DESCRIPTIONS OF MEDICAL FUNGI

THIRD EDITION
(revised November 2016)

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Front cover: *Cryptococcus neoformans*, and montages including *Syncephalastrum, Scedosporium, Aspergillus, Rhizopus, Microsporum, Purpureocillium, Paecilomyces and Trichophyton.* Back cover: the colours of *Trichophyton* spp.

PREFACE

The first edition of this book entitled Descriptions of Medical QAP Fungi was published in 1992 by David Ellis, Steve Davis, Helen Alexiou, Tania Pfeiffer and Zabeta Manatakis. The original concept was to provide all laboratories in the Royal College of Pathologists of Australasia (RCPA) Mycology Quality Assurance Program (QAP) with a set of description sheets covering medically important fungi. A second edition entitled Descriptions of Medical Fungi was released in 2007 by David Ellis, Steve Davis, Helen Alexiou, Rosemary Handke and Robyn Bartley. We now provide an updated third edition which includes new and revised descriptions. We have endeavoured to reconcile current morphological descriptions with more recent phylogenetic studies, however nomenclature changes in mycology are ongoing. To search for current accepted fungal names go to Index Fungorum (www.indexfungorum.org) and Mycobank (www.mycobank.org).

Morphological Descriptions: These descriptions have by necessity been kept brief and many have been based on descriptions by other authors. For further information regarding any of the mycoses or pathogenic fungi mentioned, the reader is referred to the citations provided. For the precise definitions of the mycological terminology used, the reader is referred to Ainsworth and Bisby's Dictionary of the Fungi (Kirk *et al*. 2008).

Classification of the Fungi

Kingdom	**Fungal Phyla**	**Examples**
Protozoa	Myxomycota	Slime moulds
Chromista	Oomycota	*Pythium*
Eumycota	Ascomycota	*Candida, Aspergillus, Scedosporium, Fusarium, Paecilomyces, Penicillium, Cladophialophora, Bipolaris,* and other hyphomycetes, including the dimorphic fungi, dermatophytes, and *Pneumocystis* (Taphrinomycotina).
	Basidiomycota	*Cryptococcus, Trichosporon, Malassezia.*
	Chytridiomycota	Chytrids
	Glomeromycota	Endomycorrhizal on plants
	Microsporidia	170 genera, 1300 species
	Zygomycota	*Apophysomyces, Lichtheimia, Mucor, Saksenaea, Rhizomucor, Rhizopus.*

Fungi are now classified across three Kingdoms. Descriptions in this book are limited to the Eumycota and include medically important representatives from the Ascomycota, Basidiomycota and Zygomycota.

PREFACE

Key Morphological Characters

Culture Characteristics:

- Surface texture [glabrous, suede-like, powdery, granular, fluffy, downy, cottony]
- Surface topography [flat, raised, heaped, folded, domed, radial grooved]
- Surface pigmentation [white, cream, yellow, brown, pink, grey, black etc]
- Reverse pigmentation [none, yellow, brown, red, black, etc]
- Growth rate [colony diameter <5 cm in 14 days or >5 cm in 15 days]
- Growth at 37^{O}C, 40^{O}C, 45^{O}C.

Zygomycota. Sporangia characteristics:

- Arrangement of sporangiospores [multispored, sporangiola, merosporangium]
- Arrangement of sporangiophores [unbranched often in groups or frequently branched]
- Sporangium shape [pyriform, spherical, flask-shaped etc]
- Sporangium size [<100 µm diam. or >100 µm diam.]
- Columella [Present or Absent]
- Apophyses [Present or Absent]
- Sporangiophore height [<0.8 mm or >1 mm]
- Rhizoids [Present or Absent] (look in the agar)
- Sporangiospore size [<6 µm or >6 µm]

Hyphomycetes - Conidial Moulds

1. Conidial characteristics:

- Septation [one-celled, two-celled, multicelled with transverse septa only, or multicelled with both transverse and longitudinal septa]
- Shape [spherical, sub-spherical, pyriform, clavate, ellipsoidal, etc]
- Size [need a graduated eyepiece, length <10 µm or >10 µm]
- Colour [hyaline or darkly pigmented]
- Wall texture [smooth, rough, verrucose, echinulate]
- How many conidial types present? [i.e. micro and macro]

2. Arrangement of conidia as they are borne on the conidiogenous cells:

- Solitary [single or in balls]
- Catenulate (in chains) [acropetal (youngest conidium at the tip) or basipetal (youngest conidium at the base]

3. Growth of the conidiogenous cell:

- Determinant (no growth of the conidiophore after the formation of conidia)
- Sympodial (a mode of conidiogenous cell growth which results in the development of conidia on a geniculate or zig-zag rachis)

4. Type of conidiogenous cell present:

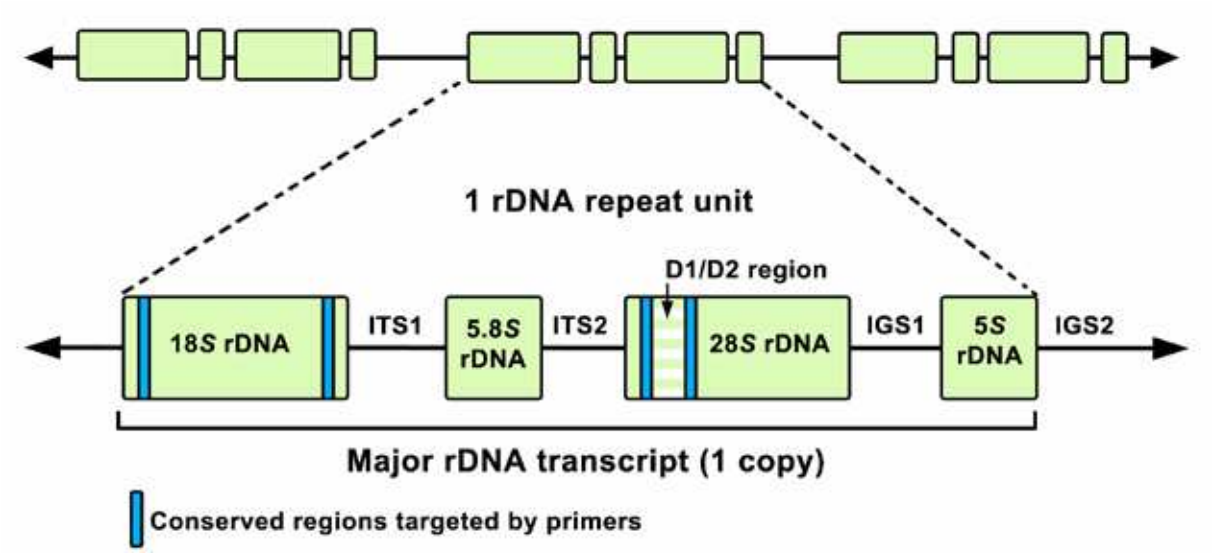
- Non-specialised
- Phialide (specialised conidiogenous cells that produces conidia in basipetal succession without increasing in length)
- Annellide (specialised conidiogenous cell producing conidia in basipetal succession by a series of short percurrent proliferations (annellations). The tip of an annellide increases in length and becomes narrower as each subsequent conidium is formed)

5. Any additional features present:

- Hyphal structures [clamps, spirals, nodular organs, etc]
- Synnemata, Sporodochia, Chlamydoconidia, Pycnidia
- Confirmatory tests for dermatophytes

PREFACE

Molecular and/or MALDI-TOF MS Identification: The use of PCR-based assays, DNA sequencing, and other molecular methods, including those incorporating proteomic approaches such as matrix assisted laser desorption ionization time of flight mass spectroscopy (MALDI-TOF MS) have shown promising results to aid in accurate species identification of fungal cultures. These are used mainly to complement conventional methods since they require standardisation before widespread implementation can be recommended (Halliday *et al.* 2015). Molecular-based fungal identification is particularly helpful for fungi that lack distinguishing morphological features, e.g. *Apophysomyces elegans*, or to distinguish between species of the *Aspergillus fumigatus* complex. Comparative sequence analysis is now the 'gold standard' for identification of fungi. Methods are referenced where available and in many instances are recommended for more definitive identifications.



Schematic diagram of the fungal rDNA gene cluster (adapted from CLSI MM18-A and Halliday *et al.* 2015). The 18*S*, 5.8*S* and 28*S* rDNA genes are separated by the two internal transcribed spacers. The 28*S* and 5*S* rDNA genes are separated by the intergenic spacer 1 (IGS1). The intergenic spacer 2 (IGS2) separates the rDNA repeat units from each other.

Regardless of the genetic locus selected, accurate sequence-based identification is dependent upon database accuracy and adequate species representation. GenBank is well known to contain numerous errors in sequences and the species names attributed to the sequences, which are rarely corrected. Therefore caution must be used when interpreting sequencing comparisons against this database, and the use of multiple sequence databases is encouraged. Well-curated databases that are helpful for species identification include:

1. International Society for Human and Animal Mycoses (ISHAM) ITS database (http://its.mycologylab.org/).
2. CBS-KNAW Fungal Biodiversity Centre database (http://www.cbs.knaw.nl).

PREFACE

Frequently used molecular targets for species identification are outlined below:

Molecular Target		Application
ITS	Internal transcribed spacer regions (ITS1-5.8*S*-ITS2)	Species level identification of wide range of fungi
D1/D2	D1/D2 variable domains of the 28*S* rDNA gene	Species identification of many of the Mucorales
β-tubulin	Beta tubulin II	Accurate species resolution of *Aspergillus*.
Cal	Calmodulin	Species discrimination of *Alternaria*.
EF-1α	Elongation factor alpha subunit	Species complex identification of *Fusarium*.
RPB1 ***RPB2***	RNA polymerase I subunit RNA polymerase II subunit	Species complex identification within genera of *Fusarium, Penicillium* and *Talaromyces*.
ACT	Actin	Species discrimination of *Aspergillus, Cladosporium, Coniochaeta, Verticillium, Verruconis.*
GPDH	Glycerol-3-phosphate dehydrogenase	Species discrimination of *Bipolaris, Curvularia, Verticillium.*
CHS	Chitin synthase	Species discrimination of *Sporothrix*
Chi18-5	Chitinase 18-5	Species discrimination of *Trichoderma*

Antifungal Susceptibility: For many species, antifungal susceptibility data has also been provided. This has been derived from both the literature and data from Australian clinical isolates generated by using the CLSI M27-A Standard for yeasts and the CLSI M38-A Standard for moulds. This composite data is provided as a guide only. In many cases the clinical relevance of *in vitro* antifungal susceptibility results remains difficult to interpret, and expert advice from a consulting microbiologist or infectious disease specialist may be required.

CLSI M27-S4 clinical breakpoints are marked where available (green for susceptible, yellow for susceptible dose dependant or intermediate, red for resistant).

Abbreviations: Amphotericin B (AmB), Fluconazole (FLU), Itraconazole (ITRA), Posaconazole (POSA), Voriconazole (VORI), Anidulafungin (ANID), Caspofungin (CAS), Micafungin (MICA), 5-Fluorocytosine (5FC), Terbinafine (TERB).

Risk group (RG) recommendations are based on published data and on current definitions in accordance with the Australian/New Zealand Standard AS/NZS 2243.3:2010. Safety in laboratories Part 3: Microbiological safety and containment. **Note:** International biosafety guidelines vary in their RG ratings of fungal species.

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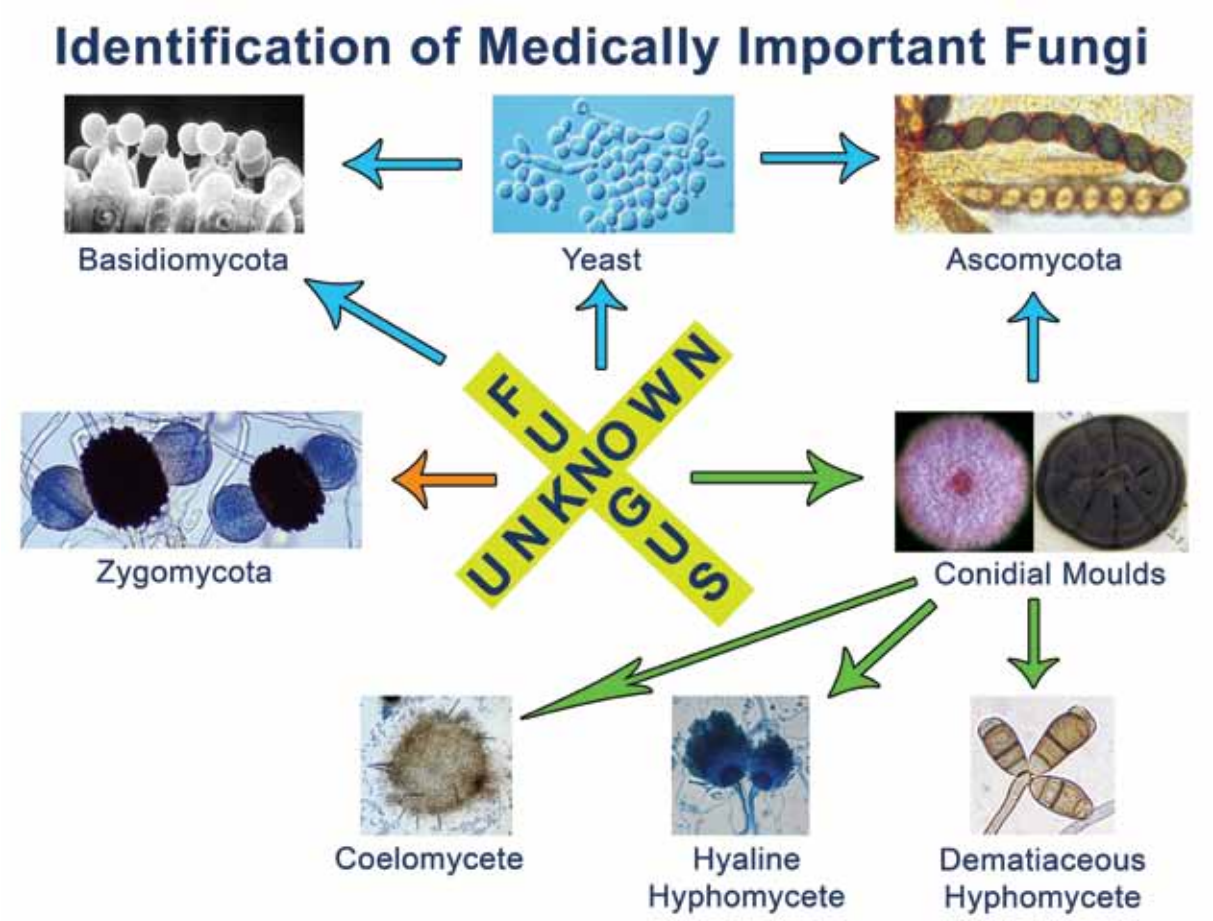


Schematic for the identification of medically important fungi.

Acremonium Link ex Fries

Key Features: Hyphomycete with solitary, erect, hyaline, awl-shaped phialides producing single-celled, globose to cylindrical conidia, mostly in slimy heads.

Antifungal Susceptibility: *Acremonium* spp. data from about 60 isolates (Perdomo *et al.* 2011 and Australian National data); **MIC µg/mL.**					
Antifungal	**Range**	**MIC_{90}**	**Antifungal**	**Range**	**MIC_{90}**
AmB	**0.25-16**	**16**	**VORI**	**0.06-8**	**8**
ITRA	**0.25-16**	**16**	**POSA**	**0.125-8**	**16**

References: Gams (1971), Domsch *et al*. (2007), Samson *et al*. (1995), de Hoog *et al*. (2000, 2015), Glenn *et al*. (1996), Perdomo *et al*. (2011a), Summerbell *et al.* (2011).

Acremonium Link ex Fries

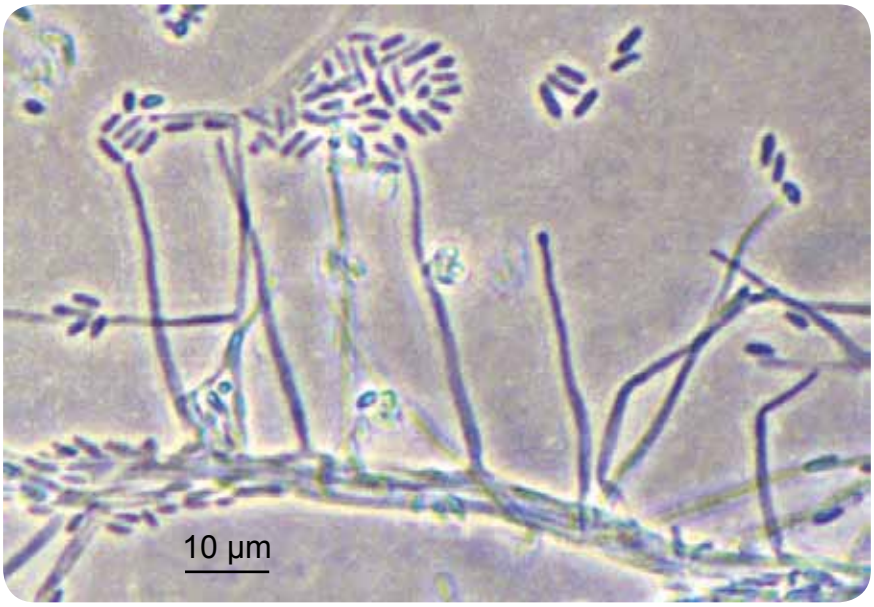
The genus *Acremonium* contains many species; most are saprophytic being isolated from dead plant material and soil. Several species including *A. recifei* and *A. alabamense* are recognised as opportunistic pathogens of man and animals, causing mycetoma, mycotic keratitis and onychomycosis. Recently, several *Acremonium*-like species recognised as opportunistic pathogens have been transferred to other genera; *Fusarium falciforme* (formerly *A. falciforme*), *Sarocladium kiliense* (formerly *A. kiliense*), *Gliomastic roseogriseum* (formerly *A. roseogriseum*) and *Sarocladium strictum* (formerly *A. strictum*) (Glenn *et al*. 1996, Summerbell *et al*. 2011).

RG-2 for species isolated from humans.

Morphological Description: Colonies are usually slow growing, often compact and moist at first, becoming powdery, suede-like or floccose with age, and may be white, grey, pink, rose or orange in colour. Hyphae are fine and hyaline and produce mostly simple awl-shaped erect phialides with inconspicuous collarettes. Conidia are usually one-celled, hyaline or rarely pigmented, globose to cylindrical, and mostly aggregated in slimy heads at the apex of each phialide. Chlamydospores may be present.

Comments: Microconidial *Fusarium* isolates may be confused with *Acremonium*, but they usually grow faster and have colonies with a characteristic fluffy appearance. *Phialemonium* species differ by having short, tapering phialides, mostly lacking a basal septum. *Coniochaeta* is characterised by having sessile phialidic collarettes that are formed directly on the hyphae.

Molecular Identification: Summerbell *et al*. (2011) revised the genus on the basis of 18*S* and D1/D2 sequence phylogeny. Sequence based identification may be performed using the D1/D2 or the ITS region. Caution must be exercised in the interpretation of database sequence comparisons due to the scarcity of database sequences from well-characterised strains, and some sequences may have been attributed to species that have been reclassified (Perdomo *et al*. 2011a).



Acremonium spp. showing long awl-shaped phialides producing cylindrical, one-celled conidia mostly aggregated in slimy heads at the apex of each phialide.

Acrophialophora fusispora (S.B. Saksena) Samson

The genus *Acrophialophora* contains 16 species that are most commonly associated with soil, especially from India (Zhang *et al.* 2015a). Two species have been reported as human pathogens, *A. fusispora* and *A. levis* (Sandoval-Denis *et al*. 2015). ITS sequencing is recommended for species identification.

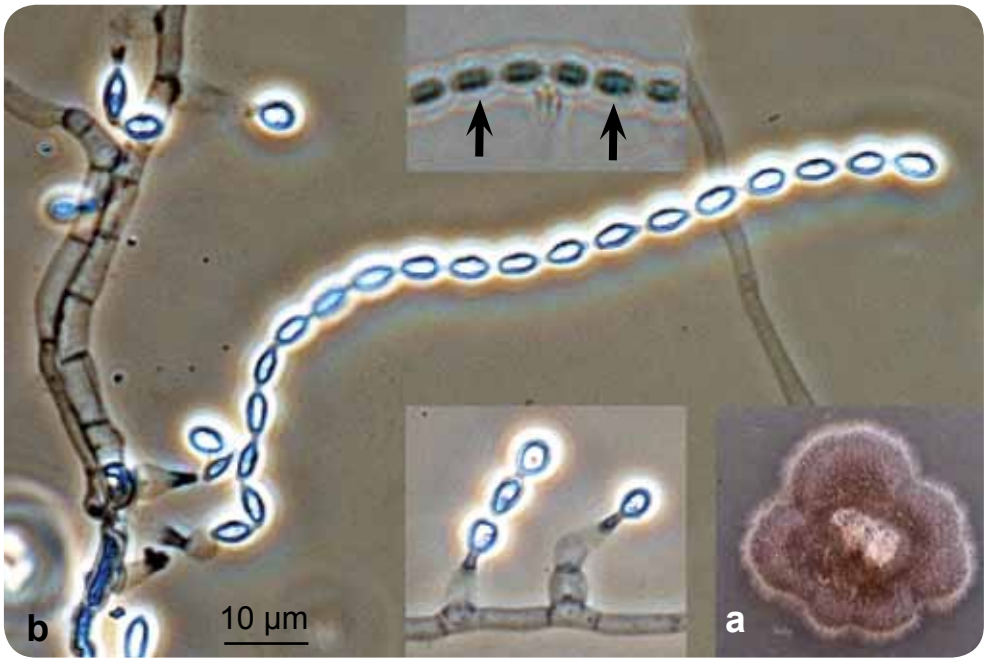
RG-1 organism.

Morphological Description: Colonies fast growing, greyish-brown with a black reverse. Conidiophores arising singly, terminally and laterally from the hyphae, erect, straight or slightly flexuose, tapering towards the apex, pale brown, rough-walled, up to 15 µm long, 2-5 µm wide, with whorls of phialides on the upper part. Phialides flask-shaped with a swollen base and a long, narrow neck, hyaline, smooth-walled or echinulate, 9-15 x 3-4.5 µm in the broadest part. Conidia in long chains, limoniform, one-celled, pale brown 5-12 x 3-6 µm, smooth to finely echinulate with indistinct spiral bands. Temperature: optimum 40^{O}C; maximum 50^{O}C**.**

Key Features: Hyphomycete with flask-shaped phialides producing long chains of one-celled, limoniform, pale brown conidia, with indistinct spiral bands. **Note:** In *A. levis* the conidia lack spiral bands and the phialides have verruculose walls.

Molecular Identification: D1/D2, ITS, 18*S* and β-tubulin sequences have been reported by Sandoval-Denis *et al*. (2015) and Zhang *et al* (2015a).

References: Domsch *et al.* (2007), de Hoog *et al.* (2000, 2015), Al-Mohsen *et al.* (2000), Guarro *et al*. (2007), Sandoval-Denis *et al*. (2015), Zhang *et al.* (2015a).



Acrophialophora fusispora (a) culture and (b) phialides and conidia with spiral striations (arrows).

Antifungal Susceptibility: ***A. fusispora*** data from about 40 isolates (Sandoval-Denis *et al.* 2015 and Australian National data); **MIC µg/mL**.					
Antifungal	**Range**	**MIC$_{90}$**	**Antifungal**	**Range**	**MIC$_{90}$**
AmB	**1-32**	**16**	**VORI**	**0.06-0.5**	**0.25**
ITRA	**0.125-4**	**1**	**POSA**	**0.25-1**	**1**

Alternaria Nees ex Fries

A ubiquitous genus containing common saprophytes in soil and air, and plant pathogens. *A. infectoria* is the most common clinical species (Pastor and Guarro, 2008). Although usually seen as saprophytic contaminants, *Alternaria* species in particular *A. alternata* and *A. infectoria* are recognised causative agents of subcutaneous phaeohyphomycosis and mycotic keratitis. They are a rare cause of onychomycosis, usually following trauma to the nail.

RG-1 organisms.

Morphological Description: Colonies are fast growing, black to olivaceous-black or greyish, and are suede-like to floccose. Microscopically, branched acropetal chains (blastocatenate) of multicellular conidia (dictyoconidia) are produced sympodially from simple, sometimes branched, short or elongate conidiophores. Conidia are obclavate, obpyriform, sometimes ovoid or ellipsoidal, often with a short conical or cylindrical beak, pale brown, smooth-walled or verrucose. Temperature: optimum 25-28^{O}C; maximum 31-32^{O}C.

Molecular Identification: Multilocus genotype studies have shown the *Alternaria* complex currently comprises nine genera and eight *Alternaria* sections (Woudenbert *et al*. 2013). ITS sequencing is sufficient for genus and usually species level identification and can clearly differentiate *A. alternata* and *A. infectoria* (Pastor and Guarro, 2008). However, it is estimated that >14% of GenBank sequences of *Alternaria* species are misclassified, so unknown sequences should be compared to those of well-characterised reference strains (Woudenberg *et al*. 2013).

Comments: *Alternaria* species soon lose their ability to sporulate in culture. Potato dextrose agar and cornmeal agar are the most suitable media to use, and incubation under ultra-violet light is recommended to maintain sporulation.

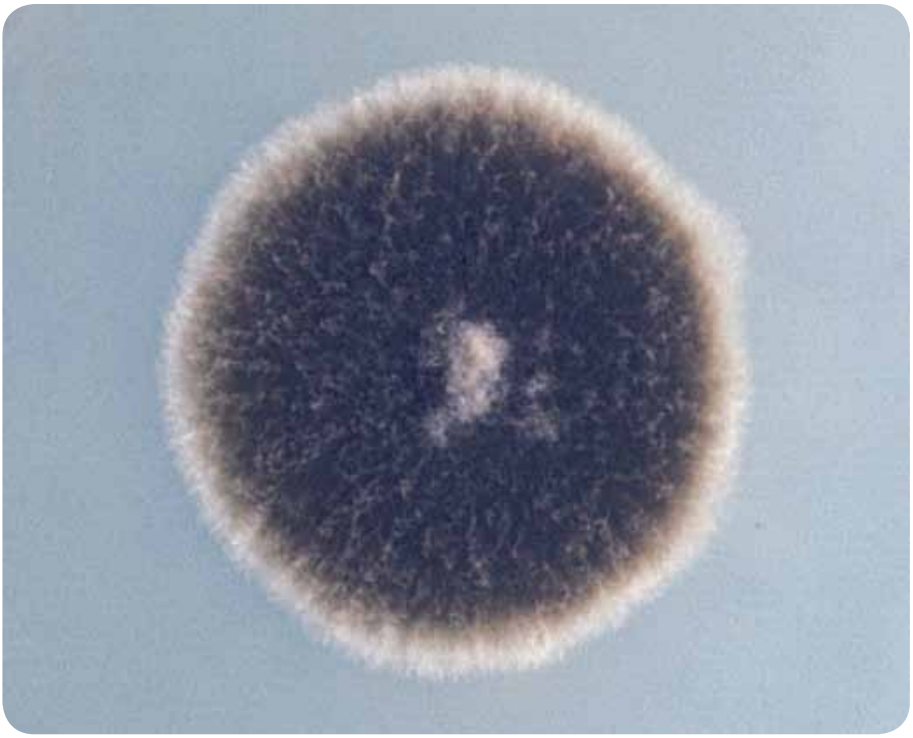
Key Features: Dematiaceous hyphomycete producing chains of darkly pigmented, ovoid to obclavate dictyoconidia, often with short conical or cylindrical beaks.

References: Simmons (1967, 2007), Ellis (1971), Domsch *et al.* (2007), Samson *et al.* (1995), de Hoog *et al.* (2000, 2015), Pryor and Gilbertson (2000), de Hoog and Horre (2002), Pastor and Guarro (2008), Woudenberg *et al.* (2013).

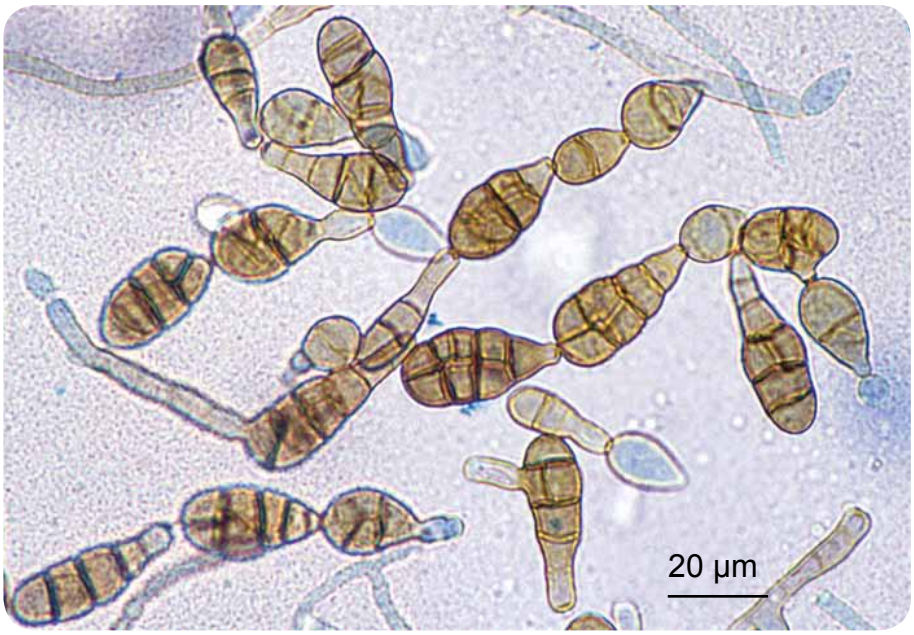
Antifungal Susceptibility: *Alternaria* spp. (Australian National data); **MIC µg/mL**.

	No	≤0.008	0.016	0.03	0.06	0.125	0.25	0.5	1	2	4	≥8
AmB	**10**					**3**	**1**	**4**	**2**			
VORI	**10**						**1**	**3**	**5**	**1**		
POSA	**9**			**2**	**1**	**5**	**1**					
ITRA	**10**			**1**	**1**	**2**	**5**	**1**				

Alternaria Nees ex Fries



Alternaria alternata colonies are black to olivaceous-black or greyish, and are suede-like to floccose.



Alternaria alternata showing branched acropetal chains and multicelled, obclavate to obpyriform conidia with short conical beaks.

Aphanoascus fulvescens (Cooke) Apinis

Aphanoascus fulvescens is a soil-borne keratinolytic ascomycete that occasionally causes dermatomycosis in humans and animals.

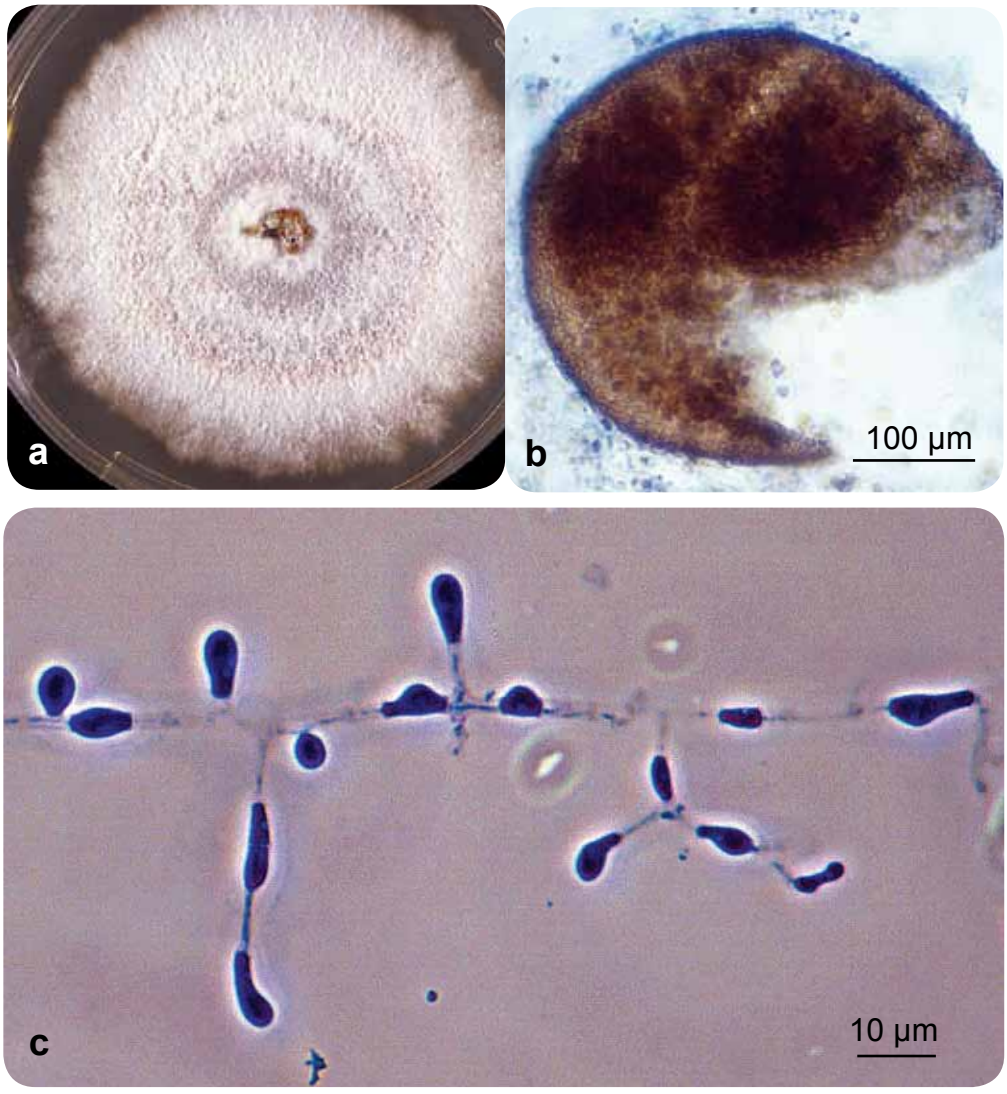
RG-2 organism.

Morphological Description: Colonies are moderately fast growing, white to tan with the production of numerous spherical, pseudoparenchymatous, buff to light brown cleistothecia (non-ostiolate ascocarps). Asci are subspherical to ellipsoidal and eight-spored. Ascospores light brown, yellowish to pale brown in mass, irregularly reticulate, lens-shaped, 3.5-4.7 x 2.5-3.5 µm. *Aphanoascus fulvescens* has a *Chrysosporium* anamorph showing typical pyriform to clavate-shaped conidia with truncated bases, 15-17.5 x 3.7-6 µm, which are formed either intercalary, laterally or terminally.

Molecular Identification: ITS sequencing will differentiate most species. The calmodulin gene may also be useful (Cano *et al*. 2002, Halliday *et al.* 2015).

Key Features: Keratinolytic, cleistothecia, and a *Chrysosporium* anamorph.

References: Domsch *et al.* (2007), McGinnis (1980), de Hoog *et al.* (2000, 2015), Cano and Guarro (1990), Cano *et al.* (2002).



Aphanoascus flavescens (a) culture, (b) cleistothecium and (c) conidia.

Apophysomyces complex

Historically the genus *Apophysomyces* was considered to be monotypic and *A. elegans* was reported to be an important human pathogen in immunocompetent patients following traumatic implantation. A phylogenetic revision of the genus has identified three additional species, *A. ossiformis, A. trapeziformis* and *A. variabilis*. Many isolates previously identified as *A. elegans*, now appear to be *A. variabilis (*Alvarez *et al.* 2010a).

Molecular identification is required to accurately differentiate these species. Morphological characteristics overlap so identification and reporting of *Apophysomyces* complex is recommended for most diagnostic laboratories.

Molecular Identification: The ITS region and D1/D2 domain may provide for accurate species identification (Halliday *et al*. 2015). ITS restriction fragment length polymorphism analysis has also been described (Chakrabarti *et al*. 2003).

Apophysomyces elegans Misra, Srivastava & Lata

RG-2 organism.

Morphological Description: Colonies are fast growing, white, becoming brownish grey with age, downy with no reverse pigment, and are composed of broad, sparsely septate (coenocytic) hyphae. Sporangiophores are unbranched, straight or curved, slightly tapering towards the apex, up to 540 µm long, 3-6 µm in width near the apophysis, and hyaline when young but developing a light to dark brown pigmentation and a conspicuous subapical thickening 10-16 µm below the apophysis with age. Sporangiophores arise at right angles from the aerial hyphae and often have a septate basal segment resembling the “foot cell” commonly seen in *Aspergillus*. Rhizoids are thin-walled, subhyaline and predominantly unbranched. Sporangia are multispored, small (20-58 µm diameter), typically pyriform in shape, hyaline at first, sepia-coloured when mature, with distinct apophyses and columellae. Columellae are hemispherical in shape and the apophyses are distinctly funnel or bell-shaped. Sporangiospores are smooth-walled, subspherical to cylindrical, (5-8 x 4-6 µm), subhyaline to sepia in mass. Good growth at 26^{O}C, 37^{O}C and 42^{O}C.

Apophysomyces variabilis Alvarez *et al.*

RG-2 organism.

Morphological Description: Colonies are fast growing, whitish with scarce aerial mycelium and no reverse pigment. Sporangiophores are erect, generally arising singly, unbranched, slightly tapering towards the apex, up to 100-400 µm long, 2-3.5 µm in width near the apophysis, hyaline when young but developing a light greyish brown pigmentation with age. Sporangia are multispored, small (15-50 µm diameter), typically pyriform in shape, hyaline at first, sepia-coloured when mature, with distinct apophyses and columellae. Columellae are hemispherical in shape and the apophyses are short and distinctly funnel-shaped. Sporangiospores are smooth-walled, variable in shape, trapezoid, ellipsoidal, sub-triangular or claviform, (5-14 x 3-6 µm), subhyaline to sepia in mass. Good growth at 26^{O}C, 37^{O}C and 42^{O}C.

Apophysomyces complex

Apophysomyces elegans/variabilis (a) young, multispored, pyriform shaped sporangium showing a typical funnel-shaped apophysis but without the subapical thickening of a more mature sporangiophore, and (b) mature sporangium showing distinct funnel-shaped apophyses, columellae, and a conspicuous pigmented subapical thickening which constricts the lumen of the sporangiophore below the apophysis (arrow). Sporangiospores are smooth-walled, oblong and subhyaline.

Apophysomyces complex

Comment: *Apophysomyces* complex is readily distinguished from other zygomycetes, especially the morphologically similar, strongly apophysate pathogen *Lichtheimia corymbifera*, by having sporangiophores with distinctive funnel or bell-shaped apophyses and hemispherical-shaped columellae. In addition, there is a conspicuous pigmented subapical thickening, which constricts the lumen of the sporangiophore below the apophysis, and distinctive foot cells.

Laboratory identification of this fungus may be difficult or delayed because of the mould's failure to sporulate on primary isolation media or on subsequent subculture onto potato dextrose agar. Sporulation may be stimulated by the use of nutrient deficient media, like cornmeal-glucose-sucrose-yeast extract agar, Czapek Dox agar, or by using the agar block method described by Ellis and Ajello (1982) and Ellis and Kaminski (1985). Molecular-based identification is particularly helpful for the definitive identification of poorly sporulating cultures.

Key Features: Soil fungus with a tropical to subtropical distribution. Characteristic "cocktail glass" apophysate sporangial morphology with conspicuous subapical thickening of the sporangiophore. Resistance to cycloheximide. Rapid growth at 42^{O}C, no growth at 50^{O}C.

References: Misra *et al*. (1979), Ellis and Ajello (1982), Padhye and Ajello (1988), Wieden *et al.* (1985), Lawrence *et al.* (1986), Cooter *et al.* (1990), Holland (1997), de Hoog *et al*. (2000, 2015), Ellis (2005b), Alvarez *et al.* (2010a), Chakrabarti *et al.* (2003, 2010), Guarro *et al.* (2011).

Antifungal Susceptibility: ***A. variabilis*** limited data (Espinel-Ingroff *et al*. 2015a, and Australian National data); **MIC µg/mL**.

	No	≤0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	≥64
AmB	10		1		1		3	5					
POSA	10				1	1	7	1					

A. variabilis data from 20 isolates (Alvarez *et al.* 2010 and Chakrabarti *et al.* 2010); **MIC µg/mL.**

AmB	Range 0.5-4; MIC_{90}= 2	VORI	Range 8-16; MIC_{90}= 16
ITRA	Range 0.25-2; MIC_{90}= 2	POSA	Range 0.5-2; MIC_{90}= 1

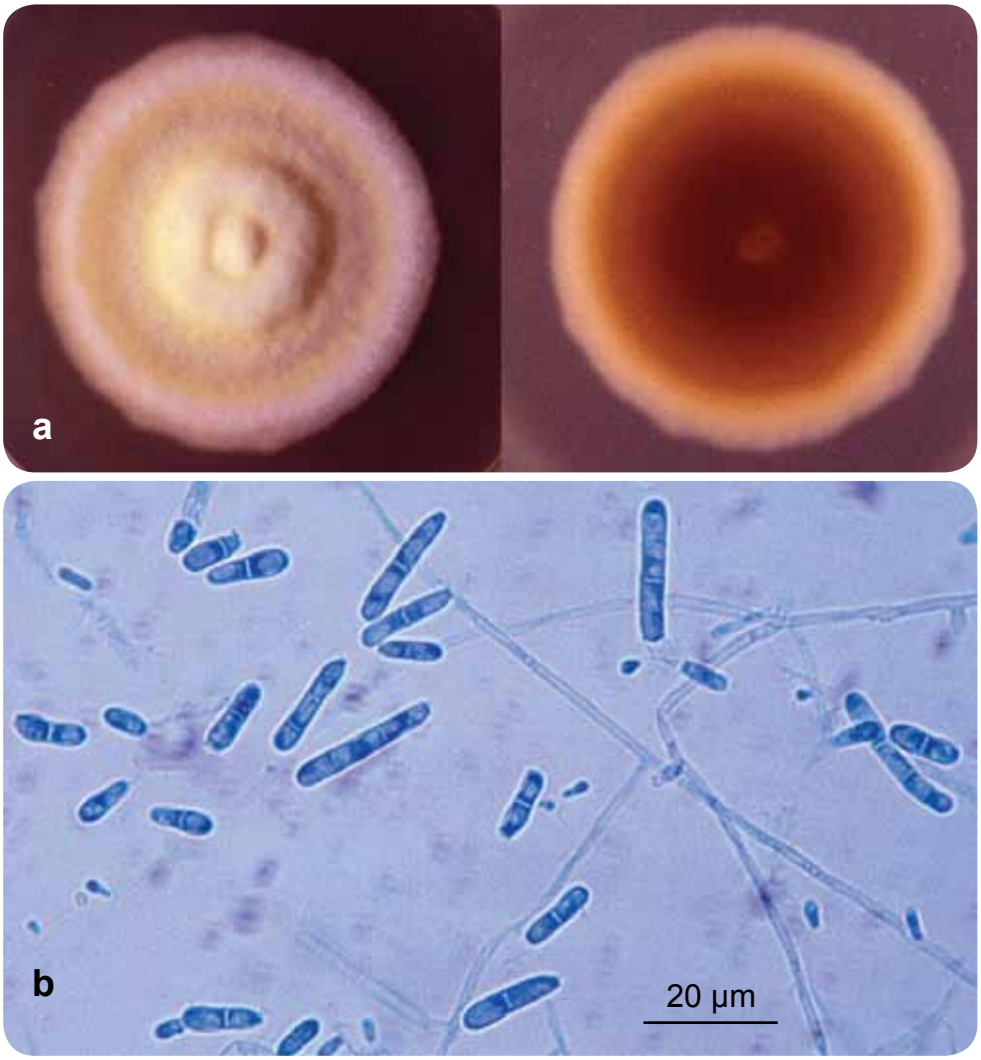
Arthroderma insingulare Padhye and Carmichael

Synonymy: *Trichophyton terrestre* Durie and Frey.

Arthroderma insingulare is a geophilic fungus of worldwide distribution which may occur as a saprophytic contaminant on humans and animals. Durie and Frey (1957) first described this soil fungus as *Trichophyton terrestre* from New South Wales, Australia. Since then *T. terrestre* has been described as an anamorph of three different species of *Arthroderma*; *A. insingulare*, *A. lenticulare* and *A. quadrifidum* (Padhye and Carmichael, 1972). However, ITS and D1/D2 sequencing of the original isolates obtained from the Mycology Laboratory at Royal North Shore Hospital, Sydney has now identified this fungus as *Arthroderma insingulare*. **RG-1 organism.**

Morphological Description: Colonies are usually flat to downy with a suede-like to granular texture resembling *T. mentagrophytes.* The surface colour may range from white to cream, buff to yellow, or greenish-yellow. Reverse pigmentation is usually yellowish-brown although some variants have a deep rose red reverse. Microconidia are large, clavate or pedicellate, usually exhibiting transition forms to more or less abundant lateral macroconidia. Macroconidia are clavate to cylindrical with rounded ends, smooth and thin-walled, and are two to six-celled. Chlamydospores, hyphal spirals, racquet mycelium and antler hyphae may also be present. No growth at 37^{O}C.

Molecular Identification: ITS and D1/D2 sequencing is recommended for definitive identification of isolates.



Arthroderma insingulare (a) culture and (b) macroconidia.

Arthroderma uncinatum Dawson & Gentles

Synonymy: *Trichophyton ajelloi* (Vanbreuseghem) Ajello.

Arthroderma uncinatum is a geophilic fungus with a worldwide distribution which may occur as a saprophytic contaminant on humans and animals but infections are doubtful. Not known to invade hair *in vivo*, but produces hair perforations *in vitro*.

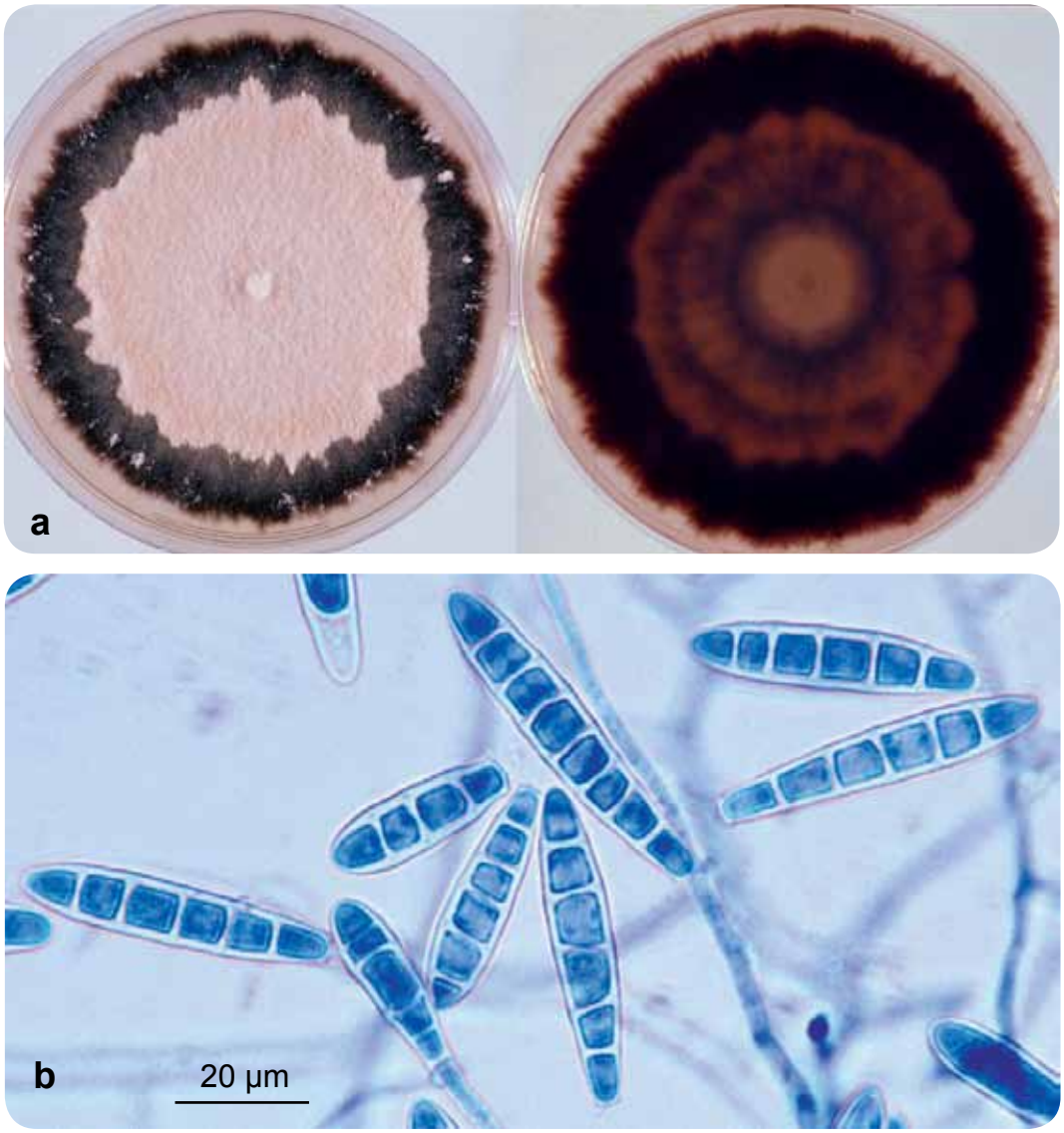
RG-1 organism.

Morphological Description: Colonies are usually flat, powdery, cream, tan to orange-tan in colour, with a blackish-purple submerged fringe and reverse. Macroconidia are numerous, smooth, thick-walled, elongate, cigar-shaped, 29-65 x 5-10 µm, and multiseptate with up to nine or ten septa. Microconidia are usually absent, but when present are ovate to pyriform in shape.

Key Features: Culture characteristics, macroconidial morphology, urease positive and good growth on Sabouraud's 5% salt agar.

Molecular Identification: ITS sequencing recommended (Gräser *et al*. 2008).

References: Rebell and Taplin (1970), Rippon (1988), de Hoog *et al.* (2015, 2016).



Arthroderma uncinatum (a) culture and (b) macroconidia.

Arthrographis kalrae (Tewari & Macpherson) Sigler & Carmichael

Arthrographis is an arthroconidial mould comprising four species: *A. kalrae, A. lignicola, A. pinicola* and *A. alba.* These fungi are commonly found in environmental samples (soil, wood, air and water), but are isolated rarely from clinical specimens (Sandoval-Denis *et al.* 2014a).

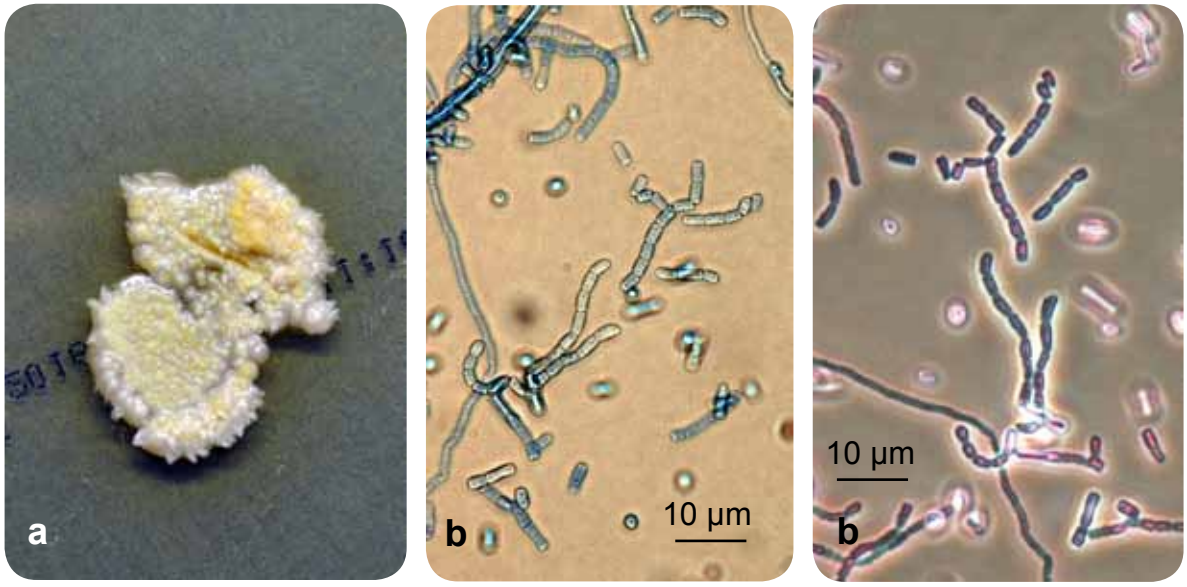
RG-1 organism.

Morphological Description: Colonies with slow to moderate growth, creamy white to tan-coloured. Initially, yeast-like then developing hyphal growth and conidiophores subhyaline, narrow, branched, often in bundles, occasionally forming whitish, large 0.5 cm, linear synnemata. Arthroconidia are one-celled, hyaline, smooth-walled, oblong to cylindrical, with truncate ends, 2.5-9x 1-2 µm. Spherical blastoconidia 2-4 x 2-3 µm, may also be formed laterally and sessile on undifferentiated hyphae. Chlamydospores may also be present. Very rarely immature ascomata submerged in the agar are produced. Growth at 42^{O}C, and on media containing cycloheximide.

Molecular Identification: ITS and D1/D2 sequencing may be used for accurate species identification (Sugiura and Hironaga 2010, Halliday *et al.* 2015).

Key Features: Keratinolytic, *in vitro* hair perforation positive, growth at 37^{O}C and tolerance to cycloheximide.

References: Sugiura and Hironaga (2010), Giraldo *et al.* (2014), Sandoval-Denis *et al.* (2014a), de Hoog *et al*. (2015).



Arthrographis kalrae (a) culture and (b) arthroconidia.

Antifungal Susceptibility: *A. kalrae* (Australian National data); **MIC µg/mL**.											
	No	**≤0.016**	**0.03**	**0.06**	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**≥8**
AmB	**7**					**1**	**5**	**1**			
VORI	**7**		**1**	**3**	**2**		**1**				
POSA	**7**		**1**		**2**	**2**	**2**				
ITRA	**7**		**1**			**4**	**2**				

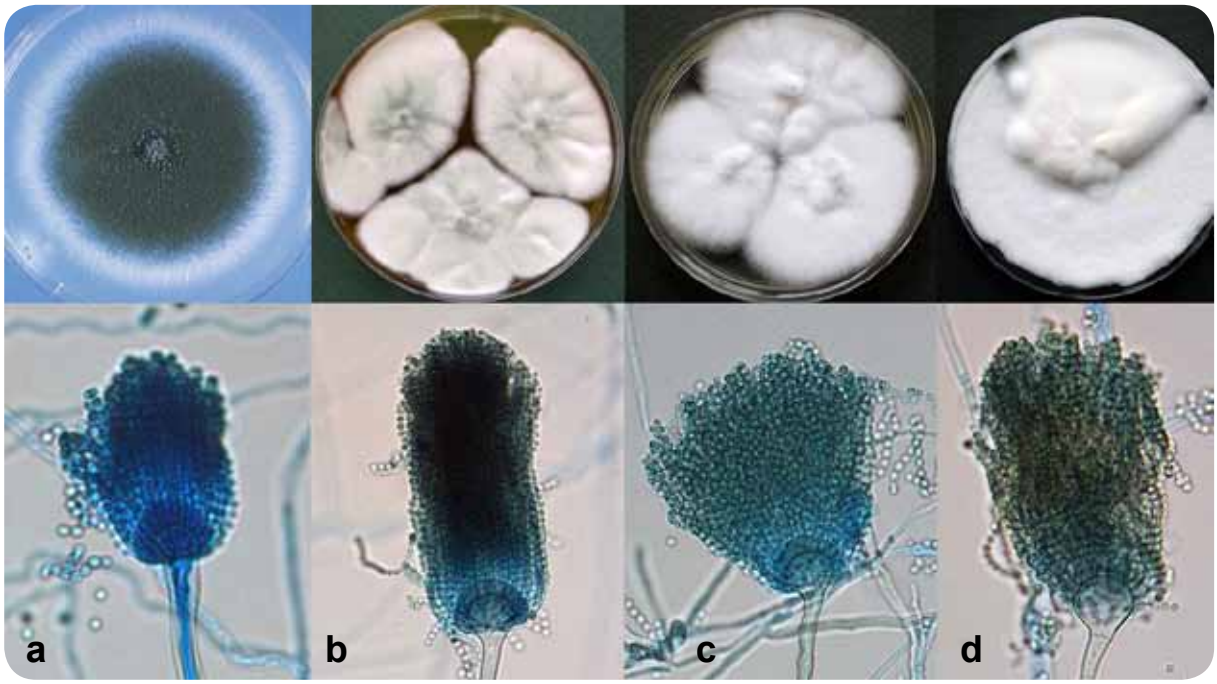
Aspergillus Micheli ex Link

Aspergillus is a very large genus containing about 250 species, which are currently classified into seven subgenera that are in turn subdivided into several sections comprised of related species (Raper and Fennell 1965, Gams *et al*. 1985, Geiser *et al.* 2007). Traditionally, clinical microbiology laboratories have relied heavily on morphology-based identification methods to differentiate *Aspergillus* species. However many species, especially members of the section *Fumigati* have overlapping morphological characteristics, which has allowed several genetically distinct species to be misidentified (Balajee *et al.* 2005, 2007). This has led to the clustering of species with overlapping morphologies into “species complexes”, so that laboratories may report more accurately morphology-based identifications.

Identification of clinical isolates of *Aspergillus* to species level may be important given that different species have variable susceptibilities to multiple antifungal drugs. For example, *in vitro* and *in vivo* studies have demonstrated that *A. terreus* isolates are largely resistant to the antifungal drug amphotericin B, *A. ustus* isolates appear to be refractory to azoles, and *A. lentulus* and *Petromyces alliaceus* have low *in vitro* susceptibilities to a wide range of antifungals including amphotericin B, azoles, and echinocandins (Balajee *et al*. 2005, 2007).

Molecular Identification: Recommended barcoding gene: β-tubulin. General criteria for identification were outlined by Balajee *et al*. (2007). Phylogenetic relationships of the entire genus were presented by Wang *et al*. (1999) and Peterson (2000, 2008).

MALDI-TOF MS: A comprehensive ‘in-house’ database of reference spectra allows accurate identification of species of *Aspergillus* even within complexes e.g. *A. fumigatus sensu stricto* and *A. lentulus* (Lau *et al*. 2013, Sleiman *et al*. 2015).



Four species in the *Aspergillus fumigatus* complex showing overlapping morphological characteristics; (a) *Aspergillus fumigatus,* (b) *Aspergillus lentulus,* (c) *Neosartorya fischeri* and (d) *Aspergillus felis*.

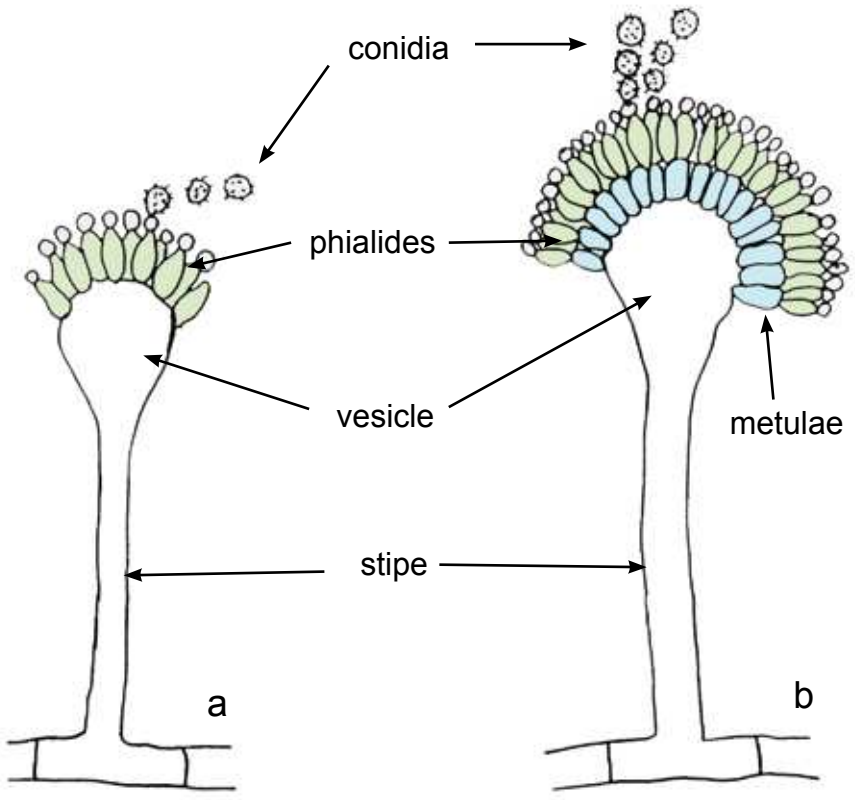
Aspergillus Micheli ex Link

Morphological Description: Colonies are usually fast growing, white, yellow, yellow-brown, brown to black or shades of green, mostly consisting of a dense felt of erect conidiophores. Conidiophores terminate in a vesicle covered with either a single palisade-like layer of phialides (uniseriate) or a layer of subtending cells (metulae) which bear small whorls of phialides (the biseriate structure). The vesicle, phialides, metulae (if present) and conidia form the conidial head. Conidia are one-celled, smooth or rough-walled, hyaline or pigmented, are produced in long dry chains which may be divergent (radiate) or aggregated in compact columns (columnar). Some species may produce Hülle cells or sclerotia.

For morphological identification, isolates are usually inoculated at three points on Czapek Dox agar and 2% malt extract agar and incubated at 25^{O}C. Most species sporulate within 7 days. Descriptions are primarily based on colony pigmentation and morphology of the conidial head. Microscopic mounts are best made using cellotape flag or slide culture preparations mounted in lactophenol cotton blue. A drop of alcohol is usually needed to remove bubbles and excess conidia.

Key Features: Hyaline hyphomycete showing distinctive conidial heads with flask-shaped phialides arranged in whorls on a vesicle.

References: Raper and Fennell (1965), Domsch *et al.* (1980), McGinnis (1980), Onions *et al.* (1981), Samson and Pitt (1990, 2000), Samson *et al.* (1995), Samson (1979), Vanden Bossche *et al.* (1988), Klich (2002), Steinbach *et al.* (2005), Samson *et al*. (2011a, 2014), de Hoog *et al.* (2000, 2015).



Conidial head morphology in *Aspergillus* (a) uniseriate, (b) biseriate.

Aspergillus flavus complex

Aspergillus section *Flavi* historically includes species with conidial heads in shades of yellow-green to brown and dark sclerotia. Hedayati *et al.* (2007) reviewed the *A. flavus* complex and included 23 species or varieties, including two sexual species, *Petromyces alliaceus* and *P. albertensis.* Several species of section *Flavi* produce aflatoxins, among which aflatoxin B1 is the most toxic of the many naturally occurring secondary metabolites produced by fungi. Aflatoxins are mainly produced by *A. flavus* and *A. parasiticus*, which coexist and grow on almost any crop or food (Varga *et al.* 2011). Within the complex, *A. flavus* is the principle medically important pathogen of both humans and animals. However, some other species in the *A. flavus* complex, notably *A. oryzae, A. avenaceus, A. tamari, A. alliaceus* and *A. nomius,* may cause rare mostly superficial infections (Hedayati *et al.* 2007, de Hoog *et al.* 2015).

Note: Accurate species identification within *A. flavus* complex remains difficult due to overlapping morphological and biochemical characteristics. For morphological identifications, it is recommended to report as *Aspergillus flavus* complex.

Molecular Identification: ITS sequence analysis is sufficient to identify to species complex level only. Definitive identification requires analysis of β-tubulin, calmodulin and actin genes (Samson *et al*. 2007, Balajee *et al*. 2005a).

Aspergillus flavus Link ex Grey

Aspergillus flavus has a worldwide distribution and normally occurs as a saprophyte in soil and on many kinds of decaying organic matter, however, it is also a recognised pathogen of humans and animals. It is a causative agent of otitis, keratitis, acute and chronic invasive sinusitis, and pulmonary and systemic infections in immunocompromised patients. *A. flavus* is second only to *A. fumigatus* as the cause of human invasive aspergillosis (Hedayati *et al.* 2007).

RG-2 organism.

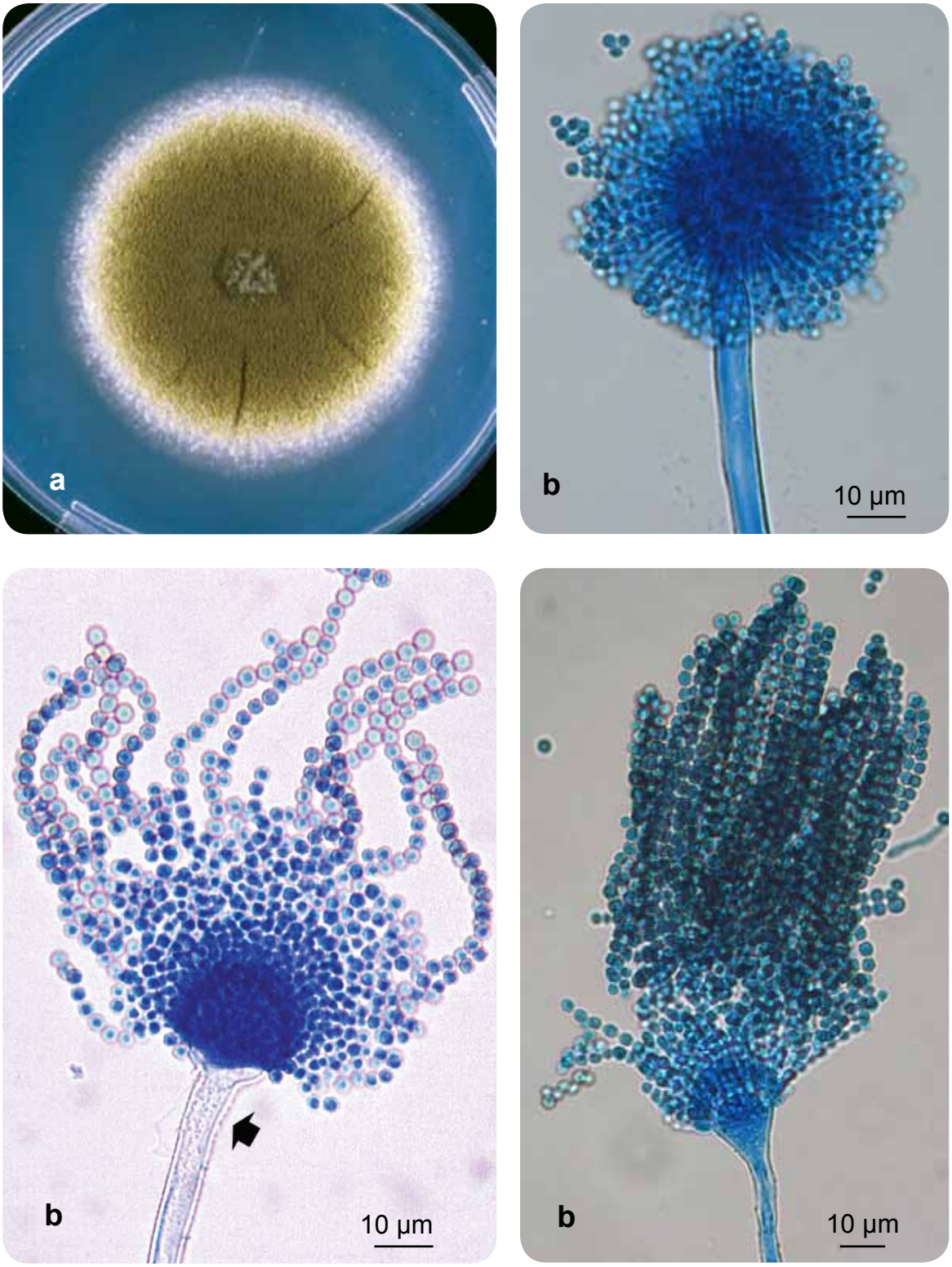
Morphological Description: On Czapek Dox agar, colonies are granular, flat, often with radial grooves, yellow at first but quickly becoming bright to dark yellow-green with age. Conidial heads are typically radiate, later splitting to form loose columns (mostly 300-400 µm in diameter), biseriate but having some heads with phialides borne directly on the vesicle (uniseriate). Conidiophore stipes are hyaline and coarsely roughened, often more noticeable near the vesicle. Conidia are globose to subglobose (3-6 µm in diameter), pale green and conspicuously echinulate. Some strains produce brownish sclerotia.

