

## *Stachybotrys eucylindrospora*, sp. nov. resulting from a re-examination of *Stachybotrys cylindrospora*

De-Wei Li<sup>1</sup>

The Connecticut Agricultural Experiment Station,  
Valley Laboratory, 153 Cook Hill Road, Windsor,  
Connecticut 06095

**Abstract:** The holotype of *Stachybotrys cylindrospora* was examined and the morphological characters were found to fit the description of *Stachybotrys chartarum*. Thus *Stachybotrys cylindrospora* is a synonym of *S. chartarum*. However a number of isolates and specimens subsequently described and studied by several mycologists have typical cylindrical conidia with longitudinal striations. The conidia are much longer than those of *S. chartarum*. These conidial characters showed that those isolates and specimens are notably different from *S. chartarum* and of the holotype of *S. cylindrospora*. Therefore a new name, *Stachybotrys eucylindrospora* sp. nov., is proposed to accommodate these isolates and specimens.

**Key words:** *Stachybotrys chartarum*, synonym, type

### INTRODUCTION

Jensen (1912) collected an isolate of *Stachybotrys* from arable soil in North Cohocton, New York, in Aug 1911 and described it as a new species, *S. cylindrospora*, in 1912. Bisby (1943) suggested that *S. cylindrospora* might be a synonym of *S. chartarum*. Rayss and Borut (1958) demoted *S. cylindrospora* to *Stachybotrys atra* var. *cylindrospora* (C.N. Jensen) Rayss & Borut. Barron (1961) isolated several strains in Canada, which were thought to fit the description of *S. cylindrospora*. However the unusually wide range of *S. cylindrospora* conidia size, which spanned the range of conidial size of *S. chartarum*, raised the question about validity of the species. The holotype specimens of *S. cylindrospora* of C.N. Jensen, the specimen of *Stachybotrys atra* var. *cylindrospora* of Rayss and Borut and additional specimens of *S. cylindrospora* and *S. chartarum* were borrowed from several major herbaria. The objective of the present study was to re-examine *S. cylindrospora* and to determine its taxonomic status.

### MATERIALS AND METHODS

The holotype of *Stachybotrys cylindrospora* (CUP-005925) was examined (TABLE I). The specimen IMI 76515 of *Stachybotrys atra* var. *cylindrospora*, which Borut deposited in IMI was examined (TABLE I). Specimens of *S. cylindrospora* and *S. chartarum* were borrowed from IMI, DAOM, CUP and BPI for comparative examination (TABLE I).

A small sample (1 × 1 mm) was taken with a needle from the surface of the type material of *S. cylindrospora* (CUP-005925) in a dried slant test tube. A quantitative polymerase chain reaction (QPCR) test of the type material of *S. cylindrospora* (CUP-005925) was conducted with Applied Biosystems Prism® model 7300 using the primers and probe of the analysis specific for detecting *S. chartarum sensu lato*. Their sequences are forward primer StacF4: 5'-TCCCAAACCCCTTATGTGAACC, reverse primer StacR5: 5'-GTTTGCCACTCAGAGAATACTGAAA, and probe StacP2: 5'-CTGCGCCCCGATCCAGGC. The procedure and technique were developed by Haugland and co-authors using sequence data of *Stachybotrys chartarum*, *S. albipes*, *S. bisbyi* (= *S. elegans*), *S. cylindrospora* (ATCC 18851), *S. dichroa*, *S. elegans*, *S. kampalensis*, *S. longispora*, *S. microspora*, *S. nephrospora*, *S. oenanthes*, *S. parvispora*, *S. theobromae*, *Memnoniella echinata* and *M. subsimplex* (Haugland and Heckman 1998, Haugland et al 1999).

An isolate, ATCC 18851 (=IMI 85334 and OAC 8603), under the name of *S. cylindrospora* (TABLE I) was cultured on malt-extract agar (MEA: 15 g malt-extract broth (Difco), 15 g agar (Oxoid), 0.075 g chloramphenicol (Fisher), 750 mL distilled water, 0.75 mL trace metal solution (1 g ZnSO<sub>4</sub>·7H<sub>2</sub>O, 0.5 g of CuSO<sub>4</sub>·5H<sub>2</sub>O, 100 mL distilled water, 1 mL 1N NaOH) and cornmeal agar (CMA: 12.75 g cornmeal agar (Difco), 0.075 g chloramphenicol (Fisher), 750 mL distilled water) at 25 C for 15 d to observe its characteristics. It is the same isolate used by Haugland et al (1999, 2001) in their studies of QPCR and phylogenetic relationships of *Stachybotrys* spp.

