# IS YOUR STACHYBOTRYS CHARTARUM A GENUINE S. CHARTARUM?

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## ABSTRACT
The fungus *Stachybotrys chartarum* is the type species of the genus *Stachybotrys*. Certain strains of the species are known to produce trichothecene mycotoxins. It is a cellulolytic saprophyte with worldwide distribution and frequently discovered in water-damaged buildings. Evidences of the detrimental effects on human health due to respiratory exposure to this fungus have been reported. However, morphological and mycotoxin profile studies showed that this species is not well delineated. This presentation reviews and summarizes data and evidences regarding the taxonomic status of the epithet *S. chartarum*.

## INDEX TERMS
Conidia, Morphology, Mycotoxin profile, *Stachyborys chartarum*, Taxonomic status.

## INTRODUCTION
*Stachybotrys chartarum* (Ehrenberg ex Link) Hughes (=*Stachybotrys atra* Corda), is a cellulolytic saprophyte with worldwide distribution. *Stachybotrys chartarum* is frequently discovered from paper, wallpapers and gypsum boards in the buildings or residences, which experienced water intrusion problem. The fungus produces several mycotoxins, highly toxic macrocyclic trichothecenes and related trichoverroids as well as immunosuppresants and endothelin receptor antagonists (Jarvis and Hinkley, 1999). Its negative effects on animal and human beings have been studied since the 1930's (Haugland and Heckman, 1998; Kendrick, 2000). It was demonstrated to be associated with "sick building syndrome" in wet buildings. It has increasingly attracted public attention to its effect on human health following the reports about its association with idiopathic pulmonary hemorrhage in infants from Cleveland, Ohio (Dearborn et al. 1999). The health issue related to the presence of *Stachybotrys chartarum* in buildings or residences has been covered by several major news media, such as New York Times and Wall Street Journal. However, *S. chartarum* as a species 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 *Stachybotrys atra* by Corda in 1837.

The objective of the paper is to discuss the problematic aspects of *S. chartarum*.

## METHODS
Four isolates (M 3N-530, M 21024, M100, and Cylindrical) with two forms of conidia isolated from indoor samples submitted to P & K Microbiology Services, Inc. were used for the study. The isolates were transferred onto MEA medium for one week at 25°C. Agar pieces of 5 × 5 mm with fungal mycelia were cut off from MEA medium and placed into test tubes containing 10 ml sterilized water. The test tubes were vortexed for 30 s to dispense conidia into water. The conidium suspension was diluted 10 times by pipetting 1 ml into 9 ml of sterile water. Following the same procedure, the conidium suspension we further diluted to

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100 x dilutions. Test tubes with conidia suspensions diluted at 0, 10, and 100 times were vortexed for 10 s to evenly suspended conidia in water. Ten µl conidia suspension was pipetted from each dilution onto MEA. The conidia suspensions on the media spread with a triangle metal bar. The Petri dishes were placed in an incubator at 25 °C. One day later the Petri dishes were examined for single conidium under a dissecting microscope at 60 x. Five individual conidia were marked. Subsequently hyphal fragments of the geminated conidia were cut out and transferred onto MEA 24 hours later for morphological comparisons.

The data were analyzed with COSTAT, statistical software.

## RESULTS AND DISCUSSIONS

### Taxonomic history and current status of *S. chartarum*

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 *Stachybotrys 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 bicellular conidia is one of the controversial aspects. Up to present all accepted members of *Stachybotrys* are unicellular conidia without exception (Jong and Davis, 1976). Later Hughes' reexamination of type material and recombination showed that Corda' description was inaccurate. This inaccuracy led to the revision of Corda' 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 descriptions from two-celled conidia to one-celled conidia and kept the name *Stachybotrys atra*. Since Bisby did not reexamine the type material, he speculated that the guttate in the conidia misled Corda to believe the conidia were bicellular. Our observation showed that some isolates developed biguttulate conidia (having two two tear-like drops in the conidia) He also reduced the number of species from over 20 to two based on his belief that a great variability existed in the species of *S. atra*.

After reexamining the type material of *S. atra*, Hughes (1956) 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 published in1966 according to the smaller sizes of conidia (6-8 × 4-5 µm) and the shape (elliptical or pyriform to globose) with the name *S. atra* Corda var. *microspora* Mathur and Sankhla.

