*, the conidiophore-delimiting cross wall can be treated as with connectives (Fig. 12). Its conidial chain could be considered a true-chain type (Minter et al. 1982) because
Figs. 20–30. TEM of longitudinal sections at different stages of the conidial development in *S. nigroseptatum*. Fig. 20. Mature conidial wall. Note the thick, middle, electron-dense amorphous wall layer (ML) embedded with fibrillar electron-dense deposits, positioned between the inner wall layer (IL) and the outer wall layer (OL). Fig. 21. Developing conidial wall with mucilaginous substance (MS), inner wall layer, and outer wall layer. Fig. 22. Section illustrating septal pore, inner septal layer (IS), and outer septal layer (OS). Figs. 23 and 26. Polar region of immature (Fig. 23) and mature (Fig. 26) conidia. Note the thickened layer of the conidial-delimiting septum (DS) between conidia and the outer wall layer, which is continuous between adjacent conidia. Also note the electron-dense middle wall layer, which is thinner at the periclinal wall of the polar cells. The outer septum (S1) of the mature conidium is bilamellate (Fig. 26). Figs. 24 and 27. Middle region of immature (Fig. 24) and mature (Fig. 27) conidia illustrating the central septum (S3). The central septum is derived from the inner wall layer and becomes bilamellate at maturity. Figs. 25 and 29. Immature conidia with a plug-like protrusion (PG) joining to the socket-like depression (SC) of an adjacent mature conidium. Note that only the inner and the middle wall layers of the periclinal conidial wall are found at both ends of the conidia. Figs. 28 and 30. Mature conidia with plug-and-socket-like structures. Note that the conidium-delimiting septum lyases longitudinally and the outer wall layer of the periclinal conidial wall is absent at both ends of the conidia. Scale bars = 0.5 μm in Figs. 20–22 and 1 μm in Figs. 23–30.

(i) the primary conidial wall is a continuous wall layer (Fig. 14) and (ii) this wall layer, which later becomes the outer conidial wall layer, apparently retains its continuity at maturity (Figs. 23 and 26).

In summary, the developmental process of the conidial chains in *S. nigroseptatum* can be described as follows: endogenous conidial ontogeny, with primary conidial walls formed by de novo synthesis and apical wall-building (sensu Hawksworth et al. 1995; Fig. 32A), followed by retrogressive delimitation; apical wall-building then ceases and is replaced by ring wall-building at the base of the conidigenous cell, adding more retrogressively delimited conidia (sensu Hawksworth et al. 1995; Fig. 32B). The outer (original) conidiogenous cell wall breaks as a true conidial chain is formed (Fig. 32C). Maturation of conidia involves deposition of two inner wall layers, formation of five distosepta (progressively S1, S2, and then S3 septa; Figs. 1–4, 7, and 31), and formation of plug-and-socket-like structures at the poles (Figs. 7, 12, and 31). Conidia in chains secede schizolytically (Figs. 7, 12, 25, and 28) and with a fringe of residual conidium-delimiting cross wall material at each end (Figs. 6, 9, and 12).

**Plug-and-socket-like structures**

Among the known species of *Sporoschisma* (Goh et al. 1997), *S. nigroseptatum* is the only species that has conidial polar structures that can be observed using light microscopy. Although this species has been frequently recorded in various countries, including Australia, Costa Rica, Hong Kong, India, Japan, and New Zealand (Rao and Rao 1964; Hughes 1966; Morris 1972; Goh et al. 1997), the structure has never been described. The SEM of *S. saccardoi* published by Nakagiri and Ito (1995) illustrates a minute, socket-like depression on a conidium, but this structure was not discussed.

