



Novel fungal genera and species associated with the sooty blotch and flyspeck complex on apple in China and the USA

H.L. Yang¹, G.Y. Sun¹, J.C. Batzer², P.W. Crous³, J.Z. Groenewald³, M.L. Gleason²

Key words

anamorph
SBFS
taxonomy

Abstract Fungi in the sooty blotch and flyspeck (SBFS) complex cause blemishes on apple and pear fruit that result in economic losses for growers. The SBFS fungi colonise the epicuticular wax layer of pomaceous fruit but do not invade the cuticle. Fungi causing fuliginous and punctate mycelial types on apple are particularly difficult to identify based on morphological criteria because many species in the SBFS complex share the same mycelial phenotypes. We compared the morphology and nuclear ribosomal DNA phylogeny (ITS, LSU) of 11 fungal strains isolated from SBFS blemishes on apple obtained from two provinces in China and five states in the USA. Parsimony analysis, supported by cultural characteristics and morphology in vitro, provided support to delimit the isolates into three novel genera, representing five new species. *Phaeothecoidiella*, with two species, *P. missouriensis* and *P. illinoisensis*, is introduced as a new genus with pigmented endoconidia in the *Dothideomycetes*. *Houjia* (*Capnodiales*) is introduced for *H. pomigena* and *H. yanglingensis*. Although morphologically similar to *Stanjehughesia* (*Chaetosphaeriaceae*), *Houjia* is distinct in having solitary conidiogenous cells. *Sporidesmajora* (*Capnodiales*), based on *S. pennsylvaniensis*, is distinguished from *Sporidesmium* (*Sordariomycetes*) in having long, multiseptate conidiophores that frequently have a subconical, darkly pigmented apical cell, and very long, multi-euseptate conidia.

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INTRODUCTION

Fungi in the sooty blotch and flyspeck (SBFS) complex occur in humid temperate regions and blemish the appearance of pomaceous fruits, resulting in economic losses for growers. The SBFS fungi colonise the epicuticular wax layer of apple fruit. Colonies that form dark mycelial mats are referred to as 'sooty blotch', whereas 'flyspeck' designates clusters of shiny, black, round to ovoid, sclerotium-like bodies lacking a visible mycelial mat.

Progress in understanding the disease has been slowed by difficulty in isolating, maintaining and identifying SBFS fungi in culture, as well as paucity of fruiting structures on fruit and in culture (Hickey 1960). Prior to combining DNA sequencing with culture-based morphology studies, the SBFS complex was assumed to be comprised of fewer than five species (Colby 1920, Johnson et al. 1996, 1997). When anamorph states of purified isolates collected from blemished apples in the USA and China were studied and parsimony analysis of rDNA genotypes was conducted, it became clear that the SBFS complex comprised > 60 species of fungi, mostly within the *Capnodiales* (Batzer et al. 2005, 2008, Sun et al. 2008, Zhai et al. 2008, Díaz Arias et al. In press, Li et al. In press, Ma et al. In press).

Despite the recent discovery that the SBFS complex is highly diverse, little is known about the geographic range of individual species. Surveys in eastern USA revealed that some SBFS species were ubiquitous whereas others were localised to sub-regions (Díaz Arias et al. In press). The SBFS fungus *Zygophiala cryptogama* occurs in Asia and as well as North

America (Batzer et al. 2008, Li et al. In press), and *Peltaster fructicola* and *Schizothyrium pomi* occur in both the USA and the Balkans (Ivanović et al. In press), but the range of most species in the complex has not been defined. However, recent surveys in China revealed several fungi that were highly similar genetically and morphologically to isolates from the USA (Sun & Gleason, unpubl. data). The aim of this study was to describe a novel group of closely related SBFS fungi in China and the USA by assessing nuclear ribosomal DNA sequences and morphology.

MATERIALS AND METHODS

Isolates

During the fall of 2007 apples infected with SBFS were collected from orchards in Jingning County, Gansu Province, and Lingboa, Henan Province, China. Thalli were transferred from colonies on the apple surface to potato-dextrose agar (PDA; Crous et al. 2009c) slants and cultured at 25 °C in darkness (Sun et al. 2003). Two isolates from China were selected for this study, along with nine isolates sampled in 2000 and 2005 from five orchards in Missouri, Illinois, Kentucky, Tennessee and Pennsylvania, USA (Batzer et al. 2005, Díaz Arias et al. In press). All isolates were purified and stored in glycerol at -80 °C at Iowa State University. Segments of apple peels exhibiting colonies with sooty blotch morphology were preserved by pressing the thallus and supporting peel between paper towels until dry. Specimens on apple peels were deposited at the Iowa State University Herbarium, Ames, Iowa.

Single-conidial isolates were established on malt extract agar (MEA; 20 g/L Biolab malt extract, 15 g/L Biolab agar) using the technique of Crous (1998). Cultures were plated onto fresh MEA, 2 % PDA and oatmeal agar (OA; Crous et al. 2009c), and subsequently incubated at 25 °C under near-ultraviolet light to promote sporulation. Reference strains are maintained in the culture collection of the Centraalbureau voor Schimmelcultures

¹ College of Plant Protection & Shaanxi Key Laboratory of Molecular Biology for Agriculture, Northwest A&F University, Yangling, Shaanxi, 712100, China; corresponding author e-mail: sgy@nwsuaf.edu.cn

² Department of Plant Pathology, Iowa State University, Ames Iowa 50011, USA.

³ CBS-KNAW Fungal Biodiversity Centre, P.O. Box 85167, 3508 AD Utrecht, the Netherlands.

Table 1 Collection details and GenBank accession numbers of isolates for which novel sequences were generated in this study.

Species	Strain no. ¹	Substrate	Country	Collector	GenBank Accession number (ITS, LSU) ²
<i>Houjia pomigena</i>	CBS 125224; CPC 16109; CMG UIF2b	<i>Malus</i> sp. (fuliginous morphology on apple)	Illinois, USA	M. Gleason	AY598885, AY598925
<i>Houjia yanglingensis</i>	CBS 125225; CPC 16114; CMG YHJB13 CBS 125226; CPC 16113; CMG LB20 CBS 125227; CPC 16111; CMG TN1 2.2F1d CBS 125228; CPC 16110; CMG KY3 13F1d	<i>Malus</i> sp. (fuliginous morphology on apple) <i>Malus</i> sp. (fuliginous morphology on apple) <i>Malus</i> sp. (fuliginous morphology on apple) <i>Malus</i> sp. (fuliginous morphology on apple)	Jingning, China Lingbao, China Tennessee, USA Kentucky, USA	G.Y. Sun G.Y. Sun S. Bost J. Hartman	GQ433628, GQ433631 GQ433629, GQ433630 FJ438378, FJ471466 FJ438377, FJ471465
<i>Phaeotheca fissurella</i>	CBS 520.89; ATCC 44385; CBS 158.81; DAOM 178454; IMI 254604; UAMH 4245	<i>Pinus contorta</i>	Canada	J. Petty	AJ244255, GU117900
<i>Phaeothecoidea intermedia</i>	CBS 124994; CPC 13711	<i>Eucalyptus globulus</i>	Australia	B.A. Sumnerell	GQ852754, GQ852628
<i>Phaeothecoidea minutispora</i>	CBS 124995; CPC 13710	<i>Eucalyptus globulus</i>	Australia	B.A. Sumnerell	GQ852755, GQ852629
<i>Phaeothecoidea illinoisensis</i>	CBS 118947; CMG UIE5 CBS 125223; CPC 16115; CMG UIE3a CMG TN1_2.4E1d	<i>Malus</i> sp. (punctate morphology on apple) <i>Malus</i> sp. (punctate morphology on apple) <i>Malus</i> sp. (punctate morphology on apple)	Illinois, USA Illinois, USA Tennessee, USA	M. Gleason M. Gleason S. Bost	AY598879, AY598918 GU117897, GU117901 GU117898, GU117902
<i>Phaeothecoidea missouriensis</i>	CBS 118959; CMG AHE7a CBS 125222; CPC 16116; CMG AHE7c	<i>Malus</i> sp. (punctate morphology on apple) <i>Malus</i> sp. (punctate morphology on apple)	Missouri, USA Missouri, USA	M. Gleason M. Gleason	GU117899, GU117903 AY598878, AY598917
<i>Sporidesmajora pennsylvaniensis</i>	CBS 125229; CPC 16112; CMG PA1_9F1a	<i>Malus</i> sp. (fuliginous morphology on apple)	Pennsylvania, USA	J.W. Travis	FJ438379, FJ471467

¹ ATCC: American Type Culture Collection, Virginia, USA; CBS: CBS Fungal Biodiversity Centre, Utrecht, The Netherlands; CMG: Culture collection of P.W. Crous, housed at CBS; DAOM: Plant Research Institute, Department of Agriculture (Mycology), Ottawa, Canada; IMI: International Mycological Institute, CAB International, Egham, Surrey, UK; UAMH: University of Alberta Microfungus Collection, Alberta, Canada.

