Phylogenetic placement of plant pathogenic *Sclerotium* species among teleomorph genera

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Abstract: Phylogenetic analyses and morphological characteristics were used to assess the taxonomic placement of eight plant-pathogenic *Sclerotium* species. Members of this genus produce only sclerotia and no fruiting bodies or spores, so *Sclerotium* species have been difficult to place taxonomically. Sequences of rDNA large subunit (LSU) and internal transcribed spacer (ITS) regions were determined for isolates of *Sclerotium cepivorum*, *S. coffeicola*, *S. denigrans*, *S. hydrophilum*, *Ceratorhiza oryzae-sativae*, *S. perniciosum*, *S. rhizodes*, *S. rolfsii* and *S. rolfsii* var. *delphinii*. Parsimony analysis grouped two species previously thought to be in the Basidiomycota, *S. denigrans* and *S. perniciosum*, within the Ascomycota; these species were found to have affinities with the teleomorph genera *Sclerotinia* and *Stromatinia* and the asexual *Sclerotium cepivorum*, which was known earlier to be related to *Sclerotinia* species. The other *Sclerotium* species were placed in one of two basidiomycetous groups, genera *Athelia* or *Ceratobasidium*. Based on rDNA analysis and morphology the basidiomycetous *Sclerotium hydrophilum* and *S. rhizodes* were transferred to genus *Ceratorhiza*, the anamorph of *Ceratobasidium* species. *Sclerotium coffeicola* was found to be close to *S. rolfsii* var. *delphinii* and *S. rolfsii* var. *rolfsii*, which was shown earlier to have an *Athelia* teleomorph.

Key words: disease management strategies, pathogen

INTRODUCTION

Fungi in genus *Sclerotium* Tode form sclerotia and sterile mycelia but no spores (Saccardo 1899). *Sclerotium* includes more than 40 plant-pathogenic species (Farr 2008). Many *Sclerotium* species do not or rarely reproduce sexually and are known only from their asexual stage (Punja 1988, Punja and Rahe 2001, Kohn 2004). The name *Sclerotium* was first introduced

by Tode (1790) to describe eight species. Fries (1821) sanctioned genus *Sclerotium* and the first-listed species, *Sclerotium complanatum* Tode, was suggested as the type by Clements and Shear (1973). *S. complanatum* may be the anamorph of *Typhula phacorrhiza* Reichard ex Fries (Remsberg 1940), which produces basidiocarps from sclerotia (Donk 1962). *S. rolfsii* Sacc., the best known species in this genus, has the teleomorph *Athelia rolfsii* (Curzi) Tu and Kimbrough, which forms resupinate basidiocarps and has hyphal strands emerging from germinating sclerotia (Tu and Kimbrough 1978).

Although no sexual stage is known for *S. cepivorum* Berk., based on similarity of the sclerotia to those seen in the ascomycete *Stromatinia gladioli* (Drayt.) Whetzel, *Sclerotium cepivorum* was transferred to the teleomorph genus *Stromatinia* Boud. by Whetzel (1945). However Kohn and Grenville (1989) argued that the teleomorph name of *S. cepivorum* should not be based on sclerotium morphology. Species of *Sclerotinia* form apothecia arising from a tuberoid sclerotium, whereas *Stromatinia* species form two kinds of sclerotia, apothecia arising from a sclerotium with a thin, black, subcuticular, effuse covering or mantle or sclerotia that are small black sphaerules borne free on the mycelium and not giving rise to apothecia (Whetzel 1945). *S. cepivorum* forms only the latter type of sclerotia. Kohn and Grenville (1989) found that sclerotia of *S. cepivorum* are similar to those of *Botrytis* both anatomically and histochemically. *S. cepivorum* also produces a *Myrioconium*-like microconidial state, which is probably spermatial. Analysis of rDNA sequences suggested that *Sclerotium cepivorum* was closely aligned with *Sclerotinia sclerotiorum* (Lib) Debary, another important species within the Sclerotiniaceae (Carbone and Kohn 1993). Analysis of sclerotial protein also suggested the affinities of *S. cepivorum* with species in the Sclerotiniaceae (Novak and Kohn 1991). Some *Sclerotium* species may be related to the anamorph genus *Rhizoctonia* (de Candolle 1815), which form sclerotia and sterile mycelia with hyphae branching at right angles (Donk 1962). *Rhizoctonia solani* J.G. Kühn is believed to be the anamorph of the basidiomycete *Thanatephorus cucumeris* (Frank) Donk, but most taxonomists agree that *R. solani* is not a single species but instead a species complex and there are more than 30 synonyms for *R. solani* (Bridge 2002, Carling and Sumner 2001, Gonzalez et

