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Comparative morphology and phylogenetic placement of two microsclerotial black fungi from *Sphagnum*

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Abstract: *Capnobotryella renispora* and *Scleroconidioma sphagnicola* form black, irregularly shaped microsclerotia that are indistinguishable in gross morphology on leaves of *Sphagnum fuscum*. In culture, microsclerotia of these fungi were similar, in that mature component cells possessed thick, highly melanized cell walls, poorly defined organelles, large lipid bodies and simple septa. They were different in morphogenesis, in the way their component cells were organized and in disseminative propagules. Microsclerotia of *S. sphagnicola* formed phialidic conidiogenous cells on their surface, whereas in *C. renispora*, adjacent cells in mature microsclerotia often separated from each other by septum schizolysis and formed chlamydospores. The identification of *C. renispora* from *Sphagnum* is provisional despite a 100% ITS sequence match with data for a culture derived from the type strain. No holoblastic, reniform conidia typical of the species were formed in nature or in culture, and the SSU sequence for a separately preserved culture of the ex-type strain was markedly divergent. Parsimony analyses of nuclear ribosomal DNA sequences showed that these two fungi were related to separate orders of Dothideomycetes. Both SSU and ITS data supported a close relationship for *S. sphagnicola* to the Dothideales sensu stricto, while the closest ITS match was to *Rhizosphaera* spp. In the SSU analyses, *C. renispora* was nested within the Capnodiales.

Key words: black yeasts, *Capnobotryella*, Capnodiales, dematiaceous hyphomycetes, Dothideales, Dothideomycetes, meristematic ascomycetes, *Scleroconidioma*, ultrastructure

INTRODUCTION

Black, irregularly shaped microsclerotia were collected on the leaves of *Sphagnum fuscum* (Schimp.) Klinggr growing in a southern boreal bog in Alberta, Canada. While these structures appeared similar under a dissecting microscope, once cultured they clearly represented two different fungi. One fungal species produced mycelial colonies with abundant black microsclerotia, many of which bore papillate conidiogenous cells that produced successive hyaline, bacilliform, conidia, while the other formed black, cerebriform colonies devoid of blastic conidia. The former fungus was named *Scleroconidioma sphagnicola* gen. nov. & sp. nov. (Tsuneda et al 2000, 2001b), and is a pathogen of *Sp. fuscum* (Tsuneda et al 2001a). The colony characteristics of the latter fungus were reminiscent of *Phaeosclera dematioides* Sigler, Tsuneda & Carmichael (Sigler et al 1981) and *Capnobotryella renispora* Sugiyama (Sugiyama and Amano 1987), dematiaceous hyphomycetes that also are cerebriform in culture. *Phaeosclera dematioides* is a nonsporulating taxon isolated from the pith of *Pinus contorta* Dougl. and is known only in vitro. Dematiaceous septate hyphae convert to thick-walled bulbil-like masses of sclerotic cells analagous to microsclerotia. *Capnobotryella renispora* is a sooty mold producing dark, moniliform, tapered hyphae that give rise to two-celled, reniform, blastic conidia directly from distal cells. Strains have been isolated from subicula of another sooty mold, *Capnobotrys neesii* Hughes, on *Abies veitchii* Lindl. (Sugiyama and Amano 1987) and also from roof tiles (Titze and de Hoog 1990). In the absence of more detailed morphological data, however, neither name could be applied with confidence to the cerebriform fungus.

The striking similarity between the microsclerotia of *S. sphagnicola* and the cerebriform fungus on *Sphagnum* prompted a comparative examination of the development of these structures. In addition, our inability to assign a name to the cerebriform fungus, or to suggest phylogenetic placement for both *S. spagnicola* and the cerebriform fungus, on the basis of morphology alone, led to a comparison of DNA

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sequence data derived from the nuclear ribosomal gene, specifically the small-subunit operon (SSU) and the internal transcribed spacer region (ITS). During these investigations, gene sequence comparisons indicated that the cerebriform fungus is identical to the ex-type culture of *C. renispora*, deposited at the Centraalbureau voor Schimmelcultures (CBS). Despite the lack of conidiation, this name is applied here to the strain of the cerebriform fungus isolated from *Sphagnum*. Because the development of the microsclerotia of *S. sphagnicola* has been reported elsewhere (Tsuneda et al 2000, 2001b), the emphasis of the developmental work reported here is on the strain of *C. renispora* from *Sphagnum*.

MATERIALS AND METHODS

Fungal strains and microscopy.—Subcultures of *C. renispora* UAMH 9870 and *S. sphagnicola* UAMH 9731, both isolated from leaves of *Sp. fuscum*, were used throughout this study. For comparison, three additional strains of *C. renispora*, CBS 214.90 (IAM13014; ex-type strain), CBS 215.90 (IAM13015) and CBS 572.89, were examined by light microscopy. *Scleroconidioma sphagnicola* was grown on cornmeal agar with dextrose (CMAD; Difco, Detroit, Michigan) (Tsuneda et al 2000), while all strains of *C. renispora* were grown on potato-dextrose agar (PDA, Difco) or 2% malt-extract agar (MEA, Difco). Incubation was at 20 C in the dark. Methods for (cryogenic and ordinary) scanning electron and transmission electron microscopy (SEM and TEM, respectively), including methods for material preparation, were the same as those used in Tsuneda et al (2001a, b).

DNA sequencing.—DNA sequences of portions of the nuclear rRNA gene were determined for the five strains examined morphologically. Genomic DNA was extracted from mycelium grown on PDA using a FastDNA® Extraction Kit (BIO 101 Inc., Carlsbad, California) and a FastPrep™ Cell Disruptor machine (BIO 101, Carlsbad, California). DNA amplification and cycle sequencing reactions were performed on a Techne Genius thermocycler (Princeton, New Jersey). PCR reactions were performed in 25 (L volumes using Ready-To-Go™ PCR Beads (Amersham Pharmacia Biotech Inc., Piscataway, New Jersey) and 2 μL of template DNA. PCR cycling parameters included 30 cycles of denaturation at 95 C for 1.5 min, annealing at 56 C for 1 min and extension at 72 C for 2 min, with an initial denaturation of 4 min and a final extension step of 10 min. Primers NS1 and ITS4 (White et al 1990) were used to amplify more than 1700 basepairs of the small subunit (SSU) as well as the complete ITS region, including the ITS1, 5.8S and ITS2. Amplified products were purified using the Wizard™ PCR Preps DNA Purification System (Promega Corp., Madison, Wisconsin), and DNA concentrations were estimated from fragments stained by ethidium bromide and separated by agarose gel electrophoresis.

Sequencing reactions were performed using the BigDye™ Terminator Cycle Sequencing System (Applied Biosystems, Foster City, California) with the recommended cycling parameters. The reaction mixture comprised one-eighth strength BigDye™ sequencing mix, an equal volume of the reaction booster *half*BD (Bio/Can Scientific, Mississauga, Ontario, Canada), 0.5 μL sequencing primer (5(M); 20–50 ng purified DNA and sterile water to a final volume of 5 μL. Reactions were purified after bringing the volume to 20 (L, by ethanol/sodium acetate precipitation and resuspended as recommended for processing on an ABI PRISM™ 310 DNA sequencer (Applied Biosystems, Foster City, California). Sequencing primers were selected from the range of SSU and ITS primers given in White et al (1990) and Landvik et al (1997) to get sequence data for the whole region. Consensus sequences were determined from overlapping sequence data for both DNA strands using the software Sequencher™ (Gene Codes Corp., Ann Arbor, Michigan).

Phylogenetic analysis.—The initial SSU data matrix included 86 small-subunit sequences retrieved from nucleotide sequence databases (GenBank; http://www.ncbi.nlm.nih.gov) and chosen to represent the phylogenetic diversity of Ascomycetes. The SSU and ITS data matrices used in the final analyses included accessions identified with Gapped-BLAST (Altschul et al 1997) as having a high sequence similarity to the two fungi under study and other taxa suspected of being closely related, based on preliminary analyses of the larger SSU dataset. Accession numbers for the GenBank sequences not listed in TABLE I are given on FIGS. 26–28. Sequence alignments were generated with the PileUp option of GCG 10.1 (Genetics Computer Group, Wisconsin Package, Madison, Wisconsin; http:www.cbr.nrc.ca) and then optimized by eye with Se-Al (Sequence Alignment Program v1. d1; http://evolve.zoo.ox.ac.uk/software/Se-Al/main.html). Alignments were trimmed to remove the characters at each end, for which there was missing data because of minor differences in sequence length. The final ITS alignment included the complete ITS1, 5.8S and ITS2 regions. The data matrices were subjected to parsimony analysis using the heuristic search option of PAUP* version 4.0b8 (Swofford 1999) with stepwise addition of taxa, tree bisection-reconnection (TBR) branch swapping and gaps treated as missing data. Support for the branching topologies was evaluated by bootstrap analysis.

RESULTS

Morphological observations.—FIGURES 1–9 show the typical process of microsclerotium development in *Capnobotryella renispora* (UAMH 9870). Two distinct modes of cell multiplication occur. In the first mode, cells become enlarged more or less isodiametrically with subsequent subdivisions (FIGS. 1, 2, arrows), accompanied by the frequent formation of bubble-like outgrowths or blebs (FIGS. 1, 2, arrowheads). Blebs also were subdivided by septa. In the second mode, apical and polar cell multiplication resulted in the production of acropetal chains of globose cells (moniliform hyphae) (FIG. 4). The first formed new cell in this mode often erupted through the outer cell

TABLE I. Dothideomycetous fungi included in phylogenetic analyses of SSU sequence data with GenBank accession, strain accession notes, and reference citation information