² ITS: Internal transcribed spacers 1 and 2 together with 5.8S nrDNA; LSU: 28S nrDNA.

(CBS), Utrecht, the Netherlands, and at Iowa State University (Table 1). Descriptions, nomenclature, and illustrations were deposited in MycoBank (Crous et al. 2004).

Genomic DNA was isolated from fungal mycelium grown on MEA, using the UltraClean™ Microbial DNA Isolation Kit (Mo Bio Laboratories, Inc., Solana Beach, CA, USA) according to the manufacturer's protocols. The Primers V9G (de Hoog & Gerrits van den Ende 1998) and LR5 (Vilgalys & Hester 1990) were used to amplify part of the nuclear rDNA operon spanning the 3' end of the 18S rRNA gene (SSU), the first internal transcribed spacer (ITS1), the 5.8S rRNA gene, the second ITS region (ITS2) and the first 900 bases at the 5' end of the 28S rRNA gene (LSU). The primers ITS4 (White et al. 1990) and LR0R (Rehner & Samuels 1994) were used as internal sequence primers to ensure good quality sequences over the entire length of the amplicon. The PCR conditions, sequence alignment and subsequent phylogenetic analysis followed the methods of Crous et al. (2006). Alignment gaps were treated as new character states. Novel sequence data were deposited in GenBank (Table 1) and the alignment in TreeBASE (www.treebase.org).

Morphology

Signs of SBFS on preserved apple peels are described, including mycelial growth patterns and fruiting body size and density. Morphological descriptions are based on cultures sporulating on synthetic nutrient-poor agar (SNA; Crous et al. 2009c) in vivo. Wherever possible, 30 measurements ($\times 1\,000$ magnification) were made of all taxonomically informative structures mounted in lactic acid, with the extremes of spore measurements given in parentheses. Colony colours (surface and reverse) were assessed after 1 mo on MEA, PDA and OA at 25 °C in the dark, using the colour charts of Rayner (1970).

RESULTS

Phylogenetic analysis

Amplification products of approximately 1 700 bases were obtained for the isolates listed in Table 1. The LSU region of these sequences was used to obtain additional sequences from GenBank, which were added to the alignment. Due to the inclusion of the shorter LSU sequences of 'AHE7c' (AY598917), *Stomiopeltis* sp. (AY598919) and *Stomiopeltis versicolor* (FJ147163) in the alignment, it was not possible to subject the full length of the determined LSU sequences (Table 1) to the analysis. The manually adjusted LSU alignment contained 85 sequences (including the outgroup sequence) and, of the 568 characters used in the phylogenetic analysis, 271 were parsimony-informative, 62 were variable and parsimony-uninformative, and 235 were constant. Fourteen equally most parsimonious trees (TL = 1 737 steps; CI = 0.349; RI = 0.749; RC = 0.261), the first of which is shown in Fig. 1, were obtained from parsimony analysis of the LSU alignment. The results of the phylogenetic analysis are highlighted below under the taxonomic notes or in the Discussion, where applicable. Although the ITS sequences were not used in phylogenetic analyses, they were lodged in GenBank for future studies and identification purposes.

Taxonomy

The present study resulted in discovery of several novel genera and species that are associated with SBFS on apples. These taxa are treated below:

Phaeothecoidea Batzer & Crous, *gen. nov.* — MycoBank MB514394

Mycelio ex hyphis brunneis, laevibus vel subtiliter exasperatis, ramosis, atro-brunneis, cum vagina crassa mucilaginis; endoconidiis phragmosporioidibus, in hyphis evolutis, pallide vel modice brunneis, aseptatis, tenuitunicatis,

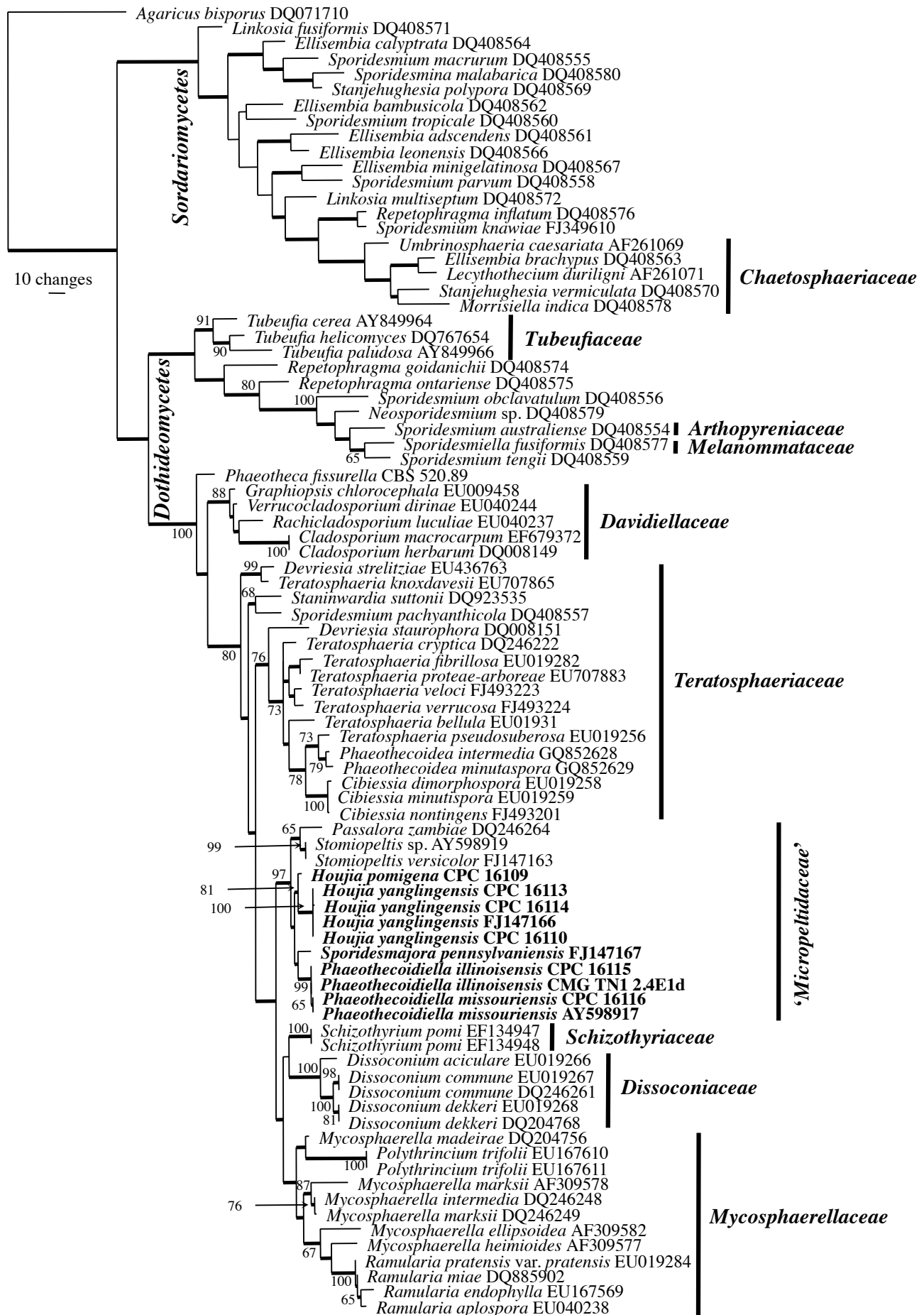


Fig. 1 The first of 14 equally most parsimonious trees obtained from a heuristic search with 100 random taxon additions of the LSU sequence alignment. The scale bar shows 10 changes, and bootstrap support values > 64 % from 1 000 replicates are shown at the nodes. Novel species from *Malus* treated in this study are indicated in **bold**. Branches present in the strict consensus tree are thickened. Classes are shown to the left of the tree and families, where known, to the right. The tree was rooted to a sequence of *Agaricus bisporus* (GenBank accession DQ071710).

subcylindraces vel late ellipsoideis, sed deinde (post liberationem) subglobosis, atro-brunneis, verruculosus, interdum cum septo tenui inconspicuo.

