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Microfungi of Windmill Island, Antarctica: Diversity and ultrastructure studies of Soil Fungi

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ABSTRACT Soil materials were collected during two summer expeditions to the Windmill Island, Antarctic in 2002 and 2004 to determine occurrence and distribution of culturable fungi using a modified Warcup's soil plate method. A total of 10 fungi consisting of eight species of Ascomycetes, one Zygomycete and one yeast were isolated. The most common species isolated from Windmill Island were *Cadophora malorum*, *Geomyces cretaceous* and *Thelebolus microsporus* while *Aureobasidium* sp., *Antarctomyces* sp., *Mucor* sp., *Mrakia frigida*, *Trichosporiella cerebriformis*, and *Phoma* sp. were less common. Ultrastructure studies of the fungi were also undertaken. This is the first report of *Trichosporiella cerebriformis* isolated from Continental Antarctic soils.

ABSTRAK Material dari tanah telah dikutip semasa ekspidisi musim panas ke Pulau Windmill, Antartika pada tahun 2002 dan 2004 bagi mengkaji kehadiran dan taburan kulat yang boleh dikultur dengan menggunakan kaedah "Warcup's soil plate". Sejumlah 10 spesies kulat telah ditemui di mana lapan spesies ascomycetes, satu zygomycetes dan satu yis telah diasingkan. Spesies yang paling kerap ditemui dari Pulau Windmill adalah *Cadophora malorum*, *Geomyces cretaceous* dan *Thelebolus microsporus,* sementara *Aureobasidium* sp., *Antarctomyces* sp., *Mucor* sp., *Mrakia frigida*, *Trichosporiella cerebriformis*, dan *Phoma* sp. adalah jarang ditemui. Kajian ultrastruktur kulat tersebut juga telah dijalankan. Ini adalah laporan pertama kehadiran *Trichosporiella cerebriformis* di dalam tanah benua Antartika.

INTRODUCTION

Terrestrial continental Antarctica is considered as an extreme environment, characterized by high-stress or high-disturbance conditions [1] that are exemplified by low temperatures, high aridity, high incidence of ultra-violet radiation, low availability of nutrients and by strong thermal excursions, especially at the microhabitat level [2]. Microbial colonization of new habitats in the Antarctic depends on the presence of moisture, warmth and nutrients [3], factors which also affect the persistence and activity of established colonies.

Microbiological investigations in the southern cold temperate to polar regions have concentrated largely on the sub-Antarctic islands, the Antarctic Peninsula and the McMurdo Dry Valleys region of Southern Victoria Land [1]. Outside these area, mycological studies on the Antarctic continent are limited to reports from Ongul Islands [4, 5], Mac.Robertson and Enderby Lands [6, 7], Vestfold Hills [7, 8], Bunger Hills [9], Cape Hallett [10], Northern Victoria Land [11], Trans-Antarctic Mountain [12], and McMurdo Sound region[13, 14, 15]. There have been few reports on fungi from Windmill Islands [16, 17, 18]. These studies suggest a low diversity of indigenous fungi in continental Antarctica with around 150 taxa recognized in the literature.

MATERIALS AND METHODS

Soil samples were collected from Windmill Island during summer expeditions in February 2002 and 2004. The collection sites include Thalla Valley, penguin rookeries on Clark Peninsula at Whitney Point, Clark Peninsula SSSI17 and a melt lake (Figure 1a-e). The Windmill Islands are centered on the Australian Casey Station (66°18'S, 110°32'E) on Bailey Peninsula and offer a wide variety of habitats, including bird colonies particularly Adelie penguins, mosses, algae and lichens, and mineral soils and lakes. The former American Wilkes

Station is located on Clark Peninsula, the most northerly peninsula[18].

Fungi were isolated from soil using a modified soil plate method [19]. Approximately 0.1g of soil was placed on the agar surface and distributed using 1 ml of sterile distilled water and rotating the plate. Five replicates were plated on the isolation agar medium which was OXOID Potato Dextrose Agar (PDA), and incubated at 4°C. All plates were examined daily to determine number of colonies and to prevent overgrowth of the fungal colonies. Fungal isolates were sub-cultured for identification. Filamentous fungi were identified to species where possible. Previous studies (Azmi and Seppelt, 1998 and unpublished data) returned around 150 isolates of fungi with some 45% of these as mycelia sterilia. Ultrastructure studies were also undertaken in the laboratory in Malaysia. Isolates for scanning electron microscopy (SEM) were prepared as described by Moss and Jones (1977) [20].