### RESULTS

The original illustration of *Stachybotrys cylindrospora* by Jensen (1912) is reproduced (FIG. 1), while his original description of the species Jensen (1912) is quoted here: "Colonies round, thin, diffuse, becoming black with age; mycelium branched, septate, hyaline, 0.5–3 μ; conidiophores hyaline at base, fuliginous toward apex, branched, septate, attenuate toward tip, 40–65 μ high, bearing on the summit from 3.9 sterigmata; sterigmata subclavate, with or without short papilla, 8–11 by 4–5 μ; conidia borne singly, smooth, subcylindrical to sometimes ovate, 6–16 by

3.8–5  $\mu$ , hyaline when young, becoming fuliginous and nearly opaque with age.”

The holotype specimen (CUP-005925) of *S. cylindrospora* collected by Jensen showed the characteristics of *S. chartarum* and fit the description of this fungus (FIGS. 2, 3). Most conidia oval, ellipsoid, (7.4–)7.5–11.1(–12.8) (mean =  $9.8 \pm 1.3$ )  $\times$  (2.4–)3.5–5.6(7.4) (mean =  $4.5 \pm 1.1$ )  $\mu$ m, length/width ratio 2.3; while a few were subcylindrical, or globose. Mature conidia were roughened with ridged ornamentation on the surface, olivaceous (FIGS. 2, 3), while immature conidia were lighter in color and smooth or minutely roughened. The conidia were not truly cylindrical. It is unambiguous that these characteristics match that of *S. chartarum* because the conidia of *S. chartarum* are colorless when young, becoming dark olivaceous when mature, ellipsoid, smooth or verrucose with ridged ornamentation on the surface, 7–12  $\times$  4–6  $\mu$ m (Jong and Davis 1976).

A total of 34 000 spore equivalents of *S. chartarum* were detected from the sample of the type material of *S. cylindrospora* with QPCR analysis. The result of the QPCR test verified that the holotype of *S. cylindrospora* is actually *S. chartarum*.

Rayss and Borut (1958) described *Stachybotrys atra* var. *cylindrospora* as conidiophores 40–70  $\times$  2.5–3.5  $\mu$ m; phialides 8–11  $\times$  3.5–4.5  $\mu$ m; conidia cylindrical, 9–11  $\times$  2.5–5  $\mu$ m. There is no indication that Rayss and Borut (1958) observed the type materials of *S. cylindrospora*. According to their description and the photograph in their paper, the isolate appears to be *S. chartarum*. An examination of IMI 76515, deposited by Borut, confirmed that *S. atra* var. *cylindrospora* is in fact *S. chartarum*. The conidia of IMI 76515 had a ridged surface, most were not cylindrical and a few were subcylindrical (FIG. 4). The significant variation among the isolates of *S. chartarum* and the conidial characters of IMI 76515 do not warrant its separation as a variety of *S. chartarum*.

An examination of the isolate ATCC 18851 (=IMI 85334) under the name *S. cylindrospora* showed that the morphological characters of its conidia are different from the ones of the holotype of *S. cylindrospora* (CUP-005925) and the specimen IMI 76515 of *S. atra* var. *cylindrospora*. Conidia of the isolate ATCC 18851 have three major characters: (i) they are cylindrical; (ii) have delicate longitudinal striations on the conidial surface; (iii) and are 12.8–16  $\times$  3.4–5.5  $\mu$ m and ratio of length/width 2.6–4.2, which is much longer than that of *S. chartarum*.

The comparative examination of specimens under the name of *S. cylindrospora* from BPI, CUP, DAOM and IMI showed that six out of eight specimens matched the isolate ATCC 18851 but not the holotype of *S. cylindrospora* (CUP-005925). DAOM208271 is *S.*

*chartarum*. IMI 152718 and BPI 525146 do not fit either ATCC 18851 or CUP-005925. Conidia of IMI 152718 are smooth, oval, not cylindrical or subcylindrical. BPI 525146 is a mounted slide, which appears to match the isolate ATCC 18851 in size and shape but has no striation on the surface of conidia. The true identity of these two specimens remains to be clarified. Specimens DAOM 70309 and IMI 79062 originally identified as *S. atra* were found to match the isolate ATCC 18851.

According to the morphological characters, and QPCR analysis of the type of *S. cylindrospora*, the author concluded that the name *S. cylindrospora* proposed by Jensen is a synonym of *S. chartarum*. The specimen, description and photograph of *S. atra* var. *cylindrospora* all are reminiscent of *S. chartarum*. In the author's opinion the conidial dimensions of IMI 76515 are within the variation of *S. chartarum* and do not warrant it as a new variety. However the isolate ATCC 18851 collected by Barron represents a distinct species of *Stachybotrys*. DNA sequence data of the isolate ATCC 18851 and analysis of phylogenetic relationships among 12 *Stachybotrys* and two species of *Memnoniella* supported the finding of the isolate ATCC 18851 as a new species (Haugland and Heckman 1998, Haugland et al 2001). Therefore the author proposes a new species.

