Evolution, taxonomy and ecology of the genus *Thelebolus* in Antarctica

G.S. de Hoog[1,2]*, E. Göttlich[3], G. Platas[4], O. Genilloud[4], G. Leotta[5] and J. van Brummelen[6]*

[1]*Centraalbureau voor Schimmelcultures, P.O. Box 85167, NL-3508 AD Utrecht, The Netherlands*
[2]*Institute of Biodiversity and Ecosystem Dynamics, University of Amsterdam, Kruislaan 318, NL-1098 SM Amsterdam, The Netherlands*
[3]*Rheinisch Westfählisches Institut für Wasserforschung, Moritzstr. 26, D-45476 Mülheim an der Ruhr, Germany;*
[4]*Merck Sharp & Dohme Espaňa, Josefa Valcarcel, 38, E-28027 Madrid, Spain*
[5]*Cátedra de Micología Médica e Industrial, Universidad Nacional de La Plata, La Plata, Argentina*
[6]*Nationaal Herbarium Nederland, P.O. Box 9514, NL-2300 RA Leiden, The Netherlands*

**Correspondence*: G.S. de Hoog, de.hoog@cbs.knaw.nl and J. van Brummelen, brummelen@nhn.leidenuniv.nl

Abstract: An investigation of the biodiversity of some Antarctic lakes revealed that *Thelebolus microsporus* was one of the preponderant species. The genus *Thelebolus* is known to combine psychrophily with growth on dung and guano, suggesting that strains in lake biomats have originated from bird vectors. A natural association with skuas, petrels and other birds was confirmed by an analysis of bird cadavers, which showed frequent intestinal colonisation by *T. microsporus*. Strains did not produce conidia but sporulated *in vitro* with ascomata on Carrot Agar (CA) at temperatures below 15 °C. The asci found were 8-spored and had forcible spore-discharge. Microsatellite data showed that several genotypes were recognisable, some of which had a very limited geographic distibution, suggesting the occurrence of local evolution in the Antarctic. Two undescribed species were encountered in lakes where birds are practically absent. These strains had reduced morphology, lacking forcible spore discharge, but produced conidia in abundance. The development of this morphology is probably attributed to a dramatic change in the life cycle due to the loss of the bird vector. Judging from ß-tubulin data, the species emerged 30–40 million years ago, when the Antarctic continent reached its current position.

In an SSU rDNA tree of *Thelebolus* and purported relatives the genus was shown to have a distant position to remaining cup fungi. The maintenance of a separate order for *Thelebolus* and similar genera is therefore justified. The psychrotolerant genus *Hyphozyma* was found to be related, while *Antarctomyces* appeared closely similar to *Thelebolus* on the basis of rDNA ITS data; psychrophily apparently is a mainstay in the evolution of *Thelebolus*.

About eighty strains and 181 herbarium collections of the genus *Thelebolus*, among which most of the ex-type cultures and type specimens, are studied for the morphology of the teleomorph and eventual anamorph. A key is provided for the morphological identification of the four species recognised. Polysporic strains are untited under the name *T. stercoreus*. *Thelebolus microsporus* has a wide distribution in the Antarctic and elsewhere. *Thelebolus globosus* Brumm. & de Hoog and *Thelebolus ellipsoideus* Brumm. & de Hoog are newly described. Both are endemic to Antarctica. A hypothesis on the possible course of evolution in Antarctic *Thelebolus* is put forward.

Taxonomic novelties: *Thelebolus ellipsoideus* Brumm. & de Hoog sp. nov., *Thelebolus globosus* Brumm. & de Hoog sp. nov.
Key words: Antarctica, DNA Walk, DNAWD, ecology, endosaprobe, halotolerance, ITS rDNA, microsatellite fingerprinting, phylogeny, psychrophily, SSU rDNA, taxonomy, ß-tubulin.

INTRODUCTION

The genus *Thelebolus* Thode : Fr. comprises a group of simply structured cleistohymenial ascomycetes with small ascomata and clavate asci containing 8 to over 2000 hyaline ascospores. The phylogenetic position of this genus is still unresolved. Until recently it has been listed as a member of the *Pezizales* (Kimbrough & Korf 1967, Eckblad 1968), but this classification has been questioned on the basis of rDNA sequence data (Momol & Kimbrough 1994; Momol *et al*. 1996). Also the ecology of members of the genus is still poorly understood. This is partly caused by a lack of criteria for reliable species distinction (Kimbrough & Korf 1967), hampering the link of interbreeding populations to particular habitats. Two habitats have nevertheless consistently been reported, as members of the genus frequently have been found (1) in association with animal dung, as well as (2) in cold (micro) climates. The frequent isolation of *Thelebolus* species from Arctic and Antarctic climate zones became particularly striking with the studies of Kobayasi *et al*. (1967) and Montemartini *et al*. (1993).

In the framework of the EU-sponsored project MICROMAT, biomats in several lakes in Eastern Antarctica were studied from the point of view of microbial species diversity. An overview of the fungi found during this study was given by Göttlich *et al*. (2003). The slow-growing, pink, filamentous cultures which

were preponderant in all lakes investigated initially were all sterile or showed simple conidiation from undifferentiated hyphae. These fungi were phenotypically identified with the genus *Hyphozyma*, known to contain species from water and from cold environments (De Hoog & Smith 1981). After incubation at 10–15 °C on low-strength media like carrot agar (CA), however, many strains produced very small, brownish "apothecioid" ascomata consisting of interwoven hyphae and containing clavate asci with ellipsoidal ascospores. Hence the fungi were subsequently recognised to be *Thelebolus* spp.

The high frequency of members of *Thelebolus* from Antarctic biomats in divergent types of lakes was unexpected. Antarctic lakes usually lack any significant zooplankton and there are no fish. Instead the systems are dominated by microbial plankton composed of viruses, bacteria, protozoa and algae (Laybourn-Parry *et al*. 1997). Algal or cyanobacterial mats can be present. In order to be able to interpret the results from the point of view of ecology, we started multilocus genotyping. Identification of strains was performed with the aid of ITS rDNA sequencing, with reference to strains of *Thelebolus* species from the CBS culture collection and from elsewhere in the Antarctic (Leotta *et al.* 2002), and including supposedly related genera of simply-structured ascomycetes such as *Antarctomyces* Stchigel *et al*. (Stchigel *et al*. 2001) and *Calyptrozyma* Boekhout & Spaay (Boekhout *et al*. 1995). Data on the population structure of the main species were generated using microsatellite typing. In addition, we aimed to establish the phylogeny of *Thelebolus* at higher taxonomic levels by applying 18S rDNA sequencing.

MATERIALS AND METHODS

Sampling sites in Antarctica

Biomats were sampled (Figs 1–2) from Fryxell and Hoare Lakes in McMurdo Dry Valleys in Southern Victoria Land, from Ace, Druzhby, Highway, Organic and Watts Lakes in the Vestfold Hills and from Manning, Reid and Sarah Tarn Lakes in the Larsemann Hills on Princess Elizabeth Land. The coastal area separating the Larsemann and Vestfold Hills contain a large Emperor Penguin colony at Amanda Bay and flying bird colonies at the Rauer Islands.

Essential data on the lakes are summarised in Table 1; their locations are indicated in Figs 1–2. Strain data are specified in Table 3. Additional strains, donated by G. Leotta, were isolated from the cloacae, intestines, tracheae and feathers of carcasses of local birds at King George Island at South Shetland Islands, Laurie Island at South Orkney Islands and Hope Bay at Antarctic Peninsula (Leotta *et al*. 2002). Reference strains were taken from the CBS culture collection.

Table 1. Essential features of lakes sampled in Southern and Eastern Antarctica*.

Salinity	Max. depth	Stratification	Coverage	
McMurdo Dry Valleys:				
Fryxell	moderate	20 m	meromictic	permanent
Hoare	fresh	34 m	mixed	permanent
Vestfold Hills:				
Ace	saline	25 m	meromictic	seasonal
Druzhby	fresh	40 m	mixed	seasonal
Highway	brackish	17 m	mixed	seasonal
Organic	hypersaline	7 m	meromictic	seasonal
Watts	brackish	29 m	mixed	seasonal (occasional overflows from lake Druzhby)
Larsemann Hills:				
Manning	fresh	shallow	mixed	seasonal
Reid	fresh	3.8 m	mixed	seasonal
Sarah Tarn	brackish	2.5 m	mixed	seasonal

*At King George, Hope Bay and Laurie Island only bird cadavers were sampled.

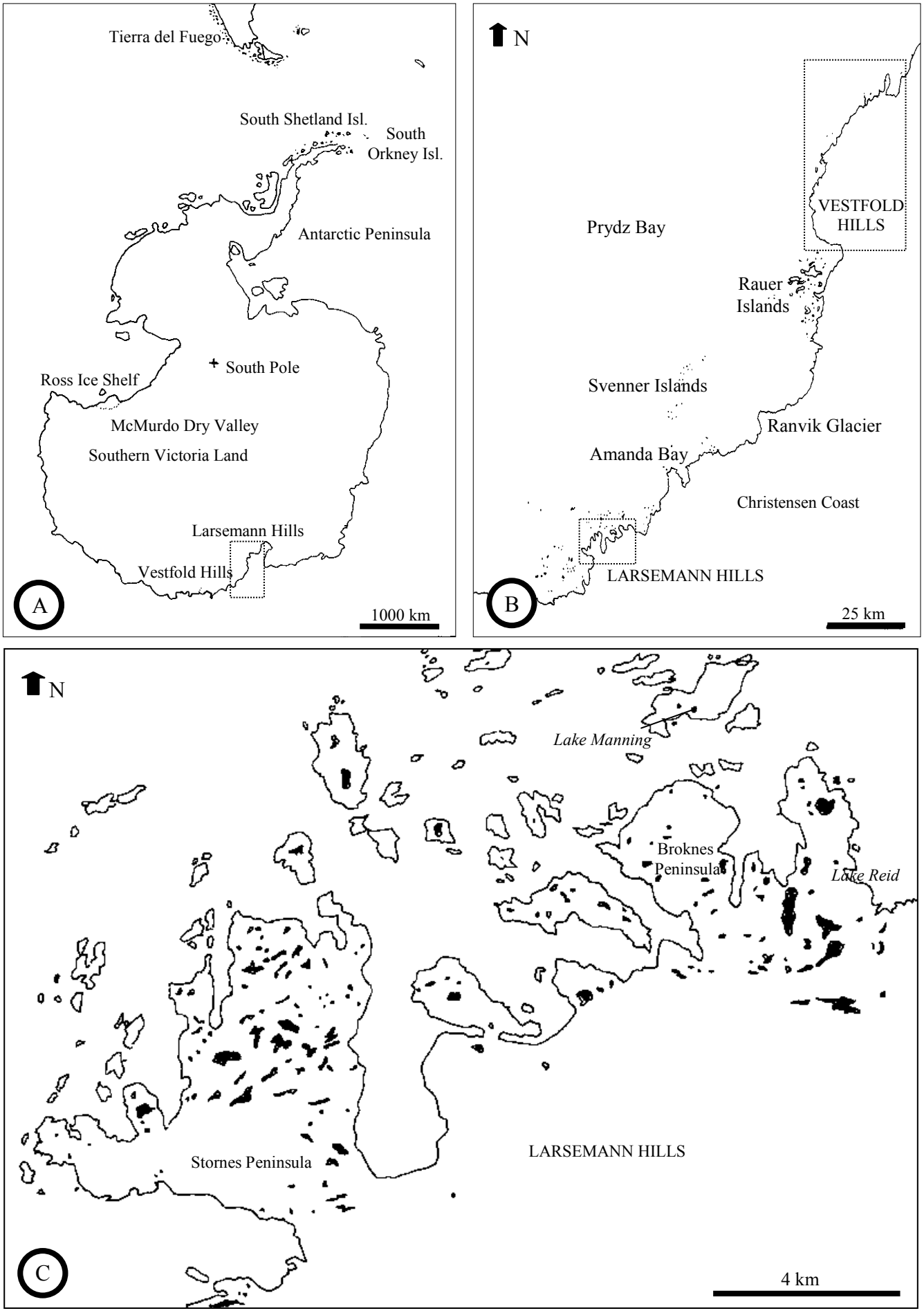


Fig. 1. Overview of sample locations. A. Geography of Antarctica with main sampling areas; B. overview of Vestfold Hills and Larsemann Hills; C. detail of Larsemann Hills (lakes in black).

McMurdo Dry Valley: Lake *Fryxell* is saline, with abundant microbial mats and permanently stratified water columns (Laybourn-Parry *et al*. 1997, Bell & Laybourn-Parry 1999, Roberts & Laybourn-Parry 1999, 2000). The lower water zone is anaerobic (Lawrence & Hendry 1985). It has a perennial ice cover with cyanobacteria, as has Lake *Hoare* located at short distance (Hawes & Schwarz 1999, Wharton *et al.* 1993). The two lakes are separated by the Canada Glacier (Spaulding *et al*. 1997). Photosynthesis is very low (Fritsen & Priscu 1998). Lake Hoare has a seasonally ice-free zone with a microbial mat (Hawes & Schwarz 1999).

Vestfold Hills: Lake *Ace* (Laybourn-Parry *et al*. 2002) has layers of moderate salinity (41 g/kg), is stratified (Bell & Laybourn-Parry 1999) and contains bacteriochlorophyl from photosynthetic *Chlorobium* spp.; algal phytoplankton is present (Volkman *et al*. 1988). Maximum depth was 23 m (Hand & Burton 1981) but in 15 years has increased to 24.5 m (Gibson & Burton 1996). Lake *Druzhby* is one of the larger freshwater lakes with a catchment area of about 100 km^2 and a depth of up to 40 m (Laybourn-Parry *et al*. 2001). It may be frequented by skuas (Dartnall 2000). *Highway* is small, slightly brackish (Laybourn-Parry *et al*. 2001). Lake *Organic* is meromictic having permanently stratified water columns with upper oxygenated water and lower anoxic water. Because its upper waters are usually of similar or lower conductivity to seawater, it develops ice-cover, which may be annual or perennial. Hence it is not or poorly flushed by glacial and snow melt (Burton 1981) and it has evolved into a hypersaline lake at 199.4 g/kg. It does not, therefore, contain any photosynthetic bacteria (Burke & Burton 1988). The water temperature usually remains well below 0 °C (Gibson & Burton 1996). Maximum depth is 7 m. Lake *Watts* is a holomictic lake with a maximum depth of 29.5 m (Roberts & McMinn 1996). The water is slightly brackish but contains freshwater flora with extensive algal mats (Dartnall 2000).

Larsemann Hills: Lakes *Manning* and *Reid*, about 200 km from the Vestfold Hills, are geologically young, shallow, extremely oligotrophic freshwater lakes and are ice-covered for 9–11 mo each year (Ellis-Evans *et al*. 1998). Water temperature ranges up to 6.6 °C and water is well-mixed by katabatic winds (Gillieson *et al*. 1990). *Sarah Tarn* is a small, shallow lake subjected to sea spray with a well-mixed water column, alkaline (pH 8.3), with temperatures up to 5.9 °C and brackish (2.9 g/kg) (Gasparon *et al*. 2002).

Sampling

Samples were collected from the littoral region of lakes by hand or from within the upper mixolimnion. During the study period Lake Ace remained ice-covered with a cover up to 2 m thick; those samples were collected with a custom-made scooping device at the end of an extendible pole, through holes drilled in the ice-cover with a motorised Jiffy drill. Aliquots of the mat material were taken aseptically and stored either deep frozen (–20 °C) or refrigerated for return to Europe via Australia. The interval between collection and delivery to European laboratories was 6 wk.

Isolation

Fungi were isolated using standard spread plate and pour plate procedures with or without enrichment. For spread plates without enrichment, aliquots of 0.5 mL of the homogenized samples were dispersed on the low-carbon media R2A (Oxoid), R2A with Tween 80 (1 %, v/v) and Sea Water Agar (SWA) (Atlas & Parks 1993), and on a high-carbon medium, potato dextrose agar (PDA; Merck). For enrichment prior to spread plating, aliquots of 0.5–1.0 g of homogenized samples were placed in 20 mL of enrichment broth in 50 mL Erlenmeyers and subsequently incubated at 4 °C and 10 °C over a period of up to several months. Enrichment broths were plated on R2A (Oxoid) with Tween 80 (1 %, v/v), R2A with NaCl (0.8 %, w/v) and R2A with NaCl (8 %, w/v). Broth volumes of 0.5 mL were subsequently spread in triplicate on the surface of culture plates with the same media as used for enrichment. All media were supplemented with penicillin G (50 mg/L; Merck), chloramphenicol (50 mg/L; Serva) and streptomycinsulphate (100 mg/L; Merck).

For pour plates, aliquots of 0.1–0.4 g of sample were immersed in 0.5 mL tap water with 0.02 % Tween 80. Aliquots of 0.1 mL solution were dispersed over the bottom of a 9 cm culture plate and covered with 10 mL of cool, molten isolation agar (SWA or R2A). Replicates were incubated at 4, 10 and 20 °C and examined after intervals of 3, 5, 7 or 21 d and up to 3 mo (4 °C). Representative isolates were then transferred onto agar slants with PDA (Merck) and stored at 10 °C. A selection of the isolates was later deposited in the culture collection of the Centraalbureau voor Schimmelcultures. Abbreviations used: *s. dat. – sine dato* (without date); *s.n. – sine numero* (without collecting number).

Cultivation, herbarium material and microscopy

For molecular identification, strains were grown on MEA and PDA slants incubated at 20 °C, and on PCA at 15 °C to obtain sporulation.

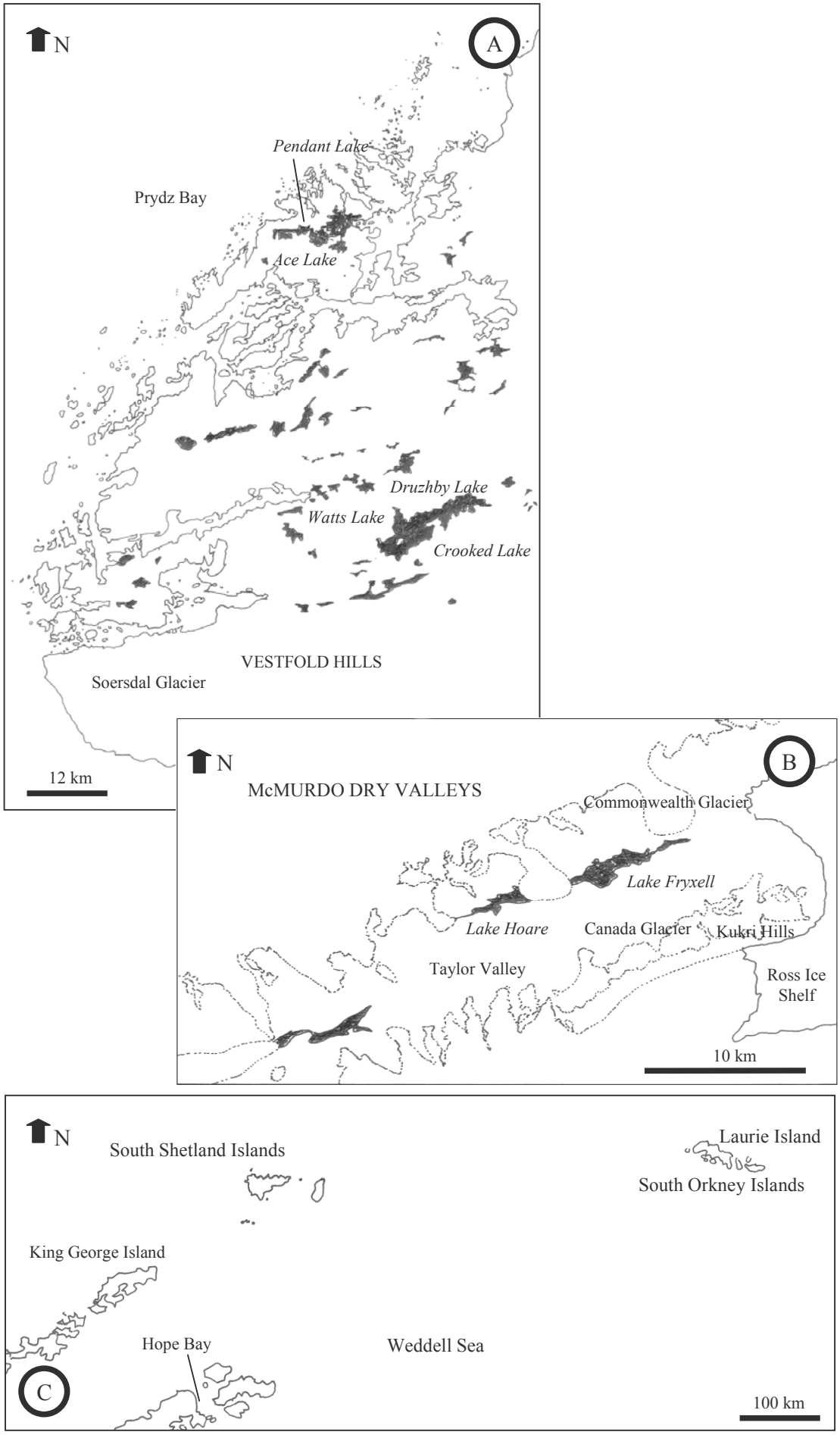


Fig. 2. Overview of sample locations. A. Detail of Vestfold Hills (lakes in black); B. McMurdo Dry Valleys (lakes in black, glaciers indicated with dotted lines); C. King George Island, South Shetland and Orkney Islands.

Slides were made in water for vital observations and in methyl blue in polyvinyl-lactophenol.

For morphological studies by the last author strains were grown on filtered (clear) CA in Petri-dishes at 15 °C. Optimal production of ascomata was reached with alternating periods of light and darkness of 12 h. A light intensity of about 370 fc (= 4100 lux) proved to be convenient. Fresh material was mounted for microscopic study in tap water. Herbarium material was studied from the acknowledged institutes. Small fragments of dried specimens were fully rehydrated in a small tube with water. Often rehydration and swelling were better when a few drops of a wetting agent, like Photo-Flo (Kodak) or Invadine (Geigy) were added to 100 mL of water. The use of mounting media with a low water content, like lactic acid, lacto-phenol or Melzer's reagent often lead to poor preparations with all kinds of artefacts. Living and rehydrated material was at first studied using bright-field or differential-interference contrast optics.

Measurements of elements and ascospores were made on mature structures and liberated spores in water.

Exact ascospore counts

Clean sterile microscopic cover-glasses were mounted for some time over sporulating ascomata. The spore heaps on the glass were checked for eventual irregularities and carefully pressed with the help of a rubber stopper in a small drop of warm glycerin-gelatin on a slide, so that all spores were situated in a single layer. After solidifying the medium by cooling, the spore shots were photographed under a microscope. Spores could exactly be counted by marking them in a photo-enlargement.

Ecophysiology

Growth at 0 and 25 °C was tested on MEA and PDA. Strains were inoculated by transfer of blocks (4 mm^2) from 10-days-old precultures grown at 20 °C onto C- and N-free Czapek Agar (magnesium sulphate 0.5 g/L, potassium chloride 0.5 g/L, Fe(II)-sulphate 0.01 g/L, di-potassium hydrogen phosphate 1 g/L, agar 13 g/L), as well as PDA (Merck) and MEA (Merck) plates in duplicate. Plates were incubated at 0 °C ± 1 and 25 °C ± 1 for 3 wk.

DNA extraction

About 1 g of mycelium was transferred to a 2 : 1 mixture of silica gel and Celite 545 with 300 µL CTAB-buffer added (Tris·HCl, 200 mM, pH 7.5; Na-EDTA, 200 mM; NaCl 8.2 %; CTAB 2 %). The material was ground with a micropestle (Eppendorf). After adding 200 µL CTAB-buffer and vigorously shaking the sample was incubated for 10 min in a 65 °C waterbath. 500 µL Chloroform was added, vortexed shortly and centrifuged for 5 min at 14 000 r.p.m. After transferring the aqueous supernatant to a new Eppendorf tube, 2 volumes (~800 µL) ethanol 96 %, –20 °C were added and mixed gently. The DNA was precipitated at –20 °C for at least 30 min. The pellet, obtained by centrifugation for 5 min at 14 000 r.p.m., was washed twice with 500 µL ethanol 70 % at –20 °C. DNA was dried overnight at room temperature and suspended in 97.5 µL TE-buffer (10 mM Tris, 10 mM Na-EDTA, pH 8.0) with 2.5 µL RNAse-solution (10 mg pancreatic RNAse 20 U/mg was added to 1 mL 0.01 M Na-acetate, heated at 100 °C during 15 min and cooled slowly to room temperature; the pH was adjusted to 7.4 by adding 100 µL Tris·HCl). The sample was incubated for 5–30 min at 37 °C and then stored in a refrigerator.

Microsatellite fingerprinting

PCR was performed using primer $(GTG)_5$ (Longato & Bonfante 1997) in a Perkin Elmer DNA Thermocycler 480 (40 cycles of 0.5 min at 95 ºC, 0.5 min at 53 ºC and 2 min at 72 ºC followed by a final 10 min at 72 ºC). Amplicons were analysed in 4–20 % acrylamide gradient gels (Criterion Precast Gel, Biorad) or in 12.5 % pre-cast polyacrylamide gels (Excel GeneGel, Pharmacia), on a GenePhor Electrophoresis Unit (Pharmacia), in the latter case followed by silver staining. Band patterns were compared with the GelCompar software package v. 4.0 (Applied Maths, Kortrijk, Belgium). A similarity matrix was generated using the Pearson product moment correlation coefficient and the dendrogram was built using the UPGMA clustering algorithm.

Sequencing

Primers used are listed in Table 2. SSU amplicons were generated with primers NS1 and NS24 and were sequenced with primers Oli1, Oli5, Oli9, Oli10, BF951, BF963, Oli2, Oli3, Oli13, Oli14, BF 1419, BF 1438 and Oli15. ITS amplicons were generated with primers V9G and LS 266 and were sequenced with primers ITS1 and ITS4. β-Tubulin amplicons were generated with primers T-1 and sequenced with primers T-22. All amplicons were purified using the Gel Band Purification Kit (Amersham Pharmacia, Roosendaal, The Netherlands). DNA was bound to GFX-columns, eluted according to protocols given by the supplier, and collected with TE-buffer. Concentrations of amplicons were estimated by comparison with SmartLadder markers (Eurogentec, Seraing, Belgium) on 1 % agarose gels.

Reactions were carried out with 15–50 ng of DNA for a 10 µL reaction mixture including 4 pmol primer and 4 µL BigDye RR Mix (Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands). Subsequently DNA was precipitated with ethanol and sequenced using an ABI PrismTM 310 Genetic Analyzer (Applied Biosystems).

Table 2. Primers used in this study.

Abbreviation	Primer sequence (5'>3')	Gene	Position (corr. *S. cerevisiae*)	Reference
NS1:	gTAgTCATATgCTTgTCT→	18S:	20–38	(White *et al*. 1990)
Oli5:	gAAACTgCgAATggCTCATT→	18S:	83–102	(Hendriks *et al*. 1989)
NS3:	gCAAgTCTggTgCCAgCAgCC→	18S:	553–573	(White *et al*. 1990)
NS2:	←ggCTgCTggCACCAgACTTgC	18S:	553–573	(White *et al*. 1990)
Oli9:	CgCggTAATTCCAgCTCCA→	18S:	573–591	(Hendriks *et al*. 1989)
Oli10:	←TggYRAATgCTTTCgC	18S:	935–951	(Hendriks *et al*. 1989)
BF951:	←TTggCRAATgCTTTYgC	18S:	936–951	(this study)
BF963:	TTAATCAgTgAACgAAAgT→	18S:	963–981	(this study)
Oli14:	ATAACAggTCTgTgATgCCC→	18S:	1419–1438	(Hendriks *et al*. 1989)
BF1419:	AKAACAggTCTgKgATgCCC→	18S:	1419–1438	(this study)
BF1438:	←gggCATCMCAgACCTgTTMT	18S:	1419–1438	(this study)
Oli15:	TTTgTACACACCgCCCgTCg→	18S:	1624–1643	(Hendriks *et al*. 1989)
Oli2:	←AACTTAAAggAATTgACgg	18S:	1127–1147	(Hendriks *et al*. 1989)
V9G:	TTAAgTCCCTgCCCTTTgTA→	18S:	1609–1627	(de Hoog & Gerrits van den Ende 1998)
Oli3:	←gTACACACCgCCCgTC	18S:	1626–1642	(Hendriks *et al*. 1989)
NS24:	←AAACCTTgTTACgACTTTTA	18S:	1750–1769	(Gargas & Taylor 1992)
ITS1:	TCCgTAggTgAACCTgCgg→	18S:	1769–1787	(White *et al*. 1990)
ITS4:	←TCCTCCgCTTATTgATATgC	26S:	41–60	(White *et al*. 1990)
ITS5:	ggAAgTAAAAgTCgTAACAAgg→	18S:	1745–1767	(White *et al*. 1990)
LS266:	←gCATTCCCAAACAACTCgACTC	26S:	266–287	(Masclaux *et al*. 1995)
T1:	AACATgCgTgAgATTgTAAgT→	ß-tub:	exon 1	(O'Donnell & Cigelnik 1997)
T10:	ACgATAggTTCACCTCCAgAC→	ß-tub:	exon 2	(O'Donnell & Cigelnik 1997)
Bt2a:	ggTAACCAAATCggTgCTgCTTTC→	ß-tub:	exon 3	(Glass & Donaldson 1995)
Bt2b:	←ACCCTCAgTgTAgTgACCCTTggC	ß-tub:	exon 4	(Glass & Donaldson 1995)
T22:	←TCTggATgTTgTTgggAATCC	ß-tub:	exon 5	(O'Donnell & Cigelnik 1997)

PCR conditions for rDNA Small Subunit (SSU) and Internal Transcribed Spacer (ITS) were 96 ºC, 10 s; 50 ºC, 5 s; 60 ºC, 4 min; 25 cycles. PCR conditions for β-tubulin were 94 °C, 30 s; 68 °C, 90 s; 72 °C, 2 min; 5 cycles, subsequently 94 °C, 1 min; 64 °C, 90 s; 72 °C, 2 min; with final elongation 72 °C, 10 min.

