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# Taxonomic history and current status of *Stachybotrys chartarum* and related species

**Abstract** The fungus *Stachybotrys chartarum* is the type species of the genus *Stachybotrys*. It is a cellulolytic saprophyte with a worldwide distribution and is frequently recovered in water-damaged buildings. Three isolates of *S. chartarum* were studied morphologically from single-spore isolations. Significant differences were found with the sizes, lengths, width, and L/W ratio of conidia and phialides among the isolates. QPCR analysis on *S. chartarum*, *S. yunnanensis*, *S. chlorohalonata*, *S. elegans*, *S. microspora*, and *S. nephrospora* showed that the primers and probe for detecting *S. chartarum* used by commercial laboratories were not able to differentiate *S. chartarum* from *S. chlorohalonata* and *S. yunnanensis*. Results suggested that *S. chartarum* may not be well delineated even after *S. chlorohalonata* was recently segregated from the species complex. Further study on the taxonomic status of the epithet *S. chartarum* is necessary.

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**Practical Implications**
Six species of *Stachybotrys* are present indoors. Differentiation of *Stachybotrys chartarum* from *S. chlorohalonata*, and *S. yunnanensis* can be challenging using either morphological or QPCR methods. Caution should be taken to identify *S. chartarum* and closely related species and to explain their health effects implication for indoor air quality investigations.

## Introduction

*Stachybotrys chartarum* (Ehrenberg ex Link) Hughes (*S. atra* Corda) is a cellulolytic saprophyte with worldwide distribution. *Stachybotrys chartarum* is frequently isolated from paper, wallpaper and gypsum wallboard in buildings or residences that experienced water damage. The fungus produces several mycotoxins (highly toxic macrocyclic trichothecenes and related trichoverroids) as well as immunosuppressants and endothelin receptor antagonists (Jarvis and Hinkley, 1999). Its harmful effects on animals and human beings have been studied since the 1930s (Haugland and Heckman, 1998; Kendrick, 2000). It was demonstrated to be associated with 'sick building syndrome' in wet buildings (Dearborn et al., 1999; Johanning et al., 1999). It has increasingly attracted public attention to its effect on human health following reports of its association with idiopathic pulmonary hemorrhage in infants from Cleveland, OH, USA (Dearborn et al., 1999). Subsequently, *S. chartarum* was reportedly isolated for the first time from the lung of a child diagnosed with pulmonary hemosiderosis in Houston, TX, USA (Elidemir et al., 1999). Another case of infant pulmonary hemorrhage associated with the presence of *S. atra* (*S. chartarum*) was reported in Kansas City, MO, USA, and mycotoxin analysis demonstrated that the isolate was highly toxigenic (Flappan et al., 1999). Vesper and Vesper (2002) studied and hypothesized that stachylysin, a hemolysin, produced by *S. chartarum* could be a contributing factor to infant pulmonary hemorrhage and hemosiderosis. The health issue related to the presence of *S. chartarum* in buildings or residences has attracted the attention of news media. However, the species concept of *S. chartarum* is not well delineated. It has been subject to controversy since it was proposed as the type species of genus *Stachybotrys* under the name of *S. atra* by Corda in 1837. In the last several years, studies (Kong, 1997; Cruse et al., 2002; Andersen et al., 2003) showed that *S. chartarum sensu lato* included several closely related species and cryptic species, which raise the question concerning common identification and reports of *S. chartarum*. The objective of this paper is to discuss morphological

studies of several isolates of four *Stachybotrys* species and the problematic aspects of *S. chartarum* identification.