RESULTS AND DISCUSSION

Table 1 lists frequency of occurrence of fungal taxa from study sites. In total, 10 species were collected and included eight ascomycetes, one zygomycete and one basidiomycetous yeast. Geomyces cretaceus is the most commonly isolated fungus followed by Cadophora malorum and Thelebolus sp. (Table 2). An unidentified fungi sp. 9 was the least common fungus isolated. Thalla Valley showed the highest number of fungal taxa while soils from SSSI-17 on Clark Peninsula had the lowest number of taxa isolated.

The fungi isolated in Thalla Valley were diverse (Table 1). They included *Geomyces cretaceus* (Figure 2(g-j)) *Aureobasidium* sp. (Figure 2a-c) and *Cadophora malorum* (Figure 2d-f), *Trichosporiella cerebriformis* (Figure 3a-b), *Mucor* sp., unidentified sp. 9 (Figure 4 (c)), *Thelebolus* sp. (Figure 3 (f-i)), *Antarctomyces* sp. (Figure 3 (c-e)) and a basidiomycetous yeast, *Mrakia frigida* (Figure 4 (a-b)). Geomyces cretaceous and Aureobasidium sp. were the most commonly encountered species in Thalla Valley. The isolation of *Trichosporiella cerebriformis* (Figure 3a-b) from Thalla Valley represents the first record from continental Antarctica.

At Whitney Point, six species were identified including yeast. They were *Aureobasidium* sp., *Cadophora malorum, Geomyces cretaceous, Antarctomyces* sp., *Thelebolus* sp. and *Mrakia frigida.* Isolates of the genus Antarctomyces were first found by Stichgel et al. [21] from soil samples taken in King George Island, maritime Antarctic. They were identified as *Antarctomyces* psychrotrophicus. The presence of large numbers of Adélie penguins at Whitney Point influenced the fungal taxa isolated [16]. At this site, *Geomyces cretaceus,* a keratinolytic fungus, *Antarctomyces* sp. and *Thelebolus* sp. were the most common isolates. *Thelebolus* spp. are generally guaniferous [22] and frequently associated with bird colonies in Antarctica [9, 10, 16, 23]. *Thelebolus* sp., *Antarctomyces* sp. and *Geomyces* cretaceous were the most commonly isolated fungi. We recorded fewer taxa than previously reported by Azmi and Seppelt (1998) [16]. *Antarctomyces* sp., *Aureobasidium* sp. and *Cadophora malorum* are newly recorded species in this area.

Isolated fungal taxa appear non-uniformly distributed throughout the sampling region (Table 1). *Cadophora malorum* and *Mrakia frigida* were isolated from SSSI-17 (Table 1). *Geomyces cretaceus, Antarctomyces* sp. and *Thelebolus* sp. were found in samples from Peterson Island in low frequency of occurrence. However, the frequency of occurrence and numbers of taxa isolated in this study are lower than that of the previous study [16].

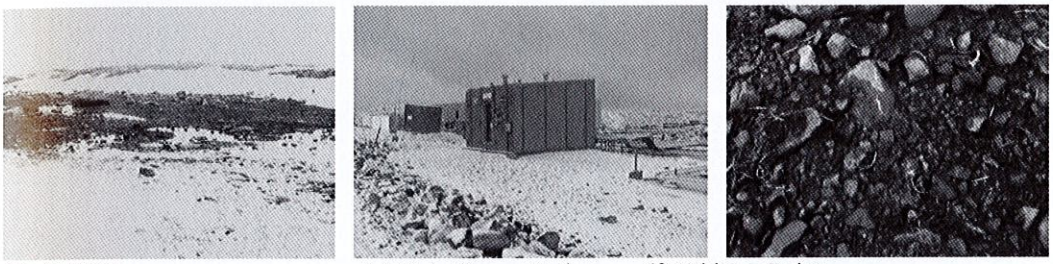
The Basidiomycete yeast, *Mrakia frigida* (Figure 4a-b) was isolated from soils at four localities: Thalla Valley, SSSI17, Whitney Point and at Wilkes Station (Table 1). This species observation records an extension of its known range [24, 25]. The rare isolation of yeast may indicate low numbers of this microorganism in the region or its selective preclusion by the particular isolation medium.