Alignment and Phylogenetic Analysis

Sequences were adjusted using SeqMan of Lasergene software (DNAstar, Madison, Wisconsin). SSU sequences were aligned and analysed in an aligned data base containing about 3000 fungal sequences in the ARB beta-package (v. 22-08-2003) developed by W. Ludwig (www.mikro.biologie.tu-muenchen.de /pub/ARB). SSU trees were made with neighbour-joining and the Parsimony/ML algorithms with 100 bootstrap replications. Alignment of ITS and β-tubulin was done using BioNumerics (Applied Maths, Kortrijk, Belgium) guided by iterative production of trees based on Ward's averaging algorithm. Distance trees were constructed with neighbour-joining with Kimura-2 correction using the Treecon (v. 1.3b) software package (Van de Peer & De Wachter 1993), and phylogenetic trees using PAUP v. 4.0b8 with heuristic search option. Bootstrap values were calculated from 100 resampled datasets. In both genes the entire amplicon could be used for alignment.

Homology analysis by DNA-walk Divergence is defined by incrementing walk steps for each nucleotide in the sequence (for example a positive step for purines, and negative for pyrimidines). The DNA Walk Divergence method (DNAWD) (Licinio & Caligiorne 2004) makes simultaneous comparisons of the three-dimensional walks (representing three composition skews): AG-TC, AC-TG, and AT-CG for each pair of sequences. One sequence slides against the other until the minimum squared walk difference is found, corresponding to a global alignment. This is then taken as a measure of their distance since statistically independent mutations and indels increase the mean square walk differences linearly. The resulting distance matrices are then fed to the FITCH program of the PHYLIP package (v. 3.572c) (Felsenstein 1996), which generates phylogenetic trees. Analyses were carried out with ITS rDNA and β-tubulin data.

Statistics

Average infraspecific divergence (d_i) of sequences was calculated according to Koufopanou *et al*. (1997) for ITS domains, β-tubulin introns and exons, the latter for coding regions and third-base positions separately, using the formula $(d_i) = 2/n\ (n - 1)$ x v / r,

where v = the number of variable positions, r = the number of loci, and n = the number of strains in that group. The infraspecific variation is compared with average pairwise divergence between species (d_s). Times of reproductive isolation of taxa (u) can be calculated from the substitution rate at third positions, u being estimated 10^{-9}/bp/yr (Nei 1987).

F-Statistics (theta) were calculated with Fstat for Windows (J. Goudet, Lausanne, Switzerland, v. 2.9.3.2) according to Weir & Cockerham (1984). Eight populations were distinguished using a combination of geographic (minimum distance between populations 200 km) and molecular data (criterion for separation indicated in β-tubulin distance tree). Variables tested were ITS rDNA genotype, β-tubulin genotype, number of ascospores (four, eight, >eight) and presence / absence of anamorph. Three strains with strongly deviating β-tubulin introns were excluded from the analysis.

RESULTS

The near-complete SSU rDNA gene was sequenced for fourteen strains of *Thelebolus*, *Hyphozyma* and *Basifimbria* and aligned with ~2500 comparable sequences taken from GenBank and held at CBS, supplemented with strains sequenced because of proven or supposed relationship with *Thelebolus*. *Hyphozyma* (yeast-like hyphomycetes) was included because of its morphological similarity to *Thelebolus* anamorphs, and *Basifimbria* ("*Dicyma*"; hyphomycetes) because of surmised discomycete affinity and psychrophilic character. A parsimony tree containing 97 of the most similar species is presented in Fig. 3. The nine strains of *Thelebolus* sequenced were found as a part of a well supported clade (93 %) containing members of *Ascozonus* (*Thelebolaceae*) and *Hyphozyma* (mitotic fungi). *Ascozonus woolhopensis* and *Hyphozyma variabilis* were found amidst *Thelebolus*, though bootstrap values were low within the clade. *Thelebolus stercoreus* strains proved to be rather dissimilar.

A heterogeneous sister group of this clade contains *Bulgaria*, *Monilinia*, *Spathularia* and *Leotia*, which all are members of the *Leotiales*. Also *Erysiphe* (*Erysiphales*) clustered among these species. Many within-clade bootstrap values were high with poorly defined clades, indicating that long branch attraction of unrelated fungi is concerned. The simply structured ascomycete *Calyptrozyma arxii* (phylogenetic position undetermined) was located among these species. Strain dH 12143, which did not sporulate but was provisionally listed as "*Thelebolus* sp." on the basis of cultural similarity, was found among the heterogenous fungi and should be regarded as being unrelated to *Thelebolus*.

The order *Pezizales* was found to be very heterogeneous, falling apart into two entities. *Peziza* and relatives formed a robust clade (*Pezizaceae*, 100 % bootstrap support). Other members of the order, with members of *Pyronemataceae*, *Ascodesmidaceae*, *Sarcosomataceae*, *Sarcoscyphaceae*, *Rhizinaceae*, *Helvellaceae*, *Tuberaceae*, *Caloscyphaceae*, *Morchellaceae*, *Orbiliaceae* and *Ascobolaceae* were found in another main cluster. This group also contained the psychrophilic hyphomycete *Basifimbria peruviana*. A clade with nematode-trapping anamorphs plus some *Orbilia* species (*Orbiliaceae*, *Orbiliales*) stood out from the remaining taxa, with high bootstrap support. Members of the *Dothideales* were found as as sister group to heterogeneous clusters of members of *Helotiales* and *Pezizales*.

On the basis of rDNA ITS data, analysed with neighbour-joining algorithm (Fig. 4), two main groups (I–II) were distinguishable within *Thelebolus*. β-Tubulin groups C and D were closer to each other than to G and H, although the number of variable positions was very low over the entire genus. Four strains had deviating β-tubulin introns and exons and thus could not be identified using that gene. Three of them belonged to ITS group I and one to ITS group II, but further characterization down to the species level was as yet impossible. The β-tubulin groups C, D, G and H were recognisable at a very low level of diversity (Table 4), with a minimum distance between two species on a single nucleotide. Groups C and D were not unambiguously distinct on the basis of ITS, but proved to have consistent differences in β-tubulin (Fig. 6) and morphology (Table 3). Heterogeneity was mainly due to single-base strain-specific indels, due to which particularly *T. stercoreus* was found to be heterogeneous. All Antarctic strains of complex C/D had a 2-base insertion at position 78/79 (Table 4), which was absent from non-Antarctic strains. This did not entirely match with a point mutation on position 83 (Table 4), but coincided with ascospore number per ascus (Table 3).

Group H, with six Antarctic strains from Druzhby and Watts lakes (Vestfold Hills), and a reference strain, CBS 734.68, from nest soil near Hallett Station (Hallett Peninsula, Victoria Land) in the Antarctic, were strictly identical and deviated in a single informative position from group G (Table 3).

Antarctomyces psychrotrophicus and *Ascozonus woolhopensis* could well be aligned and clustered paraphyletically to *Thelebolus*. The simply structured discomycete-like ascomycetes *Calyptrozyma arxii* (unclassified) could only partially be aligned.

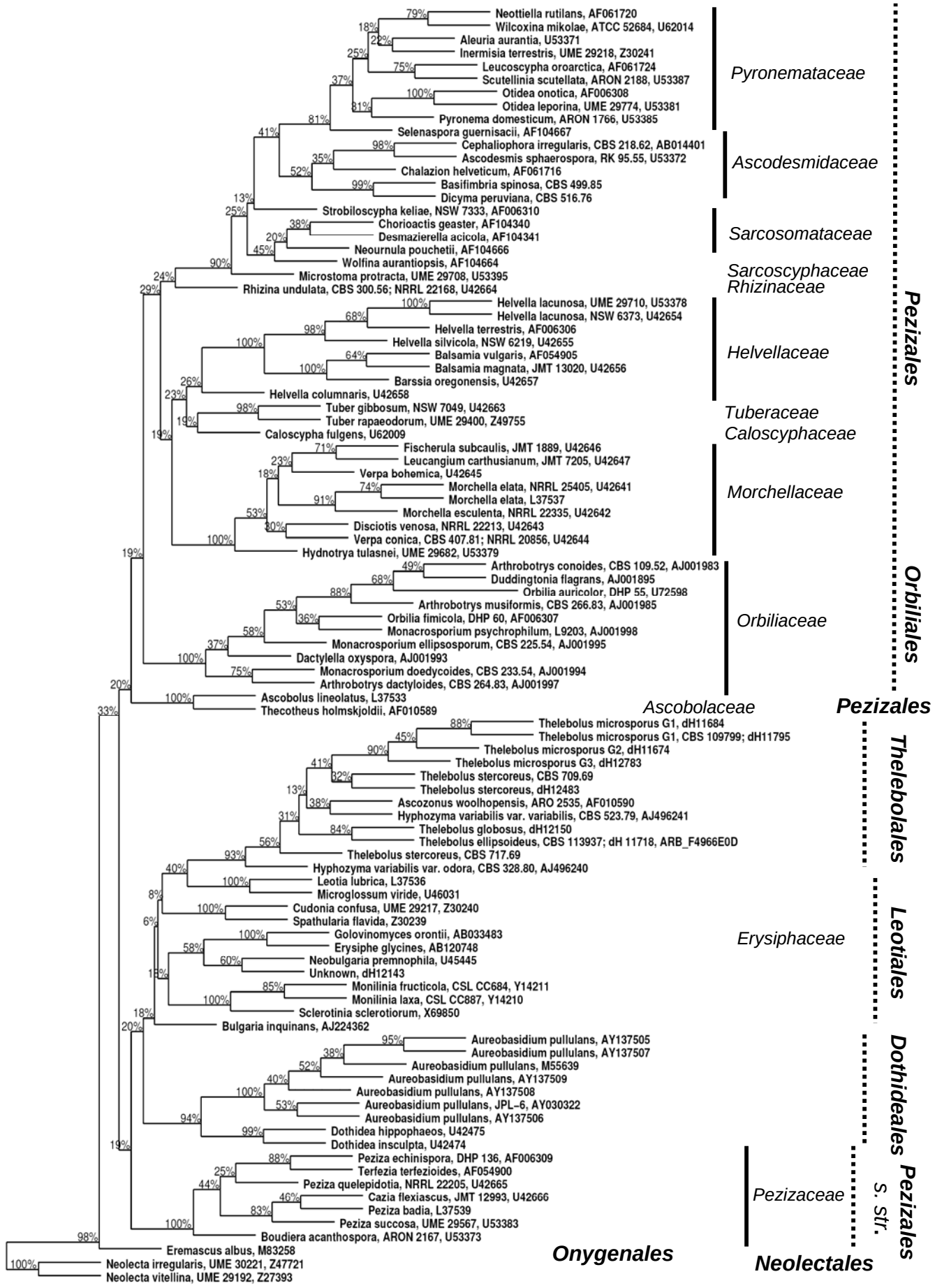


Fig. 3. SSU rDNA Phylogeny generated with the ARB package using parsimony with 1000 replications. Bootstrap values are indicated with the branches. *Neolecta* (*Neolectales*) is taken as outgroup.

Table 3. Strains and summary of results.

dH	CBS	Geography, source	25 °C (MEA / PDA)	4 °C	ITS	Tubulin	Teleo [1]	Ana	GenBank SSU	GenBank ITS	GenBank TUB
Thelebolus stercoreus:											
-	375.58	Soil, Sweden	+-	++	1	C	32	-			
-	710.69	Soil, U.S.A.	++	++	1	C	32	-			
-	711.69	Carnivore dung, Canada	++	++	1	C	256	-			
-	714.69	Herbivore dung, Tanzania	++	+w	1	?	64	-			
-	715.69	Carnivore dung, Uganda	++	++	1	C	64	-			
-	717.69	Deer dung, Canada	++	++	1	C	>2000	-	AY942193	AY957548	AY957540
-	256.78	Dung, Venezuela	++	+-	1	C	32	-			
-	709.69	Dung, Uganda	-+	-+	1	intron1	32	-	AY942192	AY957549	AY957541
12483		Soil, Caucasus	++	+w	1	C/D	32	-	AY942194		
Thelebolus ellipsoideus:											
12162	-	Hoare / Dry Valleys	++	++	1	D	*	-			
12163	-	Hoare / Dry Valleys	++	++	1	D	-	+			
11717	-	Fryxell / Dry Valleys	++	++	1	D	-	+			
11718	113937	Fryxell / Dry Valleys	++	++	1	D	-	+	DQ067574	AY957550	AY957542
11722	-	Fryxell / Dry Valleys	-+	-+	1	D	-	+			
11724	-	Fryxell / Dry Valleys	-+	-+	1	D	-	+			
11726	-	Fryxell / Dry Valleys	++	++	1	D	-	+			
11727	-	Fryxell / Dry Valleys	++	++	1	D	-	+			
12149 (T)	113938	Druzhby / Vestfold Hills	++	++	1	D	8	+			
11719	113939	Manning / Larsemann Hills	++	++	1	D	8	+			
Thelebolus ITS group 1, tubulin unclassified:											
12165		Sarah Tarn / Larsemann Hills	NN	NN	1	intron3	ND	ND			
12164		Fryxell / Dry Valleys	NN	NN	?	intron2	ND	ND			
Thelebolus microsporus:											
-	115.53	Lactuca, Switzerland	++	++	2	G1	8	-			
-	734.68	skua nest, Victoria Land	NN	NN	2	G1	8	-			
11684	109909	Organic / Vestfold Hills	++	++	2	G1	8	-	AY942189	AY957551	AY957543
11686	-	Organic / Vestfold Hills	++	++	2	ND	8	-			
12618	-	Organic / Vestfold Hills	++	++	2	ND	-	-			
12619	-	Organic / Vestfold Hills	++	++	2	ND	-	-			
12620	-	Organic / Vestfold Hills	++	++	2	ND	8	-			
12418	-	Highway / Vestfold Hills	++	++	2	G1	8	-			
11795	109799	Ace / Vestfold Hills	++	++	2	G1	8	-	AY942188	AY957552	AY957544
-	716.69	deer dung, U.S.A.	++	++	2	G1	8	-			
-	716.92	moss, King George	+-	++	2	G1	8	-			
11672	-	Reid / Larsemann Hills	+-	+-	2	G2	8	?			
11673	-	Reid / Larsemann Hills	w+	w-	2	G2	*	-			
11674	109908	Reid / Larsemann Hills	+-	+-	2	G2	8	-	AY942190	AY957553	AY957545
11675	-	Reid / Larsemann Hills	w-	++	?	ND	8	-			
11676	-	Reid / Larsemann Hills	++	++	2	G2	8	-			

Table 3 (Contd.)

dH	CBS	Geography, source	25°C (MEA / PDA)	4°C	ITS	Tubulin	Teleo [1]	Ana	GenBank SSU	GenBank ITS	GenBank TUB
11677	-	Reid / Larsemann Hills	++	++	2	G2	8	-			
11681	-	Reid / Larsemann Hills	-+	-+	2	G2	8	-			
11687	-	Reid / Larsemann Hills	-+	-+	2	G2	8	-			
11688	-	Reid / Larsemann Hills	w-	++	2	G2		-			
11689	-	Reid / Larsemann Hills	++	++	2	G2	8	-			
11690	-	Reid / Larsemann Hills	++	++	2	G2	8	-			
11691	-	Reid / Larsemann Hills	+-	--	?	?		-			
11692	-	Reid / Larsemann Hills	++	++	2	G2	8	-			
11694	-	Reid / Larsemann Hills	++	++	2	G2	8	-			
11714	-	Reid / Larsemann Hills	++	++	2	G2	8	-			
11716	-	Reid / Larsemann Hills	++	+-	2	G2	8	-			
11695	-	Manning / Larsemann Hills	++	++	2	G2	-	-			
11715	114077	Manning / Larsemann Hills	+-	++	2	G2	8	-			
11679	-	Ace / Vestfold Hills	+-	++	?	?	8	-			
11683	-	Ace / Vestfold Hills	++	++	2	G2	8	-			
11696	114076	Ace / Vestfold Hills	+-	++	2	G2	8	-			
11697	-	Ace / Vestfold Hills	++	++	2	G2	8	-			
11698	-	Ace / Vestfold Hills	-+	-+	2	G2	8	-			
12783	113943	Cloaca, skua, King George	++	++	?	G3	8	-	AY942191	AY957554	AY957546
12784	-	Cloaca, skua, King George	+-	++	2	G3	8	-			
12787	-	Trachea, skua, King George	- -	++	2	G3	8	-			
12799	-	Cloaca, petrel, King George	+-	++	?	G3	8	-			
12805	-	Feather, skua, Hope Bay	- -	++	2	G3	8	-			
12807	-	Trachea, gull, Hope Bay	+-	++	?	G3	8	-			
12811	-	Feather, egret, Hope Bay	- -	++	?	G3	8	-			
12812	113945	Cloaca, gull, Hope Bay	- -	++	?	G3	8	-			
12801	113944	Cloaca, petrel, Laurie Island	++	++	2	G3	8	-			
Thelebolus globosus:											
12150 (T)	113940	Druzhby / Vestfold Hills	++	++	2	H	8	+	AY942187	DQ028268	AY957547
12154	-	Druzhby / Vestfold Hills	NN	NN	2	H	ND	ND			
12411	-	Druzhby / Vestfold Hills	+-	++	2	H	-	+			
12152	-	Watts / Vestfold Hills	++	++	2	H	-	+			
12153	113941	Watts / Vestfold Hills	++	++	2	H	-	+			
Thelebolus ITS group 2, tubulin unclassified:											
12151	113942	Druzhby / Vestfold Hills	++	++	2	intron 4	-	+			
Unrelated:											
12143		Manning / Larsemann Hills	++	++	3	A	-	+			

[1]Number of ascospores per ascus. *Ascoma initials present, but ascospores not developed. ND = not determined.

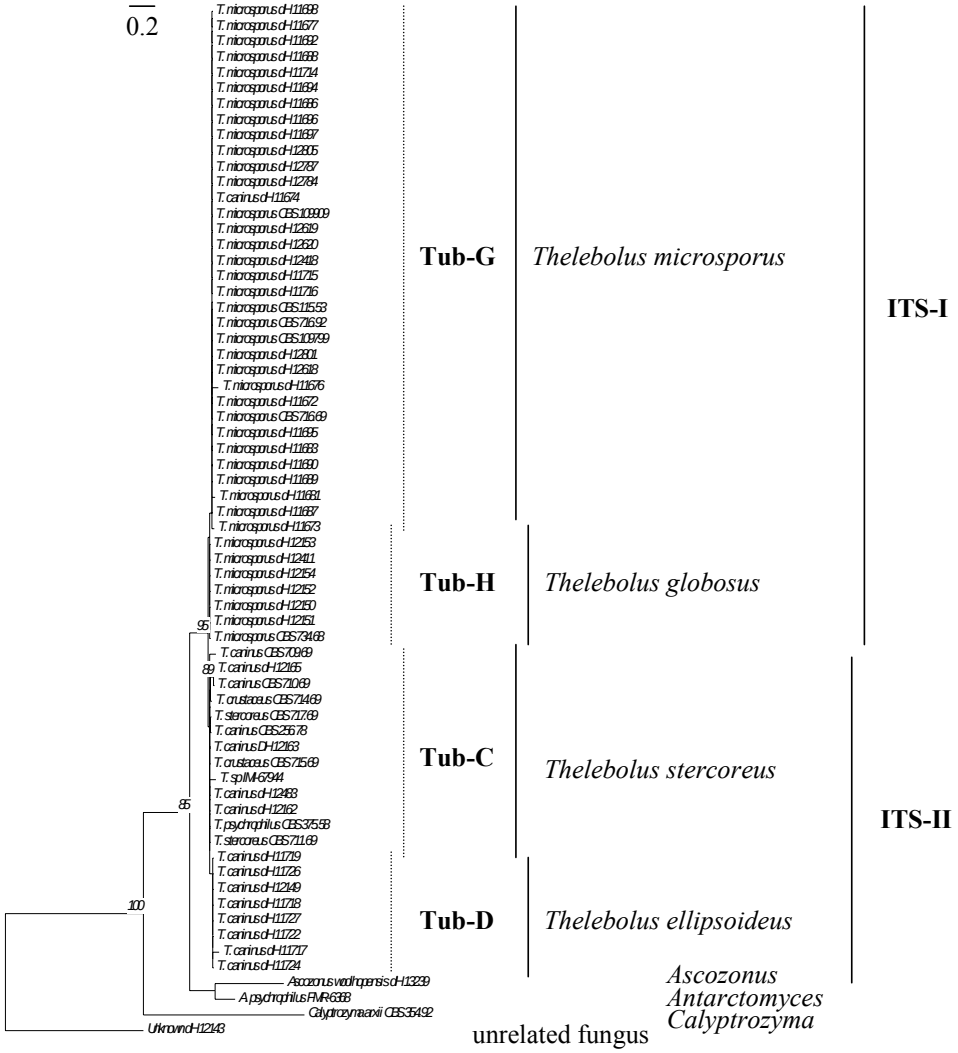


Fig. 4. Consensus tree of 63 strains of *Thelebolus* and related fungi based on confidently aligned complete rDNA ITS sequences, using the neighbour-joining algorithm in the Treecon package with Kimura-2 correction. *Calyptrozyma arxii*, CBS 354.92 was used as outgroup. Bootstrap values from 100 resampled data sets are shown. Taxon names used are based on original morphological identifications. Dotted line: grouping mainly on the basis of β-tubulin sequence data, straight line: final IDs, bold line: grouping on the basis of ITS sequence data.

With the DNAWD algorithm (Fig. 5) the differences between *Thelebolus* strains could be displayed sharply, as demonstrated by the external position of *Antarctomyces psychrophilus*. Strain dH 12143 (unknown species) could not be confidently aligned. Strains of the mitotic genus *Hyphozyma* could only partially be aligned to *Thelebolus*.

A phylogenetic tree based on β-tubulin sequence data based on Neighbour joining algorithm is presented in Fig. 6. In main traits the same grouping was found as the one based on ITS data, but at a significantly higher degree of divergence. This was particularly obvious with DNAWD algorithm (Fig. 7), where *T. stercoreus* was found as a relatively heterogeneous species. A large group (G) contained 34 strains from Antarctic Reid and Manning lakes in the Larsemann Hills, and Organic, Ace and Highway lakes in the Vestfold Hills. In addition, all strains from dead birds in King George Island, and two reference strains from the U.S.A. and Switzerland clustered in this group (Table 3). Group G was found to be subdivided into three main entities, G1, G2 and G3. The subgroups are differentiated by 9 informative positions in the β-tubulin gene. The subgroups nevertheless have a low bootstrap support (Fig. 6) because the differences were randomly distributed: G1 and G2 shared one informative position, G1 and G3 five, G2 and G3 two, while one mutation was unique to subgroup G2. Subgroup G1 was mainly found in the Vestfold Hills, but also contained the two strains from outside Antarctica (Table 3). Subgroup G2 contained 20 practically identical strains from Reid, Manning and Ace lakes, located in Larsemann and Vestfold Hills. Subgroup G3 contained a set of closely similar strains from internal organs and external bodies of dead birds at King George Island in Western Antarctica; as a cluster they are found at the largest distance to subgroups 1 and 2 containing isolates from Eastern Antarctic lakes in the Vestfold Hills. All strains of group G were morphologically identical, producing asci with 8 ascospores (Table 3).

Table 4. ITS informative diversity of groups C/D and correspondence with tubulin groups.

	ITS1			ITS2	TUB	Spore	Geography
	37	**45**	**78/79**	**83**	**113**	**Group**	**Number**	
Thelebolus microsporus:	T	T	- -	C	A	G	8	Worldwide
Thelebolus globosus:	T	T	- -	C	G	H	8	Antarctica
ITS Group II								
ITS Group I								
Ined.:								
dH 12164	A	-	CA	C	G	intron2	8	Antarctica
dH 12165	A	-	CA	C	G	intron3	ND	Antarctica
Thelebolus stercoreus:								
CBS 375.58	A	-	- -	C	G	C	multi	Sweden
CBS 711.69	A	-	- -	C	G	C	multi	Canada
CBS 715.69	A	-	- -	C	G	C	multi	Uganda
CBS 256.78	A	-	- -	C	G	C	multi	Venezuela
CBS 717.69	A	-	- -	C	G	C	multi	Canada
CBS 709.69	A	-	- -	C	G	intron1	multi	Uganda
CBS 710.69	A	-	- -	C	G	C	multi	USA
dH 12483	A	-	- -	C	G	C/D	multi	Caucasus
Thelebolus ellipsoideus:								
dH 12162	A	-	CA	C	G	D	8	Antarctica
dH 12163	A	-	CA	C	G	D	8	Antarctica
dH 11727	A	-	CA	T	G	D	8	Antarctica
dH 12149	A	-	CA	T	G	D	8	Antarctica
dH 11722	A	-	CA	T	G	D	8	Antarctica
dH 11719	A	-	CA	T	G	D	8	Antarctica
dH 11726	A	-	CA	T	G	D	8	Antarctica
dH 11718	A	-	CA	T	G	D	8	Antarctica
dH 11724	A	-	CA	T	G	D	8	Antarctica
dH 11717	A	-	CA	T	G	D	8	Antarctica

Cluster C/D (ITS) with β-tubulin clearly falls apart into two separate entities, C and D. Most strains of β-tubulin group D have a CA-indel on ITS position 78/79, a T rather than a C on ITS position 83 (Table 4), and have 8 ascospores per ascus (Table 3); the 8-spored strains of this group are limited to the Antarctic. The group C members lack the indel, have a C at position 83, and have multispored asci. However, strain dH 12483 was exceptional in having multisporic asci and the ITS structure of group C, but on the basis of β-tubulin it was a member of group D (Fig. 6).

Sequence data of β-tubulin of two strains, dH 12164 and CBS 709.69 was totally deviant, showing not only remarkable deviation from all other *Thelebolus* strains sequenced, but also from each other. Particularly the introns showed much diversity. For the introns no match was found in Genbank, while the exons revealed similarities to genera such as *Gibberella*, *Cercospora* and *Fusarium*, rather than to any discomycete. Thus, despite ITS similarity to *Thelebolus*, they could not properly be classified, as probably paralogues of the β-tubulin gene were sequenced. CBS 709.69 was morphologically identical to members of *Thelebolus* group C, while dH 12164 died before its morphology could be studied in detail.

The topology of ITS and β-tubulin trees are in conflict where group H is concerned. This is illustrated by the fact that with ITS the closest group to H is G, at 0.2 % minimum dissimilarity, while group D is found at 3.4 %. In contrast, on the basis of β-tubulin data, group D is nearest to group H at 9.4 % difference while group G shows 14.4 % minimum dissimilarity.

Microsatellite data with $(GTC)_5$ showed nearly identical patterns (Fig. 8) for 19 strains analysed that with β-tubulin data were found to belong to group G2. Among these was strain dH 11691 which failed in the sequencing procedure (Table 3). The group contains strains from Lakes Reid, Manning, Organic, Fryxell and Ace, demonstrating that this genotype has a wide distribution in the Antarctic.

Three samples from Organic, a hypersaline lake in the Vestfold Hills, yielded consistently different patterns by having an extra band, thus representing a local subtype. A more remote cluster contained strains of genotype G2. Strains of group D clustered together. The majority of the strains originate from Lakes Fryxell and Hoare (Victoria Land), while strain dH 12149 (= CBS 113938) from Druzhby is different, representing a local subtype. Strains of group H were found to cluster together.