Key Features: Spreading yellow-green colonies, rough-walled stipes, mature vesicles bearing phialides over their entire surface and conspicuously echinulate conidia.

Antifungal Susceptibility: *A. flavus* complex (Australian National data); **MIC µg/mL.**

	No	≤0.008	0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	≥16
AmB	68					1	5	7	30	22	3		
VORI	68			1	1	6	25	24	11				
POSA	57		2	1	5	16	26	7					
ITRA	68			1	3	11	43	10					

Aspergillus flavus Link ex Grey



Aspergillus flavus (a) culture and (b) conidial heads.
Note: Rough-walled stipe near vesicle (arrow) and both uniseriate and biseriate conidial heads may be present.

Aspergillus fumigatus complex

Aspergillus section *Fumigati* includes species characterised by uniseriate aspergilla with columnar conidial heads in shades of blue-green and flask-shaped vesicles (Raper and Fennell, 1965). Teleomorphic species belonging to the "*Aspergillus fischeri* series" of the *A. fumigatus* group (Raper and Fennell, 1965) were placed in the genus *Neosartorya* (family Trichocomaceae) by Malloch and Cain (1972). Section *Fumigati* includes more than 23 *Neosartorya* species and 10 anamorphic species (Samson *et al.* 2007).

Although *A. fumigatus* is recognised as the major human pathogen within the complex, recent phylogenetic studies have demonstrated that some human and animal infections may be caused by *A. lentulus, A. fumigatiaffinis, A. fumisynnematus, A. felis, Neosartorya fischeri, N. pseudofischeri, N. udagawae, N. hiratsukae* and *N. spinosa* (Coriglione *et al*. 1990; Summerbell *et al*. 1992; Padhye *et al.* 1994a; Lonial *et al*. 1997; Jarv *et al.* 2004; Balajee *et al.* 2005, 2006; Barrs *et al.* 2013).

Aspergillus felis Barrs, van Doorn, Varga & Samson

Aspergillus felis has been reported as a causative agent of invasive aspergillosis and rhinosinusitis in humans, dogs and cats. Disease in all host species is often refractory to aggressive antifungal therapeutic regimens.

RG-1 organism.

Morphological Description: Colonies of *A. felis* are suede-like to floccose, white with interspersed grey green patches of conidia (conidiation is slow to poor). Conidial heads of *A. felis* are short, columnar and uniseriate. Conidiophore stipes are smooth-walled and vesicles are usually subglobose in shape. Conidia globose (2-3 µm in diameter), smooth to finely roughened.

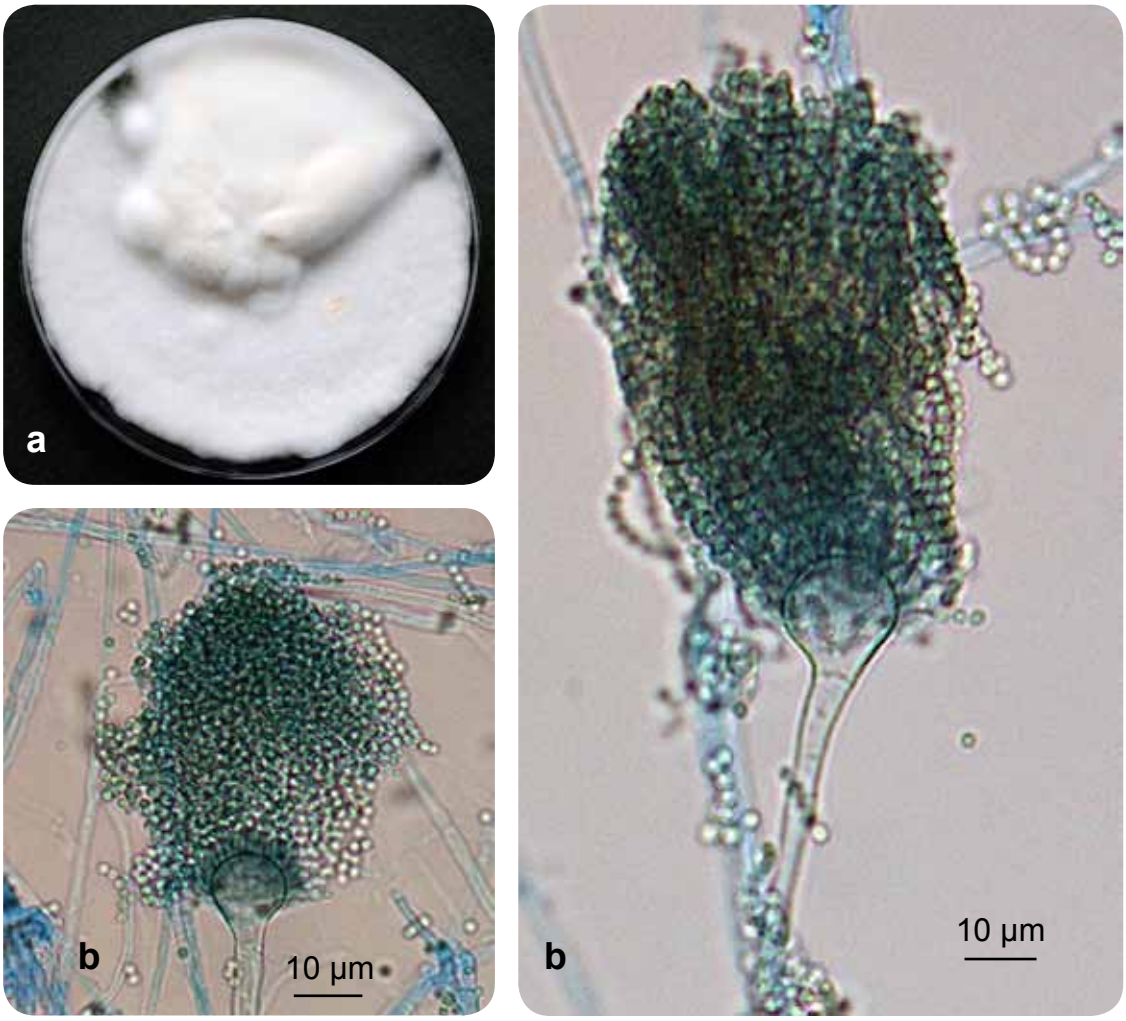
Molecular Identification: *A. felis* can be distinguished from other members of the section *Fumigati* by sequence analysis of β-tubulin, calmodulin and actin genes (Barrs *et al*. 2013). ITS sequencing is not recommended.

Comment: *A. felis* is phenotypically similar to *Aspergillus viridinutans*, but differs by its ability to grow at 45°C. This species is phylogenetically related to *Neosartorya aureola* and *N. udagawae* and differs to *N. aureola* in having a heterothallic mode of reproduction.

Antifungal Susceptibility: *A. felis* (Barrs *et al*. 2013); **MIC µg/mL**.

	No	≤0.008	0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	≥16
AmB	13						1	11	1				
VORI	13						1	3	1	5	3		
POSA	13			4	3	2	1	2	1				
ITRA	13			1	2	2	4	1	3				

Aspergillus felis Barrs, van Doorn, Varga & Samson



Aspergillus felis (a) culture and (b) conidial head morphology.

Aspergillus fumigatus Fresenius

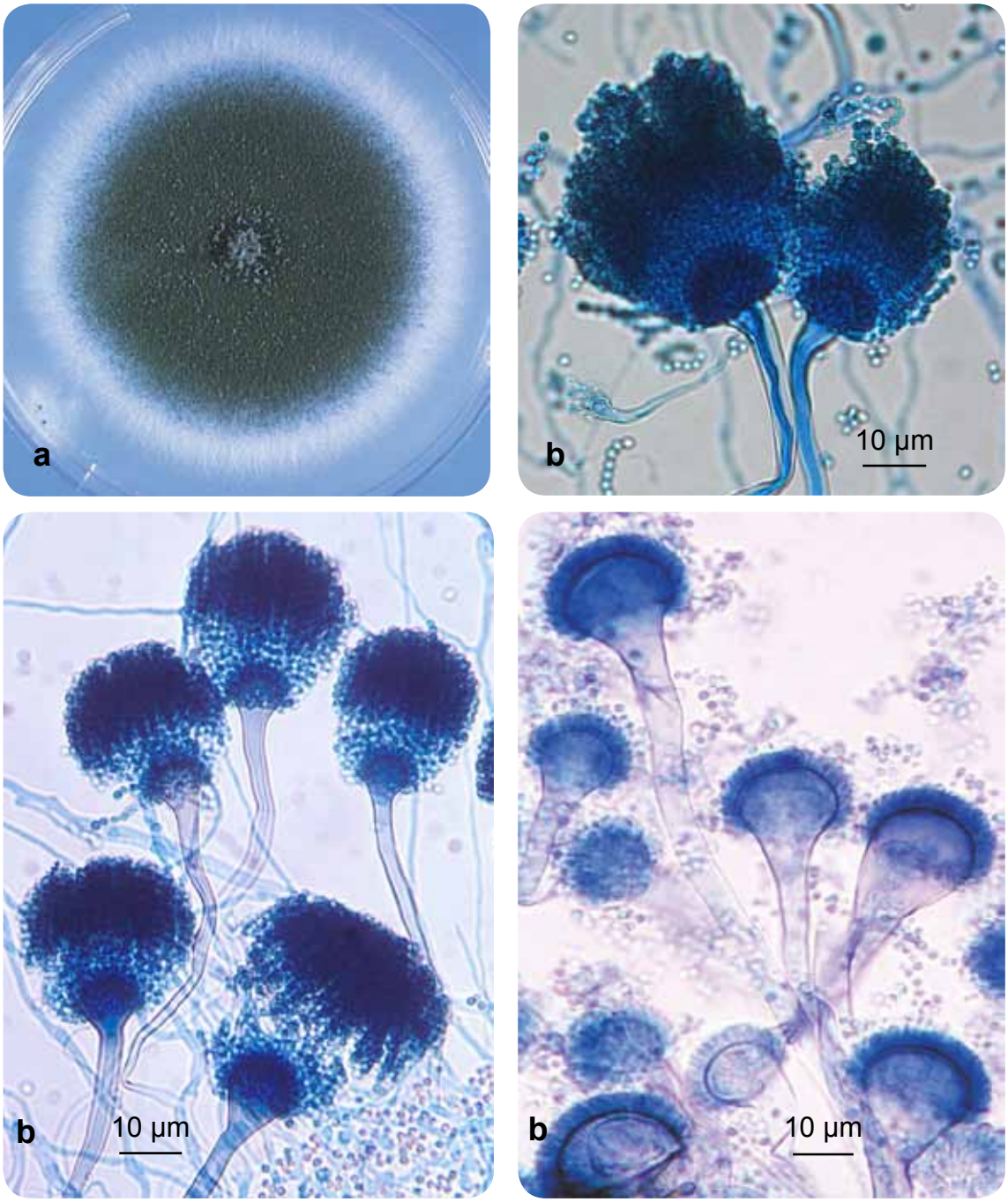
RG-2 organism.

Morphological Description: On Czapek Dox agar, colonies are typically blue-green with a suede-like surface consisting of a dense felt of conidiophores. Conidial heads are typically columnar (up to 400 x 50 µm but often much shorter and smaller) and uniseriate. Conidiophore stipes are short, smooth-walled and have conical-shaped terminal vesicles which support a single row of phialides on the upper two thirds of the vesicle. Conidia are produced in basipetal succession forming long chains and are globose to subglobose (2.5-3.0 µm in diameter), green and finely roughened. **Note:** This species is thermotolerant with a maximum growth temperature of 55^{O}C.

Key Features: Uniseriate and columnar conidial heads with the phialides limited to the upper two thirds of the vesicle and curving to be roughly parallel to each other.

Molecular Identification: Sequence analysis of ITS is sufficient to identify to species complex level only. For definitive identification analysis, β-tubulin, calmodulin and actin genes is required (Samson *et al*. 2007; Balajee *et al*. 2005).

Aspergillus fumigatus Fresenius



Aspergillus fumigatus (a) culture and (b) conidial head morphology.
Note: Uniseriate row of phialides on the upper two thirds of the vesicle.

Antifungal Susceptibility: *A. fumigatus* complex (Australian National data); **MIC µg/mL**.

	No	≤0.008	0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	≥16
AmB	523				2	40	122	167	130	58	3	1	
VORI	486		1	1	27	112	259	54	11	13	5	3	
POSA	415	7	27	50	69	162	79	13	7	0	0	1	
ITRA	523	1	6	17	41	74	244	115	15	5	1		4
ANID	249	5	170	52	18	1		1		2			
MICA	249	91	95	48	12	2		1					
CAS	264	2	22	91	106	24	12		1		1	5	

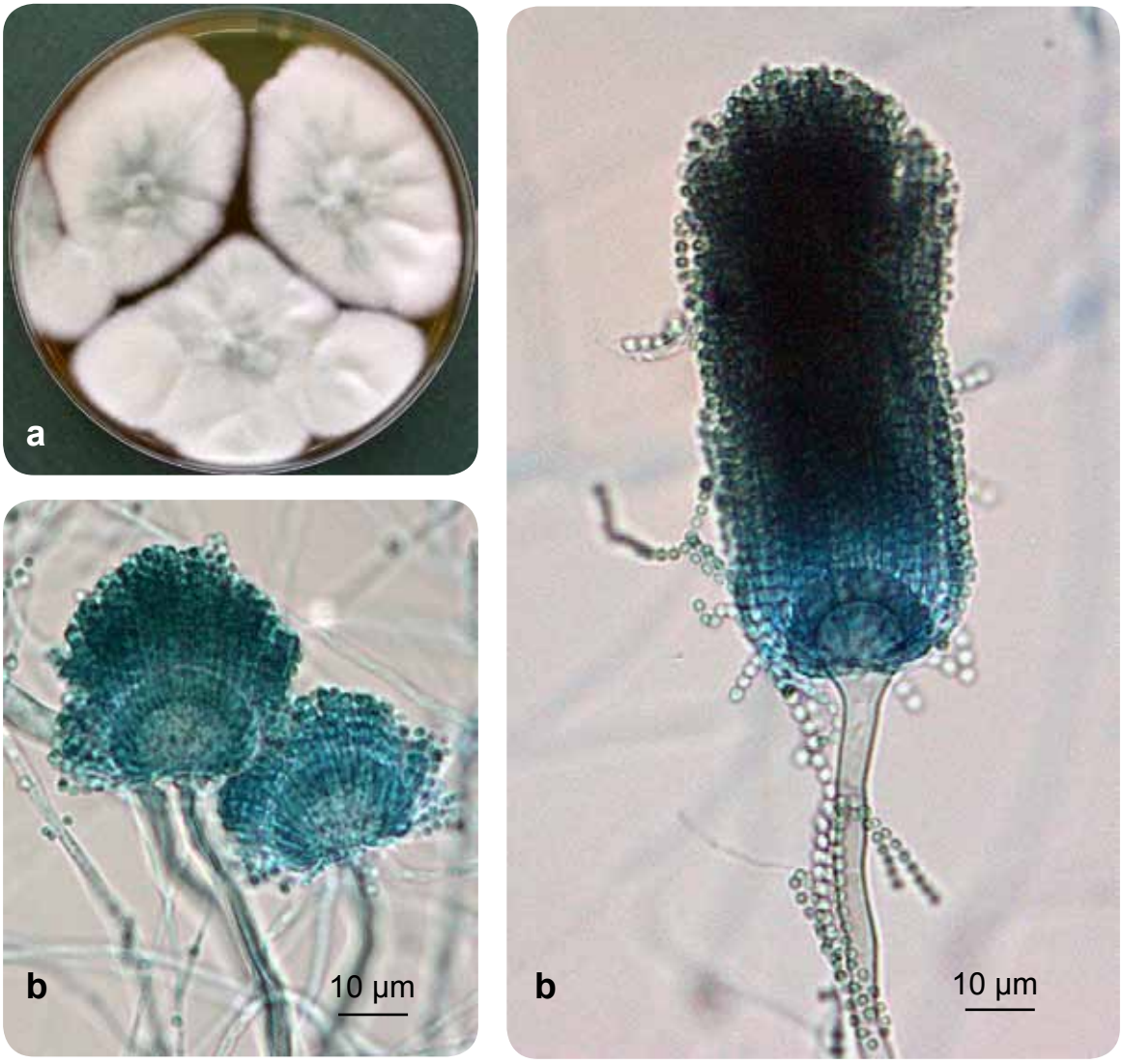
Aspergillus lentulus Balajee & Marr

Aspergillus lentulus appears to be widely distributed in soil and is now well documented as a causative agent of invasive aspergillosis in immunosuppressed patients. It is part of the *A. fumigatus* complex.

RG-2 organism.

Morphological Description: Colonies of *A. lentulus* are suede-like to floccose, white with interspersed grey-green patches of conidia (conidiation is slow to poor in most strains). Conidial heads are short, columnar and uniseriate. Conidiophore stipes are smooth-walled, sometimes sinuous and are often constricted at the neck. Vesicles are usually subglobose in shape. Conidia globose to broadly ellipsoidal (2-3.2 µm in diameter), smooth to finely roughened.

Molecular Identification: *A. lentulus* can be distinguished from other members of the section *Fumigati* by sequence analysis of β-tubulin, calmodulin and actin genes (Samson *et al*. 2007, Balajee *et al*. 2005b). ITS sequencing is not recommended.



Aspergillus lentulus (a) culture and (b) conidial head morphology.

Aspergillus lentulus Balajee & Marr

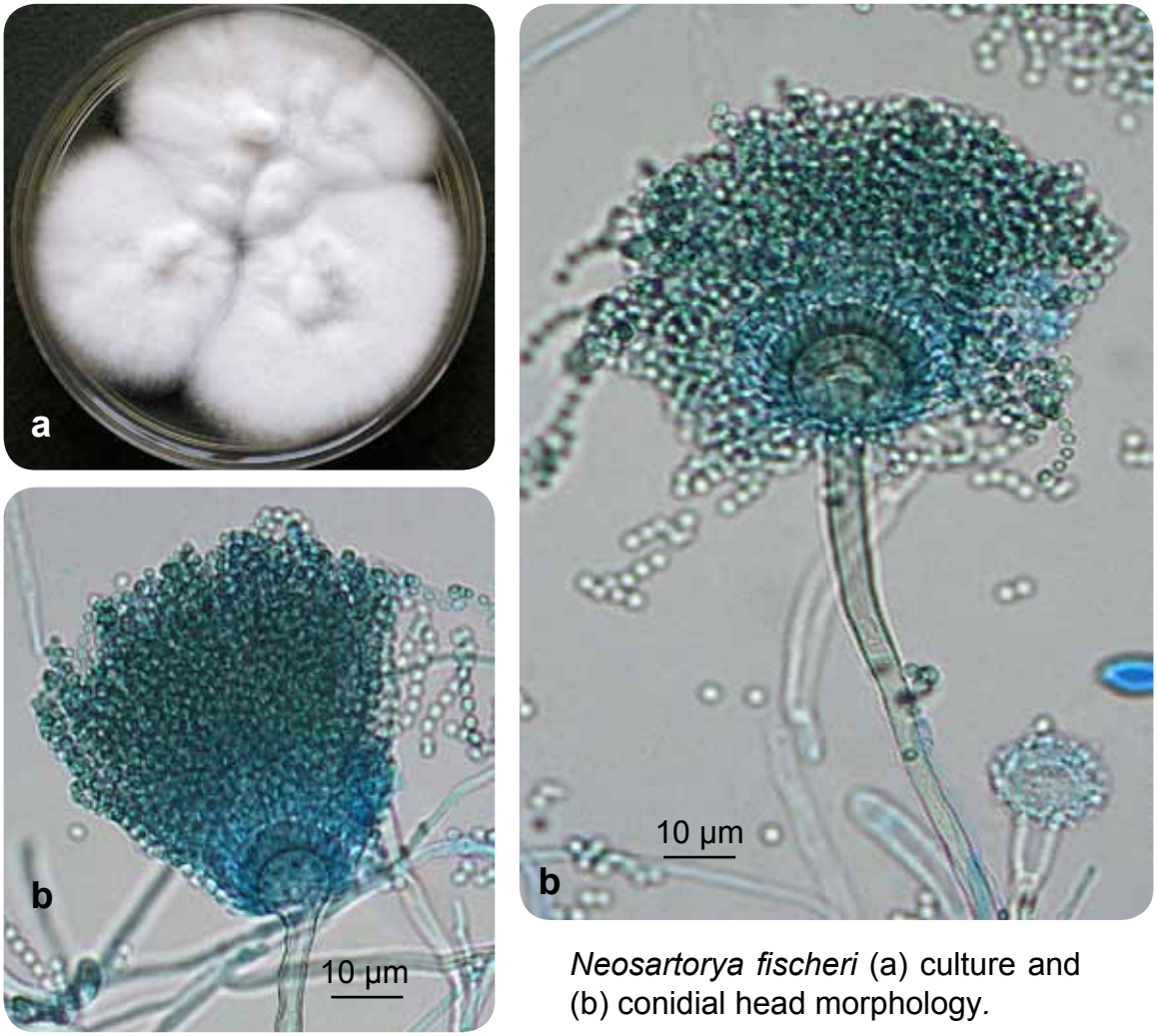
Antifungal Susceptibility: *A. lentulus* (Australian National data); **MIC µg/mL**.													
	No	**≤0.008**	**0.016**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**≥16**
AmB	**5**						**1**	**1**		**3**			
VORI	**5**								**3**	**2**			
POSA	**5**					**1**	**1**	**3**					
ITRA	**5**						**2**	**1**	**1**				**1**

Neosartorya fischeri (Wehmer) Malloch & Cain

Neosartorya fischeri is mostly found in canned foodstuffs and is now documented as a causative agent of invasive aspergillosis in immunosuppressed patients.

RG-1 organism.

Morphological Description: Colonies of *N. fischeri* are suede-like to floccose, white to pale yellow with slow to poor conidiation. Conidial heads are short, columnar and uniseriate. Conidiophore stipes are smooth-walled and vesicles are usually subglobose to flask-shaped. Conidia globose to subglobose (2-2.5 µm in diameter), smooth to finely roughened. Good growth at 37^{O}C.



Neosartorya fischeri (a) culture and (b) conidial head morphology.

Neosartorya fischeri (Wehmer) Malloch & Cain

Molecular Identification: *N. fischeri* can be distinguished from other members of the section *Fumigati* by sequence analysis of β-tubulin, calmodulin and actin genes (Samson *et al*. 2007; Balajee *et al*. 2005b). ITS sequencing is not recommended.

Antifungal Susceptibility: *N. fischeri* (Australian National data); **MIC µg/mL**.											
	No	**≤0.03**	**0.06**	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**≥16**
AmB	**8**				**1**		**5**	**2**			
VORI	**8**					**1**	**3**	**3**	**1**		
POSA	**8**			**4**	**1**	**1**	**2**				
ITRA	**8**			**1**	**2**	**2**	**3**				

Aspergillus nidulans complex

Aspergillus subgenus *Nidulantes;* Gams *et al.* (1985) includes species with biseriate conidial heads, brown pigmented often short stipes, and green conidia. Cleistothecia are soft-walled, surrounded by Hülle cells, and ascospores are red to purple in colour. Section *Nidulantes* is one of the largest subgenera of the genus *Aspergillus,* and includes about 80 species. Several species have been reported as medical pathogens principally *Aspergillus nidulans,* but also *A. sydowii, A. unguis, A. rugulovalvus* and *A. tetrazonus.*

Molecular Identification: ITS sequencing is sufficient to identify to species complex only. *A. nidulans* can be distinguished from other members of the section *Nidulantes* by sequence analysis of β-tubulin, calmodulin and actin genes.

Aspergillus nidulans (Eidam) Wint.

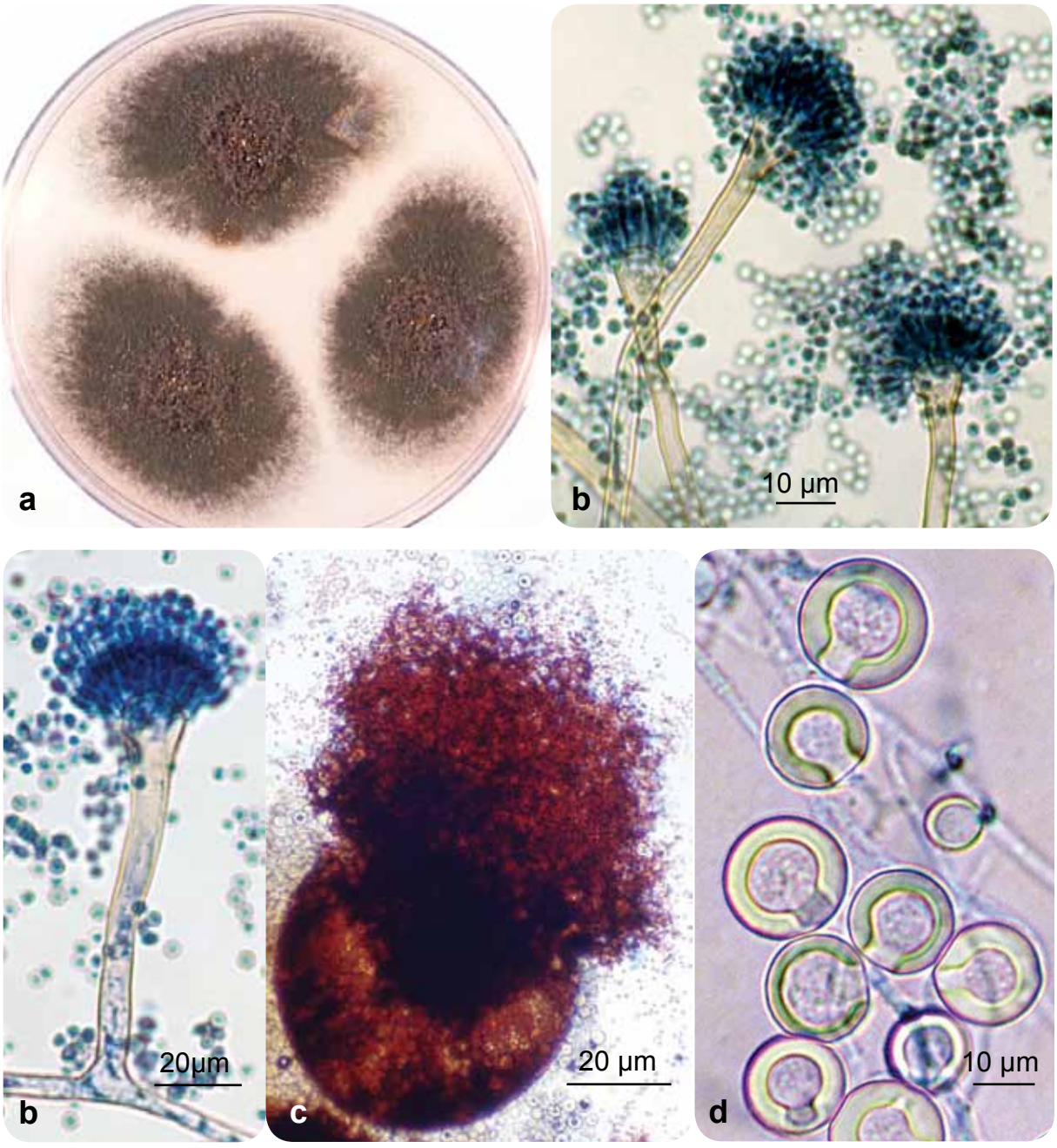
Aspergillus nidulans is a typical soil fungus with a worldwide distribution, it has also been reported to cause disease in human and animals.

RG-1 organism.

Morphological Description: On Czapek Dox agar, colonies are typically plain green in colour with dark red-brown cleistothecia developing within and upon the conidial layer. Reverse may be olive to drab-grey or purple-brown. Conidial heads are short, columnar (up to 70 x 30 µm in diameter) and biseriate. Conidiophore stipes are usually short, brownish and smooth-walled. Conidia are globose (3-3.5 µm in diameter) and rough-walled.

Key Features: Conidial heads are short, columnar and biseriate. Stipes are usually short, brownish and smooth-walled. Conidia are globose and rough-walled.

Aspergillus nidulans (Eidam) Wint.



Aspergillus nidulans (a) culture and (b) conidial head morphology, (c) cleistothecium of *Emericella nidulans* (anamorph *A. nidulans*) showing numerous reddish-brown ascospores and (d) thick-walled Hülle cells.

Antifungal Susceptibility: *A. nidulans* (Australian National data); **MIC µg/mL**.

	No	≤0.008	0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	≥16
AmB	24					5	10	1	6	2			
VORI	23			3	7	13							
POSA	20		1	6	6	5	2						
ITRA	24		1	3	6	10	4						

Aspergillus niger complex

The black aspergilli, *Aspergillus* section *Nigri* (Gams *et al.* 1985) includes species with uniseriate or biseriate conidial heads, spherical to pyriform vesicles, smooth-walled stipes and black or near black-coloured conidia. This group contains about 26 species with *Aspergillus niger* being the most common species isolated. *A. niger* can be isolated from all continents and is not very selective with respect to environmental conditions. Other species within this group that have been linked to human and animal infection include *A. acidus, A. aculeatus, A. brasiliensis* and *A. tubingensis.*

Molecular Identification: In *Aspergillus* section *Nigri*, all species can be distinguished from each other using calmodulin sequence data, and all except one can be distinguished using β-tubulin sequence data. ITS sequencing can only be used for a rough classification of the uni- and biseriate species (Samson *et al*. 2007).

Aspergillus niger van Tieghem

Aspergillus niger is one of the most common and easily identifiable species of the genus *Aspergillus*, with its white to yellow mycelial culture surface later bearing black conidia. This species is very commonly found in aspergillomas and is the most frequently encountered agent of otomycosis. It is also a common laboratory contaminant.

RG-1 organism.

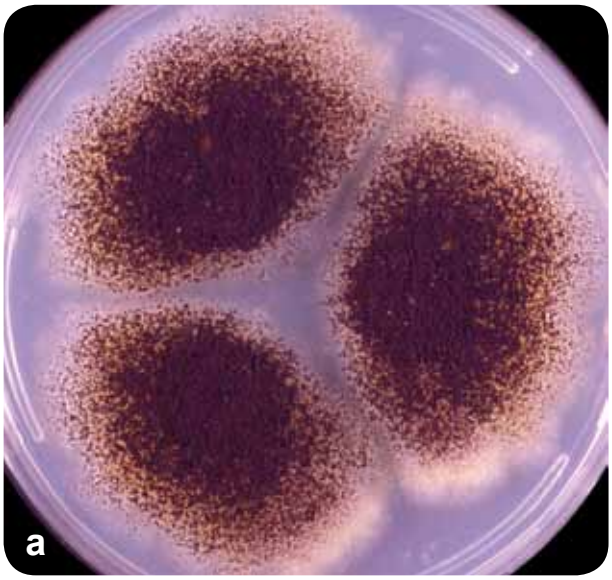
Morphological Identification: On Czapek Dox agar, colonies consist of a compact white or yellow basal felt covered by a dense layer of dark-brown to black conidial heads. Conidial heads are large (up to 3 mm by 15 to 20 µm in diameter), globose, dark brown, becoming radiate and tending to split into several loose columns with age. Conidiophore stipes are smooth-walled, hyaline or turning dark towards the vesicle. Conidial heads are biseriate with the phialides borne on brown, often septate metulae. Conidia are globose to subglobose (3.5-5 µm in diameter), dark brown to black and rough-walled.

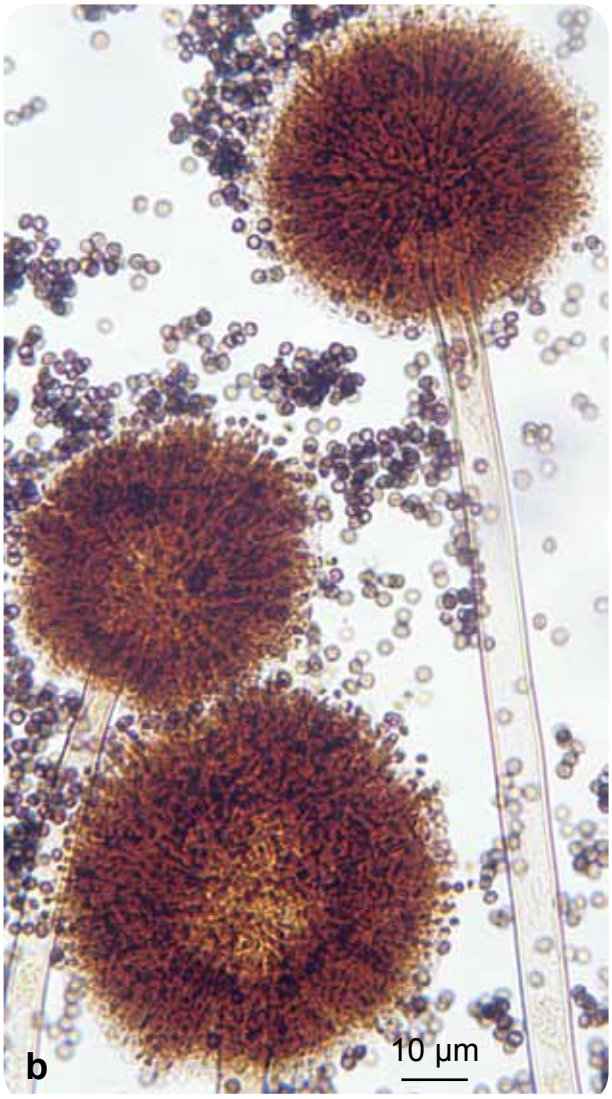
Key Features: Conidial heads are dark brown to black, radiate and biseriate with metulae twice as long as the phialides. Conidia brown and rough-walled.

Antifungal Susceptibility: *A. niger* (Australian National data); **MIC µg/mL.**

	No	≤0.008	0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	≥16
AmB	75					16	27	16	13	3			
VORI	71			3	5	8	16	28	10	1			
POSA	60		1	7	7	15	18	12					
ITRA	75			1	3	8	13	34	14			1	1

Aspergillus niger van Tieghem





Aspergillus niger (a) Culture and (b) conidial head morphology.
Note: Conidial heads are biseriate, large, globose, dark brown, becoming radiate with the phialides borne on metulae.

Aspergillus terreus complex

Aspergillus section *Terrei* (Gams *et al*. 1985); *Aspergillus terreus* complex includes species with biseriate, columnar conidial heads in shades of buff to brown (Raper and Fennell 1965). The most important species of this section is *A. terreus*, which is ubiquitous in the environment (Samson *et al.* 2011). Two other species have been reported as medical pathogens, *A. alabamensis* and *A. niveus.*

Molecular Identification: *A. terreus* can be distinguished from other members of the section *Terrei* by sequence analysis of β-tubulin, calmodulin and actin genes. ITS sequencing is sufficient to identify to species complex level only.

Aspergillus terreus Thom

Aspergillus terreus occurs commonly in soil and is occasionally reported as a pathogen of humans and animals.

RG-2 organism.

Morphological Identification: On Czapek Dox agar, colonies are typically suede-like and cinnamon-buff to sand-brown in colour with a yellow to deep dirty brown reverse. Conidial heads are compact, columnar (up to 500 x 30-50 µm in diameter) and biseriate. Metulae are as long as the phialides. Conidiophore stipes are hyaline and smooth-walled. Conidia are globose to ellipsoidal (1.5-2.5 µm in diameter), hyaline to slightly yellow and smooth-walled.

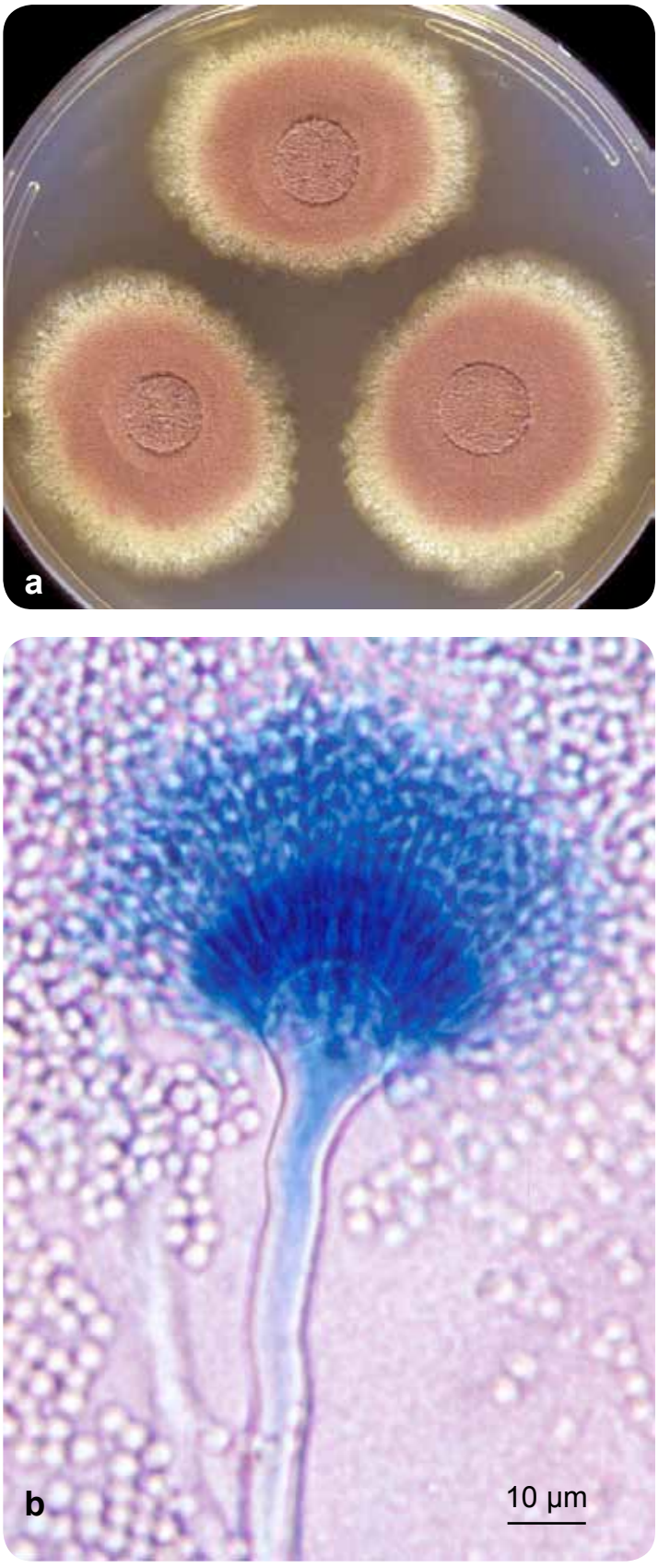
Key Features: Cinnamon-brown cultures, conidial heads biseriate with metulae as long as the phialides.

References: Raper and Fennell (1965), Domsch *et al.* (1980), McGinnis (1980), Onions *et al.* (1981), Samson and Pitt (1990), Samson *et al.* (1995), de Hoog *et al.* (2000) and Klich (2002).

Antifungal Susceptibility: *A. terreus* (Australian National data); **MIC µg/mL**.

	No	≤0.008	0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	≥16
AmB	72					1	3	11	29	24	3	1	
VORI	69				4	13	32	16	1	2		1	
POSA	59		1	10	8	23	13	2	2				
ITRA	72		4	3	9	17	35	4					

Aspergillus terreus Thom



Aspergillus terreus (a) culture and (b) conidial head morphology
Note: Conidial heads are biseriate.

Aureobasidium pullulans (de Bary) Arnaud

Aureobasidium pullulans has a worldwide distribution and is usually isolated as a saprophyte, occasionally from skin and nails. It has also been reported as a rare causative agent of phaeohyphomycosis, mycotic keratitis and peritonitis in patients on continuous ambulatory peritoneal dialysis (CAPD).

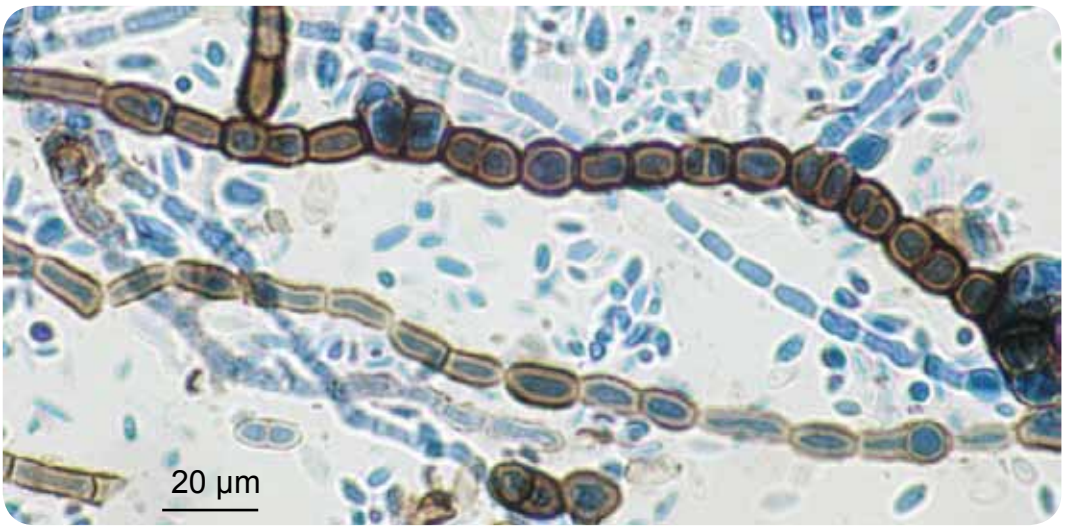
RG-1 organism.

Morphological Description: Colonies are fast growing, smooth, soon covered with slimy masses of conidia, cream or pink, later becoming brown or black. Hyphae are hyaline and septate, frequently becoming dark-brown with age and forming chains of one to two-celled, thick-walled, darkly pigmented arthroconidia. These arthroconidia actually represent the *Scytalidium* anamorph of *Aureobasidium* and are only of secondary importance in recognising members of this genus. Conidia are produced synchronously in dense groups from indistinct scars or from short denticles on undifferentiated, hyaline to subhyaline hyphae. Conidia are hyaline, smooth-walled, single-celled, ellipsoidal but of variable shape and size (8-12 x 4-6 µm), often with an indistinct hilum (i.e. a mark or scar at the point of attachment). Temperature: optimum 25^{O}C; maximum 35-37^{O}C.

Molecular Identification: Recommended barcoding genes are ITS, *EF-1α* and D1/D2 (de Hoog *et al*. 2015).

Key Features: Hyphomycete (so-called black yeast) producing hyaline blastoconidia simultaneously from the vegetative hyphae, which may also form chains of darkly pigmented, thick-walled arthroconidia.

References: Hermanides-Nijhof (1977), Domsch *et al.* (2007), McGinnis (1980), de Hoog *et al.* (2000, 2015), Najafzadeh *et al*. (2014).



Aureobasidium pullulans showing one to two-celled, darkly pigmented arthroconidia and hyaline, single-celled, ovoid-shaped conidia which are produced on short denticles.

Antifungal Susceptibility: *A. pullulans* data from 108 isolates (Najafzadeh *et al.* 2014 and Australian National data); **MIC µg/mL.**

Antifungal	Range	MIC_{90}	Antifungal	Range	MIC_{90}
AmB	**0.008-16**	**1**	**VORI**	**0.008-16**	**2**
ITRA	**0.008-16**	**0.5**	**POSA**	**0.008-4**	**0.5**

Basidiobolus ranarum Eidem

Synonymy: *Basidiobolus meristosporus* Drechsler.
Basidiobolus heterosporus Srinivasan & Thirumalachar.
Basidiobolus haptosporus Drechsler.

Basidiobolus ranarum is commonly present in decaying fruit and vegetable matter, and as a commensal in the intestinal tract of frogs, toads and lizards. It has been reported from tropical regions of Africa and Asia including India, Indonesia and Australia.

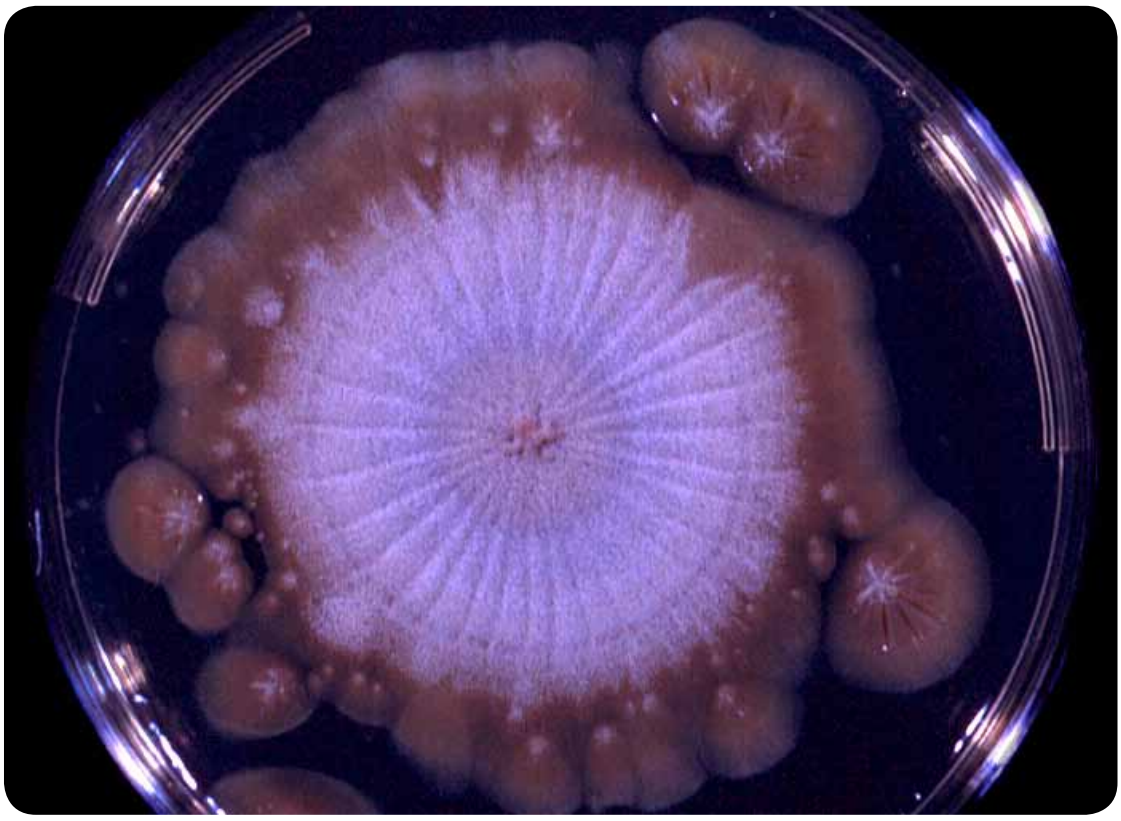
RG-2 organism.

Morphological Description: Colonies are moderately fast growing at 30^{O}C, flat, yellowish-grey to creamy-grey, glabrous, becoming radially folded and covered by a fine, powdery, white surface mycelium. Satellite colonies are often formed by germinating conidia ejected from the primary colony. Microscopic examination usually shows the presence of large vegetative hyphae (8-20 µm in diameter) forming numerous round (20-50 µm in diameter), smooth, thick-walled zygospores that have two closely appressed beak-like appendages. The production of “beaked” zygospores is characteristic of the genus. Two types of asexual conidia are formed, although isolates often lose their ability to sporulate with subculture. Special media incorporating glucosamine hydrochloride and casein hydrolsate may be needed to stimulate sporulation (Shipton and Zahari, 1987). Primary conidia are globose, one-celled, solitary and are forcibly discharged from a sporophore. The sporophore has a distinct swollen area just below the conidium that actively participates in the discharge of the conidium. Secondary (replicative) conidia are clavate, one-celled and are passively released from a sporophore. These sporophores are not swollen at their bases. The apex of the passively released spore has a knob-like adhesive tip. These spores may function as sporangia, producing several sporangiospores.

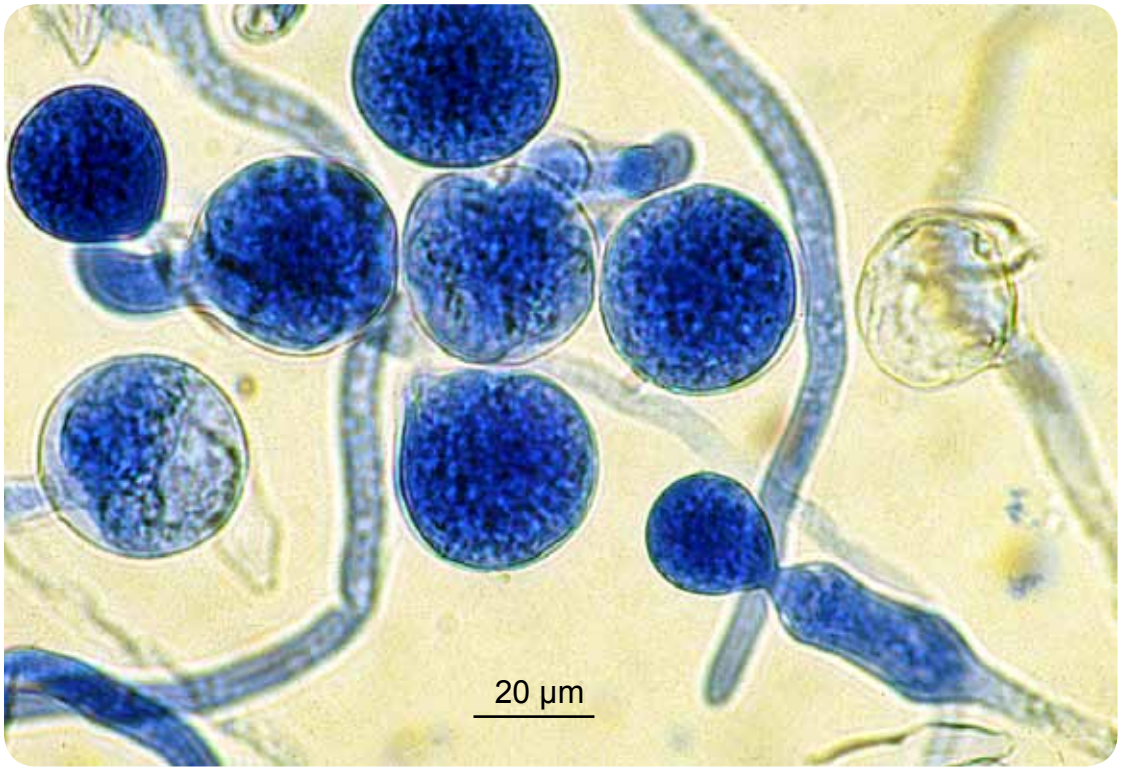
References: Strinivasan and Thirumalachar (1965), Greer and Friedman (1966), Dworzack *et al*. (1978), McGinnis (1980), King (1983), Rippon (1988), Davis *et al.* (1994), Jong and Dugan (2003), de Hoog *et al.* (2000, 2015) and Ellis (2005a).

Basidiobolus ranarum showing thick-walled zygospores.

Basidiobolus ranarum **Eidem**



Basidiobolus ranarum culture showing satellite colonies formed by germinating conidia ejected from the primary colony.



Basidiobolus ranarum showing conidia and a sporophore with a distinct swollen area just below the conidium (arrow).

Beauveria Vuillemin

Three species are recognised, two of which are well known pathogens of insects. *Beauvaria bassiana* is the most common species and is best known as the causal agent of muscardine disease in silkworms. *Beauveria* species are occasionally isolated in the clinical laboratory as saprophytic contaminants. Infections in humans are extremely rare.

RG-1 organism.

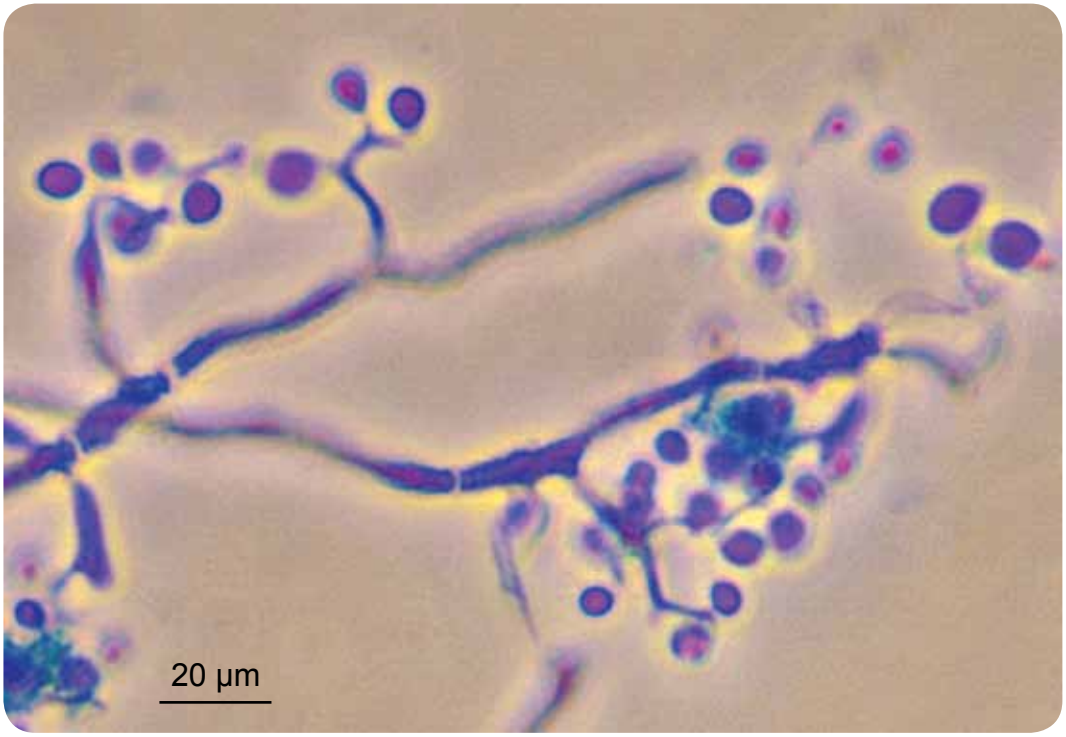
Morphological Description: Colonies are usually slow growing, usually not exceeding 2 cm in ten days at 20^{O}C, downy, at first white, but later often becoming yellow to pinkish. The genus *Beauveria* is characterised by the sympodial development of single-celled conidia (ameroconidia) on a geniculate or zig-zag rachis. Conidiogenous cells are flask-shaped, rachiform, proliferating sympodially and are often aggregated into sporodochia or synnemata. Conidia are hyaline and globose or ovoid in shape.

Key Features: Hyphomycete showing sympodial development of single-celled conidia on a geniculate or zig-zag rachis emanating from a flask-shaped conidiophore.

Molecular Identification: Specific primers were developed by Hegedus and Khachatourians (1996). Full phylogeny of the genus was provided by Rehner and Buckley (2005). Biogeography of molecular types was characterised by Ghikas *et al*. (2010).

MALDI-TOF MS: Cassagne *et al*. (2011) published a standardised procedure for mould identification in the clinical laboratory.

References: de Hoog (1972), Domsch *et al.* (2007), McGinnis (1980), de Hoog *et al.* (2000, 2015).



Beauveria bassiana showing sympodial development of conidia on a geniculate or zig-zag rachis. Conidiogenous cells are flask-shaped, rachiform, proliferating sympodially and are often aggregated into sporodochia or synnemata. Conidia are hyaline and globose or ovoid in shape, 2-3 µm diameter (phase contrast image).

Bipolaris Shoemaker

The genus *Bipolaris* contains about 45 species, which are mostly subtropical and tropical plant parasites. Recent molecular studies have recognised *Bipolaris cynodontis, B. micropus*, and *B. setariae* as species isolated from clinical samples (da Cunha *et al.* 2014). However recent phylogenetic studies have transferred several well-documented human pathogens, notably *B. australiensis, B. hawaiiensis* and *B. spicifera* to the genus *Curvularia* (Manamgoda *et al*. 2012)

RG-1 organisms.

Morphological Description: Colonies are moderately fast growing, effuse, grey to blackish brown, suede-like to floccose with a black reverse. Microscopic morphology shows sympodial development of hyaline to deep olivaceous pigmented, pseudoseptate conidia on a geniculate or zig-zag rachis. Conidia mostly curved, canoe-shaped, fusoid or obclavate, rarely straight, 2–14 pseudoseptate (usually more than 6), germinating only from the ends (bipolar).

Key Features: Dematiaceous hyphomycete producing sympodial, pseudoseptate, pale brown, long slender, gently curving conidia, which are rounded at both ends.

Comment: The genera *Drechslera, Bipolaris, Curvularia* and *Exserohilum* are all closely related. In the past, morphological differentiation of the genera relied upon a combination of characters including conidial shape, the presence or absence of a protruding hilum, the contour of the basal portion of the conidium and its hilum, the point at which the germ tube originates from the basal cell and, to a lesser degree, the sequence and location of the first three conidial septa.

However, Manamgoda *et al.* (2012) have found that there is no clear morphological boundary between genera *Bipolaris* and *Curvularia* and some species show intermediate morphology. These authors recommend using a combined ITS and *GPDH* gene analysis for definitive identification of species (Manamgoda *et al.* 2012).

Molecular Identification: ITS sequencing may be used to identify clinical species (da Cunha *et al*. 2012a). *GPDH* has been determined to be the best single phylogenetic marker of *Bipolaris* species (Manamgoda *et al.* 2012, 2014).

References: Ellis (1971, 1976), Luttrell (1978), Domsch *et al.* (2007), Alcorn (1983), McGinnis *et al.* (1986b), Sivanesan (1987), Rippon (1988), de Hoog *et al.* (2000, 2015), Manamgoda *et al*. (2012, 2014), da Cunha *et al*. (2012a).

Blastomyces dermatitidis Gilchrist & Stokes

At present the genus *Blastomyces* contains two species, *Blastomyces dermatitidis* and *Blastomyces gilchristi,* which are morphologically identical but distinguishable by sequence analysis of the ITS region (Brown *et al*. 2013). *B. dermatitidis* lives in soil and in association with decaying organic matter such as leaves and wood. It is the causal agent of blastomycosis a chronic granulomatous and suppurative disease, having a primary pulmonary stage that is frequently followed by dissemination to other body sites, typically the skin and bone. Although the disease was long thought to be restricted to the North American continent, in recent years autochthonous cases have been diagnosed in Africa, Asia and Europe.

WARNING: RG-3 organism. Cultures of *B. dermatitidis* represent a biohazard to laboratory personnel and must be handled in a Class II Biological Safety Cabinet (BSCII).

Morphological Description: Colonies at 25^{O}C have variable morphology and growth rate. They may grow rapidly, producing a fluffy white mycelium or slowly as glabrous, tan, nonsporulating colonies. Growth and sporulation may be enhanced by yeast extract. Most strains become pleomorphic with age. Microscopically, hyaline, ovoid to pyriform, one-celled, smooth-walled conidia (2-10 µm in diameter) of the *Chrysosporium* type, are borne on short lateral or terminal hyphal branches.

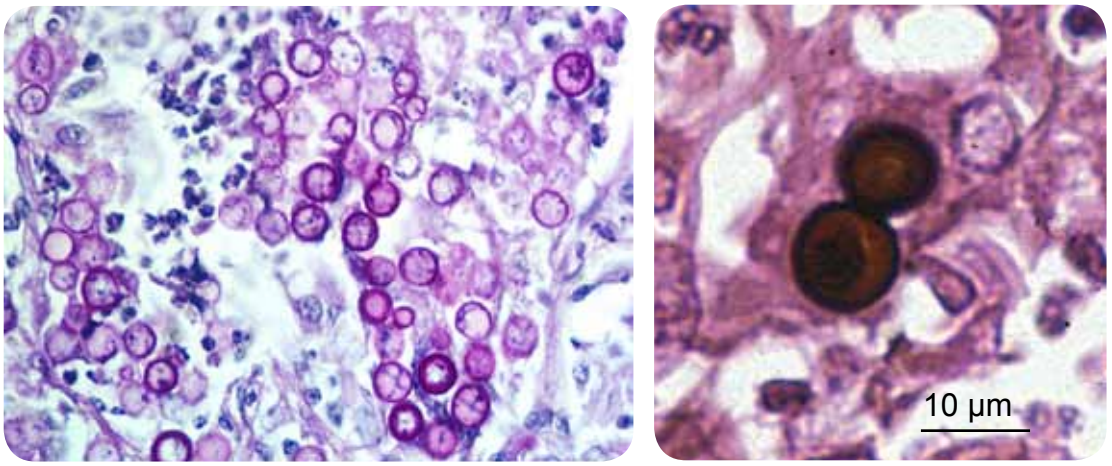
Colonies on blood agar at 37^{O}C are wrinkled and folded, glabrous and yeast-like. Microscopically, the organism produces the characteristic yeast phase seen in tissue pathology; ie. *B. dermatitidis* is a dimorphic fungus.

Comment: In the past, conversion from the mould form to the yeast form was necessary to positively identify this dimorphic pathogen from species of *Chrysosporium* or *Sepedonium.* However, culture identification by exoantigen test and/or molecular methods is now preferred to minimise manipulation of the fungus.

Key Features: Clinical history, tissue pathology, culture identification by positive exoantigen test and/or by molecular methods.

Blastomyces dermatitidis (a) culture and (b) one-celled, smooth-walled conidia borne on short lateral or terminal hyphal branches.

Blastomyces dermatitidis Gilchrist & Stokes



Histopathology: *Blastomyces dermatitidis* tissue sections show large, broad-based, unipolar budding yeast-like cells, which may vary in size from 8-15 µm, with some larger forms up to 30 µm in diameter. Tissue sections need to be stained by Grocott's methenamine silver method to clearly see the yeast-like cells, which are often difficult to observe in H&E preparations.

Molecular Diagnostics: A DNA probe assay (AccuProbe, Gen-Probe, Inc., San Diego, CA) for identification of *B. dermatitidis* in clinical isolates is available (Scalarone *et al.* 1992 and Padhye *et al.* 1994b). However this has limited application as it can be used only with pure cultures of *B. dermatitidis* (yeast or mould) (Sidamonidze *et al.* 2012). Several conventional PCR assays have been developed for the identification of *B. dermatitidis* from clinical specimens (Bialek *et al*. 2003) and soil (Burgess *et al.* 2006). Sidamonidze *et al.* (2012) developed a real-time PCR targeting the *BAD1* (formerly known as WI-1) gene for the identification of *B. dermatitidis* in culture and tissue and Morjaria *et al*. (2015) used rDNA sequencing for identification from paraffin embedded tissue.

References: McGinnis (1980), Chandler *et al*. (1980), Kaufman and Standard (1987), Rippon (1988), Brown *et al*. (2013).

Antifungal Susceptibility: *B. dermatitidis* limited data available (Sugar and Liu 1996, Espinel-Ingroff *et al.* 2001, Espinel-Ingroff 2003, Gonzales *et al.* 2005 and Sabatelli *et al.* 2006). **Antifungal susceptibility testing not recommended.** For treatment options see Clinical Practice Guidelines for the Management of Blastomycosis (Chapman *et al*. 2008); **MIC µg/mL.**

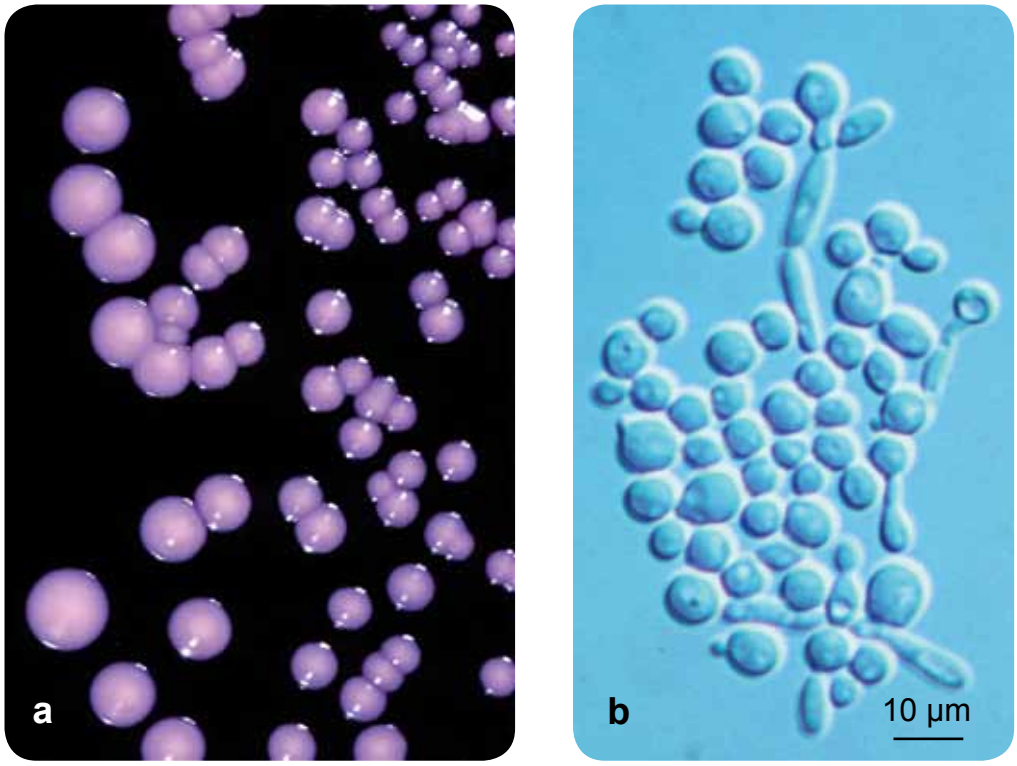
Antifungal	Range	MIC_{90}	Antifungal	Range	MIC_{90}
FLU	**0.125-64**	**4-16**	**AmB**	**0.03-1**	**0.5**
ITRA	**0.03-≥16**	**0.125-2**	**VORI**	**0.03-16**	**0.25**
POSA	**0.03-2**	**0.125**	**CAS**	**0.5-8**	**2**

Candida Berkhout

The genus *Candida* is characterised by globose to elongate yeast-like cells or blastoconidia that reproduce by narrow-based multilateral budding. Pseudohyphae and occasionally true hyphae may also be present. Colony pigmentation is usually absent. Ballistoconidia are not formed. Arthroconidia may be formed, but not extensively. Sexual reproduction is absent. Glucose may be fermented. Nitrate may be assimilated. Starch-like compounds are not produced. The diazonium blue B reaction is negative. The genus is highly polyphyletic, as it comprises mitosporic species that are devoid of special distinguishing features (Lachance *et al.* 2011).