al. 2001, Parmeter and Whitney 1970,Vilgalys and Cubeta 1994). Some binucleate (two nuclei per hyphal cell) *Rhizoctonia* species have a *Ceratobasidium* D.P. Rogers teleomorph, whereas some multinucleate species in the *R. solani* complex have a *Thanatephorus* Donk teleomorph. *Thanatephorus* and *Ceratobasidium* differ in the shape of their basidia (Staplers 1996), but recent phylogenetic studies suggest that *Cerato-basidium* and *Thanatephorus* are not mutually exclusive monophyletic groups (Gonzalez et al. 2001, 2006a, b). Some authors (Moore 1987, 1989; Shan et al. 2002; Stalpers and Andersen 1996) have classified binucleate *Rhizoctonia* species with doli-pore/parenthesome septa (O_1/P_1-type d/p) in the anamorphic genus *Ceratorhiza* Moore. *Ceratorhiza oryzae-sativae* (Saw.) Moore (*Sclerotium oryzae-sativae* Saw.) is the anamorph of *Ceratobasidium oryzae-sativae* P.S. Gunnell & R.K. Webster (Mordue 1974, Moore 1989).

Our study focused on determining phylogenetic placement of important plant-pathogenic species of *Sclerotium* that were available from the Centraalbureau voor Schimmelcultures (CBS, Utrecht, Netherlands) and other collections into teleomorph genera and families with parsimony analysis of sequences of the nuclear ribosomal DNA operon, sclerotium morphology and hyphal morphology.

MATERIALS AND METHODS

Isolates.—For each of the nine taxa studied 2–8 isolates (TABLE I) were assessed for a total of 35 isolates. The number of isolates per species was determined based on availability of isolates from CBS, Dr Zamir Punja at Simon Fraser University, Canada, and a collection at Iowa State University.

Morphology of isolates in vitro.—Cultures were grown on potato dextrose agar (PDA, Difco Laboratories, Detroit, Michigan) in 9 cm diam Petri dishes under a daily regime of approximately 8 h incandescent light and 16 h darkness at room temperature (21–24 C). Mycelial plugs (7 mm diam) were transferred to three 9 cm diam PDA plates, which were placed in an incubator in darkness at 20 C. Color of sclerotia and mycelia was observed, and number of sclerotia per Petri dish was counted after 1 and 3 wk incubation.

To determine number of nuclei per hyphal cell for isolates of *S. hydrophilum* Sacc., *Ceratorhiza oryzae-sativae* and *S. rhizodes* Auersw. mycelia from 1 wk old cultures were stained with DAPI (Polysciences Inc., Warrington, Pennsylvania) and examined under a fluorescence microscope (Olympus BH Series) at 400× magnification.

Polymerase chain reaction and sequencing.—A portion of the large subunit (LSU, 28S) region of rDNA was sequenced for each of the 35 isolates. The internal transcribed spacer region (ITS1, 5.8S rDNA gene, ITS2) was sequenced for isolates of *S. cepivorum, S. denigrans* Pape, *S. hydrophilum,*

C. oryzae-sativae, S. perniciosum Slogt. & K.S. Thomas and *S. rhizodes.* Template DNA for polymerase chain reaction (PCR) was obtained by scraping mycelium with a pipette tip from 1–2 wk old cultures grown on PDA and extracting DNA with Prepman Ultra (Applied Biosystems, Foster City, California). Primer pairs used for amplification of the LSU region were LROR and LR5, and sequencing of the LSU region used LROR and LR3 (Vilgalys and Hester 1990). The primer pair used for PCR and sequencing of the ITS region was ITS1-F (Gardes and Bruns 1993) and ITS4 (White et al. 1990). Amplification reactions consisted of 4 mM $MgCl_2$, 5× Sigma buffer, 200 μM dNTPS, 0.5 μM forward and reverse primers and 3 units Taq polymerase (Sigma Chemical Co., St Louis, Missouri). Reactions were performed in an automated thermocycler (MJ Research Inc. PTC-100 thermocycler, Waltham, Massachusetts). Cycling conditions were an initial denaturation at 94 C for 95 s, followed by 35 cycles of denaturation at 94 C for 35 s, annealing at 49 C for LSU and at 52 C for ITS for 60 s, and extension at 72 C for 2 min. The PCR product was purified with a QIAquick DNA Purification Kit (QIAGEN, Valencia, California) and quantified on a Hoefer DyNA Quant 200 Fluorometer (Amersham Pharmacia Biotech, San Francisco, California). Automated sequencing (Applied Biosystems 3730xl DNA Analyzer) was performed at the Iowa State University DNA Sequencing and Synthesis Facility.