In the present study, *Geomyces cretaceus* (Figure 2g-i) was commonly isolated (Table 2) and has been reported to have a wide range of habitats [10, 11, 13]. This species was

(a) Wilkes Land (66° 15' 63.2" S 110° 32' 26.8" E)

(b) Peterson Island (66° 24' 97.7" S 110° 53' 81.6" E)

(c) Casey Station (66° 16' 75.9" S 110° 32' 25.2" E)



(d) SSSI17 (66° 28' 20.0" S 110° 53' 87.5" E)

(e) Main Power Station (66° 28' 18.6" S 110° 52' 51.2" E)

(f) Whitney Point (66° 15' 08.0" S 110° 32' 34.1" E)

Figure 1 (a-e): Sampling sites at Windmill Islands

Table 1: Percentage of fungi occurrence from each site.

Fungal taxon	Collection sites and percentage of occurrence					
	Thalla Valley	Peterson Island	SSSI-17	Whitney Point	Casey Station	Wilkes Station
Ascomycetes						
Antarctomyces sp.	1	1	-	11	-	-
Aureobasidium sp.	7	-	-	5	-	-
Cadophora malorum (Kidd & Beaum) W. Gams	4	-	6	2	4	10
Geomyces cretaceus Traaen	8	6	-	12	14	5
Phoma sp.	-	-	-	-	-	3
Thelebolus sp.	4	4	-	11	1	5
Trichosporiella cerebriformis (de Vries & Kleine-Natrop) W. Gams	2	-	-	-	-	-
Unidentified sp. 9	2	-	-	-	-	-
Zygomycete						
Mucor sp.	3	-	-	-	-	-
Yeast						
Mrakia frigida (Fell et al.) Yamada & Komagata	2	-	2	2	-	3
Total taxa	9	3	2	6	3	5

The minus symbol (-) indicates that no fungi were detected during the observation period.

considered to be synonym of *G. pannorum* (Link) Sigler & J.W. Carmich. [26]. Strain of *G. pannorum* has been previously reported as common in low temperature soil microfloras [27] and as frequent in many parts of Antarctica, such as Vestfold Hills [7] and Signy Island [3].

The number of fungal species recovered in this study was low. Only 9 genera have been isolated from a wide range of habitats. This is consistent with the findings on Antarctic microfungi by other researchers [1, 7, 10, 11, 14, 16, 28, 29]. For example Azmi et al. [16] identified only 15 genera in their survey of the fungi of Windmill Islands. Bolter [30] and Roser et al. [31] found that yeasts and filamentous fungi are rare in soils around Casey Station in comparison with bacteria, microalgae and cyanobacteria, accounting for less than 1% of the total microbial biomass. A study on the island of South Georgia (54°S, 37°W) listed 113 fungal taxa [32]. The harsher climatic conditions and availability of nutrients appear to be a significant barrier to fungal establishment in the continental Antarctic environment [33].

In comparison with the species encountered in this study and from those reported earlier, *Geomyces, Cadophora* and *Thelebolus* can be regarded as commonly occurring in Antarctic environment. The differences in the species diversity observed in this study and those reported from the literature can be attributed to several factors. Latter [14] stated amongst the factors are the differences in soils and substrates examined, environmental conditions, the nature and location of the sites studied. No moulds of the genera *Aspergillus, Penicillium* and *Trichoderma* have been isolated in our samples, despite having reported by other workers in various substrata [5, 13, 20, 33, 34]. Detailed investigations conducted over prolonged period would provide a more comprehensive description of the fungal flora.

CONCLUSIONS

Ten fungal species from nine genera were isolated from soils around Windmill Islands, Antarctica. The fungal diversity of the Windmill Islands appears to be low. Hyphomycetes appear to dominate the fungal flora of the Windmill Islands. Most of the fungi recorded are cosmopolitan species except for *Antarctomyces* sp. The isolation of *Trichosporiella cerebriformis* is new for the fungal biodiversity in continental Antarctica.