Recently, several taxonomic rearrangements have been made and many well-known *Candida* species have been renamed and moved to other genera, notably *Pichia kudriavzevii* (formerly *Candida krusei*), *Meyerozyma guilliermondii* (formerly *Candida guilliermondii*), *Clavispora lusitaniae* (formerly *Candida lusitaniae*), *Kluyveromyces marxianus* (formerly *Candida kefyr*) and *Wickerhamomyces anomalus* (formerly *Candida pelliculosa*). *C. glabrata* and *C. parapsilosis* are now recognised as species complexes (Tavanti 2005; Correia 2006; Alcoba-Florez 2005).

Several species may be aetiological agents, most commonly *Candida albicans*, followed by *C. parapsilosis, C. glabrata, C. tropicalis* and *Pichia kudriavzevii*. Altogether, these five species account for >95% of human infections. However a number of other species may also be isolated. All are ubiquitous and occur naturally on humans.



Candida albicans showing (a) typical cream-coloured, smooth surfaced, waxy colonies and (b) narrow based budding spherical to ovoid blastoconidia.

Candida Berkhout

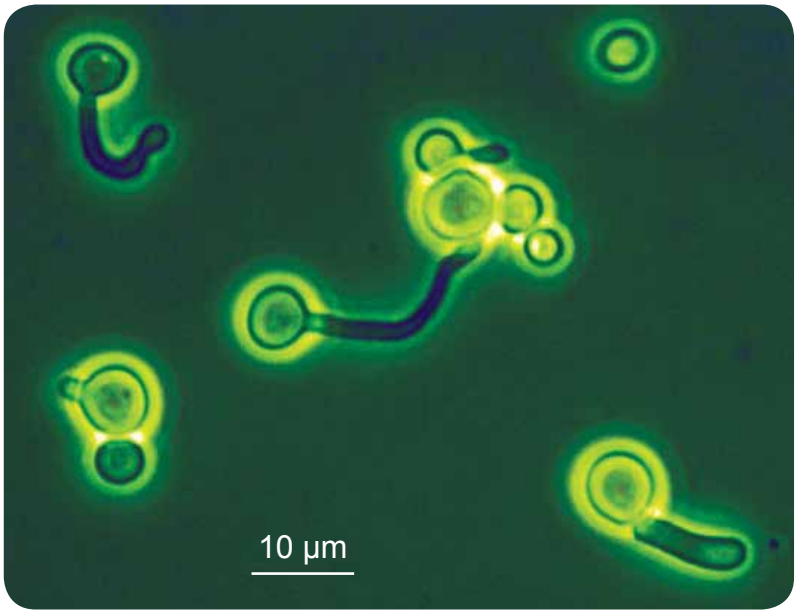
Identification: see Kurtzman, Fell and Boekhout. 2011. The Yeasts, a Taxonomic Study. 5th Edition Elsevier B.V.

Ensure that you start with a fresh growing pure culture; streak for single colony isolation if necessary.

Chromogenic Agars are used for primary isolation for rapid species identification and detection of mixed flora, especially from non-sterile sites. Depending on the brand of chromogenic media presumptive identification of *C. albicans, C. tropicalis* and *P. kudriavzevii* is possible. It is particularly useful for detection of mixed infections.

CHROMagar *Candida* plate showing chromogenic colour change for *C. albicans* (green), *C. tropicalis* (blue), *C. parapsilosis* (white) and *C. glabrata* (mauve).

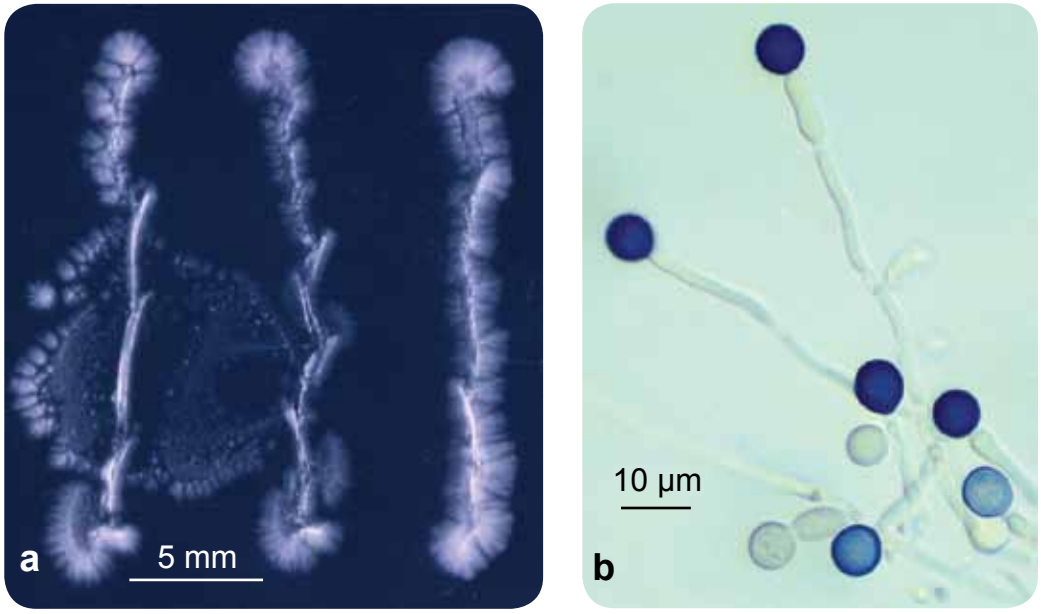
Germ Tube Test. A rapid screening test for *C. albicans* and *C. dubliniensis.* 0.5 mL of serum, containing 0.5% glucose, is lightly inoculated with the test organism and incubated at 35^{O}C for 2-3 hours. On microscopy, the production of germ tubes by the cells is presumptive for *C. albicans* and *C. dubliniensis*.



Candida albicans showing production of germ tubes.

Candida Berkhout

Dalmau Plate Culture: To set up a yeast morphology plate, dip a flamed sterilised straight wire into a culture and then lightly scratch the wire onto the surface of a cornmeal/tween 80, rice/tween 80 or yeast morphology agar plate, then place a flamed coverslip onto the agar surface covering the scratches. Dalmau morphology plates are examined *in-situ* directly under the low power of a microscope for the presence of pseudohyphae which may take up to 4-5 days at 26^{O}C to develop. *C. albicans* also produces characteristic large, round, terminal, thick-walled vesicles (often called chlamydospores). For best results a light inoculum should be scratched into the agar surface using a wire.



Dalmau plate culture of *Candida albicans* showing (a) colonies growing out from scratches on the surface of a cornmeal/tween 80 agar plate, and (b) the production of large round, thick-walled chlamydospores. **Note:** A coverslip has been placed onto the agar surface covering the scratches.

Physiological and Biochemical Tests: including fermentation and assimilation studies should be performed based on those used at the Centraalbureau voor Schimmelcultures (CBS), Delft, The Netherlands (Kurtzman *et al*. 2011). Reliable commercially available yeast identification systems are the API 20C AUX, API ID 32C, Biolog YT Station and Vitek 2 YST ID systems. However, they can only be used to identify those species in their respective databases, and may misidentify yeasts that are not represented.

Other Supplementary Tests include growth at 37^{O}C, cycloheximide resistance and hydrolysis of urea.

MALDI-TOF MS: The Bruker MALDI-TOF database is useful for identification of most clinical yeasts. The MALDI-TOF Vitek MS has been reported to misidentify some yeasts, notably *Candida metapsilosis* as *Candida parapsilosis* (Nobrega *et al*. 2014).

Molecular Identification: ITS sequencing is useful for the identification of most clinical yeasts.

References: Barnett *et al.* (1983), Kurtzman and Fell (1998, 2011), de Hoog *et al.* (2000, 2015).

Candida albicans (Robin) Berkhout

Candida albicans is a commensal of mucous membranes and the gastrointestinal tract. Environmental isolations have been made from sources contaminated by human or animal excreta, such as polluted water, soil, air and plants.

RG-2 organism.

Culture: Colonies (SDA) white to cream-coloured smooth, glabrous, yeast-like.

Microscopy: Spherical to subspherical budding blastoconidia, 2-7 x 3-8 µm in size.

India Ink Preparation: Negative - no capsules present.

Dalmau Plate Culture: Branched pseudohyphae with dense verticils of blastoconidia. Spherical chlamydospores, mostly terminal, often on a slightly swollen subtending cell, are formed near the edge of the cover slip.

Physiological Tests: + Positive, - Negative, v Variable, w Weak, s Slow

Germ Tube	+	L-Sorbose	v	L-Arabinose	v	D-Glucitol	v
Fermentation		Sucrose	v	D-Arabinose	v	α-M-D-glucoside	v
Glucose	+	Maltose	+	D-Ribose	v	D-Gluconate	v
Galactose	v	Cellobiose	-	L-Rhamnose	-	DL-Lactate	+
Sucrose	v	Trehalose	v	D-Glucosamine	v	*myo*-Inositol	-
Maltose	+	Lactose	-	*N*-A-D-glucosamine	v	2-K-D-gluconate	+
Lactose	-	Melibiose	-	Glycerol	v	D-Glucuronate	-
Trehalose	v	Raffinose	-	Erythritol	-	Nitrate	-
Assimilation		Melezitose	v	Ribitol	v	Urease	-
Glucose	+	Soluble Starch	+	Galactitol	-	0.1% Cycloheximide	+
Galactose	+	D-Xylose	+	D-Mannitol	+	Growth at 40°C	+

Key Features: Germ tube positive, production of chlamydospores on Dalmau plate culture, fermentation of glucose, sugar assimilation profile and a distinctive green colour on CHROMagar. **Note:** Germ tube negative variants (previously known as *C. claussenii*), and sucrose-negative variants (previously described as *C. stellatoidea*) may occur.

Antifungal Susceptibility: *C. albicans* (Australian National data); **MIC µg/mL.** CLSI clinical breakpoints are marked where available (Pfaller and Diekema 2012).

	No	≤0.008	0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	≥64
AmB	1725		2	16	162	644	548	305	48						
FLU	1728		2	2	2	83	468	706	314	62	12	11	18	8	40
VORI	1445	739	404	156	75	18	17	18	5	5	1	1	6		
POSA	1095	88	416	367	137	50	18	10	6	1		1			
ITRA	1728	20	122	443	661	378	43	30	10	3		1	17		
ANID	821	5	280	320	146	64	5	0	0	1					
MICA	819	470	261	73	11	4									
CAS	1171	3	13	207	490	327	112	17	2						
5FC	1728	4	147	765	362	164	171	62	16	7	5	3	3	19	

Candida catenulata Diddens & Lodder

Synonymy: *Candida brumptii* (Guerra) Langeron & Guerra.

Although most isolates of *Candida catenulata* originate from human sources, cases of candidaemia are uncommon.

RG-1 organism.

Culture: Colonies (SDA) white to cream-coloured smooth, soft and wrinkled, yeast-like.

Microscopy: Ovoid to cylindrical budding blastoconidia, 1.5-4.5 x 4-12 µm.

India Ink Preparation: Negative - no capsules present.

Dalmau Plate Culture: pseudohyphae consisting of chains of ovoid or cylindroid cells, and sometimes small verticils of ovoid blastoconidia.

Physiological Tests: + Positive, - Negative, v Variable, w Weak, s Slow							
Germ Tube	-	L-Sorbose	-	L-Arabinose	-	D-Glucitol	v
Fermentation		Sucrose	-	D-Arabinose	-	α-M-D-glucoside	-
Glucose	v	Maltose	v	D-Ribose	v	D-Gluconate	v
Galactose	-,s	Cellobiose	-	L-Rhamnose	-	DL-Lactate	-
Sucrose	-	Trehalose	v	D-Glucosamine	v	*myo*-Inositol	-
Maltose	-,s	Lactose	-	*N*-A-D-glucosamine	+	2-K-D-gluconate	v
Lactose	-	Melibiose	-	Glycerol	+	D-Glucuronate	-
Trehalose	-	Raffinose	-	Erythritol	-	Nitrate	-
Assimilation		Melezitose	-	Ribitol	v	Urease	-
Glucose	+	Soluble Starch	v	Galactitol	-	0.1% Cycloheximide	+,s
Galactose	+	D-Xylose	v	D-Mannitol	+	Growth at 37°C	v

Key Features: Separation from most physiologically similar species can be accomplished based on positive growth responses on D-mannitol, D-glucitol and resistance to 0.1% cycloheximide, combined with negative responses for sorbose and erythritol utilisation or growth in vitamin-free medium.

Antifungal Susceptibility: ***C. catenulata*** (Australian National data); **MIC µg/mL.**

	No	**≤0.008**	**0.016**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**≥64**
AmB	**3**					**2**	**1**								
FLU	**3**							**1**		**1**		**1**			
VORI	**3**	**1**	**1**		**1**										
POSA	**3**		**2**		**1**										
ITRA	**3**		**1**	**1**		**1**									
ANID	**3**				**1**	**2**									
MICA	**3**		**1**	**2**											
CAS	**3**					**1**	**2**								
5FC	**3**				**3**										

Candida dubliniensis Sullivan *et al.*

Candida dubliniensis is an occasional cause of candidaemia and mucosal infection, especially in HIV patients.

RG-2 organism.

Culture: Colonies (SDA) white to cream-coloured smooth, glabrous, yeast-like.

Microscopy: Spherical to subspherical budding blastoconidia, 3-8 x 2-7 µm in size.

India Ink Preparation: Negative - no capsules present.

Dalmau Plate Culture: Branched pseudohyphae with dense verticils of blastoconidia and spherical, mostly terminal chlamydospores.

Physiological Tests: + Positive, - Negative, v Variable, w Weak, s Slow							
Germ Tube	+	L-Sorbose	-	L-Arabinose	-	D-Glucitol	+
Fermentation		Sucrose	+	D-Arabinose	-	M-D-glucoside	+,s
Glucose	+	Maltose	+	D-Ribose	-	D-Gluconate	-
Galactose	+,s	Cellobiose	-	L-Rhamnose	-	DL-Lactate	+
Sucrose	-	Trehalose	s,+	D-Glucosamine	v	*myo*-Inositol	-
Maltose	+	Lactose	-	*N*-A-D-glucosamine	+	2-K-D-gluconate	+
Lactose	-	Melibiose	-	Glycerol	w,s,+	D-Glucuronate	-
Trehalose	v	Raffinose	-	Erythritol	-	Nitrate	-
Assimilation		Melezitose	w,+	Ribitol	+	Urease	-
Glucose	+	Soluble Starch	w,+	Galactitol	-	0.1% Cycloheximide	+
Galactose	+	D-Xylose	s,+	D-Mannitol	+	Growth at 40°C	+

Key Features: Germ tube positive, similar to *C. albicans*, except for absence of growth at 42°C; glycerol (mostly +), methyl-a-D-glucoside (-), trehalose (-), and D-xylose (-). Initial colonies dark green on CHROMagar and producing rough colonies on bird seed agar. ITS sequencing and MALDI-TOF can reliably distinguish *C. dubliniensis* from *C. albicans*.

Antifungal Susceptibility: ***C. dubliniensis*** (Australian National data); **MIC µg/mL.**															
	No	**≤0.008**	**0.016**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**≥64**
AmB	74		1	1	3	28	30	10	1						
FLU	74					24	24	21	5						
VORI	71	67	4												
POSA	63	15	19	18	8	3									
ITRA	74	6	22	14	22	6	4								
ANID	49	1	7	12	9	12	2					6			
MICA	49	4	11	18	8	2						6			
CAS	66			8	23	17	9	1		1		7			
5FC	74			12	49	7	4	1		1					

Candida glabrata complex

Recently *Candida glabrata* has been recognised as a species complex consisting of *C. glabrata, C. bracarensis* (Correia *et al.* 2006) and *C. nivariensis* (Alcoba-Flórez *et al.* 2005). These three species are phenotypically indistinguishable and are best identified by molecular methods. *C. bracarensis* was described based on PCR-fingerprints and sequence divergence in the D1/D2 domains (Correia *et al*. 2006). *C. nivariensis* was differentiated from other yeasts on the basis of ITS sequences (Borman *et al*. 2008).

Candida bracarensis Correia, P. Sampaio, James & Pais

RG-2 organism.

Culture: Colonies (SDA) white to cream-coloured smooth, glabrous, yeast-like.
Microscopy: Ellipsoidal budding blastoconidia, 3.9-6 x 2-4 µm in size. No pseudohyphae or chlamydospores produced.
India Ink Preparation: Negative - no capsules present.
Dalmau Plate Culture: No pseudohyphae produced.

Physiological Tests: + Positive, - Negative, v Variable, w Weak, s Slow							
Germ Tube	-	L-Sorbose	-	L-Arabinose	-	D-Glucitol	-
Fermentation		Sucrose	-	D-Arabinose	-	α-M-D-glucoside	-
Glucose	+	Maltose	-	D-Ribose	-	D-Gluconate	+
Galactose	-	Cellobiose	-	L-Rhamnose	-	DL-Lactate	-
Sucrose	-	Trehalose	+	D-Glucosamine	-	*myo*-Inositol	-
Maltose	-	Lactose	-	*N*-A-D-glucosamine	-	2-K-D-gluconate	-
Lactose	-	Melibiose	-	Glycerol	v	D-Glucuronate	-
Trehalose	s	Raffinose	-	Erythritol	-	Nitrate	-
Assimilation		Melezitose	-	Ribitol	-	Urease	-
Glucose	+	Soluble Starch	-	Galactitol	-	0.1% Cycloheximide	-
Galactose	-	D-Xylose	-	D-Mannitol	-	Growth at 40^{O}C	+

Key Features: *C. bracarensis* has variable API 20C patterns that overlap with *C. nivariensis* and some *C. glabrata* isolates, and has variable results with a rapid trehalose assay. **Note:** *C. glabrata* produces mauve-coloured colonies on CHROMagar, whereas isolates of *C. bracarensis, C. nivariensis, C. norvegensis* and *C. inconspicua* produce white colonies on CHROMagar (Alcoba-Flórez *et al*. 2005, Bishop *et al.* 2008).

Antifungal Susceptibility: ***C. bracarensis*** limited data (Australian National data); **MIC µg/mL.**

	No	**≤0.008**	**0.016**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**≥64**
AmB	**3**						**1**		**1**	**1**					
FLU	**3**										**2**		**1**		
VORI	**3**				**2**	**1**									
POSA	**3**						**2**	**1**							
ITRA	**3**						**2**	**1**							
ANID	**3**		**1**		**2**										
MICA	**3**	**1**	**2**												
CAS	**3**				**2**	**1**									
5FC	**3**					**1**	**1**	**1**							

Candida glabrata complex

Candida glabrata (Anderson) S.A. Meyer & Yarrow

RG-2 organism.

Culture: Colonies (SDA) white to cream-coloured smooth, glabrous, yeast-like.

Microscopy: Ovoid to ellipsoidal budding blastoconidia, 3.4 x 2.0 µm in size. No pseudohyphae or chlamydospores produced.

India Ink Preparation: Negative - no capsules present.

Dalmau Plate Culture: Ovoid budding yeast cells only. No pseudohyphae produced.

Physiological Tests: + Positive, - Negative, v Variable, w Weak, s Slow							
Germ Tube	-	L-Sorbose	-	L-Arabinose	-	D-Glucitol	-
Fermentation		Sucrose	-	D-Arabinose	-	α-M-D-glucoside	-
Glucose	+	Maltose	-	D-Ribose	-	D-Gluconate	+
Galactose	-	Cellobiose	-	L-Rhamnose	-	DL-Lactate	v
Sucrose	-	Trehalose	v	D-Glucosamine	-	*myo*-Inositol	-
Maltose	-	Lactose	-	*N*-A-D-glucosamine	-	2-K-D-gluconate	v
Lactose	-	Melibiose	-	Glycerol	v	D-Glucuronate	-
Trehalose	v	Raffinose	-	Erythritol	-	Nitrate	-
Assimilation		Melezitose	-	Ribitol	-	Urease	-
Glucose	+	Soluble Starch	-	Galactitol	-	0.1% Cycloheximide	-
Galactose	-	D-Xylose	-	D-Mannitol	-	Growth at 40°C	+

Key Features: Germ tube negative yeast and sugar assimilation pattern. *C. glabrata* is a common yeast species found on the body surface. Approximately 10% of clinical isolates show azole cross-resistance.

Antifungal Susceptibility: *C. glabrata* complex (Australian National data); **MIC µg/mL.** CLSI clinical breakpoints are marked where available (Pfaller and Diekema 2012).

	No	≤0.008	0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	≥64
AmB	**999**	**1**	**1**	**8**	**25**	**274**	**339**	**283**	**63**	**5**					
FLU	**1000**					**1**	**1**	**1**	**8**	**21**	**60**	**77**	**316**	**302**	**213**
VORI	**892**	**1**	**8**	**16**	**45**	**90**	**195**	**278**	**119**	**45**	**68**	**26**	**1**		
POSA	**770**			**3**	**8**	**15**	**40**	**115**	**228**	**231**	**5**	**125**			
ITRA	**1000**			**2**	**7**	**30**	**88**	**210**	**316**	**110**	**32**	**6**	**199**		
ANID	**629**		**50**	**215**	**208**	**134**	**5**	**2**	**3**	**10**	**1**	**1**			
MICA	**629**	**167**	**338**	**95**	**7**	**4**	**3**	**2**	**3**	**3**	**2**	**5**			
CAS	**779**		**1**	**34**	**187**	**292**	**185**	**58**	**9**	**3**	**1**	**9**			
5FC	**998**	**3**		**306**	**631**	**27**	**7**	**6**	**5**	**4**	**1**	**1**			**7**

Candida glabrata complex

Candida nivariensis Alcoba-Flórez *et al.*

RG-2 organism.

Culture: Colonies (SDA) white to cream-coloured smooth, glabrous, yeast-like.

Microscopy: Ellipsoidal budding blastoconidia, 3-5 x 1.8-3 µm in size. No pseudohyphae or chlamydospores produced.

India Ink Preparation: Negative - no capsules present.

Dalmau Plate Culture: No pseudohyphae produced.

Physiological Tests: + Positive, - Negative, v Variable, w Weak, s Slow							
Germ Tube	-	L-Sorbose	-	L-Arabinose	-	D-Glucitol	-
Fermentation		Sucrose	-	D-Arabinose	-	α-M-D-glucoside	-
Glucose	+	Maltose	-	D-Ribose	-	D-Gluconate	+
Galactose	-	Cellobiose	-	L-Rhamnose	-	DL-Lactate	-
Sucrose	-	Trehalose	-	D-Glucosamine	-	*myo*-Inositol	-
Maltose	-	Lactose	-	*N*-A-D-glucosamine	-	2-K-D-gluconate	-
Lactose	-	Melibiose	-	Glycerol	+	D-Glucuronate	-
Trehalose		Raffinose	-	Erythritol	-	Nitrate	-
Assimilation		Melezitose	-	Ribitol	-	Urease	-
Glucose	+	Soluble Starch	v	Galactitol	-	0.1% Cycloheximide	-
Galactose	-	D-Xylose	-	D-Mannitol	-	Growth at 40^{O}C	+

C. nivariensis is closely related to *C. glabrata* and *C. bracarensis*. These three species were found to differ by DNA-DNA reassociation experiments, RAPD-typing, AFLP-typing and D1/D2 and ITS sequence divergence (Alcoba-Flórez *et al.* 2005, Correia *et al.* 2006, Wahyuningsih *et al*. 2008).

Antifungal Susceptibility: ***C. nivariensis*** limited data (Australian National data); **MIC µg/mL**.

	No	**≤0.008**	**0.016**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**≥64**
AmB	**4**						**1**	**1**	**2**						
FLU	**4**										**1**	**1**	**2**		
VORI	**4**				**2**		**1**	**1**							
POSA	**4**						**1**	**1**	**2**						
ITRA	**4**							**4**							
ANID	**4**			**1**	**1**	**2**									
MICA	**4**	**2**	**2**												
CAS	**4**				**2**			**2**							
5FC	**4**					**1**		**2**	**1**						

Candida haemulonii complex

Candida haemulonii has recently been reclassified as a complex of three phenotypically identical but genotypically distinct entities: *C. haemulonii, C. duobushaemulonii* and *C. haemulonii* var. *vulnera*, based on ITS and D1/D2 sequencing. (Cendejas-Bueno *et al*. 2012, Ramos *et al*. 2015).

Candida haemulonii (van Uden & Kolipinski) Meyer & Yarrow

RG-1 organism.

Culture: Colonies (SDA) white to cream-coloured smooth, glabrous, yeast-like.

Microscopy: Ovoid to globose, budding yeast-like cells or blastoconidia, 2-7 x 2-7 µm. No pseudohyphae produced.

India Ink Preparation: Negative - no capsules present.

Dalmau Plate Culture: No pseudohyphae produced.

Physiological Tests: + Positive, - Negative, v Variable, w Weak, s Slow							
Germ Tube	-	L-Sorbose	-	L-Arabinose	-	D-Glucitol	+
Fermentation		Sucrose	+	D-Arabinose	-	α-M-D-glucoside	-
Glucose	+	Maltose	+	D-Ribose	-	D-Gluconate	+
Galactose	-	Cellobiose	-	L-Rhamnose	+,w	DL-Lactate	-
Sucrose	+	Trehalose	+	D-Glucosamine	+,s	*myo*-Inositol	-
Maltose	-	Lactose	-	*N*-A-D-glucosamine	+	2-K-D-gluconate	+
Lactose	-	Melibiose	-	Glycerol	+,s	D-Glucuronate	-
Trehalose	+,s	Raffinose	+,s	Erythritol	-	Nitrate	-
Assimilation		Melezitose	+,w	Ribitol	+,s	Urease	-
Glucose	+	Soluble Starch	-	Galactitol	-	0.1% Cycloheximide	-
Galactose	+,w	D-Xylose	-	D-Mannitol	+	Growth at 37°C	-

Key Features: Germ tube negative yeast and sugar assimilation pattern. Molecular identification may be required. *C. haemulonii* has been reported from a few cases of fungaemia but clinical isolations remain rare.

Antifungal Susceptibility: ***C. haemulonii*** (data from Cendejas-Bueno *et al*. 2012, Ramos *et al*. 2015 and Australian National data); **MIC µg/mL.**

	No	**≤0.008**	**0.016**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**≥64**
AmB	**30**						**3**	**10**	**7**	**7**	**1**	**2**			
FLU	**30**							**1**		**3**	**1**				**25**
VORI	**28**			**1**	**1**		**1**	**1**				**20**	**4**		
POSA	**23**		**1**	**2**		**1**					**1**	**3**	**16**		
ITRA	**30**			**2**	**1**	**2**			**1**				**24**		
ANID	**21**			**13**	**1**	**3**	**1**				**1**		**2**		
MICA	**21**			**2**	**10**	**3**	**2**						**4**		
CAS	**28**				**1**	**2**	**4**	**6**				**1**	**14**		
5FC	**25**			**2**	**4**	**7**	**6**	**2**	**3**	**1**					

Candida inconspicua (Lodder & Kreger-van Rij) S.A. Meyer & Yarrow

Candida inconspicua is a rare cause of candidaemia.

RG-1 organism.

Culture: Colonies (SDA) white to cream-coloured smooth, glabrous, yeast-like.

Microscopy: Ovoidal budding blastoconidia, 2.0-5 x 5.0-11.0 µm.

India Ink Preparation: Negative - no capsules present.

Dalmau Plate Culture: Spherical to ovoid budding yeast cells only. Primitive pseudohyphae may be produced after 14 days.

Physiological Tests: + Positive, - Negative, v Variable, w Weak, s Slow							
Germ Tube	-	L-Sorbose	-	L-Arabinose	-	D-Glucitol	-
Fermentation		Sucrose	-	D-Arabinose	-	α-M-D-glucoside	-
Glucose	-	Maltose	-	D-Ribose	-	D-Gluconate	-
Galactose	-	Cellobiose	-	L-Rhamnose	-	DL-Lactate	+
Sucrose	-	Trehalose	-	D-Glucosamine	+	*myo*-Inositol	-
Maltose	-	Lactose	-	*N*-A-D-glucosamine	+	2-K-D-gluconate	-
Lactose	-	Melibiose	-	Glycerol	+	D-Glucuronate	-
Trehalose	-	Raffinose	-	Erythritol	-	Nitrate	-
Assimilation		Melezitose	-	Ribitol	-	Urease	-
Glucose	+	Soluble Starch	-	Galactitol	-	0.1% Cycloheximide	-
Galactose	-	D-Xylose	-	D-Mannitol	-	Growth at 40^{O}C	+

Key Features: Germ tube negative yeast and sugar assimilation pattern and colonies are white on *Candida* CHROMagar.

Antifungal Susceptibility: ***C. inconspicua*** limited data available (Guitard *et al.* 2013, and Australian National data); **MIC µg/mL.**

	No	**≤0.008**	**0.016**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**≥64**
AmB	**16**	**2**	**2**	**1**	**1**	**3**	**6**	**1**							
FLU	**16**												**2**	**4**	**10**
VORI	**13**				**2**	**4**	**3**	**2**	**1**	**1**					
POSA	**13**				**2**	**3**	**5**	**2**				**1**			
ITRA	**15**					**2**	**6**	**6**	**1**						
ANID	**2**		**2**												
MICA	**2**		**2**												
CAS	**13**				**2**	**5**	**5**		**1**						
5FC	**14**					**1**		**1**	**2**	**4**	**3**	**1**	**1**	**1**	

Candida parapsilosis complex

Recently *Candida parapsilosis* has been recognised as a complex of four species: *C. parapsilosis, C. orthopsilosis*, *C. metapsilosis* and *Lodderomyces elongisporus* (Tavanti *et al.* 2005). These four species are phenotypically indistinguishable and are best identified by ITS sequencing or MALDI-TOF MS analysis.

Candida metapsilosis Tavanti *et al.*

RG-1 organism.

Culture: Colonies (SDA) white to cream-coloured smooth, glabrous, yeast-like.

Microscopy: Ellipsoid, subglobose to fusiform budding blastoconidia, 4 x 3-6 µm, with some larger subglobose forms present.

India Ink Preparation: Negative - no capsules present.

Dalmau Plate Culture: Abundant, much branched pseudohyphae produced.

Physiological Tests: + Positive, - Negative, v Variable, w Weak, s Slow							
Germ Tube	-	L-Sorbose	+	L-Arabinose	+	D-Glucitol	+
Fermentation		Sucrose	+	D-Arabinose	-	M-D-glucoside	+
Glucose	+	Maltose	+	D-Ribose	+	D-Gluconate	+
Galactose	-	Cellobiose	-	L-Rhamnose	-	DL-Lactate	-
Sucrose	-	Trehalose	+	D-Glucosamine	-	*myo*-Inositol	-
Maltose	-	Lactose	-	*N*-A-D-glucosamine	+	2-K-D-gluconate	+
Lactose	-	Melibiose	-	Glycerol	+	D-Glucuronate	-
Trehalose	-	Raffinose	-	Erythritol	-	Nitrate	-
Assimilation		Melezitose	+	Ribitol	+	Urease	-
Glucose	+	Soluble Starch	v	Galactitol	-	0.1% Cycloheximide	-
Galactose	+	D-Xylose	+	D-Mannitol	+	Growth at 37^{O}C	+

Key Features: *Candida metapsilosis* cannot be distinguished morphologically from *C. parapsilosis* and *C. orthopsilosis*, but can be identified by ITS sequencing (Asadzadeh *et al*. 2009, Borman *et al*. 2009, Tavanti *et al*. 2005) and MALDI-TOF MS analysis.

Antifungal Susceptibility: ***C. metapsilosis*** limited data (Diekema *et al*. 2009 and Australian National data); **MIC µg/mL.**

	No	**≤0.008**	**0.016**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**≥64**
AmB	**32**					**1**	**5**	**12**	**11**	**2**	**1**				
FLU	**32**						**1**		**19**	**10**	**2**				
VORI	**32**	**1**	**4**	**22**	**3**	**2**									
POSA	**32**		**1**	**7**	**16**	**6**	**1**								
ITRA	**2**					**1**	**1**								
ANID	**13**					**2**	**5**	**3**	**2**	**1**					
MICA	**13**						**9**	**3**	**1**						
CAS	**26**			**1**	**5**	**15**	**4**		**1**						
5FC	**2**				**2**										

Note: Additional data for **ITRA** MIC range 0.06-0.5, MIC_{90} = 0.25; and **5FC** MIC range 0.06-64, MIC_{90} = 0.5 (Gomez-Lopez *et al.* 2008, Miranda-Zapico *et al.* 2011).

Candida parapsilosis complex

Candida orthopsilosis Tavanti *et al.*

RG-1 organism.

Culture: Colonies (SDA) white to cream-coloured smooth, glabrous, yeast-like.

Microscopy: Ellipsoid to subglobose budding blastoconidia, 2-5 x 3-7 µm, with some larger elongated forms present.

India Ink Preparation: Negative - no capsules present.

Dalmau Plate Culture: Abundant, much-branched pseudohyphae produced.

Physiological Tests: + Positive, - Negative, v Variable, w Weak, s Slow							
Germ Tube	-	L-Sorbose	+	L-Arabinose	+	D-Glucitol	+
Fermentation		Sucrose	+	D-Arabinose	-	α-M-D-glucoside	+
Glucose	+	Maltose	+	D-Ribose	-	D-Gluconate	+
Galactose	-	Cellobiose	-	L-Rhamnose	-	DL-Lactate	-
Sucrose	-	Trehalose	+	D-Glucosamine	-	*myo*-Inositol	-
Maltose	-	Lactose	-	*N*-A-D-glucosamine	+	2-K-D-gluconate	+
Lactose	-	Melibiose	-	Glycerol	+	D-Glucuronate	-
Trehalose	-	Raffinose	-	Erythritol	-	Nitrate	-
Assimilation		Melezitose	+	Ribitol	+	Urease	-
Glucose	+	Soluble Starch	v	Galactitol	-	0.1% Cycloheximide	-
Galactose	+	D-Xylose	+	D-Mannitol	+	Growth at 37°C	+

Key Features: *Candida orthopsilosis* cannot be distinguished morphologically from *C. parapsilosis* and *C. metapsilosis,* but can be identified by ITS sequencing (Asadzadeh *et al.* 2009, Borman *et al.* 2009, Tavanti *et al*. 2005) and MALDI-TOF MS analysis.

Antifungal Susceptibility: *C. orthopsilosis* (Diekema *et al*. 2009, Canton *et al*. 2012, 2013 and Australian National data); **MIC µg/mL.**

	No	**≤0.008**	**0.016**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**≥64**
AmB	**224**			**3**	**3**	**30**	**45**	**60**	**48**	**23**	**9**				
FLU	**196**					**1**	**9**	**45**	**85**	**24**	**16**	**9**	**4**		**3**
VORI	**176**	**28**	**40**	**51**	**35**	**6**	**13**	**2**	**2**						
POSA	**136**		**5**	**21**	**49**	**41**	**9**	**11**							
ITRA	**94**	**1**	**2**	**14**	**30**	**32**	**15**								
ANID	**86**			**1**		**2**	**9**	**20**	**44**	**10**					
MICA	**85**		**1**	**1**		**3**	**36**	**37**	**6**	**1**					
CAS	**146**	**2**		**4**	**18**	**45**	**36**	**27**	**10**	**4**					
5FC	**92**				**56**	**22**	**8**	**3**	**1**		**1**				**1**

Candida parapsilosis complex

Candida parapsilosis (Ashford) Langeron & Talice

RG-1 organism.

Culture: Colonies (SDA) white to cream-coloured smooth, glabrous, yeast-like.

Microscopy: Predominantly small, globose to ovoid budding blastoconidia, 3-4 x 5-8 µm, with some larger elongated forms present.

India Ink Preparation: Negative - no capsules present.

Dalmau Plate Culture: Abundant, much-branched pseudohyphae in a delicate tree-like pattern with 2-3 blastoconidia in small clusters at intervals along the pseudohyphae.

Physiological Tests: + Positive, - Negative, v Variable, w Weak, s Slow							
Germ Tube	-	L-Sorbose	+,s	L-Arabinose	+	D-Glucitol	+
Fermentation		Sucrose	+	D-Arabinose	-	M-D-glucoside	+
Glucose	+	Maltose	+	D-Ribose	v	D-Gluconate	v
Galactose	+	Cellobiose	-	L-Rhamnose	-	DL-Lactate	-
Sucrose	+	Trehalose	+	D-Glucosamine	v	*myo*-Inositol	-
Maltose	-,s	Lactose	-	*N*-A-D-glucosamine	+	2-K-D-gluconate	+
Lactose	-	Melibiose	-	Glycerol	+	D-Glucuronate	-
Trehalose	-,s	Raffinose	-	Erythritol	-	Nitrate	-
Assimilation		Melezitose	+	Ribitol	v	Urease	-
Glucose	+	Soluble Starch	-	Galactitol	-	0.1% Cycloheximide	-
Galactose	+	D-Xylose	+	D-Mannitol	+	Growth at 37°C	+

Key Features: Germ tube negative yeast and sugar assimilation pattern. *C. parapsilosis* is commonly found on the skin and is a causative agent of candidaemia.

Antifungal Susceptibility: ***C. parapsilosis*** (Australian National data); **MIC µg/mL.** CLSI clinical breakpoints are marked where available (Pfaller and Diekema 2012).

	No	**≤0.008**	**0.016**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**≥64**
AmB	**607**		**2**	**8**	**28**	**131**	**142**	**210**	**80**	**2**					
FLU	**608**					**12**	**66**	**122**	**169**	**136**	**67**	**27**	**4**	**4**	**1**
VORI	**529**	**145**	**98**	**120**	**100**	**48**	**14**	**3**		**1**					
POSA	**437**	**4**	**41**	**126**	**182**	**69**	**13**	**2**							
ITRA	**608**	**1**	**15**	**80**	**134**	**279**	**84**	**12**	**3**						
ANID	**342**				**3**	**6**	**7**	**45**	**149**	**107**	**18**	**7**			
MICA	**342**		**1**		**3**		**2**	**51**	**164**	**90**	**26**	**5**			
CAS	**459**			**1**	**1**	**5**	**38**	**183**	**177**	**46**	**4**	**1**	**3**		
5FC	**608**	**2**	**1**	**31**	**193**	**176**	**158**	**38**	**5**	**2**					**2**

Candida parapsilosis complex

Lodderomyces elongisporus (Recca & Mrak) van der Walt

Lodderomyces elongisporus has been isolated from soft drinks and juice concentrates, natural fermentations of cocoa, soil, an infected fingernail, human blood infections and from baby cream. Initially isolates appeared to be atypical forms of *C. parapsilosis*, but sequence analysis identified them as *L. elongisporus*. In view of these findings, *L. elongisporus* may be more common among clinical isolates than initially thought (Lockhart *et al*. 2008a, Kurtzman).

RG-1 organism.

Culture: Colonies (SDA) white to cream-coloured smooth, glabrous, yeast-like.

Microscopy: Ellipsoid to elongate budding blastoconidia, 2.6-6.3 x 4-7.4 µm, with occasional spherical forms present.

India Ink Preparation: Negative - no capsules present.

Dalmau Plate Culture: Abundant, much-branched pseudohyphae produced.

Ascospore Formation: Asci are unconjugated, persistent, and are transformed from budding cells. Each ascus forms one, rarely two, long-ellipsoid ascospores. Ascospores observed on V8 agar after 7-10 days at 25^{O}C.

Physiological Tests: + Positive, - Negative, v Variable, w Weak, s Slow							
Germ Tube	-	L-Sorbose	+	L-Arabinose	-	D-Glucitol	+
Fermentation		Sucrose	+	D-Arabinose	-	α-M-D-glucoside	+
Glucose	+	Maltose	+	D-Ribose	-	D-Gluconate	+,w
Galactose	-	Cellobiose	-	L-Rhamnose	-	DL-Lactate	-
Sucrose	-	Trehalose	+	D-Glucosamine	-	*myo*-Inositol	-
Maltose	-	Lactose	-	*N*-A-D-glucosamine	+	2-K-D-gluconate	+
Lactose	-	Melibiose	-	Glycerol	+	D-Glucuronate	-
Trehalose	+	Raffinose	-	Erythritol	-	Nitrate	-
Assimilation		Melezitose	+	Ribitol	+	Urease	-
Glucose	+	Soluble Starch	-	Galactitol	-	0.1% Cycloheximide	-
Galactose	+	D-Xylose	+,w	D-Mannitol	+	Growth at 37^{O}C	+

Key Features: In the absence of ascospores, *L. elongisporus* cannot be distinguished physiologically from *C. parapsilosis, C. orthopsilosis* and *C. metapsilosis* but can be identified based on ITS sequencing (Asadzadeh *et al*. 2009, Borman *et al*. 2009, Tavanti *et al*. 2005) and MALDI-TOF MS analysis.

Candida rugosa complex

Candida rugosa has recently been recognised as a species complex of *C. rugosa, C. pseudorugosa* and another as yet undescribed species (Li *et al.* 2006, Paredes *et al.* 2012). These species are best identified by ITS sequencing.

Candida rugosa (Anderson) Diddens & Lodder

RG-1 organism.

Culture: Colonies (SDA) white to cream-coloured smooth, glabrous, yeast-like.

Microscopy: Ellipsoidal to elongate budding blastoconidia, 5-11 x 1.5-2.5 µm. Sometimes short pseudohyphae may be produced.

India Ink Preparation: Negative - no capsules present.

Dalmau Plate Culture: Densely branched pseudohyphae produced.

Physiological Tests: + Positive, - Negative, v Variable, w Weak, s Slow							
Germ Tube	-	L-Sorbose	v	L-Arabinose	-	D-Glucitol	v
Fermentation		Sucrose	-	D-Arabinose	-	α-M-D-glucoside	-
Glucose	-	Maltose	-	D-Ribose	-	D-Gluconate	v
Galactose	-	Cellobiose	-	L-Rhamnose	-	DL-Lactate	v
Sucrose	-	Trehalose	-	D-Glucosamine	-	*myo*-Inositol	-
Maltose	-	Lactose	-	*N*-A-D-glucosamine	+	2-K-D-gluconate	-
Lactose	-	Melibiose	-	Glycerol	+	D-Glucuronate	-
Trehalose	-	Raffinose	-	Erythritol	-	Nitrate	-
Assimilation		Melezitose	-	Ribitol	-	Urease	-
Glucose	+	Soluble Starch	-	Galactitol	-	0.1% Cycloheximide	-
Galactose	+	D-Xylose	+	D-Mannitol	v	Growth at 37^{O}C	+

Key Features: Germ tube negative yeast and sugar assimilation pattern (D-Xylose and Glycerol +ve; Ribitol -ve.). *C. rugosa* has been associated with catheter related fungaemia and has been isolated from human and bovine faeces, sea water and soil.

Antifungal Susceptibility: ***C. rugosa*** limited data (Diekema *et al*. 2009, Espinel-Ingroff *et al*. 2014 and Australian National data); **MIC µg/mL**.

	No	**≤0.008**	**0.016**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**≥64**
AmB	**21**					**1**	**1**	**5**	**5**	**7**	**1**				**1**
FLU	**97**					**1**	**3**	**15**	**24**	**29**	**8**	**8**	**6**	**1**	**2**
VORI	**80**	**4**	**8**	**38**	**16**	**4**	**7**	**3**							
POSA	**66**	**1**	**4**	**12**	**27**	**9**	**8**	**5**							
ITRA	**5**		**1**		**1**	**1**	**1**		**1**						
ANID	**20**				**3**	**4**	**4**	**4**	**3**			**2**			
MICA	**20**			**2**	**3**	**5**	**6**	**2**					**2**		
CAS	**25**			**1**	**1**		**2**	**3**	**10**	**1**	**1**		**2**		

Candida tropicalis (Castellani) Berkhout

Candida tropicalis is a major cause of septicaemia and disseminated candidiasis. It is also found as part of the normal human mucocutaneous flora and environmental isolations have been made from faeces, shrimp, kefir and soil.

RG-2 organism.

Culture: Colonies (SDA) white to cream-coloured smooth, glabrous, yeast-like.

Microscopy: Spherical to subspherical budding yeast-like cells or blastoconidia, 3.5-7 x 5.5-10 µm.

India Ink Preparation: Negative - no capsules present.

Dalmau Plate Culture: Abundant, long, wavy, branched pseudohyphae with numerous ovoid blastoconidia, budding off. Terminal vesicles (chlamydospores) are not produced.

Physiological Tests: + Positive, - Negative, v Variable, w Weak, s Slow							
Germ Tube	-	L-Sorbose	v	L-Arabinose	-	D-Glucitol	+
Fermentation		Sucrose	v	D-Arabinose	-	M-D-glucoside	v
Glucose	+	Maltose	+	D-Ribose	v,s	D-Gluconate	v
Galactose	+	Cellobiose	v	L-Rhamnose	-	DL-Lactate	v
Sucrose	v	Trehalose	+	D-Glucosamine	v	*myo*-Inositol	-
Maltose	+	Lactose	-	*N*-A-D-glucosamine	+	2-K-D-gluconate	+
Lactose	-	Melibiose	-	Glycerol	v	D-Glucuronate	-
Trehalose	+,s	Raffinose	-	Erythritol	-	Nitrate	-
Assimilation		Melezitose	v	Ribitol	v	Urease	-
Glucose	+	Soluble Starch	+	Galactitol	-	0.1% Cycloheximide	+
Galactose	+	D-Xylose	+	D-Mannitol	+	Growth at 40°C	+

Key Features: Germ tube negative yeast and sugar assimilation pattern. Colonies are dark blue on *Candida* CHROMagar.

Antifungal Susceptibility: ***C. tropicalis*** (Australian National data); **MIC µg/mL.** CLSI clinical breakpoints are marked where available (Pfaller and Diekema 2012).

	No	≤0.008	0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	≥64
AmB	284			1	8	45	94	97	38		1				
FLU	284					1	10	46	98	65	32	14	4	4	10
VORI	251	10	17	37	64	58	34	14	6	5	1	4	1		
POSA	190	4	7	28	35	53	33	17	6	2		5			
ITRA	284		2	13	22	94	98	43	2	1	1	1	7		
ANID	126		9	8	29	67	11	1				1			
MICA	126	8	28	69	16	2	1	1				1			
CAS	207		1	15	68	67	40	9	3			1			
5FC	284			49	139	54	21	5	2	1	1	2	1	2	7

Chaetomium Kunze ex Fries

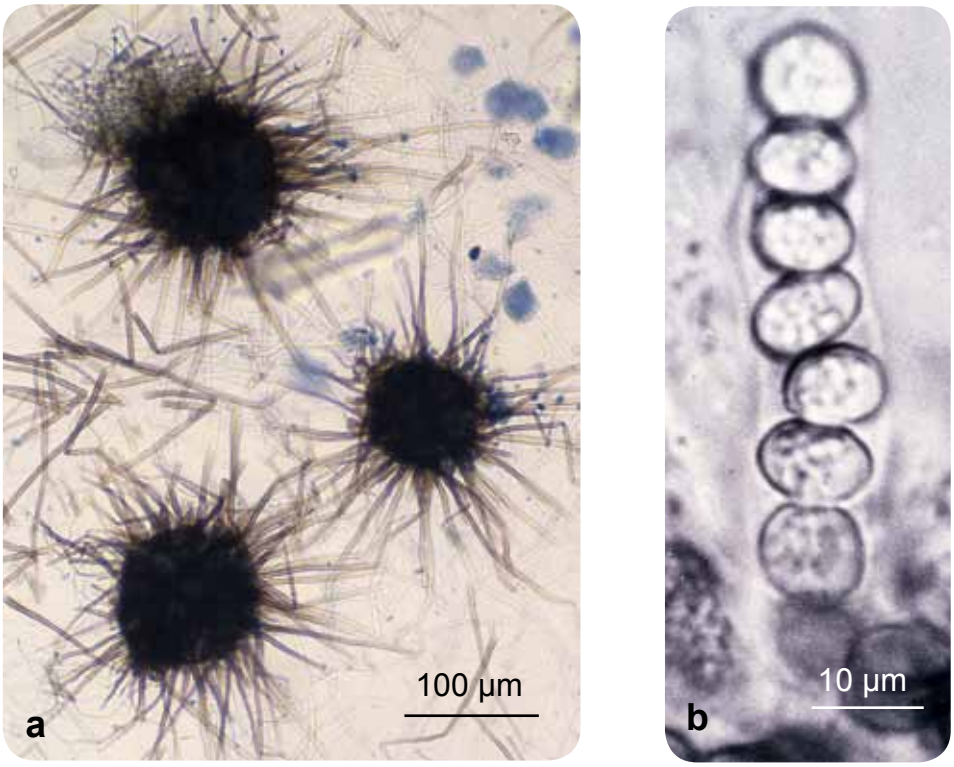
The genus *Chaetomium* contains between 160 and 180 species. All are saprophytic being isolated from soil, straw, dung and plant debris. Several species are thermophilic and can grow at temperatures above 37^{O}C. *Chaetomium* species are important agents for the decomposition of cellulose waste and plant materials, and are only rarely isolated in medical mycology laboratories. **RG-1 organisms**.

Morphological Description: *Chaetomium* is a common ascomycete characterised by the formation of darkly-pigmented, globose, ovoid, barrel to flask-shaped, ostiolate ascocarps (perithecia) beset with dark-coloured terminal hairs (setae) which are straight, branched or curved. Asci are clavate to cylindrical, typically eight-spored and evanescent. Ascospores are one-celled, darkly-pigmented, smooth-walled, of varying shape, mostly ovoid, ellipsoidal or lemon-shaped. Chlamydospores and solitary conidia may also be produced.

Molecular Identification: Lee and Hanlin (1999) established the phylogenetic relationships of *Chaetomium* based on ribosomal DNA sequences. ITS sequencing may be useful for identification of some clinical species.

Key Features: Ascomycete producing darkly-pigmented ostiolate perithecia beset with long dark terminal setae.

References: Ames (1963), Seth (1970), Millner (1975), Domsch *et al.* (2007), Ellis and Keane (1981), Ellis (1981), von Arx (1986), de Hoog *et al.* (2000, 2015).



Chaetomium spp. (a) ascocarps (perithecia) and (b) ascus with ascospores.

Antifungal Susceptibility: ***Chaetomium*** very limited data (McGinnis and Pasarell 1998a, Serena *et al.* 2003, Barron *et al.* 2003, Australian National Data); **MIC µg/mL**.

Antifungal	Range	MIC_{90}	Antifungal	Range	MIC_{90}
AmB	**0.125-16**	**4**	**VORI**	**0.125-0.5**	**0.5**
ITRA	**0.03-0.25**	**0.125**	**POSA**	**≤0.03-1**	**0.5**

Chrysosporium Corda

Species of *Chrysosporium* are occasionally isolated from skin and nail scrapings, especially from feet, but because they are common soil saprophytes they are usually considered contaminants. There are about 70 species of *Chrysosporium*, several are keratinolytic with some also being thermotolerant, and cultures may closely resemble some dermatophytes, especially *Trichophyton mentagrophytes.* Some strains may also resemble cultures of *Histoplasma* and *Blastomyces*.

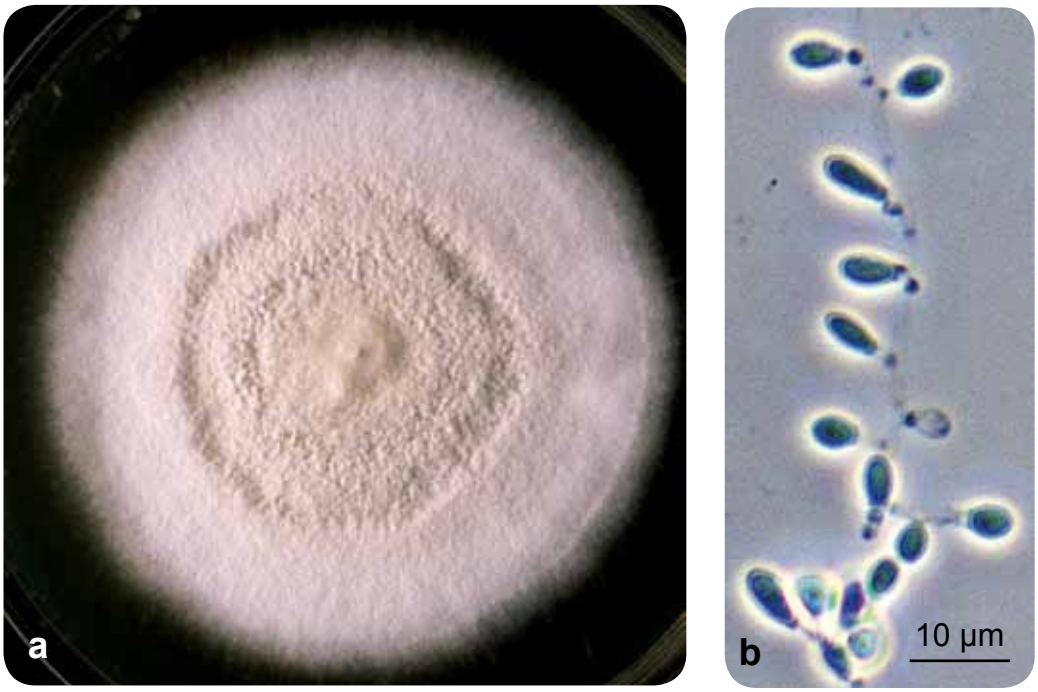
Morphological Description: Colonies are moderately fast growing, flat, white to tan to beige in colour, often with a powdery or granular surface texture. Reverse pigment absent or pale brownish-yellow with age. Hyaline, one-celled conidia are produced directly on vegetative hyphae by non-specialised conidiogenous cells. Conidia are typically pyriform to clavate with truncate bases and are formed either intercalary (arthroconidia), laterally (often on pedicels) or terminally.

Molecular Identification: *Chrysosporium* is phylogenetically heterogeneous; the polyphyletic origin of the genus was first demonstrated by Vidal *et al*. (2000) on the basis of ITS sequences, and further elaborated by Stchigel *et al.* (2014). ITS sequencing can assist in identification of clinical isolates.

Chrysosporium tropicum Carmichael

Morphological Descriptions: Colonies are flat, white to cream-coloured with a very granular surface. Reverse pigment absent or pale brownish-yellow with age. Microscopically, conidia are numerous, hyaline, single-celled, clavate to pyriform, smooth, slightly thick-walled (6-7 x 3.5-4 µm), and have broad truncate bases and pronounced basal scars. The conidia are formed at the tips of the hyphae, on short or long lateral branches, or sessile along the hyphae (intercalary). No macroconidia or hyphal spirals are seen. **RG-2 organism.**

References: Carmichael (1962), Rebell and Taplin (1970), Sigler and Carmichael (1976), van Oorschot (1980), Domsch *et al.* (2007), de Hoog *et al.* (2000, 2015).



Chrysosporium tropicum (a) culture and (b) typical pyriform to clavate-shaped conidia with truncated bases which may be formed either intercalary, laterally or terminally.

Cladophialophora Borelli

The genus *Cladophialophora* is characterised by: (1) the absence of conidiophores, "shield cells," or prominent hila (attachment points); (2) the ability to grow on media containing cycloheximide; and (3) having dry, non-fragile chains of conidia (Revankar and Sutton 2010). It has recently been re-evaluated by multilocus sequencing and currently contains seven species associated with human infection (Badali *et al.* 2008).

Cladophialophora bantiana is the causative agent of numerous cases of cerebral phaeohyphomycosis many of which occur in immunocompetent individuals and most of which are fatal (Chakrabarti *et al*. 2016). *C. carrionii* and the recently described *C. samoensis* are agents of chromoblastomycosis. Less common species occasionally implicated in deep and superficial mycoses include, *C. arxii, C. boppii, C. devriesii, C. emmonsii, C. modesta* and *C. saturnica*. *C. yegresii* is a closely related environmental sister species to *C. carrionii* (Revankar and Sutton 2010, de Hoog *et al.* 1995, 2015).

Cladophialophora bantiana (Saccardo) de Hoog *et al.*

Synonymy: *Xylohypha bantiana (*Saccardo) McGinnis, Borelli and Ajello.
Cladosporium bantianum (Sacc.) Borelli.
Cladosporium trichoides Emmons.

Cladophialophora bantiana has been isolated from soil and is a recognised agent of cerebral phaeohyphomycosis. The fungus is neurotropic and may cause brain abscess in both normal and immunosuppressed patients and is usually fatal. The fungus is likely introduced via inhalation and direct transfer to the brain via the paranasal sinuses, or traumatic head injury.

WARNING: RG-3 organism. Cultures of *C. bantiana* represent a potential biohazard to laboratory personnel and must be handled with extreme caution in Class II Biological Safety Cabinet (BSCII).

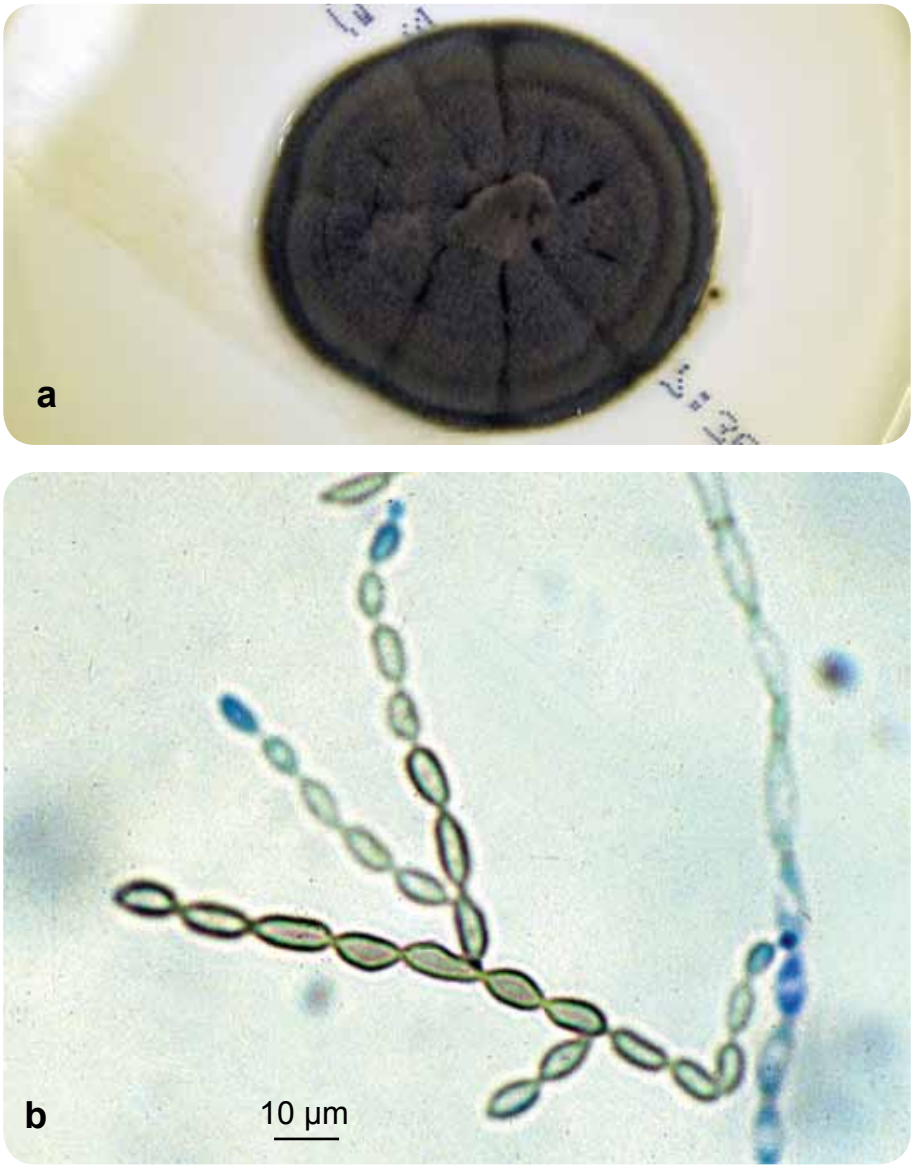
Morphological Description: Colonies are moderately fast growing, olivaceous-grey, suede-like to floccose and grow at temperatures up to 42-43^{O}C. Conidia are formed in long, sparsely branched, flexuose, acropetal chains from undifferentiated conidiophores. Conidia are one-celled (very occasionally two-celled), pale brown, smooth-walled, ellipsoid to oblong-ellipsoid and are 2-3 x 4-7 µm in size.

C. bantiana may be distinguished from *Cladosporium* species by the absence of conidia with distinctly pigmented hila, the absence of shield cells and by growth at ≥40^{O}C (compared with *C. carrionii* which has a maximum growth temperature of 35-37^{O}C, and *Cladosporium* species which have a maximum of ≤35^{O}C). *C. bantiana* may be further distinguished from *C. carrionii* by the formation of very long, sparsely branched chains of conidia.

Molecular Identification: ITS sequencing is recommended (Gerrits van den Ende and de Hoog, 1999; Badali *et al*. 2010).

References: McGinnis (1980), McGinnis and Borelli (1981), McGinnis *et al*. (1986a), Rippon (1988), Kwon-Chung and Bennett (1992), de Hoog *et al.* (2000, 2015), Chakrabarti *et al*. (2016).

Cladophialophora bantiana (Saccardo) de Hoog *et al.*



Cladophialophora bantiana (a) culture and (b) conidiophore and conidia.

Antifungal Susceptibility: ***C. bantiana*** limited data available (Australian National data); **MIC µg/mL.**

	No.	≤0.008	0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	≥64
AmB	**14**				**1**		**3**	**2**	**5**	**1**	**2**				
VORI	**14**			**3**	**2**	**7**	**1**	**1**							
POSA	**12**	**3**	**2**	**4**	**3**										
ITRA	**14**		**5**	**2**	**5**	**2**									

C. bantiana limited data (McGinnis and Pasarell 1998a, 1998b, Espinel-Ingroff *et al.* 2001, Chakrabarti *et al.* 2015); **MIC µg/mL.**

AmB	**Range 0.03-8; MIC_{90}= 2**	**VORI**	**Range 0.03-1; MIC_{90}= 0.125**
ITRA	**Range 0.03-1; MIC_{90}= 0.125**	**POSA**	**Range 0.03-0.125; MIC_{90}= 0.125**
TERB	**Range 0.03-1; Geometric Mean = 0.08**		

Cladophialophora carrionii (Trejos) de Hoog *et al.*

Synonymy: *Cladosporium carrionii* Trejos

Cladophialophora carrionii is a recognised agent of chromoblastomycosis and it has been isolated from soil and fence posts made from *Eucalyptus* spp. Cases of chromoblastomycosis caused by *C. carrionii* are commonly found in Australia, Venezuela, Madagascar and South America. Isolates from phaeomycotic cysts and opportunistic infections have also been reported.

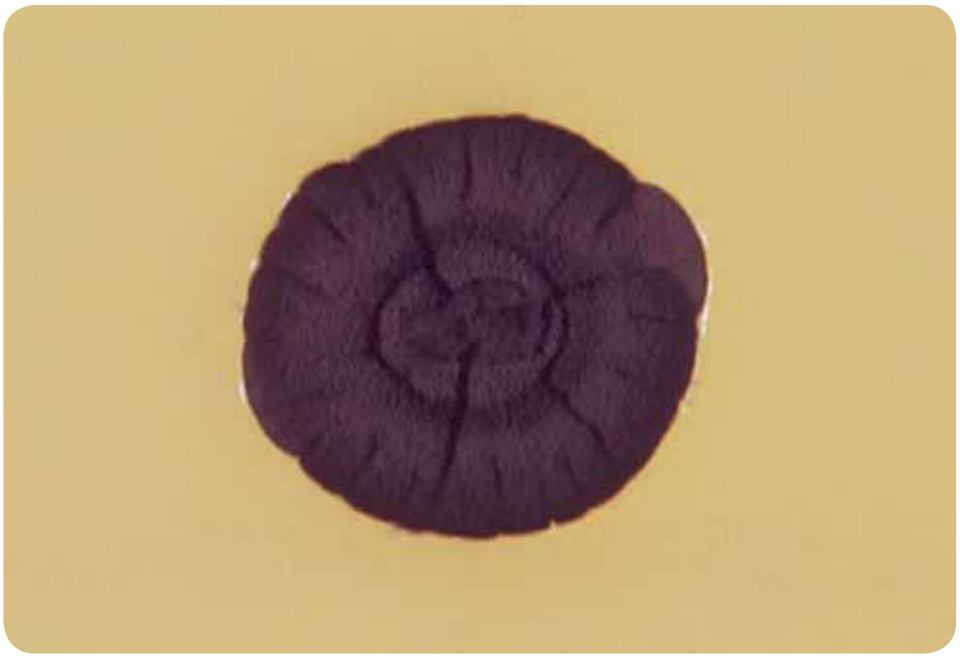
RG-2 organism.

Morphological Description: Colonies are slow growing, reaching 3-4 cm in diameter after one month, with a compact suede-like to downy surface and are olivaceous-black in colour. Microscopy shows ascending to erect, olivaceous-green, apically branched, elongate conidiophores producing branched acropetal chains of conidia. Conidia are pale olivaceous, smooth-walled or slightly verrucose, limoniform to fusiform, 1.5-3.0 x 2.0-7.0 µm in size. Bulbous phialides with large collarettes and minute, hyaline conidia are occasionally formed on nutritionally poor media. Maximum growth temperature 35-37^{O}C.

Molecular Identification: ITS sequencing is recommended (Abliz *et al*. 2004; de Hoog *et al.* 2007).

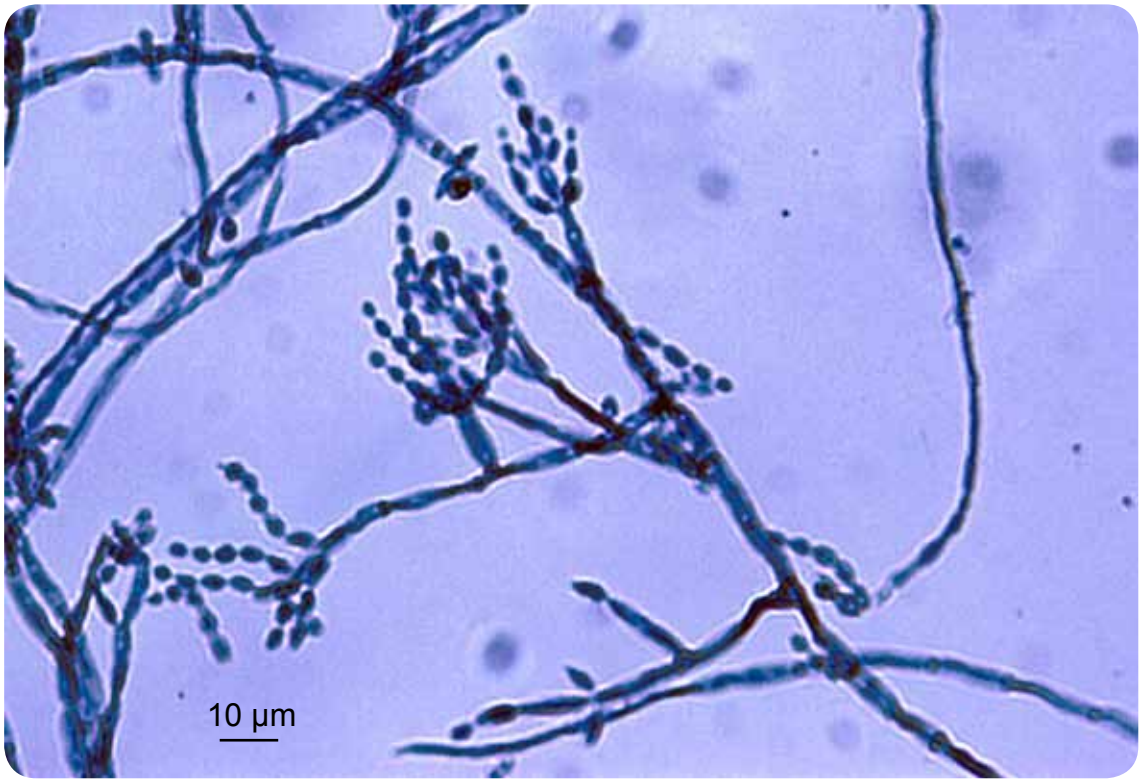
Key Features: Conidia are smaller and comprise heavily branched chains which fall apart much more easily than in the other *Cladophialophora* species.