Type species. Phaeothecoidiella missouriensis Batzer & Crous, *sp. nov.*

Etymology. Named after the genus *Phaeothecoidea*, which it resembles.

Hyphomycetous. *Mycelium* consisting of brown, smooth to finely roughened, branched, regularly septate hyphae, becoming wider, dark brown, with irregular warts, encased in a thick mucilaginous sheath. *Endoconidia* developing like phragmospores inside hyphae, pale to medium brown, aseptate, thin-walled, subcylindrical to broadly ellipsoid but becoming more globose, dark brown and roughened upon release; at times with a thin, inconspicuous septum.

Notes — *Phaeothecoidiella* resembles three other genera with pigmented endoconidia in the *Dothideomycetes*, namely *Phaeotheca*, which clusters basal to the *Davidiellaceae*, *Hyphospora* (teleomorph: *Cumminutispora*, *Planistromellaceae*), and *Phaeothecoidea*, which resides in the *Teratosphaeriaceae* (see Crous et al. 2009a). Morphologically there is little to choose among these genera, although species of *Phaeothecoidiella* tend to have endoconidia developing like phragmospores inside hyphae, rarely arranged in clusters inside hyphal cells. Furthermore, hyphae are covered in a prominent mucilaginous sheath, suggesting that taxa could occur elsewhere in nature as true sooty moulds (Hughes 1976), probably being opportunistic on the surface of apple fruit.

Phaeothecoidiella illinoisensis* Batzer & Crous, *sp. nov.

— MycoBank MB514395; Fig. 2

Mycelio ex hyphis brunneis, laevibus vel subtiliter exasperatis, ramosis, 3–4 µm latis, cum vagina mucilaginosa, ad 1.5 µm lata; endoconidiis raro in fasciculis in cellulis hypharum, pallid vel modice brunneis, aseptatis, globosis vel ellipsoideis, laevibus vel exasperatis, 5–8 × 4–5 µm.

Etymology. Named after its type locality, Illinois, USA.

On SNA. *Mycelium* consisting of brown, smooth to finely roughened, branched, regularly septate, 3–4 µm diam hyphae, becoming wider and dark-brown with endoconidia, and covered

in a mucilaginous sheath, up to 1.5 µm thick. *Endoconidia* developing like phragmospores inside hyphae, rarely in clusters inside hyphal cells, pale to medium brown, aseptate, globose to ellipsoid, smooth to roughened, 5–8 × 4–5 µm; older conidia becoming more globose to broadly ellipsoid, thin-walled, pale brown to brown, 6–10 µm diam.

Culture characteristics — Colonies after 1 mo at 25 °C in the dark on MEA erumpent, slimy, with sparse aerial mycelium and even, lobate margin; surface iron-grey, reaching 18 mm diam. Similar on PDA and OA, reaching up to 10 mm diam.

Appearance on apple — Punctate mycelia type; dark brown to black colonies; reaching 2.5 mm diam with even, feathered margins; shiny black sclerotium-like bodies scattered on the surface at a density of 15 per mm² and ranging from 28 µm to 60 µm diam.

Specimen examined. USA, Illinois, on fruit surface of apple, Sept. 2000, M. Gleason, CBS H-20323 holotype, cultures ex-type CPC 16115 = UIE3a = CBS 125223.

Phaeothecoidiella missouriensis* Batzer & Crous, *sp. nov.

— MycoBank MB514396; Fig. 3

Mycelio ex hyphis brunneis, laevibus vel subtiliter exasperatis, ramosis, 2–4 µm latis, cum vagina crassa mucilagina, ad 2 µm lata; endoconidiis medio-brunneis, aseptatis, 3–7 × 3–5 µm, tenuitunicatis, subcylindraces; conidiis veteribus ad 12 µm longis et 8 µm latis.

Etymology. Named after its type locality, Missouri, USA.

On SNA. *Mycelium* consisting of brown, smooth to finely roughened, branched, regularly septate, 2–4 µm diam hyphae, becoming wider, dark brown, with irregular warts, up to 1 µm high, or encased in a thick, mucilaginous sheath, up to 2 µm thick. *Endoconidia* developing like phragmospores inside hyphae, medium brown, aseptate, 3–7 × 3–5 µm, thin-walled, subcylindrical, but becoming more globose, dark brown and roughened upon release, up to 12 µm long and 8 µm wide, at times with a thin, inconspicuous septum.

Cultural characteristics — Colonies after 1 mo at 25 °C in the dark on MEA erumpent, spreading, with sparse aerial mycelium and uneven, crenate margin; surface uneven, folded, iron-grey,

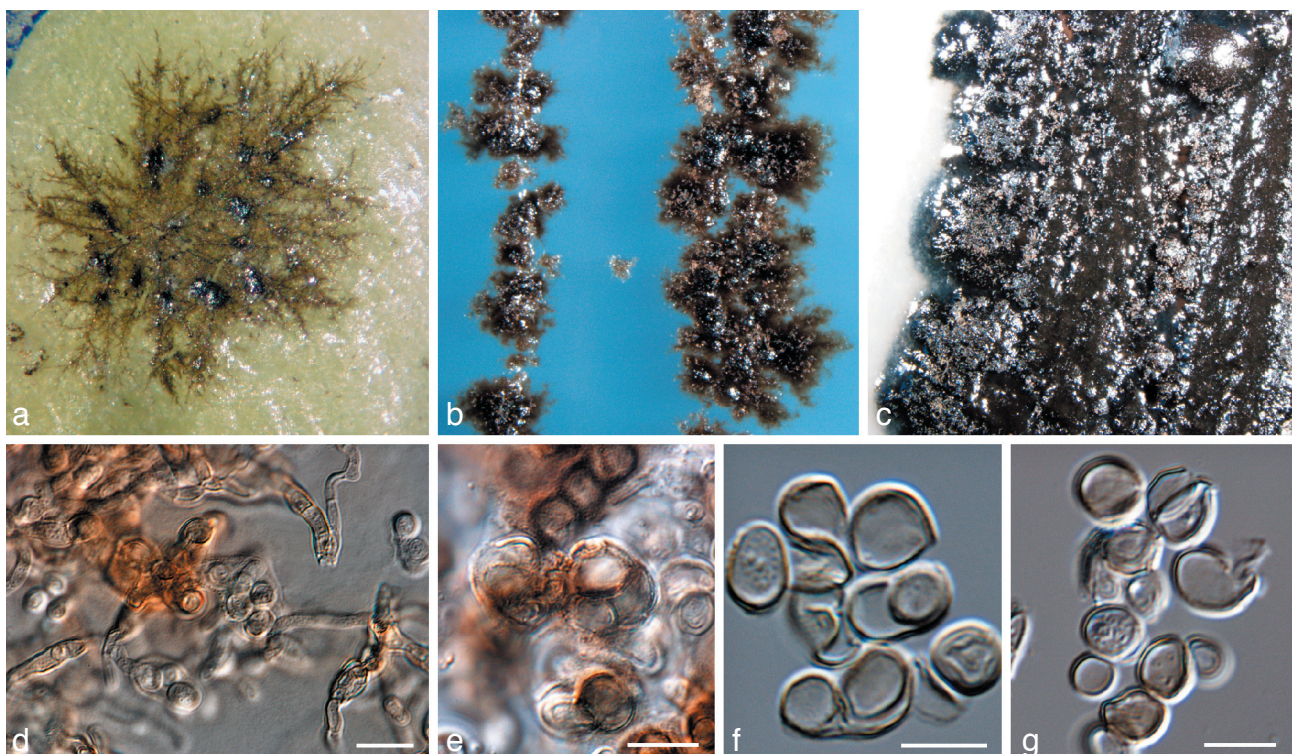


Fig. 2 *Phaeothecoidiella illinoisensis* (CPC 16115). a. Colony on apple surface; b. colony on SNA; c. colony on OA; d, e. hyphae with endoconidia; f, g. endoconidia. — Scale bars = 10 µm.