Sequence alignment and phylogenetic analysis.—To resolve phylogenetic placement for *Sclerotium* species possible relatives of each of the *Sclerotium* species were determined by subjecting ITS and LSU sequences of representative isolates to BLAST (2.2.17, National Center for Biotechnical Information, National Library of Medicine, National Institutes of Health, Bethesda, Maryland). Partial sequences of LSU and ITS highly similar to those of *Sclerotium* species were downloaded from GenBank for further phylogenetic analysis. *Saccharomyces cerevisiae* Meyen ex E.C. Hansen was used as outgroup taxon for the LSU analysis. Outgroups for two ITS analyses were chosen based on the LSU analysis.

After preliminary alignments were generated with Clustal X with gap opening and gap extension parameters of 50 : 5 (Thompson et al. 1997), aligned sequences were adjusted manually with BioEdit (Hall 1999). For LSU analysis ambiguously aligned regions (110 characters) were eliminated, resulting in 451 characters including gaps. Alignable gaps were treated as a fifth character. For ITS analysis of the basidiomycete group alignable gaps were treated as a fifth character and ambiguously aligned regions (211 characters) were eliminated, resulting in 626 characters including gaps. For ITS analysis of the discomycete group, gaps were treated as a fifth character and ambiguously aligned regions (24 characters) were eliminated, resulting in 529 characters including gaps. Maximum parsimony analysis was performed with PAUP 4.0b 10 for 32-bit Microsoft Windows. All characters were given equal weight. Heuristic searches were conducted with simple sequence addition and tree bisection-reconnection (TBR) branch swapping algorithms, collapsing zero-length branches. A strict consensus of the most parsimonious trees was generated, as well as a

Table I. Isolates of *Sclerotium* and *Ceratorhiza* species and rDNA sequence accession numbers

Species	Strain number	Geographic origin	Host	Gen Bank LSU
Sclerotium cepivorum	CBS 276.93[1]	UK	*Allium* sp.	FJ212341
	CBS 342.47[1]	Netherlands	N/A[a]	FJ212344
S. coffeicola	CBS 521.92[1]	Venezuela	*Visnia* sp.	FJ212345
	CBS 668.85[1]	Costa Rica	*Coffea* sp.	FJ212346
S. denigrans	CBS 118.43[1]	Germany	*Convallaria majalis*	FJ212347
	CBS 396.54[1]	Germany	*Convallaria majalis*	FJ212348
S. hydrophilum	CBS 201.27[1]	Netherlands	*Victoria regia*	FJ212349
	CBS 385.63[1]	Italy	submerged leaf in garden pond	FJ212350
Ceratorhiza oryzae-sativae	CBS 235.91[1]	Japan	*Oryza sativa*	FJ212351
	CBS 439.80[1]	Japan	*Oryza sativa*	FJ212352
	CBS 577.81[1]	Italy	N/A	FJ212353
S. perniciosum	CBS 268.30[1]	Netherlands	N/A	FJ212354
	CBS 274.93[1]	USA	*Allium* sp.	FJ212357
	CBS 275.93[1]	Netherlands	*Tulipa* sp.	FJ212355
	CBS 335.47[1]	Netherlands	*Allium canadense*	FJ212356
S. rhizodes	CBS 126.13[1]	Netherlands	N/A	FJ212358
	CBS 276.69[1]	Germany	*Glyceria maxima*	FJ212359
	CBS 321.68[1]	Germany	*Phalaris arundinacea*	FJ212360
S. rolfsii	Sr 1[2]	Georgia, USA	*Arachis hypogaea*	FJ212331
	Sr 2[3]	Georgia, USA	*Arachis hypogaea*	FJ212332
	WM913[4]	California, USA	*Arachis hypogaea*	FJ212338
	3088[4]	China	*Lilium* sp.	FJ212334
	185[4]	Brazil	N/A	FJ212335
	3095[4]	Italy	*Medicago sativa*	FJ212336
	3098[4]	Pakistan	*Lens culinaris*	FJ212333
	138[4]	Chile	Turfgrass	FJ212337
S. rolfsii var. *delphinii*	ATCC15200[4]	New Brunswick	*Iris* sp.	FJ212326
	3089[4]	China	*Malus domestica*	FJ212327
	Srd 1[5]	Iowa, USA	*Hosta* sp.	FJ212328
	3092[4]	China	*Arachis hypogaea*	FJ212329
	1058[4]	California	*Ajuga* sp.	FJ212330

[a] Not available.

Source:

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bootstrap analysis of 100 replications for the LSU dataset and 500 replications for the ITS dataset.

RESULTS

Morphology.—Colonies were white for all isolates of all species after 1 wk incubation. Differences in morphological characters among *Sclerotium* species, including colony color, number of sclerotia per Petri dish and in vitro sclerotia color were more distinct after 3 wk incubation. In general isolates of *S. cepivorum*, *S. denigrans* and *S. perniciosum* had darker mycelia and *S. cepivorum* and *S. perniciosum* had darker sclerotia than isolates of the other species.