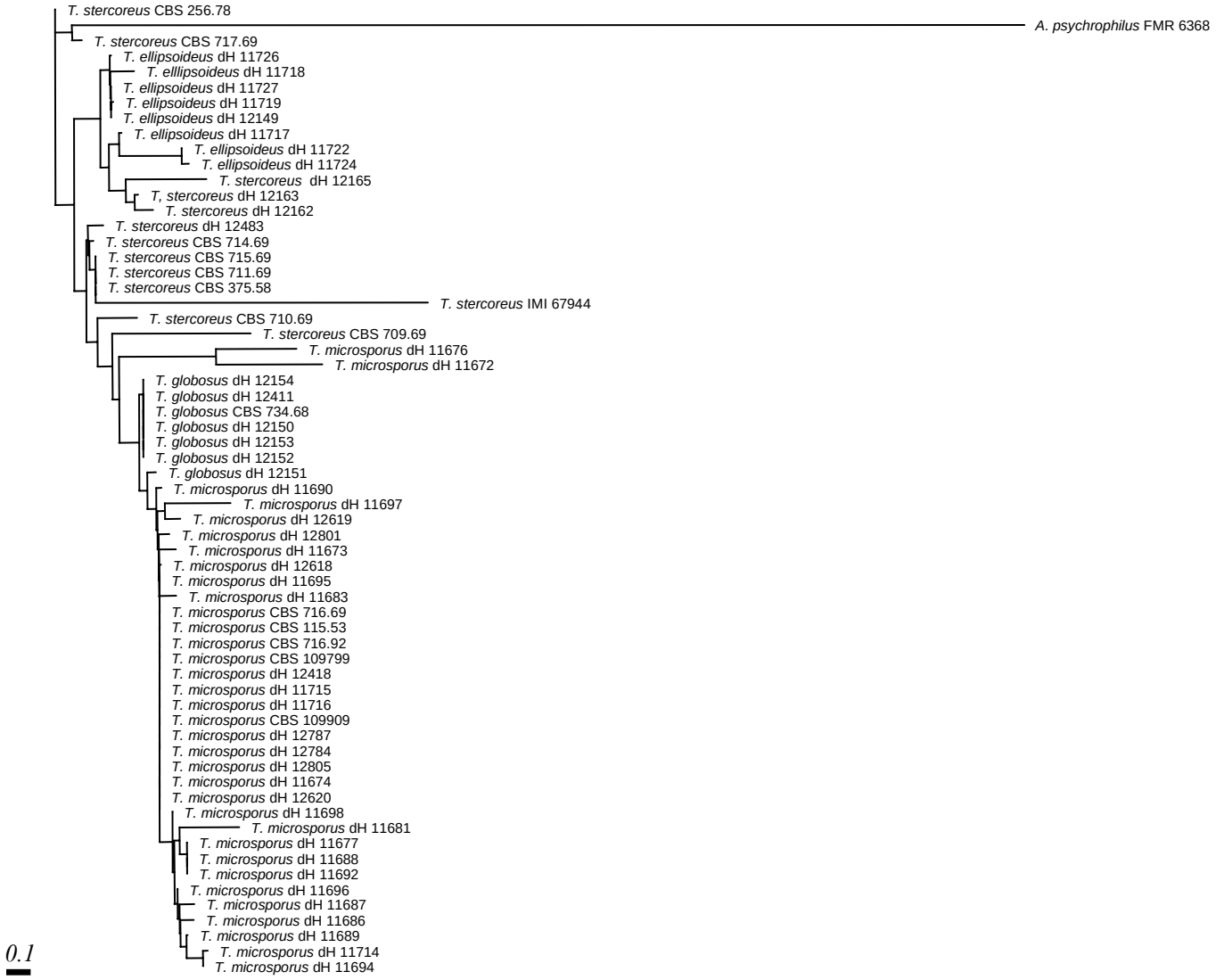


Fig. 5. DNA-Walk Divergence tree of 63 strains of *Thelebolus* and related fungi based on confidently aligned complete rDNA ITS sequences, using the DNAWD programme. Taxon names used are based on final molecular and phenetic identifications.

In a multilocus analysis using F-statistics, the groups found were remarkably well separated, yielding a theta of 0.961 over four loci analysed. As the groups were defined on a combination of molecular and phenetic data and geography (see Method section), it can be concluded that the groups C, D, G1, G2 and H represent distinct entities with reduced gene flow. Groups D and H are limited to Antarctic regions, whereas group C has a worldwide distribution outside the Antarctic (Tables 3, 6). Group G is limited to the Antarctic with the exception of subgroup G1. The slight but consistent differences found within group G and leading to the recognition of subgroups G1, G2 and G3, largely matched with distribution within the Antarctic, which was Larsemann Hills and Vestfold Hills (distance ~200 km) for subgroup G2, and Northern Antarctica (distance ~4000 km) for subgroup G3, while subgroup G1 was found in all Antarctic regions analysed (Table 6). Group D is found in the Vestfold Hills and the Dry Valleys of Victoria Land (distance 2800 km); the geographic entities within this species differ from each other in sporulating abilities (Table 3).

All *Thelebolus* strains tested were able to grow at 0 °C (Table 3). At 25 °C most strains showed similar mycelial growth, but most strains grew weakly or not at all, particularly on PDA and CA. Group G3 preponderantly was unable to grow at 25 °C on one or both media. Sporulation with ascoma formation was optimal at 15 °C.

DISCUSSION

Phylogeny

With SSU rDNA data, *Thelebolus microsporus* was found to be close to *T. stercoreus*, as expected. *Ascozonus woolhopensis* and the mitotic fungus *Hyphozyma variabilis* were found in the *Thelebolus* cluster, but with ITS data they were clearly separate, *Hyphozyma* not even entirely being alignable with confidence.

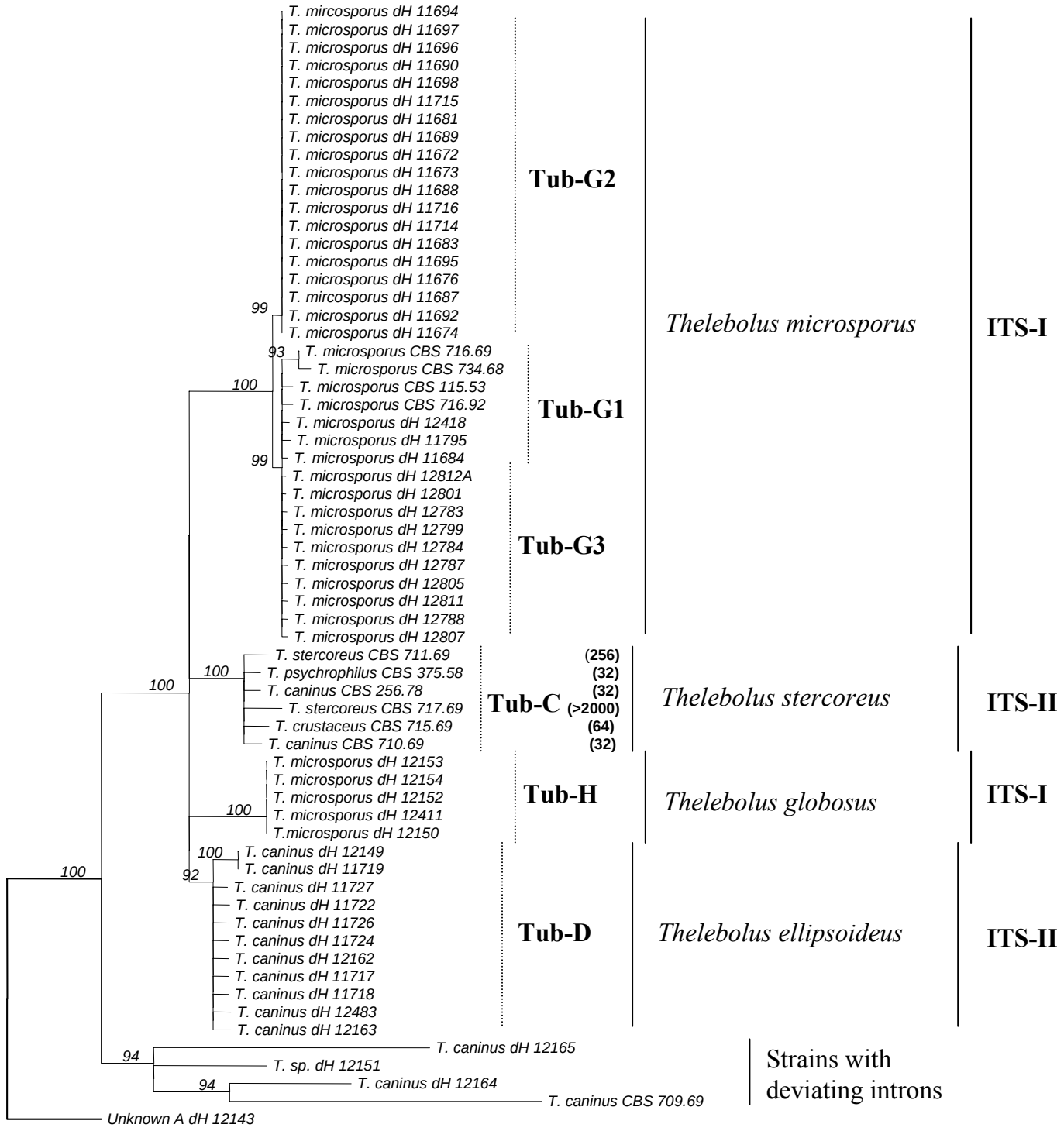


Fig. 6. Consensus tree of 63 strains of *Thelebolus* and related fungi based on confidently aligned β-tubulin sequences, using the neighbour-joining algorithm in the Treecon package with Kimura-2 correction. Strain dH 12143, with *Thelebolus*-like thallus but at larger phylogenetic distance, was used as outgroup. Bootstrap values from 100 resampled data sets are shown. Taxon names used are based on original morphological identifications. Groups recognised on the basis of combined morphological data indicated with letters; final taxonomic names listed at the right.

Thelebolus and *Ascozonus* have been treated as members of the family *Thelebolaceae* (Eckblad 1968), or of tribe *Theleboleae* of the *Pyronemataceae* (Korf 1972). The ordinal position of the group, however, is questionable. It was originally classified in the *Pezizales*, but Momol & Kimbrough (1994), using ITS rDNA sequences, found that *Thelebolus* was not closely related to any of the tested members of the *Pezizales*. This ITS domain is too variable to determine the phylogenetic position of the genus. Morphology of ascomata and the presence of inoperculate asci were reasons to surmise a relationship with *Erysiphales* (Zukal 1889, Cooke & Barr 1964) or *Pleosporales* (Samuelson & Kimbrough 1978). Using partial SSU rDNA sequences, Landvik *et al*. (1998) found an association with the *Helotiales*, but with low statistical support.

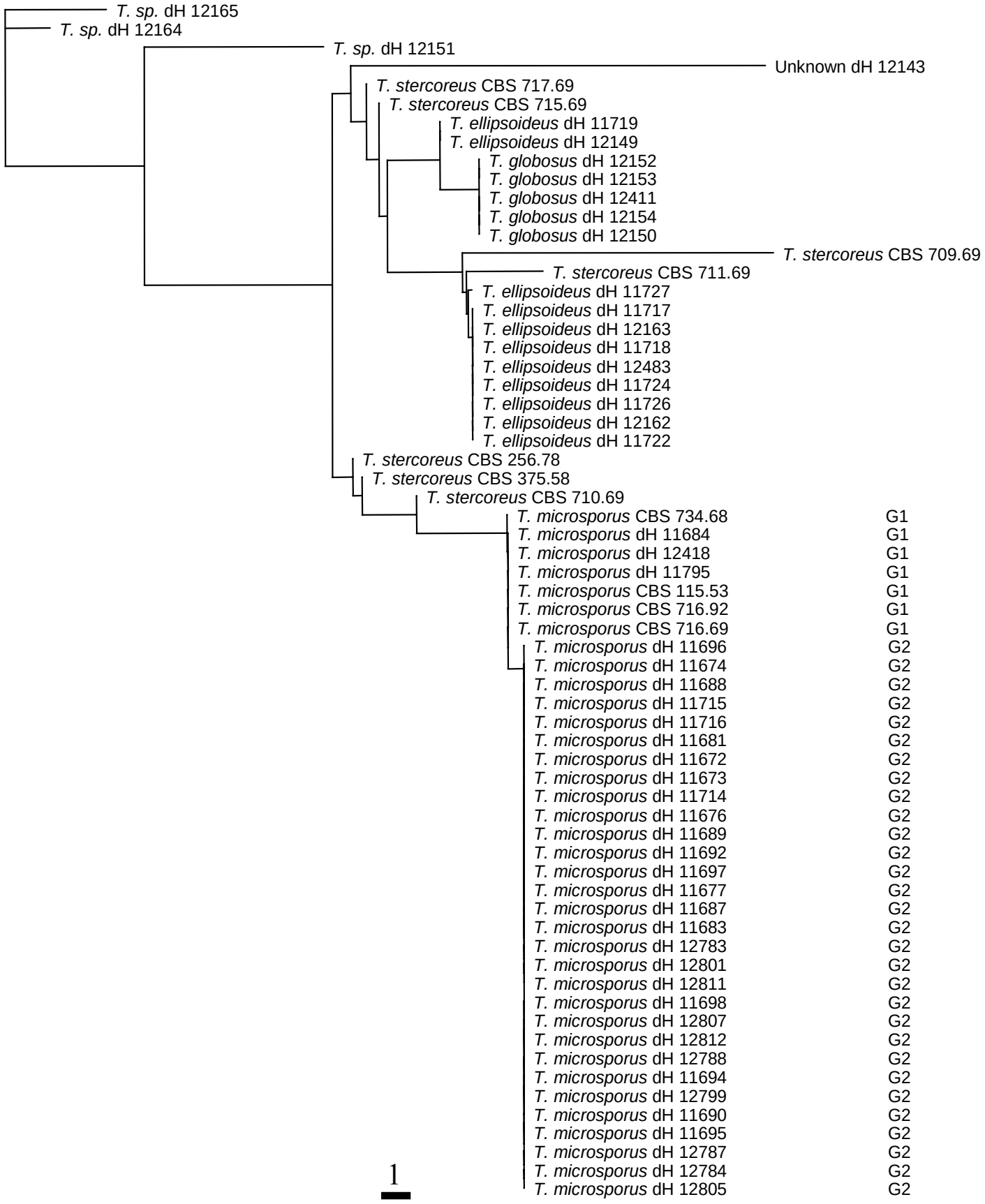


Fig. 7. DNA-Walk Divergence tree of 63 strains of *Thelebolus* and related fungi based on confidently aligned β-tubulin sequences, using the DNAWD programme. Taxon names used are based on final molecular and phenetic identifications.

Table 5. Distribution of species and populations of *Thelebolus* in Antarctica.

Lake	Salinity	Geography	Species
Hoare	Moderate	Dry Valleys	*T. ellipsoideus* (D) without ascospores
Fryxell	Fresh	Dry Valleys	*T. ellipsoideus* (D) without ascospores
Fryxell	Fresh	Dry Valleys	Unclassified (D) intron 2
Druzhby	Fresh	Vestfold Hills	*T. ellipsoideus* (D) with ascospores
Druzhby	Fresh	Vestfold Hills	*T. globosus* (H)
Druzhby	Fresh	Vestfold Hills	Unclassified (H) intron 4
Watts	Brackish	Vestfold Hills	*T. globosus* (H)
Organic	Hypersaline	Vestfold Hills	*T. microsporus* (G1)
Organic	Hypersaline	Vestfold Hills	*T. microsporus* (G2)
Organic	Hypersaline	Vestfold Hills	*T. microsporus* (G2) microsat subtype
Highway	Brackish	Vestfold Hills	*T. microsporus* (G1)
Ace	Saline	Vestfold Hills	*T. microsporus* (G1)
Ace	Saline	Vestfold Hills	*T. microsporus* (G2)
Manning	Fresh	Larsemann Hills	*T. ellipsoideus* (D) with ascospores
Manning	Fresh	Larsemann Hills	*T. microsporus* (G2)
Sarah Tarn	Fresh	Larsemann Hills	Unclassified (D) intron 3
Reid	Fresh	Larsemann Hills	*T. microsporus* (G2)
King George	Saline	Northern Antarctica	*T. microsporus* (G3)

Table 6. Summary of geographic distribution of genotypes (with reference to Table 5).

Outside Antarctica	C,	D,	G1				
Northern Antarctica			G1,		G3		
Larsemann Hills		D,		G2			
Vestfold Hills		D,	G1,	G2,		H	
Victoria Land		D,	G1				

The authors used a 580 bp fragment of the SSU rDNA operon. Gernandt *et al*. (2001), using a 1000 bp fragment of the same gene, found the family *Thelebolaceae* to be a member of the order *Helotiales*. In our extended SSU alignment file containing over 2500 sequences, an association with fungi with leotialean relationship, like *Bulgaria*, *Cyttaria*, *Leotia*, *Microglossum* and *Rhytisma*, as found earlier by Tehler *et al*. (2000) was confirmed (Fig. 3). This underlines the supposition of Landvik et al. (1998) that the partial sequence leads to the same conclusion as the entire 18S sequence. The *Helotiales* are a heterogeneous assemblage and thus poorly demarcated as a fungal order. The *Pezizales s. str*. (*Pezizaceae* and *Ascobolaceae*), containing genera such as *Ascobolus*, *Peziza* and *Thecotheus*, was located at considerable distance and at robust branching. This confirms earlier findings by Landvik *et al.* (1997). Hansen *et al*. (2001) found *Ascobolus* as a sister group of *Pezizaceae*. Members of *Erysiphales* (*Blumeria*, *Erysiphe*) were one of the consensus branches of the leotialean group, clustering with *Amylocarpus* and paraphyletic to *Neobulgaria*, though Saenz *et al*. (1994) did not find any close relative of this order. The *Pleosporales* compose a separate, well-resolved monophyletic branch, as was found by several authors (e.g. Sterflinger *et al*. 1997). The position of the *Dothideales*, amidst the cup fungi is remarkable; similar findings were reported by Theler *et al*. (2003) and Rossman *et al*. (2004).

In conclusion, *Thelebolus* being found together with *Ascozonus* relatively near to leotialean fungi and far from any of the orders to which it has been assigned previously, we concur with Cannon (Kirk *et al.* 2001) in classifying the genera in a separate order *Thelebolales*, comprising a single family *Thelebolaceae*.

Based on ascus ultrastructure van Brummelen (1998) recognised *Thelebolus*, *Caccobius*, *Ascozonus*, *Leptokalpion*, *Ramgea* and *Pseudascozonus* as belonging to the *Thelebolaceae sensu stricto*. Molecular analyses of the nuclear SSU rDNA gene by Landvik *et al.* (1998) supported the placement of *Thelebolus*, *Caccobius* and *Ascozonus* within a separate family *Thelebolaceae*. In Table 7 a morphological comparison between *Thelebolus* and other genera of the *Thelebolaceae* is presented.

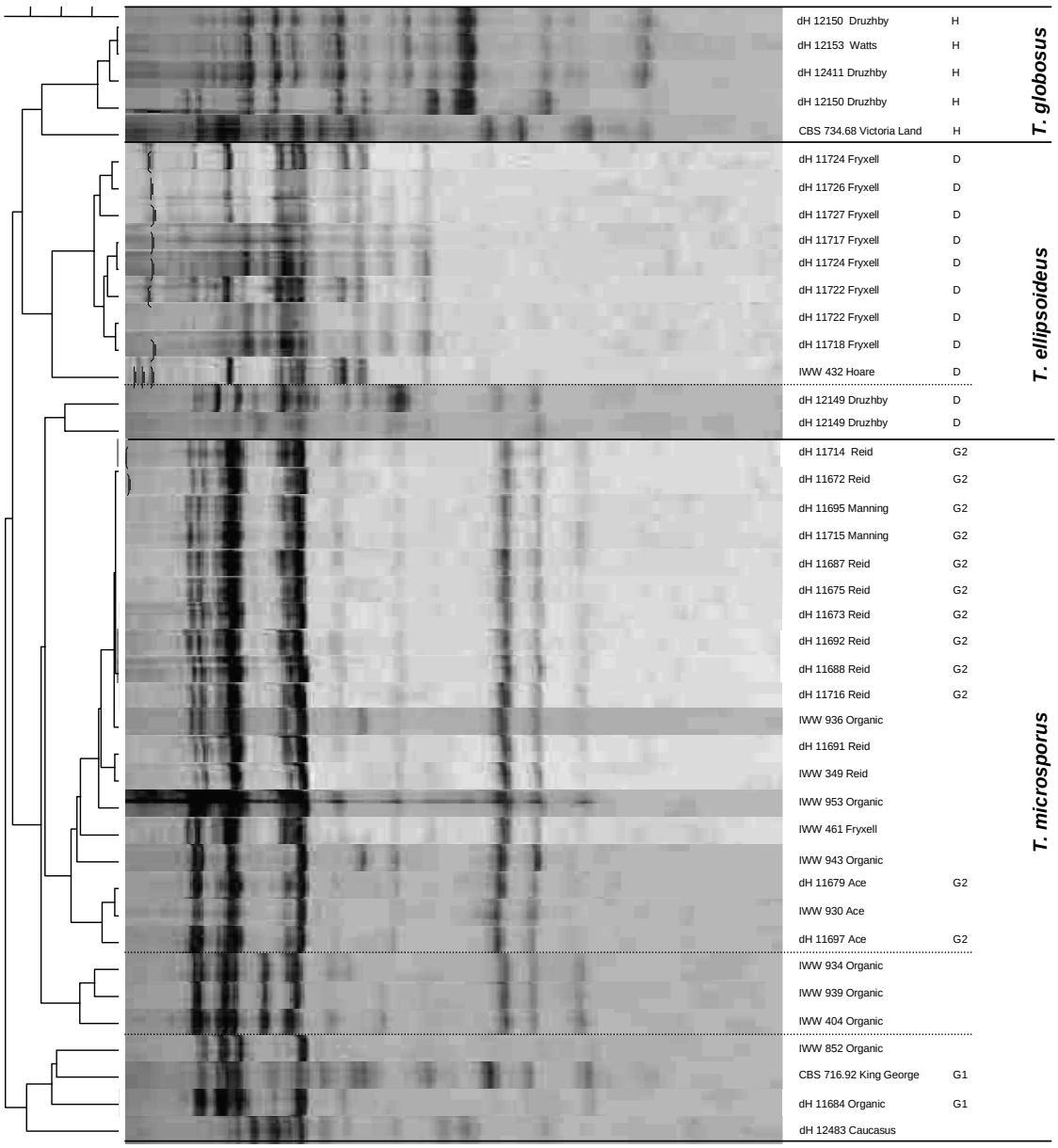


Fig. 8. UPGMA clustering of 53 strains of *Thelebolus* fingerprinted with microsatellite $(GTG)_5$ primer, including some replicates.

Taxonomic support by rDNA ITS and β–tubulin sequence data

Strains identified as *T. stercoreus* with β-tubulin sequencing (Cluster C in Fig. 6) are somewhat scattered within the *Thelebolus* clade when SSU sequence data are compared (Fig. 3). This is not surprising, because we notice much more intra-cluster variability in *T. stercoreus* than in any other *Thelebolus* species.

Differences in ITS in our data set of *Thelebolus* and related fungi were minimal. The genus *Antarctomyces*, with remarkably different teleomorph morphology in ascomata, asci and ascospores (Stchigel *et al*. 2001), was very well alignable with *Thelebolus*, differing in 20 unique positions. *Ascozonus woolhopensis* ITS was nearly identical to *Antarctomyces*, although this genus has a very characteristic teleomorph (van Brummelen 1974, 1998; van Brummelen & Richardson 2001). This close sequence similarity with high morphological diversity is in contrast to many other fungi, such as black yeasts, where species morphologically very similar species nevertheless differ from each other in > 1 % of their ITS domains (de Hoog *et al.* 2003).

Very similar species of e.g. *Geotrichum* even demonstrated an enormous ITS diversity (de Hoog & Smith 2004). The situation in *Thelebolus* resembles that in *Hypocreales*, where proven different species may have identical ITS regions (Lieckfeldt & Seifert 2000). This either indicates a recent evolution of the fungi concerned, or in *Thelebolus* and *Antarctomyces*, may be due to the psychrophilic nature of the members of the genera, leading to a very slow generation time and little accumulation of mutations, despite that large phenetic adaptations are needed in the hostile Antarctic environment. Within *Thelebolus*, only six phylogenetically informative positions were found (Table 4), which enabled the distinction of two main groups (1 and 2), though at low bootstrap support

(Fig. 4). Species, distinguished on the basis of β-tubulin sequence data and morphology, frequently differed in only a single ITS position (Table 4). With that gene, four unambiguous groups were found with bootstrap values > 90 (Fig. 6).

The two new species from the Antarctic, which are introduced below, are remarkable by having a highly reduced teleomorph morphology, accompanied by low ITS and moderate β-tubulin polymorphism. The loss of ascus top structures are generally taken to define entities at the ordinal level, but sudden evolutionary leaps with and without elaborate mechanisms are observed between closely related species of a single genus. The shift is accompanied by emergence of conidia. This suggests that dramatic climatic changes may have made forcible ascospore discharge redundant, the fungus easier being water-dispersed by simple conidia. In Hoare and Fryxell lakes, which are covered by nearly perennial ice, the teleomorph was completely lost.

Ecology

Thelebolus species are psychrophilic, with optimum temperatures for growth usually below 20 °C (Wicklow & Malloch 1971). Our strains invariably showed good growth at 0 °C but mostly also at 25 °C (Table 3), with *T. microsporus* genotype G3 as the only exception. However, production of ascomata takes place between 10 and 15 °C. *Thelebolus* is frequently isolated from Antarctica, as is apparent from our data and was also reported by Montemartini *et al*. (1993), Azmi & Seppelt (1997, 1998) and Bergero *et al*. (1999), as well as from the Arctic zone (Kobayasi *et al*. 1967).

The ecology of *T. microsporus* is puzzling. In the strains from lakes no clear association with type of water is apparent (Table 5), except for *T. ellipsoideus* (D) that is mostly found in fresh water. *T. globosus* occurs in fresh and in brackish water. The lakes of G1 are brackish to hypersaline. This genotype is also found in skua nests and on mammal faeces (Table 3). The water chemistry does not, therefore, seem to be a decisive factor in the ecology of *Thelebolus*. The genus has been isolated from mammal dung and guano (www.cbs.knaw.nl/databases). The lakes of Antarctica are ultra-oligotrophic unless they are enriched by bird droppings (Laybourn-Parry *et al*. 1997). Living communities are mostly dominated by micro-organisms, usually lack any significant zooplankton and there are no fish. Montemartini *et al*. (1993), sampling terrestrial locations, explicitly noted the association of *Thelebolus* with skuas. In the aquatic sources of isolation, a proportional association between colonies grown and frequency of skuas was apparent. The lakes sampled in the course of the present study were not or very rarely frequented by birds (J. Laybourn-Parry, pers. comm.).

Their extreme oligotrophy does not match with the expected ecology of *Thelebolus*. Debris introduced by humans as an artificial source of contamination is less likely, since Baublis *et al*. (1991) in their investigation of nutrient-rich allochthonous materials did not encounter the species. Rather, it seems likely that an occasional bird dropping in an Antarctic lake provides sufficient nutrients for *Thelebolus microsporus* to survive over prolonged periods. This suggests a considerable ecological instability of the ponds, a rare dropping being sufficient to allow proliferation of fungal invaders.

Thelebolus microsporus is equally isolated from freshwater (Reid, Manning) as well as from hypersaline water (Organic), which has water temperatures well below 0 °C over many consecutive years (Gibson & Burton 1996). Thus we must assume that the species is able to tolerate a wide ecological range, the only consistent factor for its occurrence being low temperature. The species may have a competitive advantage over other dung-associated filamentous fungi by its psychrotolerance. The isolation from the hypersaline Organic lake is further remarkable because Wright & Burton (1981) supposed that biochemical adaptations required for either cold or salt tolerance in bacteria are mutually exclusive.

Evolution

Thelebolus species show an obvious psychrophilic tendency (Wicklow & Malloch 1971), as teleomorph production is frequently enhanced by growth temperatures below 15 ºC. They are, however, also strikingly associated with animal faeces. The ability of coprophilous fungi to pass through the stomach and intestinal tract of warm-blooded animals was already shown by Janczewski (1871) and Massee & Salmon (1902). The occurrence of *Thelebolus* species from tropical to Antarctic climate zones indicate that environmental temperature appears less significant than the thermotolerance required to survive the animal body temperature. The psychrophily of several species may add to a competitive advantage at lower temperatures, suggesting that there is an extended lag between passage through the animal and subsequent animal-carried dispersal. ITS and β-tubulin data are congruent in demonstrating that *Thelebolus stercoreus* is the most variable species of the genus. It is widespread, being encountered on several continents except, so far, Antarctica. Its regular occurrence on faeces of mammals and gallinaceous birds suggests that the vector of dispersal of this species is consistent. A similar widespread occurrence on several continents, regional variability in ITS and β-tubulin, as well as occurrence on animal faeces is noted in *T. microsporus*. These species are, therefore, likely to have an ancestral position within the genus *Thelebolus*.