#### TAXONOMY

#### *Stachybotrys eucylindrospora* Li, sp. nov. FIGS. 5–8

Fungi mitosporici, Hyphomycetes.

Coloniae in MEA, 24–26 mm diam in 15 diebus ad 25 C, flaviae; marginae irregulariae.

Conidiophora erecta, simplicia, septata, macronemata, solitaria vel interdum fasciculata, determinata, recta vel exigue curvata, deinde ramosa, prope apicem verrucosa et fusca, prope basi hyalina et latvia, usque ad 200  $\mu$ m longa et 3–5  $\mu$ m crassa.

Cellulae conidiogenae phialidicae, determinatae, discretiae, laeviae, subclavatae, prope apicem fuscae olivaceae, (11.1–)12.4–14.6(–15) (Med. 13.5)  $\times$  (2.5–)2.9– 4.5(–5.2) (Med. 3.6)  $\mu$ m, collulo conspicuo praeditae, 4–7 in verticillo dispositae.

Conidia cylindrica vel cylindro-ellipsoidea, primo hyalina et laeviae, deinde atro-olivaceobrunnea, basi  $\pm$  truncate, longitudinaliter striata, (10.3–)12.8–16(–18.5) (Med. =  $14.4 \pm 1.6$ , n = 51)  $\times$  (2.5–)3.4– 5.5(–6.6) (Med.  $4.4 \pm 1$ )  $\mu$ m, biguttata, in massam mucosam nigram lecta.

Teleomorphosis ignota.

Holotypus DAOM 87664 isolatus per GL Barron de humus, palus, ad Guelph, Ontario, Canada, de Nov 1960. Isotypus IMI 85334, CBS 203.61, et OAC 8603. Vivita cultura sustentare apud ATCC (ATCC 18851).

Colonies attaining 24–26 mm diam in 15 d at 25 C on malt-extract agar, becoming light yellow where no

TABLE I. Current and original identification, source number and origin of specimens and cultures examined