S. cepivorum isolate CBS276.93 produced 595–638 spherical to hemispherical sclerotia per Petri dish for the three replicate plates, whereas no sclerotium was observed for isolate CBS342.47. Isolates of *S. denigrans* produced 80–89 crustose sclerotia per Petri dish. The four isolates of *S. perniciosum* produced spherical to hemispherical sclerotia; isolates CBS268.30, CBS274.93 and CBS275.93 produced 500–600 brown to blackish-mouse-gray sclerotia per Petri dish, whereas isolate CBS335.47 produced 10 mouse-gray sclerotia per Petri dish.

The two isolates of *S. hydrophilum* produced 596–610 spherical sclerotia. Two isolates, CBS235.91 and CBS439.80, of *Ceratorhiza oryzae-sativae* produced 79–

190 spherical sclerotia per Petri dish, whereas isolate CBS577.81 of *C. oryzae-sativae* produced six sclerotia per Petri dish.

S. coffeicola Stahel, *S. rolfsii* and *S. rolfsii* var. *delphinii* (Welch) Boerema & Hamers produced respectively 3–4, 75–133 and 10–77 sclerotia per Petri dish. Sclerotia of *S. coffeicola* and *S. rolfsii* were spherical, whereas *S. rolfsii* var. *delphinii* produced spherical to irregular sclerotia. Sclerotia of *S. coffeicola* (2.0–2.7 mm diam) and *S. rolfsii* var. *delphinii* (1.3–2.6 mm diam) were larger than those of *S. rolfsii* (1.0–1.2 mm diam).

Clamp connections were found exclusively in isolates of *S. coffeicola*, *S. rolfsii* and *S. rolfsii* var. *delphinii.* Mycelial branching at right angles was seen in *S. hydrophilum*, *Ceratorhiza oryzae-sativae* and *S. rhizodes*, and these species formed hyphae that were slightly constricted at the branch origin, often with a septum near the origin. No clamp connections were found on hyphae of *S. hydrophilum*, *Ceratorhiza oryzae-sativae* and *S. rhizodes*, but these species had hyphae with two nuclei per cell based on DAPI staining and fluorescent microscopy.

Phylogenetic placement.—Maximum parsimony analysis of the LSU sequences resulted in 368 equally most parsimonious (MP) trees. An MP tree (FIG. 1) was selected to illustrate phylogenetic placement of the *Sclerotium* species. *Sclerotium* species are found in three major clusters, designated S1, S2 and S3. *Sclerotium cepivorum*, *S. denigrans* and *S. perniciosum* formed cluster S1, which was grouped within the Sclerotiniaceae (Helotiales, Ascomycota) with a bootstrap support of 89%. Cluster S2 included *S. hydrophilum*, *S. rhizodes* and *Ceratorhiza oryzae-sativae*, which was grouped within the Ceratobasidiaceae (Basidiomycota). Cluster S3, consisting of *S. rolfsii*, *S. rolfsii* var. *delphinii* and *S. coffeicola* Stahel, was grouped within *Athelia* (Atheliales, Basidiomycota) with 100% bootstrap support. Both S2 and S3 clusters were grouped within the Agaricomycetes.

The ITS sequence of *S. cepivorum* isolate CBS 342.47 grouped with three isolates of *S. perniciosum* (CBS 268.30, 274.93 and 275.93) with 55% bootstrap support (FIG. 2). These four isolates grouped with *S. cepivorum* isolate CBS 276.93 with 85% bootstrap support. *Sclerotium cepivorum* isolate CBS 276.93 and *S. perniciosum* isolates CBS 274.93 and 275.93 were similar in colony color, number of sclerotia per plate and sclerotia color. Parsimony analysis of ITS sequences grouped *Sclerotium denigrans* with *Stromatinia rapulum* (FIG. 2) with 98% bootstrap support. Unlike other *Sclerotium* isolates that produced sclerotia, *S. denigrans* produced crustose pseudosclerotia on PDA.

All isolates of *S. hydrophilum*, *S. rhizodes* and *Ceratorhiza oryzae-sativae* were grouped with *Ceratobasidium* spp. and *Thanatephorus cucumeris* with a 95% bootstrap support (FIG. 1). Parsimony analysis of the LSU (FIG. 1) grouped *S. hydrophilum* with *Ceratobasidium* sp. (sequence AF354092), and analysis of the ITS region grouped *S. hydrophilum*, *Ceratobasidium* sp. and *Rhizoctonia* sp. with 78% bootstrap support (FIG. 3).