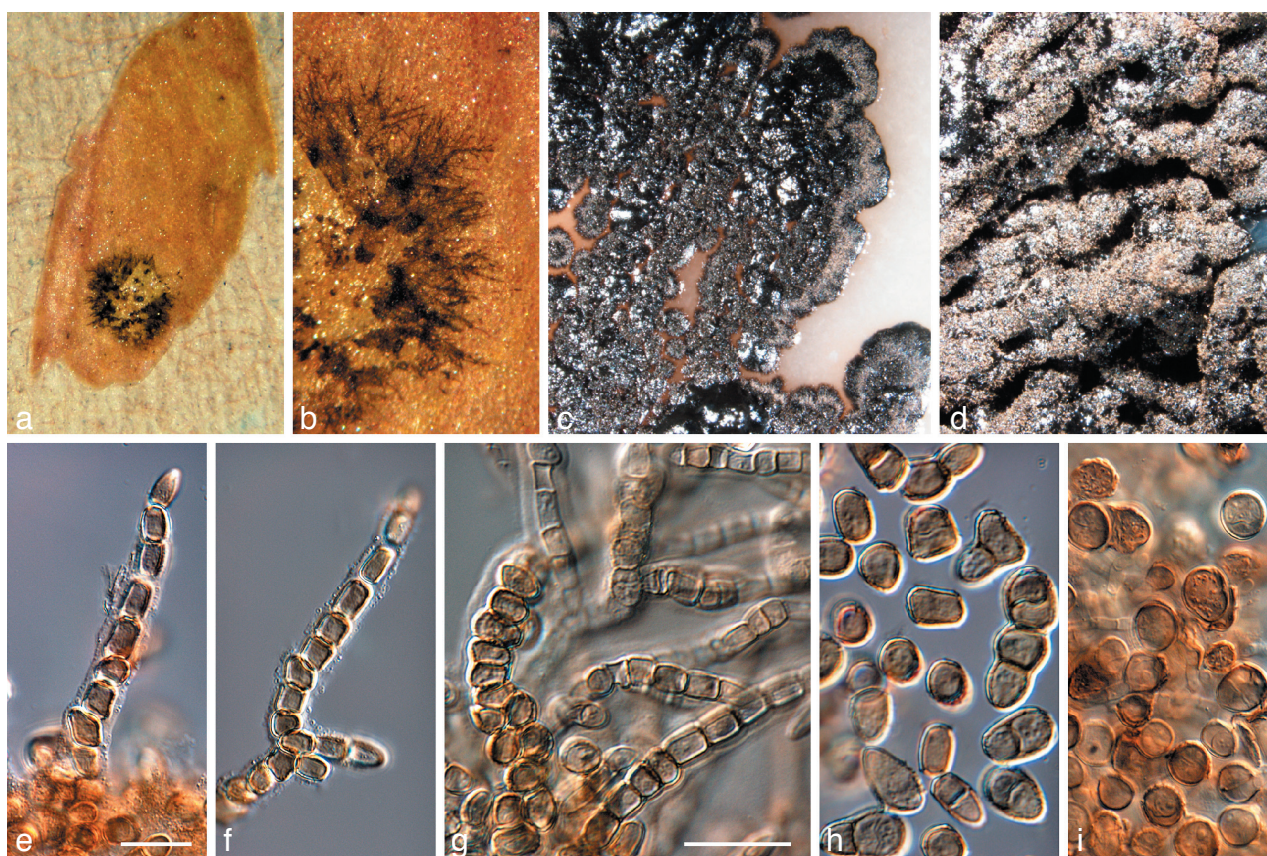


Fig. 3 *Phaeothecoidiella missouriensis* (CPC 16116). a, b. Colonies on apple surface; c. colony on OA; d. colony on SNA; e–g. hyphae with phragmo- and endoconidia (note sheath); h, i. conidia. — Scale bars = 10 μ m.

reaching up to 20 mm diam. Similar on PDA, reaching up to 15 mm diam, as well as on OA, reaching up to 10 mm diam.

Appearance on apple — Punctate mycelial type (Batzler et al. 2005); dark brown to black colonies; diameter reaching 2 mm with irregular feathered margins; shiny black sclerotium-like bodies scattered on the surface at a density of 10 per mm^2 and ranging in diameter from 17 μ m to 47 μ m.

Specimens examined. USA, Missouri, on fruit surface of apple, Sept. 2000, M. Gleason, CBS H-20322 holotype, cultures ex-type CPC 16116 = AHE7c = CBS 125222; AHE7a = CBS 118959.

Notes — Both species of *Phaeothecoidiella* have endoconidia that resemble phragmospores inside hyphae, and are rarely arranged in clusters. Conidia of *P. missouriensis* are 3–7 \times 3–5 μ m, while those of *P. illinoisensis* are somewhat larger, 5–8 \times 4–5 μ m. It would be difficult, however, to separate these taxa without the aid of molecular data (Table 1).

***Houjia* G.Y. Sun & Crous, gen. nov.** — MycoBank MB514397

Mycelio ex hyphis ramosis, septatis, brunneis, exasperatis vel verrucatis, ad septa saepe constrictis; conidiophoris in cellulis conidiogenis reductis, intercalaribus, atro-brunneis, subcylindraceis vel cuneiformibus, crassitunicatis, latitudine maximo basali, apice truncato; formatione conidiorum holoblastica vel phialidica; conidiis late ellipsoideis-subcylindraceis vel obclavatis, basi truncata, apice obtuse rotundato, transverse et oblique septatis, ad septa leniter constrictis, subtiliter verruculosi, medio-brunneis, tenuitunicatis, basi cuneiforme attenuata, hilo truncato.

Type species. *Houjia yanglingensis* G.Y. Sun & Crous, sp. nov.

Etymology. Named after Hou Ji, the creator of the Yellow River Farming Civilization in central China and a famous agriculture educator 4 000 years ago, who gave lectures in Yangling, China.

Hyphomycetous. *Mycelium* consisting of branched, septate, brown, roughened to warty hyphae, frequently constricted at septa. *Conidiophores* reduced to conidiogenous cells, intercalary, dark-brown, subcylindrical to cuneiform, thick-walled,

widest at the base, apex truncate; conidiogenesis unclear, varying from holoblastic to phialidic. *Conidia* broadly ellipsoid to subcylindrical or obclavate, base truncate, apex obtusely rounded, transversely and obliquely septate, slightly constricted at septa, finely verruculose, medium brown, thin-walled, tapering to a cuneiform basal part with a truncate hilum.

Notes — Morphologically the genus resembles *Janetia*, *Stanjehughesia* and *Linkosia*. It can be distinguished from *Stanjehughesia* by having solitary conidiogenous cells that are not aggregated in clusters (Hughes & Ilman 1974). Furthermore, phylogenetically it clusters in the *Capnodiales*, whereas the type strain of *Stanjehughesia*, *S. hormiscioides*, has *Umbrinosphaeria caesariata* as teleomorph (Réblová & Winka 2001), and resides in the *Chaetosphaeriaceae*. *Houjia* is distinct from *Linkosia* by having euseptate conidia, and from *Janetia* by having monoblastic conidiogenous cells (Wu & Zhuang 2005).

Hughes (1983) was one of the first to remark on the fact that sporidesmium-like taxa that were foliicolous and have monoblastic conidiogenous cells needed to be placed elsewhere. At this time he chose to widen the concept of *Janetia*, placing these taxa there. His description of *Janetia capnophila* appears to be allied to *Houjia*. The latter taxon occurred on leaves of a range of diverse plant hosts, and was associated with various species of sooty moulds.