Current Name	Original Name	Specimen/Culture No.	Geographic Origin
<i>Melanopsamma pomiformis</i>	<i>M. pomiformis</i> (Ana: <i>S. albipes</i> )	BPI 612161	Papia, Italy
<i>Stachybotrys albipes</i>	<i>S. albipes</i>	UAMH 7750(=ATTC18873) <sup>a,d</sup>	Yorkshire, UK
<i>S. chartarum</i>	<i>S. alternans</i>	DAOM 82255	Toronto, Canada
<i>S. chartarum</i>	<i>S. alternans</i>	DAOM 82256	Toronto, Canada
<i>S. chartarum</i>	<i>S. alternans</i>	BPI 422099	Kansas, USA
<i>S. chartarum</i>	<i>S. alternans</i>	BPI 422101	Ontario, Canada
<i>S. chartarum</i>	<i>S. alternans</i>	BPI 422102	New Haven, CT, USA
<i>S. chartarum</i>	<i>S. alternans</i>	BPI 422107	Vittorio, Italy
<i>S. chartarum</i>	<i>S. alternans</i>	BPI 422109	Saxony, Germany
<i>S. chartarum</i>	<i>S. alternans</i>	BPI 422110	Bohemia, Czechoslovakia
<i>S. chartarum</i>	<i>S. alternans</i>	BPI 422111	Massachusetts, USA
<i>S. chartarum</i>	<i>S. alternans</i>	BPI 422112	New Jersey, USA
<i>S. chartarum</i>	<i>S. alternans</i>	BPI 422116	Kansas, USA
<i>S. chartarum</i>	<i>S. atra</i>	DAOM 116282	Copenhagen, Denmark
<i>S. chartarum</i>	<i>S. atra</i>	DAOM 145122	Uppsala, Sweden
<i>S. chartarum</i>	<i>S. atra</i>	DAOM 191211	Ottawa, Canada
<i>S. chartarum</i>	<i>S. atra</i>	DAOM 18072	Lothringgen, Germany
<i>S. chartarum</i>	<i>S. atra</i>	DAOM 45879	Tennessee, USA
<i>S. chartarum</i>	<i>S. atra</i>	DAOM 129714	Saskatchewan, Canada
<i>S. chartarum</i>	<i>S. atra</i>	DAOM 41247	Michigan, USA
<i>S. chartarum</i>	<i>S. atra</i>	DAOM 59837	Rhode Island, USA
<i>S. chartarum</i>	<i>S. atra</i>	DAOM 39991	Lyndomville, NY, USA
<i>S. chartarum</i>	<i>S. atra</i>	DAOM 6078	Quebec, Canada
<i>S. chartarum</i>	<i>S. atra</i>	DAOM 106075	Saskatchewan, Canada
<i>S. chartarum</i>	<i>S. atra</i>	DAOM 75756	Ottawa, Canada
<i>S. chartarum</i>	<i>S. atra</i>	BPI 422120	Lahore, Pakistan
<i>S. chartarum</i>	<i>S. atra</i>	BPI 422121	New Guinea
<i>S. chartarum</i>	<i>S. atra</i>	BPI 422124	Hawaii, USA
<i>S. chartarum</i>	<i>S. atra</i>	BPI 422126	Surrey, UK
<i>S. chartarum</i>	<i>S. atra</i>	BPI 422129	Louisiana, USA
<i>S. chartarum</i>	<i>S. atra</i>	BPI 421929	Cerro Bola, Mexico
<i>S. chartarum</i>	<i>S. atra</i>	BPI 422123	New York, USA
<i>S. chartarum</i>	<i>S. chartarum</i>	DAOM 105737	Uppland, Sweden
<i>S. chartarum</i>	<i>S. chartarum</i>	DAOM 185730	Ontario, Canada
<i>S. chartarum</i>	<i>S. chartarum</i>	DAOM 183175a	Ontario, Canada
<i>S. chartarum</i>	<i>S. chartarum</i>	DAOM 183175c	Ontario, Canada
<i>S. chartarum</i>	<i>S. chartarum</i>	DAOM 45879	Tennessee, USA
<i>S. chartarum</i>	<i>S. chartarum</i>	DAOM 189389	Ottawa, Canada
<i>S. chartarum</i>	<i>S. cylindrospora</i>	CUP 005925 Type <sup>b</sup>	New York, USA
<i>S. chartarum</i>	<i>S. cylindrospora</i>	DAOM208271	Manitoba, Canada
<i>S. chartarum?</i>	<i>S. cylindrospora</i>	IMI 152718	Pokhara, Nepal
<i>S. chartarum</i>	<i>S. lobulata</i>	IMI 163489	Kuwait
<i>S. chartarum</i>	<i>S. lobulata</i>	DAOM 208232	Manitoba, Canada
<i>S. chartarum</i>	<i>S. lobulata</i>	DAOM 14292	Minneapolis, MN, USA
<i>S. chartarum</i>	<i>S. lobulata</i>	DAOM 20752	Idaho, USA
<i>S. chartarum</i>	<i>S. lobulata</i>	DAOM 25916	Vancouver, BC, Canada
<i>S. chartarum</i>	<i>S. lobulata</i>	DAOM 15188	San Francisco, CA, USA
<i>S. chartarum</i>	<i>S. lobulata</i>	BPI 422148	Pennsylvania, USA
<i>S. chartarum</i>	<i>S. lobulata</i>	BPI 422149	Lyon, France
<i>S. chartarum</i>	<i>S. lobulata</i>	BPI 422159	Netherlands
<i>S. chartarum</i>	<i>S. lobulata</i>	BPI 422184	Kew, UK
<i>S. chartarum</i>	<i>S. lobulata</i>	BPI 422172	Greece
<i>S. chartarum</i>	<i>S. lobulata</i>	BPI 422155	Washington DC, USA
<i>S. chartarum</i>	<i>S. dakotensis</i>	DAOM 51008 Type	Mandan, North Dakota
<i>S. chartarum</i>	<i>Stilbospora chartarum</i>	DAOM 51026	Unknown
<i>S. chlorohalonata</i>	<i>S. chlorohalonata</i>	From KF Nielson	Denmark