References: McGinnis (1980), Rippon (1988), de Hoog *et al.* (1995, 2000, 2015).



Cladophialophora carrionii culture.

Cladophialophora carrionii (Trejos) de Hoog *et al.*



Cladophialophora carrionii conidiophores and conidia.

Antifungal Susceptibility: *C. carrionii* limited data available (Australian National data); **MIC µg/mL**.

	No.	≤0.008	0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	≥64
AmB	**2**								**1**	**1**					
VORI	**2**		**1**	**1**											
POSA	**2**	**1**		**1**											
ITRA	**2**		**1**	**1**											

C. carrionii limited data available (McGinnis and Pasarell 1998a,1998b, Espinel-Ingroff *et al.* 2001, Gonzales *et al.* 2005); **MIC µg/mL**.

AmB	**Range 0.06-4; MIC_{90}= 1**	**VORI**	**Range 0.03-0.5; MIC_{90}= 0.25**
ITRA	**Range 0.06-0.5; MIC_{90}= 0.125**	**POSA**	**Range 0.06-0.5; MIC_{90}= 0.25**
TERB	**Range 0.03-0.125; Geometric Mean = 0.03**		

Cladosporium Link ex Fries

Cladosporium species are ubiquitous worldwide, and commonly isolated from soil and organic matter. They represent the most frequently isolated airborne fungi. The genus has undergone a number of revisions. The well-known thermotolerant 'true human-pathogenic species, formerly known as *C. bantiana, C. carrionii* and *C. devriesii*, characterised by the absence of conidiophores, and unpigmented conidial scars, were reclassified in *Cladophialophora* (de Hoog *et al.* 1995, Bensch *et al.* 2012). The remaining species of medical interest were *C. cladosporioides, C. herbarum, C. oxysporum,* and *C. sphaerospermum*. More recently, extensive revisions based on polyphasic approaches have recognised 169 species, and demonstrated that *C. cladosporioides, C. herbarum* and *C. sphaerospermum* are species complexes encompassing several sibling species that can only be distinguished by phylogenetic analyses (Crous *et al*. 2007, Schubert *et al*. 2007, Zalar *et al*. 2007, Bensch *et al*. 2010, 2012).

Sandoval-Denis *et al.* (2015) analysed 92 clinical isolates from the United States using phenotypic and molecular methods, which included sequence analysis of the ITS and D1/D2 regions, partial *EF-1α* and actin genes. Surprisingly, the most frequent species was *Cladosporium halotolerans* (15%), followed by *C. tenuissimum* (10%), *C. subuliforme* (6%), and *C. pseudocladosporioides* (5%). However, 40% of the isolates did not correspond to any known species and were deemed to represent at least 17 new lineages for *Cladosporium*. The most frequent anatomic site of isolation was the respiratory tract (55%), followed by superficial (28%) and deep tissues and fluids (15%). Species of the two recently described *Cladosporium*-like genera *Toxicocladosporium* and *Penidiella* were also reported for the first time from clinical samples (Sandoval-Denis *et al*. 2015).

RG-1 organisms.

Morphological Description: Colonies are slow growing, mostly olivaceous-brown to blackish-brown but also sometimes grey, buff or brown, suede-like to floccose, often becoming powdery due to the production of abundant conidia. The reverse is olivaceous-black. Vegetative hyphae, conidiophores and conidia are equally pigmented. Conidiophores are more or less distinct from the vegetative hyphae, being erect, straight or flexuose, unbranched or branched only in the apical region, with geniculate sympodial elongation in some species. Conidia are produced in branched acropetal chains, being smooth, verrucose or echinulate, one to four-celled, and have a distinct dark hilum. The term blastocatenate is often used to describe chains of conidia where the youngest conidium is at the apical or distal end of the chain. **Note:** The conidia closest to the conidiophore, and where the chains branch, are usually "shield-shaped". The presence of shield-shaped conidia, a distinct hilum, and chains of conidia that readily disarticulate, are characteristic of the genus *Cladosporium.*

Key Features: Dematiaceous hyphomycete forming branched acropetal chains of conidia, each with a distinct hilum.

Molecular Identification: Genus level identification is usually sufficient and morphological identification can be confirmed by ITS and D1/D2 sequence analysis. Multilocus gene analysis of the ITS, D1/D2, *EF-1α* and actin gene loci is necessary for accurate species identification (Bensche *et al*. 2012).

Cladosporium Link ex Fries

Cladosporium cladosporioides complex (a) culture, (b) conidiophores and conidia.

Antifungal Susceptibility: *Cladosporium* species (Sandoval-Denis *et al*. 2015 and Australian National data); **MIC µg/mL.**					
Antifungal	**Range**	**MIC_{90}**	**Antifungal**	**Range**	**MIC_{90}**
AmB	**0.03-2**	**2**	**POSA**	**0.03-4**	**0.5**
ITRA	**0.03-16**	**0.5**	**VORI**	**0.25-16**	**4**

References: Ellis (1971, 1976), Domsch *et al.* (1980), McGinnis (1980), de Hoog *et al.* (2000, 2015), Crous *et al.* (2007), Schubert *et al.* (2007), Zalar *et al.* (2007), Bensch *et al*. (2010 and 2012), Sandoval-Denis *et al*. (2015).

Clavispora lusitaniae Rodrigues de Miranda

Synonymy: *Candida lusitaniae* van Uden & do Carmo-Sousa.

Clavispora lusitaniae is a known cause of disseminated candidiasis, including septicaemia and pyelonephritis. *C. lusitaniae* was first isolated from the digestive tract of warm-blooded animals and environmental isolations have been made from cornmeal, citrus peel, fruit juices, and milk from cows with mastitis. **RG-2 organism.**

Culture: Colonies (SDA) white to cream-coloured smooth, glabrous, yeast-like.

Microscopy: Ovoid to ellipsoidal budding blastoconidia, 1.5-6.0 x 2.5-10µm.

India Ink Preparation: Negative - no capsules present.

Dalmau Plate Culture: Abundant pseudohyphae with short chains of blastoconidia.

Molecular Identification: ITS sequencing recommended.

MALDI-TOF MS: Able to accurately identify this species.

Physiological Tests: + Positive, - Negative, v Variable, w Weak, s Slow, nd No Data							
Germ Tube	-	L-Sorbose	+	L-Arabinose	v	D-Glucitol	+
Fermentation		Sucrose	+	D-Arabinose	-	α-M-D-glucoside	v
Glucose	+	Maltose	+	D-Ribose	-	D-Gluconate	s
Galactose	v	Cellobiose	+	L-Rhamnose	v	DL-Lactate	+,w
Sucrose	v	Trehalose	+	D-Glucosamine	-	*myo*-Inositol	-
Maltose	v	Lactose	-	*N*-A-D-glucosamine	+	2-K-D-gluconate	+
Lactose	-	Melibiose	-	Glycerol	+	D-Glucuronate	nd
Trehalose	v	Raffinose	-	Erythritol	-	Nitrate	-
Assimilation		Melezitose	+	Ribitol	s	Urease	-
Glucose	+	Soluble Starch	-	Galactitol	-	0.1% Cycloheximide	-
Galactose	+	D-Xylose	+	D-Mannitol	+	Growth at 40^{O}C	+

Key Features: Germ tube negative yeast and sugar assimilation pattern.
Note: *C. lusitaniae* may also be difficult to distinguish from *Candida tropicalis* using some yeast identification systems.

Antifungal Susceptibility*: C. lusitaniae* (Diekema *et al.* 2009, Pfaller *et al.* 2013, Australian National data); **MIC µg/mL**.

	No.	**≤0.008**	**0.016**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**≥64**
AmB	**214**			**1**	**9**	**31**	**86**	**74**	**10**	**1**		**1**	**1**		
FLU	**279**				**12**	**33**	**66**	**82**	**33**	**21**	**8**	**4**	**1**	**5**	**4**
VORI	**262**	**169**	**53**	**15**	**12**	**3**	**5**	**3**	**2**						
POSA	**252**	**5**	**40**	**77**	**83**	**26**	**15**	**2**	**4**						
ITRA	**99**	**1**	**1**	**3**	**21**	**33**	**20**	**15**	**4**	**1**					
ANID	**175**			**1**	**13**	**32**	**56**	**59**	**13**	**1**					
MICA	**159**	**1**	**2**	**13**	**22**	**57**	**46**	**11**	**6**	**1**					
CAS	**247**		**2**	**4**	**12**	**86**	**91**	**38**	**12**	**1**	**1**				
5FC	**44**			**15**	**20**	**1**	**2**	**2**				**1**		**1**	**2**

Coccidioides immitis/posadasii complex

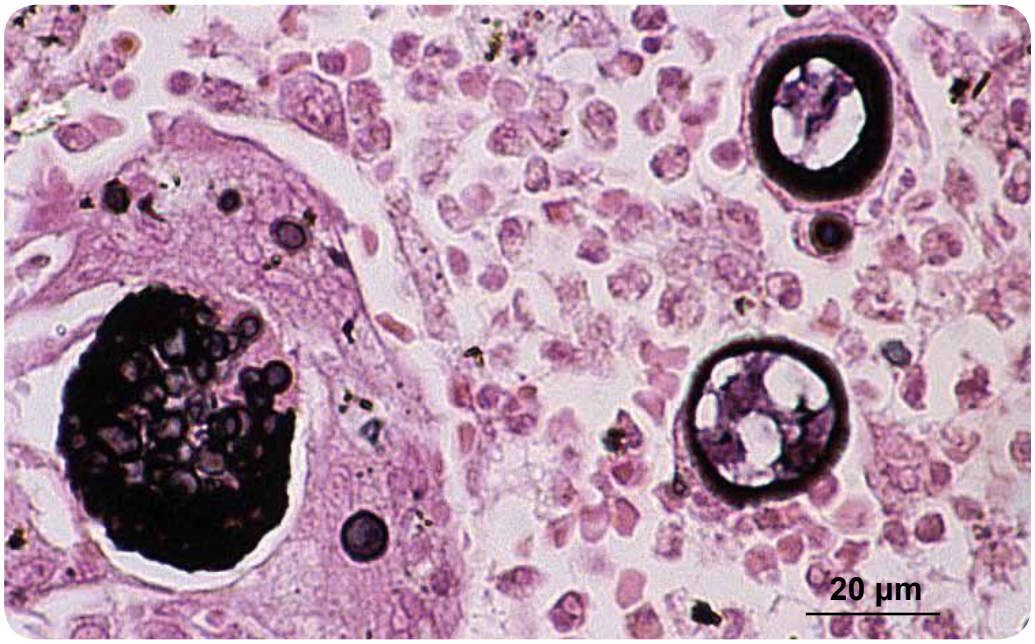
WARNING: RG-3 organism. Cultures of *Coccidioides immitis/posadasii* represent a severe biohazard to laboratory personnel and must be handled with extreme caution in Class II Biological Safety Cabinet (BSCII).

Coccidioides immitis has been separated into two distinct species: *C. immitis* and *C. posadasii* (Fisher *et al.* 2002). The two species are morphologically identical and can be distinguished only by genetic analysis and different rates of growth in the presence of high salt concentrations (*C. posadasii* grows more slowly). *C. immitis* is geographically limited to California's San Joaquin Valley region and Mexico, whereas *C. posadasii* is found in California, Arizona, Texas, Mexico and South America.

Morphological Description: Colonies of *C. immitis and C. posadasii* grown at 25^{O}C are initially moist and glabrous, but rapidly become suede-like to downy, greyish-white with a tan to brown reverse, however considerable variation in growth rate and culture morphology has been noted. Microscopy shows typical single-celled, hyaline, rectangular to barrel-shaped, alternate arthroconidia, 2.5-4 x 3-6 µm in size, separated from each other by a disjunctor cell. This arthroconidial state has been classified in the genus *Malbranchea* and is similar to that produced by many non-pathogenic soil fungi, e.g. *Gymnoascus* species.

Comment: *Coccidioides immitis and C. posadasii* dimorphic fungi, existing in living tissue as spherules and endospores, and in soil or culture in a mycelial form. Culture identification by either exoantigen test or DNA sequencing is preferred to minimise exposure to the infectious propagule.

Key Features: Clinical history, tissue pathology, culture identification by ITS sequence analysis.



Coccidioides immitis tissue morphology showing typical endosporulating spherules. Young spherules have a clear centre with peripheral cytoplasm and a prominent thick-wall. Endospores (sporangiospores) are later formed within the spherule by repeated cytoplasmic cleavage. Rupture of the spherule releases endospores into the surrounding tissue where they re-initiate the cycle of spherule development.

References: Ajello (1957), Steele *et al*. (1977), McGinnis (1980), Chandler *et al.* (1980), Catanzaro (1986), Rippon (1988), de Hoog *et al.* (2015), Fisher *et al.* (2002).

Coccidioides immitis/posadasii complex

Molecular Identification: In endemic areas a DNA probe for recognition of the species is commercially available (Padhye *et al.* 1994b). ITS sequencing is recommended for differentiation of species (Tintelnot *et al.* 2007, Binnicker *et al.* 2011).

Coccidioides immitis (a) culture and (b) arthroconidia with disjunctor cells.

Antifungal Susceptibility: ***C. immitis*** (Ramani and Chaturvedi 2007); **MIC µg/mL**. **Antifungal susceptibility testing not recommended.** For treatment options see Clinical Practice Guidelines for the Management of Coccidioidomycosis (Galgiani *et al*. 2005).

	No.	≤0.03	0.06	0.125	0.25	0.5	1	2	4	8	≥16
AmB	**45**	**7**	**25**	**8**	**4**	**1**					
FLU	**45**						**1**	**4**	**10**	**27**	**3**
ITRA	**45**	**13**	**9**	**15**	**8**						
POSA	**45**	**25**	**13**	**3**	**4**						
VORI	**45**	**23**	**22**								

Colletotrichum coccodes (Wallroth) S. Hughes

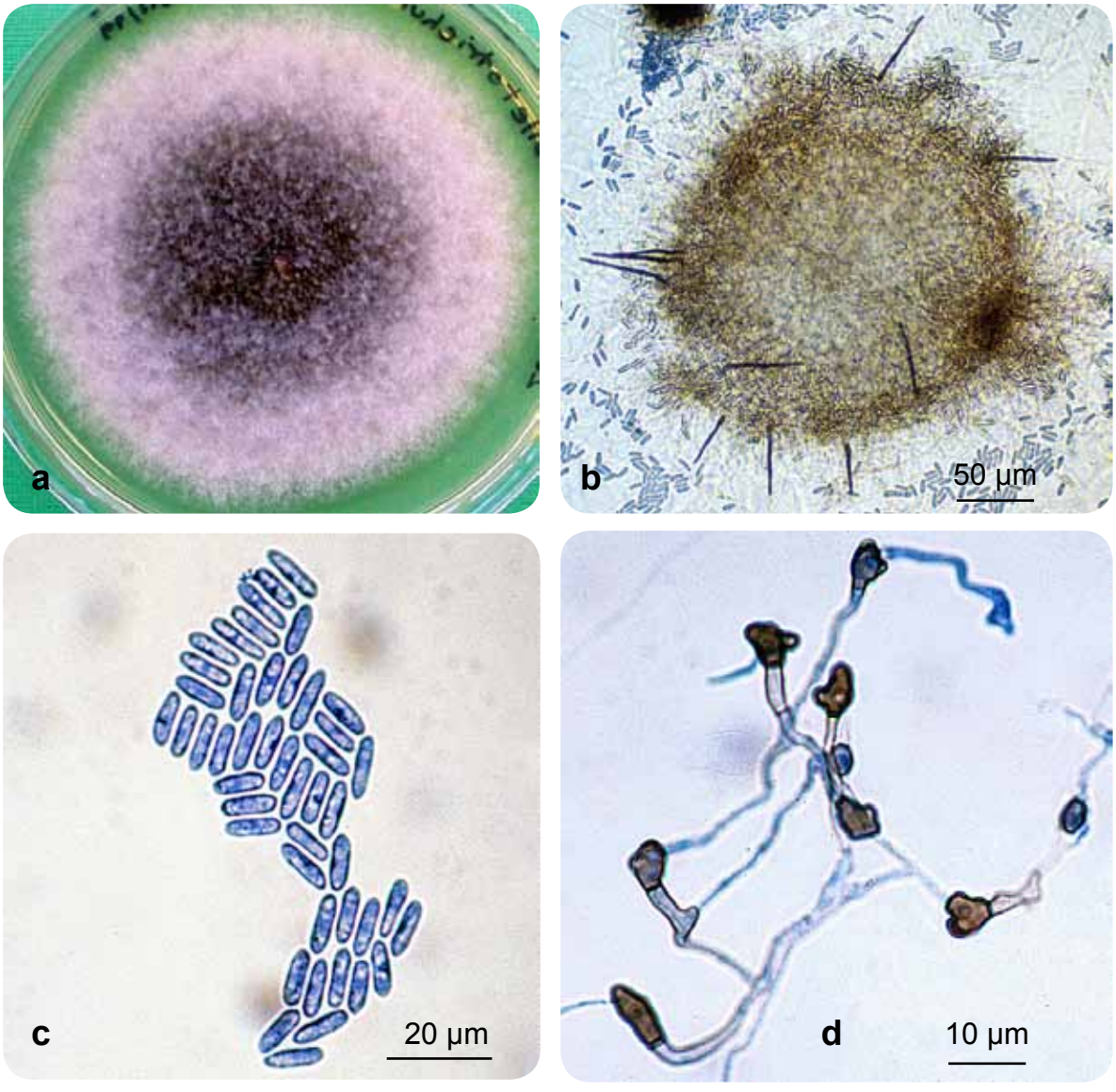
Over 500 *Colletotrichum* species have been reported. *C. coccodes* is a common soil and plant pathogen widely distributed in Africa, Asia, Australasia, Europe, and the Americas. It has been reported from a case of human mycotic keratitis.

RG-1 organism.

Morphological Description: Colonies usually darkly pigmented with white aerial mycelium, consisting of numerous black sclerotia and light brown-coloured conidial masses, reverse is dark brown. Sclerotia are usually abundant, setose, spherical and are often confluent. Conidia are straight, fusiform, attenuated at the ends, 16-22 x 3-4 µm. Appressoria are common, clavate, brown, 11-16.5 x 6-9.5 µm, variable in shape.

Molecular Identification: ITS and/or D1/D2 sequencing may be used for species identification (Cano *et al.* 2004).

References: Domsch *et al.* (1980), McGinnis (1980), de Hoog *et al*. (2000, 2015).



Colletotrichum coccodes (a) culture, (b) sclerotia with setae, (c) conidia and (d) appressoria.

Conidiobolus coronatus (Costantin) Batko

Synonymy: *Entomophthora coronata* (Costantin) Kevorkian.

The species of the genus *Conidiobolus* produce characteristic multinucleate primary and secondary (replicative) conidia on top of unbranched conidiophores. Each subspherical conidium is discharged as a result of the pressure developed within the conidium, and each bears a more or less prominent papilla after discharge (King 1983). The genus contains 27 species, however only *Conidiobolus coronatus, C. incongruus* and *C. lamprauges* have been reported as causative agents of human and animal infection. A morphological identification key for clinical isolates was given by Vilela *et al.* (2010).

RG-2 organism.

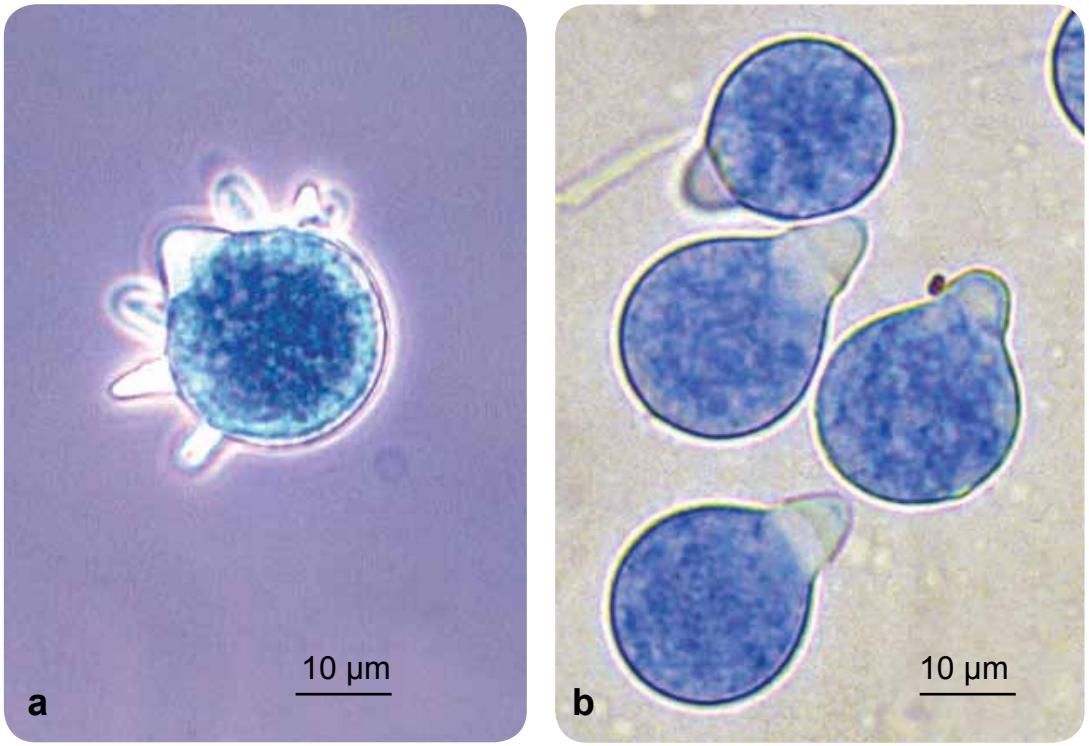
Morphological Description: Colonies of *C. coronatus* grow rapidly and are flat, cream-coloured, glabrous becoming radially folded and covered by a fine, powdery, white surface mycelium and conidiophores. The lid of the petri dish soon becomes covered with conidia, which are forcibly discharged by the conidiophores. The colour of the colony may become tan to brown with age. Conidiophores are simple forming solitary, terminal conidia which are spherical, 10 to 25 µm in diameter, single-celled and have a prominent papilla. Conidia may also produce hair-like appendages, called villae. Conidia germinate to produce either: (1) single or multiple hyphal tubes that may also become conidiophores which bear secondary conidia; or (2) replicate by producing multiple short conidiophores, each bearing a small secondary conidium.

C. coronatus is commonly present in soil and decaying leaves. It has a worldwide distribution especially in tropical rain forests of Africa. Human infections are usually restricted to the rhinofacial area. However, there are occasional reports of dissemination to other sites. All human infections have been confined to the tropics.

References: Emmons and Bridges (1961), King (1983), McGinnis (1980), Rippon (1988), Kwon-Chung and Bennett (1992), de Hoog *et al.* (2000, 2015), Ellis (2005a).

Conidiobolus coronatus culture showing satellite colonies formed by germinating conidia ejected from the primary colony.

Conidiobolus coronatus (Costantin) Batko



Conidiobolus coronatus (a) spherical conidium with hair-like appendages (villae) and (b) conidia with prominent papillae.

Coniochaeta hoffmannii (J.F.H. Beyma) Z.U. Khan, Gené & Guarro

Synonymy: *Lecythophora hoffmannii* (J.F.H. Beyma) W. Gams & McGinnis.
Phialophora hoffmannii (J.F.H. Beyma) Schol-Schwarz.

In response to recent changes in the nomenclature for pleomorphic fungi, Khan *et al*. (2013) transferred all *Lecythophora* species to *Coniochaeta*. The genus *Coniochaeta* is morphologically similar to *Phialemonium*. It also forms adelophialides, but in *Coniochaeta*, these conidiogenous cells show conspicuous collarettes, and the colonies are usually pink-salmon to dark brown, although discrete phialides like those of *Acremonium* may also be present (Perdomo *et al*. 2011b). *Coniochaeta hoffmannii* has been associated with cases of subcutaneous infections, keratitis, sinusitis, peritonitis, and canine osteomyelitis. *Coniochaeta mutabilis* has been described to be a causative agent of human peritonitis, endocarditis, endophthalmitis, and keratitis (Perdomo *et al.* 2011b).

RG-1 organism.

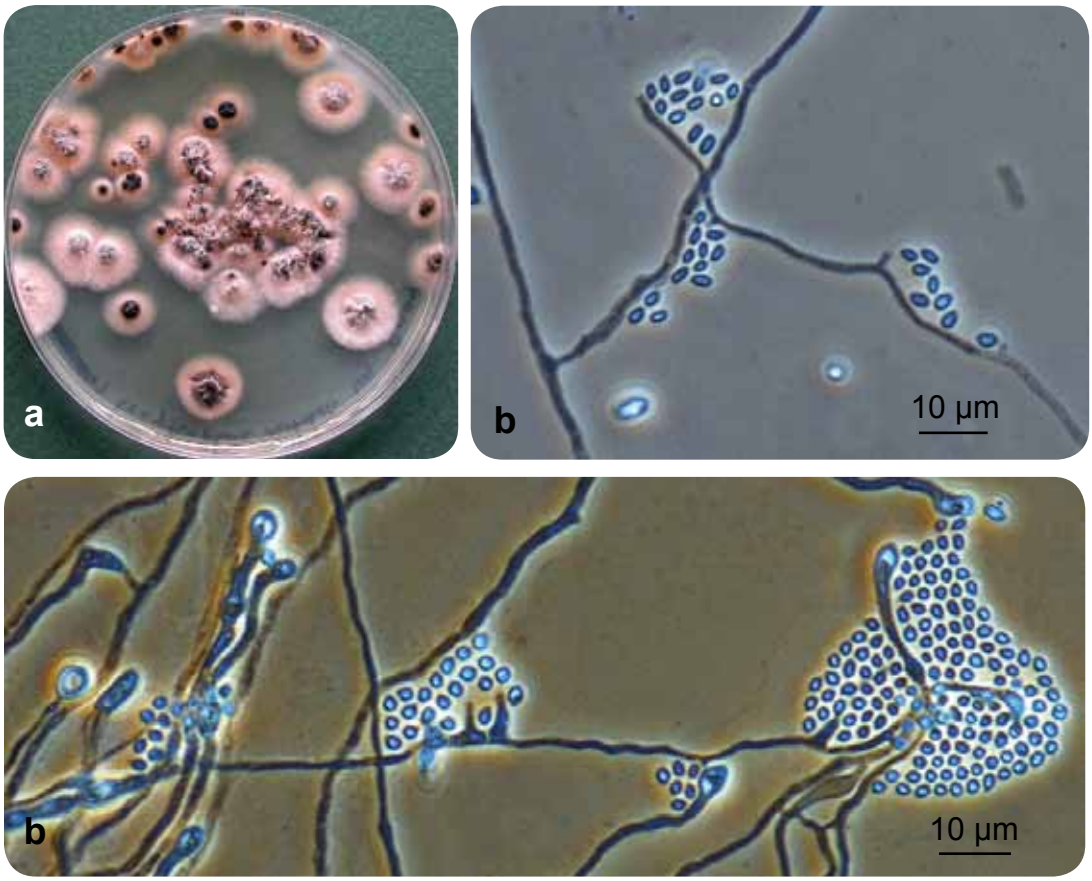
Morphological Description: Colonies are flat, smooth, moist, pink to orange, with regular and sharp margin; reverse pink. Hyphae are narrow, hyaline, producing conidia laterally from small collarettes directly on the hyphae, or from lateral cells which are sometimes arranged in dense groups; lateral cells flask-shaped or nearly cylindrical. Collarettes are unpigmented, about 1.5 µm wide. Conidia are hyaline, smooth and thin-walled, broadly ellipsoidal to cylindrical or allantoid, 3.0-3.5 x 1.5-2.5 µm, produced in slimy heads.

Coniochaeta hoffmannii (J.F.H. Beyma) Z.U. Khan, Gené & Guarro

Note: *Coniochaeta* and *Phialemonium* species are poorly differentiated morphologically, and are difficult to identify. They may also be confused with poorly sporulating *Fusarium* or *Acremonium* species.

Molecular Identification: Khan *et al*. (2013) used a combined sequence data set of the ITS region, D1/D2, actin and β-tubulin genes to resolve the unique phylogenetic status of this species.

References: de Hoog (1983), de Hoog *et al.* (2000, 2015), Perdomo *et al.* (2011b), Khan *et al*. (2013).



Coniochaeta hoffmannii (a) culture, (b) hyphae with small collarettes and conidia.

Antifungal Susceptibility: *Coniochaeta hoffmannii* very limited data (McGinnis and Pasarell 1998a, Perdomo *et al*. 2011b and Australian National data); **MIC µg/mL.**

Antifungal	Range	MIC_{90}	Antifungal	Range	MIC_{90}
AmB	0.06-2	2	POSA	0.125-1	1
ITRA	0.06-32	1	VORI	0.25-1	1

Cryptococcus Kützing emend. Phaff & Spencer

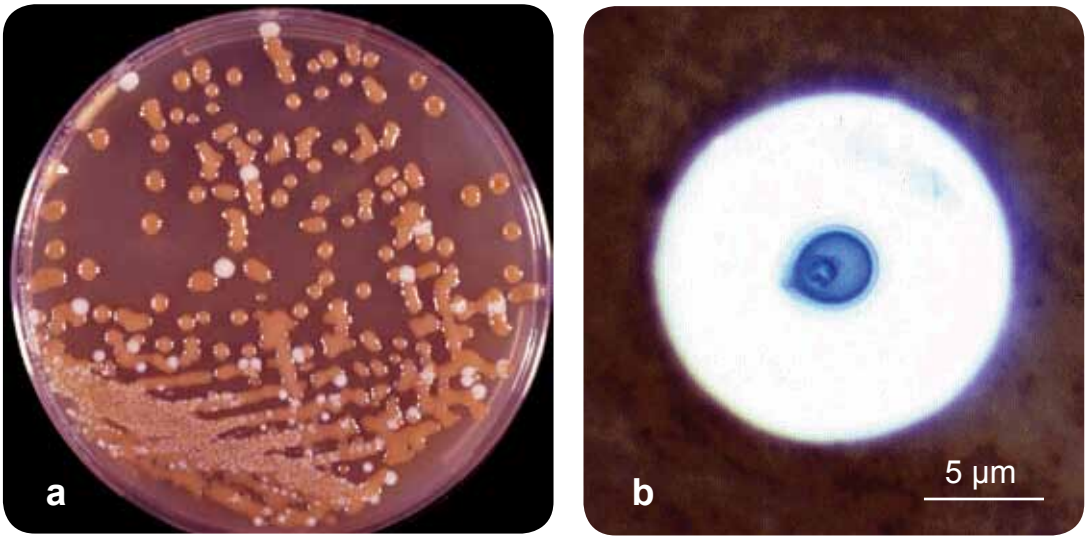
The genus *Cryptococcus* is characterised by globose to elongate yeast-like cells or blastoconidia that reproduce by narrow-necked budding. Pseudohyphae are absent or rudimentary. Most species are encapsulated, although the extent of capsule formation depends on the medium. Under certain conditions of growth, the capsule may contain starch-like compounds, which are released into the medium by many strains. Within tissue sections, mucicarmine or Alcian blue stains the capsule of *Cryptococcus* species to distinguish it from other yeasts with similar morphologies.

On solid media the cultures are generally mucoid or slimy in appearance; red, orange or yellow carotenoid pigments may be produced, but young colonies of most species are usually non-pigmented, and cream in colour. All *Cryptococcus* species produce urease and are non-fermentative. Nitrate may be assimilated or not; inositol assimilated. The genus *Cryptococcus* differs from the genus *Rhodotorula* in its inositol assimilation.

Cryptococcosis is a chronic, subacute to acute pulmonary, systemic or meningitic disease, initiated by the inhalation of infectious propagules (basidiospores and/or desiccated yeast cells) from the environment. Primary pulmonary infections have no diagnostic symptoms and are usually subclinical. On dissemination, the fungus usually shows a predilection for the central nervous system, however skin, bones and other visceral organs may also become involved. Although *C. neoformans* and *C. gattii* are regarded as the principle pathogenic species, *Cryptococcus albidus* and *C. laurentii* have on occasion also been implicated in human infection.

Molecular Identification: Requires ITS and/or D1/D2 sequencing, particularly for identification of unusual species.

MALDI-TOF MS: Can provide reliable species and subspecies level identification of *Cryptococcus* species, but its accuracy is dependent on database quality (Arendrup *et al.* 2014).



Cryptococcus neoformans (a) culture appearances on bird seed agar (brown colonies) and *Candida albicans* (white colonies) and (b) India Ink preparation of *C. neoformans* surrounded by a characteristic wide gelatinous capsule.

References: Rippon (1982), Barnett *et al.* (1983), Kurtzman *et al*. (2011), Casadevall and Perfect (1998), de Hoog *et al.* (2000, 2015), McTaggart *et al*. (2013).

Cryptococcus albidus (Saito) Skinner

Synonymy: *Cryptococcus diffluens* (Zach) Lodder & Kreger-van Rij.

RG-1 organism.

Culture: Colonies (SDA) are cream-coloured smooth, mucoid, glabrous, yeast-like.

Microscopy: Globose to ovoid budding yeast-like cells, 3.5-8.8 x 5.5-10.2 µm. Pseudohyphae are absent.

India Ink Preparation: Positive - distinct thin capsules are present.

Physiological Tests: + Positive, - Negative, v Variable, w Weak, s Slow							
Germ Tube	-	L-Sorbose	v	L-Arabinose	+	D-Glucitol	+
Fermentation		Sucrose	+	D-Arabinose	v	α-M-D-glucoside	v
Glucose	-	Maltose	+	D-Ribose	v	D-Gluconate	+
Galactose	-	Cellobiose	+	L-Rhamnose	v	DL-Lactate	v
Sucrose	-	Trehalose	+,w	D-Glucosamine	-	*myo*-Inositol	+
Maltose	-	Lactose	v	*N*-A-D-glucosamine	-	2-K-D-gluconate	+
Lactose	-	Melibiose	v	Glycerol	v	D-Glucuronate	+
Trehalose	-	Raffinose	+	Erythritol	v	Nitrate	+
Assimilation		Melezitose	+	Ribitol	v	Urease	+
Glucose	+	Soluble Starch	v	Galactitol	v	0.1% Cycloheximide	-
Galactose	v	D-Xylose	+	D-Mannitol	+	Growth at 37^{O}C	v

Cryptococcus albidus has variable growth at 37^{O}C, and infections in humans are rare. Along with *C. laurentii, C. albidus* accounts for 80% of the non-*neoformans/gattii* infections. Impaired cellular immunity is the most common risk factor with HIV infection and low CD4 counts a common comorbidity.

Antifungal Susceptibility*: C. albidus* (Australian National data); **MIC µg/mL.**
Note: All *Cryptococcus* species are intrinsically resistant to echinocandins.

	No.	**≤0.03**	**0.06**	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**≥64**
AmB	**1**						**1**						
FLU	**1**											**1**	
VORI	**1**						**1**						
POSA	**1**						**1**						
ITRA	**1**					**1**							
5FC	**1**												**1**

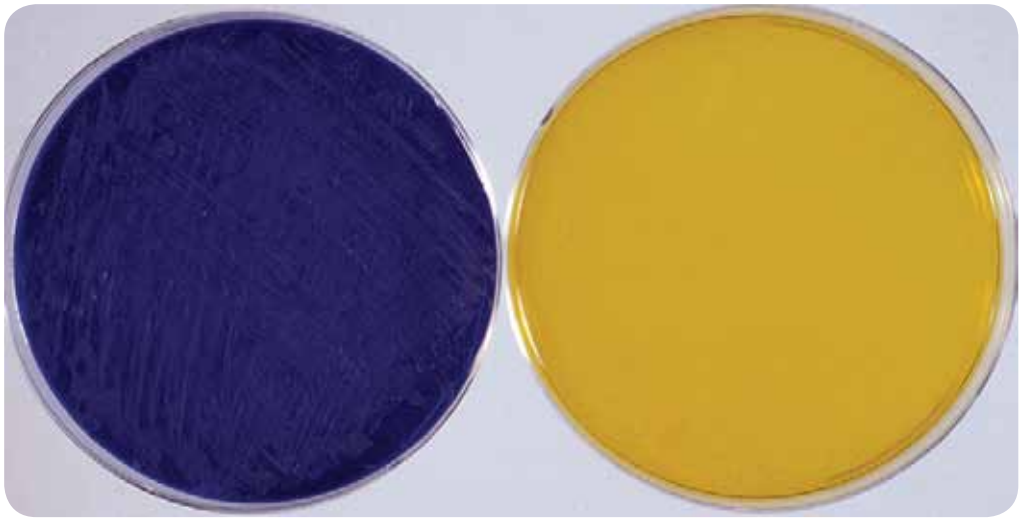
Cryptococcus gattii (Vanbreus. & Takashio) Kwon-Chung & Boekhout

Synonymy: *Filobasidiella bacillispora* Kwon-Chung.
Cryptococcus neoformans var. *gattii* Vanbreus. & Takashio.

RG-2 organism.

Cryptococcus gattii has two serotypes (B and C) and was reclassified as a separate species from *C. neoformans* in 2002 (Kwon-Chung *et al.* 2002). *C. gattii* generally has a more restricted geographical distribution than *C. neoformans*, causing human disease in climates ranging from temperate to tropical Australia, Papua New Guinea, parts of Africa, India, Southeast Asia, Mexico, Brazil, Paraguay and Southern California, although recent infections have also been reported from Vancouver Island, Canada and in the Pacific Northwest, USA (Pfaller & Diekema, 2010, Espinel-Ingroff and Kidd, 2015). *C. gattii* has a specific ecological association with numerous species of *Eucalyptus* trees, although the Canadian isolates are associated with a range of native non-*Eucalyptus* species (Kidd *et al.* 2007). Historically considered a pathogen in immunocompetent hosts, a recent review in Australia noted an increase in *C. gattii* infections in HIV-negative immunocompromised patients (Chen *et al*. 2012). Cryptococcosis caused by *C. gattii* is often associated with large mass lesions (cryptococcomas) in the lung and/or brain (Sorrell, 2001).

Canavanine glycine bromothymol blue (CGB) agar (Kwon-Chung *et al*. 1982) is the media of choice to differentiate *C. gattii* from *C. neoformans*. This simple biotype test is based on the ability of *C. gattii* isolates to grow in the presence of L-canavanine and to assimilate glycine as a sole carbon source. A heavy inoculum is important.



Cryptococcus gattii turns CGB agar blue within 2-5 days;
Cryptococcus neoformans does not grow on this medium

Cryptococcus gattii (Vanbreus. & Takashio) Kwon-Chung & Boekhout

Culture: Colonies (SDA) cream-coloured smooth, mucoid, yeast-like colonies.

Microscopy: Globose to ovoid budding yeast-like cells 3.0-7.0 x 3.3- 7.9 µm.

India Ink Preparation: Positive - distinct, wide gelatinous capsules are present. Some strains may not produce apparent capsules from culture.

Dalmau Plate Culture: Budding yeast cells only. No pseudohyphae present.

Bird Seed Agar: Colonies turn dark brown in colour as colonies selectively absorb a brown pigment from this media. Colonies are often more mucoid when compared with *C. neoformans* (Staib, 1987).

Canavanine Glycine Bromothymol Blue (CGB) Agar: Turns blue within 2-5 days.

Physiological Tests: + Positive, - Negative, v Variable, w Weak, s Slow, nd No Data							
Germ Tube	-	L-Sorbose	-	L-Arabinose	+,w	D-Glucitol	+
Fermentation		Sucrose	+	D-Arabinose	+	M-D-glucoside	+
Glucose	-	Maltose	+	D-Ribose	v	D-Gluconate	+
Galactose	-	Cellobiose	+,w	L-Rhamnose	+	DL-Lactate	-
Sucrose	-	Trehalose	+	D-Glucosamine	v	*myo*-Inositol	+
Maltose	-	Lactose	-	*N*-A-D-glucosamine	v	2-K-D-gluconate	nd
Lactose	-	Melibiose	-	Glycerol	-	D-Glucuronate	nd
Trehalose	-	Raffinose	+,w	Erythritol	-	Nitrate	-
Assimilation		Melezitose	+	Ribitol	v	Urease	+
Glucose	+	Soluble Starch	+	Galactitol	+	0.1% Cycloheximide	-
Galactose	+	D-Xylose	+	D-Mannitol	+	Growth at 37^{O}C	+

Key Features: Encapsulated yeast; absence of pseudohyphae; growth at 37^{O}C; positive hydrolysis of urea; negative fermentation of sugars and positive assimilation of glucose, maltose, sucrose, galactose, trehalose, raffinose, inositol, cellobiose, rhamnose, arabinose, melezitose and xylose, and negative assimilation of nitrate, lactose, melibiose, erythritol and soluble starch; growth on bird seed (*Guizotia abyssinica* seed) or caffeic acid agar - colonies turn a dark brown colour; growth on CGB agar turning it blue within 2-5 days.

Antifungal Susceptibility*: C. gattii* (Australian National data); **MIC µg/mL.**
Note: All *Cryptococcus* species are intrinsically resistant to echinocandins.

	No.	**≤0.008**	**0.016**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**≥64**
AmB	**152**		**1**	**3**	**18**	**52**	**55**	**12**	**10**	**1**					
FLU	**152**							**2**	**13**	**29**	**48**	**38**	**19**	**2**	**1**
VORI	**130**	**5**	**12**	**32**	**44**	**20**	**13**	**4**							
POSA	**90**	**2**	**9**	**12**	**23**	**26**	**16**	**1**	**1**						
ITRA	**152**		**7**	**22**	**38**	**61**	**23**	**1**							
5FC	**152**					**2**	**10**	**38**	**52**	**39**	**7**	**4**			

Cryptococcus laurentii (Kufferath) Skinner

RG-1 organism.

Culture: Colonies (SDA) are cream-coloured, often becoming a deeper orange-yellow with age, with a smooth mucoid texture.

Microscopy: Spherical and elongated budding yeast-like cells or blastoconidia, 2.0-5.5 x 3.0-7.0 µm. No pseudohyphae present.

India Ink Preparation: Positive - narrow but distinct capsules are present.

Physiological Tests: + Positive, - Negative, v Variable, w Weak, s Slow							
Germ Tube	-	L-Sorbose	v	L-Arabinose	+	D-Glucitol	+
Fermentation		Sucrose	+	D-Arabinose	+	α-M-D-glucoside	+
Glucose	-	Maltose	+	D-Ribose	+	D-Gluconate	+
Galactose	-	Cellobiose	+	L-Rhamnose	+	DL-Lactate	v
Sucrose	-	Trehalose	+	D-Glucosamine	-	*myo*-Inositol	+
Maltose	-	Lactose	+	*N*-A-D-glucosamine	-	2-K-D-gluconate	+
Lactose	-	Melibiose	+	Glycerol	v	D-Glucuronate	+
Trehalose	-	Raffinose	+	Erythritol	v	Nitrate	-
Assimilation		Melezitose	+	Ribitol	+	Urease	+
Glucose	+	Soluble Starch	v	Galactitol	+	0.1% Cycloheximide	-
Galactose	v	D-Xylose	+	D-Mannitol	+	Growth at 37^{O}C	-,w

Note: Some strains of *C. laurentii* may develop brown pigment on bird seed agar and turn CGB media blue, similar to *C. gattii,* however *C. laurentii* assimilates both lactose and melibiose while *C. gattii* does not. Along with *C. albidus, C. laurentii* accounts for 80% of the non-*neoformans/gattii* infections. Impaired cellular immunity is the most common risk factor with HIV infection and low CD4 counts a common comorbidity. Invasive devices are an additional risk factor.

Antifungal Susceptibility*: C. laurentii* (Australian National data); **MIC µg/mL. Note:** All *Cryptococcus* species are intrinsically resistant to echinocandins.

	No.	**≤0.03**	**0.06**	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**≥64**
AmB	**7**			**1**	**2**	**3**	**1**						
FLU	**6**							**1**	**1**		**3**		**1**
VORI	**6**		**2**			**2**	**1**			**1**			
POSA	**5**	**1**	**1**	**2**		**1**							
ITRA	**7**	**1**		**2**	**2**	**1**					**1**		
5FC	**7**												**7**

Cryptococcus neoformans (Sanfelice) Vuillemin

Synonymy: *Filobasidiella neoformans* Kwon-Chung.
Cryptococcus neoformans var. *neoformans* (San Felice) Vuill.

RG-2 organism.

This species comprises two varieties: *C. neoformans* var. *grubii* (serotype A) and *C. neoformans* var. *neoformans* (serotype D).

C. neoformans var. *grubii* has a worldwide distribution, causing 95% of all *C. neoformans* infections. It has been isolated from various sources in nature and is noted for its association with accumulations of avian guano, especially with pigeon excreta. The fungus has also been isolated from the dung of caged birds including canaries, parrots and budgerigars. Other environmental isolations of *C. neoformans* var. *grubii* include rotting vegetables, fruits and fruit juices, wood, dairy products and soil.

C. neoformans var. *neoformans* has a more restricted distribution with infections being more prevalent in Europe, including France, Italy and Denmark, where it accounts for 30% of isolates. Moreover, *C. neoformans* var. *neoformans* infections are more strongly correlated with older patients, the skin, and the use of corticosteroids (Franzot *et al.* 1999).

Culture: Colonies (SDA) cream-coloured smooth, mucoid, yeast-like colonies.

Microscopy: Globose to ovoid budding yeast-like cells 3.0-7.0 x 3.3-7.9 µm.

India Ink Preparation: Positive - distinct, wide gelatinous capsules are present on direct microscopy. Some strains may not produce apparent capsules from culture.

Dalmau Plate Culture: Budding yeast cells only. No pseudohyphae present.

Bird Seed Agar: Colonies turn dark brown in colour as colonies selectively absorb a brown pigment from this media (Staib, 1987).

Canavanine Glycine Bromothymol Blue (CGB) Agar: No growth or colour change.

Creatinine Dextrose Bromothymol Blue Thymine (CDBT) Agar may be used to differentiate *C. neoformans* var. *neoformans* and *C. neoformans* var. *grubii. C. neoformans* var. *neoformans* grows as bright red colonies, turning the medium a bright orange after 5 days while no colour change is observed for *C. neoformans* var. *grubii* (Irokanulo *et al.* 1994).

Cryptococcus neoformans (Sanfelice) Vuillemin

Physiological Tests: + Positive, - Negative, v Variable, w Weak, s Slow, nd No Data							
Germ Tube	-	L-Sorbose	-	L-Arabinose	+,w	D-Glucitol	+
Fermentation		Sucrose	+	D-Arabinose	+	α-M-D-glucoside	+
Glucose	-	Maltose	+	D-Ribose	v	D-Gluconate	+
Galactose	-	Cellobiose	+,w	L-Rhamnose	+	DL-Lactate	-
Sucrose	-	Trehalose	+	D-Glucosamine	v	*myo*-Inositol	+
Maltose	-	Lactose	-	*N*-A-D-glucosamine	v	2-K-D-gluconate	nd
Lactose	-	Melibiose	-	Glycerol	-	D-Glucuronate	nd
Trehalose	-	Raffinose	+,w	Erythritol	-	Nitrate	-
Assimilation		Melezitose	+	Ribitol	v	Urease	+
Glucose	+	Soluble Starch	+	Galactitol	+	0.1% Cycloheximide	-
Galactose	+	D-Xylose	+	D-Mannitol	+	Growth at 37°C	+

Key Features: Encapsulated yeast; absence of pseudohyphae; growth at 37°C; positive hydrolysis of urea; negative fermentation of sugars and positive assimilation of glucose, maltose, sucrose, galactose, trehalose, raffinose, inositol, cellobiose, rhamnose, arabinose, melezitose and xylose, and negative assimilation of nitrate, lactose, melibiose, erythritol and soluble starch; growth on bird seed (*Guizotia abyssinica* seed) or caffeic acid agar - colonies turn a dark brown colour; does not grow on CGB agar (no colour change).

Antifungal Susceptibility: ***C. neoformans*** (Australian National data); **MIC µg/mL.**
Note: All *Cryptococcus* species are intrinsically resistant to echinocandins.

	No.	**≤0.008**	**0.016**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**≥64**
AmB	**236**		**2**	**5**	**17**	**82**	**57**	**57**	**16**						
FLU	**236**					**1**		**10**	**32**	**40**	**65**	**65**	**16**	**6**	**1**
VORI	**197**	**27**	**25**	**54**	**63**	**23**	**4**	**1**							
POSA	**152**	**1**	**9**	**29**	**39**	**46**	**24**	**4**							
ITRA	**236**	**2**	**10**	**37**	**72**	**90**	**20**	**4**	**1**						
5FC	**236**				**1**	**1**	**5**	**20**	**45**	**66**	**61**	**33**	**2**		**2**

Cunninghamella bertholletiae Stadel

Synonymy: *Cunninghamella elegans* Lendner.
Cunninghamella echinulata var. *elegans* (Lendner) Lunn & Shipton.

The genus *Cunninghamella* is characterised by white to grey, rapidly growing colonies, producing erect, straight, branching sporangiophores. These sporangiophores end in globose or pyriform-shaped vesicles from which several one-celled, globose to ovoid, echinulate or smooth-walled sporangiola develop on swollen denticles. Chlamydospores and zygospores may also be present.

Cunninghamella species are mainly soil fungi of the Mediterranean and subtropical zones; they are only rarely isolated in temperate regions. The genus now contains seven species with *C. bertholletiae* the only known species to cause disease in humans and animals, often in association with trauma and immunosuppression.

RG-2 organism.

Morphological Description: Colonies are very fast growing, white at first, but becoming dark grey and powdery with sporangiola development. Sporangiophores up to 20 µm wide, straight, with verticillate or solitary branches. Vesicles subglobose to pyriform, the terminal ones up to 40 µm and the lateral ones 10-30 µm in diameter. Sporangiola are globose (7-11 µm diameter), or ellipsoidal (9-13 x 6-10 µm), verrucose or short-echinulate, hyaline singly but brownish in mass. Temperature: optimum 25-30^{O}C, maximum up to 50^{O}C.

Key Features: Mucorales, clinical isolates grow at 40^{O}C, one-celled, globose to ovoid, echinulate sporangiola borne on swollen terminal or lateral globose to clavate fertile vesicles.

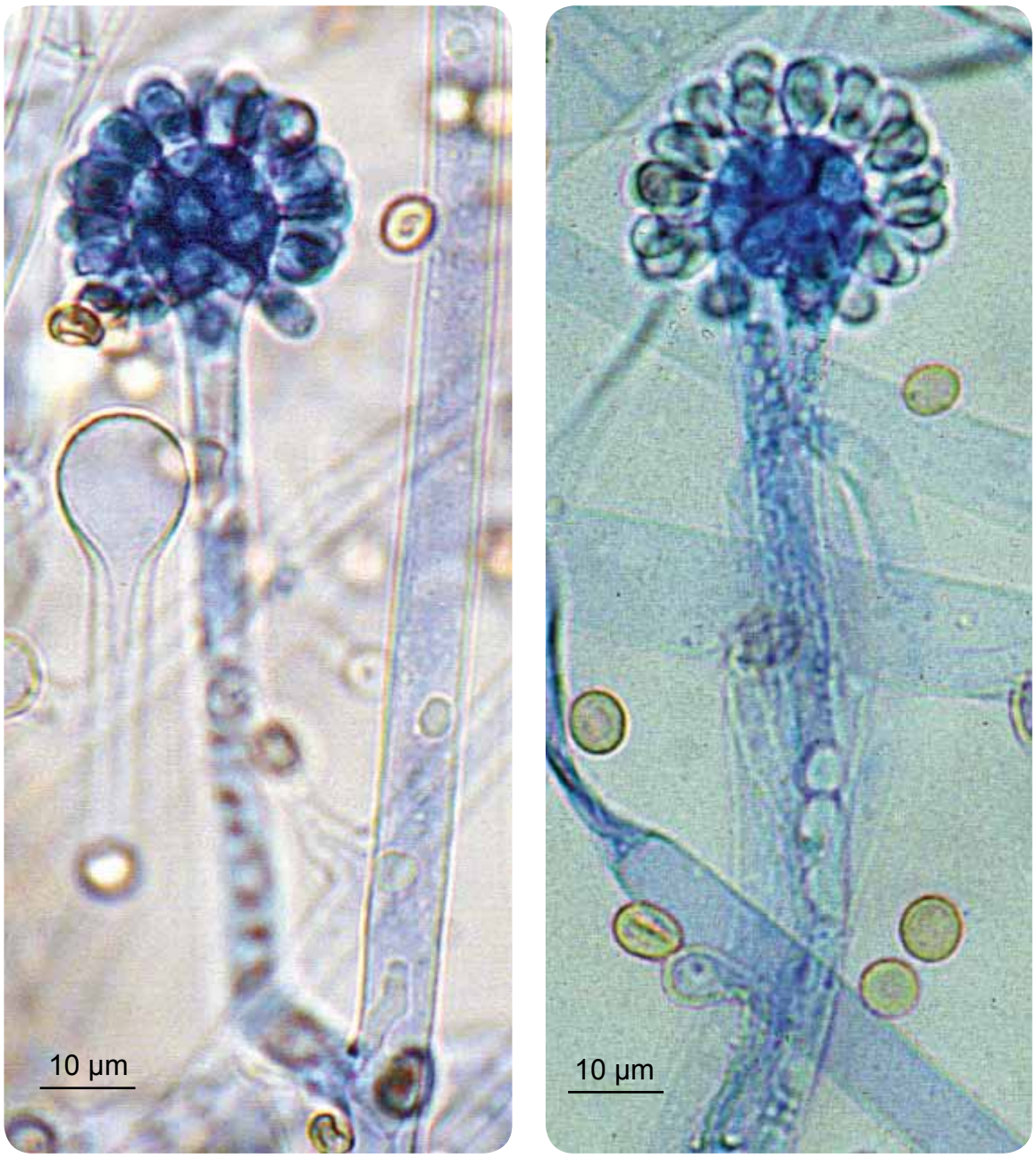
Molecular Identification: ITS sequencing is recommended (Yu *et al.* 2015).

References: McGinnis (1980), Weitzman and Crist (1980), Weitzman (1984), Lunn and Shipton (1983), Domsch *et al*. (1980), Samson (1969), de Hoog *et al.* (2000, 2015), Ellis (2005b), Zheng and Chen (2001).

Antifungal Susceptibility: ***C. bertholletiae*** (Espinel-Ingroff *et al*. 2015a, includes Australian data); **MIC µg/mL**.

	No.	≤0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	≥16
AmB	**32**					**1**	**1**	**5**	**16**	**8**	**1**	
POSA	**30**					**4**	**18**	**8**				
ITRA	**25**					**4**	**4**	**10**	**7**			

Cunninghamella bertholletiae Stadel



Cunninghamella bertholletiae showing simple sporangiophores forming a swollen, terminal vesicle around which single-celled, globose to ovoid sporangiola develop on swollen denticles.

Curvularia Boedijn

The genus *Curvularia* contains about 80 species, which are mostly soil or plant pathogens. Recent studies have shown that morphological identification does not correlate with molecular identification (Manamgoda *et al*. 2012; Yanagihara *et al.* 2010, da Cunha *et al.* 2013). A phylogenetic analysis of the genera *Bipolaris* and *Curvularia* has resulted in a re-alignment of several species. In particular, clinical isolates previously identified as *Bipolaris* species, notably *B. australiensis, B. hawaiiensis* and *B. spicifera* have now been transferred to *Curvularia* (Manamgoda *et al.* 2012).

Previously *Curvularia lunata* was the most frequently reported clinical species, however other species, such as *C. americana, C. brachyspora, C. chlamydospora, C. clavata, C. hominis, C. inaequalis, C. muehlenbeckiae, C. pallescens, C. pseudolunata, C. senegalensis* and *C. verruculosa* have now also been reported from clinical cases (Revankar and Sutton, 2010, da Cunha *et al.* 2013, Madrid *et al.* 2014).

RG-1 organisms.

Morphological Description: Colonies are fast growing, suede-like to downy, brown to blackish brown with a black reverse. Conidiophores erect, straight to flexuous, septate, often geniculate (producing conidia in sympodial succession) sometimes nodulose. Conidia are ellipsoidal, often curved or lunate, rounded at the ends or sometimes tapering slightly towards the base, pale brown, medium reddish brown to dark brown, 3–10 (usually 3–5) septa, conidial wall smooth to verrucose. Hilum protuberant in some species.

Key Features: Dematiaceous hyphomycete producing sympodial, pale brown, cylindrical or slightly curved phragmoconidia.

Comment: There is no clear morphological boundary between the genera *Bipolaris* and *Curvularia* and some species show intermediate morphology (Manamgoda *et al.* 2012). ITS and *GPDH* gene analysis is recommended for definitive identification of species (Manamgoda *et al.* 2012).

Molecular Identification: *GPDH* (Manamgoda *et al.* 2012) and/or ITS (da Cunha *et al.* 2013, Irinyi *et al.* 2015).

References: Ellis (1971), McGinnis (1980), Rippon (1988), de Hoog *et al.* (2000, 2015), Revankar and Sutton (2010), Yanagihara *et al.* (2010), Manamgoda *et al*. (2012); da Cunha *et al.* (2013), Madrid *et al. (*2014).

Antifungal Susceptibility: *Curvularia australiensis* (da Cunha *et al*. 2012a and Australian National data); **MIC µg/mL**.

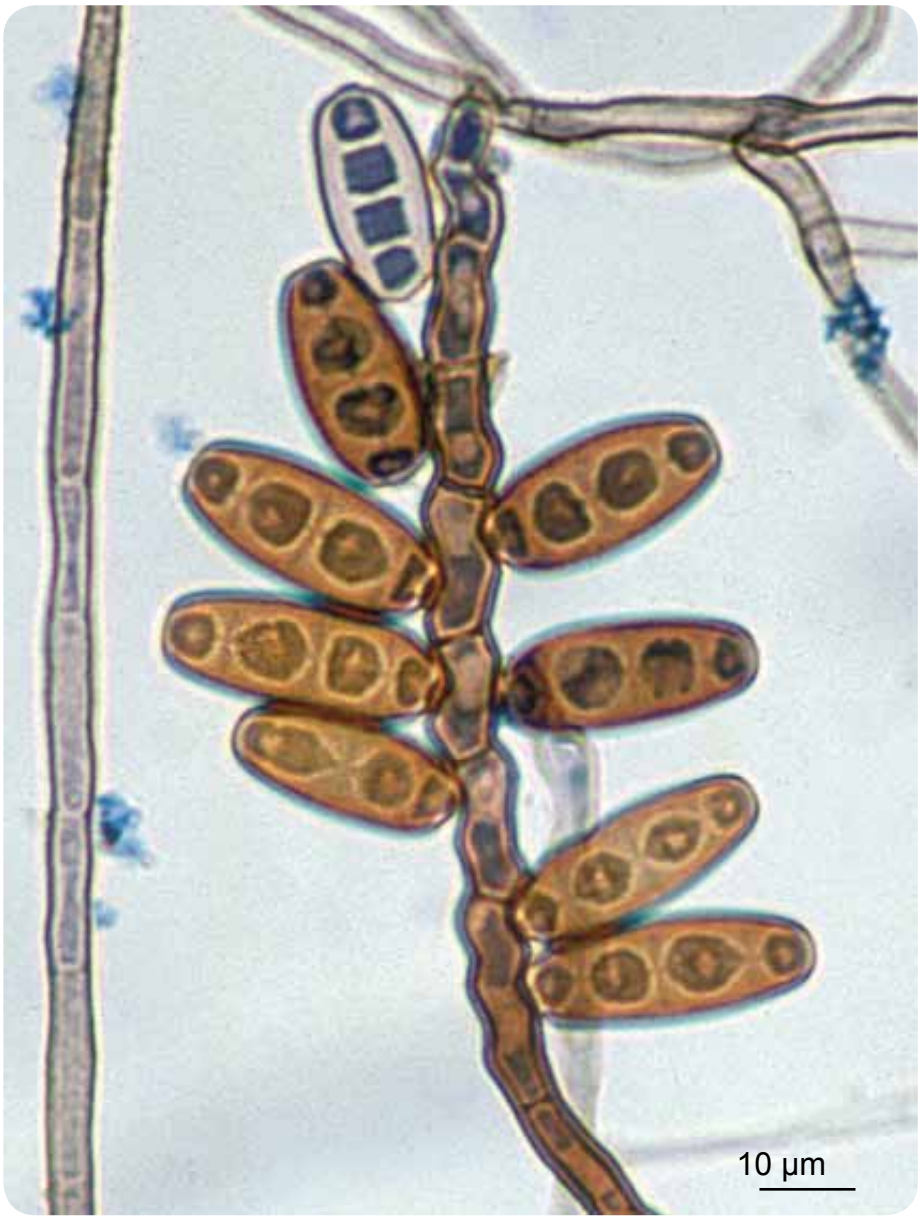
	No	≤0.008	0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	≥16
AmB	13			1	1	6	3		2				
VORI	13				1	2	6	3	1				
POSA	11	1		2	6	2							
ITRA	13			1	3	2	6	1					

Curvularia australiensis (M.B. Ellis) Manamgoda, L.Cai. & K.D. Hyde

Synonymy: *Bipolaris australiensis* (M.B. Ellis) Tsuda & Ueyama.

RG-1 organism.

Morphological Description: Colonies grey to blackish-brown, suede-like. Conidiophores brown, solitary, flexuose or geniculate, smooth-walled, up to 150 µm long, mostly 3-7 µm wide. Conidia straight, rounded at the ends, pale brown to mid reddish-brown, mostly 3-, rarely 4-5-distoseptate, ellipsoidal or oblong, 14-40 x 6-11 µm, smooth-walled to finely roughened.



Curvularia australiensis showing sympodial development of pale brown, fusiform to ellipsoidal, pseudoseptate, conidia on a geniculate or zig-zag rachis.

Curvularia hawaiiensis (Bugnic.) Manamgoda, L. Cai & K.D. Hyde

Synonymy: *Bipolaris hawaiiensis* Bugnic.

RG-1 organism.

Morphological Description: Colonies powdery to hairy, black. Conidiophores erect, unbranched, septate, apically flexuose with flat conidial scars on the edges, up to 80 µm long. Conidia smooth- and rather thick-walled, brown, with (3-) 5 (-7) distosepta, cylindrical to cigar-shaped, 18-35 × 6-9 µm.

Antifungal Susceptibility: *C. hawaiiensis* (da Cunha *et al*. 2012a); **MIC µg/mL.**					
Antifungal	**Range**	**MIC_{90}**	**Antifungal**	**Range**	**MIC_{90}**
AmB	**0.125-0.25**	**0.25**	**VORI**	**0.25-2**	**1**
ITRA	**≤0.03-0.5**	**0.5**	**POSA**	**≤0.03-0.5**	**0.25**

Curvularia lunata Boedijn

RG-1 organism.

Morphological Description: Colonies black, downy. Conidiophores erect, unbranched, septate, flexuose in the apical part, with flat, dark brown scars. Conidia smooth-walled, olivaceous brown, end cells somewhat paler; conidia obovoidal to broadly clavate, curved at the subterminal cell, 21-31 × 8.5-12.0 µm, 3-septate, the subterminal cell swollen and distinctly larger than the remaining cells.

Curvularia lunata conidiophores *and* conidia.

Curvularia lunata Boedijn

Antifungal Susceptibility: ***Curvularia lunata*** (Australian National data); **MIC µg/mL**.													
	No	**≤0.008**	**0.016**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**≥16**
AmB	**7**				**1**	**3**	**1**	**1**	**1**				
VORI	**7**				**1**	**2**	**3**	**1**					
POSA	**5**		**1**	**3**	**1**								
ITRA	**7**			**1**	**1**	**3**	**1**		**1**				

Curvularia spicifera (Bainier) Boedijn

Synonymy: *Bipolaris spicifera* Bainier.

RG-1 organism.

Morphological Description: Colonies appearing glassy with sooty powder of conidia, or hairy if sporulation is poor. Conidiophores erect, unbranched, septate, up to 250 µm long and 4-8 µm wide, regularly zig-zagged in the apical part, with flat, dark brown scars on the edges. Conidia brown, cylindrical with rounded ends, medium brown except for narrow subhyaline spots at the extremites, 20-40 × 9-14 µm, with 3 distosepta.

Antifungal Susceptibility: ***C. spicifera*** (da Cunha *et al*. 2012a); **MIC µg/mL.**					
Antifungal	**Range**	**MIC_{90}**	**Antifungal**	**Range**	**MIC_{90}**
AmB	**≤0.015-2**	**1**	**VORI**	**0.25-4**	**2**
ITRA	**≤0.03-4**	**1**	**POSA**	**≤0.03-1**	**0.5**

Cyberlindnera fabianii (Wick.) Minter

Synonymy: *Candida fabianii* (Hartmann) S.A. Meyer & Yarrow.

Cyberlindnera fabianii is a rare cause of candidaemia.

RG-1 organism.

Culture: Colonies (SDA) white to cream-coloured smooth, glabrous, yeast-like.

Microscopy: Spheroidal to ellipsoidal budding blastoconidia, 3.0-6.5 x 2-5.5 µm in size. No pseudohyphae are produced. Asci when present, are spherical, containing one to four spherical, finely roughened ascospores.

India Ink Preparation: Negative - no capsules present.

Dalmau Plate Culture: Spherical to ovoid budding yeast cells and occasional pseudohyphae produced.

Molecular Identification: ITS sequencing recommended.

MALDI-TOF MS: Able to accurately identify this species.

Physiological Tests: + Positive, - Negative, v Variable, w Weak, s Slow, nd No Data							
Germ Tube	-	L-Sorbose	-	L-Arabinose	-	D-Glucitol	+
Fermentation		Sucrose	+	D-Arabinose	-	α-M-D-glucoside	+
Glucose	+	Maltose	+	D-Ribose	-	D-Gluconate	+
Galactose	-	Cellobiose	+	L-Rhamnose	-	DL-Lactate	+
Sucrose	+	Trehalose	+	D-Glucosamine	-	*myo*-Inositol	-
Maltose	+,s	Lactose	-	*N*-A-D-glucosamine	-	2-K-D-gluconate	-
Lactose	-	Melibiose	-	Glycerol	+	D-Glucuronate	nd
Trehalose	-	Raffinose	+	Erythritol	-	Nitrate	+
Assimilation		Melezitose	+	Ribitol	-	Urease	-
Glucose	+	Soluble Starch	+	Galactitol	-	0.1% Cycloheximide	nd
Galactose	-	D-Xylose	+	D-Mannitol	+	Growth at 37°C	+

Key Features: Germ tube negative yeast and sugar assimilation pattern. Molecular identification may be required.

Antifungal Susceptibility: ***C. fabianii*** very limited data (Pfaller *et al*. 2015, Australian National data); **MIC µg/mL**.

