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Sistotrema brinkmannii, a psychrotolerant fungus from Antarctic soil

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Abstract: A psychrotolerant fungus with clamp connections was isolated from soil obtained from the Lake Fryxell Basin in the Dry Valleys of Antarctica. Phylogenetic analysis using ITS sequences of rDNA indentified the fungus as *Sistotrema brinkmannii*. Cultures produced basidia with clamp connections at their base and 4 to 8 characteristic sterigmata as well as the suballantoid basidiospores. Few filamentous basidiomycetes have been found in Antarctica and the biology and ecology of this fungus needs additional investigation.

Key words: basidiomycota, ITS sequence, phylogeny, identification

一株源于南极土壤的耐冷真菌 *Sistotrema brinkmannii*

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摘 要：从南极 Dry Valleys Feyxell Basin 湖土壤中分离获得一株具有锁状联合的耐冷担子菌，核糖体 DNA ITS 区序列分析将该菌株鉴定为 *Sistotrema brinkmannii*。形态特征研究表明该菌株产生裸担子，其上生 4－8 个小梗，每个梗上产生一个肾形担孢子，丝状担子菌在南极少有报道，该菌株的生物学及生态学值得进一步研究。

关键词：担子菌门，ITS 序列，系统分析，鉴定

INTRODUCTION

Sistotrema brinkmannii is a basidiomycete that has been previously studied because of its unusual production of basidiospores in culture (Lemke 1969; Ullrich 1973). However, the lack of easily observed characters distinguishing the species and the great morphological

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and genetic variation within this species have caused difficulty for identification. The genus *Sistotrema* was first legitimately erected by E.M. Fries (1821), with *Sistotrema confluens* Pers. 1794 as type. This genus is characterized by urniform basidia with mostly 6－8 sterigmata, smooth spores, and oil-rich hyphae, mostly with clamp connections (Hallenberg 1984). *Sistotrema brinkmannii* is characterized by the grandinioid fruitbody and the suballantoid spores (Hallenberg 1984). In early twentieth century, *Sistotrema brinkmannii* was given several names, including *Odontia brinkmannii* Bresadola 1903, *Corticium coronilla* v. Höhnel & Litschauer 1906, *Corticium octosporum* Schroeter ex v. Hohnel & Litschauer 1906, *Grandinia brinkmannii* (Bres.) Bourdot & Galzin 1914, *Corticium varians* Kniep 1915, *Sistotrema coronilla* (v. Höhn. & Litsch.) Donk ex Rogers 1935, *Trechispora brinkmannii* (Bres.) Rogers & Jackson 1943 and finally *Sistotrema brinkmannii* (Bres.) J. Erikss. 1948 (Oberwinkler 1965).

Investigations of fungal diversity in Antarctica have shown that filamentous basidiomycetes are rarely found (Arenz *et al.* 2006; Blanchette *et al*. 2004; Held *et al*. 2006). In studies at the Ross Sea region, the major decay causing fungi attacking historic huts erected by the early explorers to the South Pole were ascomycetes causing a soft rot decay (Blanchette *et al.* 2004; Held *et al*. 2006). *Sistotrema* spp. have been previously reported from maritime regions of the Antarctic Peninsula (Arenz & Blanchette 2009; Gamundi & Spinedi 1988) but has not been found in any other locations. This study was done to identify an interesting psychrotolerant fungus with clamp connections isolated from the Dry Valleys region of Antarctica. The unusual occurrence of a filamentous basidiomycete fungus at this location warranted investigations to identify the fungus.

1 MATERIALS AND METHODS

1.1 Isolation and morphology

Soils were collected from the Lake Fryxell Basin, Antarctic (77°37′S 163°15′E), By Professor Diana Wall, Colorado State University and stored frozen at -20℃ until used in August 7, 2002. The box containing soil samples was opened in a sterile hood. Each soil sample was homogenized in a beaker with a glass stick. The fungi were isolated by the sprinkle method (Barron 1977) since the original intention was to detect nematophagous fungi. The isolates were cultured on PDA (Potato Dextrose Agar) and CMA (Corn Meal Agar) plates at 25℃ and 4℃ respectively. Observations and measurements of fungal colony and morphology were recorded after incubation of seven days. Photographs of hyphae and the spores were taken with a Nikon 80i microscope with differential interference contrast (DIC).

1.2 DNA extraction, PCR amplification and sequencing

Fresh mycelium was collected from cultures growing on cellophane membranes placed on the PDA surface in Petri dish plates. Genomic DNA was extracted from the mycelium according to the method of Doyle & Doyle (1987). Primers for PCR amplification of the internal transcribed spacer region of nrDNA were synthesized according to ITS5 and ITS4 described by White *et al.* (1990). The reactions were carried out in a thermal cycler with following parameters: initial denaturation at 95℃ for 3min, followed by 34 cycles consisting of denaturation at 94℃ for 1min, annealing at 54℃ for 40s and extension at 72℃ for 1min. A final extension at 72℃ for 10min followed. Primary PCR products were purified by Go3S PCR Product Purification Kit (Shenergy Biocolor BioScience & Technology Company, Shanghai, China) and sequenced in both directions with an ABI automated Sequencer (ABI 377) (Perkin Elmer) using ye-dideoxynucleotide chain-termination method.