TABLE I. Continued

Current Name	Original Name	Specimen/Culture No.	Geographic Origin
<i>S. dichroa</i>	<i>S. dichroa</i>	IMI 18006 Type	Near Little Sutton, UK
<i>S. elegans</i>	<i>S. bisbyi</i>	DAOM 87338	Ottawa, Canada
<i>S. eucylindrospora</i>	<i>S. atra</i>	DAOM 70309	Quebec, Canada
<i>S. eucylindrospora</i>	<i>S. atra</i>	IMI 79062	Yorkshire, UK
<i>S. eucylindrospora</i>	<i>S. cylindrospora</i>	ATCC 18851 Extype <sup>a,c,d</sup>	Ontario, Canada
<i>S. eucylindrospora</i>	<i>S. cylindrospora</i>	DAOM 172376g	Ontario, Canada
<i>S. eucylindrospora</i>	<i>S. cylindrospora</i>	DAOM 176800a	Alberta, Canada
<i>S. eucylindrospora</i>	<i>S. cylindrospora</i>	DAOM 56386d	British Columbia, Canada
<i>S. eucylindrospora</i>	<i>S. cylindrospora</i>	DAOM 186941	Ontario, Canada
<i>S. eucylindrospora</i>	<i>S. cylindrospora</i>	DAOM 87664 Holotype <sup>c</sup>	Ontario, Canada
<i>S. eucylindrospora</i>	<i>S. cylindrospora</i>	IMI 107222	Yorkshire, UK
<i>S. eucylindrospora?</i>	<i>S. cylindrospora</i>	BPI 525146	Tamazunchale, Mexico
<i>S. kampalensis</i>	<i>S. kampalensis</i>	IMI 199597	Gorakhpur, India
<i>S. longispora</i>	<i>S. longispora</i>	ATCC 32451 (=MFC 2174) Extype	Japan
<i>S. mangiferae</i>	<i>S. mangiferae</i>	IMI 215279 Type	Gorakhpur, India
<i>S. microspora</i>	<i>S. atra</i> var. <i>microspora</i>	IMI 91993 Type	Udaipur, India
<i>S. nephrospora</i>	<i>S. nephrospora</i>	IMI 71845 (a)	Njaila, Sierra Leone
<i>S. nilagirica</i>	<i>S. nilagirica</i>	IMI 297610	Negri, Malaysia
<i>S. oenanthes</i>	<i>S. oenanthes</i>	IMI 16185 Type	La Bouvée, Guernsey
<i>S. parvispora</i>	<i>S. parvispora</i>	IMI 37872(d) Type	Dodowa, Ghana
<i>S. sansevieriae</i>	<i>S. sansevieriae</i>	IMI 140909 Type	Jabalpur, India
<i>S. theobromae</i>	<i>S. theobromae</i>	IMI 207398	Allahabad, India

ATCC: American Type Culture Collection; BPI: US National Fungus Collection; DAOM: Canadian National Mycological Herbarium; IMI: International Mycological Institute (CABI Bioscience); UAMH: University of Alberta Microfungi Collection and Herbarium.

<sup>a</sup>Cultures used in Jong and Davis (1976).

<sup>b</sup>Holotype of *S. cylindrospora* described by Jensen (1912).

<sup>c</sup>Specimens used in Barron (1961).

<sup>d</sup>Cultures used in Haugland and Heckman (1998); Haugland et al (1999).

sporulation or grayish where sporulation of conidia occurred, granular, reverse light brown at the center (FIG. 5); attaining 45–46 mm diam in 15 d at 25 C on cornmeal agar, pale and velutinus, sporulation commencing within 3 d of incubation (FIG. 6).

Conidiophores determinate, macronematous, solitary or in groups, erect, straight or slightly curved, simple or irregularly branched, 3–5-septate, verrucose and slightly olivaceous at the upper part, hyaline and smooth at the lower part, variable in length, up to 200 µm, 3–5 µm wide, occasionally swollen to 7 µm toward the base (FIGS. 7, 8).

Phialides determinate, discrete, subclavate, smooth, dark olivaceous at the tip, (11.1–)12.4–14.6(–15) (mean = 13.5 ± 1.1, n = 30) × (2.5–)2.9–4.5(–5.2) (mean = 3.6 ± 0.7) µm, with or without conspicuous collarettes, unicellular, 4–7 in groups (FIGS. 7, 8).

Conidia cylindrical or cylindrical ellipsoid, rounded at the apex, rounded or truncate at the base, hyaline and smooth at first and becoming gray to dark olive gray when mature, with delicate longitudinal striations on the conidial surface (the striations visible only under oil lens), unicellular, (10.3–)12.8–16

(–18.5) (mean = 14.4 ± 1.6, n = 51) × (2.5–)3.4–5.5(–6.6) (mean = 4.4 ± 1) µm, ratio of length/width 2.6–4.2 (mean = 3.4), often biguttulate, especially when young, aggregated in slimy masses (FIG. 9).

Teleomorph unknown.

Holotype: DAOM 87664 collected by GL Barron from peat soil, cedar bog, near Guelph, Ontario, Canada, in Nov 1960. Isotypes are IMI 85334, CBS 203.61, and OAC 8603. Living cultures maintained at ATCC (ATCC 18851).

Etymology: The specific epithet is chosen to indicate the unambiguous cylindrical shape of conidia of this species. The prefix “eu” is used for the purpose of distinguishing this proposed name from *S. cylindrospora*, one of the synonyms of *S. chartarum*.

Distribution: Canada, Japan, UK, Germany, Solomon Islands (Jong and Davis 1976, Matsushima 1971, Matsushima 1975).

Habitat: on *Carya ovata* husks, *Dahlia* stems, *Heracleum* sp., stems of *Ligustrum officinale*, peat soil, cedar bog, *Populus* log.