Jong and Davis (1976) suggested that proper combination of the species should be *Stachybotrys 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 mixed two fungal entities were re-isolated and identified. Jong and Davis proposed *S. atra* var. *microspora* as a new species and recombined it as *Stachybotrys microspora* (Mathur & Sankhla) Jong & Davis.

According to Bisby and Hughes's studies, *Stachybotrys chartarum* has three homotypic synonyms:
≡*Stilbospora chartarum* Ehrenb. 1818
≡*Oidium chartarum* Ehrenb. ex Link 1824
≡*Oospora chartarum* (Ehrenb. ex Link) Wallr. 1833.

There are 16 heterotypic synonyms as well. *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 taxonomic status of *Stachybotrys chartarum* is well accepted by a majority of mycologists, but inconsistent descriptions of this species (Bisby, 1943; Ellis, 1971; Jong and Davis, 1976) has resulted in a continuation of the controversy about its delineation.

**Variations of Morphological characters**

*Stachybotry chartarum* is a species with a great variation in morphology. This may explain, inpart, the inconsistency in descriptions as aforementioned. The sizes of phialides observed in the present study (Table 1) were smaller in some cases than the ones reported in the major literature: 9-14 × 4-6 µm (Jong and Davis, 1976) and 10 -13 × 4-6 µm (Ellis, 1971), but generally were in agreement. The variations of the phialides sizes between M 3N-5 and the three other isolates were significant. The variation in phialide sizes was not as great as the variation in conidia sizes.

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

| Strain | n | Length (µm) | Width (µm) | L/W[3] Ratio (µm) |
|---|---|---|---|---|
| M 3N-5 | 30 | 8.2 ± 1.33[1]a[2] | 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 |
| Cylindrical | 30 | 8.2 ± 1.13 b | 4.2 ± 0.57 d | 2.3 ± 0.37 b |

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

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 sizes of phialides and conidia in cultures 1 and 3 were similar: phialide 10-15 × 5-7 µm, conidia 8-11 × 3.5-6 µm. In culture 2 conidia were 8-11 × 5-10 µm in one-month-old cultures. Our results were in general agreement with the conidia dimensions of Jong and Davis (1976) and Bisby (1943)'s in cultures 1 and 3 (Table 2) (Figures 1 and 2). The variations in conidia length were significant except between isolates M 21024 and M100. Significant differences in width were found among all isolates. Why did the measurements of conidial size show 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).

A number of isolates from samples submitted to the senior authors' laboratory developed cylindrical conidia similar to those of *Stachybotrys cylindrospora* Jensen, but smaller (Table 2). The length/width ratio of the isolate developing cylindrical-shaped conidia was significantly greater than the rest of the isolates (Table 2). The conidia of these isolates are hyaline or dark olive, smooth or rough without striations along the length of the conidia (Figure 3). Are isolates with such conidia an intermediate form between *S. chartarum* and *S. cylindrospora*?

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

| Strain | n | Length (μm) | Width (μm) | L/W[3] Ratio (μm) |
|---|---|---|---|---|
| M 3N-5 | 30 | 8.2 ± 0.59[1] c[2] | 4.9 ± 0.74 a | 1.7 ± 0.26 c |
| 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 c |
| Cylindrical | 30 | 9.4 ± 0.82 a | 3.8 ± 0.47 d | 2.5 ± 0.29 a |

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

Such a great variation in conidia sizes and shapes and an intermediate form adds confusion to the delineation of *S. chartarum*. It might mean that the reduction in the number of species of *Stachybotrys* proposed by Bisby might be premature for certain species, which he considered as synonyms. One or two synonyms could be revived upon further study. The existence of undescribed species could be another explanation.