1.3 Phylogenetic analysis

The determined ITS sequences were used for molecular phylogenetic analysis. Additional sequences (Table 1) were obtained from GenBank. All sequences were aligned with the Clustal X 1.81 program (Thompson *et al.* 1997) and adjusted visually where necessary in the program BioEdit version 7.0.5.3 (Hall 1999). Cladistic analyses using the neighbor-joining method (Saitou & Nei 1987) were performed with MEGA version3.1 (Kumar *et al.* 2004).

2 RESULTS

Cultures of a filamentous fungus were obtained from isolations made from the soil samples. The fungus could grow at 25℃ and 4℃ and it grew moderately rapidly on both types of agar plates; colony diameter was 39－41mm on CMA and 63－64mm on PDA at 25℃

Table 1 Fungi used in the phylogenetic analysis

Species	Strain	GenBank No. (ITS)
Sistotrema brinkmannii	3-6-2	
Sistotrema brinkmannii	286F	AY089729
Sistotrema brinkmannii	aurim1111	DQ093653
Sistotrema brinkmannii	ATCC 26295	DQ899094
Sistotrema coronilla	AFTOL-ID 618	DQ397337
Botryobasidium botryosum	AFTOL-ID 604	DQ267124
Botryobasidium subcoronatum	AFTOL-ID 614	DQ200924
Tubulicrinis hirtellus	2040b	DQ873657
Tubulicrinis globisporus	3156b	DQ873655
Sistotrema confluens	AFTOL-ID 613	DQ267125
Tulasnella calospora	MAFF P305805	DQ388045

after 7 days. The colony on PDA is sparsely floccose, white, reverse usually pale yellowish white. Hyphae hyaline to white, 2－5μm wide, branched, septate, clamp connections present. Basidia urniform, 12－22(－33)× 3.5－4.5μm (4.5－5.5μm in width at the base), with a basal clamp, producing 4－8 sterigmata; basidiospores suballantoid, 3.2－5.5×1.7－2.5μm, smooth, thin-walled (Fig. 1). Morphological observations suggested the genus was *Sistotrema* based on its characteristic sistotremoid basidia and the possession of small, suballantoid basidiospores borne on the sterigmata. ITS sequence analyses indicated this filamentous basidiomycete was *Sistotrema brinkmannii* (Fig. 2).