Specimens examined: CANADA. ONTARIO: St Lawrence Islands National Park, MacDonald Island. *Carya ovata*



FIG. 131. —  
*Stachybotrys*  
*cylindrospora*.  
Conidiophore,  
basidial cells,  
and conidia,  
x 236.6

FIG. 1. Reproduction of the original illustration and caption by Jensen (1912).

husks, 23 Jul 1979, *SJ Hughes*, DAOM 172376g. CANADA. ALBERTA: Waterton National Park, Aspen Parkland Picnic Area. On *Populus* log, 4 Aug 1980, *GP White-696*, DAOM 176800a (2 packets). CANADA, BRITISH COLUMBIA: South Burnaby. *Dahlia* stems, 9 Aug 1957, *SJ Hughes*, DAOM 56386d (2 packets). CANADA. ONTARIO: Ottawa. On stems of *Ligustrum officinale*, 26 May 1983, *WI Illman*, DAOM 186941. CANADA. ONTARIO: Nr. Guelph. From peat soil, cedar bog, Nov 1960, *GL Barron*, DAOM 87664. CANADA. QUEBEC: MacDonald College. From soil, 1 Jun 1955, *OA Olsen*, DAOM 70309 (labeled as *S. atra*). United Kingdom: ENGLAND, YORKSHIRE: Gundale. From *Angelica* sp., 18 Oct 1959, *WG Bramley*, IMI 79062 (labeled as *S. atra*). United Kingdom: ENGLAND, YORKSHIRE: Pickering. From *Heracleum* sp., 3 Jul 1964, *WG Bramley*, IMI 107222 (TABLE I). Other specimens and cultures examined are listed (TABLE I).

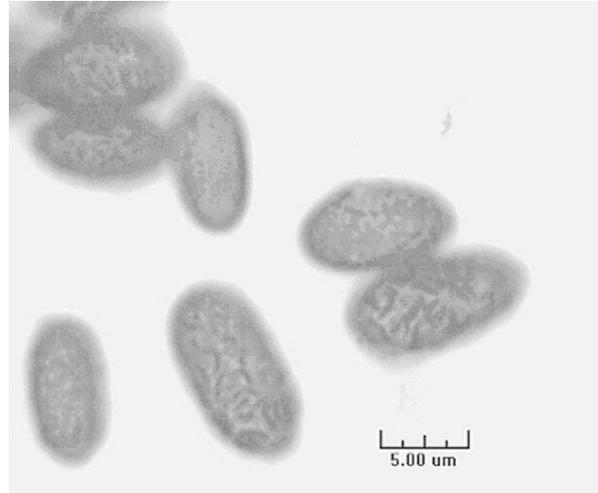
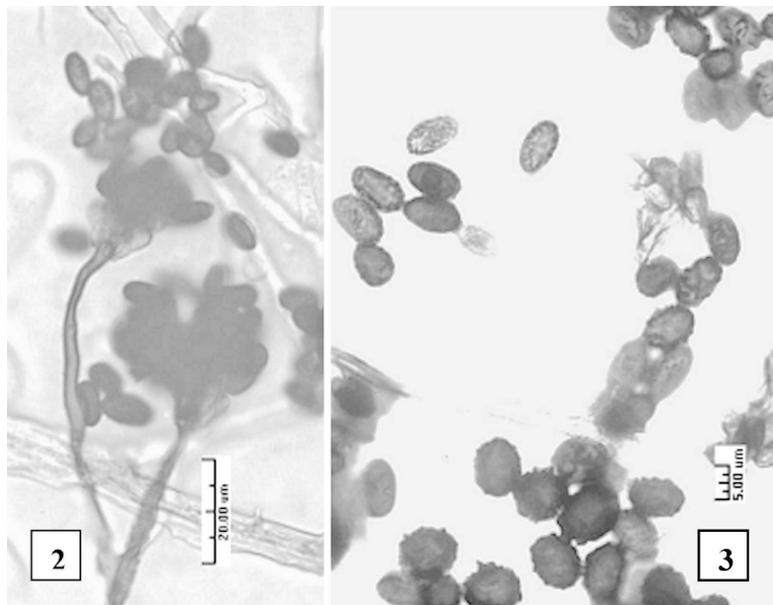