	No.	**≤0.008**	**0.016**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**≥64**
AmB	**1**					**1**									
FLU	**1**											**1**			
VORI	**5**		**1**	**3**		**1**									
POSA	**5**						**3**	**2**							
ITRA	**1**							**1**							
MICA	**4**		**2**	**1**	**1**										
CAS	**1**							**1**							
5FC	**1**			**1**											

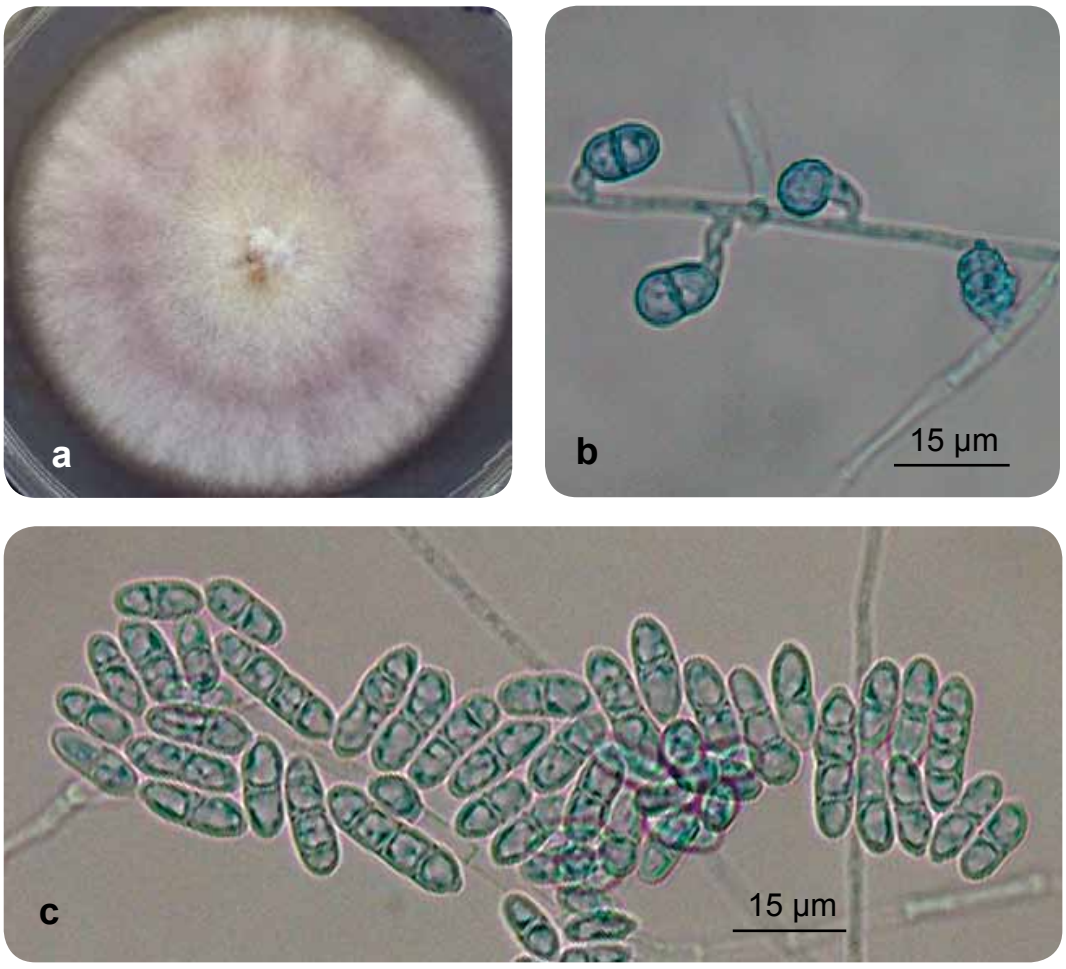
Cylindrocarpon Wollenw.

The genus contains 35 species, mostly from soil and as an occasional human and animal pathogen. *Cylindrocarpon* differs from *Fusarium* by lacking an asymmetrical foot-cell on the macroconidia.

RG-2 organism (if isolated from humans).

Morphological Description: Colonies are fast growing, hyaline or bright-coloured, suede-like or woolly. Sporodochia may occasionally be present. Conidiophores consist of simple or repeatedly verticillate phialides, arranged in brush-like structures. Phialides are cylindrical to subulate, with small collarettes producing hyaline, smooth-walled conidia, arranged in slimy masses. Two types of conidia may be produced: (1) macroconidia which are one to several septate, hyaline, straight or curved, cylindrical to fusiform, with a rounded apex and flat base; and (2) microconidia which are one-celled, and usually clearly distinct from the macroconidia. Chlamydospores may be present or absent, hyaline to brown, spherical, formed singly, in chains or in clumps, intercalary or terminal.

References: Booth (1966), Domsch *et al.* (1980), de Hoog *et al.* (2000, 2015).



Cylindrocarpon spp. (a) culture, (b) chlamydospores and (c) macroconidia.

Debaryomyces hansenii (Zopf) Lodder & Kreger-van Rij.

Synonymy: *Candida famata* (Harrison) S.A. Meyer & Yarrow.

Debaryomyces hansenii is a common environmental isolate. It may be isolated from human skin, and is only rarely recovered from blood stream infections. **RG-1 organism.**

Culture: Colonies (SDA) white to cream-coloured smooth, glabrous, yeast-like.

Microscopy: Ovoid to broadly ellipsoidal budding blastoconidia, 3.5-5 x 2-3.5 µm in size. No pseudohyphae produced. Asci when present are spherical, persistent, containing one to two spherical ascospores with rough walls.

India Ink Preparation: Negative - no capsules present.

Dalmau Plate Culture: Spherical to ovoid budding yeast cells only. Pseudohyphae are usually lacking.

Molecular Identification: ITS sequencing recommended.

MALDI-TOF MS: Able to accurately identify this species.

Physiological Tests: + Positive, - Negative, v Variable, w Weak, s Slow							
Germ Tube	-	L-Sorbose	v	L-Arabinose	+,w	D-Glucitol	+,w
Fermentation		Sucrose	+	D-Arabinose	v	α-M-D-glucoside	+
Glucose	-,w	Maltose	+	D-Ribose	v	D-Gluconate	+,w
Galactose	-,w	Cellobiose	+	L-Rhamnose	v	DL-Lactate	v
Sucrose	-,w	Trehalose	+	D-Glucosamine	v	*myo*-Inositol	-
Maltose	-,w	Lactose	v	*N*-A-D-glucosamine	v	2-K-D-gluconate	+
Lactose	-	Melibiose	v	Glycerol	+	D-Glucuronate	v
Trehalose	-,w	Raffinose	+	Erythritol	v	Nitrate	-
Assimilation		Melezitose	v	Ribitol	+	Urease	-
Glucose	+	Soluble Starch	v	Galactitol	v	0.1% Cycloheximide	v
Galactose	+	D-Xylose	+	D-Mannitol	+	Growth at 40°C	-

Key Features: Germ tube negative yeast and sugar assimilation pattern.

Antifungal Susceptibility: ***D. hansenii*** (Diekema *et al*. 2009, Beyda *et al.* 2013, Espinel-Ingroff *et al*. 2014, Australian National data); **MIC µg/mL**.

	No.	**≤0.008**	**0.016**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**≥64**
AmB	**27**				**1**	**3**	**7**	**13**	**2**		**1**				
FLU	**76**					**9**	**18**	**13**	**9**	**8**	**11**	**2**	**3**		**3**
VORI	**79**	**8**	**11**	**27**	**16**	**9**	**4**		**2**	**1**	**1**				
POSA	**72**		**8**	**11**	**20**	**12**	**8**	**8**	**2**	**3**					
ITRA	**11**			**2**		**1**	**3**	**2**	**1**				**2**		
ANID	**23**			**1**	**2**	**1**	**1**	**0**	**8**	**10**					
MICA	**23**		**1**	**3**	**1**		**2**	**8**	**5**	**3**					
CAS	**26**			**1**	**2**	**3**	**8**	**5**	**4**	**1**			**2**		
5FC	**11**			**1**	**5**	**1**	**1**	**1**							**2**

Drechslera Ito

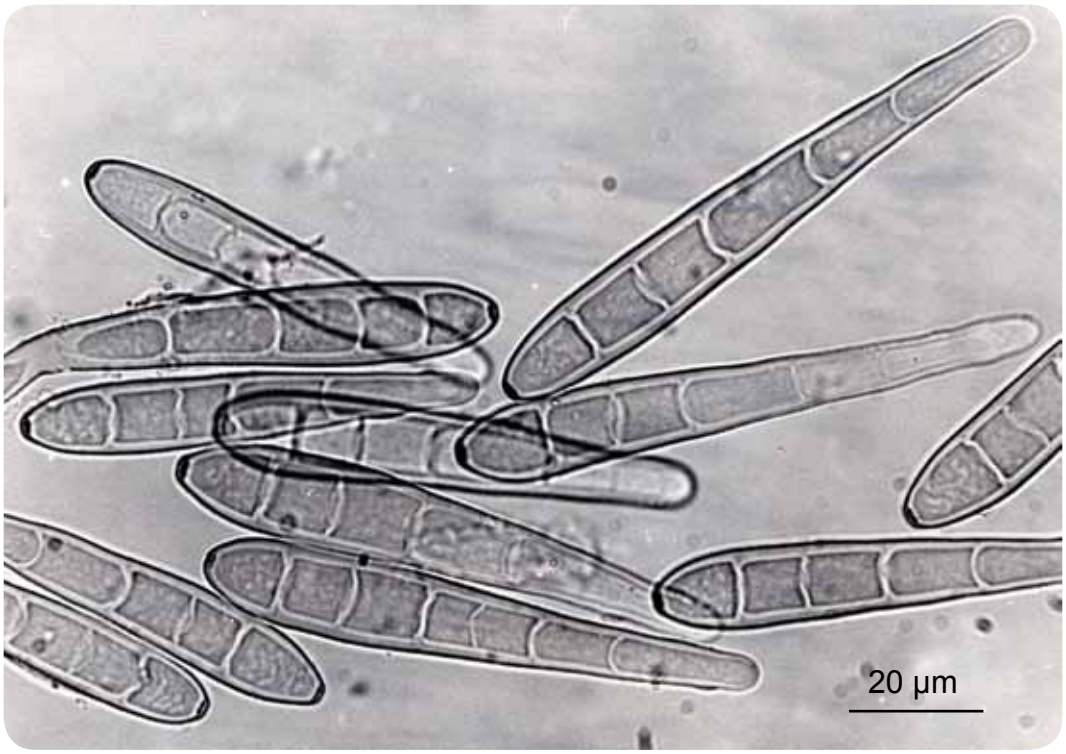
McGinnis *et al.* (1986b) have reviewed the isolates from human and animal disease purported to be *Drechslera* or *Helminthosporium* and concluded that all pathogenic isolates examined actually belong to the genera *Bipolaris* or *Exserohilum*.

RG-1 organism.

Morphological Description: Colonies are fast growing, suede-like to downy, brown to blackish brown with a black reverse. Conidia are pale to dark brown, usually cylindrical or subcylindrical, straight, smooth-walled, and are formed apically through a pore (poroconidia) in a sympodially elongating, geniculate conidiophore. Conidia are transversely septate (phragmoconidia), with the septum delimiting the basal cell formed first during conidium maturation. Germinating is from any or all cells and the hilum is not protuberant.

Key Features: Dematiaceous hyphomycete producing sympodial, pale brown, cylindrical or subcylindrical, transversely septate poroconidia.

References: Luttrell (1978), Ellis (1971, 1976), McGinnis (1980), McGinnis *et al.* (1986b), Sivanesan (1987), Rippon (1988), de Hoog *et al.* (2000, 2015). Also see Descriptions for *Bipolaris, Curvularia* and *Exserohilum*.



Drechslera spp. conidia.

Epicoccum nigrum Link

Synonymy: *Epicoccum purpurascens* Ehrenb. ex Schlecht.

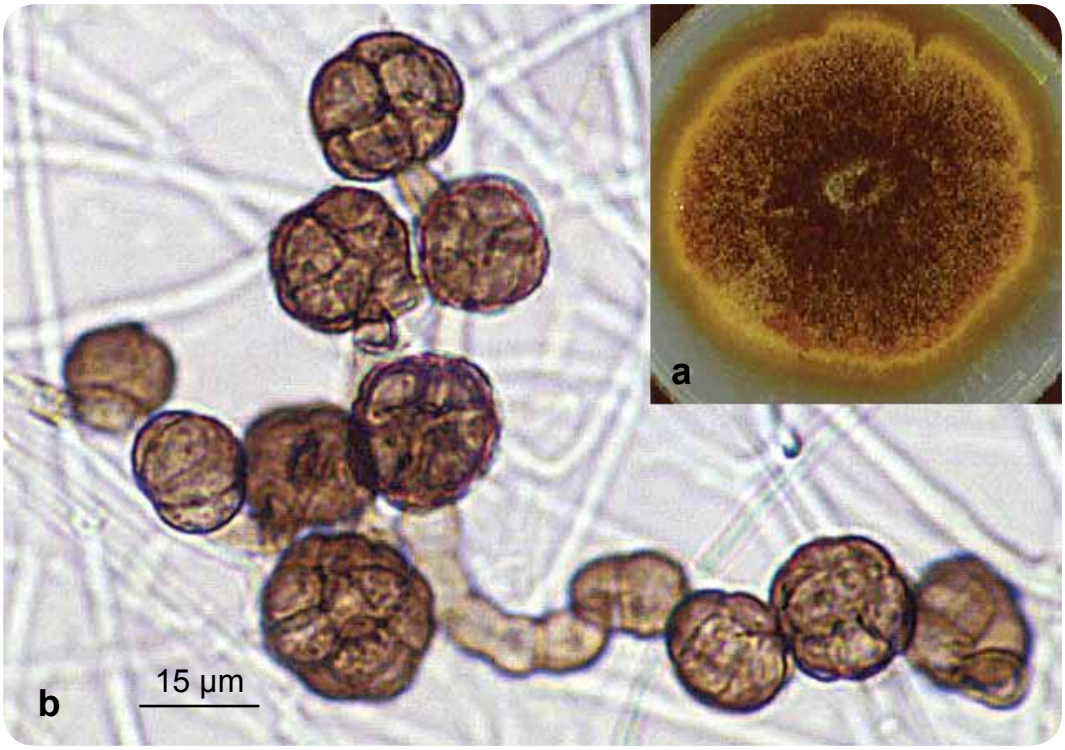
Epicoccum nigrum is a cosmopolitan saprophyte of worldwide distribution which is occasionally isolated as a contaminant from clinical specimens like skin.

RG-1 organism.

Morphological Description: Colonies are fast growing, suede-like to downy, with a strong yellow to orange-brown diffusible pigment. When sporulating, numerous black sporodochia (aggregates of conidiophores) are visible. Conidia are formed singly on densely compacted, non-specialised, determinant, slightly pigmented conidiophores. Conidia are globose to pyriform, mostly 15-25 µm diameter with a funnel-shaped base and broad attachment scar, often seceding with a protuberant basal cell; i.e. aleuric or rhexolytic dehiscence of conidia. Conidia become multicellular (dictyoconidia), darkly pigmented and have a verrucose external surface.

Key Features: Dematiaceous hyphomycete producing darkly pigmented, large globose to pyriform, verrucose dictyoconidia on a sporodochium.

References: Ellis (1971), Domsch *et al.* (1980), McGinnis (1980), Samson *et al*. (1995).



Epicoccum nigrum (a) culture and (b) conidia.

Epidermophyton floccosum (Harz) Langeron et Milochevitch

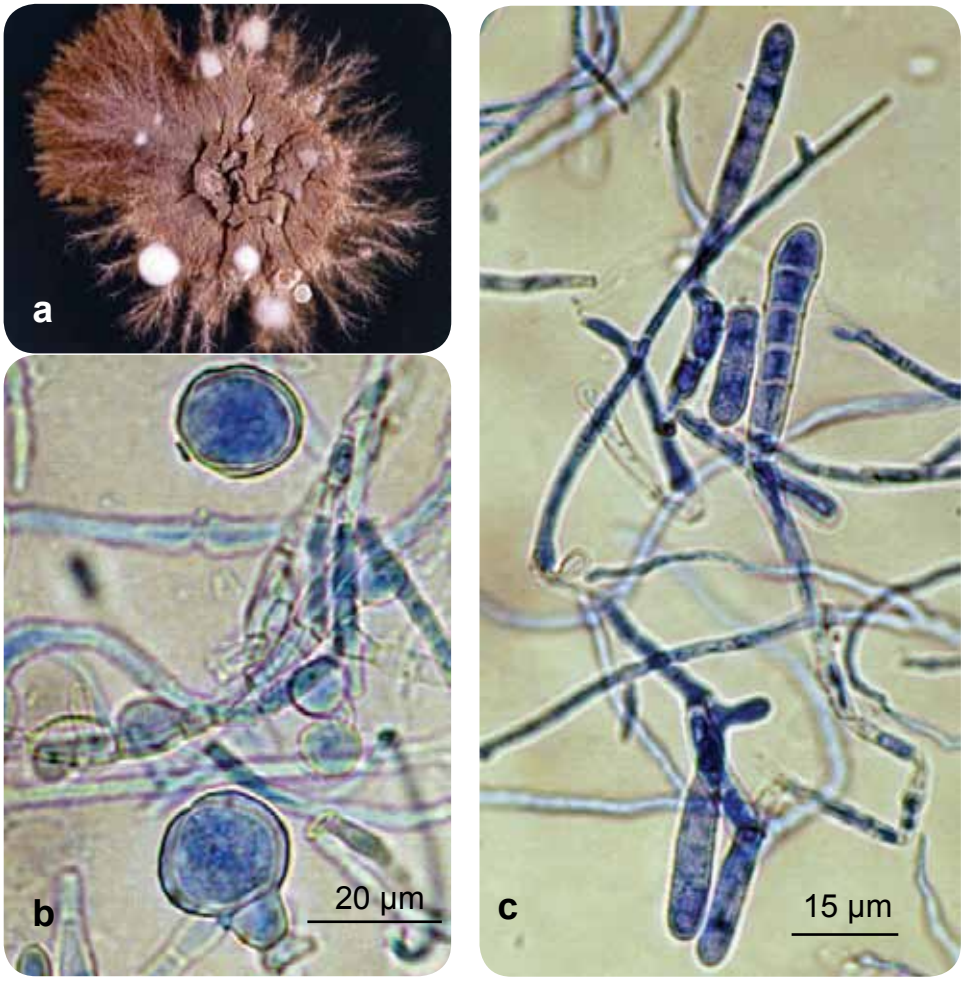
Epidermophyton floccosum is an anthropophilic dermatophyte with a worldwide distribution which often causes tinea pedis, tinea cruris, tinea corporis and onychomycosis. It is not known to invade hair *in vivo* and no specific growth requirements have been reported.

RG-2 organism.

Morphological Description: Colonies are usually slow growing, greenish-brown or khaki-coloured with a suede-like surface, raised and folded in the centre, with a flat periphery and submerged fringe of growth. Older cultures may develop white pleomorphic tufts of mycelium. A deep yellowish-brown reverse pigment is usually present. Microscopic morphology shows characteristic smooth, thin-walled macroconidia which are often produced in clusters growing directly from the hyphae. Numerous chlamydospores are formed in older cultures. Microconidia are not formed.

Key Features: Culture characteristics, microscopic morphology and clinical disease.

References: Rebell and Taplin (1970), Mackenzie *et al.* (1987), Rippon (1988), de Hoog *et al.* (2000, 2015).



Epidermophyton floccosum (a) culture, (b) chlamydospores and (c) macroconidia.

Exophiala dermatitidis (Kano) de Hoog

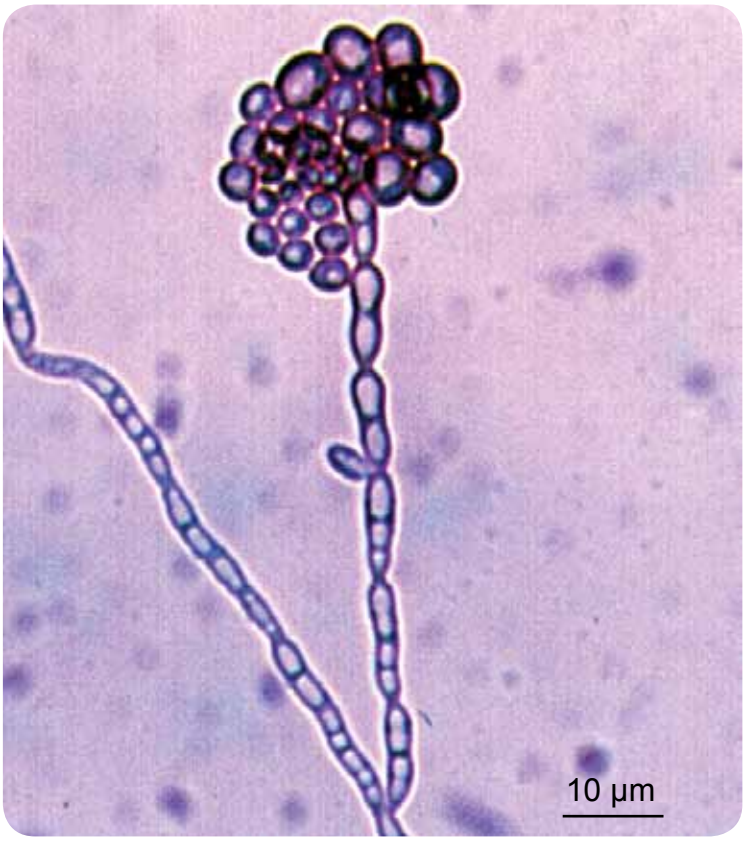
Exophiala dermatitidis has been isolated from plant debris and soil and is a recognised causative agent of mycetoma and phaeohyphomycosis in humans (Zeng *et al.* 2007).

RG-2 organism.

Morphological Description: Colonies are slow growing, initially yeast-like and black, becoming suede-like, olivaceous-grey with the development of aerial mycelium with age. A brown pigment is often produced in the agar. The initial yeast-like phase is characterised by unicellular, ovoid to elliptical, budding yeast-like cells. The yeast-like cells are hyaline and thin-walled when young becoming darkly pigmented (dematiaceous) and thick-walled when mature. With the development of mycelium, flask-shaped to cylindrical annellides are produced. Conidia are hyaline to pale brown, one-celled, round to obovoid, 2-4 x 2.5-6 µm, smooth-walled and accumulate in slimy balls at the apices of the annellides or down their sides. Cultures grow at 42^{O}C and on media containing 0.1% cycloheximide.

Molecular Identification: ITS and/or D1/D2 sequencing is recommended for species identification (Halliday *et al.* 2015).

References: de Hoog and Hermanides-Nijhof (1977), McGinnis (1980), Hohl *et al.* (1983), Nishimura and Miyaji (1983), Matsumoto *et al.* (1984), Dixon and Polak-Wyss (1991), de Hoog *et al*. (2000, 2015).



Exophiala dermatitidis annellides and conidia.

Exophiala dermatitidis (Kano) de Hoog

Antifungal Susceptibility: *E. dermatitidis* (Duarte *et al.* 2013 and Australian National data); **MIC µg/mL.**															
	No.	**≤0.008**	**0.016**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**≥64**
AmB	**44**					**1**	**2**		**25**	**16**					
VORI	**44**	**4**		**12**	**20**	**1**	**4**	**2**	**1**						
POSA	**1**				**1**										
ITRA	**44**	**10**		**13**	**6**	**9**	**4**	**1**	**1**						

E. dermatitidis data from 27 isolates (Fothergill *et al.* 2009); **MIC µg/mL.**			
AmB	**Range 0.125-1; MIC_{90}= 1**	**VORI**	**Range 0.06-1; MIC_{90}= 0.25**
ITRA	**Range <0.015-0.5; MIC_{90}= 0.5**	**POSA**	**Range <0.015-0.25; MIC_{90}= 0.06**

*Exophiala jeanselmei c*omplex

Exophiala jeanselmei has a worldwide distribution and is a recognised causative agent of mycetoma and phaeohyphomycosis in humans. Zeng *et al*. (2007) presented an overview of the medically important *Exophiala* species.

E. jeanselmei has long been recognised as heterogeneous (de Hoog 1977). Recent molecular studies have redefined *Exophiala jeanselmei* and three additional species have been identified: *E. oligosperma, E. nishimurae* and *E. xenobiotica* (Vitale and de Hoog, 2002, de Hoog *et al*. 2003, 2006). These species are morphologically very similar and can best be distinguished by genetic analysis Zeng *et al*. (2014).

Molecular Identification: ITS and/or D1/D2 sequencing is recommended for species identification (Halliday *et al*. 2015).

Morphological Description: Conidiogenous cells are predominantly annellidic and erect, multicellular conidiophores are absent. No growth at 40^{O}C.

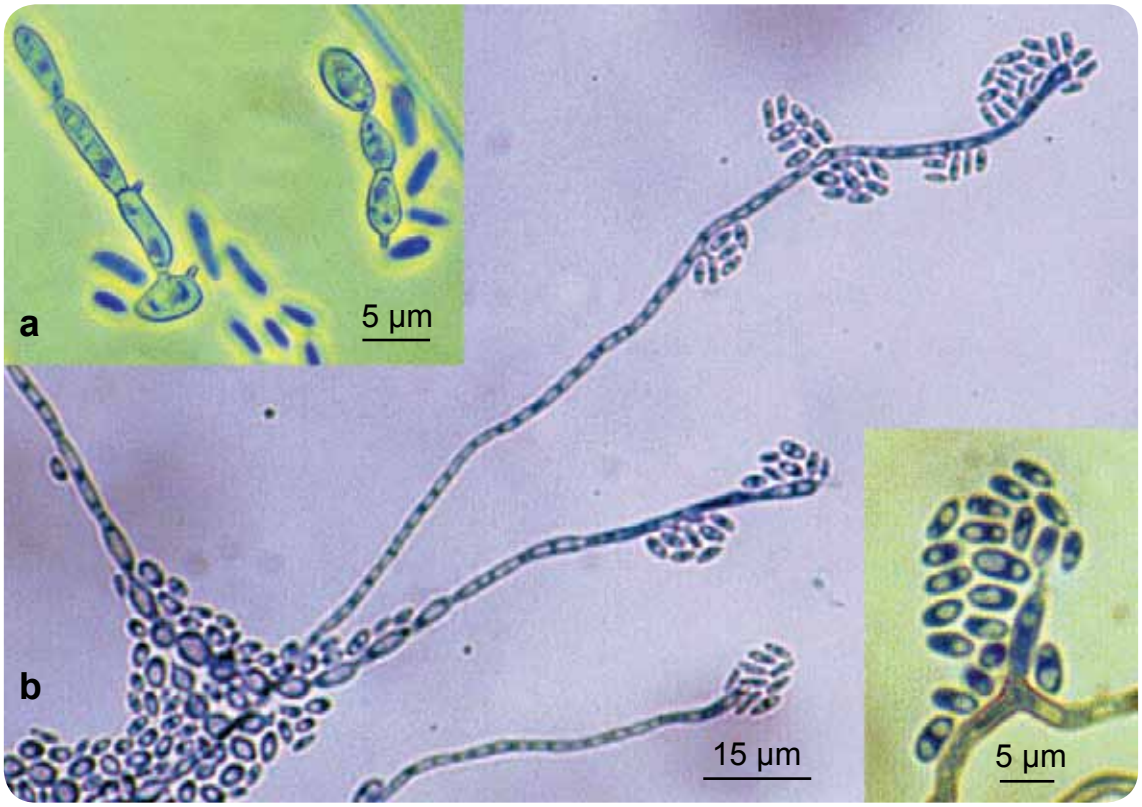
E. jeanselmei	Mature conidiogenous cells rocket-shaped, slightly darker than the supporting hyphae, with regular tapering annellated zones.
E. oligosperma	Mature conidiogenous cells remain concolorous with supporting hyphae and may be intercalary and lateral, the latter being flask or rocket-shaped. Annellated zones have the appearance of inconspicuous flat scars. Chlamydospores are absent.
E. nishimurae	Similar morphology to *E. oligosperma*, however large chlamydospore-like cells are present.
E. xenobiotica	A segregant genotype of the *E. jeanselmei* with less melanised conidiogenous cells.

Exophiala jeanselmei complex

Exophiala jeanselmei McGinnis & Padhye

RG-2 organism.

Morphological Description: Colonies are initially smooth, greenish-grey to black, mucoid and yeast-like, becoming raised and developing tufts of aerial mycelium with age, often becoming dome-shaped and suede-like in texture. Reverse is olivaceous-black. Numerous ellipsoidal, yeast-like, budding cells are usually present, especially in young cultures. Scattered amongst these yeast-like cells are larger, inflated, subglobose to broadly ellipsoidal cells (germinating cells) which give rise to short torulose hyphae that gradually change into unswollen hyphae. Conidia are formed on lateral pegs either arising apically or laterally at right or acute angles from essentially undifferentiated hyphae or from strongly inflated detached conidia. Conidiogenous pegs are 1-3 µm long, slightly tapering and imperceptibly annellate. Conidia are hyaline, smooth, thin-walled, broadly ellipsoidal, 3.2-4.4 x 1.2-2.2 µm, and with inconspicuous basal scars. Cultures grow at 37^{O}C but not at 40^{O}C.



Exophiala jeanselmei complex showing annellides, conidia and conidiogenous pegs (annellides) on (a) yeast-like cells and (b) torulose hyphae.

References: de Hoog and Hermanides-Nijhof (1977), de Hoog (1977, 1985), McGinnis and Padhye (1977), McGinnis (1978b, 1980), Domsch *et al.* (1980), Nishimura and Miyaji (1983), Matsumoto *et al.* (1987), Dixon and Polak-Wyss (1991), Badali *et al*. 2010, de Hoog *et al*. (2000, 2003, 2006, 2015).

Exophiala jeanselmei complex

Antifungal Susceptibility: *E. jeanselmei* limited data (Australian National data); **MIC µg/mL.**															
	No.	**≤0.008**	**0.016**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**≥64**
AmB	**9**				**1**	**3**	**2**	**2**		**1**					
VORI	**9**				**2**	**2**	**1**	**1**	**1**	**2**					
POSA	**6**	**1**		**2**	**2**		**1**								
ITRA	**9**		**2**		**3**	**2**		**2**							

E. jeanselmei data from eight isolates (Fothergill *et al.* 2009); **MIC µg/mL.**			
AmB	**Range 0.125-1; MIC_{90}= 1**	**VORI**	**Range 0.06-0.5; MIC_{90}= 0.5**
ITRA	**Range <0.015-0.125; MIC_{90}= 0.125**	**POSA**	**Range <0.015-0.03; MIC_{90}= 0.03**

Antifungal Susceptibility: *E. oligosperma* limited data (Australian National data); **MIC µg/mL.**															
	No.	**≤0.008**	**0.016**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**≥64**
AmB	**2**								**2**						
VORI	**2**						**1**	**1**							
POSA	**2**			**1**	**1**										
ITRA	**2**				**1**	**1**									

E. oligosperma data from 40 isolates (Fothergill *et al.* 2009); **MIC µg/mL.**			
AmB	**Range 0.125-1; MIC_{90}= 0.5**	**VORI**	**Range <0.015-4; MIC_{90}= 2**
ITRA	**Range <0.015-0.25; MIC_{90}= 0.25**	**POSA**	**Range <0.015-0.06; MIC_{90}= 0.03**

Antifungal Susceptibility: *E. xenobiotica* limited data (Australian National data); **MIC µg/mL.**															
	No.	**≤0.008**	**0.016**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**≥64**
AmB	**3**					**1**	**1**		**1**						
VORI	**3**						**3**								
POSA	**3**		**1**		**2**										
ITRA	**2**				**2**	**1**									

E. xenobiotica data from 39 isolates (Fothergill *et al.* 2009); **MIC µg/mL.**			
AmB	**Range 0.125-1; MIC_{90}= 0.5**	**VORI**	**Range <0.015-2; MIC_{90}= 1**
ITRA	**Range <0.015-1; MIC_{90}= 0.125**	**POSA**	**Range <0.015-0.06; MIC_{90}= 0.03**

Exophiala spinifera complex

Synonymy: *Phialophora spinifera* Nielsen & Conant
Rhinocladiella spinifera (Nielsen & Conant) de Hoog

E. spinifera has a worldwide distribution and is a recognised causative agent of mycetoma and phaeohyphomycosis in humans. Zeng *et al.* (2007) presented an overview of the medically important *Exophiala* species.

Recent molecular studies have re-examined *Exophiala spinifera* and have recognised two species: *E. spinifera* and *E. attenuata* (Vitale and de Hoog, 2002). These two species are morphologically very similar and can best be distinguished by genetic analysis.

Molecular Identification: ITS sequencing is recommended for accurate species identification (Zeng and de Hoog, 2008).

Morphological Description: Conidiogenous cells are predominately annellidic and erect, multicellular conidiophores that are darker than the supporting hyphae are present. No growth at 40^{O}C.

E. spinifera	Annellated zones are long with clearly visible, frilled annellations.
E. attenuata	Annellated zones are inconspicuous and degenerate.

Exophiala spinifera (Nielsen & Conant) McGinnis

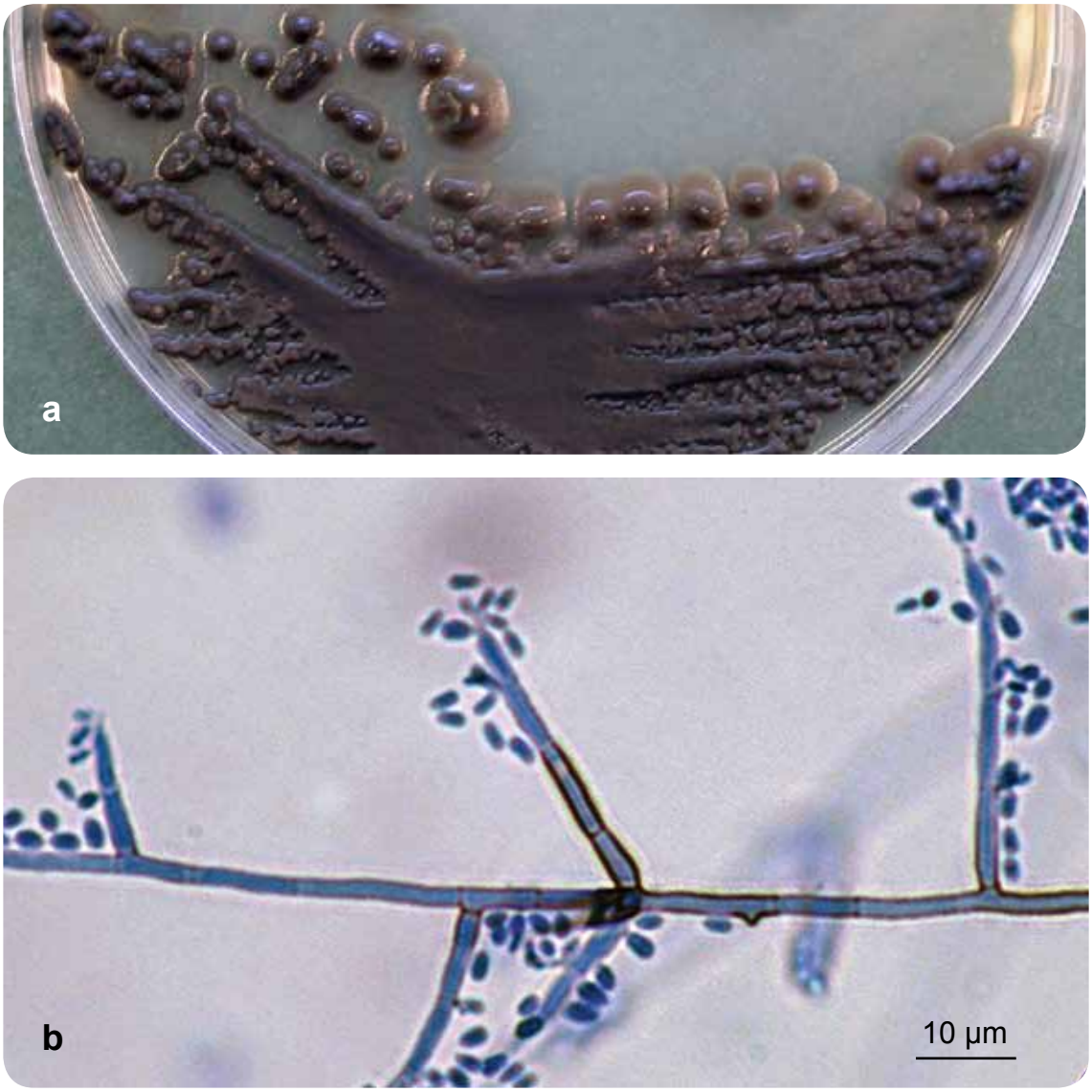
RG-2 organism.

Morphological Description: Colonies are initially mucoid and yeast-like, black, becoming raised and developing tufts of aerial mycelium with age, finally becoming suede-like to downy in texture. Reverse is olivaceous-black. Conidiophores are simple or branched, erect or sub-erect, spine-like with rather thick brown pigmented walls. Conidia are formed in basipetal succession on lateral pegs either arising apically or laterally at right or acute angles from the spine-like conidiophores or from undifferentiated hyphae. Conidiogenous pegs are 1-3 µm long, slightly tapering and imperceptibly annellate. Conidia are one-celled, subhyaline, smooth, thin-walled, sub-globose to ellipsoidal, 1.0-2.9 x 1.8-2.5 µm, and aggregate in clusters at the tip of each annellide. Toruloid hyphae and yeast-like cells with secondary conidia are typically present.

Note: Yeast cells show the presence of capsules in India Ink stained mounts and cultures will grow on media containing 0.1% cycloheximide. No growth at 40^{O}C.

References: de Hoog and Hermanides-Nijhof (1977), McGinnis and Padhye (1977), Domsch *et al.* (1980), McGinnis (1980), Nishimura and Miyaji (1983), de Hoog (1985), Matsumoto *et al.* (1987), Dixon and Polak-Wyss (1991), de Hoog *et al*. (2000, 2003, 2006, 2015).

Exophiala spinifera complex



Exophiala spinifera (a) culture showing typical black, mucoid yeast-like growth, and (b) conidia being formed on erect, multiseptate conidiophores that are darker than the supporting hyphae, with long annellated zones.

Antifungal Susceptibility: *E. spinifera* limited data (Australian National data); **MIC µg/mL.**															
	No.	**≤0.008**	**0.016**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**≥64**
AmB	**5**						**1**	**2**	**1**	**1**					
VORI	**5**					**1**	**2**	**2**							
POSA	**4**					**1**	**3**								
ITRA	**5**					**1**	**4**								

E. spinifera data from eight isolates (Fothergill *et al.* 2009); **MIC µg/mL.**			
AmB	**Range 0.25-1; MIC_{90}= 1**	**VORI**	**Range 0.06-0.5; MIC_{90}= 0.25**
ITRA	**Range <0.015-0.5; MIC_{90}= 0.125**	**POSA**	**Range <0.015-0.03; MIC_{90}= 0.03**

Exserohilum Leonard and Suggs

The genus *Exserohilum* contains about 35 species and may be differentiated from the closely related genera *Bipolaris* and *Dreschlera* by forming conidia with a strongly protruding truncate hilum (i.e. exserted hilum). In *Drechslera* species, the hilum does not protrude; in *Bipolaris* species the hilum protrudes only slightly. Several species of *Exserohilum* have been reported as agents of phaeohyphomycosis, notably *E. rostratum, E. mcginnisii* and *E. longirostratum*. Although recent molecular studies have demonstrated that the latter two species are probable synonyms of *E. rostratum* (de Cunha *et al*. 2012b, Katragkou *et al.* 2014). *E. rostratum* was recently implicated in an outbreak of fungal meningitis associated with contaminated methylprednisolone in the United States.

Exserohilum rostratum (Drechsler) Leonard & Suggs

RG-1 organism.

Morphological Description: Colonies are grey to blackish-brown, suede-like to floccose in texture and have an olivaceous-black reverse. Conidia are straight, curved or slightly bent, ellipsoidal to fusiform and are formed apically through a pore (poroconidia) on a sympodially elongating geniculate conidiophore. Conidia have a strongly protruding, truncate hilum and the septum above the hilum is usually thickened and dark, with the end cells often paler than other cells, walls often finely roughed. Conidial germination is bipolar.

Key Features: Dematiaceous hyphomycete producing sympodial, transverse septate, ellipsoidal to fusiform conidia with dark bands on both ends and a strongly protruding, truncate hilum.

Molecular Identification: ITS and D1/D2 sequencing is recommended.

References: Domsch *et al.* (1980), Alcorn (1983), Adam *et al*. (1986), McGinnis *et al.* (1986b), Rippon (1988), Burges *et al.* (1987), Dixon and Polak-Wyss (1991), de Hoog *et al.* (2000, 2015), de Cunha *et al*. (2012b), Katragkou *et al.* (2014).

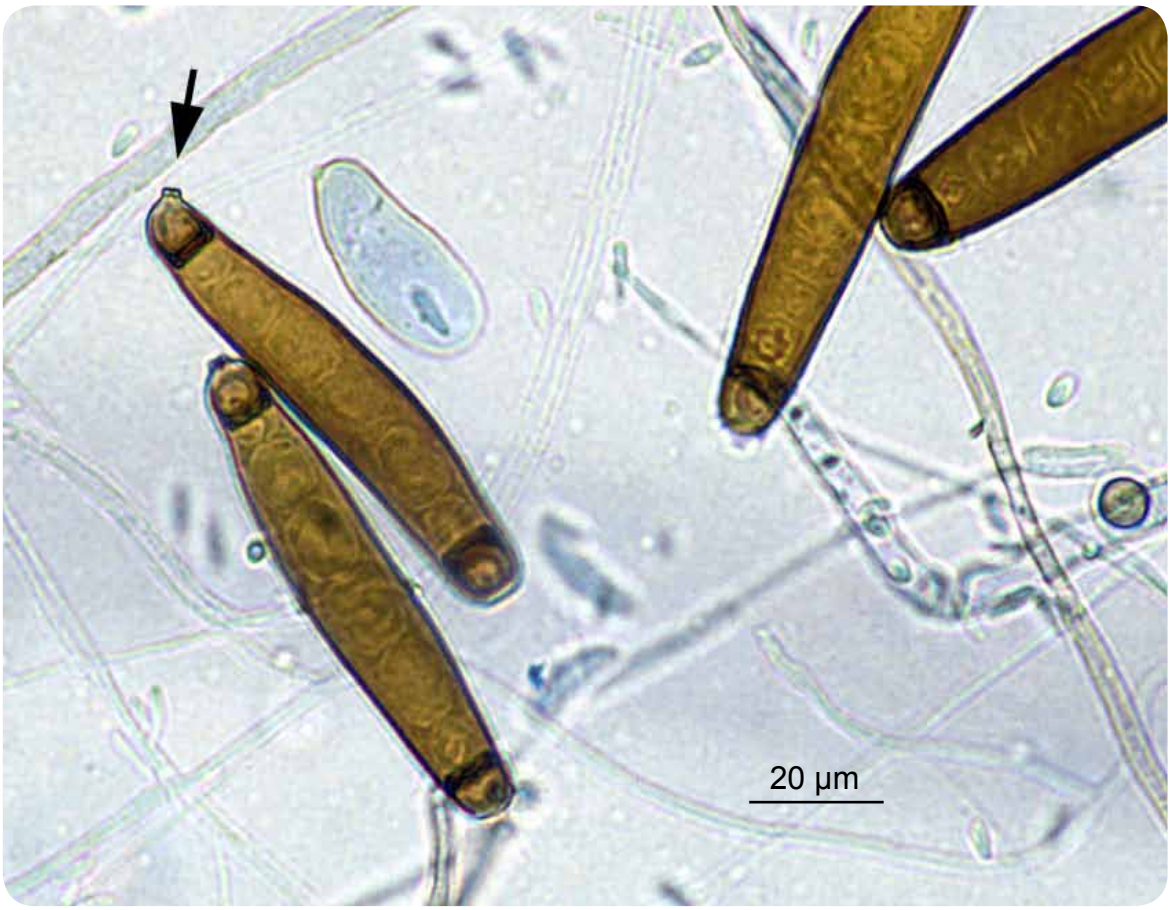
Antifungal Susceptibility: *E. rostratum* limited data (Australian National data); **MIC µg/mL.**

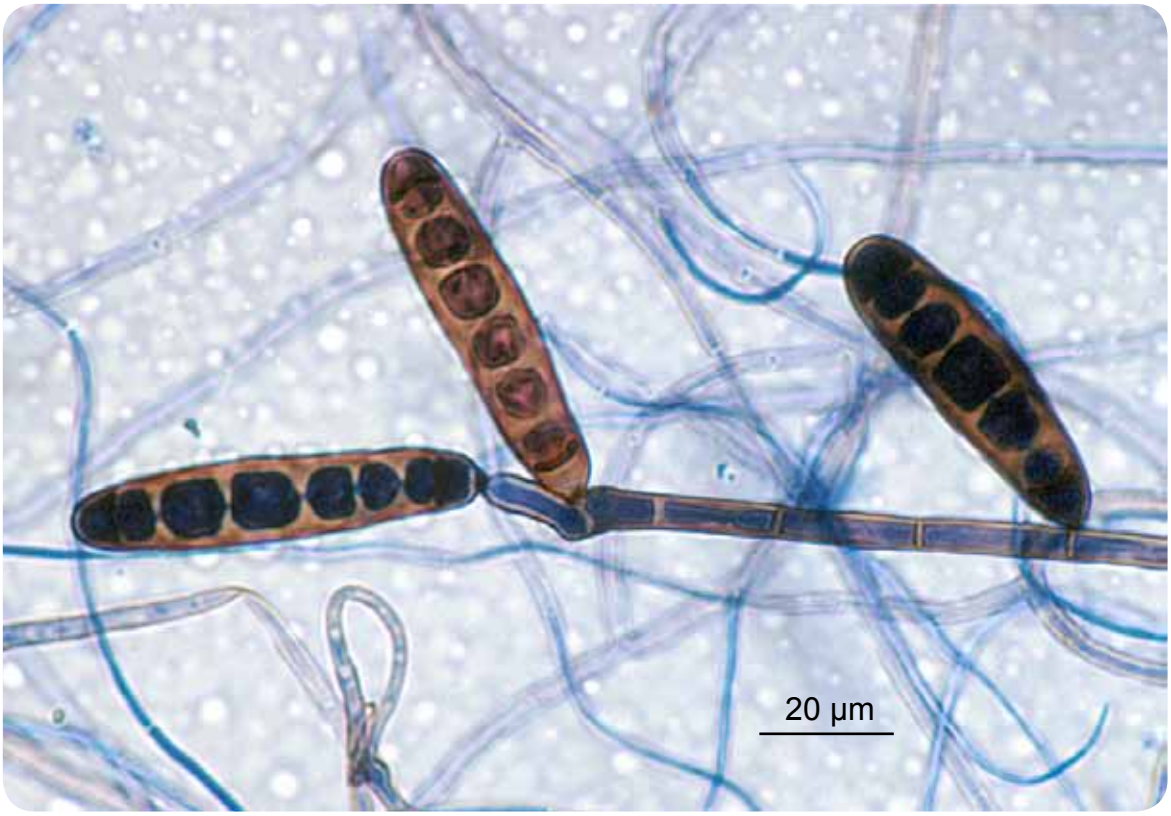
	No.	≤0.008	0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	≥64
AmB	8					4	2	2							
VORI	8				1		1	6							
POSA	6			5	1										
ITRA	8				4	4									

E. rostratum data from 34 isolates (da Cunha *et al*. 2012); **MIC µg/mL.**

AmB	Range <0.03-0.125; MIC_{90}= 0.03	VORI	Range 0.03-1; MIC_{90}= 0.25
ITRA	Range <0.03-0.125; MIC_{90}= 0.03	POSA	Range <0.03-0.5; MIC_{90}= 0.06

Exserohilum rostratum (Drechsler) Leonard & Suggs





Exserohilum rostratum conidiophores and conidia with a distinctive hilum (arrow).

Fonsecaea complex

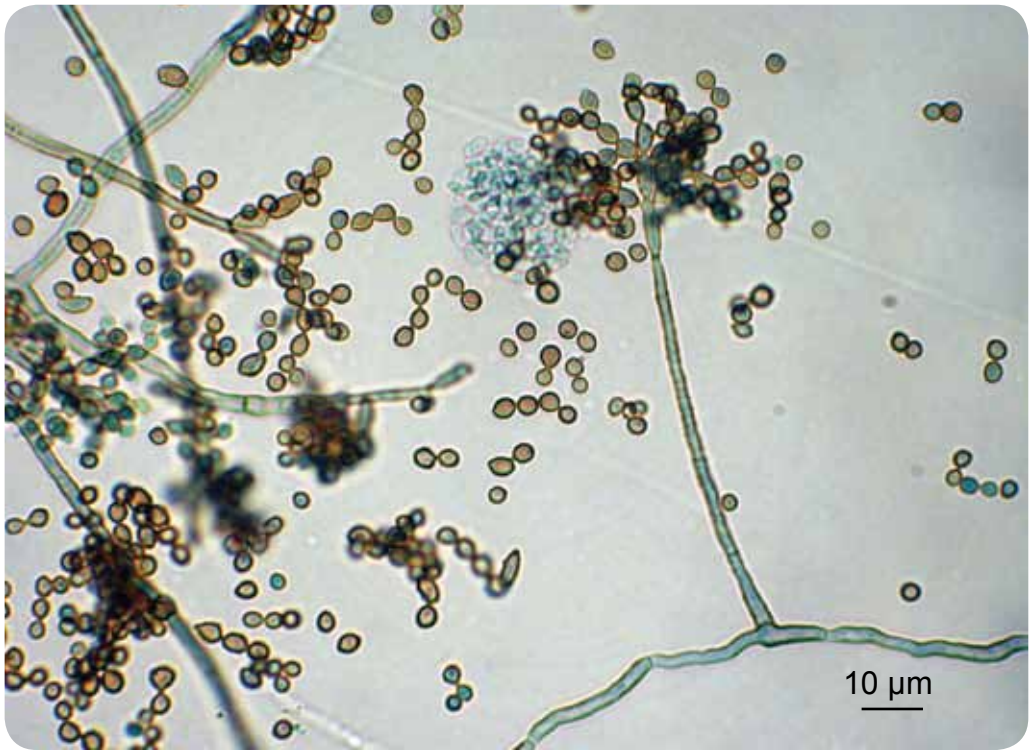
Morphologically the genus *Fonsecaea* is defined by the presence of indistinct melanised conidiophores with blunt, scattered denticles bearing conidia singly or in short chains that eventually become branched. de Hoog *et al.* (2004b) revised the genus on the basis of ITS sequencing data. Three species from humans are currently recognised, *F. monophora, F. nubica*, and *F. pedrosoi*, although they are morphologically indistinguishable (Najafzadeh *et al.* 2009, 2010b; Xi *et al*. 2009, de Hoog *et al.* 2015). All strains grow at 37^{O}C but not at 40^{O}C and the three species are recognised as aetiological agents of chromoblastomycosis.

Morphological Description: Colonies are slow growing, flat to heaped and folded, suede-like to downy, olivaceous to black with black reverse. Conidiogenous cells pale olivaceous, arranged in loosely branched systems, with prominent denticles. Conidia pale olivaceous, clavate to ellipsoidal, in short chains, subhyaline, smooth and thin-walled, 3.5-5 x 1.5-2 µm.

RG-2 organism.

Molecular Identification: ITS sequencing is recommended for species identification (Abliz *et al.* 2003, Najafzadeh *et al.* 2009, 2010b, Xi *et al.* 2009).

References: McGinnis (1980), Dixon and Polak-Wyss (1991), de Hoog *et al.* (2000, 2004b, 2015), Abliz *et al*. 2003, Najafadeh *et al.* (2009, 2010a, 2010b).



Fonsecaea complex conidiophores and conidia.

Antifungal Susceptibility: *F. pedrosoi* (Australian National data); **MIC µg/mL.**													
	No.	**≤0.008**	**0.016**	**0.03**	**0.06**	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**≥16**
AmB	**4**					**1**				**3**			
VORI	**4**			**1**		**3**							
POSA	**4**		**1**		**2**	**1**							
ITRA	**4**			**1**		**3**							

Fusarium Link ex Fries

Most *Fusarium* species are soil fungi and have a worldwide distribution. Some are plant pathogens, causing root and stem rot, vascular wilt or fruit rot. Several species have emerged as important opportunistic pathogens in humans causing hyalohyphomycosis (especially in burn victims and bone marrow transplant patients), mycotic keratitis and onychomycosis (Guarro 2013). Other species cause storage rot and are important mycotoxin producers.

Multi-locus sequence analysis of *EF-1α*, β-tubulin, calmodulin, and *RPB2* have revealed the presence of multiple cryptic species within each "morphospecies" of medically important fusaria (Balajee *et al.* 2009). For instance, *Fusarium solani* represents a complex (i.e. *F. solani* complex) of over 45 phylogenetically distinct species of which at least 20 are associated with human infections. Similarly, members of the *Fusarium oxysporum* complex are phylogenetically diverse, as are members of the *Fusarium incarnatum-equiseti* complex and *Fusarium chlamydosporum* complex (Balajee *et al*. 2009, Tortorano *et al.* 2014, Salah *et al.* 2015).

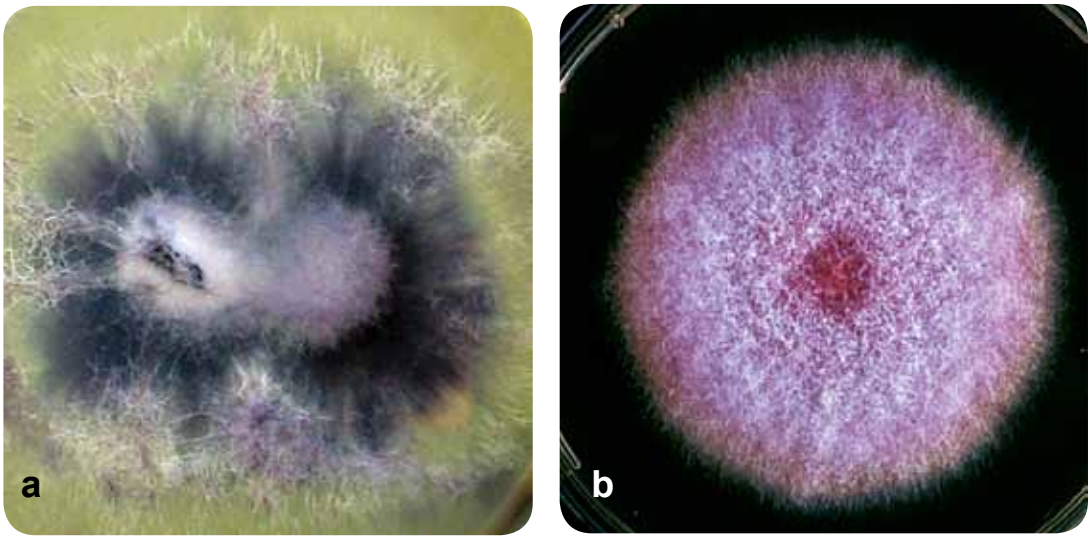
Currently the genus *Fusarium* comprises at least 300 phylogenetically distinct species, 20 species complexes and nine monotypic lineages (Balajee *et al*. 2009, O'Donnell *et al*. 2015). Most of the identified opportunistic *Fusarium* pathogens belong to the *F. solani* complex, *F. oxysporum* complex and *F. fujikuroi* complex. Less frequently encountered are members of the *F. incarnatum-equiseti*, *F. dimerum* and *F. chlamydosporum* complexes, or species such as *F. sporotrichioides* (O'Donnell *et al.* 2015, van Diepeningen *et al*. 2015).

Morphological Description: Colonies are usually fast growing, pale or bright-coloured (depending on the species) with or without a cottony aerial mycelium. The colour of the thallus varies from whitish to yellow, pink, red or purple shades. Species of *Fusarium* typically produce both macro- and microconidia from slender phialides. Macroconidia are hyaline, two to several-celled, fusiform to sickle-shaped, mostly with an elongated apical cell and pedicellate basal cell. Microconidia are one or two-celled, hyaline, smaller than macroconidia, pyriform, fusiform to ovoid, straight or curved. Chlamydospores may be present or absent.

Identification of *Fusarium* species is often difficult due to the variability between isolates (e.g. in shape and size of conidia and colony colour) and because not all features required are always well developed (e.g. the absence of macroconidia in some isolates after subculture). **Note:** Sporulation may need to be induced in some isolates and a good slide culture is essential. The important characters used in the identification of *Fusarium* species are as follows.

1. Colony growth diameters on potato dextrose agar and/or potato sucrose agar after incubation in the dark for four days at 25^{O}C.
2. Culture pigmentation on potato dextrose agar and/or potato sucrose agar after incubation for 10-14 days with daily exposure to light.
3. Microscopic morphology including shape of the macroconidia; presence or absence of microconidia; shape and mode of formation of microconidia; nature of the conidiogenous cell bearing microconidia; and presence or absence of chlamydospores.

Fusarium Link ex Fries



Cultures of (a) *Fusarium oxysporum* complex showing purple pigmentation and (b) *Fusarium fujikuroi* complex showing pink pigmentation.

Molecular Identification: Current species identification is on the basis of multilocus sequence data (Guarro 2013, O'Donnell *et al*. 2015, van Diepeningen *et al*. 2015). Internet-accessible validated databases dedicated to the identification of fusaria via nucleotide BLAST queries are available at FUSARIUM-ID at Pennsylvania State University (http://www.fusariumdb.org) and *Fusarium* MLST at the CBS-KNAW Fungal Biodiversity Centre (http://www.cbs.knaw.nl/Fusarium/).

For sequence-based identification of *Fusarium* species (O'Donnell *et al*. 2015).

1. Use *EF-1α*, *RPB1* and/or *RPB2*. Use of at least two independent loci will increase the accuracy of identification.
2. *Fusarium* MLST or FUSARIUM-ID are the recommended sequence databases, rather than GenBank.
3. Ensure sequences are carefully edited and free of ambiguities.
4. Ensure the species names associated with the top BLASTn matches are the same. If multiple species names have similar scores it may be necessary to sequence additional loci.

Note: ITS and D1/D2 sequences are too conserved to resolve species limits of most fusaria. O'Donnell *et al.* (2015) recommend avoiding ITS or D1/D2 sequences from an unknown isolate to query GenBank, because >50% of the sequences from *Fusarium* species are misidentified in this database.

Identifications based on morphology and/or ITS and D1/D2 sequences should be reported as species complexes. Sequencing of *EF-1α*, *RPB1* and/or *RPB2* is required for accurate species identification.

MALDI-TOF MS: A comprehensive 'in-house' database of reference spectra allows accurate identification of *Fusarium* species complexes (Lau *et al*. 2013).

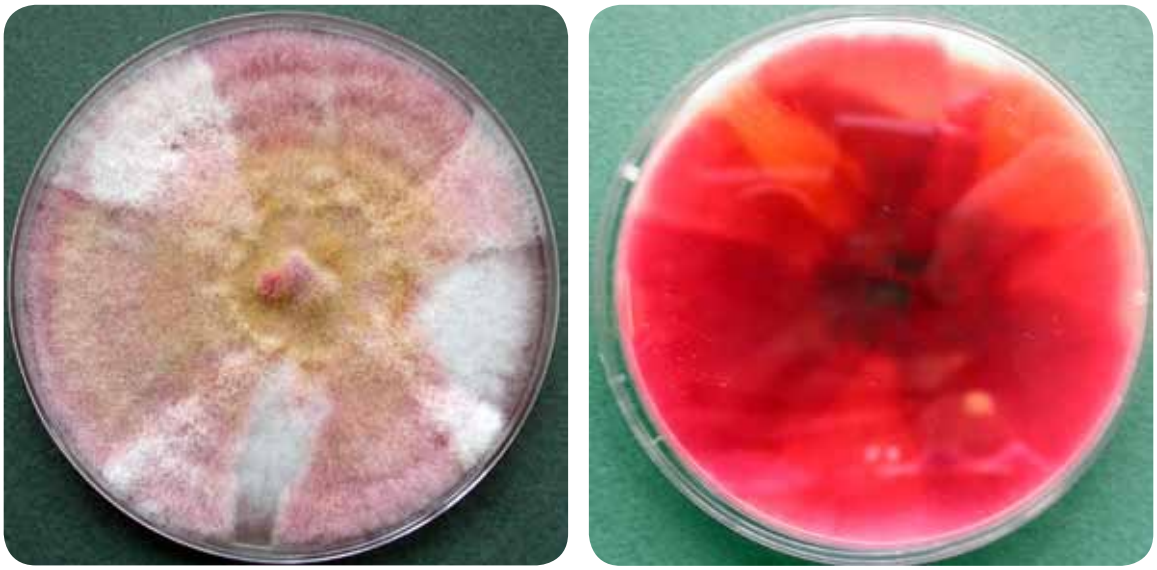
References: Booth (1971, 1977), Domsch *et al.* (1980), McGinnis (1980), Burgess and Liddell (1983), Rippon (1988), Samson *et al.* (1995), de Hoog *et al.* (2000, 2015), O'Donnell *et al.* (2008, 2009a, 2009b, 2015), Balajee *et al.* (2009), Guarro (2013), Geiser *et al.* (2013), van Diepeningen *et al.* (2015), Salah *et al.* (2015), Tortorano *et al.* (2014).

Fusarium chlamydosporum complex

Fusarium chlamydosporum complex contains five phylogenetically distinct species and is common in soils and the rhizosphere of numerous vascular plants worldwide. It is occasionally isolated from human and animal infections (O'Donnell *et al*. 2009b, Guarro 2013).

RG-1 organisms.

Morphological Description: Colonies growing rapidly, with abundant aerial mycelium, deep pink, red or ochraceous to brownish; reverse carmine red or tan to brown. Sporodochia orange, flesh-coloured or ochraceous. Conidiophores scattered over the aerial mycelium, branched; numerous polyblastic conidiogenous cells are present. Macroconidia rarely produced and appearing only on sporodochial phialides, usually three-(some up to five)-septate, slightly curved, 30-38 x 3.0-4.5 µm, with no distinct foot-shaped cell. Microconidia and blastoconidia fusiform, rounded apically and tapered towards the base, single-celled to one-(some up to three)-septate, 6-26 x 2-4 µm. Chlamydospores abundant, intercalary, often roughened.



Fusarium chlamydosporum complex, culture showing pink to ochraceous to brownish surface and a carmine red reverse.

Antifungal Susceptibility: *F. chlamydosporum* complex (Australian National data); **MIC µg/mL.**

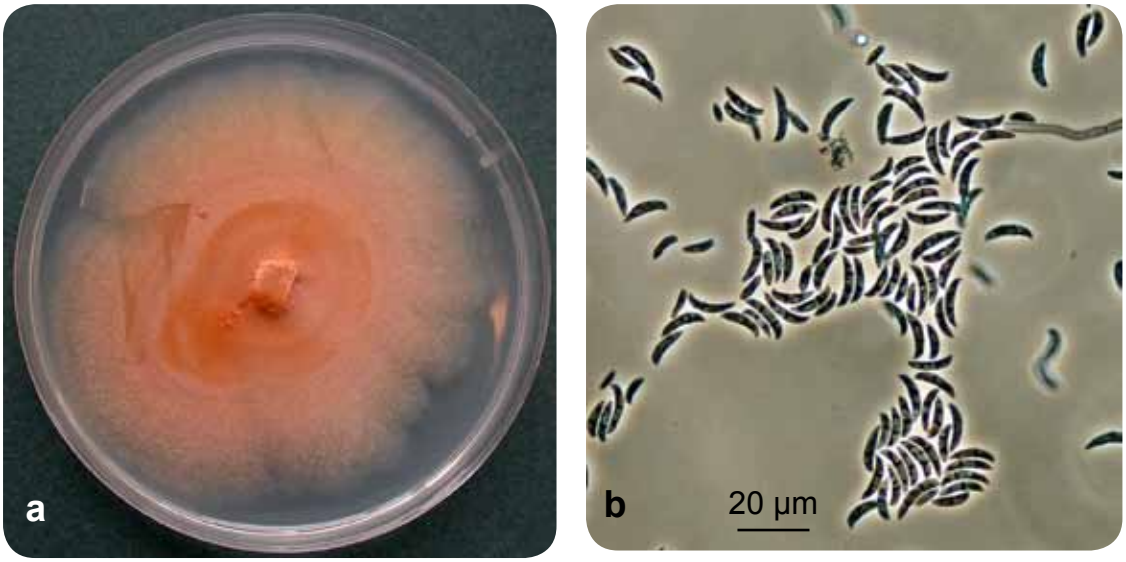
	No.	≤0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	≥64
AmB	**5**				**1**	**2**	**2**						
VORI	**5**					**1**	**2**	**2**					
POSA	**4**					**2**	**2**						
ITRA	**4**				**1**	**1**	**1**				**1**		

Fusarium dimerum complex

The *Fusarium dimerum* complex contains 12 phylogenetically distinct species including *F. delphinoides, F. penzigii* and *F. dimerum*. These are regarded as cosmopolitan saprotrophs in soil and on plant materials (Domsch *et al.* 2007). They have also been isolated from human corneal ulcers after trauma and from disseminated or localised infections in immunocompromised patients (Schroers *et al*. 2009, Guarro 2013).

RG-1 organisms.

Morphological Description: Colonies growing slowly; surface usually orange to deep apricot due to confluent conidial slime; aerial mycelium sometimes floccose and whitish. Conidiophores loosely branched, with short, often swollen phialides, 10-18 x 4-5 µm. Macroconidia strongly curved and pointed at the apex, mostly one-(some up to three)-septate, 5-25 (-32) x 1.5-4.2 µm. Microconidia absent. Chlamydospores mostly intercalary, exceptionally terminal, spherical to ovoidal, 6-12 µm diam, smooth-walled, single or in chains.



Fusarium dimerum complex (a) culture showing orange to deep apricot colour due to confluent conidial slime, and (b) macroconidia.

Antifungal Susceptibility: *F. dimerum* complex (Australian National data); **MIC µg/mL.**													
	No.	**≤0.03**	**0.06**	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**≥64**
AmB	**21**			**1**	**6**	**6**	**5**	**6**	**2**		**1**		
VORI	**20**					**5**	**8**	**6**	**3**	**3**	**1**		
POSA	**18**					**1**	**4**	**2**		**11**			
ITRA	**21**						**3**				**24**		

Fusarium fujikuroi complex

Synonymy: *Gibberella fujikuroi* complex.

Fusarium fujikuroi complex consists of 50 phylogenetically distinct species including 13 of which have been reported to cause human infection; *F. acutatum, F. ananatum, F. andiyazi, F. fujikuroi, F. guttiforme, F. napiforme, F. nygamai, F. verticillioides, F. proliferatum, F. sacchari, F. subglutinans, F. temperatum* and *F. thapsinum* (Guarro, 2013, Al-Hatmi *et al*. 2015).

RG-1 organisms.

Morphological Descriptrion: Colonies growing rapidly, pink or vinaceous to violet; aerial mycelium abundant. Sporodochia present or absent, when present they are tan to orange. Conidiophores usually erect and branched. Macroconidia abundant, falcate to rather straight, three to five-septate, with a distinct foot-cell, 27-73 x 3.4-5.2 µm. Blastoconidia straight or slightly curved, two to three-septate, fusiform to lanceolate, with a somewhat pointed, often slightly asymmetrical apical cell and a truncate basal cell, 16-43 x 3.0-4.5 µm. Microconidia produced on polyphialides and aggregating in heads, usually unicellular, ovoidal, ellipsoidal or allantoid, 4-20 x 1.5-4.5 µm. Chlamydospores absent.