Colony color of *Ceratorhiza oryzae-sativae* isolates CBS 235.91 and 439.80 differed from that of CBS 577.81, which formed fewer sclerotia per plate. The closest ITS matches to our *Ceratorhiza oryzae-sativae* isolates with BLAST were sequences from another isolate of *Ceratorhiza oryzae-sativae*, an unidentified *Ceratobasidium* sp. and an unidentified *Rhizoctonia* sp. (FIG. 3).

The ITS sequences from *S. rhizodes* isolates CBS 126.13, 276.69 and 321.68 differed by only three base pairs (FIG. 3). Isolate CBS 321.68 rarely produced sclerotia on PDA or cotton. Parsimony analysis of the ITS sequences grouped *S. rhizodes* with isolates of *Ceratobasidium cereale*, *Ceratobasidium* sp. and *Rhizoctonia* sp. with 100% bootstrap support.

Parsimony analyses of LSU (FIG.1) and ITS sequences (Xu unpubl) both suggested close relationships among *S. rolfsii*, *S. rolfsii* var. *delphinii* and *S. coffeicola*. They were grouped with *Athelia* species with 99% bootstrap support (FIG. 1). The three taxa had similar colony color, whereas they differed with regard to sclerotia size, sclerotia color and number of sclerotia per plate. Two isolates identified as *S. rolfsii* var. *delphinii* (1058 and 3092) fell into the *S. rolfsii* group based on LSU analysis, and they were similar to *S. rolfsii* isolates in size and color of sclerotia in culture.

TAXONOMY

As expected the eight anamorphic species of *Sclerotium* were found to be connected to genera of both basidiomycetes and ascomycetes. Two of the species were found to be closely related based on rDNA sequence analyses and morphological characteristics to *Ceratobasidium*, anamorphs of which have been placed in *Ceratorhiza* (Mordue 1974, Moore 1989). Two new combinations are proposed:

Ceratorhiza hydrophila (Sacc.) Xu, Harrington, Gleason, et Batzer, comb. nov.
≡ *Sclerotium hydrophilum* Saccardo, Syll. Fung. 14:1141. 1899.
MycoBank MB 514193

Ceratorhiza rhizodes (Auersw.) Xu, Harrington, Gleason, et Batzer, comb. nov.
≡ *Sclerotium rhizodes* Auerswald, Bot. Ztg. 7:294. 1849.
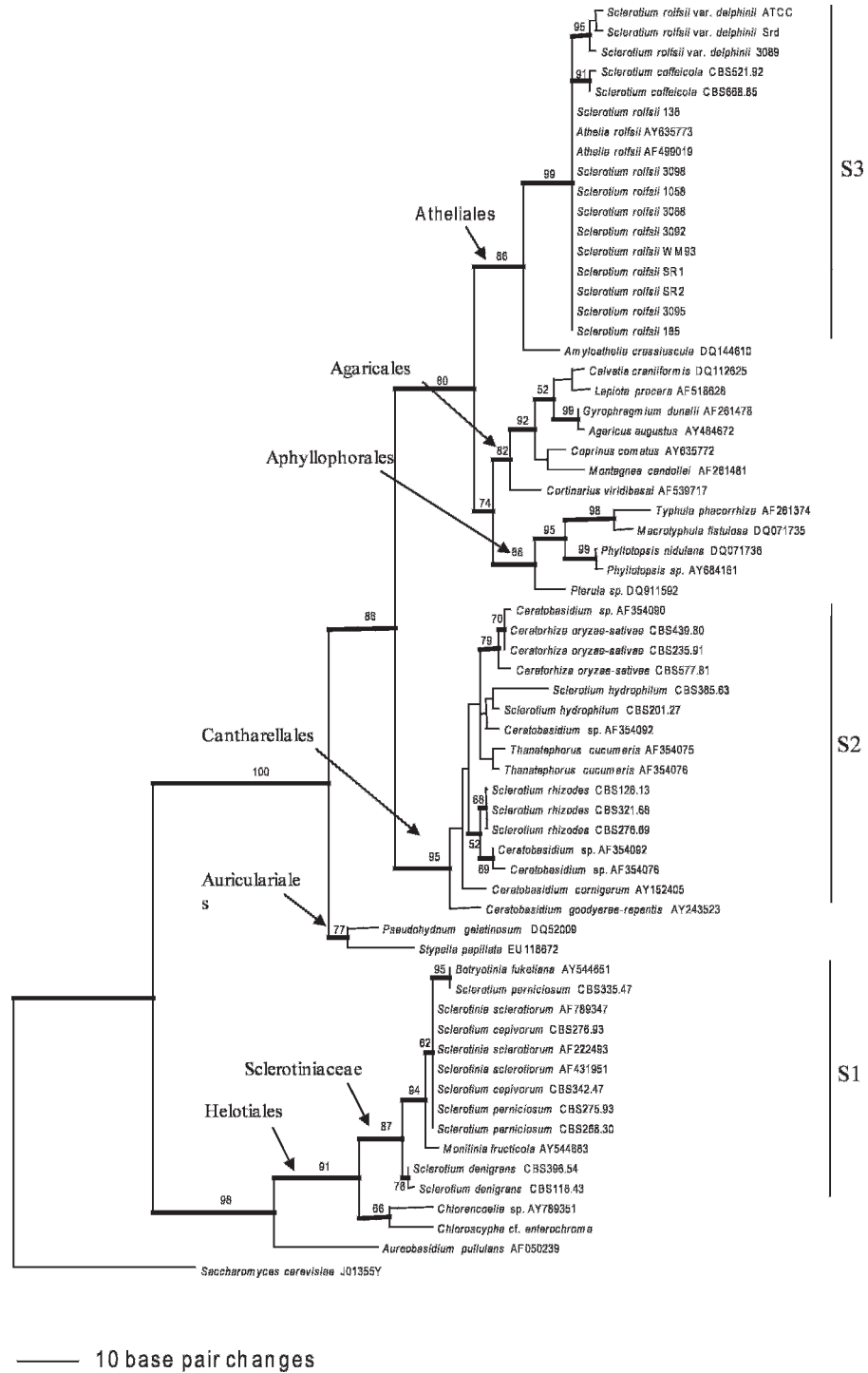MycoBank MB 514194