***Houjia pomigena* Batzer & Crous, sp. nov.** — MycoBank MB514398; Fig. 4

Mycelio ex hyphis ramosis, septatis, brunneis, exasperates vel verrucatis, 2–3 μ m latis; cellulis conidiogenis intercalaribus, atro-brunneis, subcylindraceis vel cuneiformibus, crassitunicatis, 12–16 \times 5–9 μ m; conidiis obclavatis, basi truncate, apice obtuse rotundata, 4(–5)-septata, ad septa leniter constrictis, subtiliter verruculosi, medio-brunneis, tenuitunicatis, (30–)38–40(–47) \times (5–)6–7(–8) μ m.

Etymology. Named after the fruit that it blemishes.

On SNA. *Mycelium* consisting of branched, septate, brown, roughened to warty, 2–3 μm wide hyphae, frequently constricted at septa. *Conidiophores* reduced to conidiogenous cells, intercalary, dark-brown, subcylindrical to cuneiform, thick-walled, widest at the base, 12–16 \times 5–9 μm , apex truncate, 3 μm wide, at times appearing somewhat cup-shaped with what could be periclinal thickening, but conidiogenesis unclear, varying from holoblastic to phialidic. *Conidia* obclavate, base truncate, apex obtusely rounded, 4(–5)-septate, slightly constricted at septa, finely verruculose, medium brown, thin-walled, widest in middle of basal cell, from where it tapers to a cuneiform basal part with a truncate hilum that is dark brown, 3 μm wide and long, appearing more thick-walled than the rest of the conidium, (30–)38–40(–47) \times (5–)6–7(–8) μm .

Cultural characteristics — Colonies after 1 mo at 25 °C in the dark on PDA leaden-black, erumpent, folded, lacking aerial mycelium, and with lobate margins, reaching 5 mm diam. On SNA similar, but more flattened, reaching 5 mm diam. On MEA similar, but more erumpent, reaching 10 mm diam. On OA similar, reaching 15 mm diam.

Appearance on apple — Fuliginous mycelial type; lacking sclerotium-like bodies; densely arranged ropey mycelia, arborescent margins; circular shaped, brown colonies reaching 10 mm diam.

Specimen examined. USA, Illinois, on fruit surface of apple, Sept. 2000, M. Gleason, CBS H-20324 holotype, cultures ex-type CPC 16109 = UIF2b = CBS 125224.

Houjia yanglingensis G.Y. Sun & Crous *sp. nov.* — MycoBank MB514399; Fig. 5

Mycelio ex hyphis ramosis, septatis, medi-brunneis, subtiliter exasperatis, 2–3 μm latis; cellulis conidiogenis solitariis, intercalariis, erectis, subcylindraceis, brunneis, laevibus, 0–1-septatis, 10–15 \times 5–6 μm ; conidiis brunneis, laevibus, rectis vel leniter curvatis, (18–)25–28(–30) \times (5–)6–7(–8) μm , muriforme septatis, 3–6(–10) transverse et 1–4 oblique septatis.

Etymology. Named after its type locality, the city in China where it was first isolated.

On SNA. *Mycelium* consisting of branched, septate, medium brown, finely roughened, 2–3 μm wide hyphae. *Conidiophores* solitary, intercalary, erect, subcylindrical, brown, smooth, 0–1-septate, 10–15 \times 5–6 μm . *Conidiogenous cells* brown, smooth, subcylindrical, tapering towards a subtruncate apex, 1.5–2 μm wide, 6–11 \times 3–4 μm . *Conidia* brown, smooth, straight to slightly curved, (18–)25–28(–30) \times (5–)6–7(–8) μm , muriformly septate, with 3–6(–10) transverse (eusepta or distosepta), and 1–4 oblique septa, slightly constricted at septa, broadly ellipsoid to subcylindrical or obclavate, widest in the middle; apex obtuse, tapering towards a cuneiform basal part with subtruncate base, 1.5–2 μm wide, 1–2 μm long.

Culture characteristics — Colonies after 1 mo at 25 °C in the dark on MEA erumpent, spreading, with sparse aerial mycelium and folded surface, with even, lobate margins; colonies iron-grey, reaching up to 30 mm diam. On PDA similar, with moderate aerial mycelium, reaching 30 mm diam. On OA similar, outer region more olivaceous-grey in colour, reaching 35 mm diam.

Appearance on apple — Fuliginous mycelial type; lacking sclerotium-like bodies; irregularly shaped brown colonies with even margins reaching 5 mm diam.

Specimens examined. CHINA, Jingning County, Gansu Province, on fruit surface of apple, Oct. 2007, G.Y. Sun, CBS H-20325 holotype, cultures ex-type CPC 16114 = JN13 = CBS 125225; Lingbao County, Henan Province, on fruit surface of apple, Oct. 2007, G.Y. Sun, cultures CPC 16113 = LB20 = CBS 125226. — USA, cultures CPC 16111 = TN1_2.2F1d = CBS 125227; Davidson County, Tennessee, on fruit surface of apple, Sept. 2005, S.C. Bost, cultures CPC 16110 = KY3_13F1d = CBS 125228; Paducah, Kentucky, on fruit surface of apple, Sept. 2005, J. Hartman.

Notes — *Houjia pomigena* can be distinguished from *H. yanglingensis* by having conidia that are 30–47 \times 5–8 μm , 4(–5)-septate, while those of *H. yanglingensis* are shorter (18–30 \times 5–8 μm), with 3–6(–10) transverse and 1–4 oblique septa.