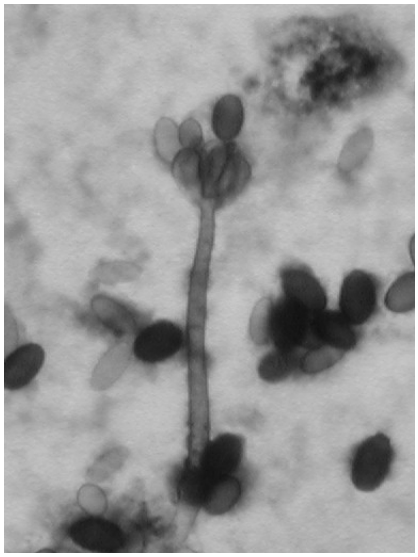

**Figure 1** *Stachybotry chartarum* M100. Scale ___ 10 μm.

**Mycotoxin profiles**

According to the mycotoxin profiles of *S. chartarum*, Andersen et al. (2002) reported that two different chemo-types 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 existed in water-damaged buildings. The undescribed species co-occurred with the two chemotypes of *S. chartarum* (Andersen et al. 2002). These results raised additional questions about the delineation of *Stachybotrys chartarum*.

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

Identifying *S. chartarum* seems to be an easy task due to its unique conidiophore, phialide arrangement, and ornamented conidia, but it can present problems due to the great variation in

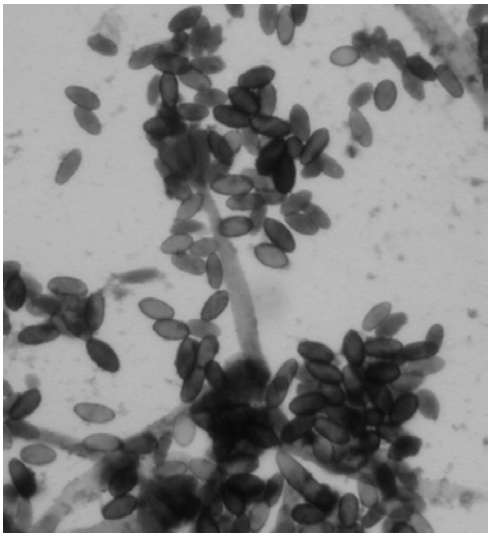

**Figure 2.** *Stachybotrys chartarum* M21024. Scale ___ 10 μm.

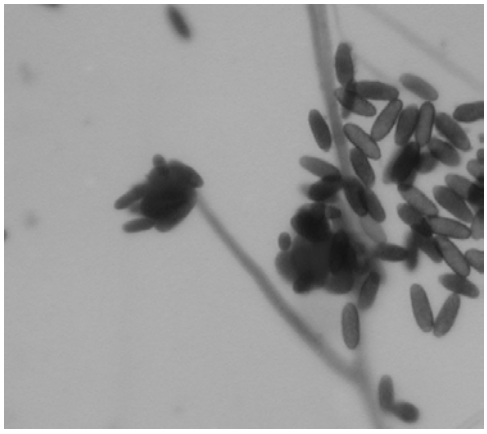

**Figure 3.** The strain of *Stachybotrys chartarum* with cylindrical shape of conidia. Scale 10 μm.

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). *S. albipes* (immature conidia hyaline, mature dark olive gray, smooth walled), *S. bisbys* (conidia hyaline, smooth-walled), and *S. cylindrospora* (conidia cylindrical, immature hyaline and smooth walled, mature dark love gray with striations running along the length of the conidia) are discovered from indoor environments occasionally. Differentiation between 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 ageing. After 7 days of incubation, certain strains of *S. chartarum* may not be fully developed. Therefore, there is a risk of misidentifying these isolates as *S. albipes* or *S. bisbyi* or vice versa. Young isolates with cylindrical conidia may be *S. cylindrospora* or *S. chartarum*. Andersen et al. (2002) has indicated that an undescribed *Stachybotrys* species can be found in water-damaged buildings in Denmark, Finland and the USA. Caution therefore should be taken when attempting to identify isolates of *Stachybotrys* from indoor sources, especially at early growth stages, because your *Stachybotrys chartarum* may not be a genuine one. An experienced mycologist should be consulted when proper identification of *Stachybotrys* species is necessary. Alternatively, molecular approaches have now been shown

to offer a reliable method for differentiating *S. chartarum* and its relatives (Haugland et al., 1999; Vesper et al. 2000; Haugland et al., 2001).

## 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. "Is your *Stachybotrys chartarum* a genuine *S. chartarum*?" The answer to this question is pending future studies on type materials (including the closely related synonyms), morphology, and phylogenetic analyses.

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