Fig. 1.   One of 128 equally most parsimonious trees of partial sequences of the 28 S large subunit (LSU) region of rDNA from *Sclerotium* species and other Basidiomycota and Ascomycota. The tree is rooted to *Saccharomyces cerevisiae.* There were 127 parsimony informative characters. Tree length = 385, consistency index (CI) = 0.6182, homoplasy index (HI) = 0.3818, CI excluding uninformative characters = 0.5751, HI excluding uninformative characters = 0.4249, retention index (RI) = 0.9177, rescaled consistency index (RC) = 0.5673. Bootstrap values > 50% are indicated above branches. Branches in boldface are supported by strict consensus of the most parsimonious trees.

FIG. 2. One of 97 equally most parsimonious trees of partial sequences of the ITS region of rDNA from isolates of *Sclerotium cepivorum, Sclerotium denigrans* and *Sclerotium perniciosum* and other Ascomycota. There were 29 parsimony informative characters. The tree is rooted to *Chloroscypha enterochrom.* Tree length = 182, consistency index (CI) = 0.9560, homoplasy index (HI) = 0.0440, CI excluding uninformative characters = 0.8298, HI excluding uninformative characters = 0.1702, retention index (RI) = 0.9208, rescaled consistency index (RC) = 0.8803. Bootstrap values > 50% are indicated above branches. Branches in boldface are supported by strict consensus of the most parsimonious trees.
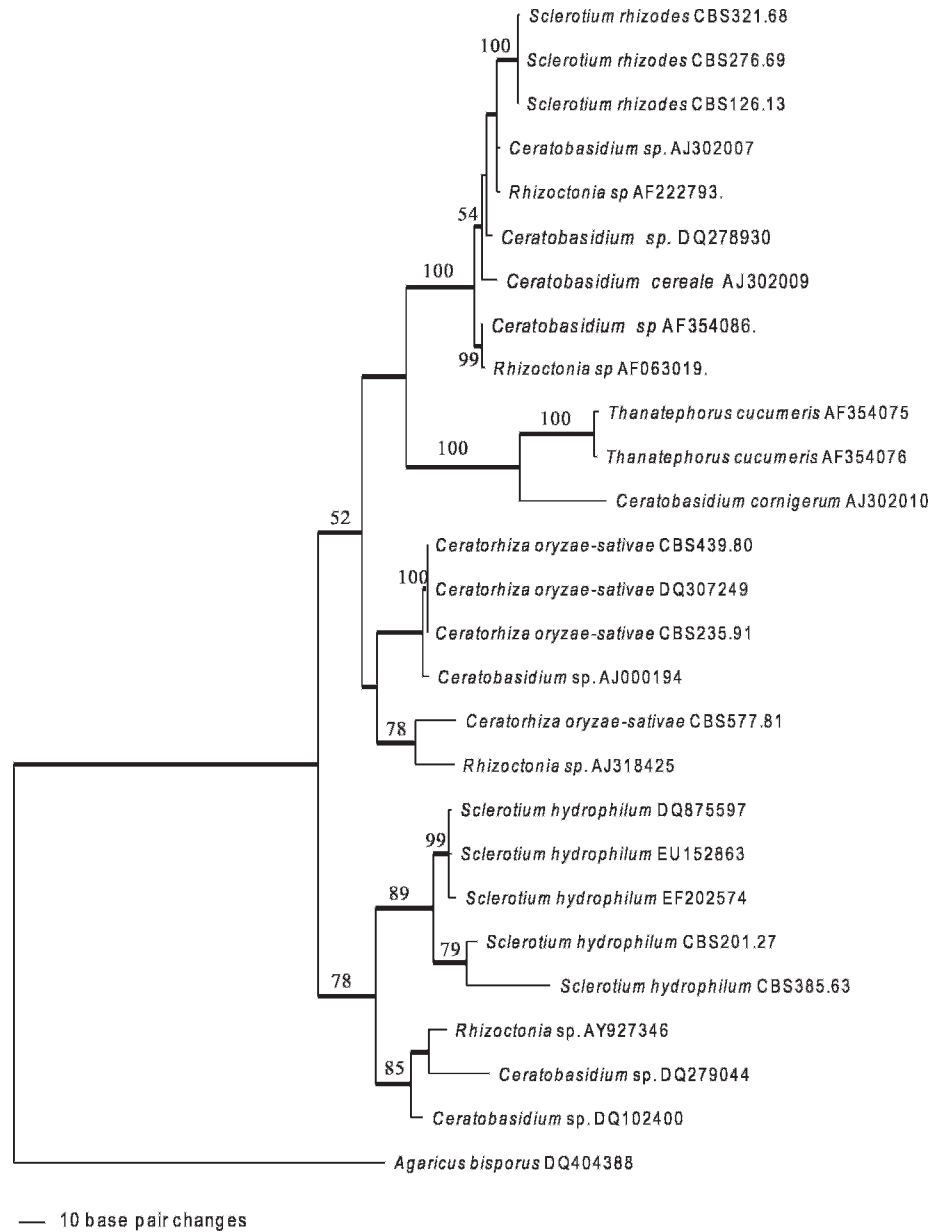
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                    ┌ Sclerotium rhizodes CBS321.68
              100 ┌─┤─ Sclerotium rhizodes CBS276.69
                  │ └ Sclerotium rhizodes CBS126.13
                  │  ┌ Ceratobasidium sp. AJ302007
                  │  ├ Rhizoctonia sp AF222793.
              54  │  ├ Ceratobasidium  sp. DQ278930
              100 │  ┤ Ceratobasidium  cereale AJ302009
                  │  ┌ Ceratobasidium  sp AF354086.
                  │ 99└ Rhizoctonia sp AF063019.
                  │       100 ┌ Thanatephorus cucumeris AF354075
                  │     100 ┌─┤ Thanatephorus cucumeris AF354076
                  │           └ Ceratobasidium cornigerum AJ302010
              52  │    ┌ Ceratorhiza oryzae-sativae CBS439.80