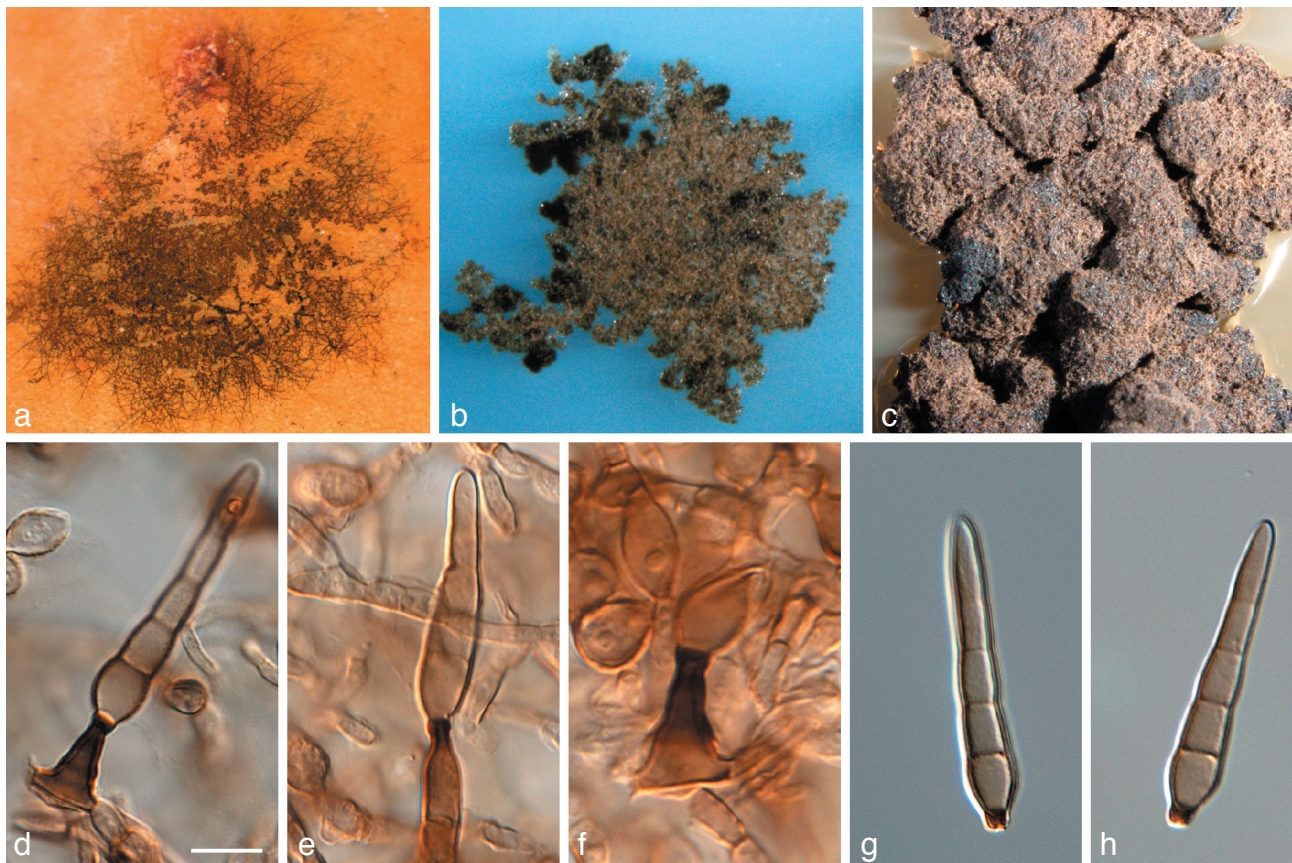


Fig. 4 *Houjia pomigena* (CPC 16109). a. Colony on apple surface; b. colony on SNA; c. colony on OA; d–f. conidiogenous cells giving rise to conidia; g, h. conidia. — Scale bar = 10 μm .

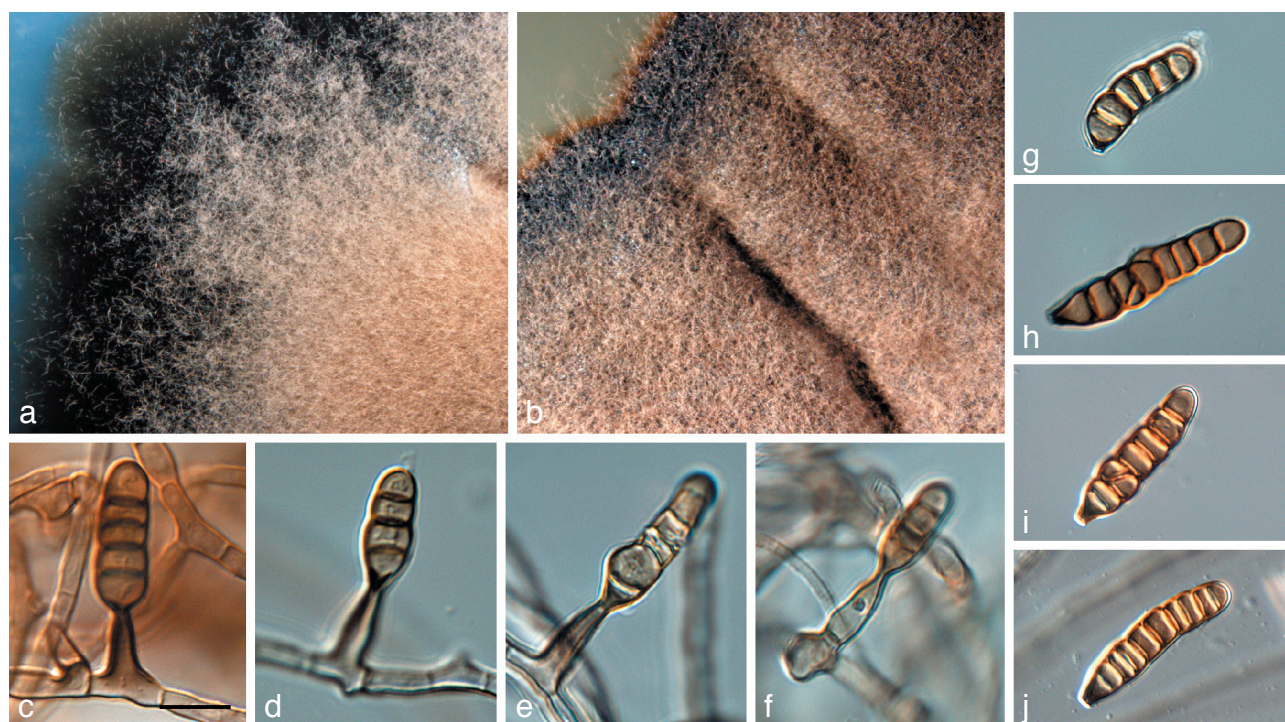


Fig. 5 *Houjia yanglingensis* (CPC 16114). a. Colony on SNA; b. colony on MEA; c–f. conidiogenous cells giving rise to conidia; g–j. conidia. — Scale bar = 10 µm.

***Sporidesmajora* Batzer & Crous, gen. nov. — MycoBank MB514400**

Mycelio ex hyphis ramosis, septatis, brunneis, subtiliter verruculosus, cum chlamydosporis brunneis in fasciculis terminalibus; conidiophoris erectis, subcylindraceis, saepe subclavatis, medio- vel atro-brunneis, subtiliter verruculosus, 1–pluriseptatis, ex celluli hypharum oriundis, pro ramulis lateralibus, vel inflatis et bulbosis; cellulis conidiogenis terminalibus, medio-brunneis, subcylindraceis vel subconicis, holoblasticis; conidiis obclavatis vel longe obclavatis, rectis vel curvatis, medio-brunneis, laevibus vel subtiliter verruculosus, guttulis, transverse pluriseptatis.

Type species. *Sporidesmajora pennsylvaniensis* Batzer & Crous, sp. nov.

Etymology. Named after its resemblance to *Sporidesmium*, but with much larger conidia.

Hyphomycetous. *Mycelium* consisting of branched, septate, brown, finely verruculose hyphae, frequently constricted at septa, and at times giving rise to terminal clusters of brown chlamydospores. *Conidiophores* erect, subcylindrical, frequently somewhat clavate, medium to dark brown, finely verruculose, at times with percurrent rejuvenation, 1–multi-septate; base arising as lateral branch directly from hyphal cell, or swollen and bulbous. *Conidiogenous cells* terminal, medium brown, subcylindrical to subconical; apex neither darkened nor thickened, truncate, holoblastic. *Conidia* obclavate to long obclavate, straight to curved, medium brown, smooth to finely verruculose, guttulate, transversely multi-euseptate; apex obtuse, base obconical, frequently darker pigmented than the rest of the conidium.

Notes — Even though the *Sporidesmium* complex was divided into numerous genera by Wu & Zhuang (2005), *Sporidesmium* remains heterogeneous. Although the type species, *S. atrum* is not known from culture, morphologically similar species such as *S. knawiae*, *S. macrusum* and *S. parvum* cluster among the *Sordariomycetes*, distant from the *Capnoidiales*. *Sporidesmajora* is quite distinct from *Sporidesmium* in having long, multiseptate conidiophores that frequently have a subconical, darker pigmented apical cell, appearing to give rise to a single holoblastic conidium. Conidia are obclavate, frequently very long, multi-euseptate, and somewhat curved, having a somewhat darker, obconical basal cell.

***Sporidesmajora pennsylvaniensis* Batzer & Crous, sp. nov. — MycoBank MB514401; Fig. 6**

Mycelio ex hyphis ramosis, septatis, brunnei, subtiliter verruculosus, 3–5 µm latis; conidiophoris erectis, subcylindraceis, saepe subclavatis, rectis vel unigeniculatis, medio- vel atro-brunneis, subtiliter verruculosus, 1–20-septatis, 35–170 × 4–6 µm; cellulis conidiogenis terminalibus, medio-brunneis (interdum atriore brunneis quam conidiophora), subcylindraceis vel subconicis, apice distincte attenuato, truncato, 10–15 × 4–6 µm; conidiis obclavatis vel longe obclavatis, rectis vel curvatis, medio-brunneis, laevibus vel subtiliter verruculosus, guttulis, 45–350 × 5–7 µm, transverse 6–plurieuseptatis.

Etymology. Named after its type locality, Pennsylvania, USA.