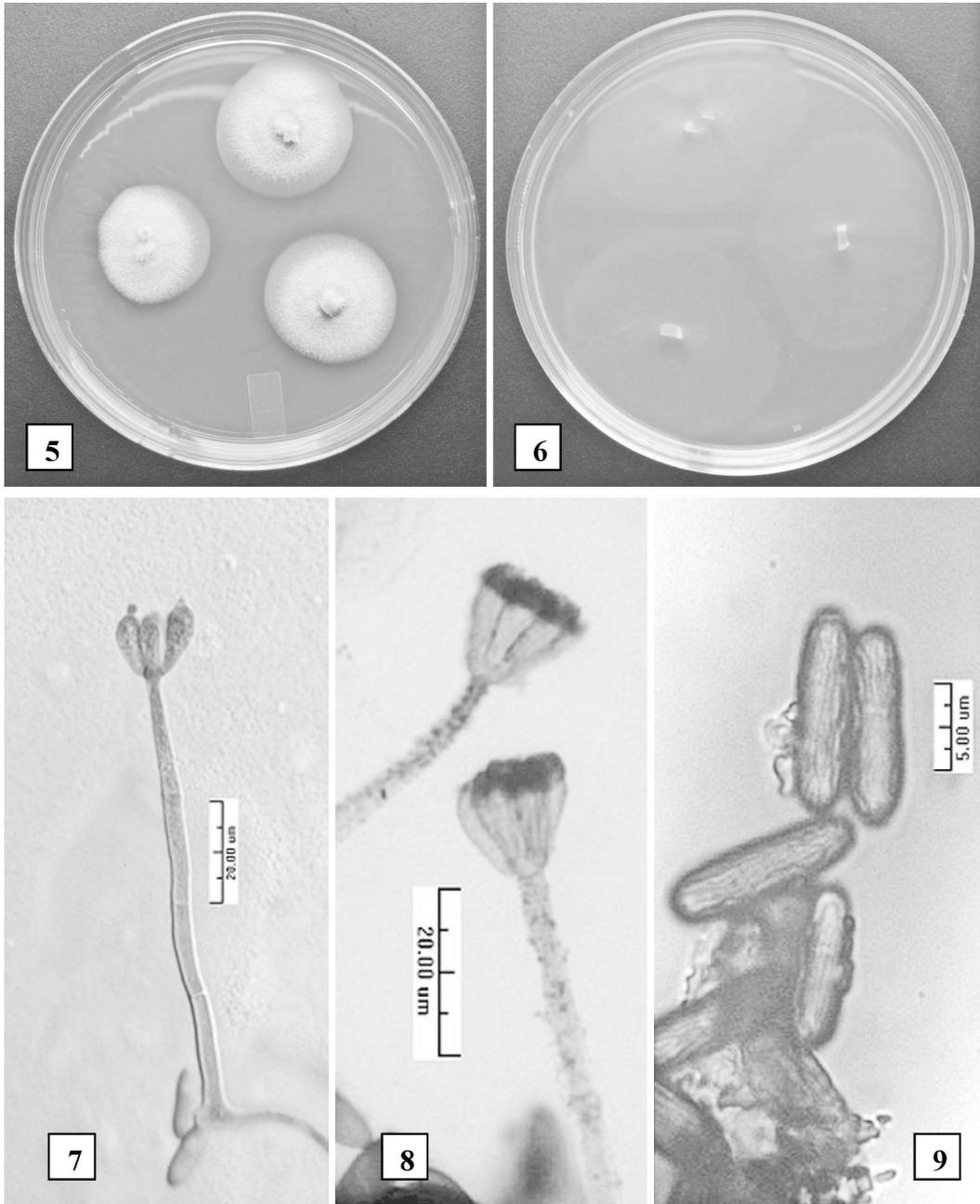
FIG. 4. Conidia from IMI 76515 deposited by Rayss and Borut for their recombination of *Stachybotrys atra* var. *cylindrospora* (Rayss and Borut 1958).

#### DISCUSSION

It is a puzzle how Jensen (1912) described his *S. cylindrospora* with such a large range in conidial size (6–16 µm) because the re-examination of his holotype showed that the size in fact is 7.5–11.1 × 3.5–5.6 µm. Jensen (1912) described these conidia as smooth and subcylindrical, not cylindrical as indicated by the epithet of *S. cylindrospora*. The conidia from his holotype, on close observation, were found to be roughened with ridged ornamentation and were



FIGS. 2–3. 2. Conidia, phialides, and conidiophores of holotype of *Stachybotrys cylindrospora*. 3. Conidia of the same holotype showing the characteristic conidia of *S. chartarum*.



FIGS. 5–9. *Stachybotrys eucylindrospora*. 5. Colonies on MEA. 6. Colonies on CMA. 7. Conidiophore, phialides, conidia. 8. Verrucose conidiophores. 9. Conidia. Bars: 7–8 = 20  $\mu\text{m}$ , 9 = 5  $\mu\text{m}$ .

mostly oval or ellipsoid. These discrepancies might have originated from use of a culture that was not mature and might not have allowed him to observe all the conidial characters, such as the roughness. Because a live culture of CUP-005925 does not exist, it prevented the author from making more extensive examinations and conducting other analyses. These discrepancies remain to be clarified.