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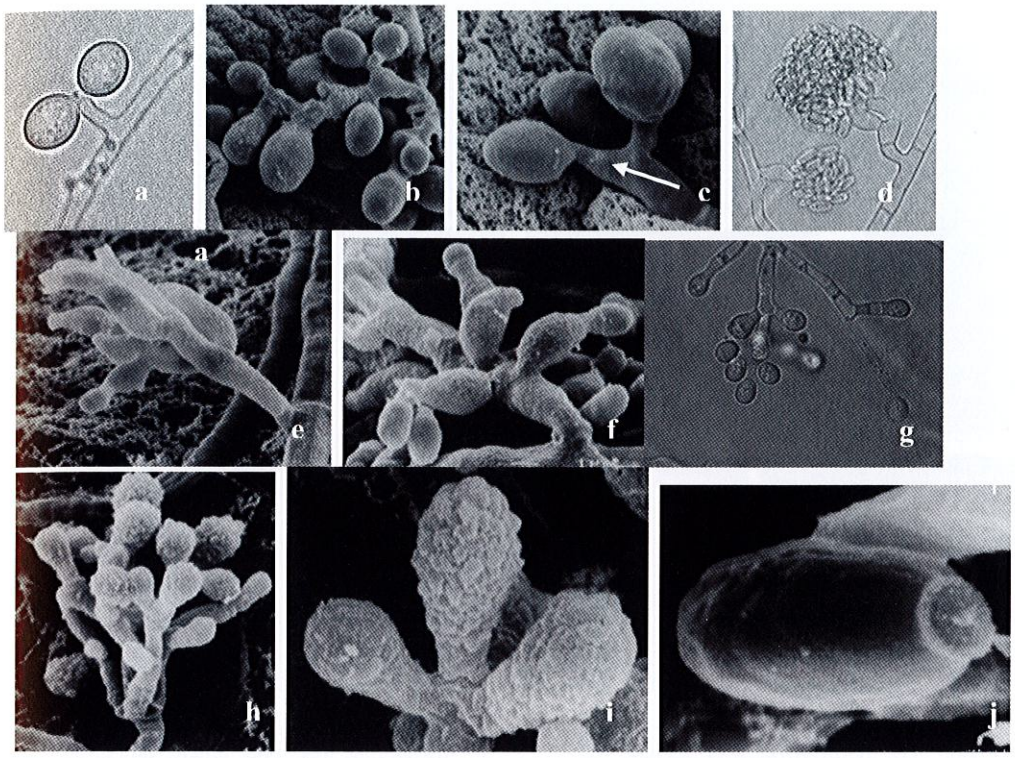
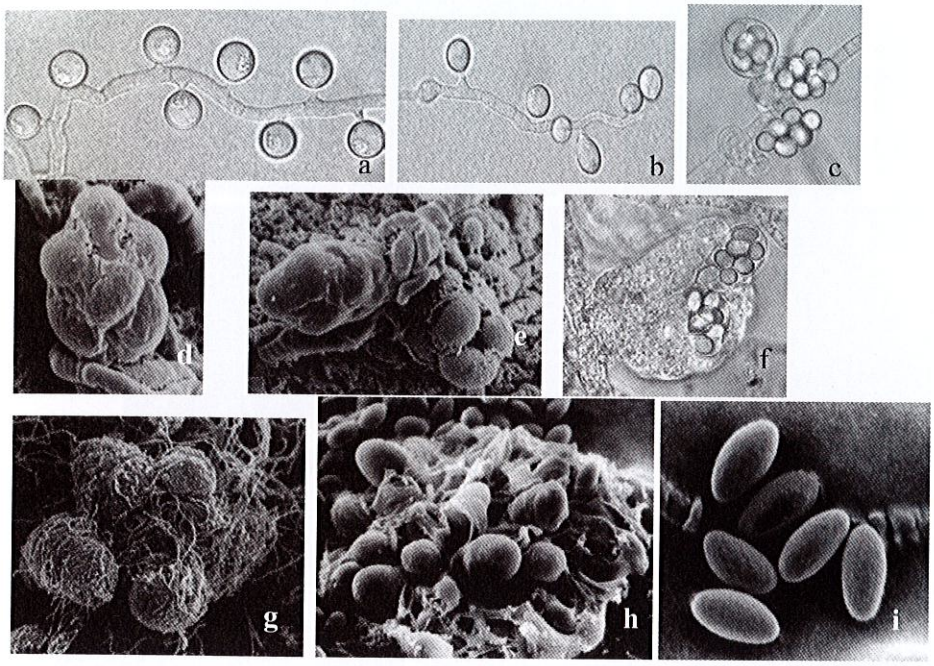
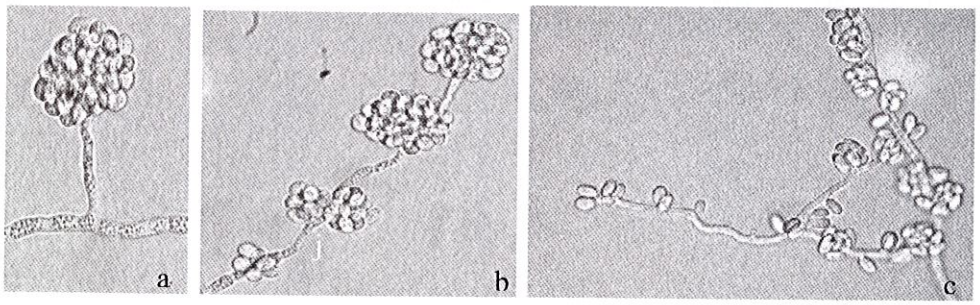