## Methods

Three isolates (M 3N-5, M 21024, M100) of *S. chartarum*, two isolates of *S. yunnanensis* (030115-063, PK401), and one isolate each of *S. chlorohalonata* (PK326), *S. elegans*, *S. microspora* (PK306), and *S. nephrospora* (PK319) recovered from indoor samples submitted to P & K Microbiology Services, Inc., Cherry Hill, NJ, USA were used for the study. For single spore isolation, the cultures were transferred onto MEA medium for 1 week at 25°C. Agar pieces of 5 × 5 mm with fungal conidia, conidiophores and mycelia were cut from the Malt Extract Agar (MEA) medium and placed into test tubes (one piece/test tube) containing 10 ml sterilized water. The test tubes were vortexed for 30 s to dispense the conidia into the water. The conidium suspension was serially-diluted 10× in sterile water. The conidium suspension was diluted up to 100× dilutions. Test tubes with conidium suspensions diluted at 1, 10, and 100× were vortexed for 10 s to evenly suspend the conidia. Ten $\mu$l of the conidium suspension was pipetted from each dilution onto MEA. The conidium suspensions were evenly spread on the media with a sterile triangle metal bar. The Petri dishes were incubated for 24 h at 25°C. Petri dishes were then examined under a dissecting microscope at 60× magnification and double-checked under 200× magnification of a compound microscope. A single-spore isolation was made by removing and transferring a single germinating spore using a surgical scalpel onto the center of an MEA plate for morphological comparisons. The sizes of conidia and phialides of *S. chartarum* isolates were measured under an Olympus BH2 compound microscope with an ocular micrometer at 600× magnification. The color and roughness of conidia were observed also. Thirty conidia and phialides were randomly chosen for the measurement, respectively. Real-time PCR detection analysis was conducted using an ABI Prism, 7000 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) for the isolates used in this study with the two primers and one probe for *S. chartarum sensu lato* (Haugland et al., 1999). The extraction of DNA and other procedures for the experiment followed the procedure described by (Haugland et al., 1999). *Geotrichum candidum* was used as internal reference for monitoring DNA recovery, PCR inhibition, and distinguishing true target negatives from PCR inhibition.

The data of the sizes of conidia and phialides were analyzed with COSTAT 6.2, statistical software (CoHort Software, Monterey, CA, USA).

## Results and discussion

### Taxonomic history and current status of *Stachybotrys chartarum* and related species

The genus *Stachybotrys* and its type species have been subject to controversy since they were proposed. *Stachybotrys chartarum* (Ehrenberg ex Link) Hughes was first described by Corda in 1837 under the name *S. atra* Corda as the type species of a new mitosporic genus *Stachybotrys*. In Corda's description, conidia of this species are two-celled (Corda, 1837). The description of two-celled conidia is one of the controversial aspects. Up to present, all accepted members of *Stachybotrys* produce unicellular conidia without exception (Jong and Davis, 1976). Later, Hughes' reexamination of type material and recombination showed that Corda's description was inaccurate. This inaccuracy led to the revision of Corda's description by Bisby (1943). Prior to critical revision of the description of the species by Bisby in 1943, over 20 species had been described. According to his extensive studies of cultures and herbarium materials, Bisby (1943) revised the species and generic description from two-celled conidia to one-celled conidia and kept the name *S. atra*. Since Bisby did not re-examine the type material, he speculated that the guttulate in the conidia misled Corda to believe the conidia were two-celled. Our observation showed that some isolates developed biguttulate (having two oil drops) conidia. He also reduced the number of species from over 20 to two based on his belief that great variability existed in the species of *S. atra*.

After re-examining the type material of *S. atra*, Hughes (1958) identified and recombined it as *S. chartarum* (Ehrenberg) Hughes. He did not write a new description for the species based on his examination of the type material. Ellis (1971) still recognized *S. atra*. He also accepted a new variety of *S. atra* proposed by Mathur and Sankhla (1966) published in 1966 having smaller conidia (6–8 × 4–5 $\mu$m) and elliptical or pyriform to globose shape, with the name *S. atra* Corda var. *microspora* Mathur and Sankhla.