Order[a]	Family	Species[b]	GenBank Accession[c]	Strain Accession[d]	Reference if available or GenBank depositor (in parentheses)
Capnodiales	Capnodiaceae	• *Antennariella californica* Bat. & Cif.	AF006722*	LAM 300855	Reynolds 1998
	Capnodiaceae	*Capnodium citri* Berk. & Desm.	AY016340	CBS 451.66	Lumbsch and Lindemuth 2001
	Capnodiaceae	*Capnodium dermatum* (V.A.M. Mill. & Bonar) D.R. Reynolds	AF006724*	LAM 20668	Reynolds 1998
	Capnodiaceae	*Scorias spongiosa* (Schwein.) Fr.	AF006726*	UAMH 4777	Reynolds 1998
	Coccodinianceae	*Coccodinium bartschii* A. Massal.	U77668	UME 30232	Winka et al 1998
	Metacapnodiaceae	• *Capnobotryella renispora* Sugiy.	AY220611	UAMH 9870	this study
	Metacapnodiaceae	• *Capnobotryella renispora* Sugiy.	AY220612	CBS 214.90	this study
	Metacapnodiaceae	• *Capnobotryella renispora* Sugiy.	AY220613	CBS 215.90	this study
	Metacapnodiaceae	• *Capnobotryella renispora* Sugiy.	AY220614	CBS 572.89	this study
	Metacapnodiaceae	• *Capnobotryella sp. nov.*	AJ301706	"NH4-3"	(Sterflinger 2000)
	Metacapnodiaceae	*Chaetasbolisia falcata* V.A.M. Mill. & Bonar	AF006725*	LAM 21988	Reynolds 1998
Dothideales	Dothideaceae	*Dothidea hippophaeos* (Pass.) Fuckel	U42475	CBS 186.58	Berbee 1996
	Dothideaceae	*Dothidea insculpta* Wallr.	U42474	CBS 189.58	Berbee 1996
	Dothideaceae	*Dothidea ribesia* Pers.	AY016343	CBS 195.58	Lumbsch and Lindemuth 2001
	Dothideaceae	*Stylodothis puccinioides* (DC.) Arx & E. Müll.	AY016353	CBS 193.58	Lumbsch and Lindemuth 2001
Patellariales	Patellariaceae	*Rhytidhysteron rufulum* (Spreng.) Speg.	AF201452	UBC F13903	Inderbitzin et al 2001
Pleosporales	Leptosphaeriaceae	*Leptosphaeria maculans* (Desm.) Ces. & De Not.	U04233	"Leroy"	Morales et al 1995
	Lophiostomataceae	*Lophiostoma crenatum* (Pers.) Fuckel	U42485	CBS 629.86	Berbee 1996
	Melanommataceae	*Melanomma sanguinarium* (P. Karst.) Sacc.	AF242265	UPSC 1924	(Winka 2000)
	Phaeosphaeriaceae	*Phaeosphaeria nodorum* (E. Müll.) Hedj.	U04236	DAOM 215173	Morales et al 1995
	Pleosporaceae	*Cochliobolus sativus* (S. Ito & Kurib.) Drechsler ex Dastur	U42479	DAOM 172489	Berbee 1996
	Pleosporaceae	*Pleospora betae* (Berl.) Nevod.	U43466	IMI 156653	(Dong 1997)
	Pleosporaceae	*Pleospora herbarum* (Pers.:Fr.) Rabenh.	U05201	DAOM 150679	Morales et al 1995.
Incertae sedis	Botryosphaeriaceae	*Botryosphaeria rhodina* (Berk. & M.A. Curti.) Arx	U42476	CBS 356.59	Berbee 1996
	Botryosphaeriaceae	*Botryosphaeria ribisi* Gross. & Duggar	U42477	CBS 121.26	Berbee 1996
	Dothioraceae	*Delphinella strobiligena* (Desmaz.) Sacc.	AY016341	CBS 735.71	Lumbsch and Lindemuth 2001
	Dothioraceae	*Discosphaerina fagi* (H.J. Hudson) Barr	AY016342	CBS 171.93	Lumbsch and Lindemuth 2001
	Dothioraceae	• *Aureobasidium pullulans* (de Bary) G. Arnaud	M55639	CBS 584.75	Illingworth et al 1991
	Mycosphaerellaceae	*Mycosphaerella mycopappi* (A. Funk & Dorworth) Crous	U43449	ATCC 64711	(Dong 1997)
	Planistromellaceae	• *Hyphospora agavaciensis* Ramaley	Y18699	CBS 619.95	Sterflinger et al 1999
	Incertae sedis	• *Hortaea werneckii* (Horta) Nishim. & Miyaji	Y18693	CBS 107.67	Sterflinger et al 1999

TABLE I. Dothideomycetous fungi included in phylogenetic analyses of SSU sequence data with GenBank accession, strain accession notes, and reference citation information

Order[a]	Family	Species[b]	GenBank Accession[c]	Strain Accession[d]	Reference if available or GenBank depositor (in parentheses)
	Incertae sedis	• *Phaeosclera dematioides* Sigler, Tsuneda & J.W. Carmich.	Y11716	CBS 157.81	Sterflinger et al 1997
	Incertae sedis	• *Phaeotheca fissurella* Sigler, Tsuneda & J.W. Carmich.	Y18697	CBS 520.89	Sterflinger et al 1999
	Incertae sedis	• *Sarcinomyces crustaceus* Lindner	Y11355	CBS 156.89	Sterflinger et al 1997
	Incertae sedis	• *Sarcinomyces petricola* Wollenz. & de Hoog	Y18702	CBS 600.93	Sterflinger et al 1999
	Incertae sedis	• *Scleroconidioma sphagnicola* Tsuneda, Currah & Thormann	AY220610	UAMH 9731	this study

[a] Classification follows Eriksson et al 2001.

[b] • denotes anamorph species (sooty moulds, meristematic fungi, and black yeasts).

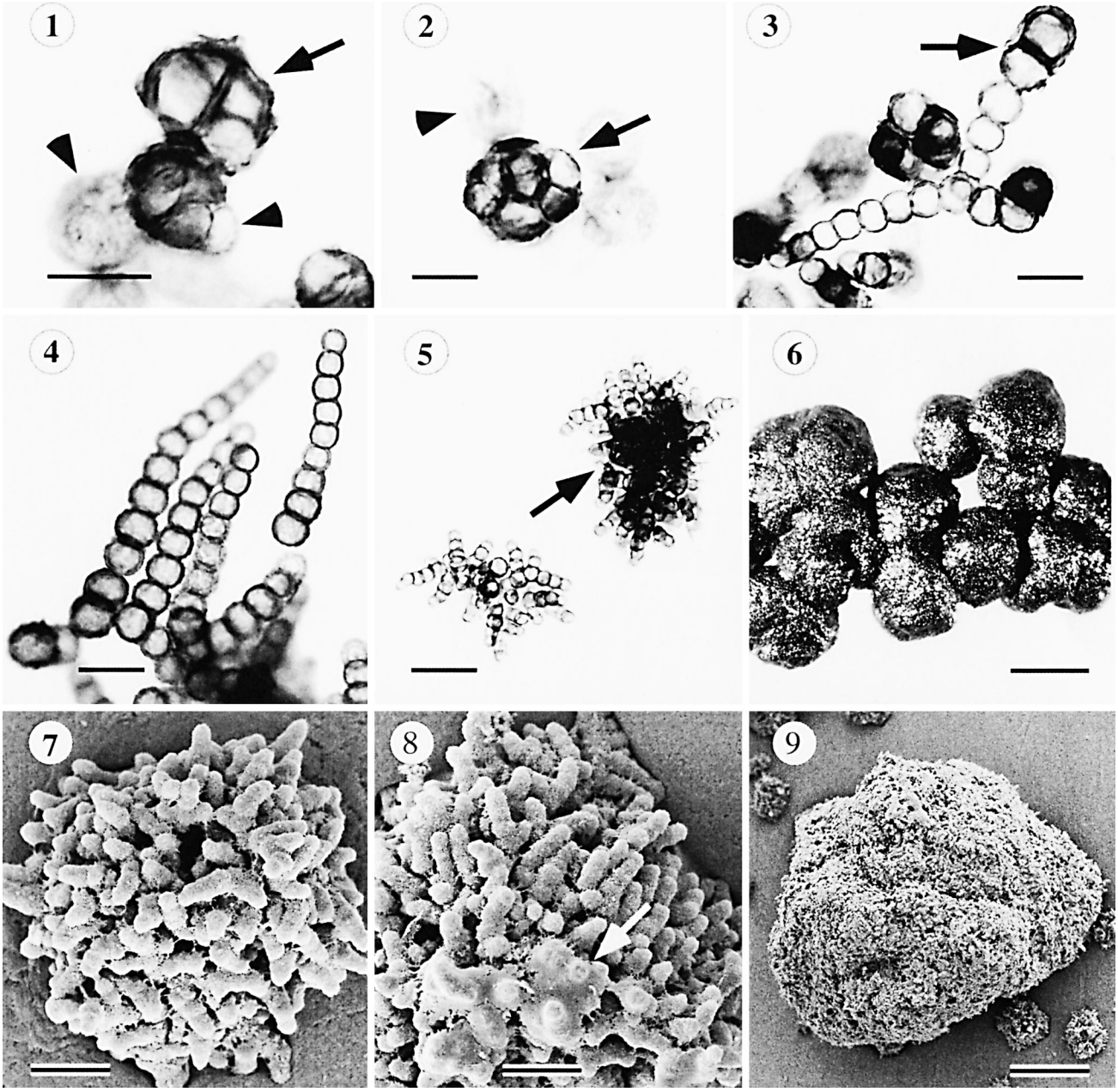
[c] unaligned sequences at least 1700 basepairs in length; *indicates a short sequence, 900–1100 basepairs.

[d] ATCC, American Type Culture Collection; CBS, Centraalbureau voor Schimmelcultures; DAOM, Canadian Collection of Fungus Cultures; IMI, International Mycological Institute (CABI Bioscience); LAM, Natural History Museum of Los Angeles County; UAMH, University of Alberta Microfungus Collection and Herbarium; UBC University of British Columbia Herbarium; UC, University of California; UME, Umeå University; UPSC Uppsala University Culture Collection; identifiers in quote marks are author designations.

wall of its mother cell (FIG. 3, arrow), and all the subsequently formed cells remained unicellular (FIG. 4). The two modes co-occurred in the initial stages of microsclerotium development (FIG. 3), however, the second mode became more dominant as the morphogenesis proceeded (FIGS. 5, 7, 8). Older cells in microsclerotium initials became darkly pigmented (FIG. 5, arrow) and exuded an amorphous coating material (FIG. 8, arrow) that made the black microsclerotium shiny (FIG. 6). Well-developed microsclerotia were cerebriform (FIG. 9) and adjacent ones frequently fused (FIG. 6). No conidia were found in culture or on microsclerotia formed in nature. These results also applied to the three older strains of *C. renispora* (CBS 214.90, 215.90 and 572.89), except that CBS 572.89 produced numerous, mostly two-celled conidia on both PDA and MEA. Morphological characters of this strain agreed well with those described by Sugiyama and Amano (1987). In CBS 214.90 and 215.90, conidium production in culture was either nil or scarce. Conidia, if present, were 4.5–6.5(–8)(3–4.5 μm in CBS 214.90 and 5–5.5 (4–5 μm in CBS 215.90, both shorter than Sugiyama's description of the species in culture, 7–10 (3–4 μm but similar to the measurements from material in vivo.

TEM of cerebriform colonies of *C. renispora* revealed that the apical cells of growing hyphae first showed apical elongation (FIG. 10) and then were subdivided by a simple septum with Woronin bodies (FIG. 11, arrows). The actively growing cells had well-defined organelles and electron-light cell walls (FIG. 10) on which melanin granules deposited rapidly (FIGS. 10, 12, arrows). In the cells delimited from the growing apices, small vacuoles appeared and subsequently enlarged. Larger vacuoles usually contained electron-dense inclusions (FIG. 13, TABLE I). Eventually, these cells became filled with large lipid bodies (FIG. 14) and possessed poorly defined organelles and heavily melanized cell walls (FIGS. 14, 15). Lateral branches often occurred (FIG. 14, arrow). Mature cells possessed a newly formed, electron-light, thin cell wall between the melanized, older cell wall and plasma membrane. In some cases, the newly formed and older cell walls were separated by a large number of melanin granules that had accumulated between them (FIG. 15, arrow).