Table 7. Morphological comparison between *Thelebolus* and other genera of the *Thelebolaceae* (after van Brummelen 1967, 1976, 1985, 1987, 1992, 1998; van Brummelen & Kristiansen 1998, Kimbrough & Korf 1967).

		***Thelebolus* Tode : Fr.**	***Caccobius* Kimbr.**	***Ascozonus* (Renny) E. Hansen**	***Leptokalpion* Brumm.**	***Ramgea* Brumm.**	***Pseudascozonus* Brumm.**
Ascomata	development:	cleistohymenial, opening late or not	eugymnohymenial	cleistohymenial, opening very early	paragymnohymenial	paragymnohymenial	eugymno-hymenial
	colour:	hyaline, yellowish, brown	hyaline, pinkish	white	white	white	hyaline, white
	hairs:	absent	hyphoid	fimbriate esp. near margin	hyphoid	absent	absent
Asci	number:	1–80(–100)	1–10	1–30	1	up to 50	4–15
	shape:	clavate, ellipsoid	cylindric-ellipsoid	'cigar-shaped' curved base	ovoid	clavate	clavate, obovoid
	length:	diverse	125–135 µm	80–145 µm	290–400 µm	27–35 µm	28–33 µm
	opening:	irregular tear above subapical ring	near thick apical plug	apical disk and bilabiata split above ring	bilabiate split or irregular	weakening of apex or irregular tear	apical thickening bilabiate split or irregular operculum
	wall-structure:	2 layers, thick	2 layers, thick	2 layers, thin	2 layers, thick	2 layers, thick	2 layers, thick
	subapical ring:	clear or prominent	not found	very prominent	not found	present	not found
Ascospores	shape:	ellipsoid, Q<2.0	ellipsoid, Q < 2.8	fusiform, Q>2.0	ellipsoid, Q<2.0	ellipsoid, Q<1.7	ellipsoid, Q>2.0
	length:	up to 9×5 µm	ca. 4.8–5.2 µm	8–18 µm	8.3–9.3 µm	7.5–8.1 µm	6.6–11.2 µm
	number per ascus:	8 to >2000	1000–1500	16–256	ca. 4000	4, 0–4 deficient	8
	ornamentation:	mostly absent	absent	absent	absent	with rings	absent
	shot away:	+/-	+	+	+	+	+
Anamorph		absent or *Hyphozyma*-like	absent	absent	absent	absent	absent
Psychrophilic		+	+	-	-	-	-

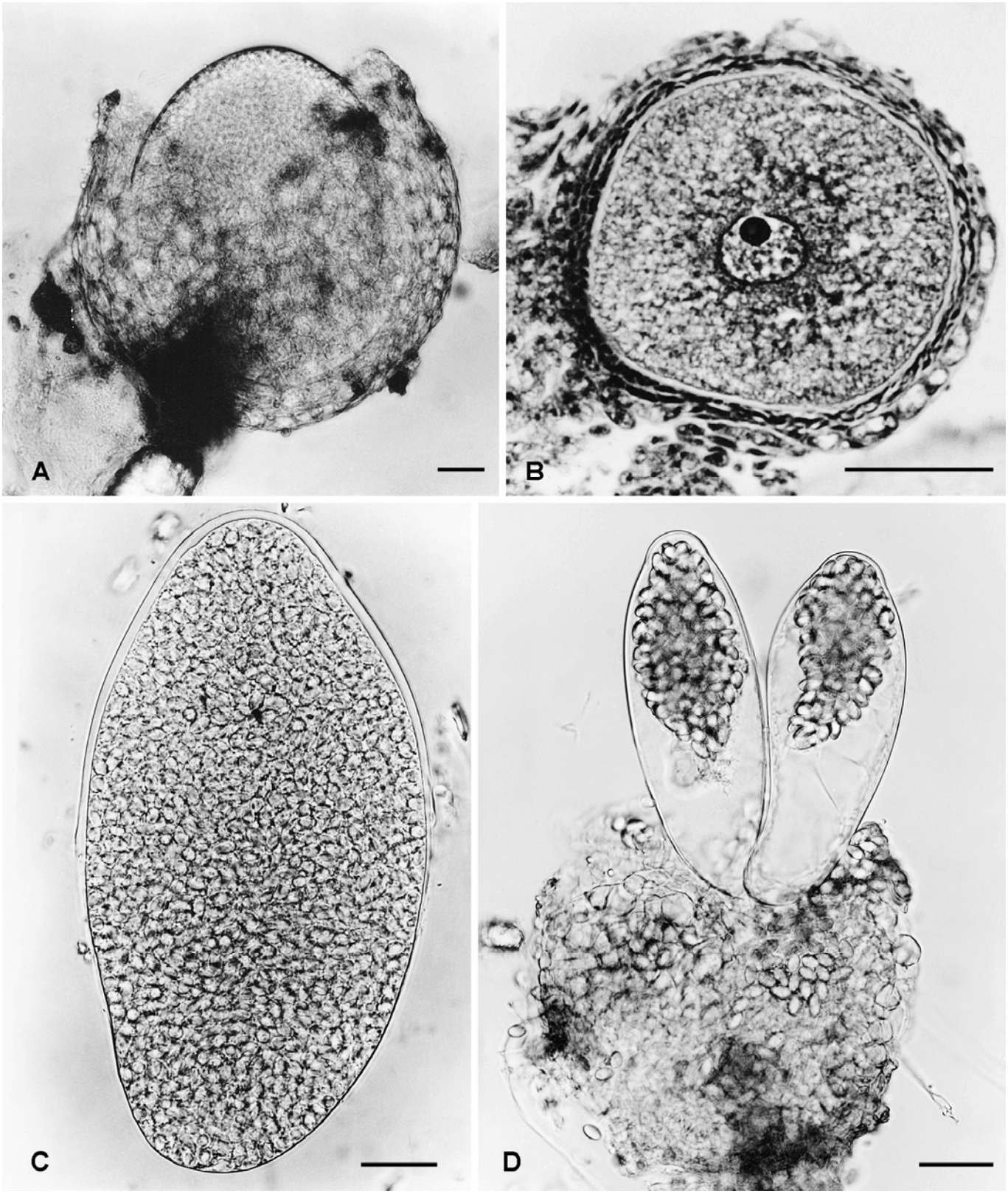


Fig. 9. *Thelebolus stercoreus.* A. Mature ascoma with single ascus with far over 2000 spores. B. Ascus at uninucleate stage with large nucleolus. C. Isolated ascus with over 2000 spores (note double ascus-wall). D. Ascoma with two mature asci, each with about 256 spores. (A, C–D after van Brummelen, 31 Aug. 1961, Kraloo, Netherlands; B after CBS 717.69, weak Flemming-haematoxylin). Scale bars = 20 µm.

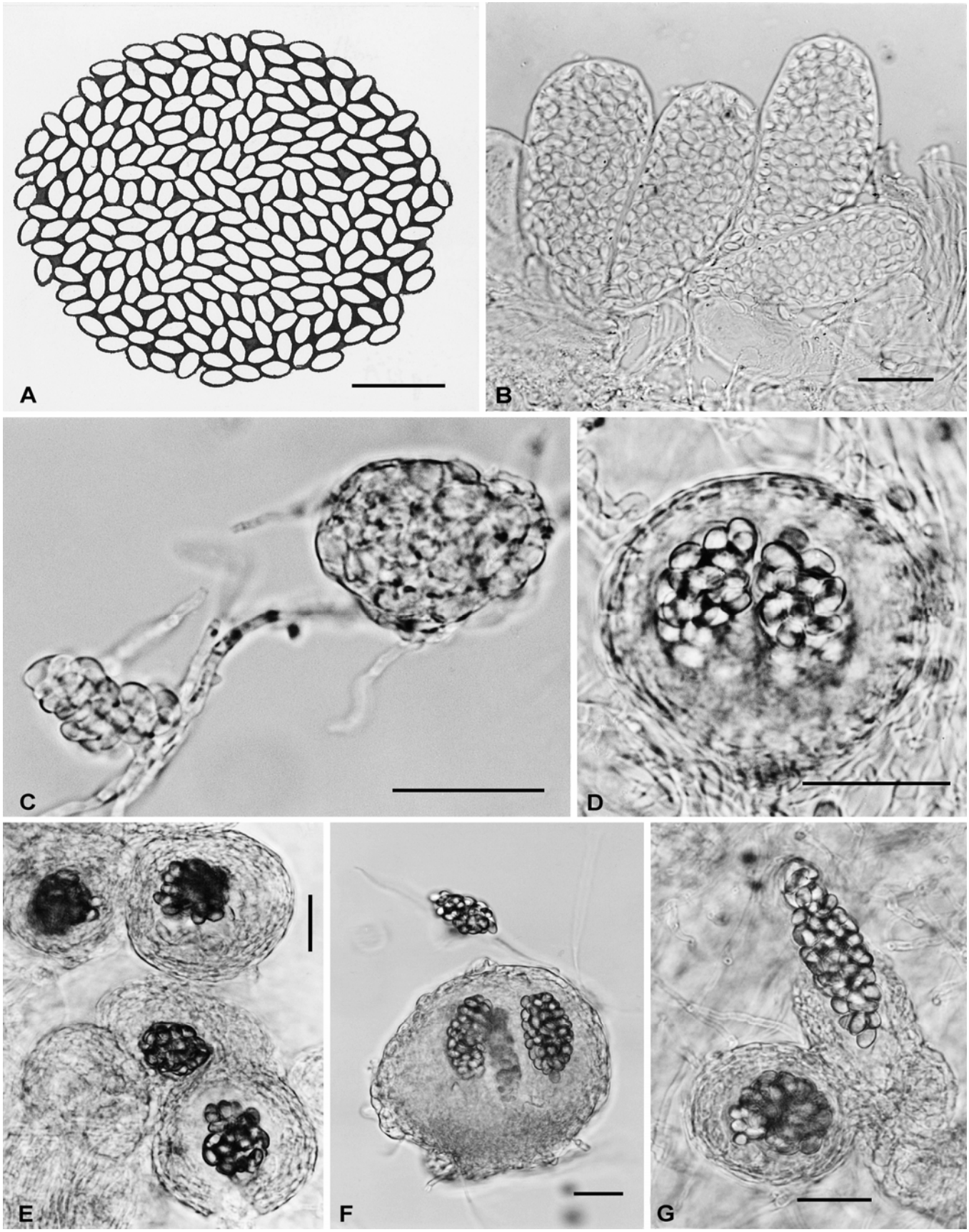


Fig. 10. *Thelebolus stercoreus*. A. Ascospore-shot, flattened to a single layer, with 242 spores (CBS 711.69). B. Asci with about 256 spores, from holotype-collection of *Ascobolus caninus* Auersw. (WRSL-A1711). C. Coiled gametangia (at left) and young ascoma (CBS 709.69). D. Still closed ascoma with two mature asci, each with about 32 spores (CBS 709.69). E–G. Cleistohymenial ascomata with about 64 mature ascospores, partly released in F and G (CBS 715.69). Scale bars = 20 µm.

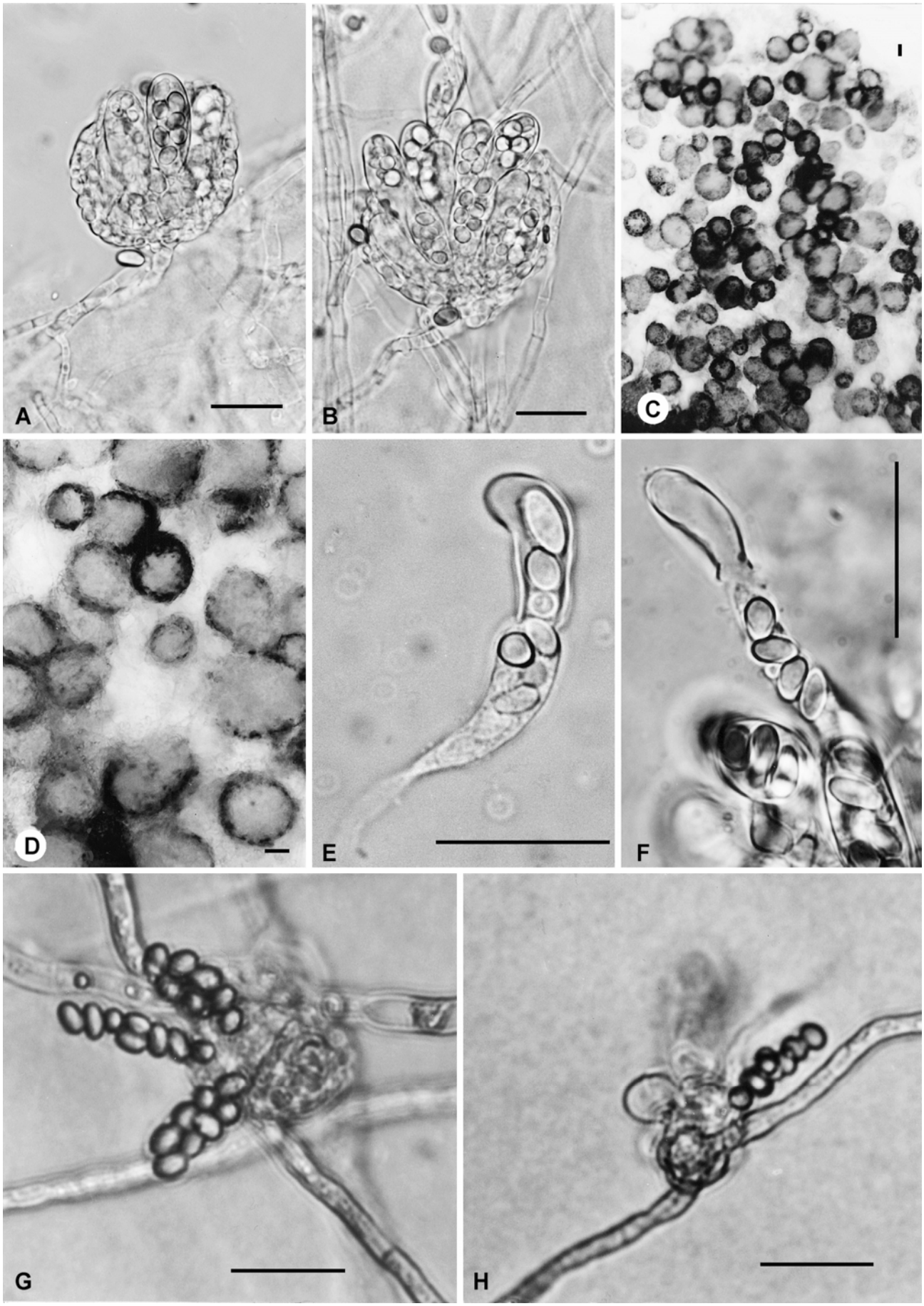


Fig. 11. *Thelebolus microsporus*. A, B. Ascomata with ripening asci, opening at the top (CBS 716.92). C–D. Mature "apothecioid" ascomata in culture, seen from the top, D in detail (dH 11674). E–F. Mature asci with easily separating rigid thick outer and flexible thin inner wall-layer (dH 11674). G–H. Ascomata with strongly reduced receptacles and naked shooting asci (CBS 115.53). Scale bars = 20 μm.

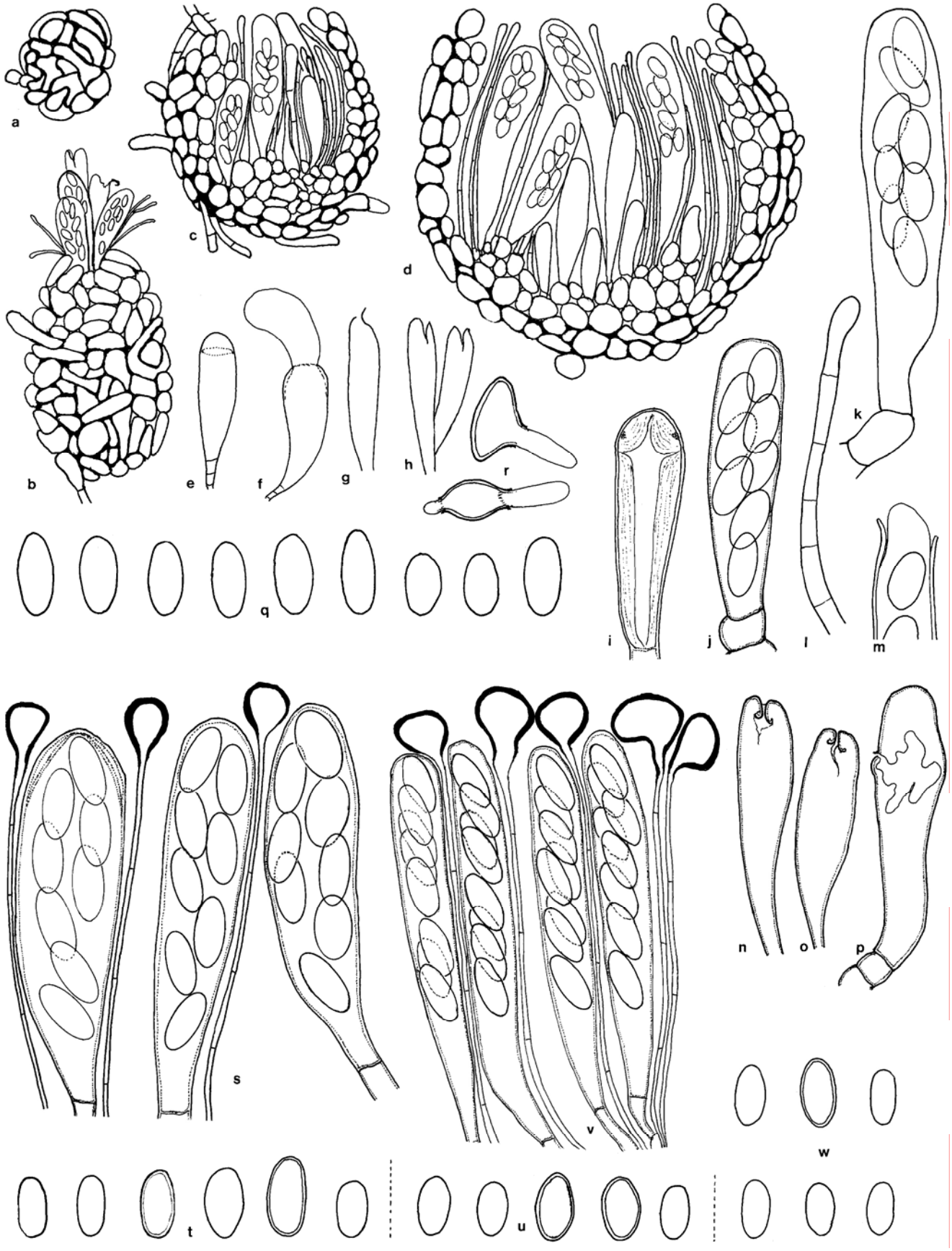


Fig. 12. *Thelebolus microsporus*. A–R. From dH 11674: A–D. Ascomata. E–H. Asci. E. Young with subapical ring. F. Inner wall-layer extruding. G, H. Emptied asci. I–P. Asci and paraphysis. I. Young with swollen inner wall-layer and subapical ring. M. Top of dehiscing ascus with ruptured outer layer and protruding inner layer (*cf.* van Brummelen 1998: Figs 1, 13a–e). N–P. Emptied asci. Q. Ascospores. R. Two germinating spores. S–U. From isotypes of *Ascobolus microsporus* in Rabenhorst, Fungi Europ. Exs. No. 977 (S, T, from ZT; U, from HBG). S. Asci and capitate paraphyses. T, U. Ascospores. V–W. From isotype of *Ascobolus cesatii* in Rabenhorst, Fungi Europ. Exs. No. 976a (HBG). V. Asci and capitate paraphyses. W. Ascospores. (A–H, × 630; I-W, × 1600.)

In contrast to *Thelebolus stercoreus*, *T. microsporus* is commonly found in the Antarctic, where it is the most widespread taxon of the genus; genotype G1 is encountered in most of the sampling locations as well as in the U.S.A. and in Europe (Table 3). (Remaining specimens analysed were herbarium specimens of which the genotype could not be established). Since several regional genotypes were revealed in the Antarctic, genotype G1 is likely to be the first genotype that has reached the continent. No large land mammals are present in Antarctica, and hence we witness a strong association with bird vectors (Leotta *et al*. 2002). The latter authors found *T. microsporus* present not only in bird cloacas but also in the digestive tube, indicating that the fungus is actively taken up by the birds and subsequently dispersed via the guano. Antarctic birds fly very long distances, e.g. the Wilson's storm petrel from pole to pole, but limited migration takes place between different Antarctic regions. This may explain why the remaining genotypes all have a localized distribution. They are likely to have emerged from genotype G1 after the species has arrived in cold Antarctic climate, which is estimated to have been after tectonic movements about 60 million years ago (Manzoni 2001). *Thelebolus microsporus* may have been distributed on the entire continent prior to this event, and subsequently was forced to adapt to changing environmental conditions. A change to a seabird vector from a mammalian and gallinaceous one is probably is not very great, but Victoria Land and Larsemann and Vestfold Hills are practically without birds. This dramatic ecological change may have led to the emergence of species adapted to life without vertebrate vectors. Independent events have twice given rise to morphologically very similar species, characterized by loss of forcible ascospore discharge, and, instead, preponderance of a simple, yeast-like anamorph. Based on 3rd codon mutations in the β-tubulin gene and given a supposed mutation frequency of 10^{-9} / base / year, these events have taken place about over 40.4 million years in the case of *T. ellipsoideus*, and 31.7 million years in the case of *T. globosus*. This matches quite well with the gradually decreasing temperature of Antarctica, marking the period in which vertebrate vectors were indeed lost.

The oldest species, *T. ellipsoideus*, is present at different bird-less coastal areas on the Antarctic. Of these, Victoria Land has the most extreme climate; Lakes Hoare and Fryxell are covered by ice during most of or even the entire year. The genotypes of these strains are identical, but the phenotypes are consistently characterized by the loss of asci. This underlines that conidia indeed become predominant when bird / air dispersal becomes increasingly unlikely. The supposed events are summarised in Fig. 17.

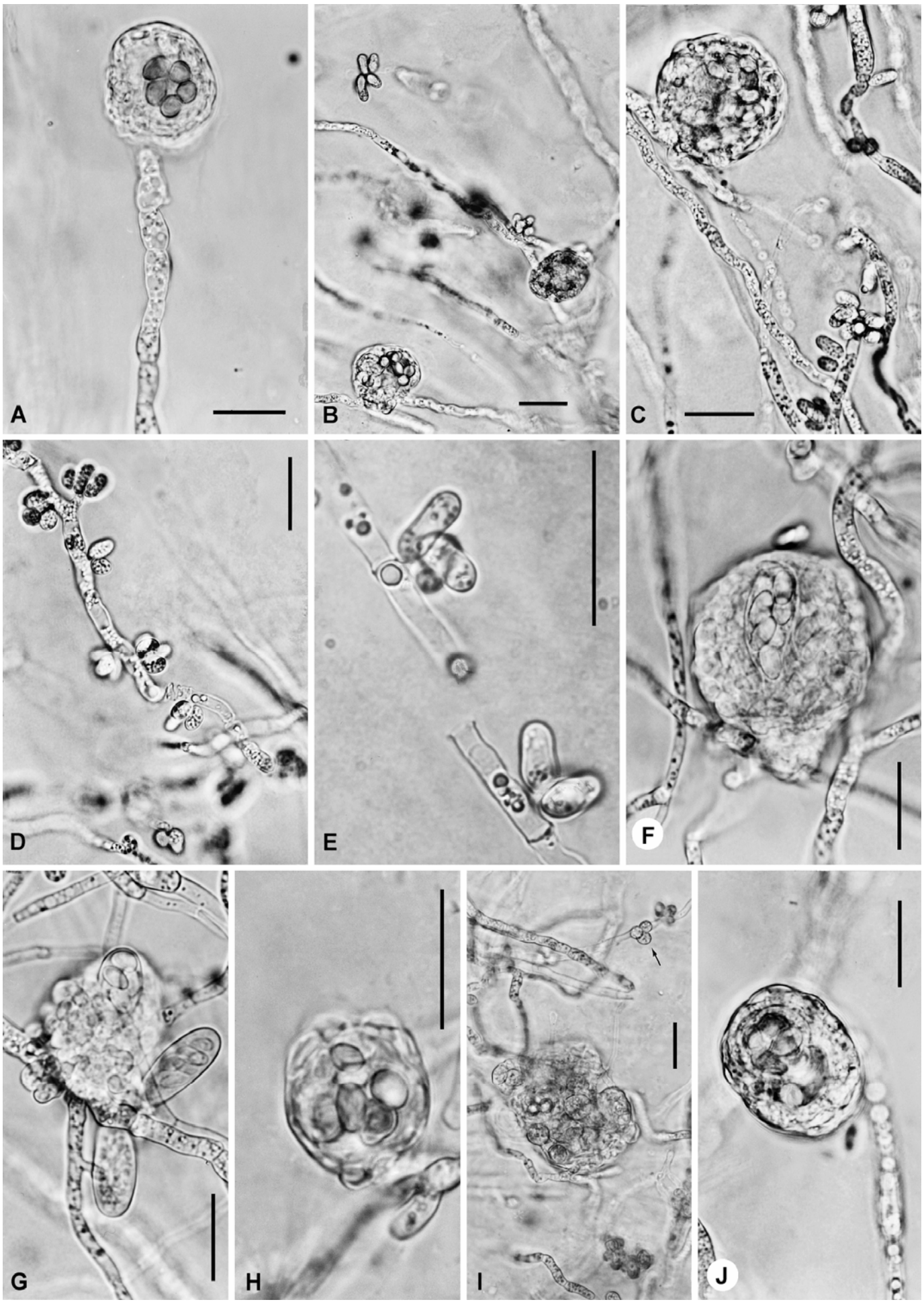
TAXONOMIC PART

THELEBOLUS Tode : Fr.

Thelebolus Tode, Fungi Mecklenb. Sel. 1: 41. 1790; Fries, Syst. Mycol. 2: 306. 1823 (name sanctioning).
Monotype: *Thelebolus stercoreus* Tode : Fr. For synonyms see Kimbrough & Korf (1967: 14).

Generic description:
Stromata absent. *Ascomata* superficial or immersed, very small, 25–400 µm diam, at first subglobose cleistohymenial, then often opening in the late mesohymenial or telohymenial phase by irregular rupturing of the cortical excipulum in the upper part and becoming lenticular to semiglobular, especially when 8- to 64-spored appearing 'apothecioid' at maturity; surface smooth, hyaline, yellowish or brown; margin not or scarcely differentiated. *Hymenium* with a palisade of asci and paraphyses dependent on the presence of light. Hypothecium absent. Medullary excipulum absent or scarcely differentiated. Cortical excipulum of varying thickness consisting of one to several layers of subglobular or flattened cells (*textura globulosa* or *epidermoidea*). *Asci*, depending on the spore number, varying from clavate-cylindrical, clavate, ellipsoid to subglobose, rounded above, rather persistent, 8- to over 2000-spored, with a wall consisting of a thick outer layer and a thin elastic inner layer easily separating at maturity, with a subapical ring-shaped wall-thickening, opening with a rather irregular split above the level of the ring, not staining blue with iodine. *Ascospores* ellipsoid with rounded ends, rarely somewhat fusoid, hyaline, rather small (up to 9 × 5 µm), without oil-drops or granules, not easily producing air-inclusions (De Bary bubbles), rather thick-walled, smooth. Paraphyses often rather scanty, slender, filiform, sparsely branched, in exposed 'apothecioid' ascomata,often with thickened apices (up to 3.5–7 µm) and covered with a thick layer of brownish or yellowish amorphous pigment. *Anamorphs* absent or simple; if present resembling *Hyphozyma*.

***Notes*:** Since the number of specific characters in *Thelebolus* is very low, the number of spores present in an ascus has played an important role in the distinction of taxa within the genus. Some authors had their doubts concerning the value of this character. Karsten distributed (Karsten 1866) and described (Karsten 1870) *Ascobolus myriadeus* P. Karst.