Jong and Davis (1976) suggested that the proper combination of the species should be *S. chartarum* (Ehrenberg ex Link) Hughes. They also reexamined the type culture of *S. atra* var. *microspora* and found that the deposited material was mixed with *S. chartarum*. The two mixed fungal entities were re-isolated and identified. Jong and Davis proposed *S. atra* var. *microspora* as a new species and recombined it as *S. microspora* (Mathur & Sankhla) Jong & Davis.

According to Bisby and Hughes's studies, *S. chartarum* has three homotypic synonyms:

- *Stilbospora chartarum* Ehrenb. 1818.
- *Oidium chartarum* Ehrenb. ex Link 1824.
- *Oospora chartarum* (Ehrenb. ex Link) Wallr. 1833.

In addition, 16 heterotypic synonyms have been listed (Jong and Davis, 1976). *Stachybotrys atra* Corda, the type species when *Stachybotrys* was proposed as a new genus in 1837, is one of the heterotypic synonyms of *S. chartarum* (Jong and Davis, 1976).

At present the name *S. chartarum* is well accepted and used by a majority of mycologists, but inconsistent descriptions of this species (Bisby, 1943; Ellis, 1971; Jong and Davis, 1976) have resulted in a continuation of the controversy concerning its species delineation. More studies have revealed the complexity of this species (Andersen et al., 2002, 2003).

Kong (1997) published *S. yunnanensis* as a new species in 1997. The key morphological character of this species is that its conidia are cylindrical to subcylindrical ($9.4 \pm 0.82 \times 3.8 \pm 0.47$ $\mu$m; L/W ratio, 2.5) (Figure 1). The author indicated that this species closely resembles *S. chartarum*. At present there has been no additional identification or report of this new species other than the 1997 publication. It is likely that isolates of *S. yunnanensis* are usually identified as *S. chartarum*. Cruse et al. (2002), using markers for three polymorphic protein-coding loci, examined 23 isolates identified as *S. chartarum* and found two cryptic species present within the isolates. The authors claimed that the two cryptic species were indistinguishable morphologically. Andersen et al. (2002) studied isolates formerly identified as *S. chartarum* and found that there were two chemotypes: one producing atranones, the other macrocyclic trichothecenes, as well as one undescribed taxon identified on the basis of morphology, growth, and, more importantly, metabolite production. The undescribed taxon has since been described as a new species, *S. chlorohalonata* (Figure 2) (Andersen et al., 2003). Morphological characters used to differentiate *S. chlorohalonata* from *S. chartarum* are that the former develops smooth conidia ($8.5 \pm 4.2 \times 5.4 \pm 0.39$ $\mu$m; L/W ratio, 1.8) (Figure 2) and more restricted colonies, and produces a green extracellular pigment on Czapek yeast agar (CYA) medium. More significantly the two species have different *tri5*, *chs1* and *tub1* gene fragments (Andersen et al., 2003).

According to the mycotoxin profiles of *S. chartarum*, Andersen et al. (2002) reported that two different chemotypes exist among the isolate identified as *S. chartarum* isolated from water-damaged buildings. One chemotype produced atranones and the other, macrocyclic trichothecene. They also found that an undescribed *Stachybotrys* species (later described as *S. chlorohalonata*) co-existed with the two chemotypes of *S. chartarum* in water-damaged buildings (Andersen et al., 2002). Studies conducted at Technical University of Denmark, Lyngby, Denmark showed that chromatograms and liquid chromatography-ultra violet light (LC-UV) profiles of isolates of *S. chartarum* were different from those of *S. microspora* and *S. nephrospora* (Nielson K. F., personal communication).