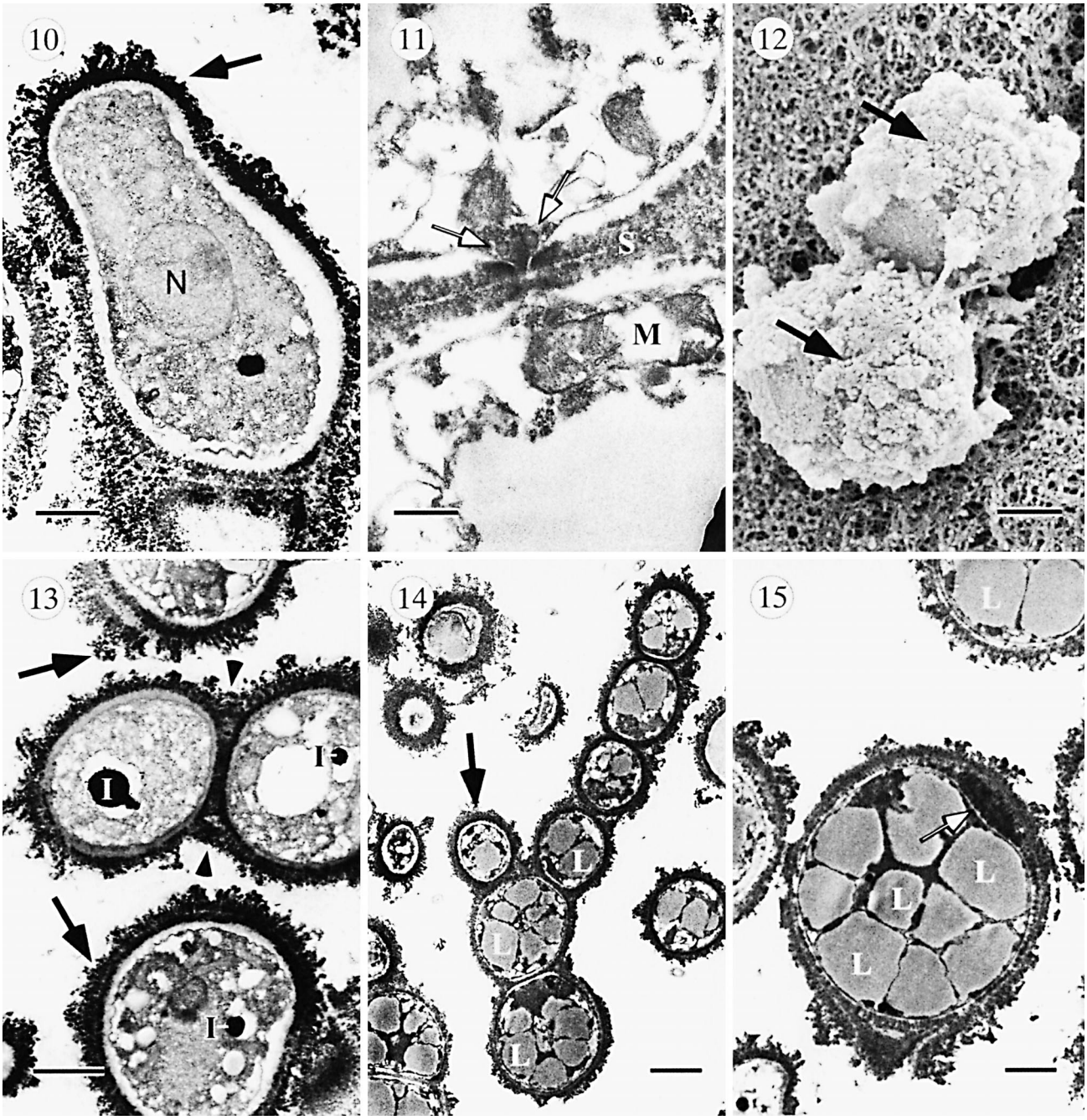
Shown in FIGS. 16–19 are cells of cryofractured, well-developed microsclerotia of *C. renispora*. Many of these cells were either in the process of septum schizolysis (FIGS. 16, 17, arrows) or had separated from adjacent cells (FIGS. 18, 19, arrows). Septal pore sites were recognized as minute protuberances (FIG. 18, arrowheads). Large lipid bodies (L) and the newly developed (or developing) cell wall (arrowheads) were evident in cells of near-median fracture (FIG.



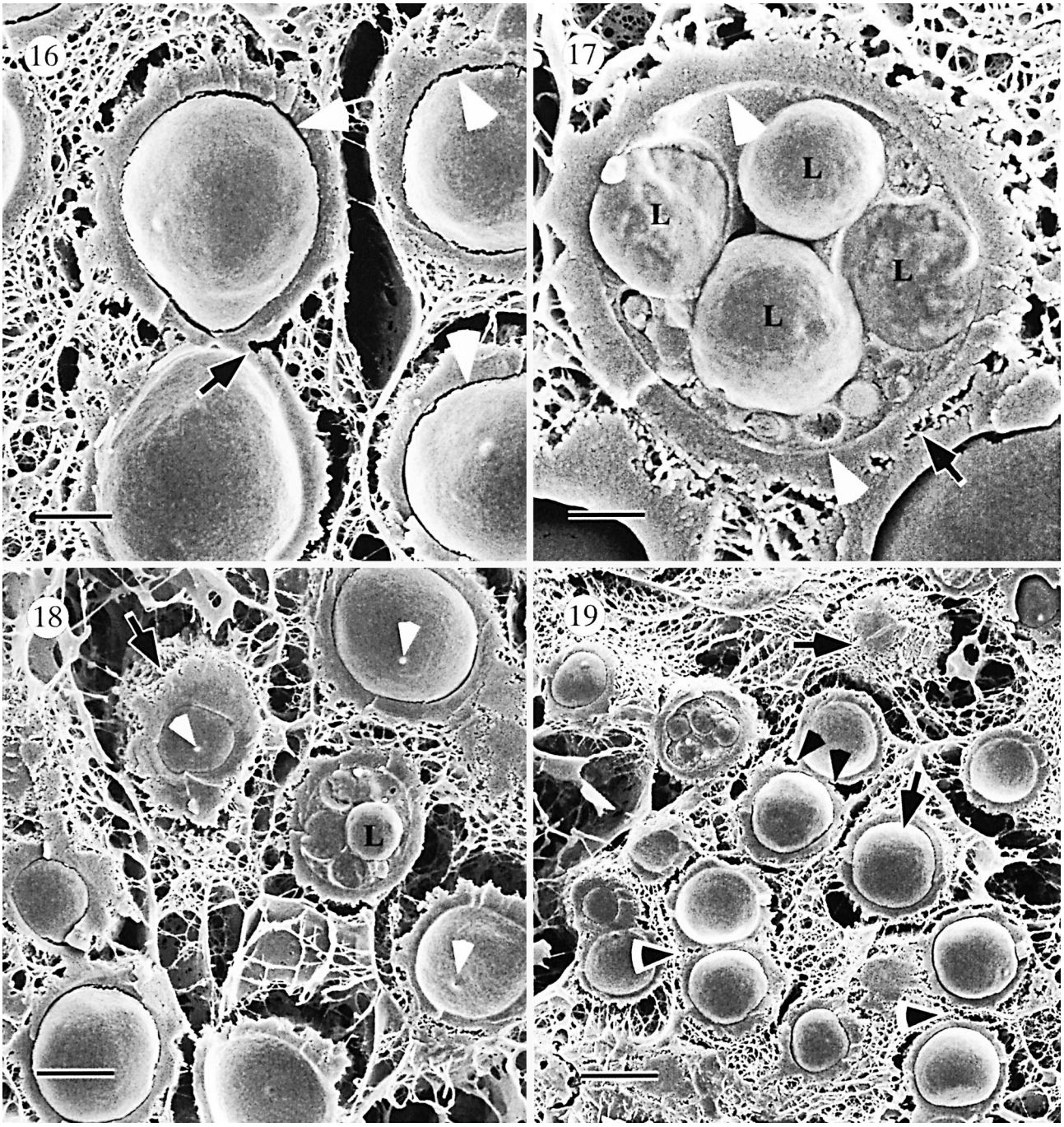
FIGS. 1–9. Typical developmental process of cerebriform colonies (microsclerotia) in *Capnobotryella renispora* UAMH 9870. 1, 2. Initial stages showing more or less isodiametric enlargement of mother cells with subdividing cells (arrows). Arrowheads indicate blebs. Bar = 10 μm. 3, 4. Formation of acropetal chains of globose cells (moniliform hyphae) from thick-walled darkly pigmented mother cells (chlamydospores). A new cell breaks out of the mother cell walls (arrow in 3). Bar = 10 μm. 5. Microsclerotial initials. Older cells are darkly pigmented (arrow). Bar = 30 μm. 6. Well-developed microsclerotia. Adjacent ones often fuse. Bar = 500 μm. 7–9. SEM micrographs showing later stages leading to the formation of a cerebriform colony (microsclerotium). No conidia are formed. The arrow in 8 indicates amorphous coating material that will eventually cover the entire microsclerotium. Bar = 20 μm in 7 and 8, 200 μm in 9.

17). In older cells, the thick, melanized outer and the newly formed inner cell walls were readily separable (FIG. 16, arrowheads) by fracturing.

In *S. sphagnicola*, mode (1) of cell multiplication, mentioned above, dominated in microsclerotium development (FIGS. 20–22). Although its cells sometimes formed moniliform chains, most of them subsequently were subdivided by septa (FIG. 20, arrow). FIGURES 23–25 are cryofractured views of mature microsclerotia showing that component cells of microsclerotia are cemented firmly together, forming a solid sclerotic mass of cells. Unlike in *C. renispora*, sep-