On OA (sterile on SNA and PDA). *Mycelium* consisting of branched, septate, brown, finely verruculose, 3–5 µm wide hyphae, frequently constricted at septa, and at times giving rise to terminal clusters of brown chlamydospores up to 10 µm diam, in chains or in clumps. *Conidiophores* erect, subcylindrical, frequently somewhat clavate, straight to once geniculate, medium to dark brown, finely verruculose, at times with percurrent rejuvenation, 1–20-septate, 35–170 × 4–6 µm; base arising as lateral branch directly from hyphal cell, or becoming swollen and bulbous. *Conidiogenous cells* terminal, medium brown (at times darker brown than conidiophores), subcylindrical to subconical, with prominent taper towards truncate apex, 10–15 × 4–6 µm; apex not darkened nor thickened, 2–2.5 µm diam; each conidiogenous cell appearing to give rise to a single conidium. *Conidia* obclavate to long obclavate, straight to curved, medium brown, smooth to finely verruculose, guttulate, 45–350 × 5–7 µm, transversely 6–multi-euseptate, at times constricted at septa, apex obtuse, tapering from the obconical basal cell to the subtruncate hilum, up to 2 µm wide; widest at basal cell, which is frequently more darkly pigmented than the rest of the conidium.

Culture characteristics — Colonies after 1 mo at 25 °C in the dark spreading on MEA, with sparse aerial mycelium; surface folded, margin even, lobate, surface leaden-grey, reverse leaden-black, reaching up to 25 mm diam. On PDA similar, but margins more feathery, reaching 25 mm diam. On OA similar, reaching 20 mm diam.

Appearance on apple — Fuliginous mycelia type; lacking sclerotium-like bodies; irregularly shaped pale brown colonies with uneven margins reaching 5 mm diam.

Specimen examined. USA, Adams County, Pennsylvania, on fruit surface of apple, Sept. 2005, J.W. Travis, CBS H-20326 holotype, cultures ex-type CPC 16112 = PA1_9F1a = CBS 125229.

DISCUSSION

All five of these newly identified fungi have been proven to cause SBFS signs on apple, based on a modified Koch's postulates (Batzer et al. 2005). These findings are among the first evidence that the same SBFS species occur in both Asia and North America. The fact that several isolates of *Houjia yanglingensis* were isolated from two provinces in China and two states in the USA suggest it is a widely distributed member of the SBFS complex. There is only one previous report of a

SBFS fungus, *Zygophiala cryptogama*, occurring in both continents (Zhai et al. 2008). Intercontinental distribution of SBFS species in Europe and North America was also documented (Wrona 2004, Ivanović et al. In press). Difficulty in isolating SBFS species from apple has impeded our understanding of the distribution of these slow-growing fungi. For example, recent use of specific primer pairs ITS1/Myc1R (Duttweiler et al. 2008) to amplify SBFS fungi directly from the thalli on apple peels has revealed that *Phaeothecoidiella* species are widely distributed throughout Iowa (Sisson 2009), despite the fact that we obtained only four pure cultures of this genus from a 30-orchard survey of the eastern USA (Batzer et al. 2005, Díaz Arias et al. In press).

The genus *Phaeothecoidiella* resembles *Hyphospora*, *Phaeotheca* and *Phaeothecoidea*, which also have endoconidia, and are placed in the *Dothideomycetes*. Morphologically they are distinct from *Phaeothecoidiella* in that the latter tends to

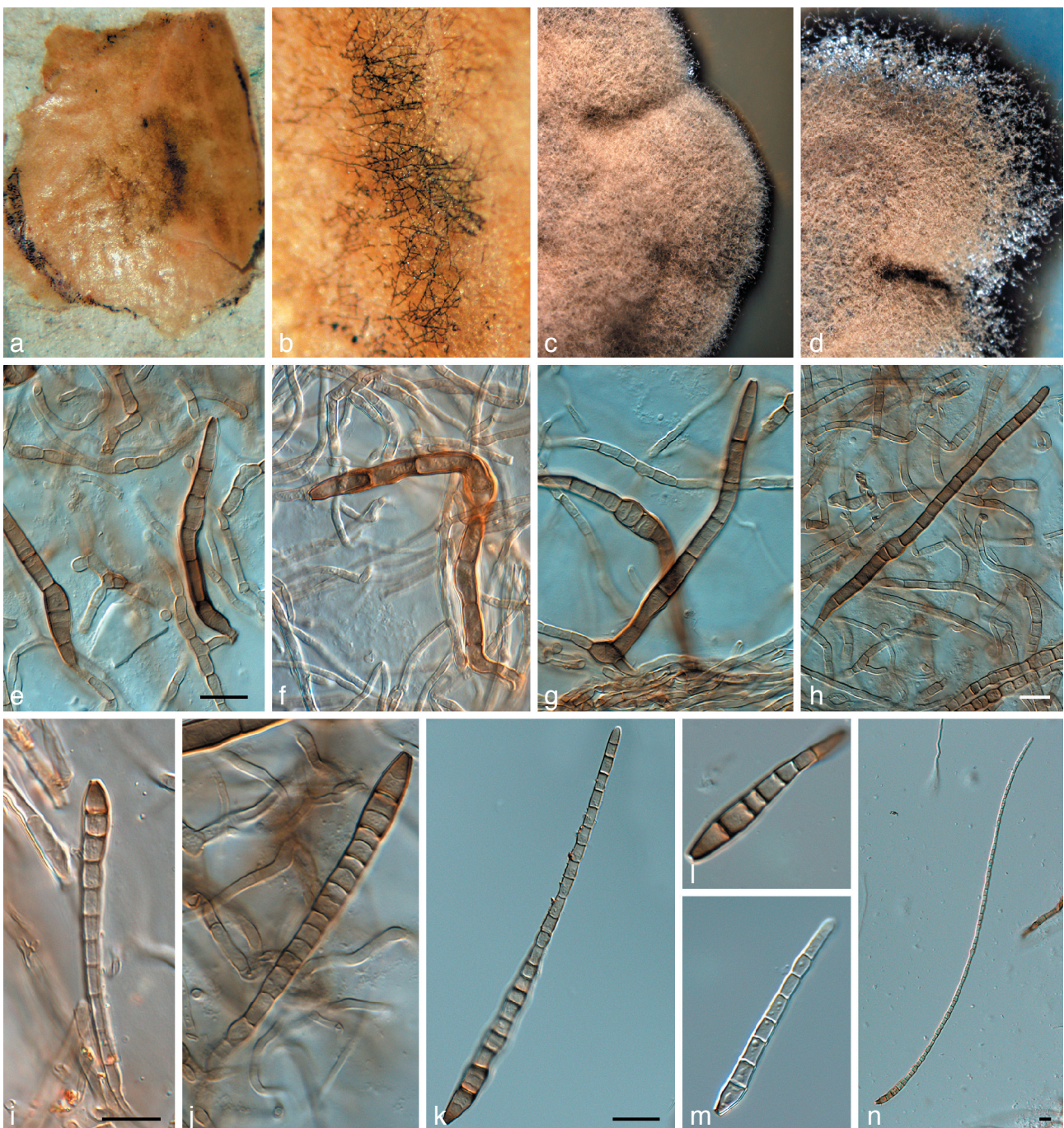


Fig. 6 *Sporidesmajora pennsylvaniensis* (CPC 16112). a, b. Colonies on apple surface. c. colony on MEA; d. colony on SNA; e–j. conidiophores; k–n. conidia. — Scale bars = 10 µm.

have more phragmospores, and hyphae that are covered in a mucoid sheath, as found in typical sooty moulds, but lacking in the former genera. Phylogenetically these genera also cluster apart.

The recent treatment of the *Sporidesmium* complex by Wu & Zhuang (2005) reiterated the numerous, as yet undefined genera present within the *Sporidesmium* complex. A complete revision of this polyphyletic complex has largely been hampered by the unavailability of cultures (Shenoy et al. 2006). Two genera in this complex are newly introduced in the present study, namely *Houjia* and *Sporidesmaja*. An important ecological feature of these genera lies in their growth habit, namely growing as epiphytes (*Capnodiales*) on apple surfaces. Using the key provided by Wu & Zhuang (2005), *Houjia*, which is morphologically similar to *Stanjehughesia* (*Chaetosphaeriaceae*), can be distinguished based on its solitary conidiogenous cells. *Sporidesmaja*, on the other hand, is distinguished from *Sporidesmium* (*Sordariomycetes*) based on its multiseptate conidiophores with darkly pigmented, subconical apical conidiogenous cells, and long, multi-euseptate conidia.