### Variations of morphological characters of *S. chartarum sensu stricto*

*Stachybotrys chartarum sensu stricto* (excluding isolates identified as *S. chlorohalonata* and *S. yunnanensis*) is still a species with a great variation in morphology. This may explain, in part, the aforementioned inconsistency in descriptions. The size of phialides observed in the present study (Table 1) was smaller in some cases than the ones reported in the major literature: $9–14 \times 4–6$ $\mu$m (Jong and Davis, 1976) and $10–13 \times 4–6$ $\mu$m (Ellis, 1971), but sizes generally were in agreement. The variation of the phialide size between

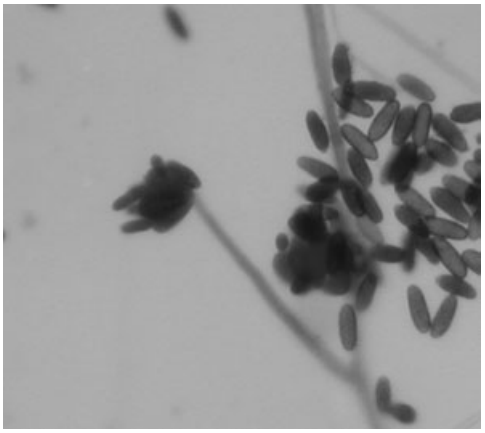

**Fig. 1** The conidia and conidiophore of *Stachybotrys yunnanensis* (scale = 10 $\mu$m)

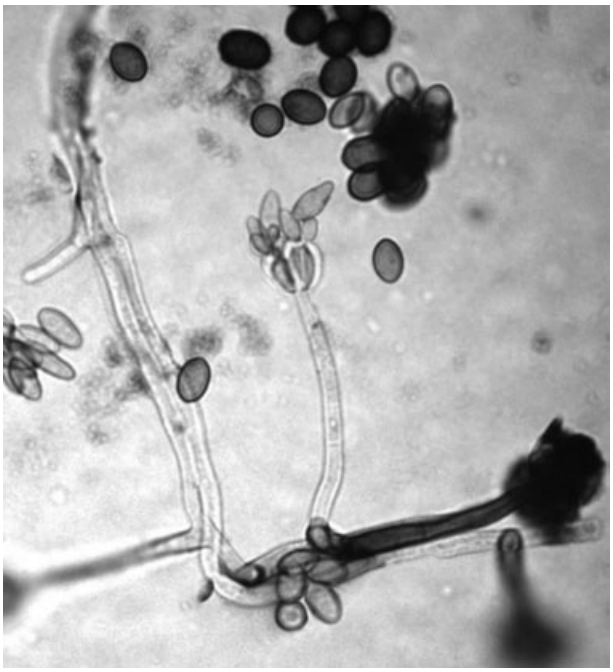

**Fig. 2** The conidia and conidiophore of *Stachybotrys chlorohalonata* (scale = 10 $\mu$m)

M 3N-5 and the two other isolates was statistically significant. The variation in the conidium size among isolates was statistically significant, especially for conidium width (Table 2).

Yang (1995) pointed out that the sizes of conidia described by Ellis (1971) and Jong and Davis (1976) had significant differences, especially the width: 7–12 × 4–6 μm (Jong and Davis, 1976; Domsch et al., 1993) and 8–11 × 5–10 μm (Ellis, 1971). The width of conidia reported in the two descriptions barely overlapped. Similar inconsistency or variation was reported in the study of Bisby (1943). In his study, the size of phialides and conidia in cultures 1 and 3 were similar: phialides 10–15 × 5–7 μm, conidia 8–11 × 3.5–6 μm. In culture 2, conidia were 8–11 × 5–10 μm in 1-month-old cultures. Our results were in general agreement with the conidia dimensions of Jong and Davis (1976) and Bisby's (1943) in cultures 1 and 3 (Table 2) (Figures 3 and 4). The variations in conidia length were significant except between isolates M 21024 and M100. Significant differences in width were found among all isolates. It is not known why the measurements of conidial size showed such a significant discrepancy. Bisby observed that conidia from young culture were much narrower. It is recognized that younger conidia are narrower than mature ones. However, the mature conidia observed in this study were not as wide as those described by Ellis (1971) and Bisby (1943).