Antifungal Susceptibility: *F. fujikuroi* complex (Castanheir *et al*. 2012, Australian National data); **MIC µg/mL.**													
	No.	**≤0.03**	**0.06**	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**≥64**
AmB	**39**						**3**	**19**	**15**	**2**			
VORI	**39**						**2**	**12**	**14**	**8**	**3**		
POSA	**39**					**4**	**9**	**7**	**3**		**16**		
ITRA	**30**							**2**	**4**	**2**	**22**		

Fusarium incarnatum-equiseti complex

Fusarium incarnatum-equiseti complex consists of 40 phylogenetically distinct species. They occasionally cause infections in humans and animals (O'Donnell *et al*. 2009b, Guarro 2013).

RG-1 organisms.

Morphological Description: Colonies growing rapidly; aerial mycelium floccose, at first whitish, later becoming avellaneous to buff-brown; reverse pale, becoming peach-coloured. Conidiophores scattered in the aerial mycelium, loosely branched; polyblastic conidiogenous cells abundant. Sporodochial macroconidia slightly curved, with foot-cell, three to seven-septate, 20-46 x 3.0-5.5 µm. Conidia on aerial conidiophores (blastoconidia) usually borne singly on scattered denticles, fusiform to falcate, mostly three to five-septate, 7.5-35 x 2.5-4.0 µm. Microconidia sparse or absent. Chlamydospores sparse, spherical, 10-12 µm diameter, becoming brown, intercalary, single or in chains.

Fusarium incarnatum-equiseti complex

Antifungal Susceptibility: *F. incarnatum-equiseti* complex (Australian National data); **MIC µg/mL.**													
	No.	**≤0.03**	**0.06**	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**≥64**
AmB	**6**				**1**	**1**	**3**			**1**			
VORI	**6**			**1**			**1**		**3**	**1**			
POSA	**5**						**1**	**3**			**1**		
ITRA	**4**				**1**						**3**		

Fusarium oxysporum complex

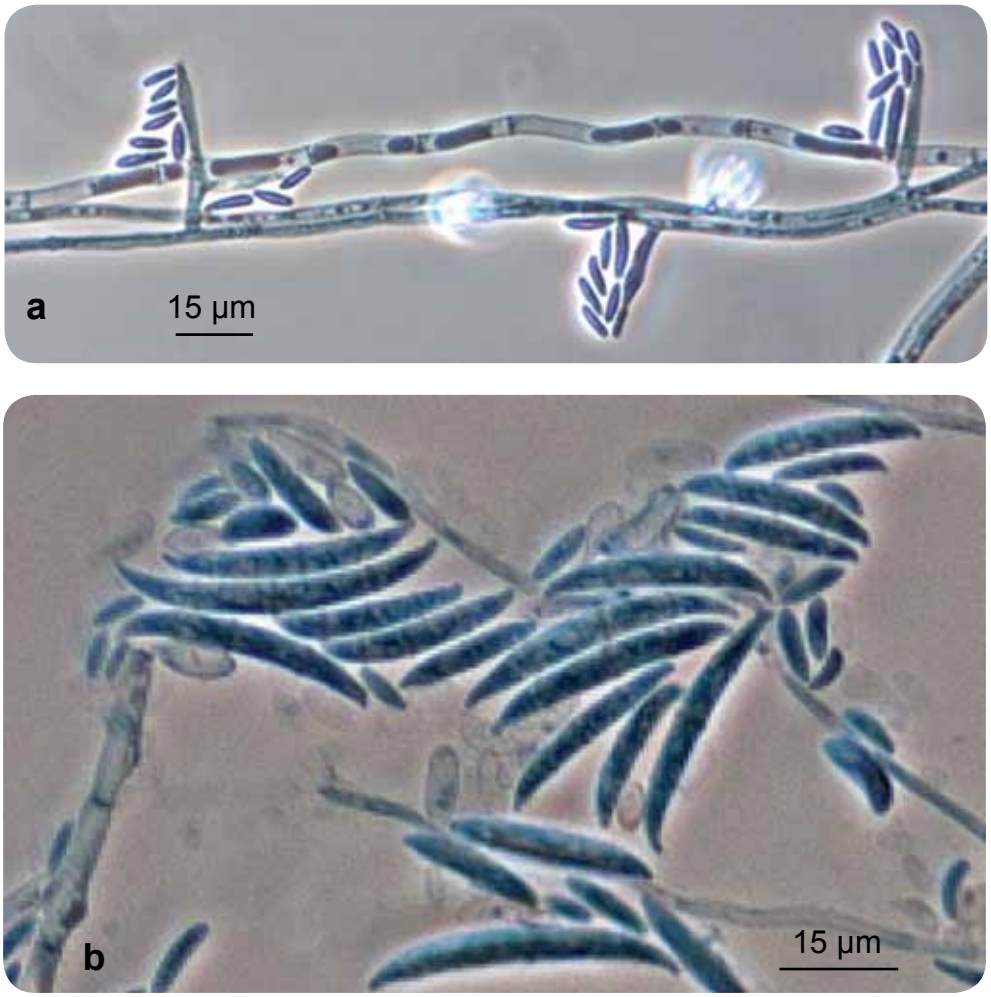
This complex contains at least five phylogenetically distinct species and accounts for about 20% of human infections caused by fusaria (Guarro 2013, Tortorano *et al*. 2014, Salah *et al*. 2015). All are ubiquitous soil borne pathogens responsible for vascular wilts, rots, and damping-off diseases of a broad range of plants. A number of these fusaria are also clinically important, causing localised or deeply invasive life threatening infections in humans and other animals (O'Donnell *et al.* 2009a). Mortality in patients who are persistently and severely neutropenic is typically 100% (Nucci and Anaissie, 2007).

RG-2 organisms.

Morphological Description: Colonies growing rapidly, 4.5 cm in four days, aerial mycelium white, becoming purple, with discrete orange sporodochia present in some strains; reverse hyaline to dark blue or dark purple. Conidiophores are short, single, lateral monophialides in the aerial mycelium, later arranged in densely branched clusters. Macroconidia are fusiform, slightly curved, pointed at the tip, mostly three septate, basal cells pedicellate, 23-54 x 3-4.5 µm. Microconidia are abundant, never in chains, mostly non-septate, ellipsoidal to cylindrical, straight or often curved, 5-12 x 2.3-3.5 µm. Chlamydospores are terminal or intercalary, hyaline, smooth or rough walled, 5-13 µm. In contrast to *F. solani* complex, the phialides are short and mostly non-septate.

Antifungal Susceptibility: *F. oxysporum* complex (Australian National data); **MIC µg/mL.**													
	No.	**≤0.03**	**0.06**	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**≥64**
AmB	**52**			**1**	**4**	**6**	**12**	**14**	**9**	**4**	**2**		
VORI	**49**					**5**	**6**	**10**	**19**	**7**	**3**		
POSA	**37**				**1**	**1**	**7**	**6**	**1**	**15**	**6**		
ITRA	**39**					**3**	**1**			**1**	**34**		

Fusarium oxysporum complex



Fusarium oxysporum complex (a)microconidia on short phialides and (b) macroconidia.

Fusarium solani complex

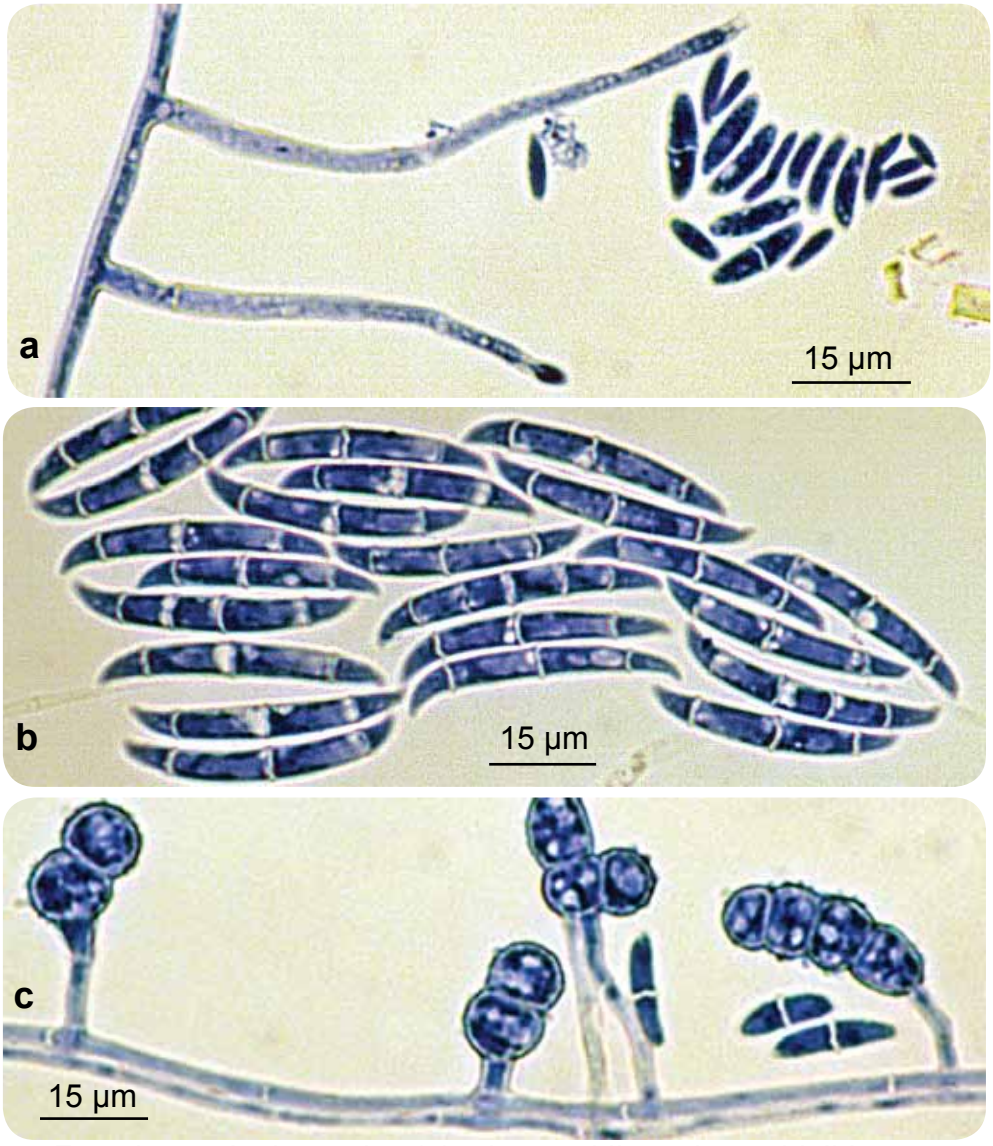
Antifungal Susceptibility: *F. solani* complex (Australian National data); **MIC µg/mL.**													
	No.	**≤0.03**	**0.06**	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**≥64**
AmB	**130**			**1**	**2**	**18**	**29**	**40**	**29**	**11**			
VORI	**126**						**7**	**24**	**36**	**34**	**25**		
POSA	**114**				**2**		**1**	**2**	**1**	**71**	**37**		
ITRA	**93**				**2**					**1**	**90**		

Fusarium solani complex

The *Fusarium solani* complex contains at least 60 species and accounts for about 50% of human infections caused by fusaria (Guarro 2013, Tortorano *et al*. 2014, Salah *et al*. 2015). All are ubiquitous soil borne pathogens responsible for vascular wilts, rots, and damping-off diseases of a broad range of plants. A number of these fusaria, notably *F. keratoplasticum, F. petroliphilum, F. lichenicola* and *F. solani* are clinically important, causing localised or deeply invasive life threatening infections in humans and other animals (Guarro 2013, O'Donnell *et al.* 2008).

RG-2 organisms.

Morphological Description: Colonies growing rapidly, 4.5 cm in four days, aerial mycelium white to cream, becoming bluish-brown when sporodochia are present. Macroconidia are formed after 4-7 days from short multiple branched conidiophores which may form sporodochia. They are three to five-septate (usually three-septate), fusiform, cylindrical, often moderately curved, with an indistinct pedicellate foot cell and a short blunt apical cell, 28-42 x 4-6 µm. Microconidia are usually abundant, cylindrical to oval, one to two-celled and formed from long lateral phialides, 8-16 x 2-4.5 µm. Chlamydospores are hyaline, globose, smooth to rough-walled, borne singly or in pairs on short lateral hyphal branches or intercalary, 6-10 µm.



Fusarium solani complex (a) microconidia on long phialides, (b) macroconidia and (c) chlamydospores.

Geotrichum candidum Link.

Synonymy: *Galactomyces candidus* de Hoog & M.Th. Smith.

The genus *Geotrichum* and related species have undergone extensive taxonomic revision (de Hoog and Smith 2004, 2011a, 2011b, 2011c). The three species of prime interest to medical mycology are *Geotrichum candidum* (*Galactomyces candidus*), *Magnusiomyces capitatus* (previously known as *Geotrichum capitatum*), and *Saprochaete clavata* (previously known as *Geotrichum clavatum)*.

Geotrichum candidum is an extremely common fungus with a worldwide distribution. It is commonly isolated from soil, air, water, milk, silage, plant tissues, and the digestive tract in humans and other mammals (Pottier *et al.* 2008). Pulmonary involvement is the most frequently reported form of the disease in humans and animals, but bronchial, oral, vaginal, cutaneous and alimentary infections have also been noted (Arendrup *et al*. 2014).

RG-1 organism.

Morphological Description: Colonies are fast growing, flat, white to cream, dry and finely suede-like with no reverse pigment. Hyphae are hyaline, septate, branched and break up into chains of hyaline, smooth, one-celled, subglobose to cylindrical arthroconidia. Arthroconidia are 6-12 x 3-6 µm in size and are released by the separation of a double septum.

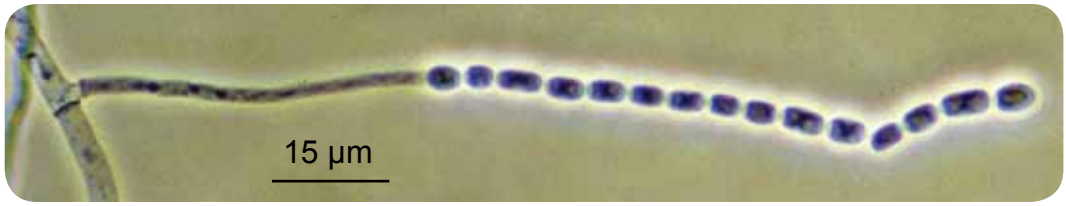
Note: True blastoconidia production is not found in this genus. This characteristic distinguishes *Geotrichum* from *Trichosporon*, which usually does produce blastoconidia.

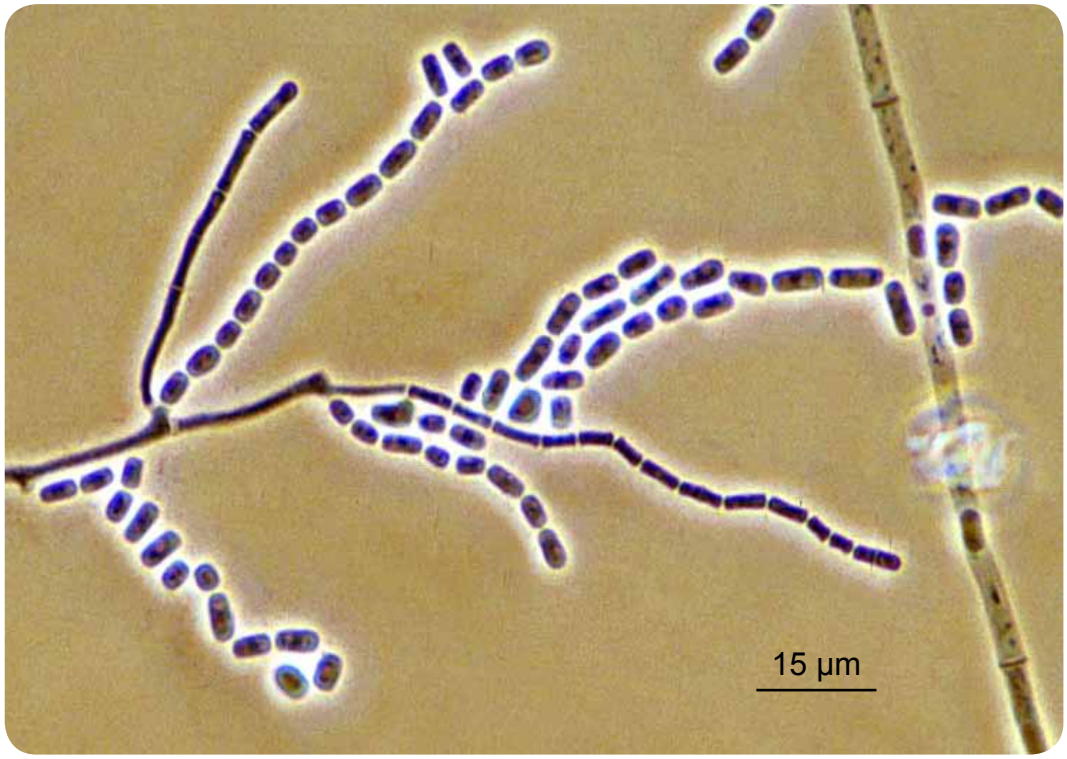
Molecular Identification: ITS sequencing recommended for accurate species identification (de Hoog and Smith 2004).

Physiological Tests: + Positive, - Negative, v Variable, w Weak, s Slow, nd No Data							
Germ Tube	-	L-Sorbose	+	L-Arabinose	-	D-Glucitol	+
Fermentation		Sucrose	-	D-Arabinose	-	α-M-D-glucoside	-
Glucose	v	Maltose	-	D-Ribose	-	D-Gluconate	-
Galactose	v	Cellobiose	-	L-Rhamnose	-	DL-Lactate	+
Sucrose	-	Trehalose	-	D-Glucosamine	-	*myo*-Inositol	-
Maltose	-	Lactose	-	*N*-A-D-glucosamine	nd	2-K-D-gluconate	-
Lactose	-	Melibiose	-	Glycerol	+	D-Glucuronate	nd
Trehalose	-	Raffinose	-	Erythritol	-	Nitrate	-
Assimilation		Melezitose	-	Ribitol	v	Urease	-
Glucose	+	Soluble Starch	-	Galactitol	-	0.1% Cycloheximide	nd
Galactose	+	D-Xylose	+	D-Mannitol	+	Growth at 37^{O}C	v

References: Gueho (1979), Domsch *et al*. (1980), McGinnis (1980), Barnett *et al.* (1983), Buchta and Otcenasek (1988), Samson *et al.* (1995), de Hoog *et al*. (1986, 2015), de Hoog and Smith (2004, 2011a, 2011b, 2011c).

***Geotrichum candidum* Link.**





Geotrichum candidum arthroconidium formation. Hyphal elements are progressively compartmentalised by fragmentation of septa. Conidial secession is by the centripetal separation (schizolysis) of a so called double septum and concomitant rupture of the original outer hyphal wall layer.

Antifungal Susceptibility: ***Geotrichum candidum*** limited data (Sfakianakis *et al.* 2007, Henrich *et al*. 2009, Australian National data); **MIC µg/mL.**

	No.	≤0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	≥64
AmB	6					2	4						
FLU	5									4	1		
VORI	6	2		2	1	1							
POSA	3					3							
ITRA	5	1			2	2							
ANID	2									2			
MICA	2									2			
CAS	3						1			2			
5FC	5	1	3										1

Gliocladium Corda

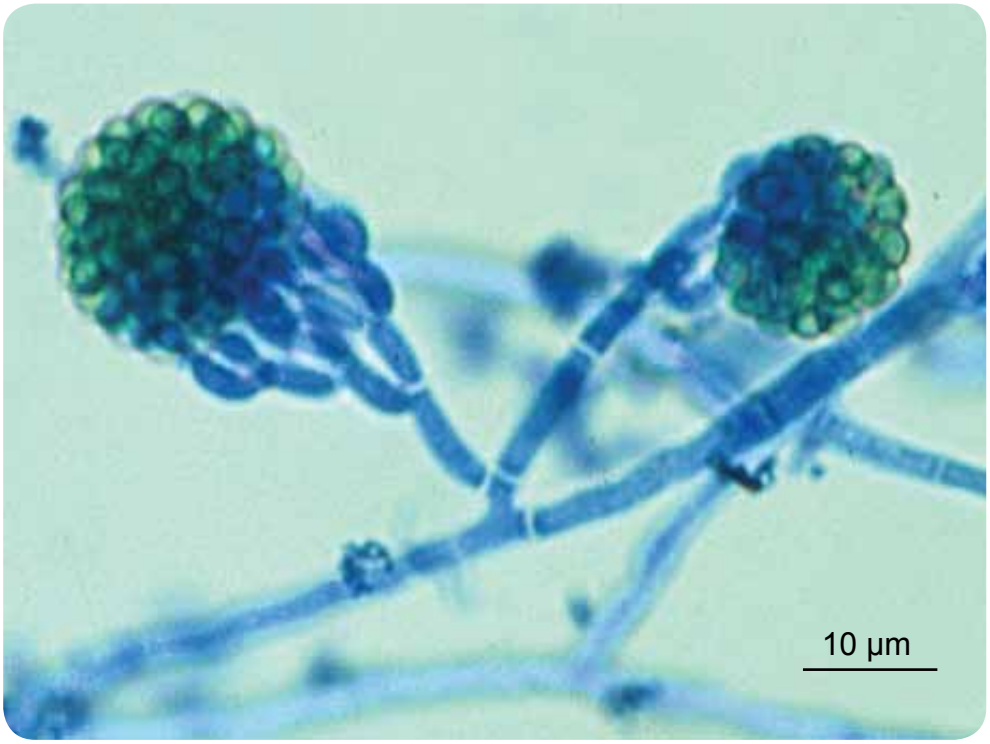
Gliocladium species have a worldwide distribution and are commonly isolated from a wide range of plant debris and soil.

RG-1 organism.

Morphological Description: The genus *Gliocladium* is often described as a counterpart of *Penicillium* with slimy conidia. Colonies are fast growing, suede-like to downy in texture, white at first, sometimes pink to salmon, becoming pale to dark green with sporulation. The most characteristic feature of the genus is the distinctive erect, often densely penicillate conidiophores with phialides which bear slimy, one-celled hyaline to green, smooth-walled conidia in heads or columns. Although, penicillate conidiophores are always present, *Gliocladium* species may also produce verticillate branching conidiophores which can be confused with *Verticillium* or *Trichoderma*.

Key Features: Hyphomycete producing distinctive erect penicillate conidiophores with phialides bearing clusters of single-celled conidia.

References: Domsch *et al*. (2007), McGinnis (1980), Onions *et al.* (1981), Rippon (1988), de Hoog *et al*. (2000).



Gliocladium spp. conidiophore and conidia.

Graphium Corda

The genus *Graphium* is characterised by the formation of synnemata which consist of a more or less compact group of erect conidiophores that are cemented together, usually splaying out and bearing conidia at the apex. *Graphium* species are commonly found on woody plant material. *Graphium basitruncatum* has been reported as causing fungaemia in an immunosuppressed child post stem-cell transplantation (El Feghaly *et al*. 2012).

Note: Many other fungi such as *Scedosporium* species may also produce synnemata.

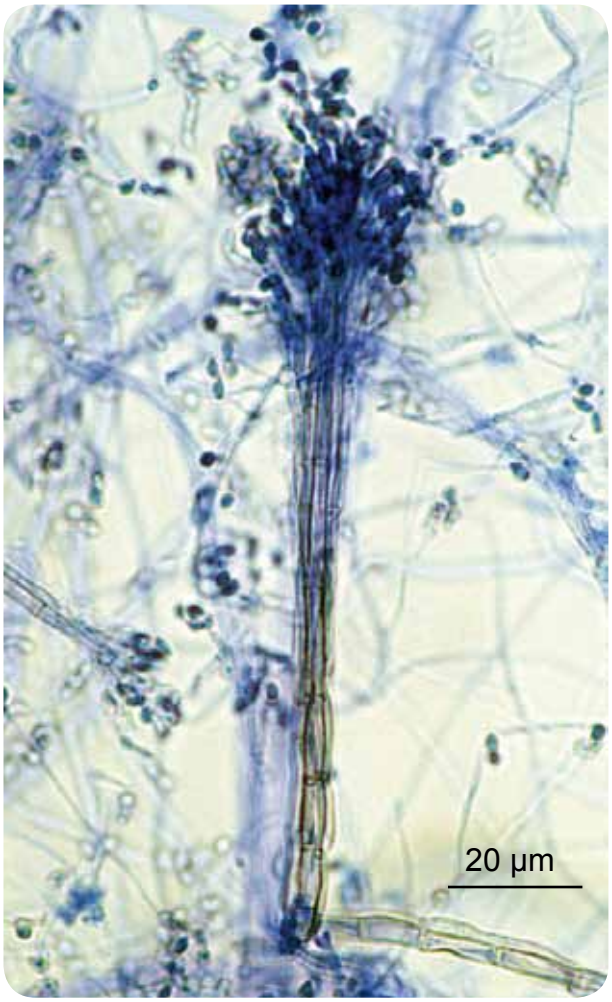
RG-1 organism.

Morphological Description: Synnemata are darkly pigmented, erect and occur solitarily or in clusters. Conidia are hyaline, one-celled, smooth, subglobose to ovoid and are usually aggregated in slimy heads at the apex of the synnemata. Colonies are effuse, grey, olivaceous brown or black.

Molecular diagnostics: The genus is phylogenetically close to *Scedosporium* but ITS sequencing can be used to resolve all species (Okada *et al*. 2000, Lackner and de Hoog 2011).

Key Features: Dematiaceous hyphomycete producing erect synnemata with apical aggregates of single-celled conidia in slimy heads.

References: Barron (1968), Ellis (1971), McGinnis (1980), de Hoog *et al*. (2000, 2015).



Graphium spp. synnemata and conidia.

Histoplasma capsulatum Darling

WARNING: RG-3 organism. Cultures of *Histoplasma capsulatum* represent a severe biohazard to laboratory personnel and must be handled with extreme caution in a Class II Biological Safety Cabinet (BSCII).

Histoplasma capsulatum has a worldwide distribution, however the Mississippi-Ohio River Valley in the USA is recognised as a major endemic region. Environmental isolations of the fungus have been made from soil enriched with excreta from chicken, starlings and bats. Histoplasmosis is an intracellular mycotic infection of the reticuloendothelial system caused by the inhalation of the fungus. Approximately 95% of cases of histoplasmosis are inapparent, subclinical or benign. The remaining 5% of cases may develop chronic progressive lung disease, chronic cutaneous or systemic disease or an acute fulminating fatal systemic disease. All stages of this disease may mimic tuberculosis. Sporadic cases have been reported in Australia.

Morphological Description: *Histoplasma capsulatum* exhibits thermal dimorphism growing in living tissue or in culture at 37^{O}C as a budding yeast-like fungus and in soil or culture at temperatures below 30^{O}C as a mould.

Colonies at 25^{O}C are slow growing, white or buff-brown, suede-like to cottony with a pale yellow-brown reverse. Other colony types are glabrous or verrucose, and a red pigmented strain has been noted (Rippon, 1988). Microscopic morphology shows the presence of characteristic large, rounded, single-celled, 8-14 µm in diameter, tuberculate macroconidia formed on short, hyaline, undifferentiated conidiophores. Small, round to pyriform microconidia, 2-4 µm in diameter, borne on short branches or directly on the sides of the hyphae may also be present.

Colonies at 37^{O}C grown on brain heart infusion (BHI) agar containing blood are smooth, moist, white and yeast-like. Microscopically, numerous small round to oval budding yeast-like cells, 3-4 x 2-3 µm in size are observed.

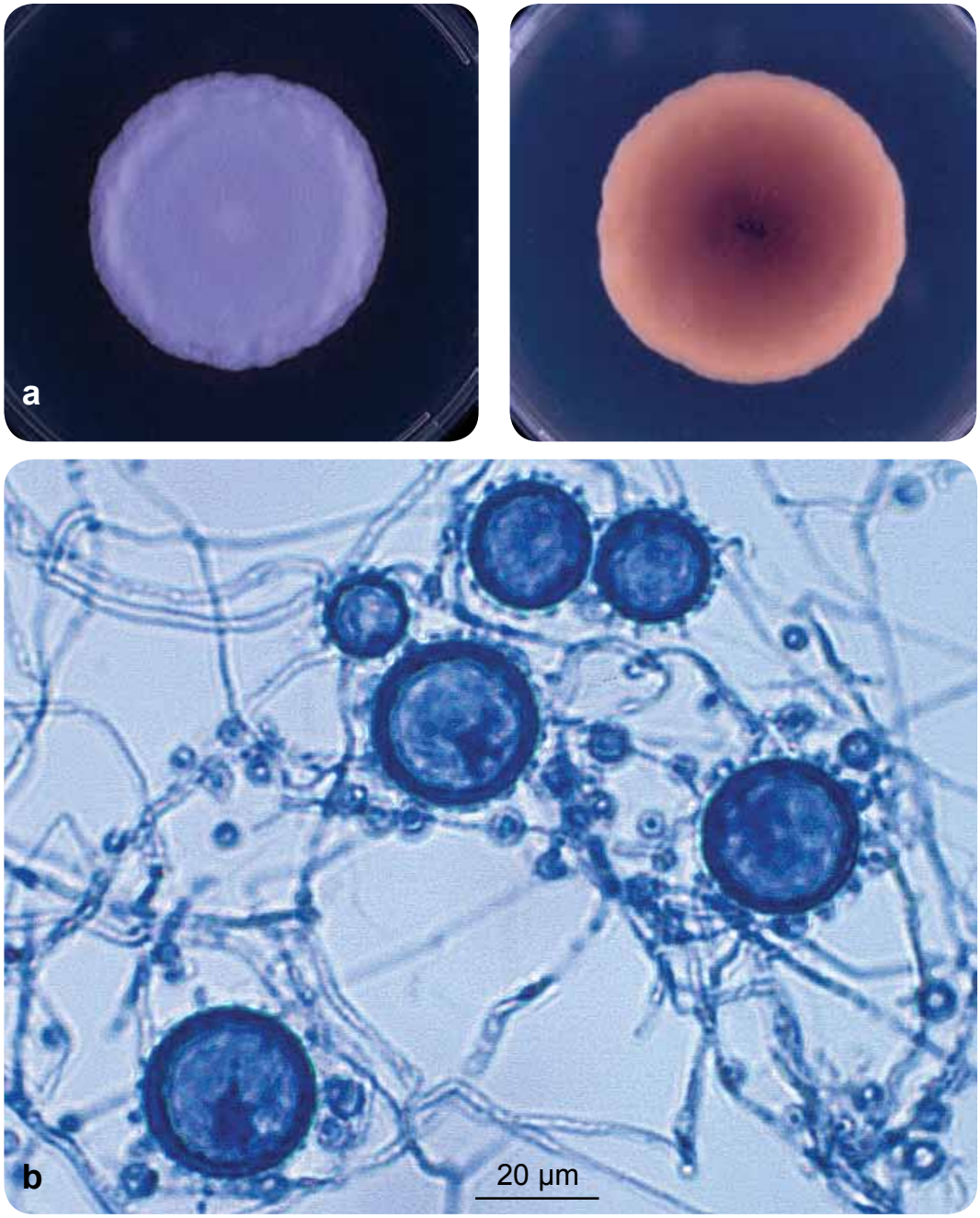
Three varieties of *Histoplasma capsulatum* are recognised, depending on the clinical disease: var. *capsulatum* is the common cause of histoplasmosis; var. *duboisii* is the African type and var. *farciminosum* causes lymphangitis in horses. *Histoplasma* isolates may also resemble species of *Sepedonium* and *Chrysosporium*. Traditionally, positive identification required conversion of the mould form to the yeast phase by growth at 37^{O}C on enriched media, however for laboratory safety, culture identification by either exoantigen test or DNA sequencing is now preferred.

Key Features: Clinical history, tissue morphology, culture morphology and positive exoantigen test or DNA sequencing.

Molecular Identification: A probe for species recognition is commercially available (Padhye *et al.* 1992, Chemaly *et al*. 2001) and Elias *et al*. (2012) developed a multiplex-PCR for identification from cultures. Scheel *et al*. (2014) developed a loop-mediated isothermal amplification (LAMP) assay for detection directly in clinical samples which is affordable and useful in resource poor facilities. ITS sequencing may also be used for accurate identification (Estrada-Bárcenas *et al*. 2014, Irinyi *et al*. 2015).

References: McGinnis (1980), Chandler *et al*. (1980), George and Penn (1986), Rippon (1988), de Hoog *et al.* (2000, 2015).

Histoplasma capsulatum Darling



Histoplasma capsulatum (a) culture and (b) microscopic morphology of the saprophytic or mycelial form showing characteristic large, rounded, single-celled, tuberculate macroconidia and smaller microconidia.

Antifungal Susceptibility: *H. capsulatum* (Espinel-Ingroff 2003, Gonzalez *et al.* 2005, Sabatelli *et al.* 2006, Brilhante *et al.* 2012, Kathuria *et al.* 2014); **MIC µg/mL.**

Antifungal	Filamentous form		Yeast form	
	Range	MIC_{90}	Range	MIC_{90}
AmB	≤0.03-0.5	0.5	0.03-0.5	0.25
FLU	1-125	16	2-8	8
ITRA	≤0.03-1	0.125 (1)	≤0.03-0.25	0.125
VORI	≤0.03-2	0.25 (1)	≤0.03-0.5	0.5
POSA	≤0.03-2	0.125 (2)	0.03-0.5	0.25

Hortaea werneckii (Horta) Nishimura & Miyaji

Synonymy: *Phaeoannellomyces werneckii* (Horta) McGinnis & Schell; *Exophiala werneckii* (Horta) v. Arx; *Cladosporium werneckii* Horta.

Hortaea werneckii is a common saprophytic fungus believed to occur in soil, compost, humus and on wood in humid tropical and subtropical regions and is the causative agent of tinea nigra in humans. **RG-1 organism.**

Morphological Description: Colonies are slow growing, initially mucoid, yeast-like and shiny-black. However with age they develop abundant aerial mycelia and become dark olivaceous in colour. Microscopically, colonies consist of brown to dark olivaceous, septate hyphal elements and numerous two-celled, pale brown, cylindrical to spindle-shaped yeast-like cells that taper towards the ends to form an annellide. Most yeast-like cells also have prominent darkly-pigmented septa. Annellides may also arise from the hyphae. Conidia are one to two-celled, cylindrical to spindle-shaped, hyaline to pale brown and usually occur in aggregated masses. Chlamydospores also present.

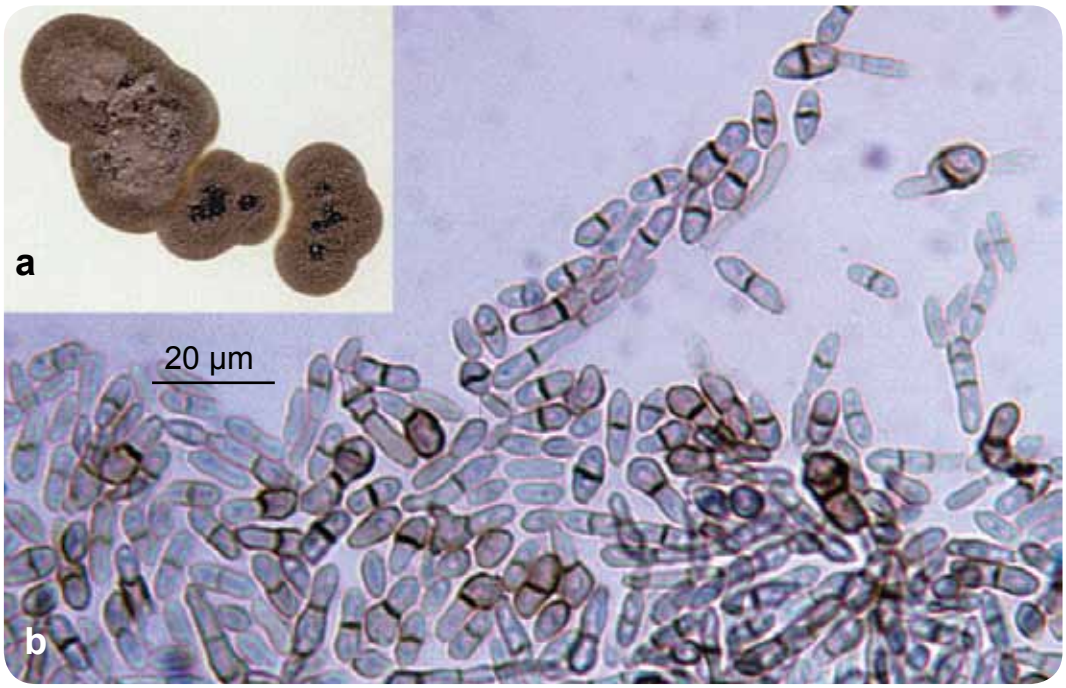
Key Features: Hyphomycete, two-celled yeast-like cells producing annelloconidia.

Molecular Identification: An ITS-primer specific for *H. werneckii* was developed by Abliz *et al.* (2003). ITS sequencing can also assist identification.

References: Mok (1982), McGinnis (1980), McGinnis *et al.* (1985), Rippon (1988), de Hoog *et al.* (2000), Abliz *et al.* (2003), Ng *et al.* (2005).

Antifungal Susceptibility: *H. wernickii* (Ng *et al.* 2005, Formoso *et al*. 2015); **MIC µg/mL.**

	No.	≤0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	≥64
AmB	6					1	1	1	1			2	
FLU	6									2	1		3
VORI	6	2	1		3								
POSA	4		2	2									
ITRA	8	2		2	2	2							



Hortaea werneckii (a) culture and (b) conidia.

Kluyveromyces marxianus (Hansen) van der Walt

Synonymy: *Candida kefyr* (Beijerinck) van Uden & Buckley.
Candida pseudotropicalis (Castellani) Basgal.

Kluyveromyces marxianus is a rare cause of candidiasis and is usually associated with superficial cutaneous manifestations rather than systemic disease. Environmental isolations have been made from cheese and dairy products. **RG-1 organism.**

Culture: Colonies (SDA) white to cream-coloured smooth, glabrous, yeast-like.

Microscopy: Short-ovoid to long-ovoid, budding blastoconidia, 3.0-6.5 x 5.5-11.0 µm, sometimes becoming elongate (up to 16.0 µm).

India Ink Preparation: Negative - no capsules present.

Dalmau Plate Culture: Abundant, long, wavy, branched pseudohyphae usually formed, with ovoid blastoconidia, budding off singly, in pairs or chains, often in a verticillated position. **Note:** In some strains pseudohyphae may be scarce or almost absent.

Molecular Identification: ITS sequencing recommended.

MALDI-TOF MS: Able to accurately identify this species.

Physiological Tests: + Positive, - Negative, v Variable, w Weak, s Slow, nd No Data							
Germ Tube	-	L-Sorbose	v	L-Arabinose	v	D-Glucitol	v
Fermentation		Sucrose	+	D-Arabinose	-	α-M-D-glucoside	-
Glucose	+	Maltose	-	D-Ribose	v	D-Gluconate	-
Galactose	+,s	Cellobiose	v	L-Rhamnose	-	DL-Lactate	+
Sucrose	+	Trehalose	-,w	D-Glucosamine	-	*myo*-Inositol	-
Maltose	-	Lactose	v	*N*-A-D-glucosamine	-	2-K-D-gluconate	-
Lactose	v	Melibiose	-	Glycerol	s	D-Glucuronate	nd
Trehalose	-	Raffinose	+	Erythritol	-	Nitrate	-
Assimilation		Melezitose	-	Ribitol	s	Urease	-
Glucose	+	Soluble Starch	-	Galactitol	-	0.1% Cycloheximide	+
Galactose	s	D-Xylose	s	D-Mannitol	v	Growth at 40^{O}C	+

Key Features: Germ tube negative yeast and sugar assimilation pattern.

Antifungal Susceptibility*: K. marxianus* (Diekema *et al.* 2009, Pfaller *et al.* 2013, Australian National data); **MIC µg/mL.**

	No.	≤0.008	0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	≥64
AmB	95						6	28	50	10					1
FLU	111				1	22	62	19	6	1					
VORI	111	82	23	4	2										
POSA	106	1	6	23	33	31	10	2							
ITRA	37		5	6	12	12	2								
ANID	86		1	14	37	29	4								
MICA	71		4	29	42	4	1	1							
CAS	106	12	70	13	5	3	2	1							
5FC	21			4	8			2		1	1	3	2		

Lasiodiplodia theobromae (Pat.) Griffon & Maublanc

Synonymy: *Botryosphaeria rhodina* (Berk & Curt) v. Arx.
Botryodiplodia theobromae Patouillard.

Lasiodiplodia theobromae is a well known plant pathogen reported from about 500 host plants, mainly confined to an area 40^{O} north to 40^{O} south of the equator. It has also been associated with ulcerated human cornea, lesions on nail and subcutaneous tissue.

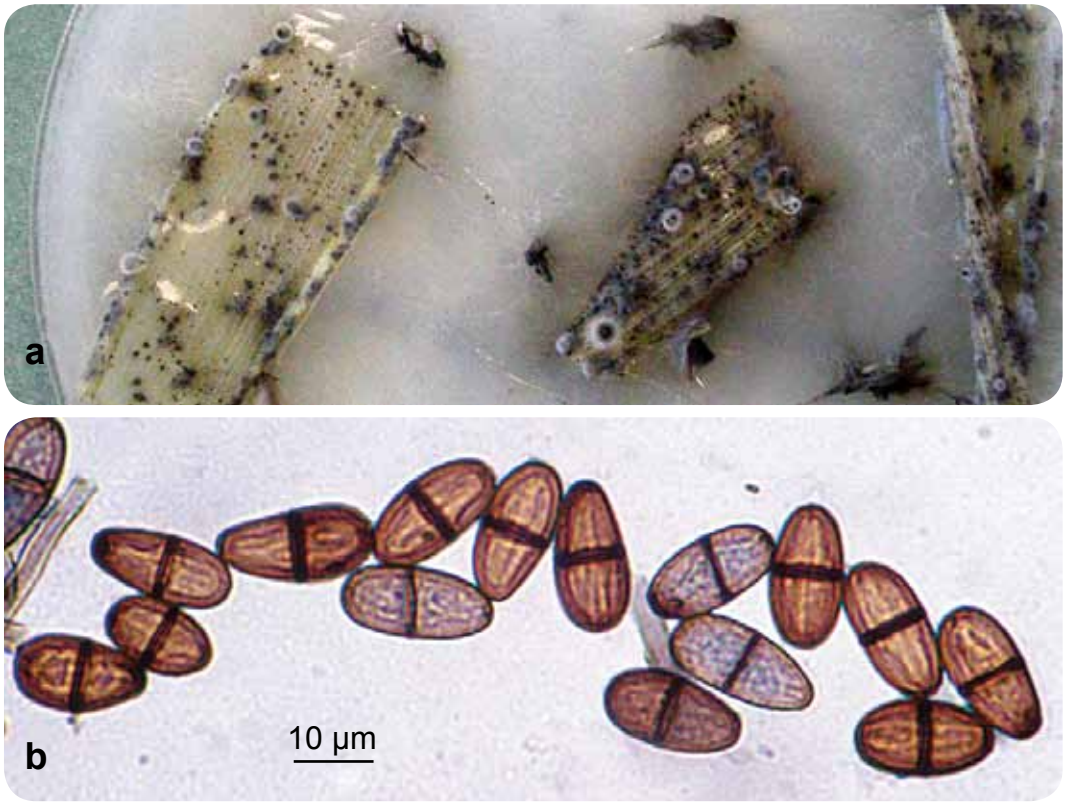
RG-1 organism.

Morphological Description: Colonies are greyish sepia to mouse grey to black, fluffy with abundant aerial mycelium; reverse fuscous to black. Pycnidia are simple or compound, often aggregated, stromatic, ostiolate, frequently setose, up to 5 mm wide. Conidiophores are hyaline, simple, sometimes septate, rarely branched cylindrical, arising from the inner layers of cells lining the pycnidial cavity. Conidiogenous cells are hyaline, simple, cylindrical to sub-obpyriform, holoblastic, annellidic. Conidia are initially unicellular, hyaline, granulose, sub-ovoid to ellipsoid-oblong, thick-walled, base truncate; mature conidia one-septate, cinnamon to fawn, often longitudinally striate, 20-30 x 10-15 µm. Paraphyses when present are hyaline, cylindrical, sometimes septate, up to 50 µm long.

Key Features: Coelomycete, with pycnidia producing characteristic two-celled, dark brown, striated conidia.

Molecular Identification: Recommended genetic marker: *EF-1α* (de Hoog *et al.* 2015). ITS sequencing is useful for identifying clinically important species (Bagyalakshmi 2008).

References: de Hoog *et al.* (2000, 2015), Liu *et al.* (2012), Phillips *et al*. (2013).



Lasiodiplodia theobromae (a) pycnidia growing on carnation leaf agar, and (b) mature two-celled dark brown conidia with typical longitudinal striations.

Lichtheimia corymbifera (Cohn) Vuill.

Synonymy: *Mycocladus corymbifera* (Cohn) J.H. Mirza.
Absidia corymbifera (Cohn) Saccardo & Trotter.
Mucor corymbifera Cohn.

Recent taxonomic revisions of the genus *Absidia* have placed the thermotolerant species into the genus *Lichtheimia* (Hoffmann *et al.* 2007, 2009). The genus *Lichtheimia* currently contains five mostly saprophytic plant decaying and soil-borne species. *Lichtheimia corymbifera* is the principle pathogen causing human and animal infections, however *L. ramosa* and *L. ornata* have also been reported as human pathogens (often misidentified morphologically as *L. corymbifera*) (Alastruey-Izquierdo *et al*. 2010).

RG-2 organism.

Morphological Description: Colonies are fast growing, floccose, white at first becoming pale grey with age, and up to 1.5 cm high. Sporangiophores are hyaline to faintly pigmented, simple or sometimes branched, arising solitarily or in groups. Sub-sporangial septa are absent or rare. Rhizoids are very sparingly produced and may be difficult to find without the aid of a dissecting microscope to examine the colony on the agar surface. Sporangia are small (10-40 µm in diameter) and are typically pyriform in shape with a characteristic conical-shaped columella and pronounced apophysis, often with a short projection at the top. Sporangiospores vary from subglobose to oblong-ellipsoidal (3-7 x 2.5-4.5 µm), hyaline to light grey and smooth-walled. Intercalary giant cells may also be present. Temperature: optimum 35-37^{O}C; maximum 46^{O}C.

Key Features: Mucorales, small pyriform-shaped sporangia with a characteristic conical-shaped columellae and pronounced apophyses, rapid growth at 40^{O}C.

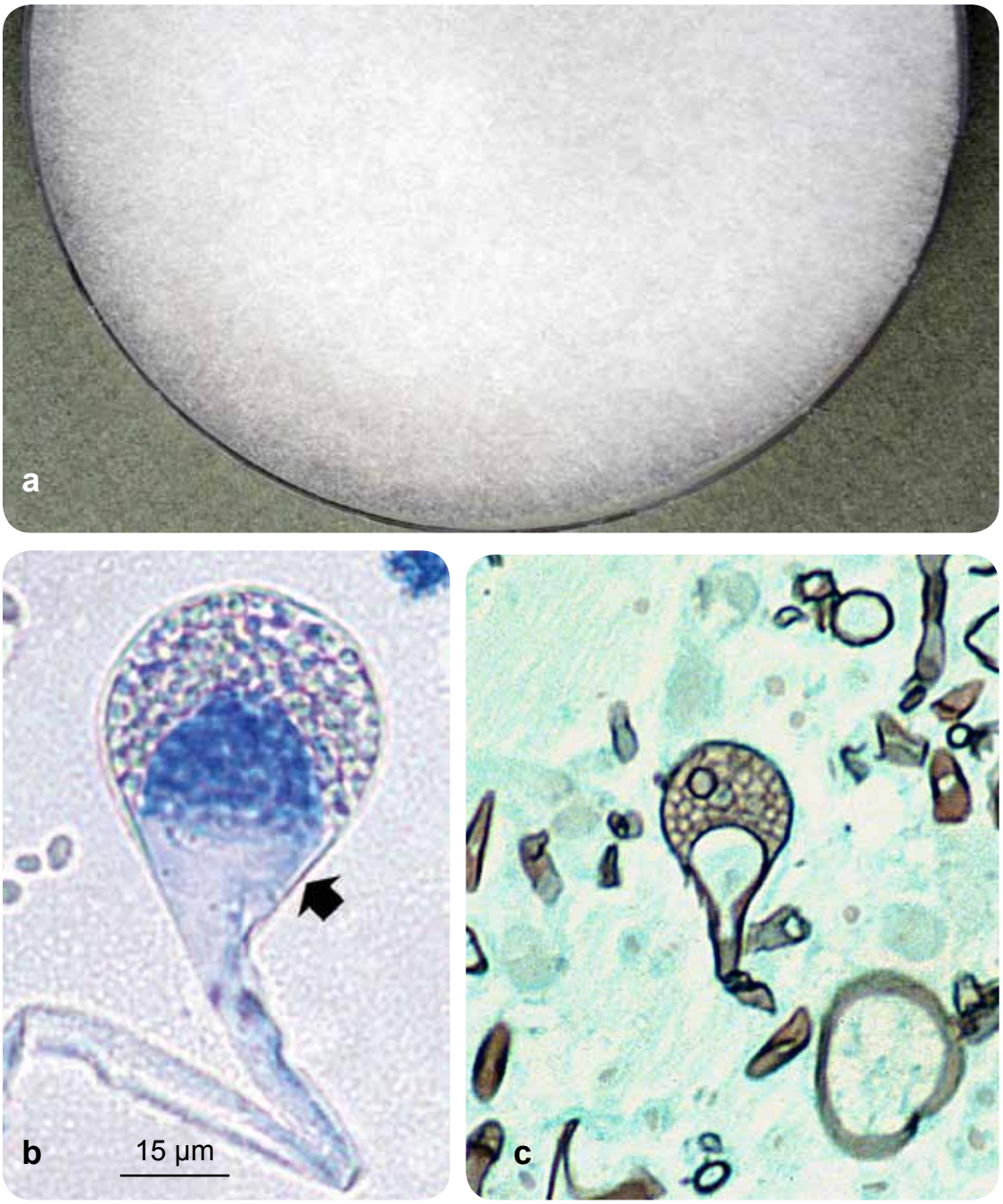
Comment: Morphological characteristics alone are not sufficient to reliably differentiate between *L. corymbifera, L. ramosa* and *L. ornata*. While *L. ornata* develops large, densely branched giant cells and *L. ramosa* has more ellipsoidal to cylindrical sporangiospores and a faster growth rate, these characters are often difficult to interpret. Molecular methods are needed to accurately separate these species.

Molecular Identification: Species recognition in *Lichtheimia* is based on ITS and/or D1/D2 sequencing (Garcia-Hermoso *et al*. 2009, Alastruey-Izquierdo *et al*. 2010).

MALDI-TOF MS: Direct identification of *Lichtheimia* species was described by Schrödl *et al.* (2012).

References: Ellis and Hesseltine (1965, 1966), Hesseltine and Ellis (1964a, 1966), Nottebrock *et al*. (1974), O'Donnell (1979), Samson *et al.* (1995), Domsch *et al*. (1980), McGinnis (1980), Ellis (2005b), de Hoog *et al.* (2000, 2015).

Lichtheimia corymbifera (Cohn) Vuill.



Lichtheimia corymbifera (a) culture and (b) typical pyriform-shaped sporangium with a conical-shaped columella and pronounced apophysis (arrow), and (c) Grocott's methenamine silver (GMS) stained tissue section from a lung biopsy showing a typical sporangium of *L. corymbifera.*

Antifungal Susceptibility: *L. corymbifera* (Espinel-Ingroff *et al*. 2015a, Australian National data); **MIC µg/mL.**

	No.	≤0.016	0.03	0.06	0.125	0.25	0.5	1	2	4	8	≥16
AmB	**136**			**7**	**17**	**36**	**53**	**19**	**3**			**1**
POSA	**112**			**3**	**9**	**26**	**51**	**21**	**1**	**1**		
ITRA	**93**			**5**	**10**	**24**	**21**	**23**	**6**	**3**	**1**	

Lomentospora prolificans Hennebert & Desai

Synonymy: *Scedosporium prolificans* (Hennebert & Desai) Gueho & de Hoog.
Scedosporium inflatum Malloch & Salkin.

Lomentospora prolificans (formerly *Scedosporium prolificans*) is phylogenetically and morphologically distinguishable from *Scedosporium* species (Lennon *et al*. 1994, Lackner *et al*. 2014a).

L. prolificans appears to occupy a restricted geographic range, with infections occurring mainly in Australia, Spain, and the United States (Heath *et al*. 2009, Revankar and Sutton, 2010). *L. prolificans* infections are refractory to antifungal therapy and are associated with high mortality. Major risk factors include malignancy, cystic fibrosis, and solid organ transplantation. The main clinical presentations are disseminated infection and pulmonary mycoses, followed by bone and joint infections (Cortez *et al*. 2008, Heath *et al*. 2009, Rodriguez-Tudela *et al.* 2009, Revankar and Sutton, 2010).

RG-2 organism.

Morphological Description: Colonies are rapid growing, flat, spreading, olive-grey to black and have a suede-like to downy surface texture. Conidia are borne in small groups on distinctive basally swollen, flask-shaped conidiophores, which occur singly or in clusters along the vegetative hyphae. Conidia are aggregated in slimy heads, single-celled, hyaline to pale-brown, ovoid to pyriform, 3-7 x 2-5 µm, and have smooth thick walls. Growth at 45^{O}C.

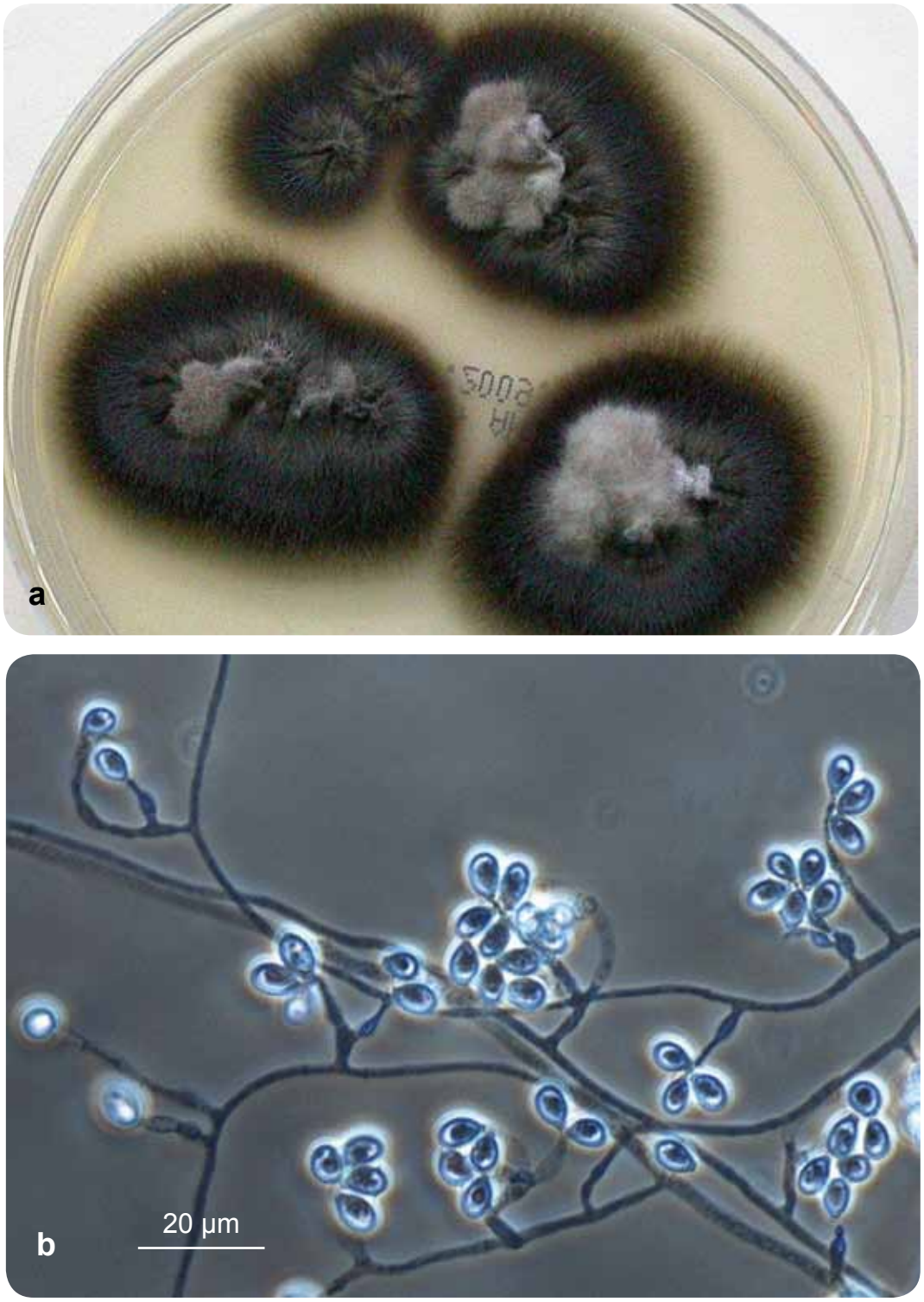
Key Features: Dematiaceous hyphomycete with initial black pasty colony, conidiophores with distinctly swollen bases, and the conidial mass forms apical aggregates of conidia. A *Graphium* synanamorph is absent and there is no growth on media containing cycloheximide (actidione).

Molecular Identification: Recommended genetic markers: ITS and β-tubulin.

References: Malloch and Salkin (1984), Salkin *et al.* (1988), Rippon (1988), Wilson *et al*. (1990), Gueho and de Hoog (1991), Lennon *et al*. (1994), Gilgado *et al.* (2005), Rainer and de Hoog (2006), Revankar and Sutton (2010), Lackner *et al*. (2014a), de Hoog *et al.* (2015).

Antifungal Susceptibility: *L. prolificans* (Australian National data); **MIC µg/mL.**													
	No.	**≤0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**≥64**
AmB	**190**				**1**		**2**	**17**	**47**	**93**	**30**		
VORI	**183**					**1**	**6**	**31**	**62**	**83**			
POSA	**105**							**1**	**1**	**103**			
ITRA	**191**							**2**	**189**				

Lomentospora prolificans Hennebert & Desai



Lomentospora prolificans (a) culture and (b) conidiophores and conidia.

Synergy testing results for *L. prolificans* (Australian National data).				
Antifungal Combination	**No.**	**∑FIC ≤ 0.5 (S)**	**∑FIC >0.5-4 (I)**	**∑FIC >4 (A)**
VORI/TERB	**109**	**94 (86%)**	**14 (14%)**	**0**
ITRA/TERB	**93**	**56 (60%)**	**37 (40%)**	**0**
S = synergy, I = indifferent (synergy or antagonism not demonstrated), A = antagonism.				

Lophophyton gallinae (Megnin) Matruchot & Dassonville

Synonymy: *Microsporum gallinae* (Megnin) Grigorakis.

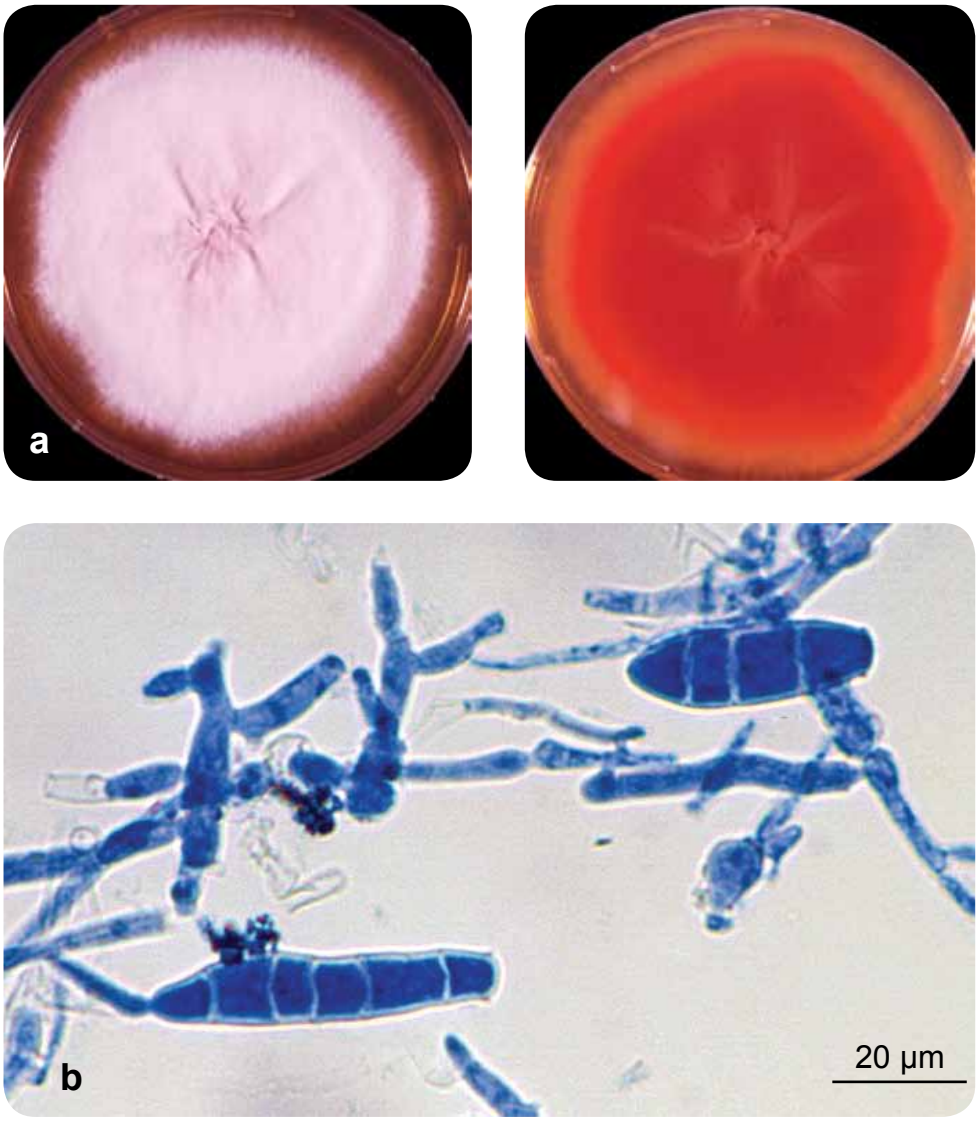
Lophophyton gallinae is a zoophilic fungus causing fowl favus in chickens and other fowl, affecting the comb and wattles producing “white comb” lesions. A rare cause of tinea in humans. Invaded hairs show a sparse ectothrix infection but do not fluoresce under Wood’s ultra-violet light. **RG-2 organism.**

Morphological Description: Colonies are flat with a suede-like texture and are white with a pinkish tinge in colour. Some cultures show radial folding. An orange-pink “strawberry” reverse pigment is usually present. Macroconidia, when present, are usually five to six celled, thin to thick-walled, slightly echinulate, cylindrical to clavate with a narrow base and blunt tip, 15-60 x 6-10 µm. Microconidia are ovoidal to pyriform in shape.

Key Features: Macroconidial morphology, culture characteristics and clinical lesions in chickens.

Molecular Identification: ITS sequencing is recommended.

References: Rebell and Taplin (1970), Rippon (1988), Gräser *et al*. (2008), Cafarchia *et al.* (2013), de Hoog *et al.* (2015, 2016).



Lophophyton gallinae (a) culture and (b) macroconidia.

Madurella complex

The genus *Madurella* was originally based on tissue morphology (mycetoma with black grains) and the formation of sterile cultures on mycological media. Initially two species were described, *M. mycetomatis* and *M. grisea*. However recent molecular studies have recognised five species: *Madurella mycetomatis, Trematosphaeria grisea* (formerly *M. grisea*), *M. fahalii, M. pseudomycetomatis* and *M. tropicana* (Desnos-Ollivier *et al*. 2006, de Hoog *et al*. 2004a, 2012). All species have been isolated from soil and are major causative agents of mycetoma. **RG-2 organism.**

Madurella mycetomatis (Laveran) Brumpt

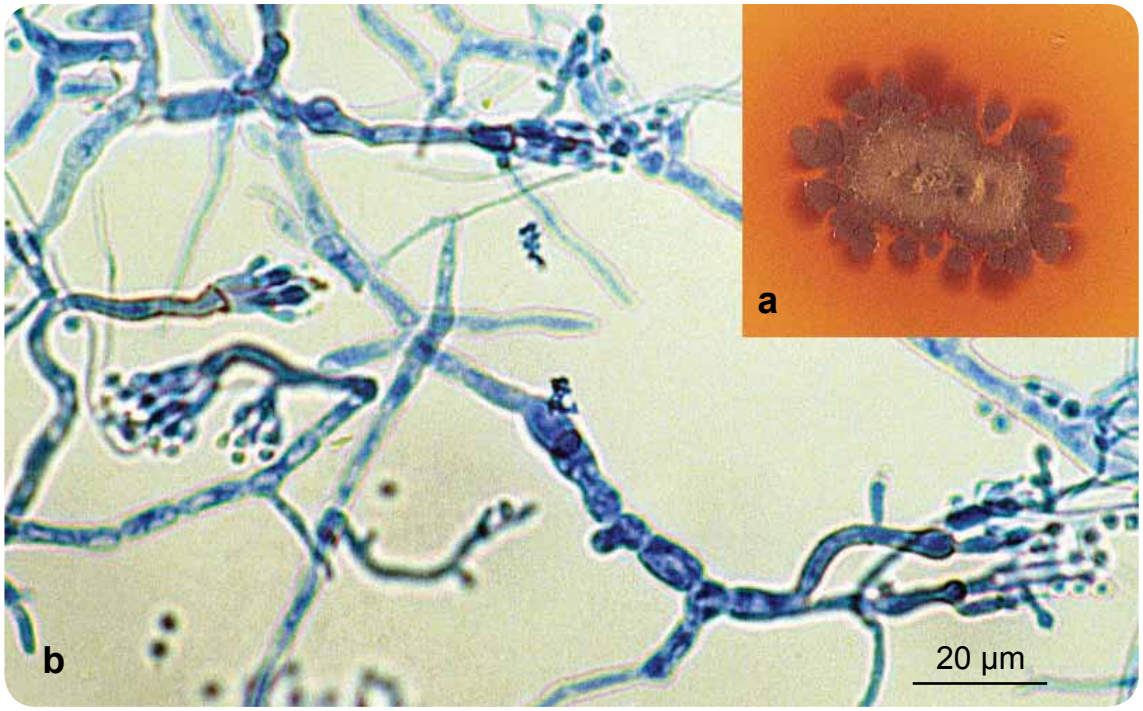
Morphological Description: Colonies are slow growing, flat and leathery at first, white to yellow to yellowish-brown, becoming brownish, folded and heaped with age, and with the formation of aerial mycelia. A brown diffusible pigment is characteristically produced in primary cultures. Although most cultures are sterile, two types of conidiation have been observed, the first being flask-shaped phialides that bear rounded conidia, the second being simple or branched conidiophores bearing pyriform conidia (3-5 µm) with truncated bases. The optimum temperature for growth of this mould is 37^{O}C.