                  │100 ├ Ceratorhiza oryzae-sativae DQ307249
                  │    ├ Ceratorhiza oryzae-sativae CBS235.91
                  │    └ Ceratobasidium sp. AJ000194
                  │ 78 ┌ Ceratorhiza oryzae-sativae CBS577.81
                  │    └ Rhizoctonia sp. AJ318425
                  │      ┌ Sclerotium hydrophilum DQ875597
                  │   99 ├ Sclerotium hydrophilum EU152863
                  │   89 └ Sclerotium hydrophilum EF202574
                  │   79 ┌ Sclerotium hydrophilum CBS201.27
              78  │      └ Sclerotium hydrophilum CBS385.63
                  │    ┌ Rhizoctonia sp. AY927346
                  │ 85 ├ Ceratobasidium sp. DQ279044
                  │    └ Ceratobasidium sp. DQ102400
                  └ Agaricus bisporus DQ404388
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── 10 base pair changes

FIG. 3. One of four equally most parsimonious trees of partial sequences of the ITS region of rDNA from isolates of *Sclerotium hydrophilum, Ceratorhiza oryzae-sativae* and *Sclerotium rhizodes* and other Basidiomycota. There were 174 parsimony informative characters. The tree is rooted to *Agaricus bisporus.* Tree length = 706, consistency index (CI) = 0.8003, homoplasy index (HI) = 0.2962, CI excluding uninformative characters = 0.7038, HI excluding uninformative characters = 0.2962, retention index (RI) = 0.8604, rescaled consistency index (RC) = 0.6886. Bootstrap values > 50% are indicated above branches. Branches in boldface are supported by strict consensus of the most parsimonious trees.

DISCUSSION

Our study resolved or confirmed taxonomic placement of eight *Sclerotium* species among genera of Ascomycota and Basidiomycota. Some of these species previously were known to have teleomorphs or close affinities to teleomorph genera, but other species have been placed among teleomorph genera for the first time.

Our phylogenetic analysis agreed with Carbone and Kohn (1993) that *S. cepivorum* belongs in the Sclerotiniaceae and indicated that *S. denigrans* and *S. perniciosum* are also in this ascomycetous family. The rDNA data contradict earlier conclusions (Kirk 2004) that *S. denigrans* and *S. perniciosum* belong in the Basidiomycota.