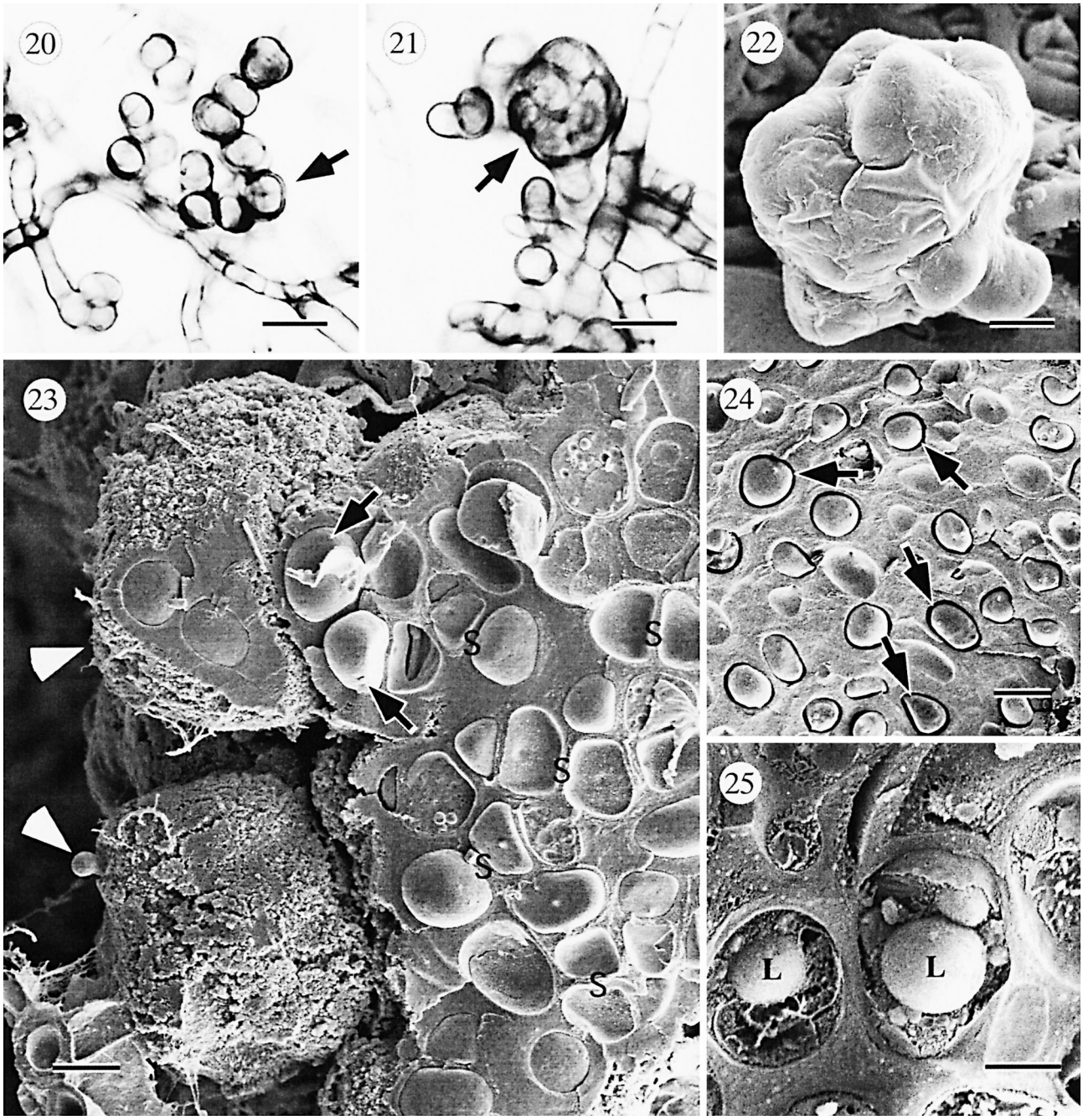


FIGS. 10–15. Ultrastructure of component cells of *Capnobotryella renispora* microsclerotia (10, 11, 13–15, TEM; 12, SEM). 10. Actively growing apex cell of a moniliform hypha. Cell organelles, including a nucleus (N), are well defined, and developing cell wall is electron light and already covered with a thick layer of melanin granules (arrow). Bar = 1 μm. 11. Simple septum (S) associated with Woronin bodies (arrows). M = mitochondrion. Bar = 0.2 μm. 12. Chlamydospore that germinated to form a new globose cell. Arrows indicate melanin granules. Bar = 2 μm. 13. Maturing cells with large vacuoles containing electron-dense inclusions (I). Note lack of large lipid bodies at this stage. Abundant melanin granules occur on the cell surface (arrows) and between adjacent cells (arrowheads). Bar = 2 μm 14. Mature cells of a hypha filled with large lipid bodies (L). The arrow indicates a side branch. Bar = 4 μm. 15. Mature cell (chlamydospore) after septal schizolysis. Cell organelles are poorly defined, and cell walls are heavily impregnated with melanin. The arrow indicates the area where newly developed and older cell walls are separated by accumulated melanin granules. The newly developed wall is thin and electron light. L = lipid bodies. Bar = 2 μm.



FIGS. 16–19. Cryo-fractured views of mature microsclerotia of *Capnobotryella renispora*. 16. Cells in the process of septum schizolysis (arrow). Arrowheads indicate a gap between the newly formed and older cell wall layers. Bar = 2 μm. 17. Cell of near-median fracture having large lipid bodies (L) and a newly developed (developing) cell wall layer (arrowheads). The arrow points to the schizolysing septum. Bar = 1 μm. 18, 19. Lower-magnification micrographs, showing separating and separated cells (chlamydospores) (arrows). Arrowheads in 18 indicate septal pore areas and those in 19 point to schizolysing septa. L = lipid body. Bar = 3 μm in 18, 5 μm in 19.

tum schizolysis did not occur between adjacent cells and microsclerotia often developed a layer of, or small masses of, conidiogenous cells on their surface (FIG. 23, arrowheads). As with *C. renispora*, however, component cells of well-developed microsclerotia possessed an inner, newly formed cell wall (Tsuneda et al 2001b) and were filled with large lipid bodies (FIG. 25). There was a distinct gap between the newly formed and older cell walls (FIG. 24, arrows), and cells possessing only the newly formed cell wall could



FIGS. 20–25. Typical developmental process of microsclerotia (conidiomata) in *Scleroconidioma sphagnicola* (UAMH 9731). 20. Chain of subglobose cells that undergo subdivision (arrow). Bar = 10 μm. 21. Microsclerotium initial consisting of actively subdividing cells (arrow). Bar = 10 μm. 22. SEM view of a microsclerotium initial. Bar = 5 μm. 23–25. Cryo-fractured views of mature microsclerotia. 23. Small masses of conidiogenous cells (arrowheads) developed on a microsclerotium. The lower arrowhead points to a conidium emerging from a conidiogenous cell. Septa (S) of component cells of the microsclerotium show no sign of schizolysis. Arrows point to cells dislodged from their older cell walls upon fracturing. Bar = 3 μm. 24. Part of a mature microsclerotium. Distinct gap is evident between newly formed and older cell walls in many component cells (arrows). Bar = 6 μm. 25. Mature component cells containing large lipid bodies (L). Bar = 3 μm.

be dislodged from their older cell walls upon fracturing (FIGS. 23, arrows).

DNA sequencing.—The lengths of the SSU sequences determined for *S. sphagnicola* UAMH 9731 and *C. renispora* UAMH 9870 were 1744 basepairs (bp). The SSU sequences were complete at the five-prime end. The length of the sequence determined for *S. sphagnicola* using primers ITS1–4 was 512 bp; the ITS1 and

ITS2 alone were 176 and 163 bp, respectively. For *C. renispora*, the total ITS sequence was 472 bp in length; the ITS1 and ITS2 alone were 146 and 153 bp. The SSU and ITS sequences for CBS 214.90, ex-type culture of *C. renispora*, and CBS 215.90 were identical to those of UAMH 9870, except for the insertion of a single nucleotide (nt) in the ITS1 sequence of CBS 215.90 (four consecutive adenine nt, rather than three, positions 143–146). The ITS sequence for CBS 572.89 (469 bp) was only 95% similar to that of the other two strains, while the SSU sequence differed at three nt positions. The newly derived SSU-ITS sequences were deposited in GenBank as AY220610 (UAMH 9731), AY220611 (UAMH 9870), AY220612 (CBS 214.90), AY220613 (CBS 215.90) and AY220614 (CBS 572.89).

The closest GenBank SSU sequence matches for *S. sphagnicola* were for taxa classified in the Dothideales and Capnodiales, while the closest ITS matches were to *Rhizosphaera* spp. with 96% similarity. The closest GenBank SSU matches for *C. renispora* were also to the Dothideomycetes, and our identical ITS sequences for UAMH 9870 and the ex-type strain, CBS 214.90 (IAM13014), were a 100% match for a published ITS sequence (AJ244238; de Hoog et al 1999) for CBS 214.90. Other ITS sequences retrieved in the BLAST search were for *Trimmatostroma* spp. and *Mycosphaerella* spp., but the matches were at 90% similarity or less.

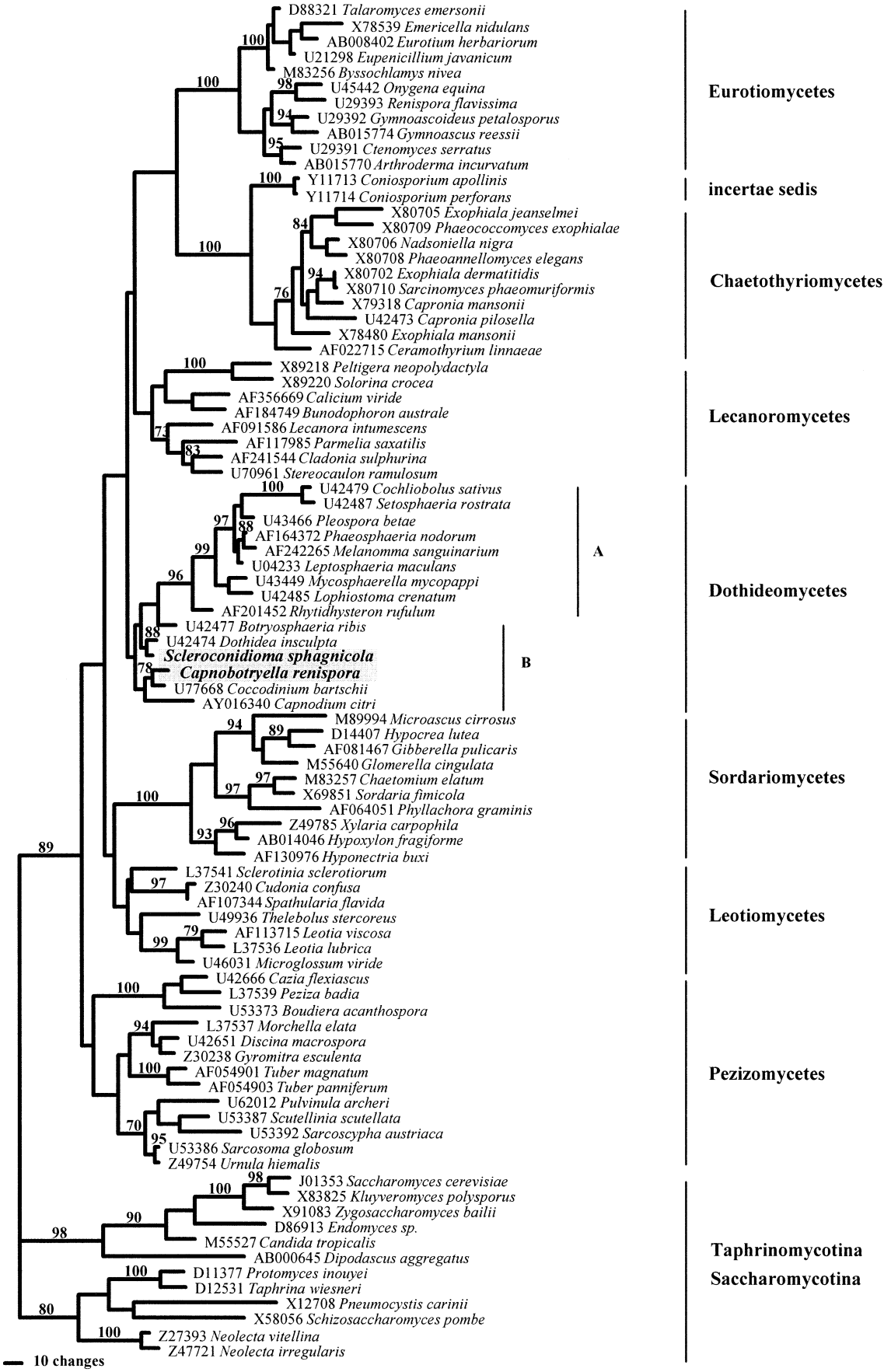
Our identical SSU sequences for CBS 214.90, CBS 215.90 and UAMH 9870 unexpectedly differed from a published SSU sequence for CBS 214.90 (Y18698; Sterflinger et al 1999) at 17 nt positions (98% similarity) and were only 91% similar to a second SSU sequence (AF006723; Reynolds 1998) for this strain of *C. renispora* separately preserved as ATCC 64891 (ITS sequence data was not available for this culture). Because of the marked discrepancies among the sequences available for the ex-type strain of *C. renispora*, only the sequences determined from the CBS cultures obtained for and examined in this study were used in the analyses.