A
B
C
D
E
F
G
H
I
J

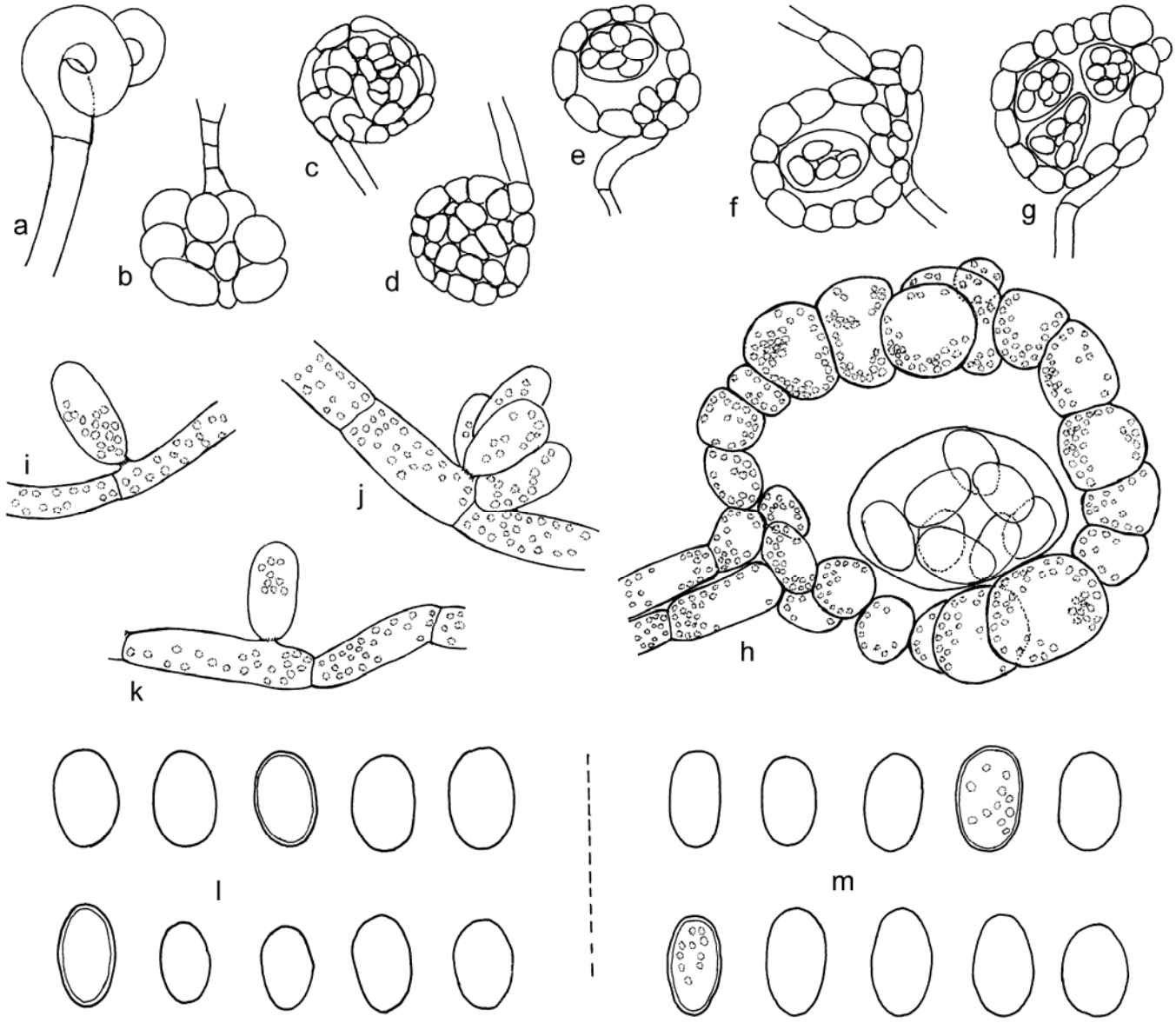


Fig. 14. (p. 59) *Thelebolus ellipsoideus*. A. Initials of ascomata; B–D. Young ascomata seen from outside. E–H. Ascomata in optical section. I–K. Conidia arising on hyphae. L. Ascospores. M. Conidia. (A–G × 1250; H–M × 3200; all from CBS 113938).

In the same sample fruit-bodies with 24-, 32-, 48-, and 64-spored asci were found growing closely together. Study of the type specimen of *Ascobolus cookei* Crouan in the Crouan-herbarium by van Brummelen (1967) also revealed the presence of asci with 32, 48 and 64 ascospores, while Le Gal (1961) found in the same package fruit-bodies with at least 150–180 spores in an ascus. Kimbrough & Korf (1967) accepted a variable number of spores per ascus in their description of *Thelebolus stercoreus*.

In contrast, after a study on a great number of cultures isolated from many samples of dung, Wicklow & Malloch (1971) claimed that the ascospore number within any strain is constant.

Fig. 13. (p. 58) A–G, J. *Thelebolus ellipsoideus*. A–E. dH 11719. A. Single cleistohymenial ascoma at the end of a hypha, with mature ascospores. B–C. Ascomata and ellipsoid conidia. D–E. Conidia. E. Id. in detail. F–G. Ripening and desintegrating ascomata with mature asci (dH 12160). J. Closed ascoma with mature ascospores (dH 12149 = CBS 113938). H–I. *Thelebolus globosus* (dH 12150 = CBS 113940). Scale bars = 20 µm. I. Conglomeration of cleistohymenial ascomata and conidia (arrow). H. Detail of closed ascoma with mature ascospores. Scale bars = 20 µm.

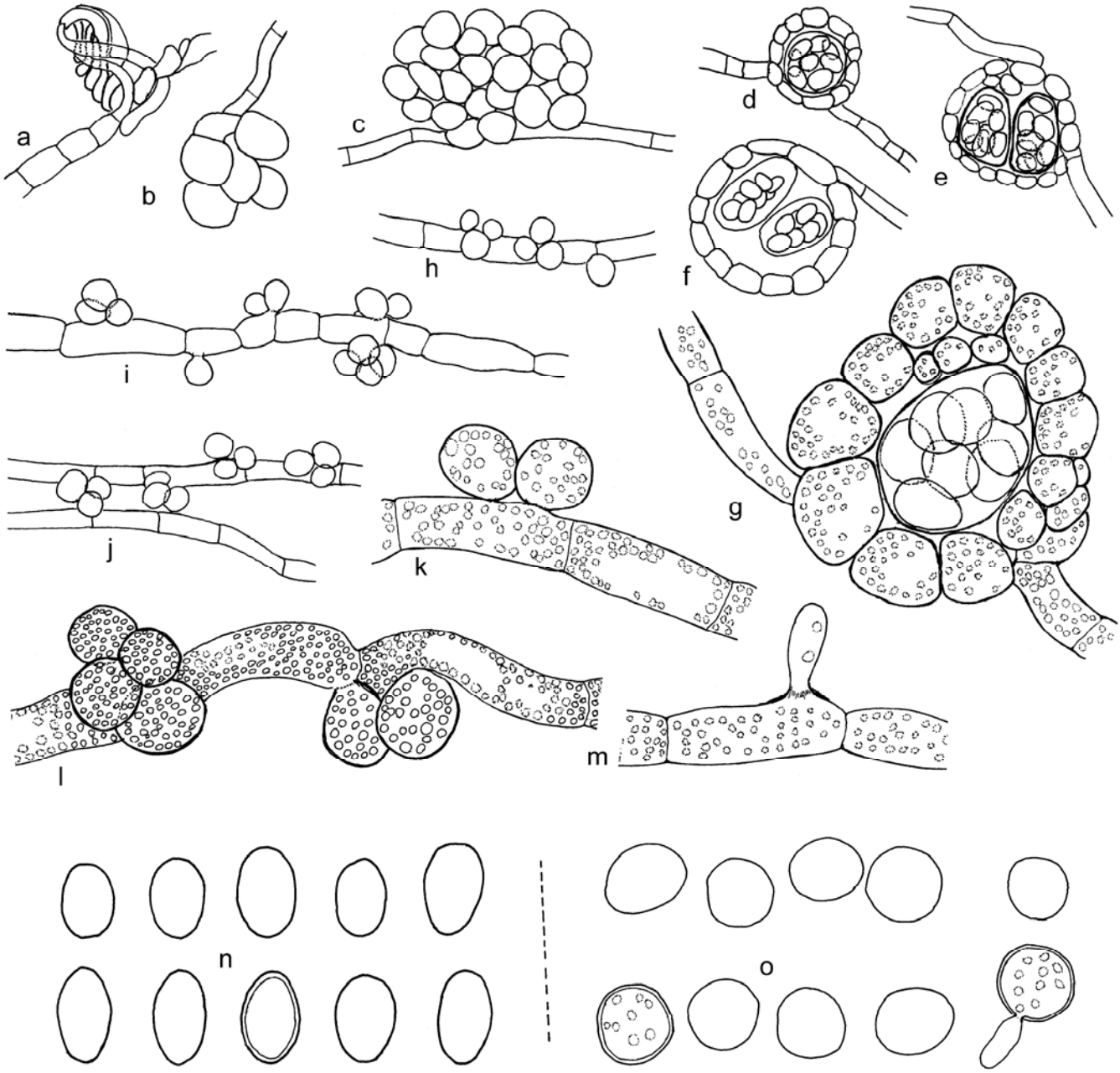


Fig. 15. *Thelebolus globosus*. A–B. Initials of ascomata. C. Ascoma seen from outside. D–G. Ascomata in optical section. H–M. Conidia arising on hyphae. N. Ascospores. O. Conidia (A–F, H–J × 1250; G, K–O × 3200; all from type dH 12150 = CBS 113940).

This has led to a revaluation of the character and the distinction of species of *Thelebolus* according to the number of spores found in an ascus (e.g. Jeng & Krug 1977, Kimbrough 1981, Cannon *et al*. 1985, Raitviir & Prokhorov 1988, Prokhorov 1998, van Brummelen 1998, Kutorga 2000, Doveri 2004).

Rather often the given spore numbers were found to be inaccurate or based on repeated estimations. It is difficult to count spores in a three-dimensional mass in an ascus under the microscope, so we developed a method for accurate counting of ascospores (see "Materials and methods") and used it in a number of tests. In our studies we regularly observed asci with different spore numbers within the same ascoma. As an example: several hundreds of spore heaps were exactly counted in mono-spore isolates of strain TRTC 45548 (= CBS 711.69), described by Wicklow & Malloch (1971) with 256-spored asci. The results (Figs 10A, 16) showed, that the spore number per ascus varied from 128 to 264 with an optimal number close to 246. The theoretically stated number of 256 was found in less than 10 % of the cases.

Molecular data in main traits support the unity of multispored species as a single, well-supported clade with ß-tubulin (Fig. 6), although some heterogeneity remains which is particularly revealed using the DNAWD algorithm (Figs 5, 7).

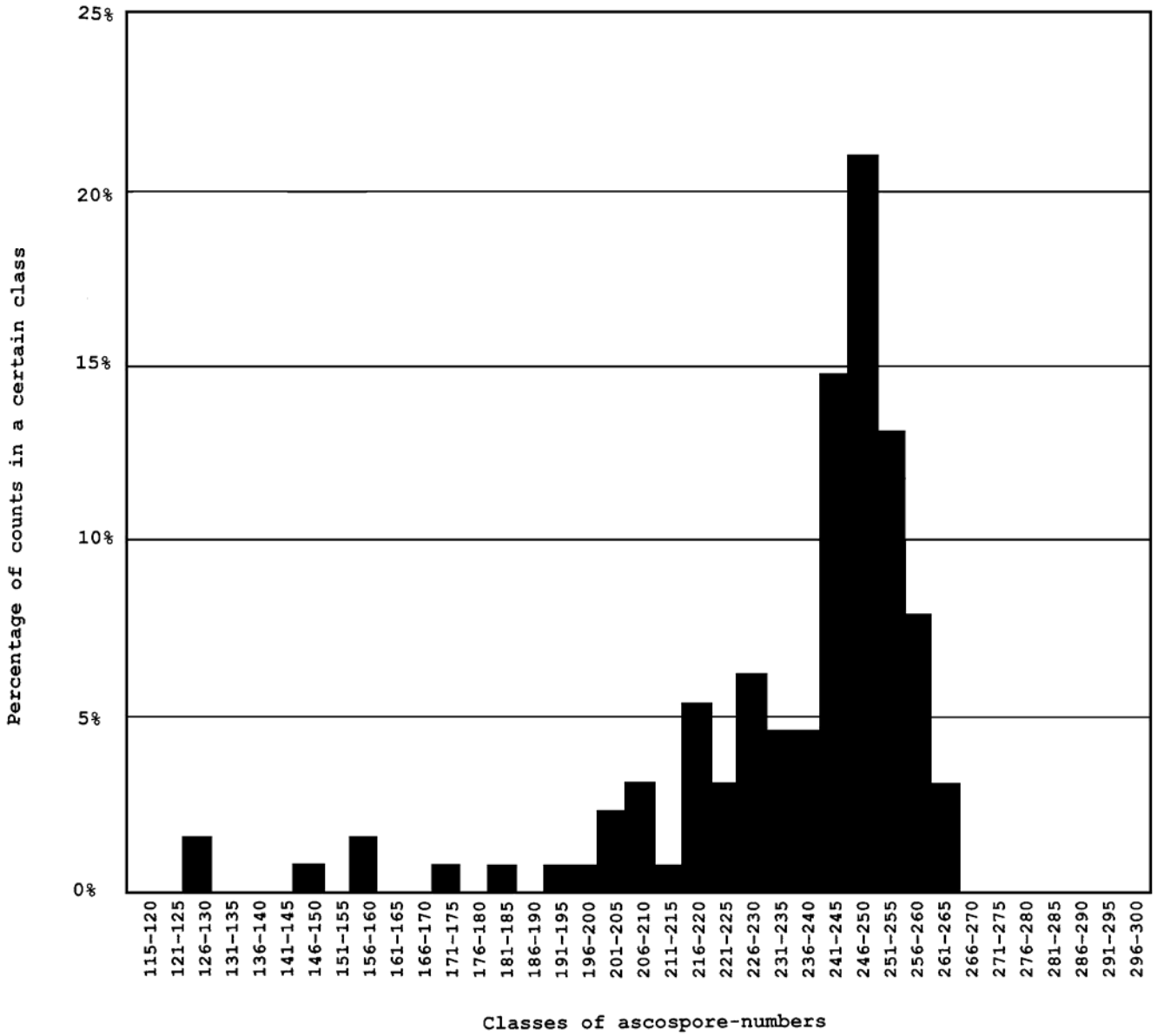


Fig. 16. Distribution diagram of spore counts (as percentages) over classes of ascospore-numbers in monospore isolates of *Thelebolus stercoreus* strain CBS 711.69.

The molecular differences observed do not clearly coincide with spore counts (Table 3, Fig. 6), which would necessitate treatment of each strain as an individual taxonomic entity. Given the high degree of molecular similarity in the entire data set combined with a high degree of phenetic diversity, it is possible that *T. stercoreus* has to be interpreted as a single species in an early state of diversification, all subpopulations having > 8 spores per ascus.

In conclusion, we found that in samples of *Thelebolus* with more than eight spores in an ascus (multispored), the spore-number is inconstant and of little value for the distinction of species. Geographically isolated populations differing in ascospore numbers may be recognised when more material will have become available for study.

On the contrary, samples with eight-spored asci were never found to produce a higher number of ascospores. Three of the species of *Thelebolus* with 8-spored asci are present in Antarctica, two of them are even endemic to that part of the world. Multi-spored samples of *Thelebolus* are not found in Antarctica, but are rather common in the rest of the world.

Other characters available for species distinction in *Thelebolus,* such as: (1) number of asci in an ascoma, (2) size of the asci, (3) size of the ascospores, (4) details of the ascospore discharge, (5) shape of the ascoma, (6) thickness of the ascus wall, directly depend on the number of ascospores formed. A diagnostic overview of *Thelebolus* and related genera is given in Table 7.

The presence of light has an important influence on the development of ascomata in species of *Thelebolus*. In the absence of light mycelial growth and pigmentation are about normal, but the production of ascomata initials is considerably retarded in 8-spored strains and even almost absent in multi-spored strains. In the presence of light usually regular hymenia with a palisade of asci and paraphyses are formed, often giving rise to "apothecioid" ascomata. Especially under the influence of directed light in species of *Thelebolus* regular apical apparatuses are formed, as illustrated by van Brummelen (1998).

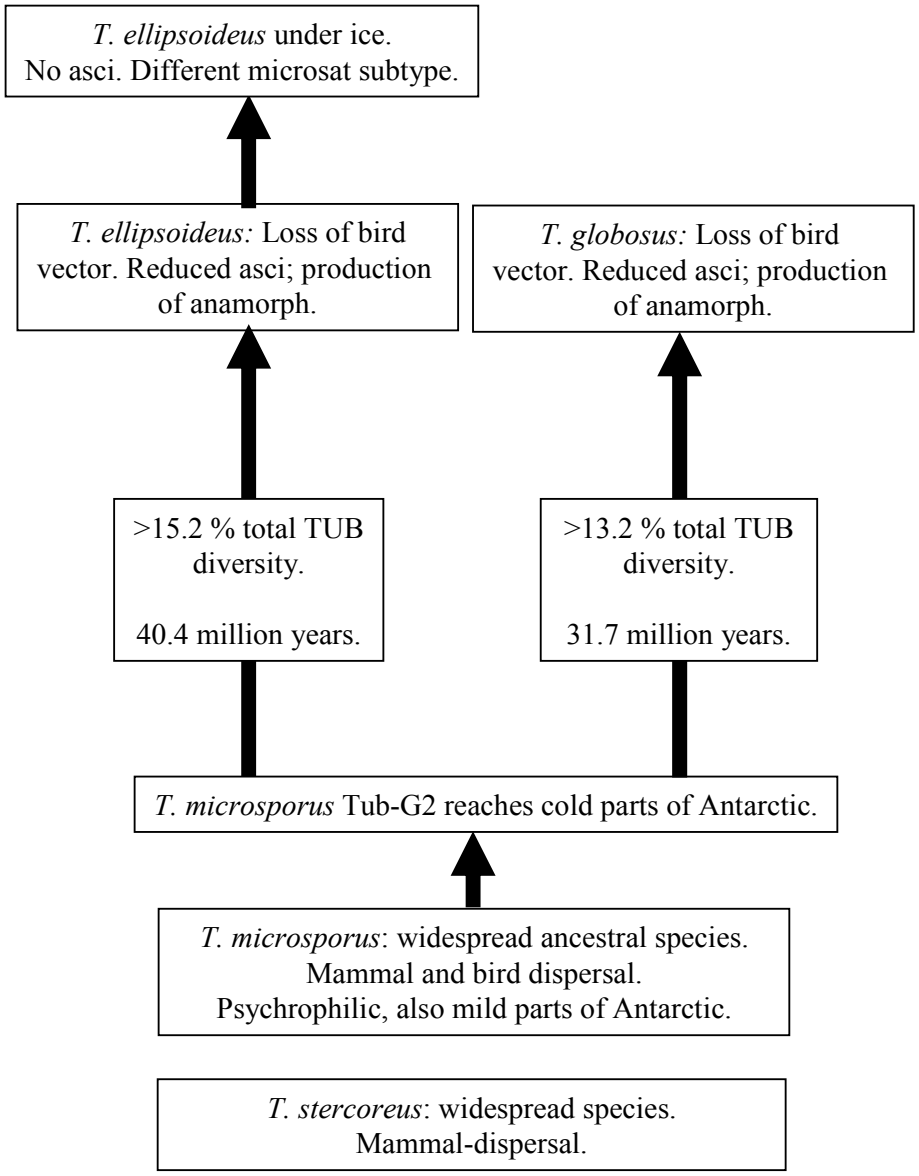


Fig. 17. Diagrammatic representation of hypothesized evolutionary steps in *Thelebolus* after the Antarctic continent reached its current position.

Key to the species of *Thelebolus*

1a. Asci with more than 8 spores, species outside Antarctic ..... ***Thelebolus stercoreus***
1b. Asci with 8 spores, species Antarctic or outside Antarctic **2**

2a. Cleistohymenial ascomata opening in the meso- or telo-hymenial phase, at first subglobular, often becoming 'apothecioid', forming a palisade of asci and paraphyses (hymenium), usually with forcible discharge of ascospores through an irregular split above a subapical ring at the ascus top; conidia not present ..... ***Thelebolus microsporus***
2b. Cleistohymenial ascomata not opening before full maturity or disintegration, subglobular, never becoming 'apothecioid', asci irregularly arranged, paraphyses absent, without forcible discharge of ascospores, abundantly forming conidia **3**

3a. Conidia subglobular or shortly ellipsoid, length/width ratio less than 1.2, 2.5–7.4 µm diam, in rather large clusters(2–40) ..... ***Thelebolus globosus***
3b. Conidia ellipsoid, length/width ratio over 1.2, 4–9 × 3.5–5.5 µm, in small clusters (1–5) ..... ***Thelebolus ellipsoideus***

Accepted species of *Thelebolus*

Thelebolus microsporus (Berk. & Br.) Kimbr. Figs 11–12.

Ascobolus microsporus Berk. & Br., Annls Mag. Nat. Hist III, 15: 449. 1865 [not *Ascobolus microsporus* Velen., Monogr. Discom. Boh. 365. 1934].

≡ *Ascophanus microsporus* (Berk. & Br.) E.C. Hansen, Vidensk. Meddr Naturhist. For. 1876: 287. 1877.

≡ *Thelebolus microsporus* (Berk. & Br.) Kimbr., in Kobayasi *et al*., Ann. Rep. Inst. Ferment., Osaka 3: 50. 1967.

Type: **Great Britain**, Batheaston, on cow dung, C.E. Broome *s.n.*, 19 Nov. 1864 (K-A2525, part of the holotype; isotypes in Rabenhorst, Fungi Europ. Exs. No. 977, as *Ascobolus microsporus*, studied from G, HBG, PRM, ZT).

Ascobolus cesatii Carest., in Rabenhorst, Fungi Europ. Exs. No. 976a. 1866.

≡ *Pezizula cesatii* (Carest.) P. Karst., Bidr. Känn. Finl. Nat. Folk 19: 83. 1871.

≡ *Rhyparobius cesatii* (Carest.) P. Karst., Acta Soc. Fauna Fl. Fenn. II, 6: 122. 1885.

≡ *Ascophanus cesatii* (Carest.) Sacc., Syll. Fung. 8: 533. 1889.

Type: **Italy**, Alto Adige, Riva, on dung of black grouse (*Tetrao tetrix*), Carestia *s.n.*, 1864, in Rabenhorst, Fungi Europ. Exs. No. 976a (isotypes of *Ascobolus cesatii*, studied from BR, G, HBG, PRM, W).

Peziza subfusca Crouan, Fl. Finistère 53. 1867.

≡ *Ascophanus subfuscus* (Crouan) Boud., Annls Sci. Nat. (Bot.), Sér. V, 10: 242. 1869.

≡ *Ascobolus subfuscus* (Crouan) Rehm, Ascomyceten Exs. No. 167. 1873.

Type: **France**, Finistère, s. loc., on dog dung, *Crouan s.n.*, 28 Feb. 1864 (holotype, studied from CO-A2432).[1]

Ascobolus punctiformis P. Karst., Fungi Fenn. Exs. 655. 1867.

≡ *Ascobolus polysporus* P. Karst. subsp. *punctiformis* (P. Karst.) P. Karst., Notis. Sällsk. Fauna Fl. Fenn. Förh. 11: 209. 1870.

≡ *Pezizula polyspora* P. Karst. subsp. *punctiformis* (P. Karst.) P. Karst., Bidr. Känn. Finl. Nat. Folk 19: 82. 1871.

≡ *Ascophanus punctiformis* (P. Karst.) Sacc., Syll. Fung. 8: 532. 1889.

Type: **Finland**, Abo region, Pisparisti skog and near Mustiala, on horse dung, *P. A. Karsten*, May, Fungi Fenn. Exs. No. 655 (isotype, studied from K; with 8-spored asci; growing together with *Ascobolus polysporus* P. Karst., with 150- to 200-spored asci).

Ascobolus fallax Auersw., Hedwigia 7: 52. 1868.

≡ *Ascobolus crustaceus* Fuckel subsp. *fallax* (Auersw.) P. Karst., Notis. Sällsk. Fauna Fl. Fenn. Förh. 11: 208. 1870.

≡ *Pezizula crustacea* (Fuckel) P. Karst subsp. *fallax* (Auersw.) P. Karst., Bidr. Känn. Finl. Nat. Folk 19: 82. 1871.

≡ *Ascophanus fallax* (Auersw.) Sacc., Syll. Fung. 8: 532. 1889.

≡ *Rhyparobius crustaceus* (Fuckel) Rehm var. *fallax* (Auersw.) Heimerl., Jber. Ober-Realschule Bezirke Sechshaus Wien 15: 26. 1889.

[1]For a more accurate indication of herbarium specimens, especially when insufficiently labelled, the usual abbreviations of the herbarium (according to Holmgren *et al*., 1990), is followed by the last author's revision-number.

≡ *Rhyparobius fallax* (Auersw.) Rehm, Ascom. No. 1016. 1891.

Type: Not known to be in existence. *Type locality*: **Germany**, Rosenthal near Leipzig.

Ascophanus coemansii Boud., Annls Sci. Nat. (Bot.), Sér. V, 10: 244, pl. 19 f. 30. 1869.

≡ *Ascobolus coemansii* (Boud.) Quél., Ench. Fung. 296. 1886.

≡ *Thelebolus coemansii* (Boud.) Brumm., Persoonia 16: 433. 1998.

Type: Type specimen not preserved; type represented by Boudier's illustration. *Type locality*: **France**, Dép. Seine-et-Oise, Montmorency.

Ascophanus minutissimus Boud., Annls Sci. Nat. (Bot.), Sér. V, 10: 243, pl. 10 f. 29. 1869.

≡ *Ascobolus minutissimus* (Boud.) Quél., Ench. Fung. 296. 1886.

Type: **France**, Dép. Seine-et-Oise, Montmorency, E. Boudier *s.n.*, *s. dat*. (isotype ex PC; slide studied from S).

Ascobolus tetricum Carestia, in Rabenhorst, Fungi Europ. Exs. No. 1236. 1869 [nomen nudum, cf. Saccardo, Syll. Fung. 8: 524. 1889]; Hedwigia 8: 88. 1869 [nom. nud.]; Bot. Ztg. 27: 431. 1869 [nom. nud.].

≡ *Ascophanus tetricum* Carestia ex Rehm, Rabenh. Kryptog.-Fl. I (3): 1087. 1895.

Type: Rabenhorst, Fungi eur. exs. No. 1236 (isotypes studied from G, K, and PRM).

Excluded

Thelebolus terrestris Alb. & Schw., Consp. Fung. Lusat. 71. Tab. II f. 4. 1805; Fries, Syst. Mycol. 2: 307. 1822 (sanctioning).

≡ *Byssonectria terrestris* (Alb. & Schw. : Fr.) Pfister, Mycologia 85: 953. 1993.

Type: **Germany**, Moholzer Haide, on the ground, Albertini & Schweinitz, *s. dat.* (Herb. C.H. Persoon, L). A psychrophilic fungus with 4–8-spored asci, belonging to *Octospora* Hedw. or *Byssonectria* P. Karst. *emend*. Korf.

Description of *Thelebolus microsporus*

Colonies on carrot agar (Montemartini 1993) attaining a diameter of 3–5 cm in two wk at 15 °C, pale pinkish; ascomata developing abundantly after a consecutive period of 2 to 3 wk at 10–15 °C, especially when growing superficially and exposed to (directed) light developing "apothecioid" ascomata with a hymenial layer and asci directed towards the maximum of light intensity; otherwise (in the dark) forming irregularly disposed hymenial elements. Ascomatal initials consisting of narrowly coiled or contorted side branches of aerial or substrate mycelium soon forming compact masses. Mycelium hyaline. Hyphae 0.8–2 µm diam, abundantly septate, branched, rich in oleaginous globules. *Ascomata* closely crowded, superficial or immersed, (25–)45–205(–270) µm diam, brown, at first closed, cleistohymenial, and subglobular, sometimes not opening, but often opening in late-mesohymenial or telohymenial phase by irregular rupturing of the wall in the upper part and becoming lenticular to semiglobular; surface almost smooth with irregularly torn fragments of the cortical excipulum covering the lateral sides; margin very irregular or absent. Hymenium about 35–50 µm thick. Hypothecium and medulla not clearly differentiated. Cortical excipulum (peridium) not very compact, 7–12 µm thick, consisting of strongly branched and septate cohering hyphae 3–5 µm wide, without interhyphal spaces (*textura epidermoidea*), at the outside covered with varying amounts of amorphous brown intercellular pigment. *Asci* 5–80(–100) in an ascoma, irregularly cylindric-clavate with a short stalk, rounded above, rather thick-walled, without an operculum, at first (17–)25–47(–65) × (5–)6–10(–11) µm, but strongly stretching shortly before dehiscence up to 80–125 × 20–26 µm, 8-spored; the wall not staining blue with iodine, consisting of a thick, rigid outer layer and a thin, elastic inner one, easily separating at maturity; finally when exposed to light forcefully discharging their contents as a single projectile through a large irregular tear in the outer ascus-wall above the level of a subapical ring-shaped wall thickening. *Ascospores* irregularly biseriate or uniseriate, ellipsoid, occasionally slightly asymmetrical (length / width ratio (1.5–)1.7–2.4(–2.7), av. 1.6–2.1), hyaline, rather variable in size and shape, (5.5–)6.1–8.0(–9.0) × (3.0–)3.5–4.5(–5.2) µm (av. 7.5 × 3.8 µm), without conspicuous oil drops or granules, not easily producing air-bubbles, without mucilaginous substance, smooth. *Paraphyses* septate, at first irregularly slender cylindrical 2.0–3.2 µm wide, branched near the base, becoming filiform 0.8–2.3 µm thick, simple, hyaline near the base, in material grown on dung from temperate regions often covered with a layer of amorphous brown pigment near the end, 2.3–3.5 µm thick, often conspicuously enlarged up to 3.5–7 µm at the tip, not embedded in mucus; when young containing many small granules and oleaginous globules. *Conidiophores* and conidia not found.