Grains of *Madurella mycetomatis* (tissue microcolonies) are brown or black, 0.5-1.0 mm in size, round or lobed, hard and brittle, composed of hyphae which are 2-5 µm in diameter, with terminal cells expanded to 12-15 (30) µm in diameter.

M. mycetomatis can be distinguished from *Trematosphaeria grisea* by growth at 37^{O}C and its inability to assimilate sucrose.

Key Features: Black grain mycetoma, growth at 37^{O}C, diffusible brown pigment produced on culture and the occasional presence of phialides.

References: McGinnis (1980), Chandler *et al*. (1980), Rippon (1988), de Hoog *et al*. (2000, 2004a, 2012, 2015), Desnos-Ollivier *et al*. (2006).



Madurella mycetomatis (a) culture showing brown diffusible pigment, and (b) phialides and conidia.

Madurella complex

Molecular Identification: ITS sequencing is recommended for species separation (Ahmed *et al.* 2014b, Desnos-Olliver *et al*. 2006, Irinyi *et al.* 2015). A five locus phylogenetic analysis was performed by Ahmed *et al*. (2014a) using the ITS, D1/D2, *RPB2* and *EF-1α* genes.

Trematosphaeria grisea (MacKinnon *et al.*) S.A. Ahmed *et al.*

Synonymy: *Madurella grisea* Mackinnon, Ferrada and Montemayer.

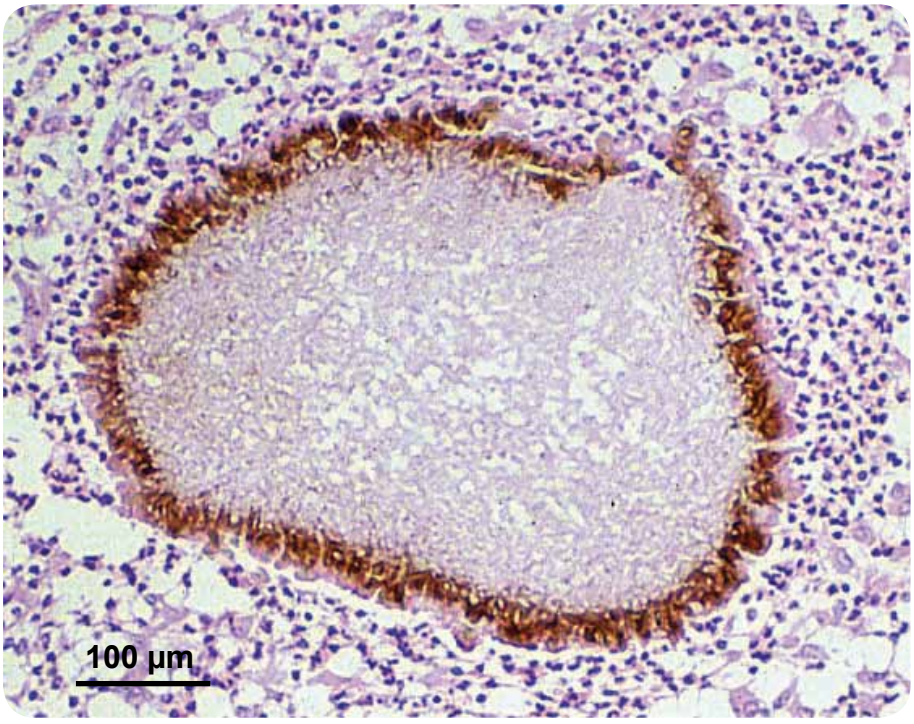
RG-2 organism.

Morphological Description: Colonies are slow growing, dark, leathery, folded with radial grooves and with a light brown to greyish surface mycelium. With age, colonies become dark brown to reddish-brown and have a brownish-black reverse. Microscopically, cultures are sterile, although hyphae of two widths have been described, thin at 1-3 µm in width or broad at 3-5 µm in width. The optimum temperature for growth of *T. grisea* is 30^{O}C; this fungus does not grow at 37^{O}C.

Trematosphaeria grisea can be distinguished from *Madurella mycetomatis* by the inability to grow at 37^{O}C and to assimilate lactose.

Key Features: Black grain mycetoma, no growth at 37^{O}C, no diffusible brown pigment produced on culture and absence of conidia.

References: McGinnis (1980), Chandler *et al*. (1980), Rippon (1988), de Hoog *et al*. (2000, 2015), Ahmed *et al.* (2014b), Desnos-Olliver *et al*. (2006), Irinyi *et al.* (2015).



Trematosphaeria grisea grains (tissue microcolonies) are black, round to lobed, soft to firm, up to 1.0 mm, with two distinctive zones, a hyaline to weakly pigmented central zone and a deeply pigmented periphery.

Magnusiomyces capitatus (de Hoog *et al*.) de Hoog & M.Th. Smith

Synonymy: *Saprochaete capitata* (Diddens & Lodder) de Hoog & M.Th. Smith; *Geotrichum capitatum* (Diddens & Lodder) v. Arx; *Trichosporon capitatum* Diddens & Lodder; *Blastoschizomyces capitis* (Diddens & Lodder) Salkin *et al.*

Based on a phylogenetic analysis of rDNA gene sequences, de Hoog and Smith (2004) transferred *Geotrichum capitatum* to the genus *Magnusiomyces. Magnusiomyces capitatus* occurs quite commonly in humans, usually as a transient component of normal skin flora and sputum. Systemic infections including pulmonary, fungaemia and endocarditis have been reported in immunosuppressed patients. **RG-1 organism.**

Morphological Description: Colonies are moderately fast growing, flat, whitish, and finely suede-like with no reverse pigment. Hyphae are profusely branched at acute angles, with terminal and intercalary conidiogenous cells which form long, cicatrised rachids on which conidia are borne. Conidia are hyaline, smooth, one-celled, cylindrical to clavate, with a rounded apex and flat base, 7-10 x 2.5-3.5 µm. Rectangular arthroconidia are also often present.

Physiological Tests: + Positive, - Negative, v Variable, w Weak, s Slow, nd No Data							
Germ Tube	-	L-Sorbose	v	L-Arabinose	-	D-Glucitol	+
Fermentation		Sucrose	-	D-Arabinose	-	α-M-D-glucoside	-
Glucose	-	Maltose	-	D-Ribose	-	D-Gluconate	-
Galactose	-	Cellobiose	-	L-Rhamnose	-	DL-Lactate	+
Sucrose	-	Trehalose	-	D-Glucosamine	-	*myo*-Inositol	-
Maltose	-	Lactose	-	*N*-A-D-glucosamine	nd	2-K-D-gluconate	-
Lactose	-	Melibiose	-	Glycerol	+	D-Glucuronate	nd
Trehalose	-	Raffinose	-	Erythritol	-	Nitrate	-
Assimilation		Melezitose	-	Ribitol	-	Urease	-
Glucose	+	Soluble Starch	-	Galactitol	-	0.1% Cycloheximide	+
Galactose	+	D-Xylose	-	D-Mannitol	+	Growth at 40^{O}C	+

Molecular Identification: ITS sequencing recommended (de Hoog and Smith 2004).

Note: *Magnusiomyces capitatus* and *Saprochaete clavata* are human pathogens that are closely related and are frequently mistaken for each other. Desnos-Ollivier *et al.* (2014) proposed species-specific carbon assimilation patterns and MALDI-TOF MS profiles to enable the identification of *S. clavata, M. capitatus* and *Geotrichum candidum* to the species level.

References: de Hoog and Smith (2004, 2011c), de Hoog *et al.* (2015), Garcia-Ruiz *et al*. (2013), Desnos-Ollivier *et al.* (2014), Arendrup *et al*. (2014).

Antifungal Susceptibility: ***M. capitatus*** limited data (Garcia-Ruiz *et al*. 2013, Australian National data); **MIC µg/mL**. **Note:** Isolates of *M. capitatus* are intrinsically resistant to echinocandins (Arendrup *et al*. 2014).

	No.	**≤0.03**	**0.06**	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**≥64**
AmB	**9**				**1**	**1**	**4**	**3**					
FLU	**9**							**1**	**1**	**2**	**3**	**2**	
VORI	**8**	**2**	**2**	**2**	**1**		**1**						
POSA	**8**		**2**	**1**	**2**	**2**	**1**						
ITRA	**9**			**1**	**4**	**4**							
5FC	**4**		**2**	**1**		**1**							

Malassezia Baillon

Malassezia species are basidiomycetous yeasts and form part of the normal skin flora of humans and animals. The genus now includes 14 species of which 13 are lipid dependent. These include *M. caprae* (goat, horse), *M. cuniculi* (rabbit), *M. dermatis* (human), *M. equina* (horse, cow), *M. furfur* (human, cow, elephant, pig, monkey, ostrich, pelican), *M. globosa* (human, cheetah, cow), *M. japonica* (human), *M. nana* (cat, cow, dog), *M. obtusa* (human), *M. pachydermatis* (dog, cat, carnivores, birds), *M. restricta* (human), *M. slooffiae* (human, pig, goat, sheep), *M. sympodialis* (human, horse, pig sheep) and *M. yamatoensis* (human) (Cabanes *et al*. 2011).

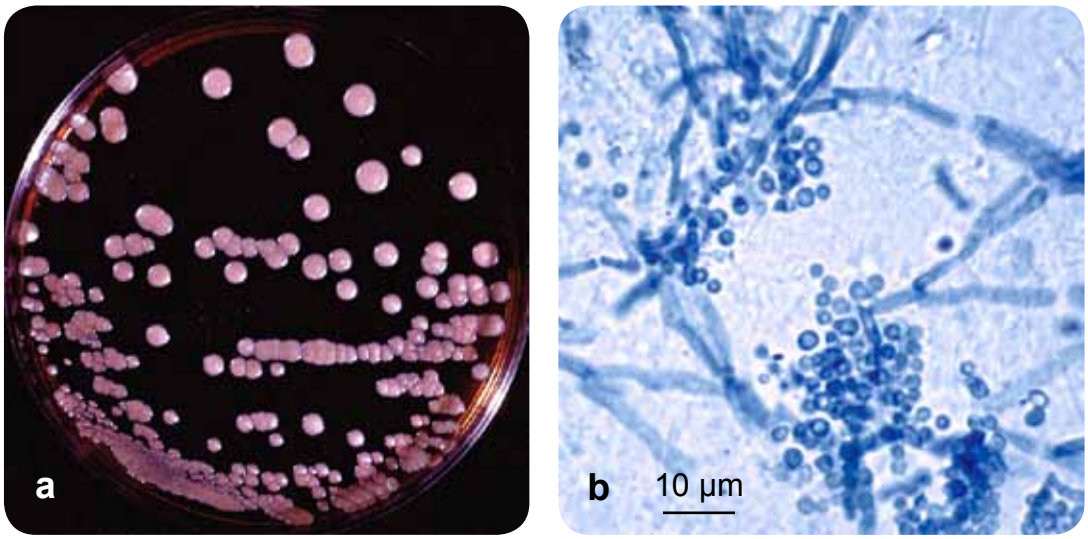
M. sympodialis, M. globosa, M. slooffiae and *M. restricta* are the most frequently found species responsible for colonisation of humans (Arendrup *et al.* 2014).

Malassezia species may cause various skin manifestations including pityriasis versicolor, seborrhoeic dermatitis, dandruff, atopic eczema and folliculitis. *M. pachydermatis* is known to cause external otitis in dogs. Fungaemia due to lipid-dependent *Malassezia* species usually occurs in patients with central line catheters receiving lipid replacement therapy, especially in infants (Tragiannides *et al.* 2010, Gaitanis *et al*. 2012, Arendrup *et al.* 2014).

Note: With the exception of *M. pachydermatis*, the primary isolation and culture of *Malassezia* species is challenging because *in vitro* growth must be stimulated by natural oils or other fatty substances. The most common method used is to overlay Sabouraud's dextrose agar (SDA) containing cycloheximide (actidione) with olive oil or alternatively to use a more specialised media like modified Leeming and Notham agar (Kaneko *et al.* 2007), or modified Dixon's agar (see specialised culture media).

However, CHROMagar *Malassezia* medium is now commercially available for the primary isolation and differentiation of the most common *Malassezia* species.

Comment: For clinical management at the level of the individual patient, species identification is less important, although it is obviously needed for epidemiological surveillance and outbreak investigation (Arendrup *et al*. 2014).



Malassezia furfur (a) culture on modified Dixon's agar and (b) direct microscopy of skin scrapings showing characteristic clusters of thick-walled round, budding yeast-like cells and short angular hyphal forms (the so called "spaghetti and meatballs" appearance) typically seen in pityriasis versicolor.

Malassezia Baillon

RG-1 organisms.

Morphological Description: On media like modified Dixon's agar, colonies are cream to yellowish, smooth or lightly wrinkled, glistening or dull, and with the margin being either entire or lobate. *Malassezia* is characterised by globose, oblong-ellipsoidal to cylindrical yeast cells. Reproduction is by budding on a broad base and from the same site at one pole (unipolar).

Molecular Identification: ITS and D1/D2 sequencing may be used for accurate species identification (de Hoog *et al*. 2015).

MALDI-TOF MS: Capable of identifying all 14 *Malassezia* species in concordance with those of ITS sequence analyses (Kolecka *et al.* 2014).

Identification criteria for the differentiation of *Malassezia* species (de Hoog *et. al.* 2015).
+ Positive, - Negative, v Variable, w Weak, s Slow, nd No Data

Species	Buds	SDA	40°C	Cremophor EL	Tween 80	Tween 60	Tween 40	Tween 20	Esculine	Catalase
M. caprae	narrow	-	-	-	+	+	+	+	+	+
M. couiculi	narrow	-	+	-	-	-	-	-	nd	+
M. dermatis	wide	-	+	nd	+	+	+	+	nd	+
M. equina	narrow	-	-	-	+	+	+	+	+	+
M. furfur	wide	-	+	+	+		+	+	w	+
M. globosa	narrow	-	-	-	-		-	-	-	+
M. japonica	wide	-	-	nd	-	+	w	-	nd	+
M. nana	narrow	-	v	nd	w	+	+	v	nd	+
M. obtusa	wide	-	-	-	-		-	-	+	+
M. pachydermatis	wide	+	+	-,w	+		+	-,w	v	v
M. restricta	narrow	-	-	-	-		-	-	-	-
M. slooffiae	wide	-	+	-	+,w		+	+	-	+
M. sympodialis	narrow	-	+	-,w	+	+	+	+	+	+
M. yamatoensis	wide	-	-	nd	+	+	+	+	nd	+

References: Guillot and Gueho (1995), Gueho *et al.* (1996), Guillot *et al*. (1996, 2000), Boekhout *et al.* (2010), Cafarchia *et al*. (2011), de Hoog *et al*. (2015).

Antifungal Susceptibility: Very limited data available. Special growth conditions are needed for antifungal susceptibility testing; data from Nakamura *et al*. (2000), Velegraki *et al.* (2004) and Miranda *et al*. (2007); **MIC µg/mL.**

Antifungal	MIC µg/mL		Antifungal	MIC µg/mL	
	Range	MIC_{90}		Range	MIC_{90}
FLU	0.125->64	4 (8)	**AmB**	0.03-16	1 (8)
ITRA	0.03-16	0.125	**VORI**	0.03-16	0.125 (1)
KETO	0.03-4	0.25	**POSA**	0.03-32	0.125 (2)

Note: There is no standardised method and results are often variable, therefore susceptibility testing for guiding treatment is not recommended.

Malbranchea pulchella Sacc. & Penz.

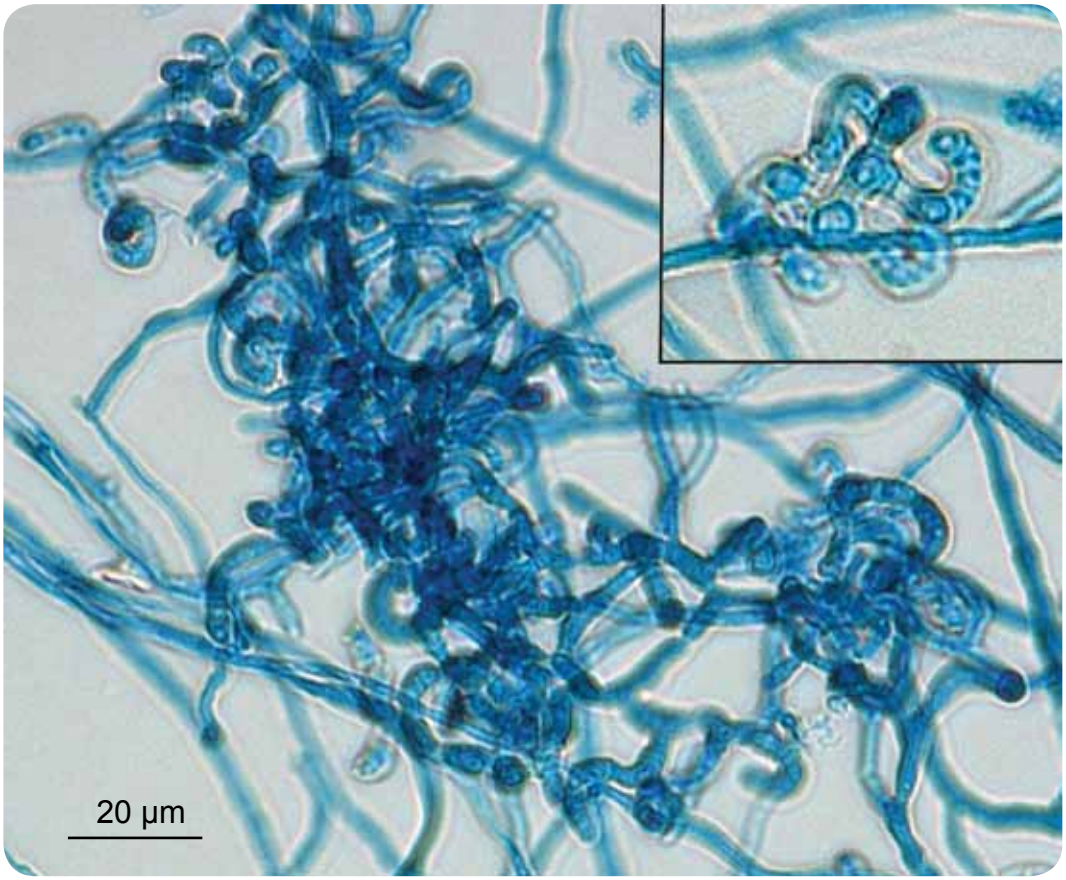
Malbranchea species are soil fungi of worldwide distribution which microscopically may resemble *Coccidioides immitis/posadasii.* **Note:** Culture identification by exoantigen test or ITS sequencing is the method of choice for identification of *C. immitis/posadasii.*

RG-1 organism.

Morphological Description: Colonies are white to sulphur-yellow to ochre-brown in colour, suede-like in texture, with a reddish-brown reverse, and often a reddish diffusible pigment. Microscopic morphology shows typical hyaline, one-celled, cylindrical, truncate, alternate arthroconidia produced in tightly coiled, terminal fertile branches of the hyphae. Arthroconidia are released by lysis of the disjunctor cells. These arthroconidia may be perceived as a yellow dust when released at maturity.

Key Features: Hyphomycete producing alternate arthroconidia with disjunctor cells.

References: Cooney and Emerson (1964), Sigler and Carmichael (1976), McGinnis (1980), Rippon (1988), de Hoog *et al.* (2000, 2015).



Malbranchea pulchella arthroconidia produced in tightly coiled, terminal fertile branches of the hyphae.

Meyerozyma guilliermondii (Wick.) Kurtzman & M. Suzuki

Synonymy: *Candida guilliermondii* (Castellani) Langeron & Guerra.

Meyerozyma guilliermondii has been isolated from numerous human infections, mostly of cutaneous origin. It is also found on normal skin and in sea water, faeces of animals, fig wasps, buttermilk, leather, fish and beer. **RG-1 organism.**

Culture: White to cream-coloured smooth, glabrous, yeast-like colonies.

Microscopy: Spherical to subspherical budding yeast-like cells or blastoconidia, 2.0-4.0 x 3.0-6.5 µm.

India Ink Preparation: Negative - no capsules present.

Dalmau Plate Culture: Branched pseudohyphae with dense verticils of blastoconidia.

Molecular Identification: ITS sequencing recommended.

MALDI-TOF MS: Able to accurately identify this species.

Physiological Tests: + Positive, - Negative, v Variable, w Weak, s Slow, nd No Data							
Germ Tube	-	L-Sorbose	v	L-Arabinose	v	D-Glucitol	v
Fermentation		Sucrose	+	D-Arabinose	v	α-M-D-glucoside	v
Glucose	+	Maltose	+	D-Ribose	+	D-Gluconate	v
Galactose	v	Cellobiose	v	L-Rhamnose	v	DL-Lactate	v
Sucrose	+	Trehalose	+	D-Glucosamine	+	*myo*-Inositol	-
Maltose	-	Lactose	-	*N*-A-D-glucosamine	+	2-K-D-gluconate	+
Lactose	-	Melibiose	v	Glycerol	+	D-Glucuronate	nd
Trehalose	+	Raffinose	+	Erythritol	-	Nitrate	-
Assimilation		Melezitose	v	Ribitol	+	Urease	-
Glucose	+	Soluble Starch	-	Galactitol	v	0.1% Cycloheximide	v
Galactose	+	D-Xylose	+	D-Mannitol	v	Growth at 37°C	v

Key Features: Germ tube negative yeast and sugar assimilation pattern.

Antifungal Susceptibility: ***M. guilliermondii*** (Diekema *et al*. 2009, Australian National data); **MIC µg/mL.** CLSI clinical breakpoints are marked where available (Pfaller and Diekema 2012).

	No.	≤0.008	0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	≥64
AmB	198	1	1	2	9	70	70	27	9	2	1	1			5
FLU	199						1	4	5	75	75	7	11	4	7
VORI	197	2	11	23	88	52	9	3	1	2			6		
POSA	194		1	9	14	47	79	30	4	4			6		
ITRA	24					6	3	12	2	1					
ANID	120		1		2	6	5	9	41	48	8				
MICA	13		3	1	4	10	14	39	29	8			1		
CAS	175			2	9	24	35	63	25	8	2	3	4		
5FC	24			8	12	1	1	1	1						

Microsphaeropsis arundinis (S. Ahmad) B. Sutton

Microsphaeropsis arundinis is a coelomycete that is ubiquitous in soil and fresh water. It typically inhabits terrestrial plant hosts and has a well-known association with *Aruno donax*, a garden escape weed known as 'giant reed' or 'elephant grass'. *M. arundinis* is an emerging cause of phaeohyphomycosis in cats and immunosuppressed humans.

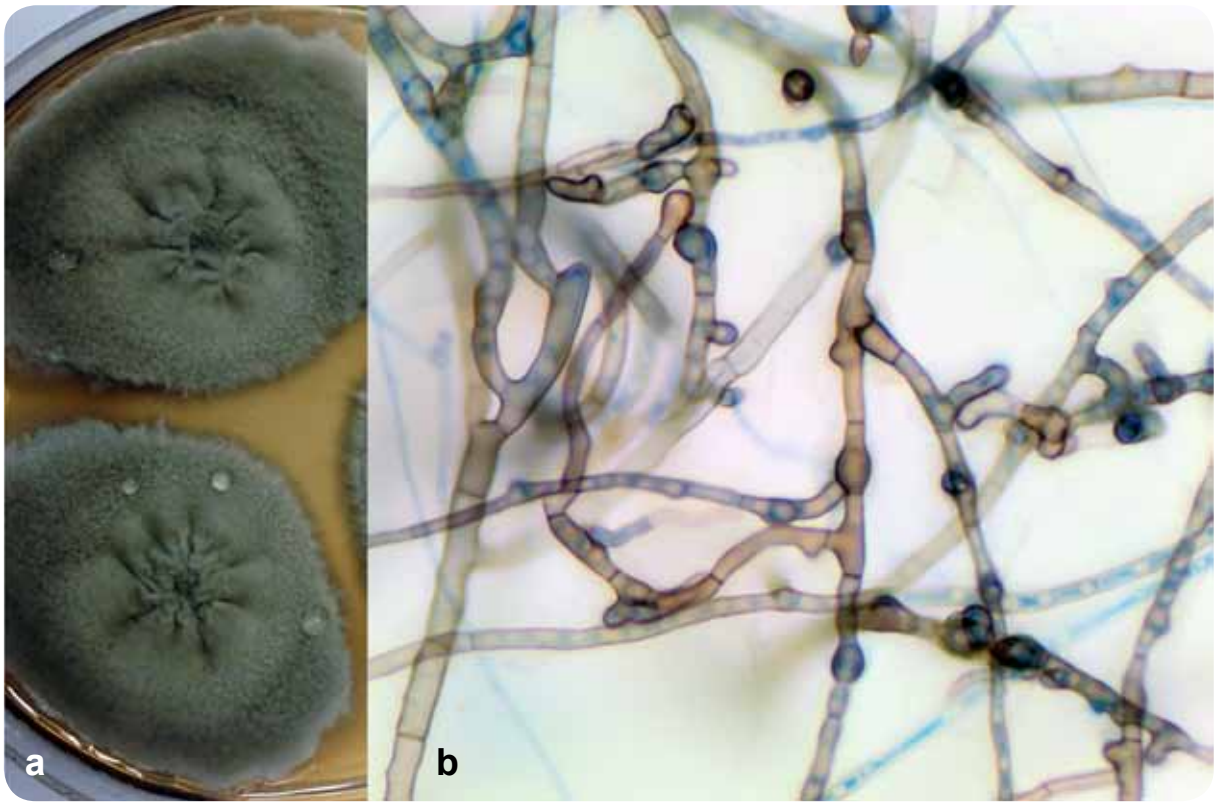
RG-1 organism.

Morphological Description: Colonies growing slowly, with dense aerial mycelium, initially greenish-grey, later becoming dark brown to grey-brown. Hyphae are septate, pigmented, and irregularly shaped, with swollen segments up to 4 µm in diameter. Pycnidia are subspherical, 250-350 µm in diameter; with a pseudoparenchymatous wall composed of very densely packed cells that appear angular in cross section (*textura angularis*). Conidiogenous cells ampulliform, up to 5 µm long. Conidia brown, thick- and smooth-walled, cylindrical, 3.5-4.5 × 1.0-1.5 µm.

Key Features: Coelomycete, with ostiolate pycnidia, ampulliform conidiogenous cells, and small, smooth-walled, brown, cylindrical conidia.

Molecular Identification: ITS and D1/D2 sequencing is recommended, especially as it may take many weeks before pycnidia are produced in culture (Reppas *et al*. 2015).

References: Kluger *et al*. (2004), Pendle *et al*. (2004), Krockenberger *et al*. (2010), Hall *et al*. (2013), Reppas *et al.* (2015), de Hoog *et al*. (2015).



Microsphaeropsis arundinis (a) culture and (b) pigmented septate hyphae, with swollen segments.

Microsporum Gruby

A recent multilocus phylogenetic study the has reviewed the taxonomy of the dermatophytes. *Arthroderma* now contains 21 species, *Epidermophyton* one species, *Lophophyton* one species, *Microsporum* three species, *Nannizzia* nine species and *Trichophyton* 16 species. In addition, two new genera have been introduced: *Guarromyces* containing one species and *Paraphyton* three species (de Hoog *et al.* 2016).

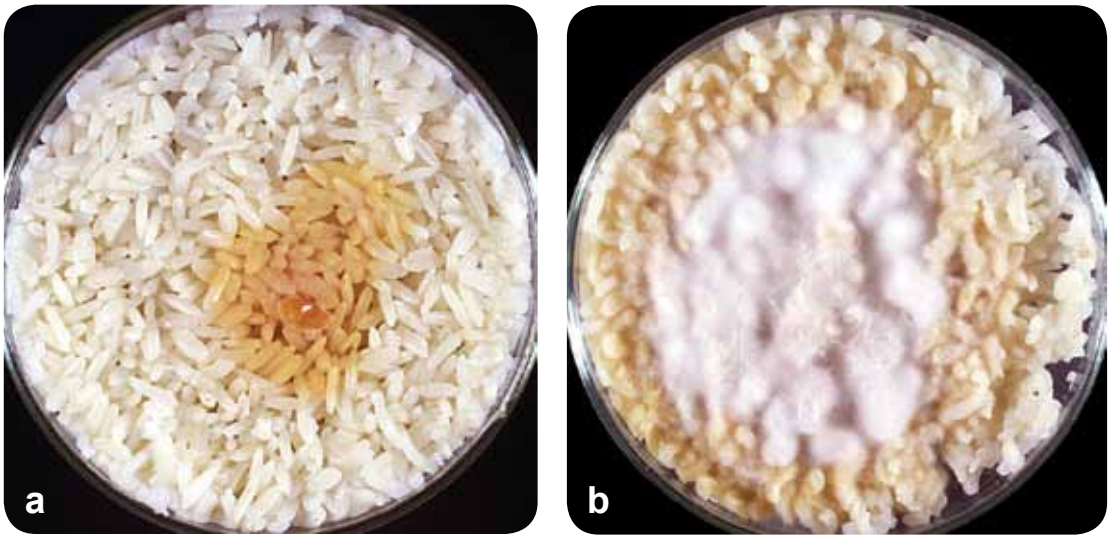
The genus *Microsporum* is now restricted to just three species: *M. audouinii, M. canis* and *M. ferrugineum*. The remaining geophilic and zoophilic species, previously considered *Microsporum* species, have been transferred to the genera *Lophophyton* and *Nannizzia*.

Microsporum species may form both macro- and microconidia, although they are not always present. Cultures are mostly granular to cottony, yellowish to brownish, with a cream-coloured or brown colony reverse. Macroconidia are hyaline, multiseptate, with thick rough cell walls, and are clavate, fusiform or spindle-shaped. Microconidia are single-celled, hyaline, smooth-walled, and are predominantly clavate in shape.

Note: Strains of *M. canis* often do not produce macroconidia and/or microconidia on primary isolation media and subcultures onto polished rice grains or lactritmel agar are recommended to stimulate sporulation. These non-sporulating strains of *M. canis* are often erroneously identified as *M. audouinii* and it is surprising just how many laboratories have difficulty in differentiating *M. canis* and *M. audouinii.*

Molecular Identification: ITS sequencing is recommended (Gräser *et al.* 1998, 2000, Brillowska-Dabrowska *et al.* 2013).

References: Rebell and Taplin (1970), Rippon (1988), McGinnis (1980), Domsch *et al.* (1980), Ajello (1977), Weitzman *et al.* (1986), Mackenzie *et al*. (1986), Kane *et al.* (1997), de Hoog *et al.* (2000, 2015), Gräser *et al*. (1999a, 2008). Cafarchia *et al.* (2013).



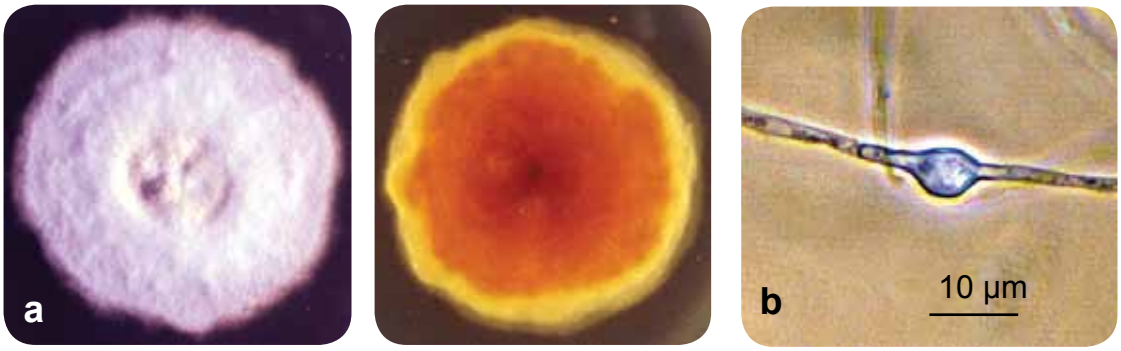
(a) *Microsporum audouinii* showing poor growth on rice grains, usually being visible only as a brown discolouration. (b) *Microsporum canis* on rice grains showing good growth, yellow pigmentation and sporulation.

Microsporum audouinii Gruby

Microsporum audouinii is an anthropophilic fungus causing non-inflammatory infections of the scalp and skin, especially in children. Once the cause of epidemics of tinea capitis in Europe and North America, it is now less common. Invaded hairs show an ectothrix infection and usually fluoresce a bright greenish-yellow under Wood's ultra-violet light. Only rarely found in Australasia, most reports are in fact misidentified non-sporulating strains of *M. canis*.

RG-2 organism.

Morphological Description: Colonies are flat, spreading, greyish-white to light tan-white in colour, and have a dense suede-like to downy surface, suggestive of mouse fur in texture. Reverse can be yellow-brown to reddish-brown in colour. Some strains may show no reverse pigment. Macroconidia and microconidia are rarely produced, most cultures are sterile or produce only occasional thick-walled terminal or intercalary chlamydospores. When present, macroconidia may resemble those of *M. canis* but are usually longer, smoother and more irregularly fusiform in shape; microconidia, when present, are pyriform to clavate in shape and are similar to those seen in other species of *Microsporum*, *Lophophyton* and *Nannizzia.* Pectinate (comb-like) hyphae and racquet hyphae (a series of hyphal segments swollen at one end) may also be present.



Microsporum audouinii (a) Culture and (b) a thick-walled intercalary chlamydospore. **Note:** Macroconidia and microconidia are only rarely produced.

Confirmatory Tests:

Growth on Rice Grains: Very poor or absent, usually being visible only as a brown discolouration. This is one of the features which distinguish *M. audouinii* from *M. canis.*

Reverse Pigment on Potato Dextrose Agar: Salmon to pinkish-brown (*M. canis* is bright yellow).

BCP Milk Solids Glucose Agar: Both *M. canis* and *M. audounii* demonstrate profuse growth, but only *M. audouinii* shows a rapid pH change to alkaline (purple colour).

Vitamin Free Agar (Trichophyton Agar No.1): Good growth indicating no special nutritional requirements. Cultures are flat, white, suede-like to downy, with a yellow-brown reverse. **Note:** Growth of some strains of *M. audouinii* is enhanced by the presence of thiamine (Trichophyton agar No.4).

Hair Perforation Test: Negative after 28 days.

Key Features: Absence of conidia, poor or no growth on polished rice grains, inability to perforate hair *in vitro,* and culture characteristics.

Microsporum canis Bodin

Microsporum canis is a zoophilic dermatophyte of worldwide distribution and is a frequent cause of ringworm in humans, especially children. Invades hair, skin and rarely nails. Cats and dogs are the main sources of infection. Invaded hairs show an ectothrix infection and fluoresce a bright greenish-yellow under Wood's ultra-violet light.

RG-2 organism.

Morphological Description: Colonies are flat, spreading, white to cream-coloured, with a dense cottony surface which may show some radial grooves. Colonies usually have a bright golden yellow to brownish yellow reverse pigment, but non-pigmented strains may also occur. Macroconidia are typically spindle-shaped with 5-15 cells, verrucose, thick-walled and often have a terminal knob, 35-110 x 12-25 µm. A few pyriform to clavate microconidia are also present. Macroconidia and/or microconidia are often not produced on primary isolation media and it is recommended that subcultures be made onto lactritmel agar and/or boiled polished rice grains to stimulate sporulation.

Confirmatory Tests:

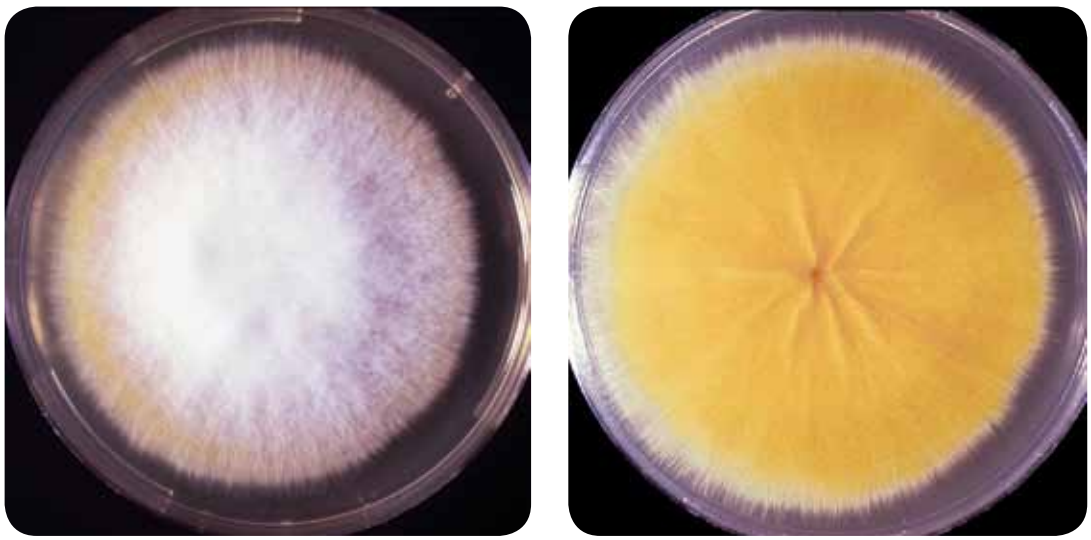
Growth on Rice Grains: good growth of white aerial mycelium with production of yellow pigment. Microscopy reveals numerous macroconidia and microconidia similar to those described above.

Reverse Pigment on Potato Dextrose Agar: Bright yellow (both *M. audouinii* and *M. canis* var. *equinum* are salmon to pinkish-brown).

Vitamin Free Agar (Trichophyton Agar No.1): Good growth indicating no special nutritional requirements. Cultures are flat, white, suede-like to downy, with a yellow to pale yellow-brown reverse.

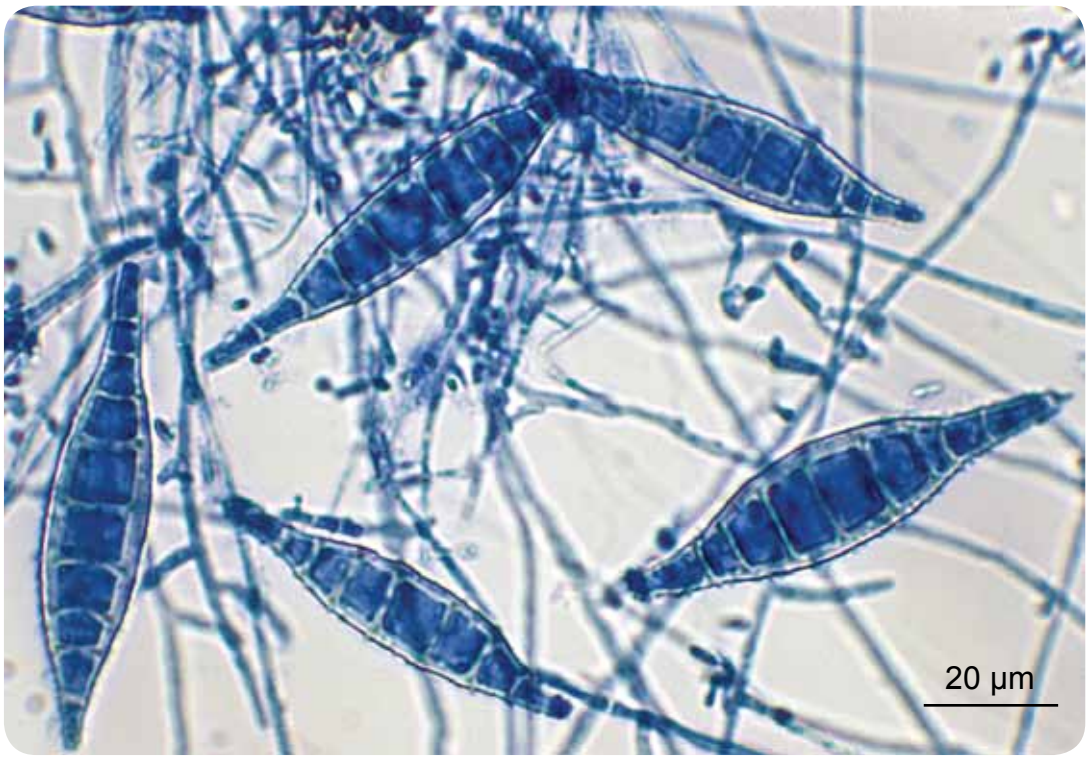
Hair Perforation Test: Positive at 14 days.

Key Features: Distinctive macroconidia and culture characteristics. Abundant growth and sporulation on polished rice grains and *in vitro* perforation of hair.



Microsporum canis culture showing a bright golden yellow reverse pigment

Microsporum canis Bodin



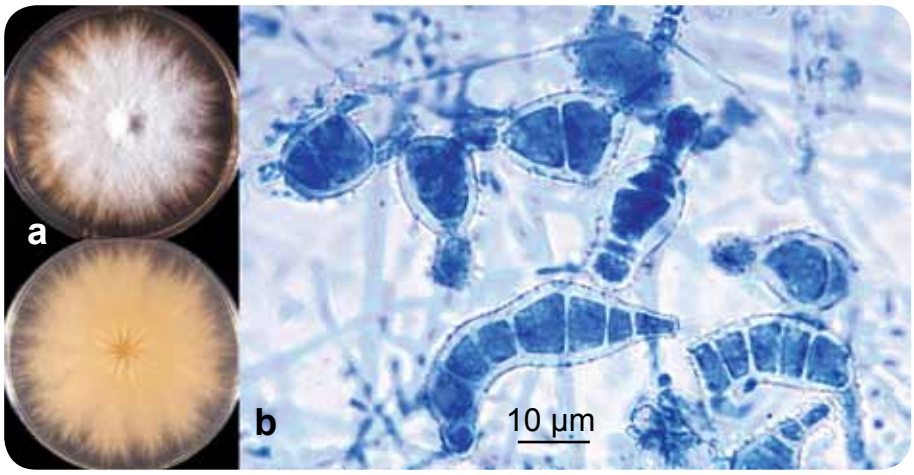
Microsporum canis typical spindle-shaped macroconidia.

Microsporum canis dysgonic strains are rare but may also occur. These dysgonic strains typically have a heaped and folded, yellow-brown thallus and macroconidia are usually absent. However, typical colonies and macroconidia of *M. canis* are usually produced by this variant when subcultured onto polished rice grains. **Note:** The dysgonic type colony of *M. canis* is similar to that of *Microsporum ferrugineum*.

Microsporum canis Bodin

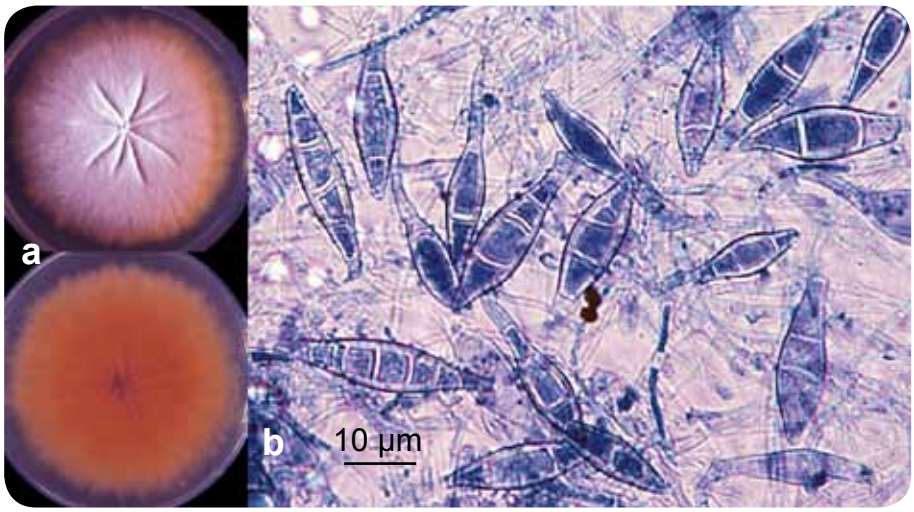
Supplementary description for *Microsporum canis* var. *distortum,* a dysgonic variant of *M. canis* with distinctive distorted macroconidia. Abundant growth and sporulation on rice grains.

Microsporum canis var. *distortum* is a zoophilic fungus known to cause infections in cats, dogs and other animals. It is a rare cause of tinea capitis in New Zealand, Australia and North America. Clinical disease is similar to *M. canis*. Invaded hairs show an ectothrix infection and fluoresce a bright greenish-yellow under Wood's ultra-violet light.



Microsporum canis var. *distortum* (a) culture and (b) distorted macroconidia.

Microsporum canis var. *equinum* is now considered to be a genotypic synonym of *Microsporum canis* (de Hoog *et al*. 2000). This variant of *M. canis* is a rare cause of ringworm of horses. Invaded hairs show an ectothrix infection and fluoresce a bright greenish-yellow under Wood's ultra-violet light. Rarely infects man or other animal species. Reported from Australia, Europe and North America.



Microsporum canis var. *equinum* (a) colonies are pale buff to pale salmon with, a buff to pinkish-buff to yellow-brown reverse. (b) Macroconidia are small, broad, irregular, spindle-shaped, 18-60 x 5-15 µm with rough thick walls and few septa. Microconidia are pyriform to clavate in shape, 3-9 x 1.5-3.5 µm, but are rarely produced.

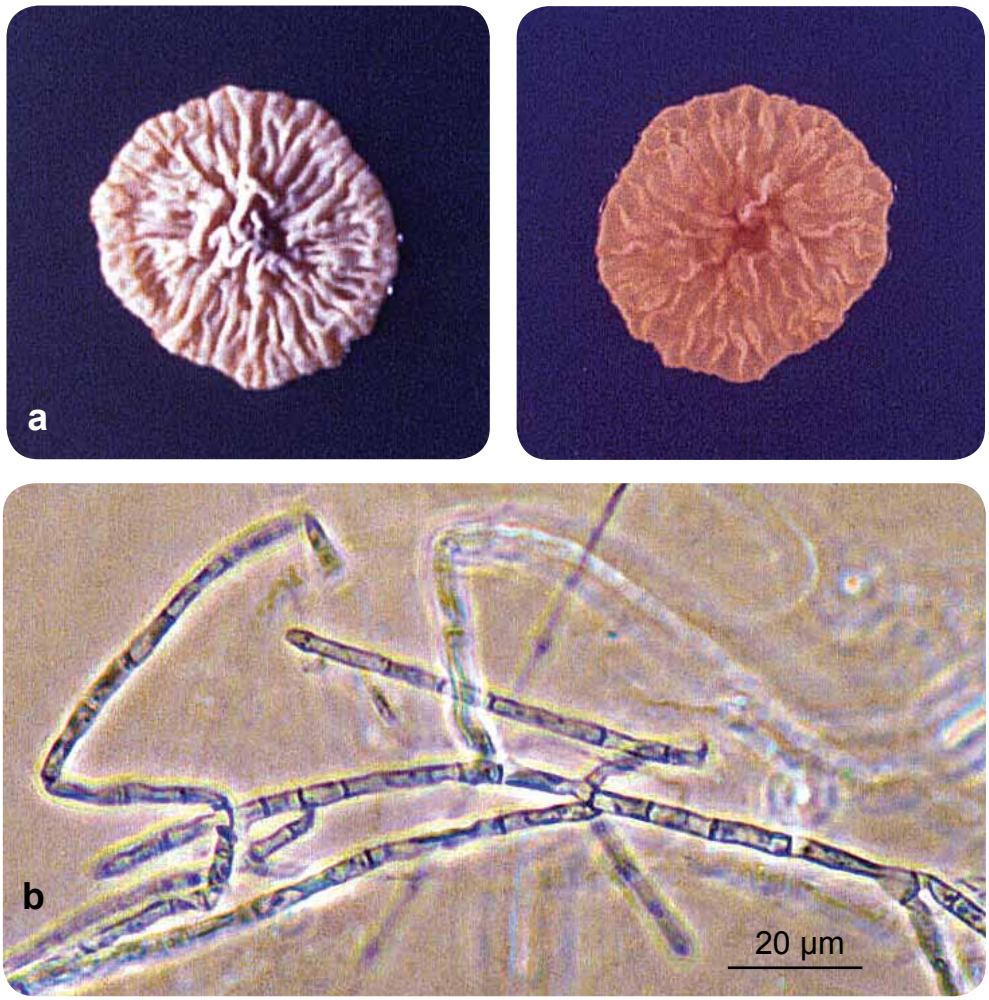
Microsporum ferrugineum Ota

Microsporum ferrugineum is an anthropophilic fungus causing epidemic juvenile tinea capitis in humans. The clinical features are similar to those of infections caused by *M. audouinii*. Invaded hairs show an ectothrix infection and fluoresce a greenish-yellow under Wood's ultra-violet light. Reported from Asia (including China and Japan), Russia, Eastern Europe and Africa.

RG-2 organism.

Morphological Description: Colonies are slow growing, forming a waxy, glabrous, convoluted thallus with a cream to buff-coloured surface and no reverse pigment. **Note:** Surface pigmentation may vary from cream to yellow to deep red and a flatter white form sometimes occurs. Cultures rapidly become downy and pleomorphic. Microscopic morphology is negative, microconidia or macroconidia are not produced. However, irregular branching hyphae with prominent cross walls ("bamboo hyphae") and chlamydospores are seen. "Bamboo hyphae" are a characteristic of this species.

Key Features: Clinical history, culture characteristics and distinctive "bamboo hyphae".



Microsporum ferrugineum (a) culture and (b) "bamboo hyphae".

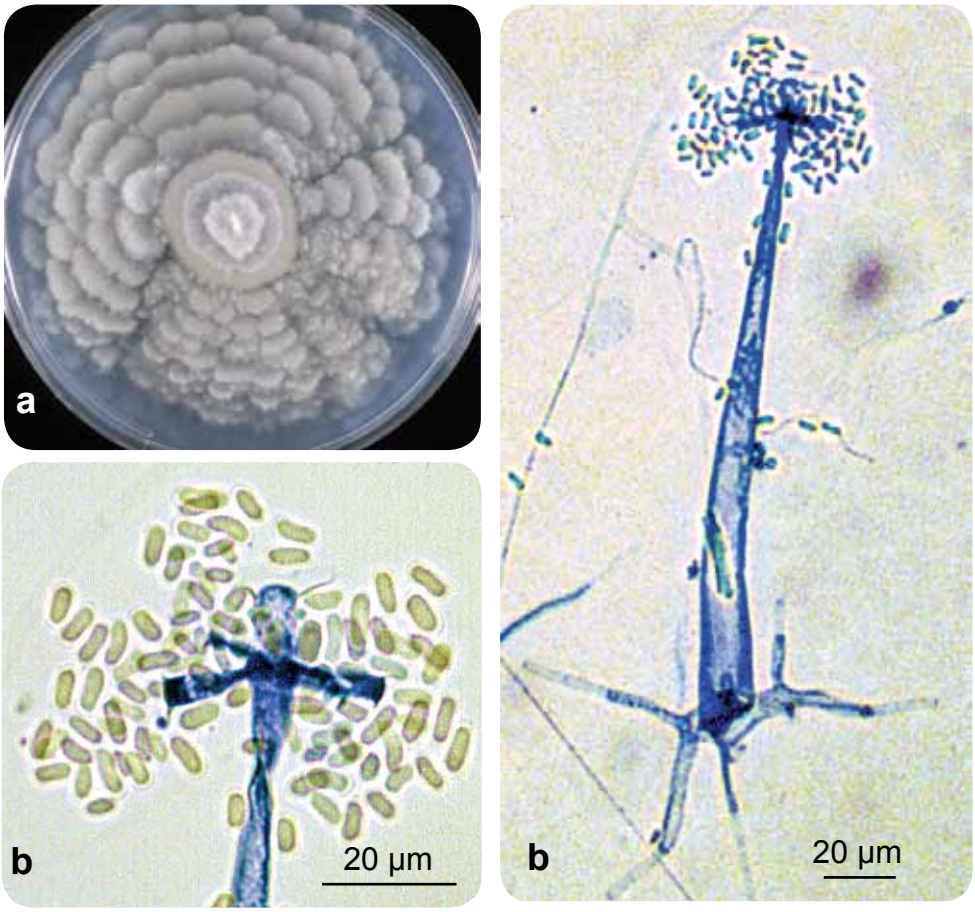
Mortierella wolfii Mehrotra & Baijal

The genus *Mortierella* has now been placed in a separate order, the Mortierellales (Cavalier-Smith 1998). The genus contains about 90 recognised species, however *Mortierella wolfii* is probably the only pathogenic species being an important causal agent of bovine mycotic abortion, pneumonia and systemic mycosis in New Zealand, Australia, Europe and USA. **RG-2 organism.**

Morphological Description: Cultures are fast growing, white to greyish-white, downy, often with a broadly zonate or lobed (rosette-like) surface appearance and no reverse pigment. Sporangiophores are typically erect, delicate, 80-250 µm in height, 6-20 µm wide at the base, arising from rhizoids or bulbous swellings on the substrate hyphae and terminating with a compact cluster of short acrotonous (terminal) branches. Sporangia are usually 15-48 µm in diameter, with transparent walls and a conspicuous collarette is usually present following dehiscence of the sporangiospores. Columellae are generally lacking and sporangiospores are single-celled, short-cylindrical, 6-10 x 3-5 µm, with a double membrane. Chlamydospores with or without blunt appendages (amoeba-like) may be present, zygospores have not been observed. Temperature: grows well at 40-42^{O}C; maximum 48^{O}C.

Key Features: Mucorales, rapid growth at 40^{O}C (thermotolerant), and characteristic delicate acrotonous branching sporangia without columellae.

References: Domsch *et al.* (1980), McGinnis (1980), Rippon (1988), de Hoog *et al.* (2000, 2015).



Mortierella wolfii (a) culture showing a broadly zonate or lobed rosette-like surface appearance, and (b) sporangium, showing a sporangiophore, wide at the base, arising from rhizoids, and acrotonous (terminal) branches, collarettes and sporangiospores.

Mucor Micheli ex Staint-Amans

The genus *Mucor* contains about 50 recognised taxa, many of which have widespread occurrence and are of considerable economic importance (Zycha *et al.* 1969, Schipper 1978, Domsch *et al.* 1980). However, only a few thermotolerant species are of medical importance and human infections are only rarely reported. Most infections reported list *M. circinelloides* and similar species such as *M. indicus*, *M. ramosissimus, M. irregularis* and *M. amphibiorum* as the causative agents. However, *M. hiemalis* and *M. racemosus* have also been reported as infectious agents, although their inability to grow at temperatures above 32^{O}C raises doubt as to their validity as human pathogens and their pathogenic role may be limited to cutaneous infections (Scholer *et al.* 1983, Goodman and Rinaldi 1991, Kwon-Chung and Bennett 1992, de Hoog *et al*. 2000, 2015).

Maximum temperature for growth of the reported pathogenic species of *Mucor.*

Species	Max temp. (^{O}C)	Pathogenicity
M. amphibiorum	36	Animals, principally amphibians
M. circinelloides	37	Animals, occasionally humans
M. hiemalis	30	Questionable cutaneous infections only
M. indicus	42	Humans and animals
M. irregularis	38	Humans
M. racemosus	32	Questionable
M. ramosissimus	36	Humans and animals

Morphological Description: Colonies are very fast growing, cottony to fluffy, white to yellow, becoming dark-grey, with the development of sporangia. Sporangiophores are erect, simple or branched, forming large (60-300 µm in diameter), terminal, globose to spherical, multispored sporangia, without apophyses and with well-developed subtending columellae. A conspicuous collarette (remnants of the sporangial wall) is usually visible at the base of the columella after sporangiospore dispersal. Sporangiospores are hyaline, grey or brownish, globose to ellipsoidal, and smooth-walled or finely ornamented. Chlamydospores and zygospores may also be present.

Key Features: Mucorales, large, spherical, non-apophysate sporangia with pronounced columellae and conspicuous collarette at the base of the columella following sporangiospore dispersal.

Molecular Identification: ITS sequencing recommended (Walther *et al.* 2012).

References: Schipper (1978), Domsch *et al.* (1980), McGinnis (1980), Onions *et al.* (1981), Scholer *et al*. (1983), Rippon (1988), Goodman and Rinaldi (1991), Samson *et al.* (1995), de Hoog *et al*. (2000, 2015), Schipper and Stalpers (2003), Ellis (2005b).

Mucor Micheli ex Staint-Amans

Mucor spp. showing sporangia, columella with inconspicuous collarette (arrow) and sporangiospores.

Mucor amphibiorum Schipper

RG-2 organism.

Morphological Description: Colonies are greyish-brown, slightly aromatic and do not grow at 37°C (maximum temperature for growth is 36°C). Sporangiophores are hyaline, erect and mostly unbranched, rarely sympodially branched. Sporangia are dark-brown, up to 75 µm in diameter, and are slightly flattened with a diffluent membrane. Columellae are subglobose to ellipsoidal or pyriform, up to 60 x 50 µm, with small collarettes. Sporangiospores are smooth-walled, spherical, and 3.5-5.5 µm in diameter. Zygospores, when formed by compatible mating types, are spherical to slightly compressed, up to 70 x 60 µm in diameter, with stellate projections.

Comment: *Mucor amphibiorum* is distinguished by poor branching of the sporangiophores and by globose sporangiospores. Ethanol and nitrates are not assimilated (Schipper 1978, Scholer *et al.* 1983, Hoog *et al.* 2000, 2015).

Antifungal Susceptibility: ***M. amphibiorum*** very limited data (Australian National data); **MIC µg/mL.**

	No.	≤0.016	0.03	0.06	0.125	0.25	0.5	1	2	4	8	≥16
AmB	**1**						**1**					
POSA	**1**						**1**					
ITRA	**1**							**1**				

Mucor circinelloides v. Tiegh

M. circinelloides is a common and variable species that includes four formae: *circinelloides, lusitanicus, griseocyanus* and *janssenii* (Schipper 1978, Scholer *et al*. 1983).

RG-1 organism.

Morphological Description: Colonies are floccose, pale greyish-brown and grow poorly at 37^{O}C (maximum growth temperature 37^{O}C). Sporangiophores are hyaline and mostly sympodially branched with long branches erect and shorter branches becoming circinate (coiled). Sporangia are spherical, varying from 20-80 µm in diameter, with small sporangia often having a persistent sporangial wall. Columellae are spherical to ellipsoidal and are up to 50 µm in diameter. Sporangiospores are hyaline, smooth-walled, ellipsoidal, and 4.5-7 x 3.5-5 µm in size. Chlamydospores are generally absent. Zygospores are only produced in crosses of compatible mating types and are reddish-brown to dark-brown, spherical with stellate spines, up to 100 µm in diameter and have equal to slightly unequal suspensor cells.

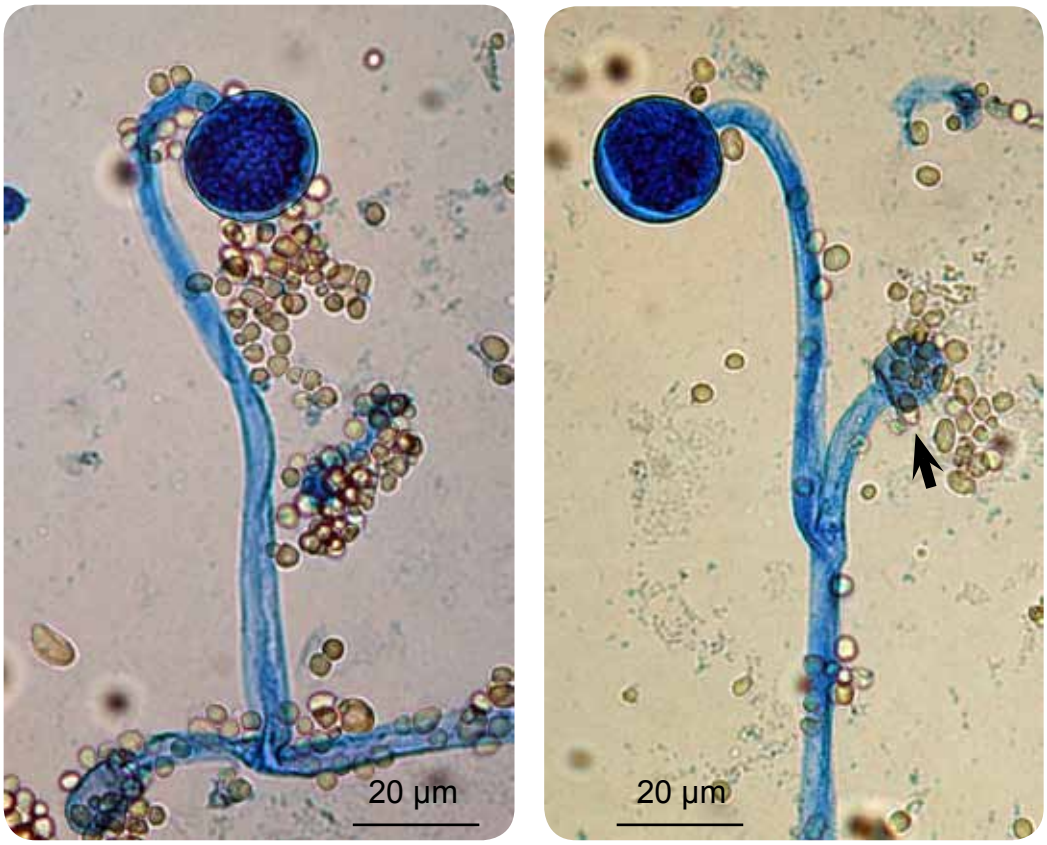
Comment: *M. circinelloides* differs from other species of *Mucor* in its formation of short circinated, branched sporangiophores bearing brown sporangia and its ability to assimilate ethanol and nitrates (Schipper 1976, Scholer *et al.* 1983, Samson *et al.* 1995, de Hoog *et al*. 2000, Schipper and Stalpers 2003).

Antifungal Susceptibility: *M. circinelloides* (Espinel-Ingroff *et al*. 2015a, Australian National data); **MIC µg/mL**.

	No.	≤0.016	0.03	0.06	0.125	0.25	0.5	1	2	4	8	≥16
AmB	123		1	4	14	42	44	18				
POSA	120			2	2	9	21	49	26	5	2	4
ITRA	48					4	3	7	12	15	5	3

Mucor circinelloides culture.

Mucor circinelloides v. Tiegh



Mucor circinelloides sporangia showing circinate sporangiophores, also note the columella with inconspicuous collarette (arrow) and sporangiospores.

Mucor indicus Lendner

Morphological Description: Colonies are characteristically deep-yellow, aromatic and have a maximum growth temperature of 42°C. Sporangiophores are hyaline to yellowish, erect or rarely circinate and repeatedly sympodially branched, with long branches. Sporangia are yellow to brown, up to 75 µm in diameter, with diffluent membranes. Columellae are subglobose to pyriform, often with truncate bases, up to 40 µm high. Sporangiospores are smooth-walled, subglobose to ellipsoidal, and 4-5 µm in diameter. Chlamydospores are produced in abundance, especially in the light. Zygospores are black, spherical up to 100 µm in diameter, with stellate spines and unequal suspensor cells. **RG-1 organism.**

Comment: *Mucor indicus* differs from other species of *Mucor* by its characteristic deep-yellow colony colour, growth at over 40°C, assimilating ethanol, but not nitrate, and thiamine dependence (Schipper 1978, de Hoog *et al.* 2000, Schipper and Stalpers 2003).

Antifungal Susceptibility: ***M. indicus*** (Espinel-Ingroff *et al*. 2015a, Australian National data); **MIC µg/mL.**

	No.	≤0.016	0.03	0.06	0.125	0.25	0.5	1	2	4	8	≥16
AmB	**10**		**1**		**3**	**4**	**1**	**1**				
POSA	**10**					**2**	**3**	**3**	**1**		**1**	

Mucor irregularis Stchigel *et al*.

Synonymy: *Rhizomucor variabilis* Zheng & Chen.

RG-2 organism.

Morphological Description: Colonies are whitish to ochraceous, with buff-ochre reverse. Sporangiophores arising from hyphae or from stolons; rhizoids abundant. Sporangiophores are hyaline, up to 2 mm long, 9-23 µm wide, simple or once branched, with branches terminating at a higher level than the main stems; branches all ending in a sporangium. Sporangia subspherical to spherical, up to 100 µm diameter. Columella spherical, ellipsoidal to cylindrical, about 40 µm wide, sometimes lobed, with or without an apophysis. Sporangiospores are hyaline, smooth-walled, very variable, mostly subspherical to ellipsoidal, 3-11 × 2-7 µm. Chlamydospores are abundant. Maximum growth temperature 38^{O}C.

Comment: *Mucor irregularis* differs from other species of *Mucor* by having abundant rhizoids of different sizes and sporangiospores of highly variable shape, mostly subspherical to ellipsoidal (Lu *et al*. 2009, 2013). This species is closely related to *Mucor hiemalis* (Voigt *et al*. 1999).

Mucor ramosissimus Samutsevich

RG-1 organism.

Morphological Description: Colonial growth is restricted, greyish and does not grow at 37^{O}C (maximum temperature for growth is 36^{O}C). Sporangiophores are hyaline, slightly roughened, tapering towards the apex and are erect with repeated sympodial branching. Sporangia are grey to black, globose or somewhat flattened, up to 80 µm in diameter and have very persistent sporangial walls. Columellae are applanate (flattened), up to 40-50 µm in size and are often absent in smaller sporangia. Sporangiospores are faintly brown, smooth-walled, subglobose to broadly ellipsoidal, 5-8 x 4.5-6 µm in size. Oidia may be present in the substrate hyphae, chlamydospores and zygospores are absent. Assimilation of ethanol is negative and that of nitrate is positive.

Comment: *Mucor ramosissimus* differs from other species of *Mucor* by its low, restricted growth on any medium, extremely persistent sporangial walls, columellae that are applanate or absent in smaller sporangia (often resembling *Mortierella* species), short sporangiophores that repeatedly branch sympodially as many as 12 times, and the occurrence of racket-shaped enlargements in the sporangiophores (Hesseltine and Ellis 1964b, Schipper 1976, Scholer *et al*. 1983, de Hoog *et al.* 2000, Schipper and Stalpers 2003).

Antifungal Susceptibility: ***M. ramosissimus*** (Espinel-Ingroff *et al*. 2015a, Australian National data); **MIC µg/mL.**

	No.	≤0.016	0.03	0.06	0.125	0.25	0.5	1	2	4	8	≥16
AmB	**19**			**2**	**4**	**3**	**6**	**1**		**1**		
POSA	**13**					**4**	**4**	**2**	**2**	**1**		

Myrmecridium schulzeri (Sacc.) Arzanlou *et al.*

Synonymy: *Ramichloridium schulzeri* Stahel ex de Hoog.

Ramichloridium schulzeri was placed in a new genus, *Myrmecridium* by Arzanlou *et al*. (2007). *M. schulzeri* is an uncommon soil saprophyte of worldwide distribution. It has also been isolated from plant detritus and as a contaminant of bronchoscopy fluid. It is the causative agent of “Golden Tongue” syndrome reported by Rippon *et al.* (1985).