Phylogenetic analysis.—The initial SSU data matrix comprised 88 taxa and 1983 aligned characters. Of these, 182 characters corresponded to short, unalignable sequence motifs unique to individual sequences (mostly for outgroup taxa); these were treated as insertions relative to the rest of the alignment and were excluded from the analyses. Of the remaining 1801 characters, 1039 were constant, 235 were parsimony uninformative and 527 were parsimony informative. An analysis using the heuristic search algorithm, with 100 replicates of random addition sequence, resulted in 18 equally parsimonious trees (MPTs) of 2648 steps and with CI = 0.432, RI = 0.711, RC = 0.308 and HI = 0.568. A bootstrap analysis (1000 replicates) was performed using the ''fast'' stepwise-addition search option to determine the level of support for the topology of the inferred phylogeny. Fungi classified in seven of the 11 classes of the subphylum Pezizomycotina were included in the analysis, while representatives of the Taphrinomycotina and Saccharomycotina served as outgroup taxa. Classification follows Eriksson et al (2001).

The initial analyses confirmed the BLAST search results that both *S. sphagnicola* UAMH 9731 and *C. renispora* UAMH 9870 were related to species classified in the Dothideomycetes (FIG. 26). The monophyly of the class was supported in the strict consensus of all MPTs (data not shown). Both fungi were excluded from the other major clades in the tree, identified by class name on FIG. 26, by virtue of their high bootstrap values and/or the number of character state changes, as evidenced by branch length. While bootstrap support was less than 50% for the Dothideomycetes clade, several subclades received higher support. Group A (FIG. 26), bootstrap 96%, comprised species in five families of the Pleosporales, *Mycosphaerella mycopappi* (Mycosphaerellaceae, *incertae sedis*) and *Rhytidhysteron rufulum* (Patellariales) in a basal position. Within the group, several suprafamilial subgroupings also received high bootstrap support. Group B comprised representatives of the Dothideales, Capnodiales and Botryosphaeriaceae (*incertae sedis*). *Scleroconidioma sphagnicola* clustered with *Dothidea insculpta* U42474 (Dothideales) with moderate bootstrap support at 88%; *Capnobotryella renispora* grouped with *Coccodinium bartschii* U77668 (Capnodiales) with moderate support of 78% in a sister group to *Capnodium citri* AY016340.

The final SSU alignment included representatives of the Group A Dothideomycetes, Dothideales, Capnodiales and Dothioraceae (*incertae sedis*), as well as several dematiaceous hyphomycetes. *Leotia lubrica* L37536, *L. viscosa* AF113715, *Microglossum viride* U46031, *Cudonia confusa* Z30240 (Leotiomycetes), *Peziza badia* L37539, *Morchella elata* L37537 and *Urnula hiemalis* Z49754 (Pezizomycetes) served as the outgroup. The ITS alignment included the sequences available for species in the Dothideaceae and Dothioraceae and closely related anamorph genera. Based on the SSU analysis, *Phaoesclera dematioides* AJ244254 and *Sarcinomyces crustaceous* AJ244258 were chosen as outgroup taxa. *Capnobotryella renispora* was not included in the ITS analysis; ITS data could not be aligned with confidence to the sequence for *S. sphagnicola* or to those sequences retrieved from GenBank as a result of the BLAST search.

The final SSU data matrix comprised 38 taxa and


D88321 Talaromyces emersonii
X78539 Emericella nidulans
AB008402 Eurotium herbariorum
U21298 Eupenicillium javanicum
M83256 Byssochlamys nivea
U45442 Onygena equina
U29393 Renispora flavissima
U29392 Gymnoascoideus petalosporus
AB015774 Gymnoascus reessii
U29391 Ctenomyces serratus
AB015770 Arthroderma incurvatum
Eurotiomycetes
Y11713 Coniosporium apollinis
Y11714 Coniosporium perforans
incertae sedis
X80705 Exophiala jeanselmei
X80709 Phaeococcomyces exophialae
X80706 Nadsoniella nigra
X80708 Phaeoannellomyces elegans
X80702 Exophiala dermatitidis
X80710 Sarcinomyces phaeomuriformis
X79318 Capronia mansonii
U42473 Capronia pilosella
X78480 Exophiala mansonii
AF022715 Ceramothyrium linnaeae
Chaetothyriomycetes
X89218 Peltigera neopolydactyla
X89220 Solorina crocea
AF356669 Calicium viride
AF184749 Bunodophoron australe
AF091586 Lecanora intumescens
AF117985 Parmelia saxatilis
AF241544 Cladonia sulphurina
U70961 Stereocaulon ramulosum
Lecanoromycetes
U42479 Cochliobolus sativus
U42487 Setosphaeria rostrata
U43466 Pleospora betae
AF164372 Phaeosphaeria nodorum
AF242265 Melanomma sanguinarium
U04233 Leptosphaeria maculans
U43449 Mycosphaerella mycopappi
U42485 Lophiostoma crenatum
AF201452 Rhytidhysteron rufulum
A
U42477 Botryosphaeria ribis
U42474 Dothidea insculpta
Scleroconidioma sphagnicola
Capnobotryella renispora
U77668 Coccodinium bartschii
AY016340 Capnodium citri
B
Dothideomycetes
M89994 Microascus cirrosus
D14407 Hypocrea lutea
AF081467 Gibberella pulicaris
M55640 Glomerella cingulata
M83257 Chaetomium elatum
X69851 Sordaria fimicola
AF064051 Phyllachora graminis
Z49785 Xylaria carpophila
AB014046 Hypoxylon fragiforme
AF130976 Hyponectria buxi
Sordariomycetes
L37541 Sclerotinia sclerotiorum
Z30240 Cudonia confusa
AF107344 Spathularia flavida
U49936 Thelebolus stercoreus
AF113715 Leotia viscosa
L37536 Leotia lubrica
U46031 Microglossum viride
Leotiomycetes
U42666 Cazia flexiascus
L37539 Peziza badia
U53373 Boudiera acanthospora
L37537 Morchella elata
U42651 Discina macrospora
Z30238 Gyromitra esculenta
AF054901 Tuber magnatum
AF054903 Tuber panniferum
U62012 Pulvinula archeri
U53387 Scutellinia scutellata
U53392 Sarcoscypha austriaca
U53386 Sarcosoma globosum
Z49754 Urnula hiemalis
Pezizomycetes
J01353 Saccharomyces cerevisiae
X83825 Kluyveromyces polysporus
X91083 Zygosaccharomyces bailii
D86913 Endomyces sp.
M55527 Candida tropicalis
AB000645 Dipodascus aggregatus
Taphrinomycotina
Saccharomycotina
D11377 Protomyces inouyei
D12531 Taphrina wiesneri
X12708 Pneumocystis carinii
X58056 Schizosaccharomyces pombe
Z27393 Neolecta vitellina
Z47721 Neolecta irregularis
10 changes

1652 aligned characters. Of these, 1285 were constant, 210 were parsimony uninformative and 157 were parsimony informative. An analysis using the heuristic search algorithm, with 100 replicates of random addition sequence, resulted in 15 MPTs of 651 steps and with CI = 0.662, RI = 0.700, RC = 0.463 and HI = 0.338. Results of a full bootstrap analysis (1000 replicates) using the heuristic search option are shown on one MPT (FIG. 27). Four sequences were short compared to the rest (TABLE I), but analysis of only that portion of the data matrix for which all sequences were complete did not alter the overall topology of the resulting tree (data not shown). The ITS alignment comprised 25 taxa and 569 aligned characters. Of these, 21 ambiguously aligned characters were excluded, 370 were constant, 69 were parsimony uninformative and 109 were parsimony informative. A branch and bound analysis resulted in 10 MPTs of 352 steps and with CI = 0.670, RI = 0.784, RC = 0.525 and HI = 0.330. Results of a full bootstrap analysis (1000 replicates) using the heuristic search option are shown on one MPT (FIG. 28).

Parsimony analysis of the final SSU alignment supported the placement of *S. sphagnicola* near the Dothideales but not within the order (FIG. 27). There was high bootstrap support (95%) for a monophyletic Dothideales sensu stricto, while *S. sphagnicola* clustered with *Delphinella strobiligena* (Dothioraceae) in an unsupported sister clade. *Aureobasidium pullulans* and its teleomorph *Discosphaerina fagi* (Dothioraceae) (Yurlova et al 1999), two identical sequences in the trimmed alignment, were in a basal position. *Capnobotryella renispora* UAMH 9870/CBS 214.90/215.90 grouped with an undescribed species of *Capnobotryella* within a large clade corresponding to the Capnodiales (FIG. 27). The clade included holomorphs in *Capnodium*, *Scorias* (Capnodiaceae) and *Coccodinium* (Coccodiniaceae) and the allied anamorph genera *Antennariella* (Antennulariaceae) and *Chaetasbolisia* (Metacapnodiaceae) but also the dematiaceous hyphomycete genera *Hyphospora* and *Hortaea*. Although the Capnodiales clade was retained in the strict consensus of all MPTs, in general, relationships within the order were unsupported by the bootstrap analysis, and terminal branch lengths were long, indicating a substantial genetic distance between species. The relationships of the other dematiaceous hyphomycetes included in the analysis, *Phaeotheca*, *Phaeosclera* and *Sarcinomyces*, were unresolved. Two species of *Botryosphaeria* clustered together with high bootstrap support, forming a sister clade with *Sarcinomyces petricola* to the well-supported clade comprising Group A Dothideomycete species.