Habitat

On dung of cow, horse, sheep, deer, elk, moose, dog, porcupine, rabbit, human, and black grouse, also on mud or soil polluted with skua dung, and leaves of *Lactuca sativa*. Also isolated from tracheae, cloacae, intestines, and feathers of Antarctic birds like skua, gull, egret, and giant petrel.

Distribution

Known from Europe, North-America and Antarctica, especially during the cold season and from colder regions.

Selected illustrations

Boudier (1869): pl. 10 f. 28 (*Ascophanus subfuscus*); Id.: pl. 10 f.29 (*A. minutisimus*); Id.: pl. 10 f. 30 (*A. coemansii*); Kimbrough (1972): pl. 16 fs. 7–8, pl. 17 fs. 9–11 (*T. microsporus*); Kimbrough & Luck-Allen (1974): fs. 1, 2, 4–7 (*Lasiothelebolus oblongisporus*, f. 3 excluded = anamorph of *Chalara* sp.); Montemartini *et al*. (1993): fs. 1–9; van Brummelen (1998): f. 1, 14 a–e (*T. microsporus*); Id.: f. 2, 14 f, g (*T. coemansii*).

Additional specimens and cultures examined

Great Britain, England: s. loc., C.E. Broome *s.n.*, 1864 (K-A2525, probably part of the type of *Ascobolus microsporus*, in Herb. M.J. Berkeley). W. Yorkshire, Bradford, on dung, H.I. Soppitt *s.n.*, *s. dat.* (NY). Shropshire, Shrewsbury, on cow dung, W. Phillips *s.n.*, *s. dat.*, distributed in M.C. Cooke, F. Brit. Exs. Ed. 2, No. 657, as *Ascobolus subfuscus* (PAD). Cheshire, on sheep dung, J. Holland *s.n.*, VI.1900 (NY); id. VI.1902 (NY). Somerset, Batheaston, on dung of cow and sheep, C.E. Broome *s.n.*, *s. dat.*, distributed in Rabenhorst, F. europ. No. 977, as *Ascobolus microsporus* (BR, HBG, PRC, isotypes of *A. microsporus* Berk. & Br.; NY, drawing by G. Massee). Essex, Warley Common, on sheep dung, C.E. Broome *s.n.*, 15 Oct. 1878 (K-A2996). London, Zoo, on deer dung, G. Massee, 1900 (NY, drawing). S. loc., on cow dung, M.C. Cooke, distributed in M.C. Cooke, F. Brit. Exs. Ed. 2 No. 657, as *Ascophanus subfuscus* Boud. (NY, drawing).

Sweden, Knivsta near Stockholm, on horse dung, 1897, *J. Vleugel* (S-A788, as *Ascobolus glaber*). Nacka near Stockholm, on dung of elk, von Lagerheim*, s. dat*., in Rehm, Ascom. Exs. No. 1271, sub *Saccobolus depauperatus* f. *denigratus* Rehm (PAD, W).

Finland, Abo region, Pisparisti skog and near Mustiala, on horse dung, P.A. Karsten, May *s. dat.*, distributed in Fungi Fenn. Exs. No. 655 (isotype of *Ascobolus polysporus* P. Karst. subsp. *punctiformis* P. Karst., K).

Denmark, Amager, on human dung, E.C. Hansen *s.n., s. dat*., distributed in Rabenhorst, F. europ. No. 2019, as *Ascophanus subfuscus* (NY).

Netherlands, Prov. N.-Holland: Overveen, on horse dung, J. van Brummelen *s.n.*, 21 June 1973 (L). Prov. Overijssel: Ommen, Eerder Achterbroek, on cow dung, J. van Brummelen *1567*, 1569, 13–14 Oct. 1962 (L). Prov. Gelderland: Angerloo near Lathum, on bird's dung, J. van Brummelen *5212*, 5 May1978 (L). Prov. Limburg: Echt, De Doodt, on cow dung, J. van Brummelen *1529*, 6 Oct. 1962 (L).

France, Finistère (near Brest), on dog dung ('*album graecum*'), Crouan *s.n.*, 20 Feb. 1868 (CO-A2386, as *Ascobolus pygmaeus* Crouan, unpublished name); Id., Crouan *s.n.*, 6 March 1870 (CO-A2387, as *A. pygmaeus* Crouan). Finistère, s. loc., on dog dung, Crouan, 28 Feb. 1864 (PC-A2432, as *Peziza subfusca* Crouan, nov. sp.; holotype). Seine-et-Oise, Montmorency, on cow dung, É. Boudier, Oct. 1879 (PC-A4035 and PC-A4034, as *Ascophanus minutissimus* var. *vaccinus*, det. Boudier).

Poland, Prov. Wroclaw, Kamien, on cow dung, J. van Brummelen 2016, 2 Sep. 1966 (L).

Germany, Franken: Obernesselbach, on cow dung, *H. Rehm s.n.*, Apr. 1875 (S-A664, as *Ascobolus minutissimus*); Obernesselbach, on cow dung, H. Rehm, Dec. 1878 (S-A657, as *Ascobolus minutissimus*); Bavaria: Bayerischer Alpen, Reiteralp bei Berchtesgaden, on dung of black grouse, Ade, Sep. 1910, distributed in Rehm, Ascom. Exs. No. 1908, as *Ascophanus tetricum* (Ces.) Rehm (CUP, MPU, NY, and W).

Switzerland, Wädenswill, on leaf of *Lactuca sativa*, T.H. Diener *s.n.*, May 1949 (culture CBS 115.53).

Austria, Tyrol: near Proxmar, on cow dung, Aug. 1872, H. Rehm (S-A637, as *Ascobolus minutissimus*). Vienna, cultured on dung, A. Heimerl *s.n.*, 1889, distributed as microscopic slide in Rehm, Ascom. No. 1016, as *Rhyparobius fallax* (Auersw.) Heimerl (NY).

Italy, Alto Adige, Riva, on dung of black grouse (*Tetrao tetrix*), Carestia *s.n.*, 1864, distributed in Rabenhorst, F. europ. No. 976a, as *Ascobolus cesatii* Carest. in lit. (BR, G, HBG, PRM, isotypes of *A. cesatii* Carest.). Riva (Valesia), on dung of *Tetrao tetrix*, Carestia *s.n.*, Nov. 1866, distributed in Rabenhorst, F. europ. No. 1236, as Carest. (G, K, NY, isotypes of *A. tetricum* Carestia).

Canada, Ontario: Algonquin Park, Sproute Park, on porcupine dung, R.F. Cain 30485, 17 Sep. 1954 (UPS, as *Saccobolus depauperatus*).

U.S.A. Idaho: Payette Lake, on cow dung, H.E. Bigelow *s.n.*, 2 July 1954 (G-A1036). Utah: Duchesne, on rabbit dung, R.F. Cain *s.n.*, 20 Aug. 1957 (CUP 47616). Wyoming: Shell Canyon, from deer dung, E.R. Luck-Allen *s.n.*, 20 March 1962 (culture TRTC 45568, CBS 716.69). Colorado: Geneva Creek Canyon, on rabbit dung, F.J. Seaver & E. Bethel 3–12, Sep. 1910 (NY); Jackson Co., Cameron Pass, on cow dung, C.T. Rogerson *3702*, 20 Aug. 1954 (NY, as *Ascophanella microspora* (Crouan) Korf, det. Korf). Castle Canyon, alt. 2700 m, on cow dung, F.E. & E.S. Clements *s.n.*, 19 July 1906 (E, in Cryptog. Form. Colorad. No. 301, as *Saccobolus obscurus*). Iowa: Decorah, on cow dung, E.W.D. Holway *s.n.*, 9.V.1886 (CUP); Iowa City, on cow dung, F. J. Seaver *s.n.*, 4 May 1904 (CUP, Herb. E.J. Durand 475); S. loc., on cow dung, F.J. Seaver *s.n.*, 4 May 1904 (NY). New York: Saranac Lake, S. of P. Smith College, on deer dung, E.R. Luck-Allen *s.n.*, 12 Sep. 1965 (TRTC 45247, only the lectotype part of the mixed type specimen of *Lasiobolus oblongisporus* Kimbr. & Luck-Allen, cf. van Brummelen, 1998: 428). Massachusetts: Cambridge, on dog dung, W.G. Sturgis *s.n.*, March 1890 (BPI, NY). New Jersey: New York City, Bronx, on cow dung, F.J. Seaver & B.O. Dodge *s.n.*, 5 May 1911 (NY).

Antarctica, Victoria Land (72–75° S. lat., 162–169° E. long.; expeditions during 1989–1991 by the Italian National Program for Antarctic Research): Edmonson Point (74° 20′ S., 165° 08′ E.), Southern Lake, Camp 115, on mud polluted with skua dung, A. Montemartini Corte 115/1 bis (culture from Instituto di Botanica dell′Università di Genova); id., Camp 56, A. Montemartini Corte 56/1 (monosporic culture from Genova); id., Lake Eneide, Meteorological Camp (74° 42′ S., 164° 06′E.), A. Montemartini Corte 65/1 (monosporic culture from Genova); id. near German Base, Gondwana Pond (74° 37′ S., 164° 13′ E.), A. Montemartini Corte Gondw. S 1 (culture from Genova); id., near German Base, Gondwana Pond, A. Montemartini Corte Gondw. S 1 bis (culture from Genova); id., Baker Rocks Pond (74° 15′ S., 165° 01′ E.), A. Montemartini Corte BR 3 (culture from Genova). Hallett Peninsula (72° 30' S., 170° 10' E.), Hallett Station, from soil near nest of skua, O.L. Lange *s.n.*, 1 Nov. 1966 (culture CBS 734.68).

Notes

We sequenced some CBS strains (CBS 734.68, CBS 115.53 and CBS 716.69) which were analysed morphologically by Montemartini *et al*. (1993).

Thelebolus microsporus was the most frequently isolated species in the course of our study, with a total of 43 strains from Antarctica whose identity was confirmed by ITS sequencing and/or microsatellite typing. The species was isolated repeatedly from sampling sites in Ace, Organic, Manning and Reid lakes, and has also been confirmed from King George Land about 4000 km away. The species is known to have a world-wide distribution, as there are confirmed strains from North-America and Europe. Judging from their morphology, several type materials of previously described species were included as synonyms. These were collected on various types of mammal dung in the Northern temperate zone. Hence it is likely that the species is indeed widely distributed in temperate climates. The vector of distribution is probably avian, by intestinal passage and dissemination via faeces. This is underlined by the absence of other obvious ecological parameters: the species occurs in Antarctica as well as in temperate climates (Table 3), and the lakes from which it was recovered vary from fresh to hypersaline (Tables 1, 5).

No ITS difference was detected between strains of population G1 from Northern and Southern hemispheres, but consistent differences were noted between three Antarctic populations G1, 2 and 3. The wide distribution of G1 in Antarctica and on other continents suggests panmixis; the group consistently reproduces with ascospores (Table 3). As *Thelebolus* has an association with sea bird intestines (Leotta *et al*. 2002) it is likely to have been carried over very large distances. For example, the petrels from which strains of group G3 were isolated are known to migrate between Arctic and Antarctic climatic zones. There is, however, limited bird migration between the different regions of Antarctica, and thus little gene flow between populations after they are established. Thus genotypes may have been brought to the Antarctic on separate occasions, and may subsequently have gone through a regional process of speciation. This proves that this *Thelebolus* species is able to grow and survive under Antarctic conditions, and the populations can, therefore, be regarded as endemic psychrophiles. Populations G2 and G3 show a limited distribution. In Northern Antarctica (G1 + G3) as well as in the Vestfold Hills (G1 + G2) mixed populations occur. Taking into account the likeliness of sampling effects, we suggest that the three populations are present all over Antarctica, albeit regionally with different frequencies.

Microsatellite data correctly identified three Antarctic species recognised on the basis of ITS and β-tubulin data, but proved to be more sensitive in that infra- as well as inter-specific entities were clearly different (Fig. 8). Genotype G2 strains from Reid and Manning lakes (Larsemann Hills) had identical microsatellite profiles; this genotype was also found in Organic lake with very different ecological conditions (hypersaline) and in Fryxell lake with another geography (Victoria Land). Organic lake has the highest diversity of local subtypes of *T. microsporus* (Table 5). This suggests a slow, local evolution of *T. microsporus* from this lake. Transport of the fungus by visiting birds is less likely, since this would lead to near-even distribution of genotypes across the lakes. In contrast, Druzhby lake has the highest diversity, including different species, suggesting that bird-transport to the lake is the most frequent. This matches with the observation that this is the largest lake frequented by skuas.

Thelebolus stercoreus Tode : Fr. Figs 9–10.

Thelebolus stercoreus Tode, Fungi Mecklenb. Sel. 1: 41, t. 7, f. 56. 1790; Fries, Syst. Mycol. 2: 307. 1823 (name sanctioning).

Type: Type specimen not preserved. *Type locality*: **Germany**, Mecklenburg.

Ascobolus crustaceus Fuckel, Fungi rhen. No. 1858. 1866; Hedwigia 5: 4. 1886.

≡ *Pezizula crustacea* (Fuckel) P. Karst., Bidr. Känn. Finl. Nat. Folk 19: 81.1871

≡ *Rhyparobius crustaceus* (Fuckel) Rehm, Ascom. Exs. No. 52b. 1872.

≡ *Thelebolus crustaceus* (Fuckel) Kimbr., in Kobayasi *et al*., Ann. Rep. Inst. Fermentation, Osaka 3: 50. 1967.

Type: Fuckel, Fungi Rhen. No. 1858 (isotype studied from B). In the type asci with 64 spores per ascus are found.

≡ *Ascobolus myriadeus* P. Karst., Fungi Fenn. Exs. 552. 1866 (*pro parte*: the fungus with 48 to 64 spores in an ascus).

Lectotype: **Finland**, near Wasa, on dung of unidentified animal, P.A. Karsten *s.n.*, IV.1864, distributed in Fungi Fenn. Exs. 552 (*pro parte*: the fungus with 48 to 64 spored asci; studied from K). This fungus was found identical with *Rhyparobius crustaceus* (Fuckel) Rehm, with 64-spored asci, by both Karsten (1871: 81) and Rehm (1895: 1103).

Ascobolus myriadeus P. Karst., Fungi Fenn. Exs. No. 552. 1866 (*pro parte*: the fungus with 24 to 32 spores in an ascus).

≡ *Ascobolus crustaceus* Fuckel subsp. *myriadeus* P. Karst., Notis. Sälsk. Fenn. Förh. 11: 208. 1870 ≡ *Pezizula myriadea* (P. Karst.) P. Karst., Bidr. Känn. Finl. Nat. Folk. 19: 81. 1871.

≡ *Rhyparobius crustaceus* (Fuckel) Rehm var. *myriadeus* (P. Karst.) Sacc., Syll. Fung. 8: 935. 1889.

Type: **Finland**, near Wasa, P.A. Karsten *s.n.*, Apr. 1864, distributed in Fungi Fenn. Exs. No. 552F (isotype; studied from K). This fungus was found growing together with the closely related fungus with 64-spored asci identical with *Rhyparobius crustaceus* (Fuckel) Rehm from which it was said to differ only in the number of spores in an ascus. A

study of the type revealed that ascomata with 64, and 256 spores per ascus were found growing closely together.

Ascobolus cookei Crouan, Fl. Finistère 56. 1867.

≡ *Rhyparobius cookei* (Crouan) Boud., Annls. Sci. Nat. (Bot.), Sér. V, 10: 238. 1869.

Type: **France**, Finistère, s. loc., on *Album graecum,* Crouan *s.n.*, 7 Apr. 1860 (in pencil also: III.1862; studied from CONC-A2391). In the type specimen asci with 32, 48 and 64 ascospores were found. Le Gal (1961) found in the same package also fruit-bodies with at least 150–180 ascospores in an ascus.

Ascobolus polysporus P. Karst., Fungi Fenn. Exs. 656. 1867 (not *Ascobolus polysporus* Auersw., Hedwigia 7: 51. 1868).

≡ *Pezizula polyspora* (P. Karst.) P. Karst., Bidr. Känn. Finl. Nat. Folkr. 19: 82. 1871.

≡ *Rhyparobius polysporus* (P. Karst.) Speg., An. Soc. Cient. Argent. 10: 24. 1880.

≡ *Thelebolus polysporus* (P. Karst.) Otani & Kanzawa, Trans. Mycol. Soc. Japan 11: 45. 1970.

Type: **Finland**, Abo region, Pisparisti skog and near Mustiala, on horse dung, P.A. Karsten *s.n.*, Mai *s. dat.*, in P. Karsten, Fungi Fenn. Exs. 656 (isotype, studied from K). According to Karsten (1871) this fungus has 150 to 200 spores in an ascus.

Ascobolus polysporus Auersw., Hedwigia 7: 51. 1868 (not *Ascobolus polysporus* P. Karst., Fungi Fenn. Exs. 656. 1867).

Type: Type specimen not preserved. *Type locality*: **Germany**, Rosenthal near Leipzig. A *Thelebolus* described with 60 or more spores in an ascus.

Ascobolus caninus Auersw., Hedwigia 7: 52. 1868 (not *Ascobolus caninus* Fuckel, Hedwigia 5: 3. 1866).

≡ *Rhyparobius caninus* (Auersw.) Sacc., Syll. Fung. 8: 539. 1889.

≡ *Rhyparobius crustaceus* (Fuckel) Rehm var. *caninus* (Auersw.) Boud., Hist. Class. Discom. Eur. 78. 1907.

≡ *Thelebolus caninus* (Auersw.) Jeng & Krug, Can. J. Bot. 55: 2998. 1977.

Type: **Germany**, Rosenthal near Leipzig, on rabbit dung, B. Auerswald *s.n.*, *s. dat*. (WRSL-A1711, holotype; B-A3291, isotype). The holotype specimen shows a *Thelebolus* with 32 smooth spores per ascus, as described by Auerswald, but ascomata with over 250 spores per ascus are also found (Fig. 12B).

Rhyparobius brunneus Boud., Annls Sci. Nat. (Bot.), Sér. V, 10: 237, pl. 9 f. 23. 1869.

≡ *Ascobolus brunneus* (Boud.) Cooke, Grevillea 5: 153. 1876 (not *Ascobolus brunneus* Cooke, Handb. Brit. Fungi 728. 1871).

Type: Type specimen not preserved; type represented by Boudier's illustration. *Type locality*: **France**, Dép. Seine-et-Oise, Montmorency. A *Thelebolus* described by Boudier (l.c.) with 32 spores per ascus.

Rhyparobius dubius Boud., Annls Sci. Nat. (Bot.), Sér. V, 10: 240, pl. 10 f. 26. 1869.

≡ *Ascobolus dubius* (Boud.) Quél., Ench. Fung. 296. 1886.

≡ *Thelebolus dubius* (Boud.) Doveri var. *dubius* (Boud.) Doveri, Fungi Fimic. Ital. 527. 2004.

Type: Type specimen not preserved; type represented by Boudier's illustration. *Type locality*: **France**, Dép. Seine-et-Oise, Montmorency. A *Thelebolus* described with 128 spores per ascus.

Rhyparobius felinus Boud., Annls Sci. Nat. (Bot.), Sér. V, 10: 238, pl. 10 f. 25. 1869.

≡ *Ascobolus felinus* (Boud.) Quél., Ench. Fung. 296. 1886.

Type: Type specimen not preserved; type represented by Boudier's illustration. *Type locality*: **France**, Dép. Seine-et-Oise, Montmorency. A *Thelebolus* described with 64 spores per ascus.

Pezizula cesatii (Carest.) P. Karst. subsp. *hyalinella* P. Karst., Bidr. Känn. Finl. Nat. Folk 19: 83. 1871.

≡ *Rhyparobius hyalinellus* (P. Karst.) Sacc. forma *hyalinellus* (P. Karst.) Sacc., Syll. Fung. 8: 542. 1889.

Type: **Finland**, Haarankorpi forest near Mustiala, on dung of black grouse (*Tetrao tetrix*), P.A. Karsten *s.n.*, 8.IX.1870. A fungus with 72-spored asci, growing together with the type of *Pezizula cesatii* subsp. *promiscua* P. Karst., with 32-spored asci.

Pezizula cesatii (Carest.) P. Karst. subsp. *promiscua* P. Karst., Bidr. Känn. Finl. Nat. Folk 19: 83. 1871.

≡ *Rhyparobius crustaceus* (Fuckel) Rehm forma *promiscua* (P. Karst.) Rehm, Rabenh. Kryptog.-Fl. I (3): 1104. 1896

≡ *Rhyparobius hyalinellus* (P. Karst.) Sacc. forma *promiscuus* (P. Karst.) Sacc., Syll. Fung. 8: 542. 1889.

≡ *Thelebolus hyalinellus* (Fuckel) Rehm forma *promiscua* (P. Karst.) Rehm, Rabenh. Kryptog.-Fl. I (3): 1104. 1896.

≡ *Thelebolus hyalinellus* (P. Karst.) Doveri var. *promiscuus* (P. Karst.) Doveri, Fungi Fimic. Ital. 528. 2004.

Type: **Finland**, Haarankorpi forest near Mustiala, on dung of black grouse (*Tetrao tetrix*), P.A. Karsten *s.n.*, 8 Nov. 1870 (not studied; *fide* Rehm). A *Thelebolus* described with 32-spored asci, growing together with the type of *Pezizula cesatii* (Carest.) P. Karst. subsp. *hyalinella* P. Karst., with 72-spored asci.

Rhyparobius monascus Mouton, Bull. Soc. R. Bot. Belg. 25: 141. 1886

≡ *Thelebolus monascus* (Mouton) Boud., Hist. Class. Discom. Eur. 79. 1907.

Type: **Belgium**, Angleur, near Liège, on deer dung, V. Mouton 254, IV.1884 (BR, lectotype). A *Thelebolus* with over 500 spores per ascus.

Rhyparobius pachyascus Zukal, in Rehm, Ascom. Exs. No. 914b. 1887; Hedwigia 27: 167. 1888; Heimerl, Jber. K.K. Ober-Realschule Bezirke Sechshaus Wien 15: 27. 1889; Rehm, in Rabenh., Kryptog.-Fl. I (3): 1105 .1895.

Type: **Austria**, near Vienna, on dung of horse and rabbit, H. Zukal *s.n.*, *s. dat*., distributed as microscopic slides in

Rehm, Ascom. Exs. No. 914b (isotype, studied from W). A *Thelebolus* with 64 to 200 spores per ascus.

Thelebolus nanus Heimerl, Jber. K.K. Ober-Realschule Bezirke Sechshaus Wien 15: 30, pl. 1 f. II. 1889.
Holotype: **Austria**, Vienna, Pressbaum, on hare dung, A. Heimerl, Summer 1889 (W, slide collection Heimerl No. 42). A representative of *Thelebolus* with 1000 to 1500 spores per ascus.

Rhyparobius dubius Boud. var. *lagopi* Boud. ex Rea, Trans. Br. Mycol. Soc. 3: 378. 1912.
≡ *Thelebolus dubius* (Boud.) Doveri var. *lagopi* (Boud. ex Rea) Doveri, Fungi Fimic. Ital. 527. 2004.
Type: **Great Britain**, England, Perthshire, Dunkeld, Inver Woods, on dung of black grouse (*Tetrao tetrix*), Ch. McIntosh *s.n.*, 28 Oct. 1911 (not studied). A *Thelebolus* with 128-spored asci (Rea 1912).

Streptotheca obscura Seaver, N. Am. Cup-fungi (Operc.) 143, pl. 12 f. 4. 1928.
≡ *Thelebolus obscurus* (Seaver) Eckbl., Nytt Mag. Bot. 15 (1, 2): 23. 1968.
Type: **U.S.A.**, New York, Tarrytown, on rabbit dung, F.J. Seaver *s.n.*, 19 May 1916 (studied from NY). A representative of *Thelebolus* with 64 spores per ascus (Eckblad, 1968).

Streptotheca psychrophila Bergman, in Bergman & Shanor, Mycologia 49: 879. 1957.
≡ *Thelebolus psychrophilus* (Bergman) Eckbl., Nyt. Mag. Bot, 15: 23. 1968.
Type: **Sweden**, Stockholm, isolated from soil of pine forest by Mrs. D. Fennell A-£230, 1952 (studied from K, part of a dried culture; living cultures NRRL A-5230, CBS 375.58, CBS 718.69). A *Thelebolus* described with 32 spores per ascus.

Rhyparobius cookei (Crouan) Boud. var. *maritimus* Apinis & Chesters, Trans. Br. Mycol. Soc. 47: 433. 1964.
Type: **Great Britain**, England, Lincolnshire, Gibraltar Point, isolated from soil, A.E. Apinis *s.n.*, Dec. 1960 (isotype: culture IMI 102473 = CBS 356.66, studied from CBS). A *Thelebolus* described with 48-spored asci.

Rhyparobius diaphanus Faurel & Schotter, Rev. Mycol. 30: 148 f. 3. 1965.
Type: Type specimen not preserved. Type represented by Faurel & Schotter's illustration. *Type locality*: **Algeria**, Central Sahara, Tefedest, along the stream Adenek, alt. 1600 m.

Excluded
Ascobolus solms-laubachii Rabenh., F. Europ. Exs., Cent. V, No. 420. 1862; Rabenh., Bot. Ztg. 20: 198. 1862.
≡ *Rhyparobius solms-laubachii* (Rabenh.) Rehm, Rabenh. Kryptog.-Fl. I (3): 1101. 1895.
≡ *Ascozonus solms-laubachii* (Rabenh.) Brumm., Persoonia 16: 432. 1998.
Lectotype: Rabenhorst, F. Europ. Exs. No. 420 (Herb. Rehm, S). An *Ascozonus* with about 32 spores per ascus.

Ascobolus niveus Fuckel, Hedwigia 5: 4 pl.1 f. 3. 1866; Fuckel, Jber. Nassau. Ver. Naturk. 23–24: 289. 1870; Fuckel, Fungi Rhen. No. 2375. 1871 (not *Ascobolus niveus* Quél., C. R. Ass. Fr. Avanc. Sci. (Congr. Reims, 1880) 9: 674 pl. 9 f. 18. 1881).
≡ *Rhyparobius niveus* (Fuckel) Sacc., Syll. Fung. 8: 544. 1889.
≡ *Ascozonus niveus* (Fuckel) Boud., Hist. Class. Discom. Eur. 79. 1907.
≡ *Coprotus niveus* (Fuckel) Kimbr. *et al*., Canad. J. Bot. 50: 967. 1972.
Type: **Germany**, Mt. Rabenkopf, on dog dung, L. Fuckel *s.n.*, *s.dat.* (Winter), distributed in Fuckel, F. rhen. No. 2375 (cf. Fuckel, 1870: 289) (isotypes studied from BPI, HBG, L, W, ZT). Boudier (1907) included this in *Ascozonus*, although Fuckel (1866) described a large operculum at the end of the ascus (*"denum operculo magno rumpentibus")*. The presence, however, of fine white marginal hairs (*'extus margineque pilis concoloribus subtilissime puberulis'*), curved asci, and fusiform ascospores (l.c. pl. 1 f. 3c) all indicate a species of *Ascozonus*. In all isotype samples studied material of a 64-spored representative of *Ascozonus* was found. Empty asci of *Ascozonus* often show a truncate shape, especially when after spore release, the torn remains of the apex turn inwards below the level of the subapical ring. Several authors have concluded that the top of the ascus was shot off (*cf.* van Brummelen, 1998). Others considered the ring as the margin of the operculum, as did Fuckel. *Ascobolus niveus* Fuckel is a 64-spored representative of *Ascozonus* and becomes as such a synonym of *Ascozonus solms-laubachii* (Rabenh.) Brumm. The fungus described as *Coprotus niveus* by Kimbrough *et al*. (1972) is in need of another name.