Altogether we described three new genera and five new species in the SBFS complex. Although this finding is quite significant, the *Dothideomycetes*, and the *Capnodiales* in particular, still host numerous undescribed families and genera (Schoch et al. 2006, Crous et al. 2007, 2009b), suggesting that these novelties reported here represent but the tip of the iceberg. What is extremely interesting is that apple fruit surfaces appear to represent yet another unique epiphytic niche, similar to that reported by Ruibal et al. (2008) from rock surfaces. By employing morphological and DNA comparisons, we were able to resolve the status of several previously collected genera and species which would otherwise have escaped recognition.

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REFERENCES

- Batzer JC, Díaz Arias MM, Harrington TC, Gleason ML, Groenewald JZ, Crous PW. 2008. Four species of *Zygophiala* (Schizothyriaceae, Capnodiales) are associated with the sooty blotch and flyspeck complex on apple. *Mycologia* 100: 246–258.
- Batzer JC, Gleason ML, Harrington TC, Tiffany LH. 2005. Expansion of the sooty blotch and flyspeck complex on apples based on analysis of ribosomal DNA gene sequences and morphology. *Mycologia* 97: 1268–1286.
- Colby AS. 1920. Sooty blotch of pomaceous fruits. *Transactions Illinois State Academic Science* 13: 139–175.
- Crous PW. 1998. *Mycosphaerella* spp. and their anamorphs associated with leaf spot diseases of Eucalyptus. *Mycologia Memoir* 21: 1–170.
- Crous PW, Braun U, Groenewald JZ. 2007. *Mycosphaerella* is polyphyletic. *Studies in Mycology* 58: 1–32.
- Crous PW, Gams W, Stalpers JA, Robert V, Stegehuis G. 2004. MycoBank: an online initiative to launch mycology into the 21st century. *Studies in Mycology* 50: 19–22.
- Crous PW, Schoch CL, Hyde KD, Wood AR, Gueidan C, Hoog GS de, Groenewald JZ. 2009a. Phylogenetic lineages in the Capnodiales. *Studies in Mycology* 64: 17–47.
- Crous PW, Slippers B, Wingfield MJ, Rheeder J, Marasas WFO, Philips AJL, Alves A, Burgess T, Barber P, Groenewald JZ. 2006. Phylogenetic lineages in the Botryosphaeriaceae. *Studies in Mycology* 55: 235–253.
- Crous PW, Summerell BA, Carnegie AJ, Wingfield MJ, Hunter GC, Burgess TI, Andjic V, Barber PA, Groenewald JZ. 2009b. Unravelling *Mycosphaerella*: do you believe in genera? *Persoonia* 23: 99–118.
- Crous PW, Verkley GJM, Groenewald JZ, Samson RA (eds). 2009c. Fungal Biodiversity. CBS Laboratory Manual Series. Centraalbureau voor Schimmelmcultures, Utrecht, Netherlands.
- Díaz Arias MM, Batzer JC, Harrington TC, Wong AW, Bost SC, Cooley DR, Ellis MA, Hartman JR, Rosenberger DA, Sundin GW, Sutton TB, Travis JW, Wheeler MJ, Yoder KS, Gleason ML. In press. Diversity and biogeography of sooty blotch and flyspeck fungi on apple in the Eastern and Midwestern United States. *Phytopathology*.
- Duttweiler KB, Sun GY, Batzer JC, Harrington TC, Gleason ML. 2008. An RFLP-based technique for identifying fungi in the sooty blotch and flyspeck complex on apple. *Plant Disease* 92: 794–799.
- Hickey KD. 1960. The sooty blotch and flyspeck diseases of apple with emphasis on variation within *Gloeodes pomigena* (SCW.) Colby. PhD thesis. Department of Plant Pathology, The Pennsylvania State University, USA.
- Hoog GS de, Gerrits van den Ende AHG. 1998. Molecular diagnostics of clinical strains of filamentous Basidiomycetes. *Mycoses* 41: 183–189.
- Hughes SJ. 1976. Sooty moulds. *Mycologia* 68: 693–820.
- Hughes SJ. 1983. New Zealand fungi 32. *Janetia capnophila* sp. nov. and some allies. *New Zealand Journal of Botany* 21: 177–182.
- Hughes SJ, Illman WI. 1974. *Sporidesmium hormiscioides*. *Fungi Canadensis* No. 58. National Mycological Herbarium, Biosystematics Research Institute, Agriculture Canada, Ottawa, Ontario, Canada.
- Ivanović MM, Ivanović MS, Batzer JC, Tatalović N, Oertel B, Latinović J, Latinović N, Gleason ML. In press. Fungi in the apple sooty blotch and flyspeck complex from Serbia and Montenegro. *Journal of Plant Pathology*.
- Johnson EM, Sutton TB, Hodges CS. 1996. *Peltaster fructicola*: a new species in the complex of fungi causing apple sooty blotch. *Mycologia* 88: 114–120.
- Johnson EM, Sutton TB, Hodges CS. 1997. Etiology of apple sooty blotch disease in North Carolina. *Phytopathology* 87: 88–95.
- Li HY, Zhang R, Sun GY, Batzer JC, Gleason ML. In press. New species and record of *Zygophiala* on apple fruit from China. *Mycological Progress*.
- Ma YQ, Zhang R, Sun GY, Zhu HX, Tang M, Batzer JC, Gleason ML. In press. A new species of *Zygophiala* associated with the flyspeck complex on apple from China. *Mycological Progress*.
- Rayner RW. 1970. A mycological colour chart. British Mycological Society. Commonwealth Mycological Institute, Kew, Surrey.
- Réblová M, Winka K. 2001. Generic concepts and correlations in ascomycetes based on molecular and morphological data: *Lecythothecium duriligni* gen. et sp. nov. with a *Sporidesmium* anamorph, and *Ascolacicola austriaca* sp. nov. *Mycologia* 93: 478–493.
- Rehner SA, Samuels GJ. 1994. Taxonomy and phylogeny of *Gliocladium* analysed from nuclear large subunit ribosomal DNA sequences. *Mycological Research* 98: 625–634.
- Ruibal C, Platas G, Bills GF. 2008. High diversity and morphological convergence among melanised fungi from rock formations in the central mountain system of Spain. *Persoonia* 21: 93–110.
- Schoch CL, Shoemaker RA, Seifert KA, Hambleton S, Spatafora JW, Crous PW. 2006. A multigene phylogeny of the Dothideomycetes using four nuclear loci. *Mycologia* 98: 1043–1054.
- Shenoy BD, Jeewon R, Wu WP, Bhat DJ, Hyde KD. 2006. Ribosomal and RPB2 DNA sequence analyses suggest that *Sporidesmium* and morphologically similar genera are polyphyletic. *Mycological Research* 110: 916–928.
- Sisson AJ. 2009. Assessing new methods of integrated pest management for apple orchards in the midwest and phenology of sooty blotch and flyspeck fungi on apples in Iowa. Masters thesis. Department of Plant Pathology, Iowa State University, USA.
- Sun GY, Zhang R, Li H, Gleason ML. 2008. Diversity of fungi causing flyspeck-like signs on apple in China. *Phytopathology* 98: S153.
- Sun GY, Zhang R, Zhang Z, Zhang M. 2003. Isolation of sooty blotch and flyspeck fungi from apple surface by picking up the thalli. *Acta Phytopathologica Sinica* 33: 479–480 [in Chinese].
- Vilgalys R, Hester M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* 172: 4238–4246.
- 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*: 315–322. Academic Press, USA.
- Wrona BR. 2004. Etiology of apple sooty blotch in Poland. *Journal of Plant Protection Research* 44: 289–292.
- Wu W, Zhuang W. 2005. *Sporidesmium*, *Endophragmiella* and related genera from China. *Fungal Diversity Research Series* 15: 1–351.
- Zhai X, Li H, Zhang R, Sun G, Tang M, Batzer JC, Gleason ML. 2008. *Zygophiala* (hyphomycetes) – a genus newly recorded from China. *Mycotaxon* 105: 325–330.