Barron (1961) obtained several strains (Nos. 8602, 8603, 8604) from organic soils at several localities in

Ontario, Canada, and considered these isolates morphologically to match *S. cylindrospora*. He suggested that *S. cylindrospora* is a valid species. However he noticed that his isolates differed distinctively from Jensen's description in two characters. First, conidia were primarily cylindrical,  $12\text{--}16 \times 4\text{--}5.5 \mu\text{m}$  and the ratio of length/width of conidia was 2.5–4. Jensen's inaccurate description and sketchy illustration (FIG. 1) had led Barron (1961) to explain the discrepancy as strain variation. Second, mature

conidia of Barron's isolates show fine striations running obliquely lengthwise along the conidia under the oil lens (Barron 1961). The striation on the conidial surface was the important character observed by Barron and others (Barron 1961, Jong and Davis 1976, Matsushima 1975). Barron did not examine the type material of *S. cylindrospora*.

Verona and Mazzucchetti (1968) published a monograph of *Stachybotrys* and *Memnoniella*. Their description of *S. cylindrospora* was based on that of Jensen (1912). However their illustration was redrawn from Barron's illustration. There was no indication which specimens were examined.

Jong and Davis (1976) described *S. cylindrospora* according to their observation of the cultures ATCC 18851 and ATCC 16276 isolated by W. Gams from wheat field soil in Kittzeberg, Germany, and determined by G.L. Hennebert as *S. cylindrospora*. Understandably their description was in agreement with Barron's. Ellis (1971) and Matsushima (1975) also agreed with Barron (1961) that *S. cylindrospora* is valid species. The *S. cylindrospora* studied in these publications is *S. eucylindrospora*.

Two phylogenetic studies with DNA sequence data of 12 species of *Stachybotrys*, two species of *Memnoniella*, nine species of *Stachybotrys* and 3 species of *Memnoniella* respectively elucidated that the isolate ATCC 18851 under the name *S. cylindrospora* was well delineated from other *Stachybotrys* spp. including *S. chartarum* (Haugland and Heckman 1998, Haugland et al 2001). When the species specific primers and probe were developed for detecting *S. chartarum sensu lato*, the isolate ATCC 18851 was included as a species different from *S. chartarum* along with other species of *Stachybotrys* (Haugland and Heckman 1998). This confirms that the isolates observed by Barron (1961), Jong and Davis (1976) and Matsushima (1978) are different from the type material of *S. cylindrospora* and composed of a valid species, *S. eucylindrospora*. The quantitative result of 34 000 spore equivalents from QPCR analysis on the holotype of *S. cylindrospora* (CUP-005925) using *S. chartarum sensu lato* primers and probe verified the conclusion derived from morphological observation that *S. cylindrospora* is a synonym of *S. chartarum*.

Bisby's (1945) opinion that *S. cylindrospora* is possibly a synonym of *S. chartarum* was correct. As Bisby (1943) indicated *S. chartarum* has extreme variability under standard culture conditions. Such variability has led to some confusion in species delineation among *Stachybotrys*. Anderson et al (2003) studied *S. chartarum sensu lato* with morphological, chemical and phylogenetic methods and subsequently described a new species, *S. chlorohalonata* Andersen et Thrane sp. nov. *S. chlorohalonata*

differs morphologically from *S. chartarum* by developing smooth conidia that are ellipsoidal to broadly ellipsoidal and papillate at the basal end, in comparison with those of *S. chartarum*, which have a rough surface and are slightly longer, ellipsoidal (Anderson et al 2003). The holotype of *S. cylindrospora* with rough conidia matches *S. chartarum sensu stricto* and not its sister species, *S. chlorohalonata*. The study of Andersen et al (2003) refined the concept of *S. chartarum sensu stricto*. The species might not be as variable as it was considered before.

Isolates or specimens of *S. cylindrospora* also were reported from the USA (Gilman 1957, Matsushima 1975). Because specimens or cultures are not available for examination their true identities cannot be confirmed as either *S. eucylindrospora* or *S. chartarum*.

Other species of *Stachybotrys* have cylindrical and subcylindrical conidia. Conidia of *S. yunnanensis* are cylindrical or subcylindrical, but they are smaller ( $7.0\text{--}11 \times 3.5\text{--}5.5 \mu\text{m}$ ) than those of *S. eucylindrospora*, which are smooth, or occasionally rough (Kong 1997). The cylindrical conidia of *S. longispora* are  $8.8\text{--}12 \times 2\text{--}2.4 \mu\text{m}$  without ornaments or striations and L/W ratio  $>4$  (Matsushima 1971).

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