Nectria myriospora Crouan, Fl. Finistère 37, Suppl. pl. f. 15. 1867.
≡ *Rhyparobius myriosporus* (Crouan) Boud., Annls. Sci. Nat. (Bot.), Sér. V, 10: 240. 1869.
≡ *Chilonectria myriospora* (Crouan) Sacc., Michelia 1: 279. 1878.
≡ *Ascobolus myriosporus* (Crouan) Quél., Ench. Fung. 296. 1886.
Type: **France**, Finistère, Crouan *s.n.*, *s. dat.* (studied from CO-A2442; a rather poor collection). According to Crouan & Crouan (1867) this fungus has 100 to 150 ascospores in an ascus, while Boudier (1869: 241) estimated the spore number from 200 to 250. It possibly belongs to the *Eurotiales*.

Ascobolus leveillei Renny, Trans. Woolhope Nat. Field Club 1873: 130. 1873; Renny, J. Bot. (Lond.) 12: 356. 1874 (not *Ascobolus leveillei* Crouan, Fl. Finistère 57.1867).
≡ *Rhyparobius leveilleanus* (Renny) Phill., Br. Discom. 301. 1887 (name change).
≡ *Ascozonus leveilleanus* (Renny) Boud., Hist. Class. Discom. Eur. 79. 1907.

Type: Not known to be in existence. *Type locality*: **Great Britain**, Hereford. An *Ascozonus*, described with 64 to 96 spores per ascus.

Rhyparobius winteri E. Marchal, Bull. Soc. R. Bot. Belg. 24: 71. 1885.

≡ *Coprotus winteri* (E. Marchal) Kimbr., in Kimbr. & Korf, Am. J. Bot. 54: 22. 1972.

Type: **Belgium**, Ardennes, Vervoeren (Tervueren), on deer dung, *E. Marchal s.n.*, "vere 1884 et autumno 1885" (BR-B692, Herb. E. Marchal). A 256-spored representative of the genus *Coprotus* Korf & Kimbr. in Kimbr. & Korf, 1967: 21 (≡ *Leporina* Velenovsky, 1947: 154). The name *Coprotus* needs conservation against the earlier name *Leporina*.

Ascozonus oligoascus Heimerl, Jber. K.K. Ober-Realschule Bezirke Sechshaus Wien 15: 27, pl. 1 f. 1. 1889.

≡ *Rhyparobius oligoascus* (Heimerl.) Rehm, Rabenh. Kryptog.-Fl. I (3): 1105. 1896.

Lectotype (chosen here): **Austria**, Vienna, Pressbaum, on deer dung, A. Heimerl, Summer 1889 (W, slide collection Heimerl No. 33). A species of *Ascozonus*.

Ascobolus niveus Fuckel *sensu* G. Winter

≡ *Rhyparobius murinus* Rehm, Rabenh. Kryptog.-Fl. I (3): 1102. 1895.

Type: **Germany**, near Leipzig, on dung of mouse, G. Winter *s.n.*, *s. dat*. (HBG-A467). According to Rehm (1895) close to *Rhyparobius albidus* Boud. [≡ *Coprotus albidus* (Boud.) Kimbr.].

Description of *Thelebolus stercoreus*

Ascomata solitary or gregarious, superficial or immersed, at first subglobular and closed, cleistohymenial and opening in the mesohymenial or telohymenial phase; when immersed remaining closed, often without clear polarity and not opening before disintegration; especially when growing superficially and exposed to (directed) light developing ´apothecioid' ascomata with a hymenial layer and asci directed towards the maximum of light intensity; in free-living uni-ascal forms becoming subglobular, ovoid or ellipsoid, (40–)55–220 µm diam, reaching 300–400 µm; hyaline, pale yellowish or pale brown; margin absent; often remaining closed, but usually opening near the top with irregular large tears in the cortical layer exposing the hymenium. Hymenium with a variable number of asci: 10–40 when 32-spored, 7–12 when 64-spored, 2–5(–10) when 256-spored, and 1 or 2 when 500- to over 2000-spored. Hypothecium not differentiated. Cortical excipulum clearly differentiated, pale yellowish to brown, at first 20–35 µm thick of two to four layers of isodiammetric cells 4.5–12 µm diam (*textura globulosa*) at maturity 15–20 µm thick of one to three layers of more or less flattened cells 4.5–12 × 4.5–7 µm (*textura angularis*). *Asci* 32- over 2000-spored, with variable shape and size, depending on the number of spores formed, when up to 64-spored broadly clavate (40–75 × 15–25 µm), when 256-spored ellipsoid (80–150 × 40–60 µm), when 500- to over 2000-spored at first subglobular to ellipsoid and later often slightly pointed at the top (80–260 × 80–170 µm); when exposed to the surface opening by an irregular tear in the apex above the level of an enforced subapical ring in the ascus wall and forcefully discharging its contents. *Ascospores* rather firmly united in a single cluster and ejected together, ellipsoid with blunt rounded ends, rarely slightly fusiform (length/width ratio 1.2–1.7, average 1.4–1.6), hyaline, in 32- or 64-spored asci 5.8–9 × 4–5 µm, in 256-spored asci (3.8–)4.5–7 × 2.8–4 µm, in 500- to over 2000-spored asci 4.5–8 × 3–4.5 µm; contents rather homogeneous, not easily producing air-bubbles, rather thin-walled, smooth or rarely very finely verrucose. *Paraphyses* rather scarce, often absent in representatives with a single ascus only, filiform, hyaline or with amorphous intercellular brown pigment, 2–3 µm thick, straight, forked or curved above, not or gradually enlarged upwards up to 4–5 µm at the tip, especially in "apothecioid", 32- and 64-spored representatives. *Mycelium* hyaline, often forming a thin white mycelial mat on the surface of the substratum; hyphae irregularly cylindrical, 2.3–7 µm wide. *Conidiophores* and conidia not found.

Distribution

Found on dung of cow, horse, sheep, goat, Burmese goat, deer, chamois, elk, moose, dog, fox, wolf, marmot, porcupine, rabbit, hare, arctic hare, black grouse (*Tetrao tetrix*), capercaillie (*Tetrao urogallus*), and partridge (*Perdicis cineriae*). Also isolated from soil, a human tumor and hands of a man. Especially frequent in cold and temperate regions and in isolated cold localities in tropical regions. Known from Europe, Africa, Asia, North-America, but not from Antarctica.

Selected illustrations

Boudier (1869): pl. 9 f. 23 (*Rhyparobius brunneus*); Id.: pl. 9 f. 24 (*R. cookei*); Id.: pl. 10 f. 25 (*R. felinus*); Id.: pl. 10 f. 26 (*R. dubius*); Id.: pl. 10 f. 27 (*R. myriosporus*); Heimerl (1889): f. II a (*T. nanus*); Ramlow (1906): f. 3, pl. IV; Seaver (1928): pl. 12 f. 4 (*Streptotheca obscura*); Id.: pl. 12 f. 5 (*Rhyparobius polysporus*); Id.: pl. 12 f. 6 (*R. monascus*); Bergman & Shanor (1957): fs. 1–7 (*Streptotheca psychrophila*); Kimbrough & Korf (1967): f. 1 a–e; Kimbrough (1972): pl. 16 fs 1–6); Jeng & Krug (1977): fs. 26–32 (*T. caninus,* with smooth ascospores); van Brummelen (1998): fs. 3, 15 a–e (*T. caninus,* with smooth ascospores); Id.: fs. 4, 5 c, d, 14 h (*T. crustaceus*); Id.: fs. 5 a, b, 15 f (*T. polysporus*); Id.: fs. 6, 15 g–i (*T. stercoreus*); Czymmek & Klomparens (1992): all figs. (*Thelebolus crustaceus*, ultrastructure of ascosporogenesis).

Additional specimens and living cultures examined

Great Britain, Scotland: Orkney Islands, Brough of Dearness, on rabbit dung, R. W. G. Dennis *s.n.*, 4 July 1970 (K-B372, as *Thelebolus myriosporus*); W. Ross, Pass of the Cattle, Applecross Forest, on sheep dung, J. van Brummelen 6536, 21 June 1982 (L); id. Melvaig, on sheep dung, J. van Brummelen 6518, 6556, 18–25 June 1982 (L); id. Cove, on sheep dung, J. van Brummelen 6510, 6512, 18.VI.1982 (L); id. near Rassall, on dung of capercaillie, J. van Brummelen 6559, 29 June 1982 (L); id. Loch Maree, near Hotel, on sheep dung, J. van Brummelen 6506, 6536, 18–21 June 1982 (L). England: Yorkshire: Scarborough, on rabbit dung, G. Massee *s.n.*, *s. dat*. (NY, drawing). Lincolnshire: Gibraltar Point, from soil, A.E. Apinis *s.n.*, May 1966 (type culture of *Rhyparobius cookei* (Crouan) Boud. var. *maritimus* Apinis & Chesters, IMI 002.473, CBS 356.66). Norfolk: Castle Rising, on rabbit dung, C.B. Plowright *s.n.*, 23 Jan 1899 (K-B373, as *Thelebolus myriosporus*), near Sherden, on rabbit dung, J.W.H. Trail *s.n.*, 22 Nov. 1888 (K-B371, as *Thelebolus myriosporus*). Leicester, Leicester, on horse dung, C.T. Ingold *s.n.*, 17 March 1943 (NY, photographs). Surrey, Reigate, on rabbit dung, E.S. Salmon *s.n.*, Nov. 1900 (NY, drawing). London: Kew, on rabbit dung, G. Massee *s.n.*, Oct.–Nov. 1900 (NY, drawings).

Sweden, Stockholm, from pine forest soil, Dr. Shanor *s.n.*, Apr. 1958 (type culture of *Streptotheca psychrophila* Bergman, NRRL A-5230, CBS 375.58, id. comm. D. Malloch CBS 718.69). S. loc. (near Stockholm), culture on agar-medium isolated from soil of a pine forest, D. Fennell A-£230, 1952 (K, part of the type of *Streptotheca psychrophila* Bergman; with 32-spored asci).

Finland, Abo region, Pisparisti skog and near Mustiala, on horse dung, P.A. Karsten *s.n.*, May *s. dat*, distributed in Fungi Fenn. Exs 656 (isotype of *Ascobolus polysporus* P. Karst., studied from K).

Netherlands, Prov. Drenthe: Kraloo, on deer dung, J. van Brummelen *s.n.*, 31 July 1961 (L). Prov. Flevoland: Robbenoord Bos, on deer dung, J. van Brummelen 6687, 1 Oct. 1982 (L). Prov. Utrecht: "Groeneveld" near Baarn, on rabbit dung, G.S. de Hoog No. 29, Feb. 1969 (culture CBS 129.69); id. G.S. de Hoog No. 1, 1968 (culture CBS 128.69). Prov. Gelderland: Deeler Woud, on sheep dung, G.S. de Hoog No. 125, 1969 (culture CBS 216.69); Wageningen, isolated from organic particle in sand, J.H. van Emden *s.n.*, 13 March 1967 (culture CBS 265.67). S. loc., isolated from tumor near eye-lid and from hands, Dr. Janke No. 172, June 1950 (culture CBS 485.50).

Belgium, Forêt de Beaufays, Lapinière à Angleur (Park), on hare dung, V. Mouton *s.n.*, March 1884 (BR-C130, as *Ascozonus cunicularius*).

France, Finistère: Kernenez, Forêt Dom. de Clohars-Carnoët, on deer dung, J. van Brummelen 8902, 21 Nov. 2002 (L); id. J. van Brummelen 8912, 29 Nov. 2002 (L); s. loc. (prob. near Brest), on "*album graecum*", 7 Apr. 1860 (in pencil also III.1862), Crouan (CO-A2391, holotype of *Ascobolus cookei* Crouan; annot. Crouan: "Cette curieuse espèce a quarante huit spores dans chaque de ces thèques"; in fact asci with 32, 48 and 64 spores are found); s. loc., on dog dung, Crouan *s.n.*, 6 Feb. 1868 (CO-A2422, CO-A2423, as "*Ascobolus cookei* Crouan, Florule du Finistère"); s. loc., on dog dung, Crouan *s.n.*, 6 March 1870 (CO-A2442, as *Ascobolus myriasporus* Crouan, unpublished name; CO-A2446, as *Nectria myriotheca* Crouan). Haute-Garonne: Luchon, on dung of marmot, Ch. Fourcade, *s. dat.*, distributed in Roumeguère, F. Sel. Exs. No. 5712, as *"Rhyparobius" polysporus* (CUP). Haute-Loire: Loire river near Vorey, on sheep dung, J. van Brummelen 8635, 24 Nov. 1999 (L); Lac du Bouchet, on deer dung, J. van Brummelen 8625, 18 Nov. 1999 (L). Doubs: Levier Marine, on deer dung, J. van Brummelen 8812, 12 Nov. 2001 (L); Tourbière de Frasne, Borbonnet, on deer dung, J. van Brummelen 8801, 22 Oct. 2001 (L); id. J. van Brummelen 8858, 14 March 2002 (L); Gros Crêt, Mouthe, alt. 1400 m, on dung of capercaillie (*Tetrao urogallus*) (comm. Mr. Moyne), J. van Brummelen 8918, 18 Dec. 2002 (L); Forêt du Jura, Levier, on deer dung, J. van Brummelen *8842*, 14 March 2002 (L); Forêt du Jura, Rte. du Pont de la Marine, on deer dung, J. van Brummelen 8843, 4 March 2002 (L); Chassagne St. Denis, Dents de Lerii, on dung of chamois (comm. Mr. Moyne), J. van Brummelen 8852, 11 March 2002 (L). Allier: Forêt des Colettes, on cow dung, J. van Brummelen 7542, 15 Oct. 1986 (L).

Poland, Silesia: near Ziebigk-Dessau, on horse dung, R. Staritz *s.n.*, Apr. 1907 (BPI, CUP 6896, as *Rhyparobius polysporus* P. Karst. var. *staritzii* P. Henn.).

Germany, Hessen: near Oestrich, on dog dung ("*album graecum*"), Fuckel *s.n.*, *s. dat.*, distributed in F. rhen. No. 1858 (BR, L, isotype of *Ascobolus crustaceus* Fuckel; with 32-spored asci). Sachsen: Königstein, on horse dung, W. Krieger *s.n.*, Apr. 1895, distributed in Rabenhorst-Pazschke, F. Europ. et Extraeurop. No. 4174, as *Rhyparobius crustaceus* (BPI, NY, W); "Nonnenthal" near Eisleben, on dung of partridge (*Perdicis cinerieae*), J. Kunze *s.n.*, Apr. 1875, distributed in F. sel. Exs. No. 189, as *Ascobolus polysporus* Auerswald (BPI, L, LE, NY, PAD; with about 48-spored asci); Rosenthal near Leipzig, on rabbit dung, Auerswald *s.n.*, *s.dat.* (BRSL-A1711, holotype of *Ascobolus caninus* Auerswald; B-A329, isotype); Königstein, on horse dung, W. Krieger *s.n.*, 6 Apr. 1895, distributed in Krieger, F. Saxon. No. 1135, as *Rhyparobius crustaceus* (BPI, CUP, NY, W); Id. 10 Apr. 1895, distributed in id. No. 1136, as *Rhyparobius polysporus* (BPI, CUP, NY). Franken: Sugenheim, on dung, H. Rehm *s.n.*, March 1871 (NY). Bavaria: Oberpfalz, Amberg, Ammerbachs-Tal, on hare dung, K. Sarcs *9.65*, 1 Apr. 1946 (NY); Regenstauf, Bahndamm, on dog dung, H. Rehm *s.n.*, 3 Feb. 1884 (CUP 47739); id. Feb.–March 1884, distributed in Rehm, Ascom. No. 771, as *Rhyparobius crustaceus* (NY); Zugspitze, on cow dung, H. Rehm *s.n.*, Oct. 1900, distributed in Rehm, Ascom. No. 52b, as *Rhyparobius crustaceus* (BPI, CUP, W).

Switzerland, Speer near Wesen, on cow dung, S. Gloristy *s.n.*, June 1880 (NY).

Austria, Tyrol: Kühtei, Hochalpen, on cow dung, H. Rehm *s.n.*, VIII.1892 (CUP 47741); id. Aug. 1972, distributed in Rehm, Ascom No. 105, as *Rhyparobius dubius* Boud. (NY). Vienna: Vienna, on horse dung, A. Heimerl *s.n.*, Feb. 1889, distributed as microscopic slide in Rehm, Ascom. No. 967, as *Thelebolus stercoreus* (NY).

Italy, Longobardi, Santa Teresa, near Papian, on sheep dung, Cavara *s.n.*, *s. dat.* Distributed in Cavara, F. Longobard. Exs. No. 165, as "*Riparobius dubius"* (BPI, CUP, NY).

Thailand, Prov. Payap, Mt. Doi Chieng Dao, alt. 1900 m, on cultured dung of Burmese goat, J. van Brummelen 1786, 1790, 26 Nov. 1973 (L).

Uganda, Ruwenzori Mts., Mt. Speke, on carnivore dung, R.F. Cain *et al*. *s.n.*, 23 July 1969 (culture TRTC 45566,

CBS 715.69); id. Lower Bigo Bog, from carnivore dung, R.F. Cain *et al. s.n.*, 21 July 1966 (culture TRTC 66.2597a, CBS 709.69).
Tanzania, Mt. Kilimanjaro (10.500 ft.), above Kifinika, from carnivore dung, R.F. Cain *et al. s.n.*, 15 Aug. 1966 (culture TRTC 66.2379b, CBS 712.69); Usambara Mts. (5.500 ft.), N. of Lushoto, from herbivore dung, R.F. Cain *et al. s.n.*, 14 Aug. 1966 (culture TRTC 45564, CBS 714.69).
Kenya, Mt. Kenya, Timau Track (13.000 ft.), from carnivore dung, R.F. Cain *et al. s.n.*, 14 July 1966 (culture TRTC 66.639b, CBS 713.69).
Canada, Bitish Columbia: Vancouver Island, Sooke, on deer dung, E.R. Luck Allen *s.n.*, 18 Aug. 1962 (CUP 47719). Alberta: Bluff Nat. Park, Parker Ridge, on deer dung, E.R. Luck Allen *s.n.*, 9 Aug. 1962 (CUP 47722). Saskatchewan: Cypress Hill, E. Black Campsite, on deer dung, R.F. Cain *s.n.*, 25 July 1962 (CUP 47723). Ontario: Algoma Distr., Mashagama Lake, on wolf dung, R.F. Cain *et al. s.n.*, 18 June 1960 (CUP 47717); Algoma Distr., Heron Bay, on wolf dung, E.R. Luck Allen *s.n.*, 19 July 1962 (CUP 47715); Algoma Distr., Nickel Township, on moose dung, R.F. Cain *s.n.*, 21 June 1961 (CUP 47731); Algoma Distr., Aubinadong R., on dung, R.F. Cain *et al. s.n.*, 17 June 1960 (CUP 47716); Algoma Distr., Aubinadang R., on dung of carnivorous animal, R.F. Cain *s.n.*, 17 June 1960 (CUP 47711); Brant Co., New Durham, on rabbit dung, R.F. Cain *s.n.*, 22 May 1935 (CUP 47734); Brant Co., Princeton, on rabbit dung, R.F. Cain *s.n.*, 29 Aug. 1932 (CUP 47736); Gogama-Sudbury Distr., Bennewies, on dung, R.F. Cain *s.n.*, 24 June 1960 (CUP 47725 and CUP 47729); Kent Co., Cedar Springs, on cow dung, R.F. Cain *s.n.*, 13 July 1932 (CUP 47609); Mica Bay, on rabbit dung, R.F. Cain *s.n.*, 18 June 1961 (CUP 47727); Simcoe Co., Allison, on dog dung, W. Obrist *s.n.*, 15 Apr. 1960 (CUP 47707); Simcoe Co., S. of Coldwater, from deer dung, D. Malloch *s.n.*, 13 May 1968 (culture TRTC 45546, CBS 717.69). Sudbury Distr., Gogoma, on dung, R.F. Cain *et al. s.n.*, 22 June 1960 (CUP 47714, CUP 47713); Whitney Lake, N. of Temagamy, on rabbit dung, R. F. Cain *s.n.*, 30 July 1931 (CUP 47670); Lake Temagami, Bear Island, on porcupine dung, R.F. Cain *s.n.*, 21 June 1932 (CUP 47733); Lake Temagami, High Rock Island, on rabbit dung, R.F. Cain *s.n.*, 22 June 1931 (CUP 47672); Lake Temagami, Bear Island, on rabbit dung, R.F. Cain *s.n.*, 13 June 1932 (CUP 47734); Wawa, on rabbit dung, R.F. Cain *s.n.*, 19 June 1961 (CUP 47726); York Co., Nashville, from cow dung, R.F. Cain *s.n.*, 24 Dec. 1957 (culture TRTC 33513, CBS 341.58); York Co., Nashville, on rabbit dung, *R.F. Cain* s.n., 14 and 24 March 1963 (resp. CUP 47675 and 47604); York Co., Nashville, culture from rabbit dung, R. F. Cain *s.n.,* March 1963 (CUP 47702, CUP 47703); York Co., Nashville, from rabbit dung, R.F. Cain *s.n.*, March 1963 (CUP 47706); id. May 1963 (CUP 47611); York Co. Nashville, on dung, R.F. Cain *s.n.*, 14 June 1963 (CUP). Haliburton, S. of Dorset, from deer dung, D. Malloch *s.n.*, 18 Sep. 1967 (culture TRTC 45563, CBS 708.69). Nipigon Prov. Forest, Cirkel Lake, from wolf dung, R.F. Cain *s.n.*, 29 July 1967 (culture TRTC 45562, CBS 707.69). Québec: Laurentides Park, Camp Mercier, on deer dung, R.F. Cain *s.n.*, 27 Aug. 1938 (CUP 47732). Nova Scotia: Cape Breton Highlands Natl. Pk., Warren Lake, from carnivore dung, D. Malloch *s.n.*, 9 June 1967 (culture TRTC 45548, CBS 711.69).
Greenland, Nyhavn, on dung of arctic hare (comm. H. Dissing), J. van Brummelen 7190, 7191, 18 Apr. 1984 (L).
U.S.A., Alaska: South Meadow-Lake, on dung, Y. Kobayasi *s.n.*, 7 Aug. 1965 (NY, ex Herb. IFO 11575). S. loc. from tundra-soil (IFO 8585), K. Tubaki *s.n.*, June 1966 (culture CBS 349.66); South Meadon Lake, isolated from dead leaves, Y. Kobayasi *s.n.*, 7 Aug. 1965 (NY, ex IFO 11575). California: Lassen Nat. Park,, above King Creek Meadows, on deer dung, W.B. Cooke *s.n.*, 12 July 1961 (CUP 47730). Arizona: Mt. Humphrey, Flagstaff, on horse dung, R.F. Cain *s.n.*, 21 June 1955 (CUP 47677). Wyoming: Teton Co., Moran, on moose dung, R.F. Cain *s.n.*, 3 July 1955 (CUP 47709); Yellowstone Natl. Pk., on deer dung, K.H. McKnight F 7684, 4 June 1965 (NY); Shoshone Natl. Forest, Togwotee Pass, on deer dung, R.F. Cain *s.n.*, 2 July 1955 (CUP 47708); Shell Canyon, on deer dung, E.R. Luck Allen *s.n.*, 2 Sep. 1962 (CUP 47710); Shoshone Natl. Forest, Togwotee Pass, on moose dung, R.F. Cain *s.n.*, 2 July 1955 (CUP 47720). Colorado: Geneva Creek Canyon, on rabbit dung, F.J. Seaver & E. Bethel *s.n.*, 2–12 Sep. 1910 (NY); near Tolland, on horse dung, F.J. Seaver & E. Bethel *s.n.*, 24–26 Aug. 1910 (NY); Jackson Co., Cameron Pass, on cow dung, C.T. Rogerson 3702, 20 Aug. 1954 (NY, as *Ascophanella microscopica* (Crouan) Korf, det. Korf). Wisconsin: S. loc., from soil, D.T. Wicklow *s.n.*, Dec. 1969 (culture WSF 5168, CBS 710.69). New York: Tarrytown, on rabbit dung, F.J. Seaver *s.n.*, 19 May 1916 (NY, type of *Streptotheca obscura* Seaver); Ithaca, Campus Cornell Univ., on rabbit dung, A. Costonas *s.n.*, 8 Jan. 1964 (CUP 47608). Massachusetts: New Haven, on dog dung, W.C. Sturgis *s.n.*, Apr. 1892 (CUP 47738); Cambridge, on dog dung, W.C. Sturgis *s.n.*, March 1890 (CUP 47740, NY); Cambridge, on dog dung, W.C. Sturgis *s.n.*, March 1890 (NY). Connecticut: New Haven, on dog dung, W.C. Sturgis *s.n.*, Apr. 1892 (NY).

Notes

The number of spores per ascus may vary from 32 to over 2000. The majority of strains maintained in the CBS culture collection originated from large-mammal dung or were isolated from soil. The species has a world-wide distribution, including cold localities in tropical climate zones, but has not been found in Antarctica. The ex-type strain of *Thelebolus psychrophilus*, CBS 375.58 was sequenced and found to have a single base substitution in ITS2 compared to *T. microsporus* (Table 4). It was morphologically identical to a strain dH 12483 from the Caucasus (Russia) identified as *T. caninus* (Auersw.) Jeng & Krug; both strains having asci with 32 verruculose ascospores as described by Spooner (1981) and van Brummelen (1998). There is however a difference in the observed verruculosity with these authors. Spooner described small isolated warts or isolated spicules visible under oil-immersion (Spooner 1981: Fig. 35B). These are especially seen in media with a low water content, like lactophenol or Melzer's reagent, but not in water. The finely verrucose ascospore surface described by van Brummelen is visible in mature ascospores with transmission electron microscopy (van Brummelen

1998: Figs. 3b, d). The β-tubulin sequence of strain CBS 375.58 matched with that of group C.

Thelebolus globosus Brumm. & de Hoog, **sp. nov.** MycoBank 500195. Figs 13H–I, 15.

Ascomata inaequaliter subglobosa aut ovoidea ad ellipsoidea, cleistohymenialia, quae numquam "apothecioidea" fiunt; nec antequam plene maturuerunt aut soluta sunt hiascunt; ad superficiem aut introrsus crescentia, unumquodque ad singulam hypham conjunctum; (14–)22–70(–250) µm diametro: interdum ubi ad superficiem crescit, usque ad 300–520 µm; incoloria usque pallide sucina-subflavida; laevia, sine margine. Sine hymenio aut hypothecio. Corticale excipulum 6–9 µm crassum, pallide flavidum aut hyalinum, quod constitit in singula stratura plus minusve abundantium subglobosarum crassissimis parietibus cellularum (3.5–)4.5–7 (–9.5) µm diametro (*textura globulosa*). Asci inaequales, breviter ellipsoidei usque subglobosos, nec basi nec apice discriminato, tenuissimis parietibus; 12–15 × 9–12 µm; octo sporis; 1–4 asci in singula ascomata. *Ascosporae* incondite dispositae; ample ellipsoideae (longitudinis/latitudinis ratio : 1.2–1.6, medietas: 1.35), 5–7.5(–8) × 4.1–5.1(–6) µm, pallide subflavidae, crassissimis parietibus, laevs, cum homogenea continentia; haud vehementer expulsae, plerumque liberatae ubi asci tunica deliquescit.Nullae paraphysae. Mycelium filiforme, saepe curvatum et inaequaliter inflatum, cum copiosis fibulis; hyalinum aut pallide roseolum in dauci-agar; 2.8–6.8 µm in latitudinem; multa parva globulosa granula continens. Conidiophora in duo ad quadraginta et interdum usque ad centum conglomerata; directo ad mycelium, saepe apud septa vel laterales ramusculos. *Conidia* subglobosa aut brevissime ellipsoidea, saepe acuta ubi adfixa sunt (longitudinis/latitudinis ratio: 1–1.15, raro 1.2), (2.5–)3–5.5(–7.4) µm diametro; hyalina, tenuibus parietibus, laevia, copiosa parva globulosa granula continentia.

Holotype: Herb. L-986.305 257, cultura ex-typus dH 12150 (CBS 113940), **Antarctica**, Vestfold Hills, Lake Druzhby, isolated from biomats, E. Göttlich & G.S. de Hoog.