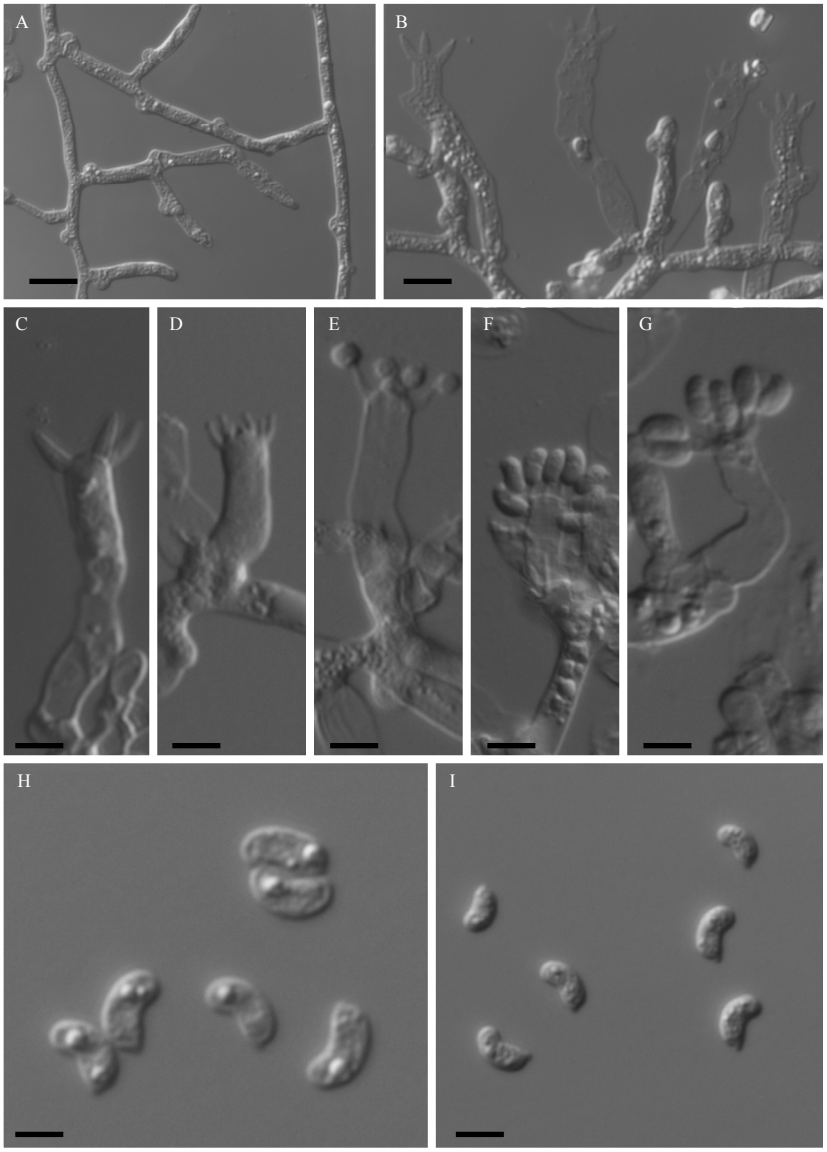

Fig. 1 *Sistotrema brinkmannii*. A: Clamp connections; B: Basidia; C-G: Sterigmata and basidiospores borne on basidia; H-I: Basidiospores; Scale bars: A-I = 5μm.

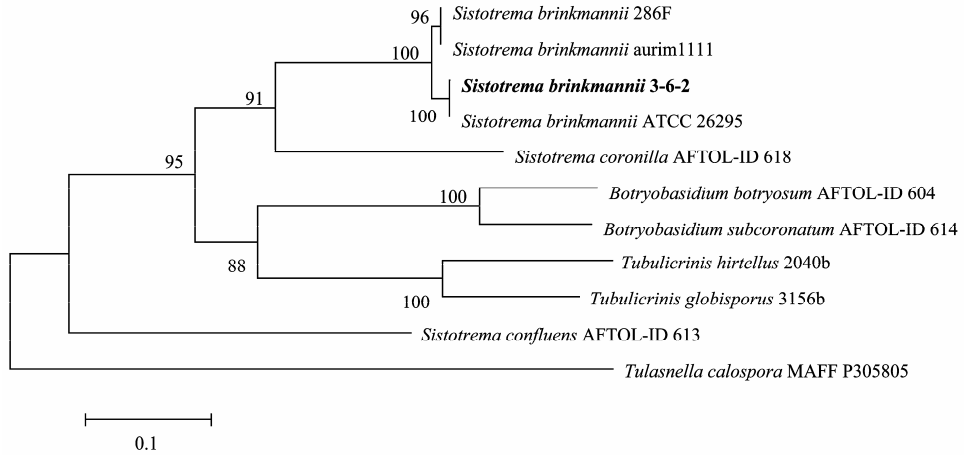

Fig. 2 Dendrogram constructed from neighbor-joining analyses based on sequences of rDNA ITS gene fragment of *Sistotrema brinkmannii* (in bold) and species of high sequences similarity, using *Tulasnella calospora* as outgroup. Bootstrap values are placed on the tree nodes.

3 DISCUSSION

The fungus we obtained from this extreme environment in Antarctic was well assigned into the genus *Sistotrema* due to its characteristics such as sistotremoid basidia and the possession of small, suballantoid basidiospores borne on the sterigmata. And the microstructure of this fungus basically fits morphological criteria for *S. brinkmannii.*

By amplifying the ITS region, our isolate generated a 641bp amplicon. A blast similarity search of this strain on the consensus sequence of the ITS region demonstrated a close relationship with the genus *Sistotrema*. Species with high sequence similarity with this strain was examined and applied in phylogenetic analysis. A cladogram of the ITS regions (Fig. 2) shows that our strain is clustered together with two strains of *S. brinkmannii*. The identification of *S. brinkmannii* could be established based on the 99% similarity of their ITS region and the clade of 100% bootstrap support value. We attempted to induce the basidiomata on cultures of the species to provide the supplementary evidences but failed.

In addition, *Sistotrema brinkmannii* apparently exists as a complex or aggregate of morphologically similar entities possessing differing patterns of sexuality that control development of the life cycle. The bipolar and tetrapolar heterothallic forms as well as homothallic forms were recognized (Biggs 1937) and confirmed (Lemke 1969). The existence of the sexuality patterns in both geographical and substratal respects were reported (Ullrich 1973). *Sistotrema brinkmannii* complex was studied and analyzed, and six species were established and described (Hallenberg 1984).

Recent studies have shown this species is broadly distributed in different regions of the world including Asia, Europe, and North and South America (Greslebin & Rajchenberg 2003; Maekawa 1993). The fungus was isolated from maritime sites in the Antarctic Peninsula but has not previously been isolated from the Ross Sea region or continental Antarctica (Gamundi & Spinedi 1988; Arenz & Blanchette 2009). The isolation of a filamentous basidiomycete from a Dry Valley location is unusual and suggests that this fungus may play a more important role in decomposition and nutrient recycling than previously realized. In other parts of the world, this fungus colonizes and degrades wood and wood products (Schroeder & French 1964). Since there are no lignocelulosic substances in the Dry Valley's of Antarctica, the substrates used by the fungus in Antarctic soils are uncertain. Its ability to produces basidiospores in culture suggests that the fungus may be able to disseminate more easily than other basidiomycetes. Information on the biology and ecology of this interesting fungus is needed to better understand why this particular fungus can exist in the extreme Antarctic environment when other filamentous basidiomycetes cannot.

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