In the ITS analysis, two major clades received high bootstrap support (FIG. 28). In the first (clade I), *S. sphagnicola* clustered with *Rhizosphaera* spp., as predicted by the BLAST search. This well-supported grouping was sister to a cluster of three groups corresponding to teleomorph genera in the Dothideaceae (*Dothidea*) and Dothioraceae (*Dothiora* and *Sydowia*). The coelomycetous genus *Rhizosphaera* has a putative teleomorph connection to *Phaeocryptopus* (Kirk et al 2001), yet *Phaeocryptopus gaumannii* (Rhode) Petrak (Venturiaceae) was excluded from the *Rhizosphaera* clade. Species of *Hormonema* clustered with *Dothiora* or *Sydowia*; both genera produce anamorphs in *Hormonema* (de Hoog et al 1999). Species of the anamorphic genus *Kabatina* were basal to the *Sydowia* and *Dothidea* clades but did not cluster together. The other major clade (II) comprised strains of the anamorphic black yeast *Aureobasidium pullulans*, [synonym *Kabatiella lini* (Lafferty) Karakulin] (Yurlova et al 1999).

DISCUSSION

On the host and in colony and morphogenic characteristics, both *S. sphagnicola* and *C. renispora* resemble the so-called ''meristematic fungi,'' fungi with growth characterized by slowly expanding, black, cauliflower-like colonies (= cerebriform colonies or microscerotia) and reproduction by isodiametric enlargement with subdividing cells (Sterflinger et al 1999). Some of these fungi possess budding cells and have a close taxonomic affinity to the black yeasts of the Chaetothyriomycetes (Haase et al 1995), while others with a yeast-like phase are allied with the Dothideomycetes (Sterflinger et al 1999). Many black yeast taxa convert to meristematic growth under stressful conditions and therefore the distinction between ''black yeasts'' and ''meristematic fungi'' is not absolute (Sterflinger and Krumbein 1995, Sterflinger et al 1999, de Hoog et al 1999). As well, the term ''black fungi'' sometimes is used to embrace both groups of fungi (Sterflinger and Krumbein 1995). Most of these fungi occur in extreme environments, tolerating stresses caused by temperature, water availability, oxygenic action, irradiation by ultraviolet rays (UV), electrolyte content and/or scarcity of nutrients (Sterflinger et al 1999, de Hoog et al 1999). Epiphytic species occur in such genera as *Aureobasidium*, *Coniosporium*, *Hormonema*, *Hyphospora*, *Phaeotheca*,

←

FIG. 26. One of 18 equally parsimonious trees (2648 steps, CI = 0.432) resulting from a maximum-parsimony analysis of the initial SSU data matrix using the heuristic search algorithm of PAUP* version 4.0b8. Bootstrap values above 65% are given adjacent to the corresponding node.

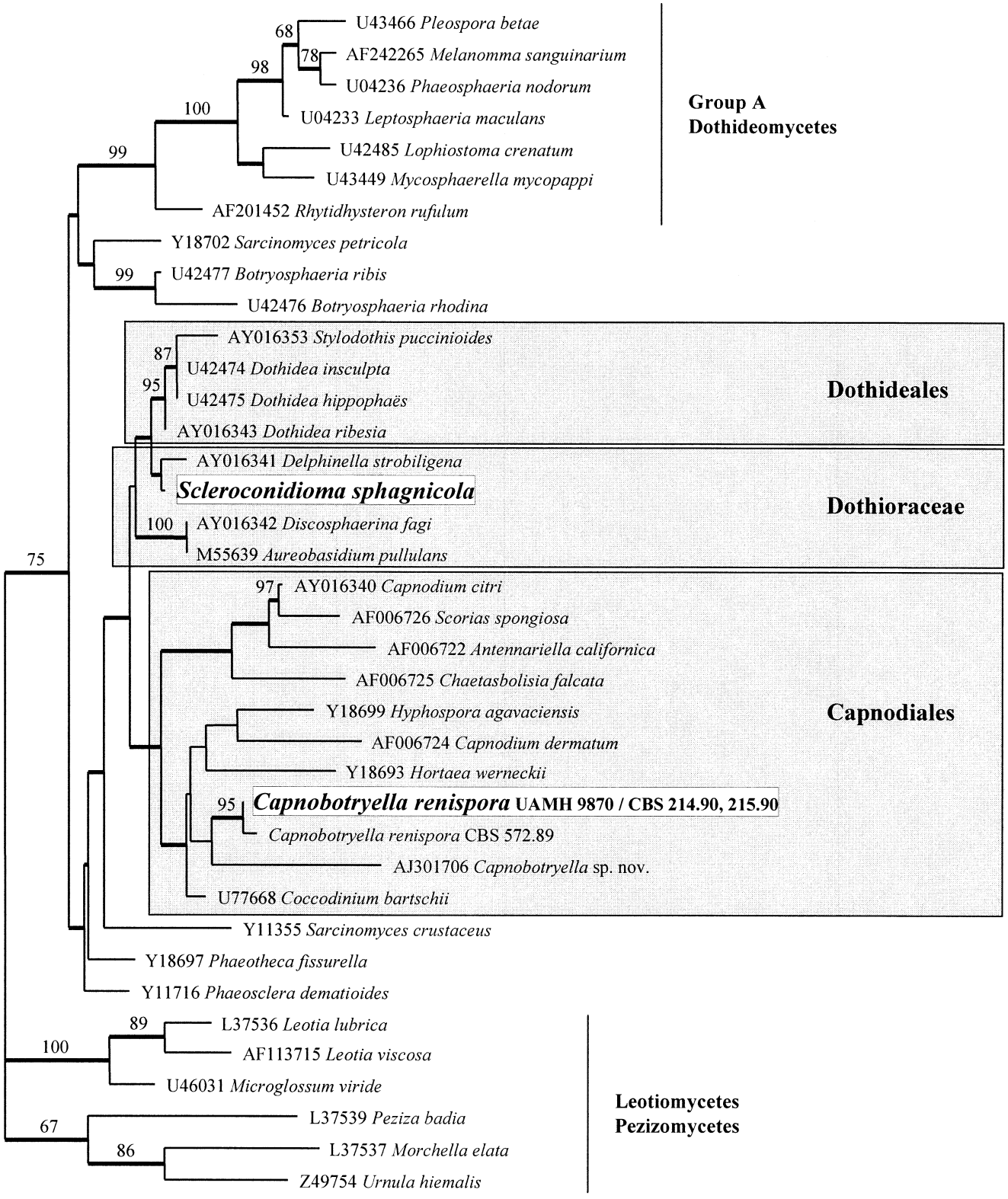


FIG. 27. One of 15 equally parsimonious trees (651 steps, CI = 0.662) resulting from a maximum-parsimony analysis of the final SSU data matrix using the heuristic search algorithm of PAUP* version 4.0b8. Bootstrap values above 60% are given adjacent to the corresponding node. Branches in bold were present in the strict consensus.

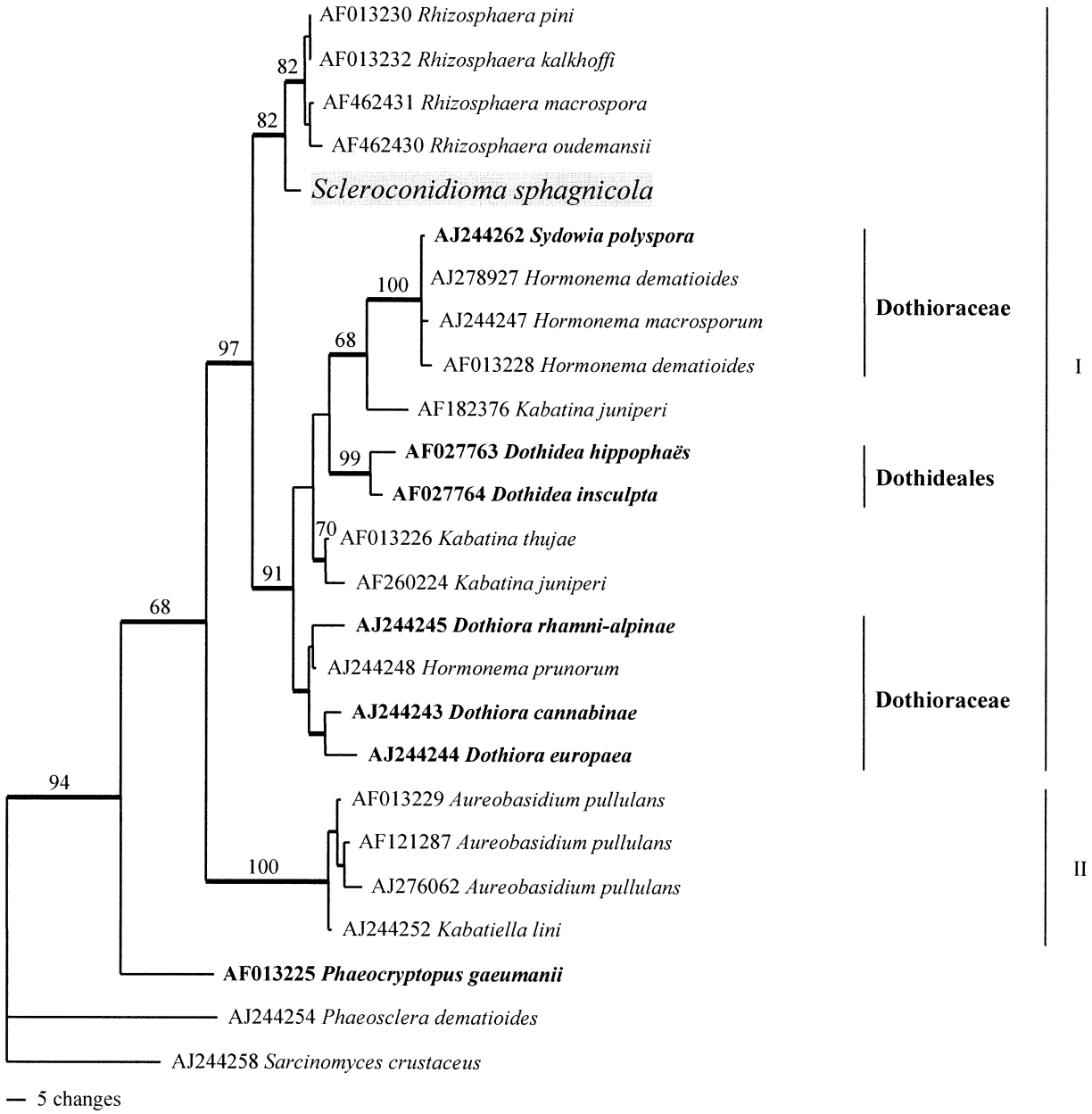


FIG. 28. One of 10 equally parsimonious trees (352 steps, CI = 0.670) resulting from a branch and bound analysis of the ITS data matrix. Bootstrap values above 65% are given adjacent to the corresponding node. Branches in bold were present in the strict consensus. Bold type indicates teleomorphic taxa.

Sarcinomyces and *Trimmatostroma* (e.g., Butin et al 1995, DesRochers and Ouellette 1994, Sigler et al 1981, Sterflinger et al 1999, Yurlova et al 1999), fungi with diverse phylogenetic affinities.