Description of *Thelebolus globosus*

Ascomata irregularly subglobular or ovoid to ellipsoid, cleistohymenial, never becoming "apothecioïd", not opening before full matutity or disintegration, growing superficially or immersed, each on a single hypha, (14–)22–70(–250) µm diam, occasionally at the surface up to 300–520 µm across, colourless to pale amber-yellowish, smooth, without a margin. Without a hymenium or hypothecium. Cortical excipulum ca. 6–9 µm thick, pale yellowish or hyaline, consisting of a single layer of more or less proliferating subglobular rather thick-walled cells (3.5–)4.5–7 (–9.5) µm diam (*textura globulosa*). *Asci* irregular shortly ellipsoid to subglobular, with base and top not differentiated, rather thin-walled, 12–15 × 9–12 µm, 8-spored, 1–4 asci per ascoma. *Ascospores* irregularly disposed, broadly ellipsoid (length/width ratio 1.2–1.6, av. 1.35), 5–7.5(–8) × 4.1–5.1(–6) µm, pale yellowish, rather thick-walled, smooth, with homogeneous contents, not forcefully discharged, usually set free by deliquescence of the ascal wall. *Paraphyses* absent. *Mycelium* filiform, often curved and irregularly swollen, frequently septate, hyaline or pale pinkish on carrot-agar, 2.8–6.8 µm wide, with contents rich in small globular granules. *Conidiophores* in small or large groups of 2 to 40, sometimes up to over 100, together direotly on the mycelium, often close to septa or on short lateral branches. *Conidia* subglobose or very shortly ellipsoid, often somewhat pointed at the side of attachment (length/width ratio 1–1.15, rarely 1.2), (2.5–)3–5.5(–7.4) µm diam, hyaline, thin-walled, smooth, with contents rich in small globular granules.

Distribution

Only known from biomats in lakes of Vestfold Hills of Antarctica only very infrequently visited by birds.

Notes

This is the species with the most limited distribution, only being found in biomats in two lakes in the Antarctic Vestfold Hills. Birds are practically absent in this region, so that distribution of the species, when once emerged, may be supposed to be very limited. The mechanism of active ascospore dispersal is lost, and simple, slimy conidia are produced in abundance from undifferentiated hyphae. The combination of these characters suggests that the species thrives in submersion for a major part of its life cycle.

Thelebolus ellipsoideus Brumm. & de Hoog, **sp. nov.** MycoBank 500196. Figs 13A–G, J, 14.

Ascomata inaequaliter subglobosa aut ovoidea, cleistohymenalia, quae numquam "apothecioidea" fiunt nec antequam plene maturuerunt aut soluta sunt hiascunt; ad superficiem aut introrsus crescentia; (10–)17–46(–55) µm diametro; hyalina, laevia aut leviter inaequalia ob in superficie emergentes ex cortice cellulas; sine margine. Sine hymenio aut hypothecio. Corticale excipulum 5–9 µm crassum, hyalinum, quod in globulosis 5.5–10 µm diametro cellulis constitit (*textura globulosa*). *Asci* inaequales, breviter ellipsoidei usque subglobosos, nec basi nec apice discriminato, tenuissimis parietibus; 15–22 × 11–16(–18) µm; octo sporis; 1–8, raro 25 asci in singula ascomata. *Ascosporae* incondite dispositae, breviter ellipsoideae, saepe plus minusve inaequali forma (longitudinis/latitudinis ratio: 1.4–2; medietas: 1.57); 5–9.2 × 4–5.3(–6) µm, hyalinae, crassissimis parietibus, laeves, cum homogenea continentia; haud vehementer expulsae, plerumque liberatae ubi asci tunica deliquescit. Nullae paraphysae. Mycelium hyalinum, (2.3–)3–6.5(–9) µm latitudine, quod multa parva globulosa granula continet. Conidiophora singula ad quina conglomerata, directo ad mycelium, saepe apud septa. Conidia ellipsoidea (longitudinis/latitudinis ratio: 1.3–2.1; medietas: 1.62); 4–9 × 3.5–5.5 µm diametro; hyalina,

tenuibus parietibus, laevibus; copiosa parva globulosa granula continentia.

Holotype: Herb. L-986.305 258, culture ex-type dH 12149 (CBS 113938), **Antarctica**, Vestfold Hills, Lake Druzhby, isolated from biomats, E. Göttlich & G.S. de Hoog.

Description of *Thelebolus ellipsoideus*
Ascomata irregularly subglobular or ovoid to ellipsoid, cleistohymenial, never becoming ´apothecioid´, not opening before full maturity or disintegration, growing superficially or immersed, (10–)17–46(–55) µm diam, hyaline, smooth or slightly irregular by protruding superficial cells of the cortex, without a margin. Without a hymenium or hypothecium. Cortical excipulum *ca*. 5–9 µm thick, hyaline, consisting of globular cells 5.5–10 µm diam (*textura globulosa*). *Asci* irregular shortly ellipsoid to subglobose, with base and top not differentiated, rather thin-walled, 15–22 × 11–16(–18) µm, 8-spored, 1–8 (rarely up to 25) asci per ascoma. *Ascospores* irregularly disposed, shortly-ellipsoid, often more or less irregularly shaped (length/width ratio 1.4–2.0, average 1.57), 5–9.2 × 4–5.3(–6) µm, hyaline, rather thick-walled, smooth, with homogeneous contents, not forcefully discharged, usually becoming free by deliquescence of the ascal wall. *Paraphyses* absent. *Mycelium* hyaline, (2.3–)3–6.5(–9) µm wide, with contents rich in small globular granules. *Conidiophores* in small groups of 1 to 5 together directly on the mycelium, often close to septa. *Conidia* ellipsoid (length/width ratio 1.3–2.1, average 1.62), 4–9 × 3.5–5.5 µm, hyaline, thin-walled, smooth, with contents rich in small globular granules.

Distribution
Only known from biomats in lakes of bird-less areas of Antarctica in Vestfold and Larsemann Hills and Victoria Land.

Notes
All strains from Fryxell and Hoare lakes (Dry Valleys) deviated from all other strains by a relatively significant number of informative ITS base differences (Table 4). With β-tubulin the group comprises a clearly separate entity (Fig. 6). As the molecular differences are more significant than within *T. microsporus / T. stercoreus*, this group is likely to represent another, hitherto undescribed species. The same genotype was observed in Manning and Druzhby lakes in the Vestfold Hills (Table 3), about 2500 km from the Dry Valley. These strains deviated from the ones in the Dry Valleys by producing a teleomorph in culture, which remained absent from the strains from Fryxell and Hoare. The loss of the ability to produce a teleomorph may be an adaptation to life under nearly permanent ice (Table 1). The species has hitherto not been observed outside the Antarctic.

GENERAL CONCLUSIONS

Psychrophily in the genus *Thelebolus* seems to be more other environmental parameters such as salinity or availability of guano. The genus is one of the preponderant fungal groups encountered in Antarctica. Two species were discovered that are endemic to the Antarctic. Given the consistent cold association of the genus, coincidental trapping in ice and subsequent cultivation after isolation, as was supposed to be the case in *Cladosporium* (Ma *et al*. 2000) is unlikely; the species probably have their natural ecological niche with competitive advantage under conditions of extreme coldness; only *T. stercoreus* is an exception to this pattern. One species was also observed outside the Antarctic, and the Antarctic population is likely to have reached distant localities on repeated, independent occasions, probably by migratory bird vectors. The dispersal of *T. globosus,* that has a localised distribution in the Antarctic zone where are only very few birds, remains poorly understood.

ACKNOWLEDGEMENTS

The EU-sponsored project “Micromat” co-ordinated by A. Wilmotte provided a framework for the present study. We are indebted to D. Hodgson and J. Laybourn-Parry for information on lake sampling sites. The Instituto Antártico Argentino for cooperation and travel support of G. Leotta to South Shetland and Antarctic Peninsula. E. Akker, A.H.G. Gerrits van den Ende, K. Luijsterburg and M. Reubzaet are thanked for technical assistance. R. Caligiorne and P. Licinio are kindly acknowledged for providing the DNAWD programme and making the respective trees. We are indebted to Y. Gräser for help with some of the calculations. The curators and directors of the following herbaria, with their standard abbreviations according to Holmgren *et al*. (1990), have been helpful in supplying specimens for study: Berlin (B), Beltsville (BPI), Meise (BR), Wroclaw (WRSL), Concarneau (CO), Ithaca (CUP), Edinburgh (E), Genève (G), Hamburg (HBG), Kew (K), Leiden (L), Petrograd (LE), New York (NY), Padova (PAD), Paris Cryptogamie (PC), Praha Mycology (PRM), Stockholm (S), Toronto (TRTC), Uppsala (UPS), Vienna (W), Zürich (ZT). Thanks are also extended to Dr. J.-P. Pirlot for the translations into Latin of the descriptions of the new species. M.J. Richardson and G. Verkley are thanked for reviewing the manuscript.

REFERENCES

Atlas RM, Parks LS (1993). *Handbook of Microbiological Media*. CRC, Boca Raton.

Azmi OR & Seppelt RD (1997). Fungi of the Windmill Islands, continental Antarctica. Effect of temperature, pH and culture media on the growth of selected microfungi. *Polar Biology* **18**: 128–134.

Azmi OR & Seppelt RD (1998). The broad-scale distribution of microfungi in the Windmill Islands region, continental Antarctica. *Polar Biology* **19**: 92–100.

Baublis JA, Wharton RA & Volz PA (1991). Diversity of micro-fungi in an Antarctic dry valley. *Journal of Basic Microbiology* **31**: 3–12.

Bell EM & Laybourn-Parry J (1999). Annual plankton dynamics in an Antarctic saline lake. *Freshwater Biology* **41**: 507–519.

Bergero R, Girlanda M, Varese GC, Intilli D & Luppi AM (1999). Psychrooligotrophic fungi from Arctic soils of Franz Joseph Land. *Polar Biology* **21**: 361–368.

Bergman PS & Shanor L (1957). A new species of *Streptotheca*. *Mycologia* **49**: 879–883.

Boekhout T, Roeijmans H & Spaay F (1995). A new pleomorphic ascomycete, *Calyptrozyma arxii* gen. et sp. nov., isolated from the human lower oesophagus. *Mycological Research* **99**: 1239–1246.

Boudier JLÉ (1869). Mémoire sur les Ascobolés. *Annales de Science Naturelle, Botanie,* Sér. V, **10**: 191–268.

Boudier JLÉ (1907). *Histoire et classification des Discomycètes d'Europe*: i–vii. P. Klincksieck, Paris, France.

Brummelen J van (1967). *A world-monograph of the genera* Ascobolus *and* Saccobolus *(*Ascomycetes, *Pezizales)*. *Persoonia* (Suppl.) **1**: 1–260.

Brummelen J van (1974). Light and electron microscopic studies of the ascus top in *Ascozonus woolhopensis*. *Persoonia* **8**: 23–32.

Brummelen J van (1978). The operculate ascus and allied forms. *Persoonia* **10**: 113–128.

Brummelen J van (1985). *Pseudascozonus*, a new genus of *Pezizales*. *Proceedings of the Indian Academy of Sciences, Plant Science* **94**: 363–367.

Brummelen J van (1987). Ultrastructure of the ascus and the ascospores in *Pseudascozonus* (*Pezizales, Ascomycotina*). *Persoonia* **13**: 369–377.

Brummelen J van (1992). *Ramgea*, a new genus of *Pezizales* from the Netherlands. *Persoonia* **14**: 577–582.

Brummelen J van (1998). Reconsideration of relationships within the *Thelebolaceae* based on ascus ultrastructure. *Persoonia* **16**: 425–469.

Brummelen J van & Kristiansen R (1998). Two rare coprophilous ascomycetes from Norway. *Persoonia* **17**: 119–125.

Brummelen J van & Richardson MJ (2001). *Ascozonus monascus*, a new species of ascomycetes from Great Britain. *Persoonia* **17**: 487–490.

Burke CM & Burton HR (1988). Photosynthetic bacteria in meromictic lakes and stratified fjords of the Vestfold Hills, Antarctica. *Hydrobiologia* **165**: 13–23.

Burton HR (1981) Chemistry, physics and evolution of Antarctic saline lakes: a review. *Hydrobiologia* **165**: 13–23.

Cannon PF, Hawksworth DL & Sherwood-Pike MA (1985). *The British* Ascomycotina*. An annotated checklist.* CMI, Slough, U.K.

Cooke JC & Barr ME (1964). The taxonomic position of the genus *Thelebolus*. *Mycologia* **56**: 763–769.

Crouan PL & Crouan HM (1867) *Florule du Finistère*. Brest, France.

Czymmek KJ & Klomparens KL (1992). The ultrastructure of ascosporogenesis in freeze-substituted *Thelebolus crustaceus*: enveloping membrane system and ascospore initial development. *Canadian Journal of Botany* **70**: 1669–1683.

Dartnall HJG (2000). A limnological reconnaissance of the Vestfold Hills. *Anare Reports* **141**: 1–54.

Dissing H & Schumacher T (1994). In: DL Hawksworth (ed.), *Ascomycete systematics: Problems and perspectives in the nineties.* Plenum Press, New York, U.S.A.

Doveri F (2004). *Fungi fimicoli Italici. A guide to the recognition of basidiomycetes and ascomycetes living on faecal material*. Fondazione Centro Studi Micologia dell'A.M.B., Vicenza, Italy.

Eckblad F-E (1968). The genera of the Operculate Discomycetes. A re-evaluation of their taxonomy, phylogeny and nomenclature. *Norwegian Journal of Botany* **15**: 1–191.

Ellis-Evans JC, Laybourn-Parry J, Bayliss PR & Perriss SJ (1998). Physical, chemical and microbial community characteristics of lakes of the Larsemann Hills, Continental Antarctica. *Archives of Hydrobiology* **141**: 209–230.

Ellis-Evans JC, Laybourn-Parry J, Bayliss PR & Perris SJ (1998). Physical, chemical and microbial community characteristics of lakes of the Larsemann Hills, Continental Antarctica. *Archives of Hydrobiology* **141**: 209–230.

Felsenstein J (1996). Phylip (Phylogeny Inference Package), v. 3.572. Department of Genetics, University of Washington, Seattle, WA.

Fritsen CH & Priscu JC (1998). Cyanobacterial assemblages in permanent ice covers on Antarctic lakes: distribution, growth rate, and temperature response of photosynthesis. *Journal of Phycology* **34**: 587–597.

Fuckel KWGL (1866). Ueber rheinische *Ascobolus*-Arten. *Hedwigia* **5**: 1–5.

Fuckel KWGL (1870). Symbolae mycologicae. Beiträge zur Kenntniss der rheinischen Pilze. *Jahrbuch des Nassauischen Vereins für Naturkunde* **23–24**: 1–459.

Gargas A. & Taylor JW (1992). Polymerase chain reaction (PCR) primers for amplifying and sequencing nuclear 18S rDNA from lichenized fungi. *Mycologia* **84**: 589–592.

Gasparon M, Lanyon R, Burgess JS & Sigurdsson IA (2002). *The freshwater lakes of the Larsemann Hills, East Antarctica: chemical characteristics of the water column*. Anare, Kingston.

Gernandt DS, Platt JL, Stone JK, Spatafora JW, Holst-Jensen A, Hamelin RC & Kohn LM (2001). Phylogenetics of *Helotiales* and *Rhytismatales* based on partial subunit nuclear ribosomal DNA sequences. *Mycologia* **93**: 915–933.

Gibson JAE & Burton HR (1996). Meromictic Antarctic lakes as recorders of climatic change: the structures of Ace and Organic lakes, Vestfold Hills, Antarctica. *Papers and Proceedings of the Royal Society of Tasmania* **130**: 73–78.

Gillieson D, Burgess J, Spate A & Cochrane A (1990). An atlas of the lakes of the Larsemann Hills, Princess Elizabeth Land, Antarctica. *Anare Research Notes* **74**: 1–173.

Glass NL & Donaldson GC (1995). Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Applied and Environmental Microbiology* **61**: 1323–1330.

Göttlich E, Hoog GS de, Genilloud O, Jones BE, Marinelli F (2003). MICROMAT: culturable fungal diversity in microbial mats of Antarctic lakes. In: *Antarctica Biology in a Global Context* (Huiskes AHL *et al*., eds). Backhuys, Leiden, The Netherlands: 251–254.

Hansen K, Laessøe & Pfister DH (2001). Phylogenetics of the *Pezizaceae*, with emphasis on *Peziza*. *Mycologia* **93**: 958-990.

Hawes I & Schwarz A-M (1999). Photosynthesis in an extreme shade habitat: benthic microbial mats from Lake Hoare, Antarctica. *Journal of Phycology* **35**: 448–459.

Heimerl A (1889). Die niederösterreichischen Ascoboleen. *Jahresberichte k.k. Oberrealschule Bezirke Sechshaus Wien* **15**: 1–32.

Hendriks L, Goris A, Neefs J-M, Peer Y van de, Hennebert G & Wachter R de (1989). The nucleotide sequence of the small ribosomal subunit RNA of the yeast *Candida albicans* and the evolutionary position of the fungi amongst the Eukaryotes. *Systematic and Applied Microbiology* **12:** 223–229.

Holmgren PK, Holmgren NH. & Barnett LC (1990). *Index herbariorum Part 1: The herbaria of the world*. Ed. 8. New York Botatical Garden, New York, U.S.A.

Hoog GS de & Gerrits van den Ende AHG (1998). Molecular diagnostics of clinical strains of filamentous Basidiomycetes. *Mycoses* **41**: 183–189.

Hoog GS de & Smith MTh (1981). *Hyphozyma*, a new genus of yeast-like Hyphomycetes. *Antonie van Leeuwenhoek* **47**: 339–352.

Hoog GS de & Smith MTh (2004). Ribosomal gene phylogeny and species delimitation in *Geotrichum* and its teleomorphs. *Studies in Mycology* **50**: 489–515.

Hoog GS de , Vicente V, Caligiorne RB, Kantarcioglu S, Tintelnot K, Gerrits van den Ende AHG & Haase G (2003). Species diversity and polymorphism in the *Exophiala spinifera* clade containing opportunistic black yeast-like fungi. *Journal of Clinical Microbiology* **41**: 4767–4778.

Janczewski E (1871). Morphologische Untersuchungen über *Ascobolus furfuraceus*. *Botanische Zeitung* **24**: 257–262.

Jeng RS & Krug JC (1977). New records and new species of coprophilous *Pezizales* from Argentina and Venezuela. *Canadian Journal of Botany* **55**: 2987–3000.

Karsten PA (1866). *Fungi Fenniae exsiccati, Sammling of Finska svampar.* Nrs. 301–600.

Karsten PA (1870). Monographia Ascobolorum Fenniae. *Notiser Societas Fauna et Flora Fennicam* **11**: 197–210.

Karsten PA (1871). Mycologia Fennica. Pars prima: Discomycetes. *Bidrag till Kännedom af Finlands Natur och Folk* **19**: 1–264.

Kimbrough JW (1972). Ascal structure, ascocarp ontogeny, and a natural classification of the *Thelebolaceae*. *Persoonia* **6**: 395–404.

Kimbrough JW (1981). Cytology, ultrastructure, and taxonomy of *Thelebolus* (Ascomycetes). *Mycologia* **73**: 1–27.

Kimbrough JW & Korf RP (1967). A synopsis of the genera and species of the tribe *Thelebolae* (*Pseudoascoboleae*). *American Journal of Botany* **54**: 9–23.

Kimbrough JW & Luck-Allen ER (1974). *Lasiothelebolus*, a new genus of the *Thelebolaceae* (*Pezizales*). *Mycologia* **66**: 588–592.

Kimbrough JW, Luck-Allen ER & Cain RF (1972). North American species of *Coprotus* (*Thelebolaceae: Pezizales*). *Canadian Journal of Botany* **50**: 957–971.

Kirk PM, Cannon PF, David JC & Stalpers JA (eds) (2001). *Ainsworth & Bisby's Dictionary of the Fungi*, 9th ed. CAB International, Egham, U.K.

Kobayasi Y, Hiratsuka N, Korf RP, Tubaki K, Aoshima K, Soneda M & Sugiyama J (1967). Mycological studies of the Alaskan Arctic. *Annual Report of the Institute for Fermentation, Osaka* **3**: 1–138.

Korf RP (1972). Synoptic key to the genera of the *Pezizales*. *Mycologia* **64**: 937–994.

Koufopanou V, Burt A & Taylor JW (1997). Concordance of gene genealogies reveals reproductive isolation in the pathogenic fungus *Coccidioides immitis*. *Proceedings of the National Academy of Sciences of the U.S.A.* **94**: 5478–5482.

Kutorga E (2000). *Lietuvos Grybai III-5. Ausūniečiai* (*Pezizales*). Bot. Inst. Vilniaus Univ., Vilnius, Lituania.

Landvik S, Kristiansen R & Schumacher T (1997). Towards a subordinal classification of the *Pezizales* (*Ascomycota*): phylogenetic analyses of SSU rDNA sequences. *Nordic Journal of Botany* **17**: 403–418.

Landvik S, Kristiansen R & Schumacher T (1998). Phylogenetic and structural studies in the *Thelebolaceae* (Ascomycota). *Mycoscience* **39**: 49–56.

Lawrence MJF & Hendry CH (1985). Water column and sediment characteristics of Lake Fryxell, Taylor Valley, Antarctica. *Journal of Geology and Geophysics* **28**: 543–552.

Laybourn-Parry J, Hofer JS & Sommaruga R (2001). Viruses in the plankton of freshwater and saline Antarctic lakes. *Freshwater Biology* **46**: 1279–1287.

Laybourn-Parry J, James MR, McKnight DM, Priscu J, Spaulding SA & Shiel R (1997). The microbial plankton of Lake Fryxell, Southern Victoria Land, Antarctica during the summers of 1992 and 1994. *Polar Biology* **17**: 54–61.

Le Gal M (1961). Les Discomycètes de l'herbier Crouan. *Annales de Science Naturelle, Botanique,* Sér. XII, **1**: 441–467.

Leotta GA, Paré JA, Sigler L, Montalti D, Vigo G, Petruccelli M & Reinoso EH (2002). *Thelebolus microsporus* mycelial mats in the trachea of wild brown skua (*Catharacta antarctica lonnbergi*) and South Polar Skua (*C. maccormicki*) carcasses. *Journal of Wildlife Diseases* **38**: 443–447.

Licinio P & Caligiorne RB (2004). Inference of phylogenetic distances from DNA-walk divergences. *Physica A* **341**: 471–481.

Lieckfeldt E. & Seifert KA (2000). An evaluation of the use of ITS sequences in the taxonomy of the *Hypocreales*. *Studies in Mycology* **45**: 35–44.

Longato S & Bonfante P (1997). Molecular identification of mycorrhizal fungi by direct amplification of microsatellite regions. *Mycological Research* **101**: 425–432.

Ma L-J, Rogers SO, Catranis CM & Starmer WT (2000). Detection and characterization of ancient fungi entrapped in glacial ice. *Mycologia* **92**: 286–295.

Manzoni M (ed.)(2001). *La Natura dell'Antartide.* Springer-Verlag Milano, Italy pp. 59–94.

Masclaux F, Guého E, Hoog GS de & Christen R (1995). Phylogenetic relationships of human-pathogenic *Cladosporium (Xylohypha)* species inferred from partial

LS rRNA sequences. *Journal of Medical and Veterinary Mycology* **33**: 327–338.
Massee G & Salmon ES (1902). Researches on coprophilous fungi. II. *Annals of Botany* **61**: 57–92.
Momol EA & Kimbrough JW (1994). Phylogenetic analysis of selected genera of *Pezizales*, inferred from 5.8S rDNA, ITS1 and ITS2 sequences. *Systema Ascomycetum* **13**: 1–12.
Momol EA, Kimbrough JW & Eriksson OE (1996). Phylogenetic relationships indicated by 18S rDNA sequence analysis. *Systema Ascomycetum* **14**: 91–100.
Montemartini A, Caretta G. & Del Frate G (1993). Notes on *Thelebolus microsporus* isolated in Antarctica. *Mycotaxon* **48**: 343–358.
Nei M (1987). *Molecular Evolutionary Genetics*. Columbia Press, New York.
O'Donnell K & Cigelnik E (1997). Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. *Molecular and Phylogenetic Evolution* **7**: 103–116.
Peer Y van de, Wachter R de (1994). Treecon for Windows: a software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment. *Computers in Applied Bioscience* **10**: 569–570.
Prokhorov VP (1998). The genera *Coprotus, Ascozonus, Thelebolus* and *Trichobolus* (*Pezizales*): The keys to the identification of species. *Mikologia i Fitopatologia* **32**: 40–43.
Raitviir A & Prokhorov V (1988). Discomycetes of Middle Asia IV. New data on *Ascobolaceae* and *Thelebolaceae*. *Proceedings of the Academy of Sciences of the Estonian SSR, Biology* **37**: 215–222.
Ramlow G (1906). Zur Entwicklungsgeschichte von *Thelebolus stercoreus* Tode. *Botanische Zeitschrift* **64**: 85–99.
Rea C (1912). New and rare British fungi. *Transactions of the British Mycological Society* **3:** 376–380.
Rehm H (1895) Ascomyceten: Hysteriaceen und Discomyceten. Rabenhorst's Kryptogamenflora 1(3) **44**: 1041–1104.
Roberts D & McMinn A (1996). Relationships between surface sediment diatom assemblages and water chemistry gradients in saline lakes of the Vestfold Hills, Antarctica. *Antarctic Science* **8**: 331–341.
Roberts EC & Laybourn-Parry J (1999). Mixotrophic cryptophytes and their predators in the Dry Valley lakes of Antarctica. *Freshwater Biology* **41**: 737–746.
Roberts EC, Laybourn-Parry J, McKnight DM & Novarino G (2000) Stratification and dynamics of microbial loop communities in Lake Fryxell, Antarctica. *Freshwater Biology* **44**: 649–661.
Rossman AY, Aime MC, Farr DF, Castlebury LA, Peterson KR & Leahy R (2004). The coelomycetous genera *Chaetomella* and *Pilidium* represent a newly discovered lineage of inoperculate discomycetes. *Mycological Progress* **3**: 275–290.
Saenz GS, Taylor JW & Gargas A (1994). 18S rRNA gene sequences and supraordinal classification of the *Erysiphales*. *Mycologia* **86**: 212–216.
Samuelson DA & Kimbrough JW (1978). Asci of the Pezizales. IV. The apical apparatus of *Thelebolus*. *Botanical Gazet* **139**: 346–361.
Seaver FJ (1928). *The North American Cup-fungi (Operculates)*. F.J. Seaver, New York, U.S.A.
Spaulding SA, McKnight DM, Stoermer EF & Doran PT (1997). Diatoms in sediments of perennially ice-covered Lake Hoare, and implications for interpreting lake history in the McMurdo Dry Valleys of Antarctica. *Journal of Paleolimnology* **17**: 403–420.
Spooner BM (1981). New records and species of British microfungi. *Transactions of the British Mycological Society* **76**: 265–301.
Sterflinger K, Baere R de, Hoog GS de, Wachter R de, Krumbein WE & Haase G (1997). *Coniosporium perforans* and *C. apollinis*, two new rock-inhabiting fungi isolated from marble in the Sanctuary of Delos (Cyclades, Greece). *Antonie van Leeuwenhoek* **72**: 349–363.
Stchigel AM, Cano J, MacCormack W & Guarro J (2001). *Antarctomyces psychrotrophicus* gen. et sp. nov., a new ascomycete from Antarctica. *Mycological Research* **105**: 377–382.
Tehler A, Farris JS, Lipscomb DL & Källersjö M (2000). Phylogenetic analyses of the fungi based on large rDNA data sets. *Mycologia* **92**: 459–474.
Tehler A, Little DP & Farris JS (2003). The full-length phylogenetic tree from 1551 ribosomal sequences of chitinous fungi, *Fungi*. *Mycological Research* **107**: 901–916.
Tode HJ (1790). *Nova fungorum genera complectens*. Fungi Mecklenburgenses selecti 1.
Velenovský J (1947). *Novitates Micologicae Novissimae*. 1–168. Praha.
Volkmann JK, Burton HR, Everitt DA & Allen DI (1988). Pigment and lipid compositions of algal and bacterial communities in Ace Lake, Vestfold Hills, Antarctica. *Hydrobiologia* **165**: 41–57.
Weir BS & Cockerham CC (1984). Estimating F-statistics for the analysis of population structure. *Evolution* **38**: 1358–1370.
Wharton RA, McKay CP, Clow GD & Andersen DT (1993). Perennial ice covers and their influence on Antarctic lake ecosystems. *Antarctic Research Series* **59**: 53–70.
White TJ, Bruns T., Lee SB, Taylor JW (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for Phylogenetics. In: *PCR Protocols, A guide to methods and applications* (Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds). Academic, San Diego, U.S.A.: 315–322.
Wicklow D & Malloch D (1971). Studies in the genus *Thelebolus* temperature optima for growth and ascocarp development. *Mycologia* **63**: 118–131.
Wright SW & Burton HR (1981). The biology of Antarctic saline lakes. *Hydrobiologia* **82**: 319–338.
Zukal H (1889). Entwicklungsgeschichtliche Untersuchungen aus dem Gebiete der Ascomyceten. *Sitzungsberichte der Köninglichen Akademie der Wissenschaften zu Wien, Mathematisch-naturwissenschaftliche Klasse* I, **98**: 520–603.