Parsimony analysis of the LSU and ITS regions both indicated that *S. perniciosum* isolates CBS 268.30,

274.93 and 275.93 were closely related to isolates of *S. cepivorum*. However one of the *S. perniciosum* isolates, CBS 335.47, was in a different clade than other isolates of *S. perniciosum*. Colony color and number of sclerotia per plate of isolate CBS 335.47 differed from those of the other three *S. perniciosum* isolates, and it might be misidentified. The ITS sequence of CBS 335.47 was more similar to that of *Botryotinia fuckeliana* isolates than other *S. perniciosum*.

S. denigrans was reported as the cause of diseases on monocots, such as dying-off of lily of the valley (*Convallaria majalis)*, by Pape in Germany (Boerema and Hamers 1988). The causal organism of smolder disease of tulip, *S. perniciosum*, first was described in 1925 and also has been recorded as a pathogen of other monocots, such as *Fritillaria* and *Allium* (Boerema and Hamers 1988). Based on analyses of LSU and ITS regions there is evidence that *S. cepivorum*, *S. denigrans* and *S. perniciosum* have affinities with the sclerotial lineage in family Sclerotiniaceae, which is consistent with the conclusion of Carbone and Kohn (1993) and Holst-Jensen et al. (1998) based on ITS sequences. The crustose-like sclerotia seen in our cultures of *Sclerotium denigrans* correspond with the description of manteloid sclerotia produced by *Stromatinia* Whetzel (1945). Sclerotia of *Sclerotium perniciosum* were morphologically similar to those of *S. cepivorum*.

Sclerotium species in group S2 of the present study generally infect aquatic or semi-aquatic plants of wet meadows and marshes (Farr 2006). *S. hydrophilum* has been reported on wild rice (*Zizania aquatica*), rice (*Oryza sativa*), fragrant water lilies (*Nymphaea odorata*) and Eurasian water milfoil (*Myriophyllum spicatum*). *Ceratorhiza oryzae-sativae* causes sheath rot of rice (*Oryza sativa*). *S. rhizodes* first was isolated from reed canary grass (*Phalaris arundinacea*) (Saccardo 1899). The hyphae of these three species have branches at right angles, suggesting that they are related to *Rhizoctonia* species (Tredway and Burpee 2006). Our LSU and ITS phylogenetic analyses are consistent with the finding of Johanson et al. (1998), who reported that a *R. solani*-specific primer pair (GMRS-4 and ITS1) amplified DNA from *S. hydrophilum*. Although septal ultrastructure of isolates of *S. hydrophilium* and *S. rhizodes* was not determined, the binucleate hyphae, right-angle branching and similarity of rDNA sequences to *Ceratorhiza* species and *Ceratobasidium* indicate that *S. hydrophilum* and *S. rhizodes* belong in the anamorph genus *Ceratorhiza*.

Based on LSU sequence analysis fungi in group S3 are closely related to the teleomorph genus *Athelia*. A study of *S. coffeicola*, *S. rolfsii* and *S. rolfsii* var. *delphinii* (Punja and Damiani 1996), comparing colony characteristics, sclerotia formation, growth response to different temperature and media, and ability to produce oxalic acid and pectinases, also concluded that these three species are closely related.

In 1892 Peter Henry Rolfs wrote the first published report of losses due to *S. rolfsii* in USA on tomato in Florida (Aycock 1966, Punja 1988). *S. rolfsii* var. *delphinii* initially was named *S. delphinii* (Welch 1924), but it was renamed *S. rolfsii* var. *delphinii* by Boerema and Hamers (1988). Isolates 1058 and 3092 of *S. rolfsii* var. *delphinii* might be misidentified because they have ITS sequences and sclerotia characteristics consistent with our other isolates of var. *rolfsii*.

Our study provided new insights into phylogenetic relationships of *Sclerotium* species as a whole and phylogenetic placement for each individual species. The information is critical in assessing disease management strategies, such as fungicide application. Some classes of fungicides have modes of action that are specific for either Basidiomycota or Ascomycota (McGrath 2004). For instance carboxin is active on basidiomycetes whereas fenhexamid is active only on Ascomycota such as *Botrytis*, *Monilinia* and *Sclerotinia*. Our results could help growers choose appropriate fungicides and therefore could improve their ability to manage economically important diseases caused by these phytopathogens.

Results from our study suggested that morphological characters (color of mycelium and sclerotia and number of and size of sclerotia) alone are insufficient to delimit species of *Sclerotium*. Coupled with DNA sequence data however morphological characters are useful tools in delineating species (Harrington and Rizzo 1999). Future studies might involve detailed comparison of morphology, physiology and sequence data.

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