Despite similarities in their morphology and habitat, molecular evidence indicates that meristematic fungi and black yeasts belong to at least three different orders, Chaetothyriales, Dothideales (sensu lato) and Pleosporales (Haase et al 1995, de Hoog et al 1999, Sterflinger et al 1999). Our parsimony analyses of small-subunit nuclear rDNA sequence data showed that *S. sphagnicola* and *C. renispora* phylogenetically were distinct from one another. Both were related to fungi classified in the Dothideomycetes but to different orders, specifically to the Dothideales and the Capnodiales, respectively, and distant from the Chaetothyriomycetes. These two dematiaceous fungi isolated from *Sphagnum* leaves also were distinct morphologically. Although they produced microsclerotia similar in gross morphology and simple septation (FIGS. 9, 11) (Tsuneda et al 2001b), further examination showed that they differed in the mode of de-

velopment, internal structure and disseminative propagules.

Morphological and ecological aspects.—In *C. renispora*, the dominant form of cell multiplication in microsclerotium formation is apical and polar, leading to the formation of acropetal chains of globose cells (moniliform hyphae; FIG. 4). Thus, growth is predominantly hyphal. Adjacent cells in mature microsclerotia separate by septum schizolysis. However in *S. sphagnicola*, growth is revealed primarily by isodiametric enlargement and subdivision of mother cells (FIGS. 21–23) (Tsuneda et al 2001b), and the cells of mature microscerotia are cemented in a solid sclerotic mass. The similar, cauliflower-like macromorphology of the microsclerotia observed on the host, then, is derived from different developmental processes.

Diverse fungi, including some parasitic ones, occur on living or decomposing *Sphagnum* (Döbbeler 1978, 1986, Redhead 1981, Redhead and Spicer 1981, Thormann et al 2001, Tsuneda et al 2001a). *Sphagnum* cell walls are highly resistant to microbial degradation, and environmental conditions prevailing in *Sphagnum* bogs, i.e., wide fluctuations in temperature, moisture and radiation, could select for fungi producing dark pigments and having the ability to form sclerotia. It is apparent that the primary function of microsclerotia in both *C. renispora* and *S. sphagnicola* is survival on the host. Component cells of mature microsclerotia in both fungi contain a large amount of reserve material (lipid bodies) and possess thick cell walls that are densely covered and heavily impregnated with melanin granules. Further, their cell organelles are not well defined (FIG. 15) (Tsuneda et al 2001b), except in actively multiplying cells. These characteristics indicate that the microsclerotia remain in a resting state until environmental conditions become favorable for growth. Melanin appears to play an important role in stress tolerance because wall-bound melanin protects fungal cells from various stress factors, including physical (e.g., extreme temperatures and desiccation) and biological ones, such as lysis caused by cell-wall degrading enzymes released by other microorganisms (Butler et al 2001). In addition, melanin granules absorb X-, gamma and UV rays (Zhdanova et al 1973) and limit the leakage of certain metabolites (Butler et al 2001). These characteristics of melanin explain the higher incidence of melanized microbes occurring on rocks, leaf surfaces and other exposed locations (Ursi et al 1997).

Microsclerotia of *S. sphagnicola* function not only as survival structures but also as a reservoir of nutrients that sustains the production of conidiogenous cells and numerous conidia, when environmental conditions are favorable (Tsuneda et al 2001a, b). Dissemination of this fungus among *Sphagnum* stands thus is primarily by conidia. In contrast, conidia never were observed for the cerebriform fungus from *Sphagnum*, although the type strain of *C. renispora* produces holoblastic, reniform conidia, both in its natural habitat and in culture (Sugiyama and Amano 1987). Then how could *C. renispora* on *Sphagnum* disseminate itself in bogs? FIGURES 15–19 clearly show that many of the component cells of mature *C. renispora* microsclerotia separate by septum schizolysis. In this way, the microsclerotium could function as a mass of thick-walled, thallic spores (= chlamydospores). It is reasonable to assume that such chlamydospores can function to disperse the fungus when the microsclerotium is weathered and/or crushed by external force. The highly melanized outer cell walls become brittle as microsclerotia age, and thus moisture can be readily supplied to the internal cells. This characteristic enables microsclerotial cells to resume growth quickly in response to favorable environmental conditions.

A striking characteristic of both *C. renispora* and *S. sphagnicola* is that each component cell of a mature microsclerotium possesses a thin layer of newly formed cell wall (FIG. 15, arrow) (Tsuneda et al 2001b). That portion of the cell with the newly formed cell wall can be separable from the highly melanized, older cell wall (FIGS. 16–18, 23, 24). In *C. renispora*, when re-growth takes place from these cells (= chlamydospores), the newly forming cell breaks out of the outer cell wall of the chlamydospore (FIG. 3, arrow). These features indicate that the outer cell wall is no longer an integral part of the ''living'' cell but serves as a physical barrier between the cell and its surroundings. The portion consisting of a protoplast and newly formed cell wall thus is analogous to an endoconidium. Endoconidiation occurs in such genera of black fungi as *Aureobasidium*, *Coniosporium*, *Hyphospora*, *Phaeotheca*, *Sarcinomyces* and *Trimmatostroma* (de Leo et al 1999, de Hoog and Hermanides-Nijhof 1977, Sigler et al 1981, Wollenzien et al 1997, Zalar et al 1999a, b). In *Phaeotheca fissurella*, whose microsclerotia resemble those of *S. sphagnicola* in ultrastructure, each cell with the newly formed cell wall subsequently is subdivided to form two to several endoconidia. These endoconidia are released by dissolution of mother-cell walls (Sigler et al 1981, Tsuneda and Murakami 1985). Microsclerotia of *C. renispora* and *S. sphagnicola* lack such an elaborate releasing mechanism.

Phylogenetic aspects.—The SSU and ITS analyses provided evidence of a close relationship between *S.*

sphagnicola and the Dothideales sensu stricto, restricted to one family (Dothideaceae) in the Eriksson et al (2001) classification, and genera in the Dothioraceae. Based on the ITS sequences available in GenBank, its closest relatives are in *Rhizosphaera*, a coelomycete genus characteristically found on conifer needles functioning as a weak parasite (Butin and Kehr 2000). These genera are similar in the shape of conidiogenous cells and conidia, and in phialidic conidiogenesis. Conidiogenous cells of *Rhizosphaera* are produced within black pycnidia, and they form the single layer of cells of the conidiomatal wall (Sutton 1980), whereas those of *Scleroconidioma* form on the surface of dematiaceous microsclerotia (FIG. 23) (Tsuneda et al 2001b), giving the impression of a pycnidium of *Rhizosphaera* turned inside-out.

There was support from the ITS analysis for a more inclusive concept of the Dothideales, that would include the Dothioraceae (anamorphs in *Hormonema*), and the anamorph genus *Kabatina*, an acervular fungus that is *Hormonema*-like in pure culture (Hermanides-Nijhof 1977). *Rhizosphaera* and *Scleroconidioma* were sister taxa, while several strains of *Aureobasidium*, its synonym *Kabatiella* and by inference its teleomorph *Discosphaerina* (FIG. 27; Yurlova et al 1999) formed a separate well-supported monophyletic group. Strong support for such a wider concept, which also would include *Aureobasidium*, was provided by maximum-likelihood and neighbor-joining analyses of combined large and small-subunit data (Lumbsch and Lindemuth 2001) but not by SSU analysis alone (this study, Berbee 1996). Our analyses raised questions about phylogenetic relationships within the Venturiaceae. Although *Rhizosphaera* spp. are considered to be anamorphic Venturiaceae (Kirk et al 2001), *Phaeocryptopus gaumannii* is far removed in the ITS tree and, in the SSU analyses, *Venturia liriodendri* Hanlin is allied with the Pleosporales clade (data not shown, Silva-Hanlin and Hanlin 1999).

Our identical sequence data for UAMH 9870 and the ex-type strain of *C. renispora* (IAM 31014) preserved as CBS 214.90 strongly suggested conspecificity. Furthermore, our sequence data for a second strain used for the original description of the species (CBS 215.90, IAM 31015; Sugiyama and Amano 1987) also were identical, except for one nucleotide difference in the ITS sequence. The genus *Capnobotryella* (Metacapnodiaceae) clustered well within the Capnodiales in the SSU analysis, as did the two dematiaceous hyphomycetes, *Hyphospora* and *Hortaea*. In a study focused on the Capnodiales, Reynolds (1998) found that the taxa sampled formed a monophyletic group based on a neighbor-joining analysis of SSU sequences. *Capnobotryella renispora* and other allied anamorphic genera clustered with the teleomorph representatives but with low bootstrap support. The results of our SSU analyses, which incorporated the relevant sequences from the studies of Reynolds (1998) and Lumbsch and Lindemuth (2001), are in agreement.

Relationships among taxa within the Capnodiales clade (FIG. 27) were unresolved and difficult to interpret. Type species have not yet been sampled, two of the four families included were represented by anamorph taxa only, and there are other capnodiaceous families for which no sequence data are available. Two species of *Capnodium* (Capnodiaceae) clustered in different subclades as did two genera of the Metacapnodiaceae. The lack of bootstrap support for the clade and the genetic distance between taxa within the clade, as evidenced by the long terminal-branch lengths, suggest that the monophyly of the sooty mold order might not hold up with increased sampling. ITS sequences available for representatives of the Capnodiales and the other anamorph genera in the clade were highly divergent and could not be aligned with confidence to sequences of *C. renispora*.

The identification of the *Sphagnum* isolate remains provisional because it did not form the holoblastic, reniform conidia that characterize *C. renispora* and because three different SSU sequences have been determined for the ex-type strain (our data, Y18698 and AF006723). The morphological characters we observed for the CBS culture agreed with those in the original description of material from the host, although conidia were rare. We were confounded by the marked differences between the SSU sequences derived from the CBS and ATCC cultures. We were unable to examine the fungus preserved as ATCC 64891 for this study, but in our analyses all three SSU sequences cluster within the Capnodiales (data not shown) and therefore it is unlikely that the discrepancies are a result of culture contamination.

Two other possible explanations are that a sequencing error occurred or that two distinct sooty mold species have been preserved inadvertently as IAM 31014. *Capnobotyrella renispora* was isolated from the subicula of another sooty mold, *Capnobotrys neesii*, for which there are no cultures and no sequence data. Sooty molds are found superficially on living plants, and several taxa frequently grow together in apparent harmony in such a way that species are not readily distinguishable from each other on the host and specimens often comprise more than one species (Hughes 1976). In addition, species are highly pleomorphic, with up to three synanamorphs produced. Careful studies of more specimens are needed to clarify the extent of morphological and molecular variation exhibited by fungi in the form genus *Capnobotryella*. Conidiation among the strains examined

ranged from prolific (CBS 572.89) to scarce or nil; the long terminal branch lengths for the three fungi included in the SSU analysis presented in FIG. 27 (*C. renispora* and *Capnobotryella* sp. nov.) suggest that genetic variation is substantial.