It is unknown whether a similar mechanism of holding conidia in chains exists in other mitosporic genera. Other than *Sporoschisma*, many hyphomycetes genera produce chains of cylindrical, cuneiform, or fusiform conidia in a phialidic manner extruding from the tubular collarettes of conidiogenous cells or conidiohyles. These include *Aescoucomitum* Seaver, *Chaetochalara* B.C. Sutton & Piroz., *Chalara* (Corda) Rabenh., *Chalaraopsis* Peyronel, *Fusichalara* S. Hughes & Nag Raj, *Phialocephala* W.B. Kendr., *Phialophora* sect. *Catenulate* W. Gams, *Sporendicladia* G. Arnaud ex Nag Raj & W.B. Kendr., and *Sporoschismopsis* Hol.-Jech. & Hemmert (Ellis 1971, 1976; Carmichael et al. 1980). On the other hand, isthmospores produced by genera such as *Speirospira* and *Wiesneriomycetes* are reported as being capable of seceding, although with difficulty, at the constricted region of the conidia (Ellis 1976). Other hyphomycetes, including some species of *Acremonium* Link and *Monocillium* S.B. Sakse, produce globular conidia with a nipple-shaped protrusion that links to adjacent conidia in the conidial chain (Carmichael et al. 1980; Matsushima 1985). Whether these hyphomycetes possess mechanisms similar to those of *S. nigroseptatum* for holding individual conidia together is not known. Further examination of these fungi using light and electron microscopy are necessary.

**Taxonomic notes**

Wingfield et al. (1995) demonstrated that a *Chalara*-like taxon exhibited conidial development by apical wall-building, resulting in a discontinuous outer wall layer between adjacent conidia (i.e., false-chain type) and a fringe of the wall layer existing only at the proximal end of the conidia. In contrast, *Sporoschisma* and *Chalara* species produce conidia by ring wall-building, resulting in a continuous wall layer between adjacent conidia (i.e., true-chain type) with a fringe of septum material at both ends of the conidia (Hawes and Beckett 1977b; Minter et al. 1982, 1983a, 1983b). Wingfield et al. (1995) did not place their taxon in the genus *Chalara* because of the different wall-building pattern and it remained unnamed. However, some *Chalara*-like taxa with only a basal fringe of wall material are still accepted as species of *Chalara* (e.g., *Chalara angustata* Kowalski & Halschlag, 1996). We agree with Wingfield et al. (1995; also Subramanian 1972) that the wall-building type of conidial development could be used as an important feature in delimiting genera of hyphomycetes. A new genus is probably needed to accommodate the *Chalara*-like species with apical wall-building.

*Sporoschisma saccardoi* is morphologically similar to *S. nigroseptatum*. The former is conventionally distinguished from the latter in having conidia with four inner cells of about equal size and with narrower black septal bands (Nag Raj and Kendrick 1975). Nakagiri and Ito (1995), however, demonstrated that these characters are inconsistent both in culture and on natural substrates. Using light microscopy, we have observed that conidia of *S. nigroseptatum* consistently possess distinct plug-and-socket-like structures at the ends, while those of *S. saccardoi* do not. We consider this is a helpful

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Figs. 31 and 32. Diagrammatic interpretations of the different developmental stages of the conidia, conidiogenous cells, and conidiophores of _S. nigroseptatum_. Fig. 31. Development of conidia in true conidial chains. (A–F) Progressive developmental stages of conidia in chains (Fig. 31A being at the earliest stage and Figs. 31E and 31F being at the latest stage) illustrating outer wall layer (OL), middle wall layer (ML), inner wall layer (IL), outer septal layer (OS), inner septal layer (IS), plug-like structure (PG), socket-like structure (SC), and conidium-delimiting septum (DS). Fig. 32. Development of conidial chains within conidiogenous cells. (A) Young conidiophore and conidiogenous cell with hypothesized apical wall-building (AWB). Primary conidial wall (asterisk) and conidium-delimiting septum formed by de novo synthesis. (B) Mature conidiophore and conidiogenous cell with retrogressively formed conidium-delimiting septum, and ring wall-building (RWB) replacing apical wall-building. (C) Mature conidiophore and conidiogenous cell. The tip of the conidiogenous cell wall breaks as the conidial chain is formed progressively by ring wall-building. AL, additional wall layer; CW, conidial wall; PW, conidiogenous cell wall; S, septum.

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