Figure. 2a-c: *Aureobasidium* sp. (a) Light micrograph showing hyaline hyphae and conidia. Conidia 7.5-10 x 5-7.5 µm, smooth wall and produced on hyphae on short denticles. (b-c) Scanning electron micrograph of conidia produced on short denticles (arrow). Figs. 2d-f: *Cadophora malorum* (d) Light micrograph of hyaline phialides developed laterally or terminally from conidiophores or directly along the hyphae itself. Elliptical conidia, 5-7.5 x 5 µm, accumulate at the apices of phialides. (e-f) Scanning electron micrographs of conidia and conidiophores (e) and; phialide and conidia (f). Figs. 2 (g-j) *Geomyces cretaceus*: (g) Light micrograph of verticillately branched conidiophores. Conidia borne on the tips of conidiophores or on sides of the hyphae, pyriform with truncate bases, 5-6.3 x 2.5 µm, heavy cell wall and may be either smooth or rough. (h-j) Scanning electron micrographs of conidia and conidiophores (h), mature conidia with verrucose wall (i) and young conidia (j).



Figures 3: (a-b) *Trichosporiella cerebriformis*: (a-b) Light micrograph of simple or branched conidiophores not clearly differentiated from the vegetative hyphae. Conidia are small, globose, smooth and hyaline, 5-8 µm in diameter. Figs. 3 (c-e) *Antarctomyces* sp.: (c) Light micrograph of naked asci, single or in groups of 2-4, arising directly from the hyphae. Paraphyses absent. Asci subglobose, unitunicate, thick walled, 6-8 spored. Ascospores hyaline, 7.5-10 x 6-7.5 µm, single-celled, smooth walled, ellipsoidal. (d-e) Scanning electron micrographs of clusters of asci containing ascospores (d), naked asci produced openly on hypha (e). Figs. 3 (f-i) *Thelebolus sp.:* (f) Light micrograph showing superficial ascomata: ascomata subglobose and hyaline. Asci are clavate, 6-8 spored and apically rounded. Ascospores are ellipsoidal, smooth walled, 7.5-12.5 x 5-7.5 µm. (g-i) Scanning electron micrograph of hyphae covered ascomata (g), matured ascoma with ascospores (h), and ascopores (i).



Figures. 4 (a-b) *Mrakia frigida*: (a,b) Light micrograph of hyaline and subglobose conidia. Conidiophores are not present, the spore masses being borne on on hyphae, proliferated by budding. Figure. 4 (c) Unidentified sp. 9.

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