Such a great variation in size and shape of phialides and conidia adds confusion to the identification of *S. chartarum*. It might mean that the species concept of *S. chartarum* proposed by Bisby is still too broad. One or two synonyms could be revived upon further study.

**Table 1** Sizes of phialides of three strains of *Stachybotrys chartarum* grown on MEA

| Strain | *n* | Length (μm) | Width (μm) | L/W[1] ratio |
|---|---|---|---|---|
| M 3N-5 | 30 | 8.2 ± 1.33[2] a[3] | 5.0 ± 1.44 a | 1.7 ± 0.53 a |
| M 21024 | 30 | 9.8 ± 1.14 b | 4.6 ± 0.47 ab | 2.1 ± 0.32 b |
| M100 | 30 | 9.4 ± 1.47 b | 4.4 ± 0.70 b | 2.2 ± 0.49 b |

[1]Length/width.
[2]Mean ± SD.
[3]Different letters in the same column indicate the statistically significant difference ($P < 0.05$).

**Table 2** Sizes of conidia of three strains of *Stachybotrys chartarum* grown on MEA

| Strain | *n* | Length (μm) | Width (μm) | L/W[1] ratio |
|---|---|---|---|---|
| M 3N-5 | 30 | 8.2 ± 0.59[2] a[3] | 4.9 ± 0.74 a | 1.7 ± 0.26 a |
| M 21024 | 30 | 8.9 ± 1.13 b | 4.6 ± 0.64 b | 2.0 ± 0.39 b |
| M100 | 30 | 8.8 ± 0.97 b | 5.3 ± 0.63 c | 1.7 ± 0.22 a |

[1]Length/width.
[2]Mean ± SD.
[3]Different letters in the same column indicate the statistically significant difference ($P < 0.05$).

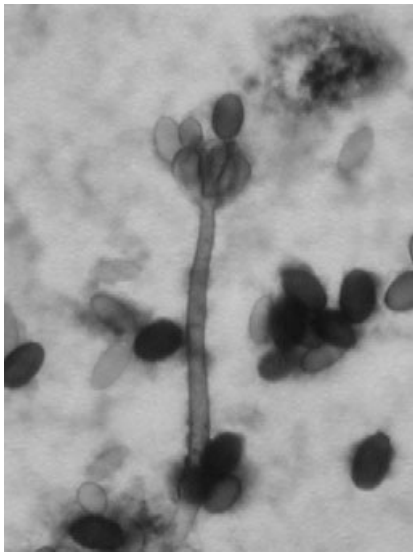

**Fig. 3** Stachybotrys chartarum M100 (scale = 10 μm)

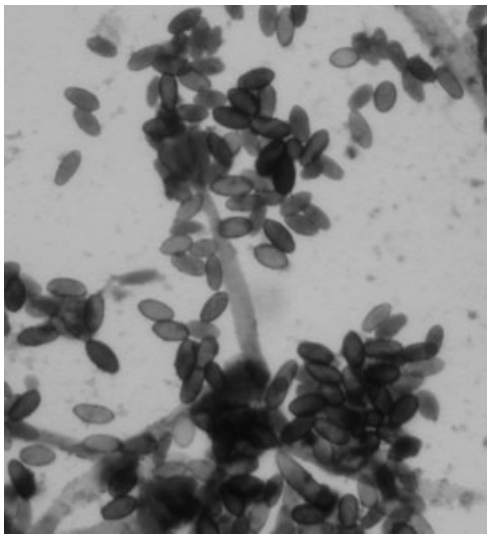

**Fig. 4** *Stachybotrys chartarum* M21024 (scale = 10 μm)

The existence of undescribed species could be another explanation.