Other meristematic fungal genera allied with the Dothideomycetes include *Aureobasidium*, *Botryomyces*, *Hormonema*, *Hortaea*, *Hyphospora*, *Phaeosclera*, *Phaeotheca* and some species of *Sarcinomyces* (de Hoog et al 1999, Sterflinger et al 1999). Of these, only *Botryomyces* is related to the Pleosporales (de Hoog et al 1999) or the Group A Dothideomycetes of our analyses. The rest clustered with Group B taxa, as shown in FIGS. 27 and 28. Prior molecular-based studies have examined relationships within the Dothideomycetes and have highlighted the division of the class into two groups, one consistently being the well-supported monophyletic Pleosporales (Berbee 1996, Silva-Hanlin and Hanlin 1999, Liew et al 2000, Lumbsch and Lindemuth 2001). In general, the groups correspond to the pseudoparaphysate taxa of the Pleosporales, including the Melanommatales, versus the aparaphysate taxa in the Dothideales and Capnodiales, thus Groups A and B of our analyses. Overall, the ascomycete phylogenetic hypotheses presented here concur with prior hypotheses of supra-ordinal taxonomic relationships (Tehler et al 2000, Eriksson et al 2001). They also reinforce the finding that relationships among Group B Dothideomycete taxa are not well resolved by parsimony analysis of larger datasets of small- subunit sequence data (Silva-Hanlin and Hanlin 1999).

Conclusions.—Using rDNA sequence data, we have shown that two dematiaceous hyphomycetes occurring together on *Sphagnum* leaves are related to separate orders of ascostromatic ascomycetes, the Dothideales and Capnodiales. They are phylogenetically distinct from other taxa that exhibit meristematic growth patterns. Their classification remains inconclusive, pending the discovery of their teleomorphs and/or the availability of sequence data for a wider range of taxa from these two orders. Comparative microscopic examinations have elucidated fundamental morphological differences that corroborate the phylogenetic distinction. *Scleroconidioma sphagnicola* and *C. renispora* differ in the development and internal structure of superficially similar microsclerotia produced on the host and in culture and in disseminative propagules, which are predominantly phialoconidia and chlamydospores, respectively. Evidence is presented that the component cells of the mature microsclerotia are analogous to the endocondia produced by other genera of black fungi of diverse phylogenetic affinities. Morphological and developmental characteristics of both species are well suited to the severe fluctuating environmental conditions of *Sphagnum* bogs.

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LITERATURE CITED

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucl Acids Res 25:3389–3402.

Berbee ML. 1996. Loculoascomycete origins and evolution of filamentous ascomycete morphology based on 18S rRNA gene sequence data. Mol Biol Evol 13:462–470.

Butin H, Kehr R. 2000. *Rhizosphaera pseudotsugae* sp. nov. and related species. Mycol Res 104:1012–1016.

———, Pehl L, Hoog GS de, Wollenzien U. 1995. *Trimmatostroma abietis* sp. nov. (Hyphomycetes) and related species. Antonie van Leeuwenhoek 69:203–209.

Butler JB, Day AW, Henson JM, Money NP. 2001. Pathogenic properties of fungal melanins. Mycologia 93:1–8.

DesRochers P, Ouellette GB. 1994. *Phaeotheca dimorphospora* sp.nov.: description et caractéristiques culturales. Can J Bot 72:808–817.

Döbbeler P. 1978. Moosbewohnende Ascomyceten I. Die pyrenocarpen, den Gametophyten besiedelnden Arten. Mitt. Bot. München 14:1–360.

———. 1986. Ein Zweitfund von *Epicoccum plagiochilae* (Hyphomycetes). Mitt Bot München 22:485–492.

Eriksson OE, Baral H-O, Currah RS, Hansen K, Kurtzman CP, Rambold G, Laessøe T. 2001. Outline of Ascomycota— 2001. Myconet 7:1–88.

Haase G, Sonntag L, van de Peer Y, Uijthof JMJ, Podbielski A, Melzer-Krick B. 1995. Phylogenetic analysis of ten black yeast species using nuclear small-subunit rRNA gene sequences. Antonie van Leeuwenhoek 68:19–33.

Hermanides-Nijhof EJ. 1977. *Aureobasidium* and allied genera. Stud Mycol 15:141–177.

Hughes SJ. 1976. Sooty Molds. Mycologia 68:693–820.

Hoog GS de, Hermanides-Nijhof EJ. 1977. The black yeasts and allied hyphomycetes. Stud Mycol 15:178–223.

———, Zalar P, Urzi C, Leo F de, Yurlova NA, Sterflinger K. 1999. Relationships of dothideaceous black yeasts and meristematic fungi based on 5.8S and ITS2 rDNA sequence comparison. Stud Mycol 43:31–37.

Illingworth CA, Andrews JH, Bibeau C, Sogin ML. 1991. Phylogenetic placement of *Athelia bombacina*, *Aureobasidium pullulans* and *Collectotrichum gloeosporioides*

inferred from sequence comparisons of small-subunit ribosomal RNAs. Experimental Mycology 15:65–75.

Inderbitzin P, Landvik S, Abdel-Wahab MA, Berbee ML. 2001. Aliquandostipitaceae, a new family for two new tropical ascomycetes with unusually wide hyphae and dimorphic ascomata. Am J Bot 88:52–61.

Kirk PM, Cannon PF, David JC, Stalpers JA. 2001. Ainsworth and Bisby's Dictionary of the fungi. 9th ed. Wallingford, UK: CAB International. 655 p.

Landvik S, Egger KN, Schumacher T. 1997. Towards a subordinal classification of the Pezizales (Ascomycota): phylogenetic analyses of SSU rDNA sequences. Nordic J Bot 17:403–418.

Leo F de, Urzì C, Hoog GS de. 1999. Two *Coniosporium* species from rock surfaces. Stud Mycol 43:70–79.

Liew ECY, Aptroot A, Hyde KD. 2000. Phylogenetic significance of the pseudoparaphyses in Loculoascomycete taxonomy. Mol Phylogenet Evol 16:392–402.

Lumbsch HT, Lindemuth R. 2001. Major lineages of Dothideomycetes (*Ascomycota*) inferred from SSU and LSU rDNA sequences. Mycol Res 105:901–908.

Morales VM, Jasalavich CA, Pelcher LE, Petrie GA, Taylor JL. 1995. Phylogenetic relationship among several *Leptosphaeria* species based on their ribosomal DNA sequences. Mycol Res 99:593–603.

Redhead SA. 1981. Parasitism of bryophytes by agarics. Can J Bot 59:63–67.

———, Spicer KW. 1981. *Discinella schimperi*, a circumpolar parasite of *Sphagnum squarrosum*, and notes on *Bryophytomyces sphagni*. Mycologia 73:904–913.

Reynolds DR. 1998. Capndiaceous sooty mold phylogeny. Can J Bot 76:2125–2130.

Sigler L, Tsuneda A, Carmichael JW. 1981. *Phaeotheca* and *Phaeosclera*, two new genera of dematiaceous Hyphomycetes and a redescription of *Sarcinomyces* Lindner. Mycotaxon 12:449–467.

Silva-Hanlin DM, Hanlin RT. 1999. Small-subunit ribosomal RNA gene phylogeny of several loculoascomycetes and its taxonomic implications. Mycol Res 103:153–160.

Sterflinger K, Krumbein WE. 1995. Multiple stress factors affecting growth of rock-inhabiting black fungi. Bot Acta 108:490–496.

———, Baere R de, Hoog GS de, Wachter R de. 1997. *Coniosporium perforans* and *C. appollinis*, two new rock-inhabiting fungi isolated from marble in the Sanctuary of Delos (Cyclades, Greece). Antonie van Leeuwenhoek 72:349–363.

———, Hoog GS de, Haase G. 1999. Phylogeny and ecology of meristematic ascomycetes. Stud Mycol 43:98–106.

Sugiyama J, Amano N. 1987. Two metacapnodiaceous sooty molds from Japan: their identity and behavior in pure culture. In: Sugiyama J, ed. Pleomorphic fungi: the diversity and its taxonomic implications. Amsterdam: Elsevier Science Publishers. p 141–156.

Sutton BC. 1980. The Coelomycetes. Slough, England: Commonwealth Mycological Institute. 696 p.

Swofford DL. 1999. PAUP*: phylogenetic analysis using parsimony (*and other methods), version 4. Sunderland, Massachusetts: Sinauer Associates.

Tehler A, Farris JS, Lipscomb DL, Källersjö M. 2000. Phylogenetic analyses of the fungi based on large rDNA datasets. Mycologia 92:459–474.

Thormann MN, Currah RS, Bayley SE. 2001. Microfungi isolated from *Sphagnum fuscum* from a Southern Boreal Bog in Alberta, Canada. The Bryologist 104:548–559.

Titze A, Hoog GS de. 1990. *Capnobotryella renispora* on roof tile. Antonie van Leeuwenhoek 58:265–269.

Tsuneda A, Murakami S. 1985. Endoconidium development and release in the hyphomycete *Phaeotheca fissurella*. Mycologia 77:433–440.

———, Chen MH, Currah RS. 2001a. Characteristics of a disease of *Sphagnum fuscum* caused by *Scleroconidioma sphagnicola*. Can J Bot 79:1217–1224.

———, ———, ———. 2001b. Conidiomatal morphogenesis and pleomorphic conidiogenesis in *Scleroconidioma sphagnicola*. Mycologia 93:1164–1173.

———, Thormann MN, Currah RS. 2000. *Scleroconidioma*, a new genus of dematiaceous Hyphomycetes. Can J Bot 78:1294–1298.

Ursi C, Wollenzien U, Criseo G, Krumbein WE. 1997. Biodiversity of the rock inhabiting microbiota with special reference to black fungi and black yeasts. In: Allsopp D, Colwell RR, Hawksworth DL, eds. Microbial diversity and ecosystem function. Kew, Surrey, UK: CAB International. p 289–302.

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds. PCR Protocols: a guide to methods and applications. New York: Academic Press. p 315–322.

Winka K, Eriksson OE, Bång Å. 1998. Molecular evidence for recognizing the Chaetothyriales. Mycologia 90:822–830.

Wollenzien U, Hoog GS de, Krumbein W, Uijthof JMJ. 1997. *Sarcinomyces petricola*, a new microcolonial fungus from marble in the Mediterranean basin. Antonie van Leeuwenhoek 71:281–288.

Yurlova NA, Hoog GS de, Gerrits van den Ende AHG. 1999. Taxonomy of *Aureobasidium* and allied genera. Stud Mycol 43:63–69.

Zalar P, Hoog GS de, Gunde-Cimerman N. 1999a. Taxonomy of the endoconidial black yeast genera *Phaeotheca* and *Hyphospora*. Stud Mycol 43:49–56.

———, ———, ———. 1999b. *Trimmatostroma salinum*, a new species from hypersaline water. Stud Mycol 43:57–62.

Zhdanova NN, Gavryushina AL, Vasilevskaya AI. 1973. Effect of gamma and UV-irradiation on survival of *Cladosporium* sp. and *Oidiodendron cerealis*. Microbiol Zhurn Kiev 35:449–452.