### Real-time PCR analysis to differentiate *S. chartarum* from closely related species

In addition to *S. chartarum*, several species of *Stachybotrys* including *S. chlorohalonata*, *S. elegans*, *S. microspora*, *S. nephrospora*, and *S. yunnanensis* were isolated from samples collected in indoor environments (Li and Yang, in preparation; Li and Yang, 2004; Andersen et al., 2003). Real-time PCR detection using *S. chartarum* primers and probes (Haugland et al., 1999) did confirm the identities of the three strains identified as *S. chartarum*. The primer and probe did not react with isolates identified as *S. elegans*,

**Table 3** Real-time PCR analysis of *Stachybotrys* species and isolates of *S. chartarum* using the primers and probe of *S. chartarum*

| *Stachybotrys* Taxon | Real-time PCR detection |
|---|---|
| *S. chartarum* | |
| M 3N-5 | + |
| M 21024 | + |
| M100 | + |
| *S. chlorohalonata* (PK326) | + |
| *S. elegans* | − |
| *S. microspora* (PK306) | − |
| *S. nephrospora* (PK319) | − |
| *S. yunnanensis* | |
| 030115-063 | + |
| PK401 | + |
| *Geotrichum candidum* (positive control) | + |

*S. microspora* and *S. nephrospora*. However, the primers and probe failed to differentiate *S. chlorohalonata* and *S. yunnanensis* from *S. chartarum* (Table 3). This suggests that the current primer and probe set is for the detection of *S. chartarum sensu lato*.

### Practical aspects in identifying *Stachybotrys chartarum* and closely related species

Identifying *S. chartarum* seems to be an easy task because of its unique conidiophore, phialide arrangement, and ornamented conidia. However, it can present problems because of the great variation in the size and shape of the conidia, and especially the color and roughness (immature conidia hyaline, mature dark olive gray, opaque; smooth-walled to coarsely roughened with warts and ridges). In addition, other species of *Stachybotrys* have been identified indoors (Li and Yang, 2004). Differentiation among these species and *S. chartarum* is mainly dependent upon the shape, color, size, and ornamented surface of their conidia (Jong and Davis, 1976). The color and ornamentation of conidia change with age. After 7 days of incubation, color and ornamentation of conidia may not be fully developed in strains of *S. chartarum*. Therefore, there is a risk of misidentifying these isolates. Young isolates with cylindrical or subcylindrical conidia may be identified as *S. yunnanensis*, *S. cylindrospora* or *S. chartarum*. It can be a challenge to differentiate *S. chlorohalonata* from *S. chartarum*, *S. albipes*, and *S. elegans* when an isolate is not fully developed. Caution therefore should be taken when identifying isolates of *S. chartarum* from indoor sources and linking them to mycotoxin production, especially at early growth stages, because the isolate may not be a genuine *S. chartarum*. The types and amount of mycotoxins produced vary with *S. taxa*. Identification of *S. chartarum sensu lato* does not necessarily indicate the production of macrocyclic trichothecenes (Andersen et al., 2002). An experienced mycologist should be consulted when identification of *Stachybotrys* cultures to species is necessary. Molecular DNA approaches have been suggested to offer an alternative method for detecting *S. chartarum* (Haugland et al., 1999; Vesper et al., 2000; Haugland et al., 2001). Real-time PCR testing conducted in the author's laboratory suggested that the primers and probe could not differentiate *S. chartarum* from *S. chlorohalonata* and *S. yunnanensis*, if they are considered three valid species.

## Conclusion and implications

Based on the present study and descriptions in major literature it appears that *S. chartarum* is at present not a well-delineated species. Questions can be raised about the identification of *S. chartarum* and the implication and complication that are associated with the species. The answer to the questions are pending future studies on type materials (including the closely related synonyms), morphology, and phylogenetic analyses. Since the PCR primers and probe designed for *S. chartarum* also detected *S. yunnanensis* and *S. chlorohalonata*, further research is needed to study the phylogenetic relationship of these closely related species to determine whether *S. yunnanensis* and *S. chlorohalonata* are valid species. If they are, the primers and probe for detecting *S. chartarum* should be redesigned.

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