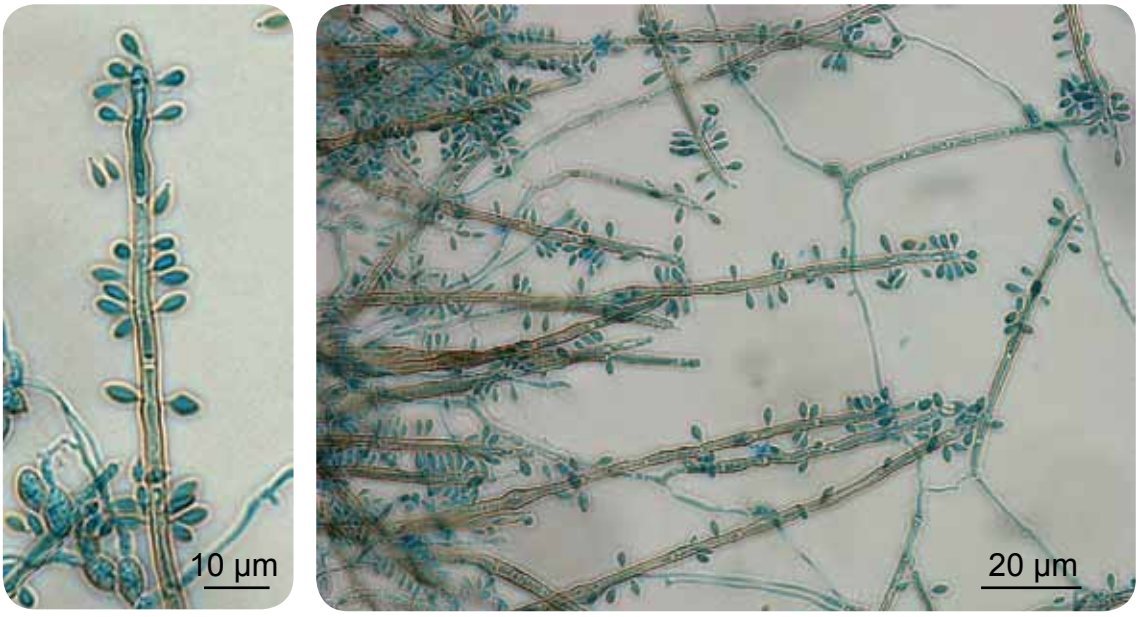
RG-1 organism.

Morphological Description: Colonies growing moderately rapidly, consisting of a rather compact, flat, submerged mycelium, pale orange, locally with some powdery, brownish aerial mycelium; reverse pink to orange. Conidiophores are erect, straight, unbranched, thick-walled, reddish-brown, up to 250 µm high, gradually becoming paler towards the apex, of variable length, elongating sympodially during conidiogenesis, with scattered, pimple-shaped conidium bearing denticles which have unpigmented scars. Conidia are subhyaline, smooth-walled or slightly rough-walled, ellipsoidal, obovoidal or fusiform, 6.5-10 x 3-4 µm, usually with an acuminate base and unpigmented scars.

Molecular Identification: ITS and D1/D2 sequencing may be used for accurate species identification (Halliday *et al.* 2015).

Note: *Myrmecridium* species can be distinguished from other *Ramichloridium*-like fungi by having entirely hyaline vegetative hyphae, and widely scattered, pimple-shaped denticles on the long hyaline rachis. The conidial sheath is also visible in lactic acid mounts with bright field microscopy Arzanlou *et al.* (2007).

References: de Hoog (1977), Rippon *et al.* (1985), de Hoog *et al.* (2000, 2015), Arzanlou *et al.* (2007).



Myrmecridium schulzeri conidiophores showing sympodial development of conidia.

Nannizzia fulva (Uriburu) Stockdale

Synonymy: *Microsporum fulvum* Uriburu.

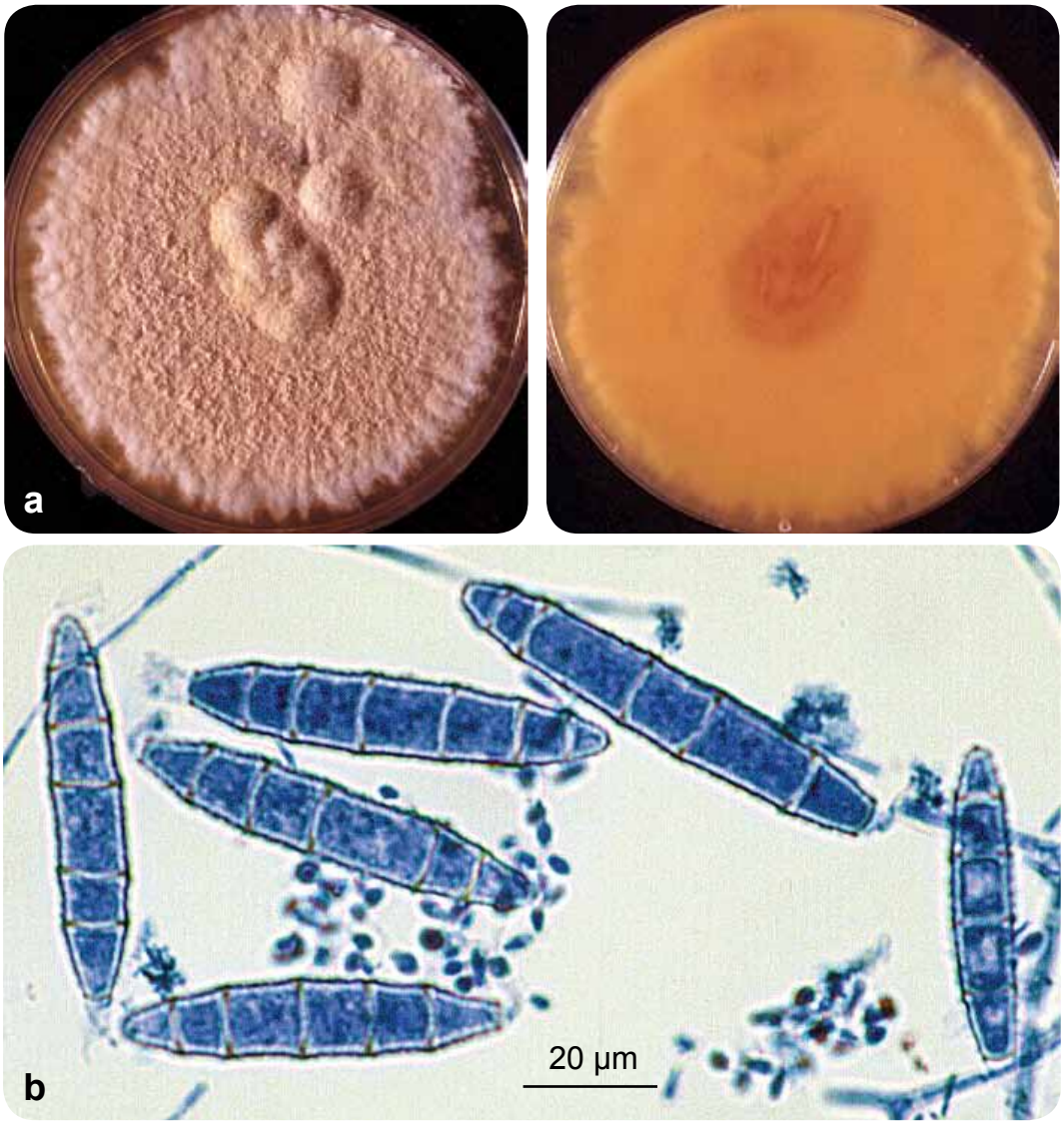
Nannizzia fulva is a geophilic fungus of worldwide distribution which may cause occasional infections in humans and animals. Clinical disease is similar to *N. gypsea* but less common. Invaded hairs show a sparse ectothrix infection but do not fluoresce under Wood's ultra-violet light. **RG-1 organism.**

Morphological Description: Colonies are fast growing, flat, suede-like, tawny-buff to pinkish-buff in colour and frequently have a fluffy white advancing edge. A dark red under surface is occasionally seen, otherwise it is colourless to yellow brown. Abundant thin-walled, elongate, ellipsoidal macroconidia are formed which closely resemble those of *N. gypsea*, except they are longer and more bullet-shaped (clavate) with three to six septa. Numerous spiral hyphae, which are often branched are seen. Numerous pyriform to clavate microconidia are also produced but these are not diagnostic.

Key Features: Macroconidial morphology and culture characteristics.

Molecular Identification: ITS sequencing is recommended.

References: Rebell and Taplin (1970), Rippon (1988), Gräser *et al*. (2008), Cafarchia *et al.* (2013), de Hoog *et al.* (2015, 2016).



Nannizzia fulva (a) culture and (b) macroconidia.

Nannizzia gypsea (Nannizzi) Stockdale

Synonymy: *Microsporum gypseum* (Bodin) Guiart & Grigorakis.

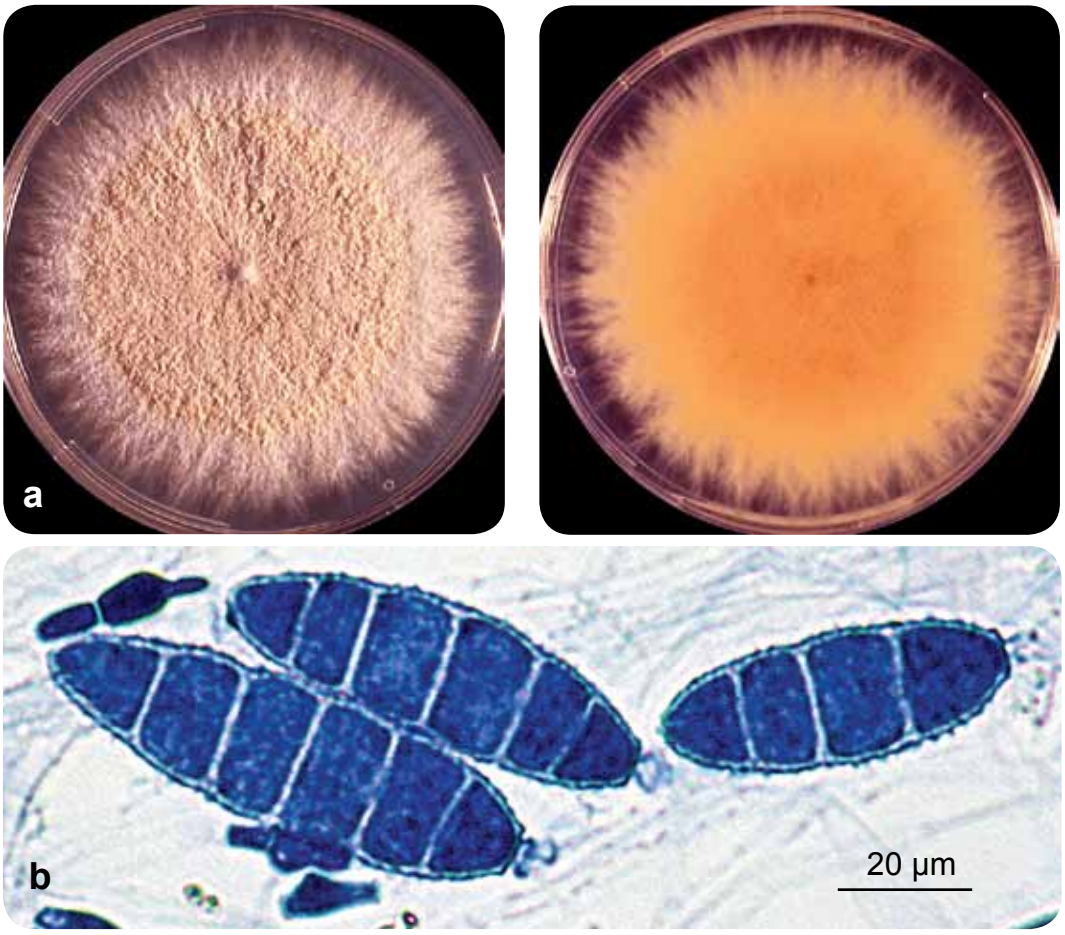
Nannizzia gypsea is a geophilic fungus with a worldwide distribution which may cause infections in animals and humans, particularly children and rural workers during warm humid weather. Usually produces a single inflammatory skin or scalp lesion. Invaded hairs show an ectothrix infection but do not fluoresce under Wood's ultra-violet light. **RG-1 organism.**

Morphological Description: Colonies are usually flat, spreading, suede-like to granular, with a deep cream to tawny-buff to pale cinnamon-coloured surface. Many cultures develop a central white downy umbo (dome) or a fluffy white tuft of mycelium and some also have a narrow white peripheral border. A yellow-brown pigment, often with a central darker brown spot, is usually produced on the reverse, however a reddish-brown reverse pigment may be present in some strains. Cultures produce abundant, symmetrical, ellipsoidal, thin-walled, verrucose, four to six-celled macroconidia. The terminal or distal ends of most macroconidia are slightly rounded, while the proximal ends (point of attachment to hyphae) are truncate. Numerous clavate-shaped microconidia are also present, but these are not diagnostic.

Key Features: Distinctive macroconidia and culture characteristics.

Molecular Identification: ITS sequencing is recommended, especially for the separation of *N. gypsea* and *N. incurvata* which are morphologically similar.

References: Rebell and Taplin (1970), Rippon (1988), Gräser *et al*. (2008), Cafarchia *et al.* (2013), de Hoog *et al.* (2015, 2016).



Nannizzia gypsea (a) culture and (b) macroconidia.

Nannizzia nana (Fuentes) Gräser & de Hoog

Synonymy: *Microsporum nanum* Fuentes

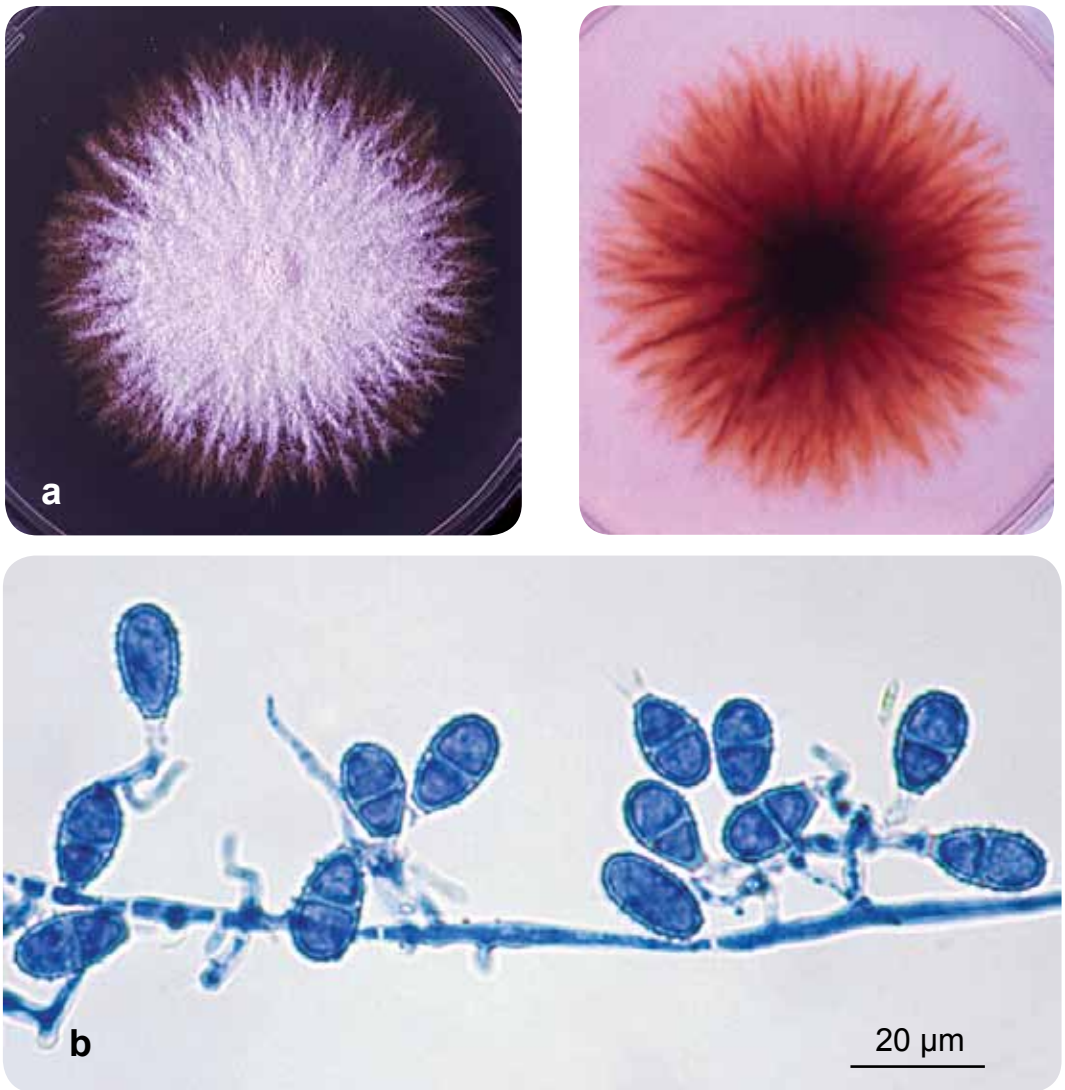
Nannizzia nana is a geophilic and zoophilic fungus frequently causing chronic non-inflammatory lesions in pigs and a rare cause of tinea in humans. Also present in soil of pig-yards. Infections in man are usually contracted directly from pigs or fomites. Invaded hairs may show a sparse ectothrix or endothrix infection but do not fluoresce under Wood's ultra-violet light. The geographical distribution is worldwide. **RG-2 organism.**

Morphological Description: Colonies are flat, cream to buff in colour with a suede-like to powdery surface texture. Young colonies have a brownish-orange pigment which deepens into a dark reddish-brown with age. Cultures produce numerous small ovoid to pyriform macroconidia with one to three (mostly two) cells, with relatively thin, finely echinulate (rough) walls, and broad truncate bases. Many macroconidia are borne on conidiophores (stalks) which do not stain readily. Occasional clavate microconidia are present, which distinguishes *N. nana* from some species of *Chrysosporium.*

Key Features: Distinctive macroconidia and culture characteristics.

Molecular Identification: ITS sequencing is recommended.

References: Rebell and Taplin (1970), Rippon (1988), Gräser *et al*. (2008), Cafarchia *et al.* (2013), de Hoog *et al.* (2015, 2016).



Nannizzia nana (a) culture and (b) macroconidia.

Nannizzia persicolor (Sabouraud) Stockdale

Synonymy: *Microsporum persicolor* (Sabouraud) Guiart & Grigorakis

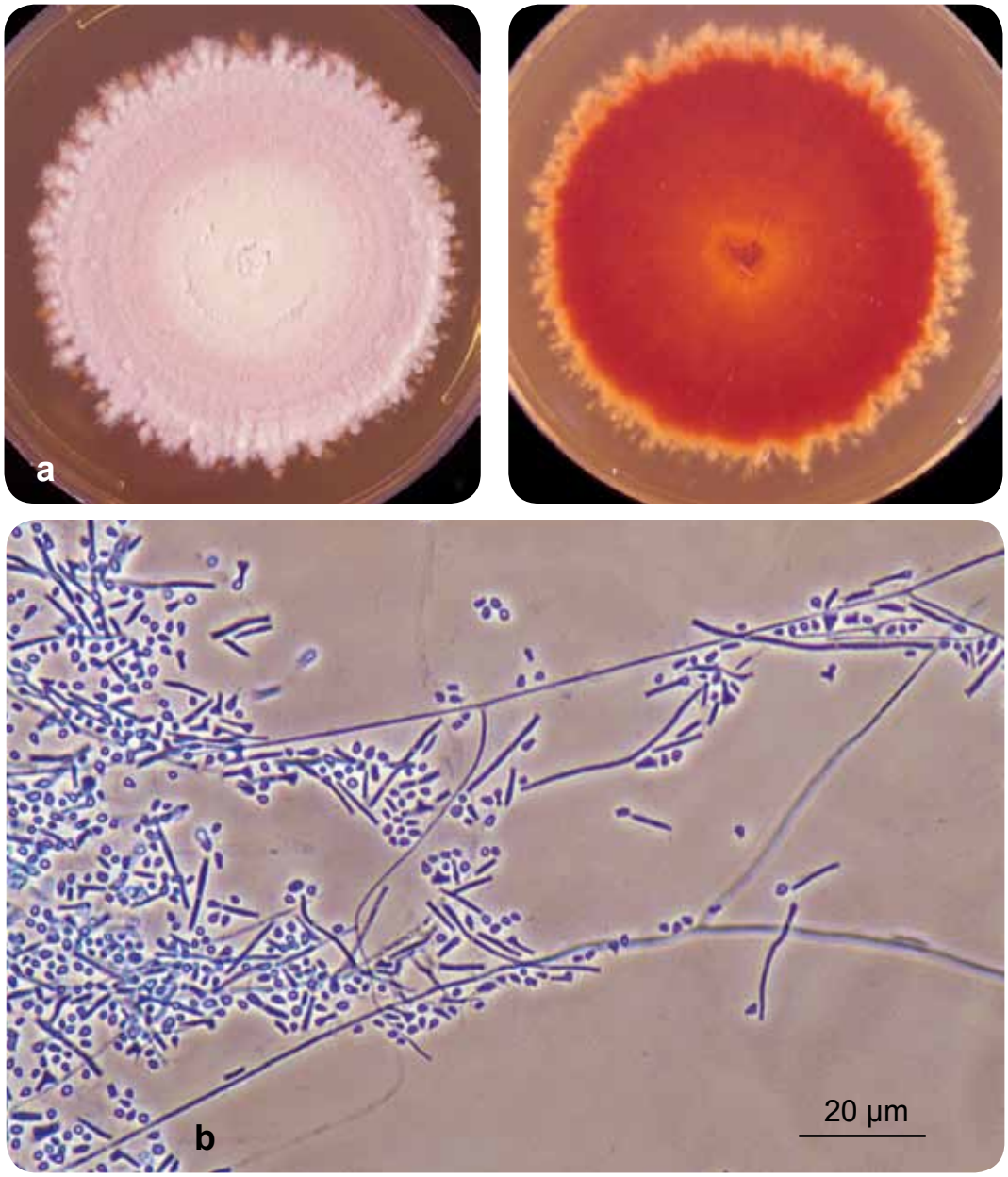
Nannizzia persicolor is a zoophilic fungus often occurring as a saprophyte on voles and bats. A rare cause of tinea corporis in humans. Not known to invade hair *in vivo*, but produces hair perforations *in vitro*. Distribution: Africa, Australia, Europe and North America. **RG-2 organism**.

Morphological Description: Colonies are generally flat, white to pinkish in colour, with a suede-like to granular texture and peripheral fringe. Reverse pigmentation is orange to red. Macroconidia are thin-walled, cigar-shaped, four to seven-celled, 40-60 x 6-8 µm but are only rarely produced. Microconidia are abundant, spherical to pyriform.

Key Features: Microscopic morphology and culture characteristics.

Molecular Identification: ITS sequencing is recommended.

References: Rebell and Taplin (1970), Rippon (1988), Gräser *et al*. (2008), Cafarchia *et al.* (2013), de Hoog *et al.* (2015, 2016).



Nannizzia persicolor (a) culture and (b) microconidia.

Neoscytalidium dimidiatum (Penzig) Crous & Slippers

Synonymy: *Hendersonula toruloidea* Nattras.
Scytalidium dimidiatum (Penzig) Sutton & Dyko.
Scytalidium hyalinum Campbell & Mulder.

Neoscytalidium dimidiatum is a coelomycete and recognised agent of onychomycosis and superficial skin infections, especially in tropical regions. The primary isolation of this fungus from clinical specimens may be difficult as isolates are sensitive to cycloheximide (actidione), which is commonly added to primary isolation media used for culturing skin scrapings.

The taxonomy of this species has been very confusing; the conidial state of *S. dimidiatum* was originally described under the name *Hendersonula toruloidea*. However it is phylogenetically remote from *Scytalidium* and has now been placed into *Neoscytalidium* (Crous *et al.* 2006, Machouart *et al.* 2012). Colourless mutants (previously known as *Scytalidium hyalinum*) often occur and have now been listed as variety *N. dimidiatum* var. *hyalinum* (Madrid *et al.* 2009).

Note: *Nattrassia mangiferae*, previously considered the teleomorph form of *S. dimidiatum*, is now considered a distinct species, and has been placed in the genus *Neofusicoccum*, based on the lack of an arthoconidial anamorph (Crous *et al*. 2006, Machouart *et al*. 2012).

RG-2 organism.

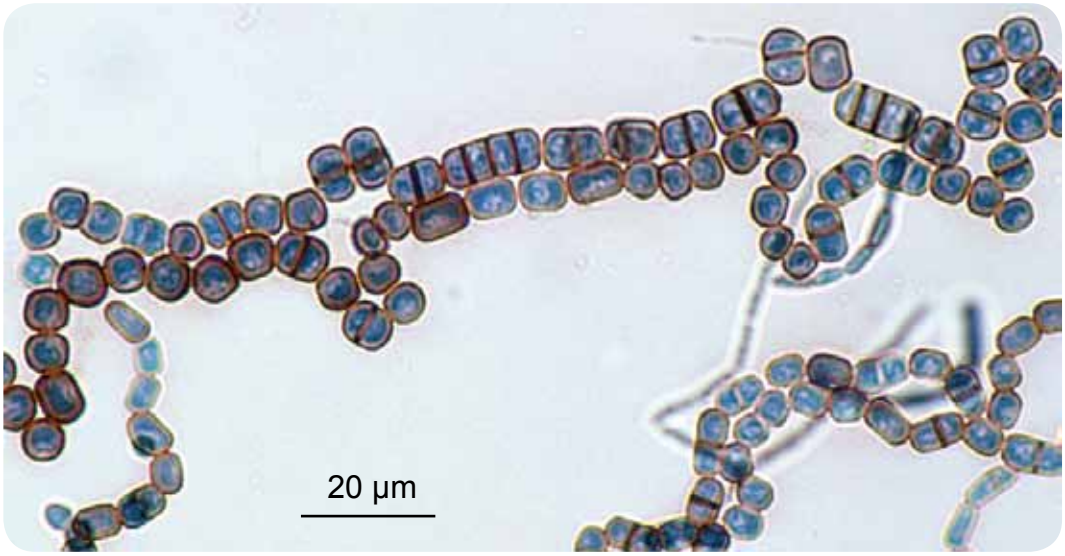
Morphological Description: Cultures are effuse, hairy, dark grey to blackish-brown, or white to greyish, with a cream-coloured to deep ochraceous-yellow reverse. Colourless mutants often occur. Arthroconidia are typically present in chains of one to two-cells, darkly pigmented, 3.5-5 x 6.5-12 µm, produced by the holothallic fragmentation of undifferentiated hyphae. Pycnidia, only occasionally formed in older cultures are black, ostiolate and contain numerous hyaline, flask-shaped phialides. Phialoconidia are at first one-celled and hyaline, later becoming three-celled, brown, with the centre cell darker than the end cells and are ovoid to ellipsoidal in shape.

Molecular Identification: ITS and D1/D2 sequencing may be used for accurate species identification (Halliday *et al*. 2015).

MALDI-TOF MS: Alshawa *et al.* (2012) developed a spectral database for 12 different species of dermatophytes which also included *Neoscytalidium dimidiatum* and *N. dimidiatum* var. *hyalinum*. Correct identification of the species was obtained for 18/21 *Neoscytalidium* isolates (85.7%).

References: McGinnis (1980), Moore (1986), Rippon (1988), Frankel and Rippon (1989), Sutton and Dyko (1989) Madrid *et al*. (2009), de Hoog *et al*. (2000, 2015), Crous *et al*. (2006), Machouart *et al*. (2012), Alshawa *et al.* (2012).

Neoscytalidium dimidiatum (Penzig) Crous & Slippers



Neoscytalidium dimidiatum showing chains of one to two celled, darkly pigmented arthroconidia.

Antifungal Susceptibility: *Neoscytalidium dimidiatum* limited data (Australian National data); **MIC µg/mL.**															
	No.	**≤0.008**	**0.016**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**≥64**
AmB	**3**				**1**	**1**			**1**						
VORI	**3**	**1**		**1**		**1**									
POSA	**3**			**1**		**1**			**1**						
ITRA	**3**		**1**						**1**				**1**		
N. dimidiatum data from 24 isolates (Madrid *et al*. 2009); **MIC µg/mL.**															
AmB	**Range 0.125-0.5; Geometric mean = 0.32**														
VORI	**Range 0.06-4; Geometric mean = 1.37**														
POSA	**Range 0.06-32; Geometric mean = 6.15**														
N. dimidiatum data from 10 isolates (Espinel-Ingroff *et al*. 2001); **MIC µg/mL.**															
ITRA	**Range 0.03-32; Geometric mean = 0.65**														

Ochroconis de Hoog and Arx

Recently, the genus *Ochroconis* has undergone taxonomic revision, and the most relevant species, *Ochroconis gallopava*, was transferred to the new genus *Verruconis* (Samerpitak *et al*. 2014). *Ochroconis* species are mesophilic saprobes, with an optimum growth temperature between 15 and 30^{O}C and an inability to grow at 37^{O}C. Nevertheless, some species have been isolated from clinical specimens; such as *Ochroconis tshawytschae, O. mirabilis, O. cordanae, O. olivacea* and *O. ramosa* (Samerpitak *et al*. 2014, Seyedmousavi *et al*. 2014, Giraldo *et al.* 2014). **Note:** Species described in the literature from clinical cases as *O. constricta* or *O. humicola* are probably *O. musae* (Samerpitak *et al*. 2014).

Giraldo *et al.* (2014) reported on the occurrence of *Ochroconis* and *Verruconis* species in clinical specimens from the United States. *V. gallopava* was the most common species (69%), followed by *O. mirabilis* (22%). Other species isolated were *O. cordanae, O. olivacea* and *O. ramosa.* The most common anatomical site of isolation was the lower respiratory tract (59%), followed by superficial (22%) and deep tissues (20%) (Giraldo *et al*. 2014).

RG-1 organisms.

Morphological Description: Colonies restricted, velvety to funiculose, brown to olivaceous, often with rust-brown reverse. Hyphae smooth- or somewhat rough-walled, pale olivaceous. Conidiophores slightly or conspicuously differentiated, cylindrical, often flexuose, producing conidia on scattered, cylindrical to conical denticles. After detachment an inconspicuous frill often remains both on the denticle and on the conidium base. Conidia one to four-celled, pale olivaceous brown, smooth- or rough-walled, ellipsoidal, cylindrical, clavate or cuneiform. Maximum growth temperature around 33^{O}C.

Molecular Identification: Giraldo *et al*. (2014) used sequence analyses of the 18*S*, ITS, D1/2, actin, and β-tubulin genes, while Seyedmousavi *et al*. (2014), used ITS sequence analyses to identify species.

References: de Hoog *et al*. (2015), Samerpitak *et al*. (2014), Seyedmousavi *et al.* (2014), Giraldo *et al.* (2014).

Antifungal Susceptibility: *Ochroconis mirabilis* variable data for posaconazole, caspofungin and anidulafungin from Seyedmousavi *et al.* (2014)[1] and Giraldo *et al* (2014)[2]; **MIC µg/mL.**

Antifungal	Range	MIC_{90}	Antifungal	Range	MIC_{90}
AmB	**1-32**	**32**	**POSA**	**0.06-0.25[1] 0.5-32[2]**	**- 32**
ITRA	**0.25-32**	**32**	**VORI**	**2-32**	**32**
CAS	**0.5-1[1] 1-32[2]**	**- 4**	**ANID**	**0.03-0.125[1] 0.015-32[2]**	**- 4**
MICA	**0.06-0.50**	**0.25**	**TERB**	**0.015-0.125**	**0.02**

Onychocola canadensis Sigler

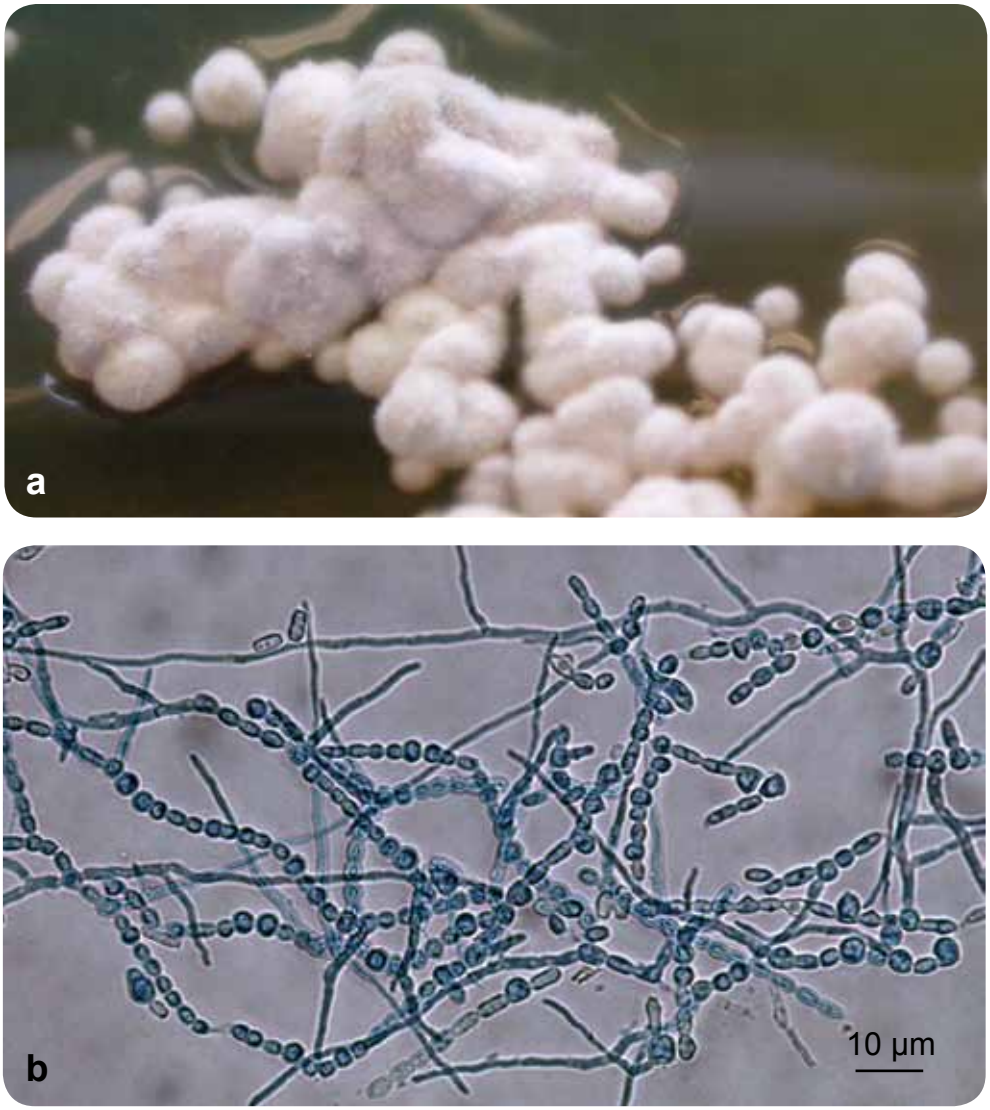
Onychocola canadensis is an uncommon cause of distal and lateral subungual or white superficial onychomycosis. However, it may sometimes be present in an abnormal-appearing nail as an insignificant finding, not acting as a pathogen.

RG-2 organism.

Morphologial Description: Colonies grow slowly and are velvety to lanose, white to yellowish, with a brownish reverse. Arthroconidia are cylindrical to broadly ellipsoidal, one to two-celled, hyaline to subhyaline, 4-16 x 2-5 µm in size, forming long chains. Older cultures may show broad, brown, rough-walled hyphae.

Key Features: Slow growing, white, arthroconidial mould isolated from nails.

References: Sigler and Congly (1990), Sigler *et al.* (1994), Gupta *et al.* (1998), de Hoog *et al.* (2000, 2015).



Onychocola canadensis (a) culture and (b) arthroconidia.

Paecilomyces Bain

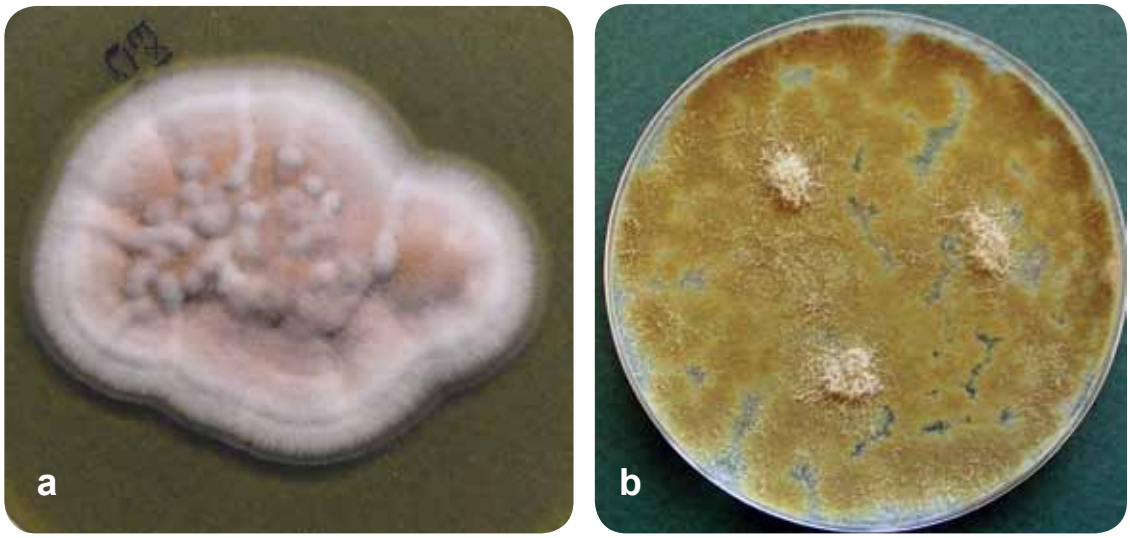
The genus *Paecilomyces* may be distinguished from the closely related genus *Penicillium* by having long slender divergent phialides and colonies that are never typically green. *Paecilomyces* species are common environmental moulds and are seldom associated with human infection. However, the species, *P. variotii and P. marquandii* are emerging as causative agents of mycotic keratitis and of hyalohyphomycosis in the immunocompromised patient. **Note:** *Paecilomyces lilacinus* has been transferred to *Purpureocillium lilacinum* (Luangsa-ard *et al*. 2011).

Morphological Description: Colonies are fast growing, powdery or suede-like, gold, green-gold, yellow-brown, lilac or tan, but never green or blue-green as in *Penicillium.* Phialides are swollen at their bases, gradually tapering into a rather long and slender neck, and occur solitarily, in pairs, as verticils, and in penicillate heads. Long, dry chains of single-celled, hyaline to dark, smooth or rough, ovoid to fusoid conidia are produced in basipetal succession from the phialides.

Molecular Identification: Molecular phylogeny based on 18*S* rDNA sequences was done by Luangsa-ard *et al*. (2004); the genus is polyphyletic.

Key Features: Long slender divergent phialides and culture pigmentation.

References: Samson (1974), Domsch *et al.* (1980), McGinnis (1980), Onions *et al*. (1981), Rippon (1988), de Hoog *et al.* (2000, 2015).



Cultures of (a) *Paecilomyces marquandii* and (b) *Paecilomyces variotii* showing colony pigmentation.

Paecilomyces marquandii (Massee) Hughes

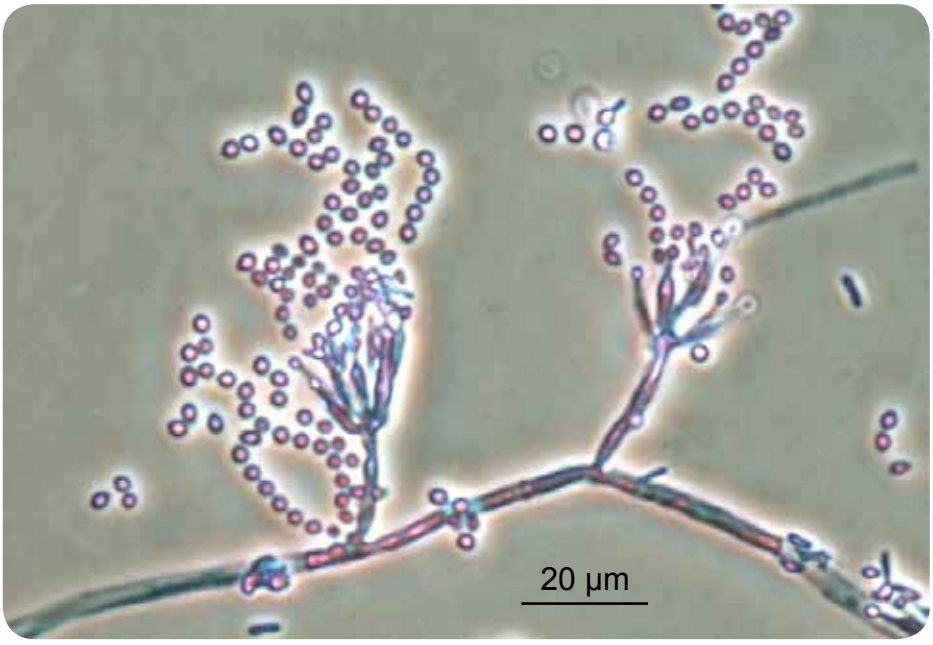
Paecilomyces marquandii is a soil fungus of worldwide distribution from temperate to tropical regions.

RG-1 organism.

Morphological Description: Colonies are fast growing, suede-like to floccose, pale vinaceous to violet-coloured, with a yellow to orange yellow reverse. Conidiophores are erect, arising from submerged hyphae, 50-300 µm in length, bearing loose whorls of branches and phialides. Conidiophore stipes are 2.5-3.0 µm wide, hyaline and smooth-walled. Phialides are swollen at their bases, tapering into a thin, distinct neck. Conidia are ellipsoidal to fusiform, smooth-walled to slightly roughened, hyaline to purple in mass, 2.5-3.0 x 2-2.2 µm. Spherical to ellipsoidal chlamydospores, 3-5 µm diameter are present. No growth at 37^{O}C.

Key Features: Colony pigmentation with yellow reverse pigment, phialides with swollen bases, smooth conidiophore stipes, presence of chlamydospores, and no growth at 37^{O}C. **Note:** *Purpureocillium lilacinum* has no yellow reverse pigment, rough-walled conidiophore stipes, absence of chlamydospores and growth at 37^{O}C.

References: Samson (1974), Domsch *et al.* (1980, 2007), de Hoog *et al*. (2000, 2015).



Paecilomyces marquandii conidiophores, phialides and conidia.

Antifungal Susceptibility: *P. marquandii* (Australian National data); **MIC µg/mL.**													
	No.	**≤0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**≥64**
AmB	**7**				**1**			**1**	**1**	**1**	**3**		
VORI	**4**		**1**	**2**			**1**						
POSA	**2**	**1**		**1**									
ITRA	**7**	**1**			**2**	**2**		**1**			**1**		

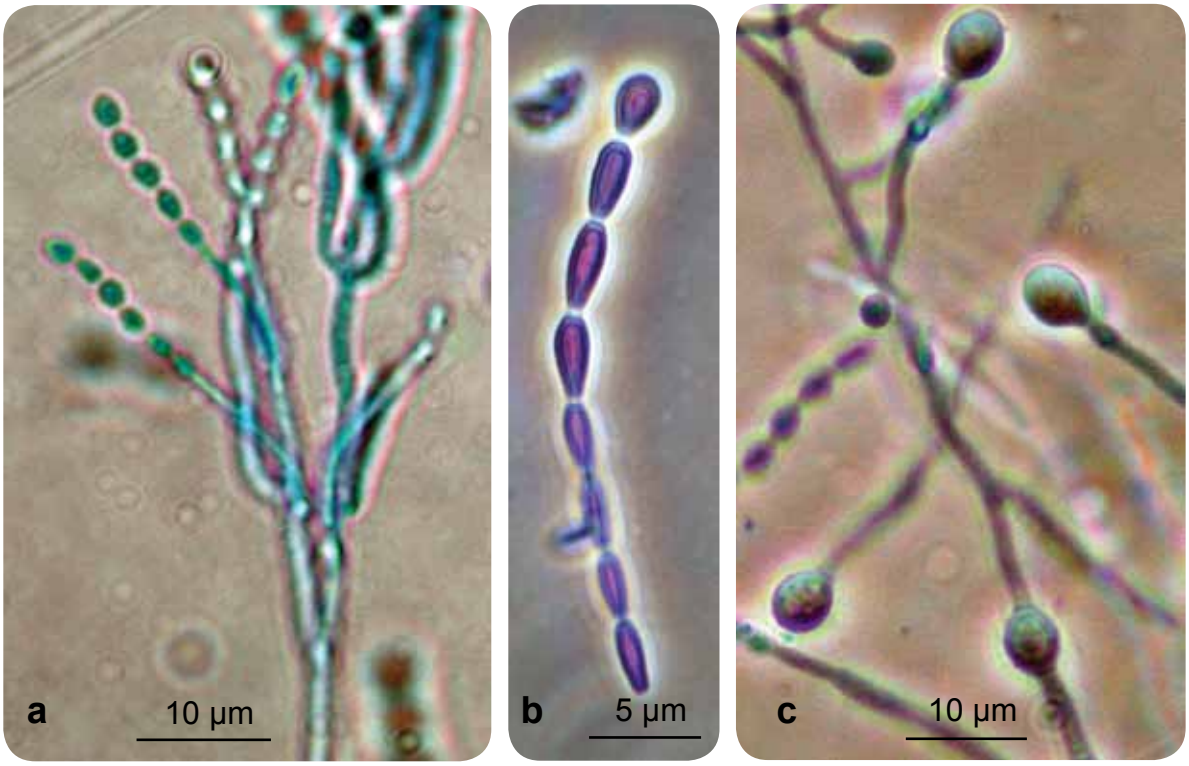
Paecilomyces variotii Bain

Paecilomyces variotii is a common environmental mould that is widespread in composts, soils and food products. It is known from substrates including food, indoor air, wood, soil and carpet dust.

RG-2 organism.

Morphological Description: Colonies are fast growing, powdery to suede-like, funiculose or tufted, and yellow-brown or sand-coloured. Conidiophores bearing dense, verticillately arranged branches bearing phialides. Phialides are cylindrical or ellipsoidal, tapering abruptly into a long and cylindrical neck. Conidia are subspherical, ellipsoidal to fusiform, hyaline to yellow, smooth-walled, 3-5 x 2-4 µm and are produced in long divergent chains. Chlamydospores are usually present, singly or in short chains, brown, subspherical to pyriform, 4-8 µm in diameter, thick-walled to slightly verrucose.

Key Features: Yellow-brown colony pigmentation, cylindrical phialides, and presence of chlamydospores.



Paecilomyces variotii (a) conidiophores, phialides, (b) conidia and (c) terminal chlamydospores.

Antifungal Susceptibility: *P. variotii* (Australian National data); **MIC µg/mL.**													
	No.	**≤0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**≥64**
AmB	**17**			**7**	**3**	**4**	**2**	**1**					
VORI	**17**						**1**	**2**	**1**	**12**	**1**		
POSA	**17**	**5**	**3**	**2**	**5**	**2**							
ITRA	**17**		**4**	**5**	**5**	**3**							

Paracoccidioides brasiliensis/lutzii Complex

WARNING: RG-3 organism. Cultures of *Paracoccidioides brasiliensis/lutzii* represent a biohazard to laboratory personnel and should be handled with extreme caution in a Class II Biological Safety Cabinet (BSCII).

Recently *P. brasiliensis* has been recognised as two species: *P. brasiliensis* and *P. lutzii* (Teixeira *et al*. 2014, Theodoro *et al*. 2012). *P. brasiliensis/lutzii* is geographically restricted to areas of South and Central America. The two species are morphologically very similar; conidia of *P. lutzii* are elongated whereas those from *P. brasiliensis* are pyriform. Molecular confirmation is recommended.

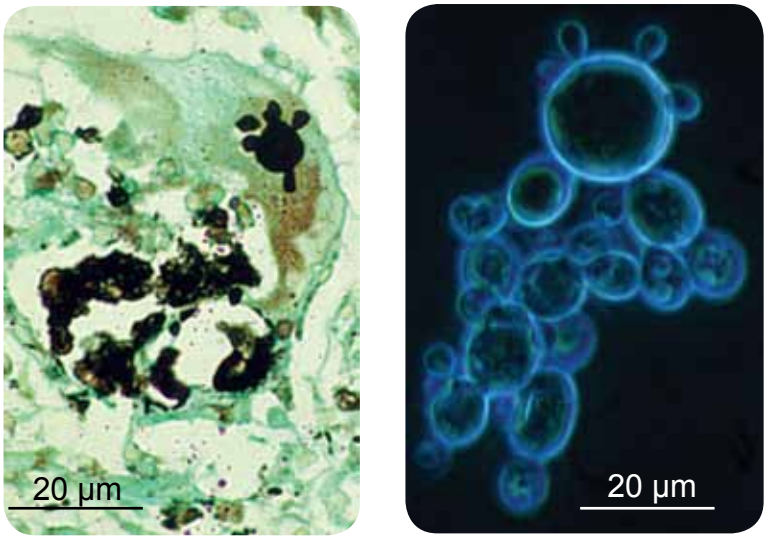
Molecular Identification: ITS sequencing is recommended (Imai *et al.* 2000)

Morphological Description: Colonies grown at 25°C are slow growing and variable in morphology. Colonies may be flat, wrinkled and folded, glabrous, suede-like or downy in texture, white to brownish with a tan or brown reverse. Microscopically, a variety of conidia may be seen, including pyriform microconidia, chlamydospores and arthroconidia. However, none of these are characteristic of the species, and most strains may grow for long periods of time without the production of conidia.

On blood agar at 37°C, the mycelium converts to the yeast phase and colonies are white to tan, moist and glabrous and become wrinkled, folded and heaped. Microscopically, numerous large, 20-60 µm, round, narrow base budding yeast cells are present. Single and multiple budding occurs, the latter are thick-walled cells that form the classical "steering wheel" or "mickey mouse" structures that are diagnostic for this fungus, especially in methenamine silver stained tissue sections.

Key Features: Clinical history, tissue pathology, culture identification with conversion to yeast phase at 37°C, however molecular identification is now recommended.

References: McGinnis (1980), Chandler *et al.* (1980), Rippon (1988), de Hoog *et al.* (2000, 2015).



Paracoccidioides brasiliensis/lutzii showing multiple, narrow base budding yeast cells "steering wheels".

Antifungal Susceptibility: *P. brasiliensis* very limited data (McGinnis *et al.* 1997).			
Antifungal	**MIC Range µg/mL**	**Antifungal**	**MIC Range µg/mL**
AmB	**0.03-4**	**ITRA**	**≤0.03-1**
FLU	**≤0.125-64**	**VORI**	**≤0.03-2**

Paraphyton cookei (Ajello) Gräser & de Hoog

Synonymy: *Microsporum cookei* Ajello; *Microsporum racemosum Borelli.*

Lophophyton cookei is a geophilic fungus which has been isolated from the hair of small mammals showing no clinical lesions. Infection has been reported in rodents, dogs and rarely in humans. It is not known to invade hair *in vivo*, but produces hair perforations *in vitro*. **RG-1 organism.**

Morphological Description: Colonies are flat, spreading, buff to pale brown, powdery to suede-like, with a slightly raised and folded centre and some radial grooves. Reverse pigment dark reddish-brown. Numerous large, very thick-walled, echinulate (rough) elliptical macroconidia with predominantly five to six septa but may be from two to eight septa. Occasional spiral hyphae may be seen. Moderate numbers of mainly slender clavate with some pyriform microconidia are present.

Key Features: The macroconidia of *L. cookei* are quite characteristic and diagnostic*;* the thick walls and usually larger size of the macroconidia distinguish it from *N. gypsea.*

Confirmatory Tests:

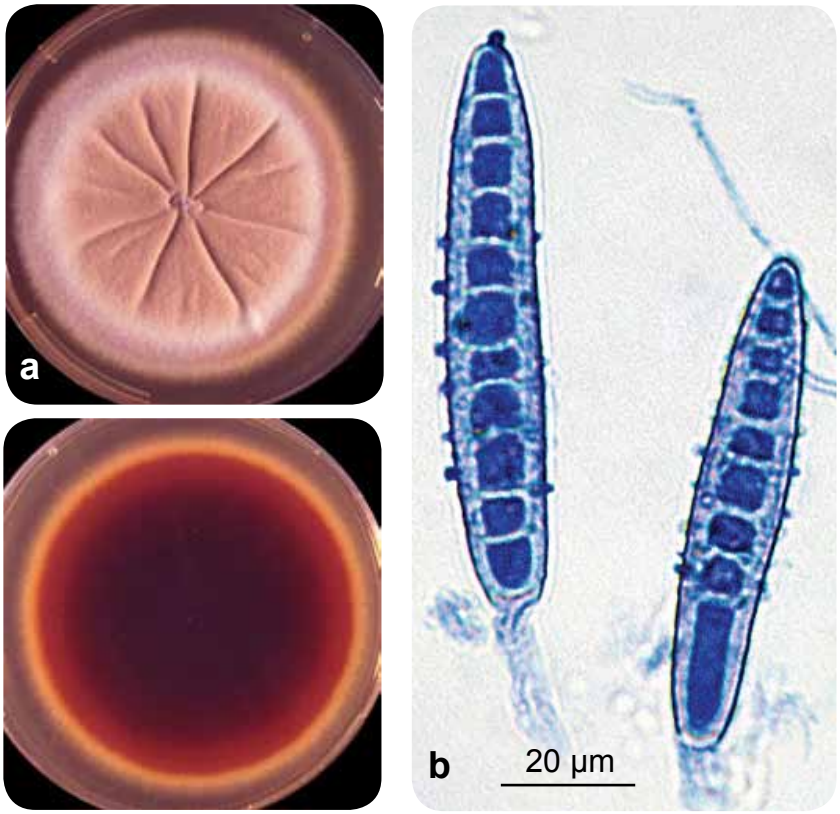
Vitamin Free Agar (Trichophyton Agar No.1): Good growth indicating no special nutritional requirements, pinkish-buff-coloured, suede-like colony with a deep magenta red reverse.

Hair Perforation Test: Positive.

Key Features: Distinctive macroconidial morphology and culture characteristics.

Molecular Identification: ITS sequencing is recommended.

References: Rebell and Taplin (1970), Rippon (1988), Gräser *et al*. (2008), Cafarchia *et al.* (2013), de Hoog *et al.* (2015, 2016).



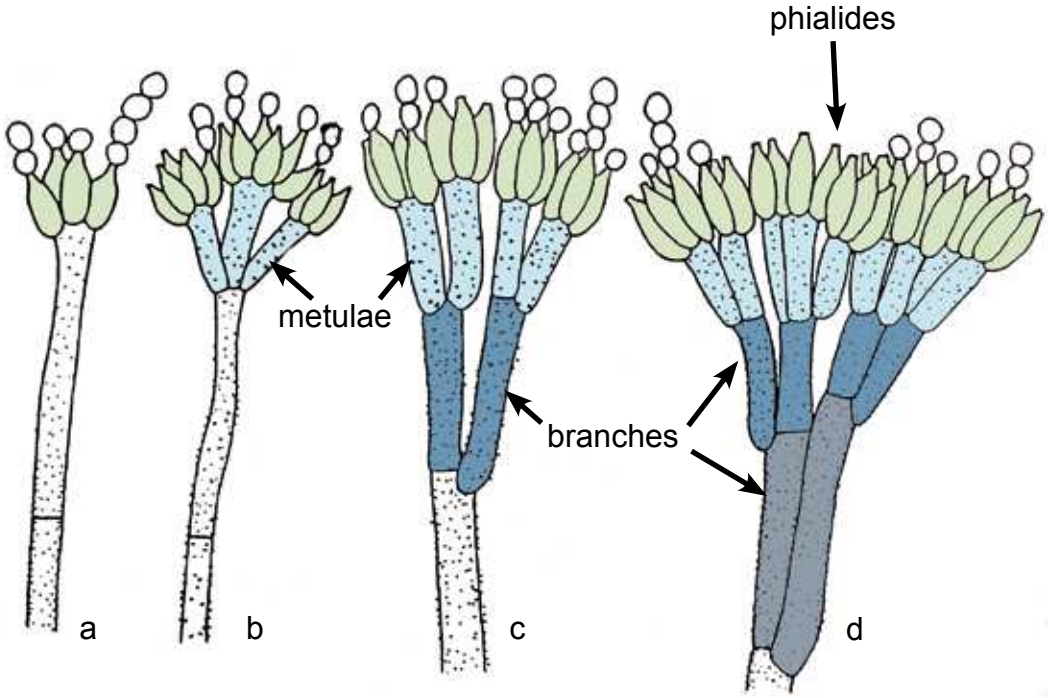
Lophophyton cookei (a) culture and (b) macroconidia.

Penicillium Link: Fries

Penicillium is a very large and ubiquitous genus which currently contains 354 accepted species (Visagie *et al*. 2014). Many species are common contaminants on various substrates and are known as potential mycotoxin producers. Correct identification is therefore important when studying possible *Penicillium* contamination of food. Human pathogenic species are rare, however opportunistic infections leading to mycotic keratitis, otomycosis and endocarditis (following insertion of valve prosthesis) have been reported (Lyratzopoulos *et al.* 2002). **Note:** *Penicillium marneffei* and other subgenus *Biverticillium* species have been transferred to the genus *Talaromyces* (Samson *et al*. 2011b).

RG-1 organisms.

Morphological Description: Colonies are usually fast growing, in shades of green, sometimes white, mostly consisting of a dense felt of conidiophores. Microscopically, chains of single-celled conidia are produced in basipetal succession from a specialised conidiogenous cell called a phialide. The term basocatenate is often used to describe such chains of conidia where the youngest conidium is at the basal or proximal end of the chain. In *Penicillium*, phialides may be produced singly, in groups or from branched metulae, giving a brush-like appearance (a penicillus). The penicillus may contain both branches and metulae (penultimate branches which bear a whorl of phialides). All cells between the metulae and the stipes of the conidiophores are referred to as branches. The branching pattern may be either simple (non-branched or monoverticillate), one-stage branched (biverticillate-symmetrical), two-stage branched (biverticillate-asymmetrical) or three- to more-staged branched. Conidiophores are hyaline, smooth or rough-walled. Phialides are usually flask-shaped, consisting of a cylindrical basal part and a distinct neck, or lanceolate (with a narrow basal part tapering to a somewhat pointed apex). Conidia are in long dry chains, divergent or in columns, are globose, ellipsoidal, cylindrical or fusiform, hyaline or greenish, smooth or rough-walled. Sclerotia are produced by some species.



Morphological structures and types of conidiophore branching in *Penicillium*. (a) Monoverticillate; (b) Biverticillate; (c) Terverticillate; (d) Quaterverticillate (see Visagie *et al.* 2014).

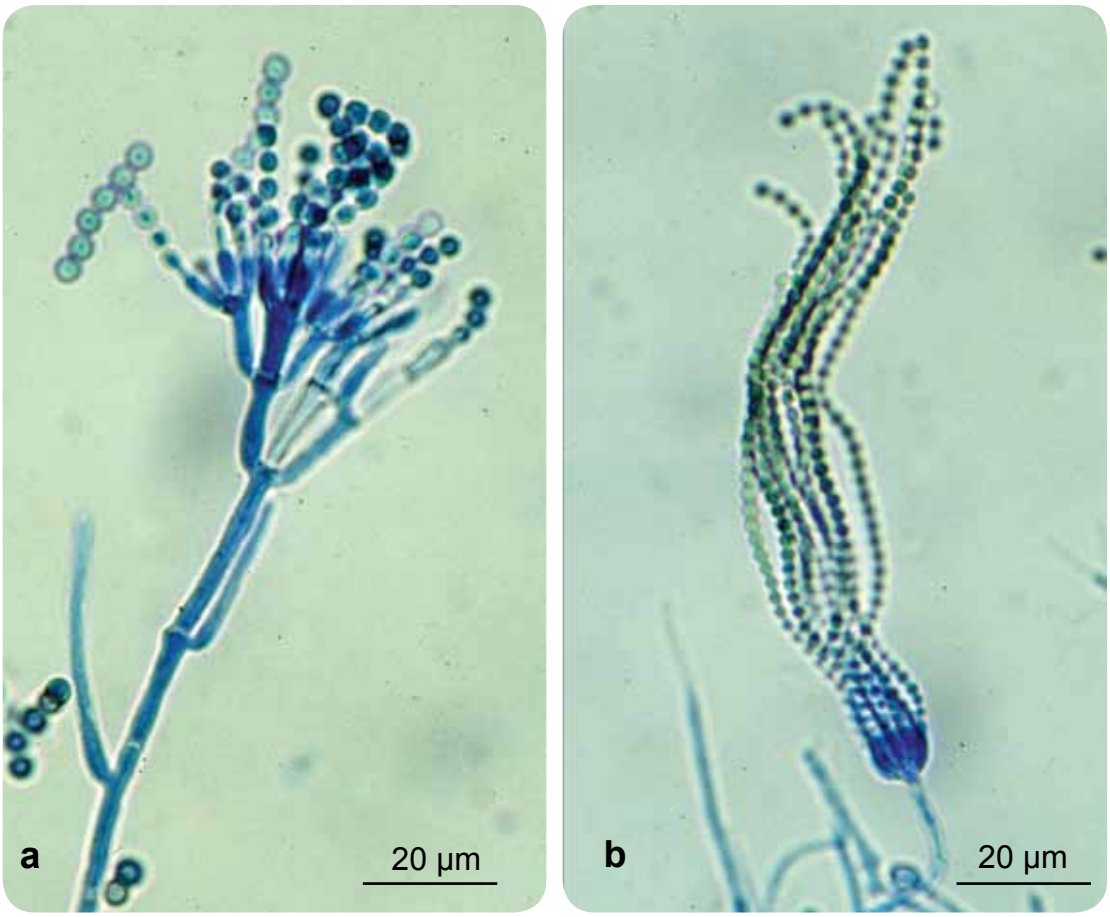
Penicillium Link:Fries

For identification, isolates are usually inoculated at three points on Czapek Dox agar and 2% Malt extract agar and incubated at 25°C. Most species sporulate within 7 days. Microscopic mounts are best made using a cellotape flag or a slide culture preparation mounted in lactophenol cotton blue. A drop of alcohol is usually needed to remove bubbles and excess conidia (Samson *et al*. 1995).

Molecular Identification: ITS and/or β-tubulin loci are recommended for identification of *Penicillium* species (Visagie *et al*. 2014, Yilmaz *et al*. 2014).

Key Features: Hyphomycete, flask-shaped phialides arranged in groups from branched metulae forming a penicillus.

References: Raper and Thom (1949), Pitt (1979), Domsch *et al*. (1980), McGinnis (1980), Onions *et al.* (1981), Ramirez (1982), Samson *et al*. (1995, 2011b), de Hoog *et al.* (2000, 2015), Visagie *et al*. (2014).



(a) *P. verrucosum* var. *cyclopium* conidiophores showing two-stage branching.
(b) *P. cheresanum* simple conidiophore showing chains of single-celled conidia.

Antifungal Susceptibility: *Penicillium sp.* (Australian National data); **MIC µg/mL.**													
	No.	**≤0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**≥64**
AmB	**28**			**2**	**3**	**5**	**12**	**5**	**1**				
VORI	**26**				**2**	**4**	**6**	**3**	**2**	**8**	**1**		
POSA	**26**	**1**	**3**	**4**	**5**	**3**	**6**	**4**					
ITRA	**27**		**3**	**4**	**1**	**6**	**8**	**1**			**4**		

Phaeoacremonium parasiticum (Ajello *et al.*) W. Gams *et al.*

Synonymy: *Phialophora parasiticum* Ajello, Gerog & Wang.

The genus *Phaeoacremonium* initially accommodated isolates with features similar to those seen in both *Acremonium* and *Phialophora*. It differs from the former by having pigmented hyphae and conidiophores and from the latter by having indistinct collarettes and warty conidiogenous cells (Revankar and Sutton, 2010).

Phaeoacremonium currently consists of 46 species with *P. parasiticum* and *P. krajdenii* recognised as the predominant species associated with human infections (Mostert *et al.* 2005). Other species have also been isolated from clinical cases i.e. *P. alvesii, P. amstelodamense, P. griseorubrum, P. minimum, P. rubrigenum, P. tardicrescens*, and *P. venezuelense*. Infections caused by *P. parasiticum* include subcutaneous abscesses, thorn-induced arthritis, and disseminated infection (Revankar and Sutton, 2010, Gramaje *et al*. 2015).

RG-2 organism.

Morphological Description: Cultures usually slow growing, suede-like with radial furrows, initially whitish-grey becoming olivaceous-grey with age. Hyphae hyaline, later becoming brown and some becoming rough-walled. Phialides are brown, thick-walled, slender, acular to cylindrical slightly tapering towards the tip, 15-50 µm long, often proliferating, with small, funnel-shaped collarettes. Conidia, often in balls, are hyaline, thin-walled, cylindrical to sausage-shaped, 3-6 x 1-2 µm, later inflating. Maximum growth temperature 40°C.

Molecular Identification: ITS and β-tubulin sequencing (Mostert *et al*. 2006, Gramaje *et al.* 2015).

Key Features: The identification of the different *Phaeoacremonium* species can be done by combining cultural, morphological and sequence data (Mostert *et al*. 2006, Gramaje *et al*. 2015).

References: de Hoog *et al.* (2000, 2015), Revankar and Sutton (2010), Mostert *et al*. (2005, 2006), Gramaje *et al.* (2015), Badali *et al*. (2015).

Phaeoacremonium parasiticum (a) colony and (b) a phialide with a small, funnel-shaped collarette.

Phaeoacremonium parasiticum (Ajello *et al.*) W. Gams *et al.*

Phaeoacremonium parasiticum phialides and conidia.

Antifungal Susceptibility: *Phaeoacremonium parasiticum* (Badali *et al.* 2015, Australian National data); **MIC µg/mL.**													
	No.	**≤0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**≥64**
AmB	**56**		**1**	**7**	**13**	**17**	**13**	**5**					
VORI	**56**		**2**	**8**	**21**	**23**	**1**	**2**					
POSA	**53**		**1**	**5**	**17**	**23**	**6**	**1**					
ITRA	**56**		**1**			**3**	**3**	**1**	**1**		**47**		

Antifungal Susceptibility: *Phialophora verrucosa* limited data (Australian National data); **MIC µg/mL.**													
	No.	**≤0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**≥64**
AmB	**4**				**1**		**1**	**2**					
VORI	**4**				**1**	**3**							
POSA	**4**			**2**	**2**								
ITRA	**4**				**1**	**3**					**1**		

P. verrucosa data from 25 isolates (McGinnis and Pasarell 1998a); **MIC µg/mL.**	
AmB	**Range 0.03-4; Geometric mean = 0.36**
VORI	**Range 0.03-0.5; Geometric mean = 0.12**
ITRA	**Range 0.03-0.5; Geometric mean = 0.07**

Phialophora verrucosa Medlar

The genus *Phialophora* contains more than 40 species, most are saprophytes commonly found in soil or on decaying wood. Some human pathogens with phialidic conidiogenesis previously assigned to *Phialophora* have been moved to other genera, namely, *Phaeoacremonium* and *Pleurostomophora*. *P. verrucosa, P. americana, P. bubakii, P. europaea* and *P. reptans* remain of medical interest (Revankar and Sutton 2010). Both *P. verrucosa* and *P. americana* produce their conidia from phialides with conspicuous darkened collarettes, however sequencing has demonstrated a close relatedness, suggesting that these species may be synonymous (de Hoog *et al*. 1999). *P. verrucosa* is primarily an agent of chromoblastomycosis although other reported infections include endocarditis, keratitis, and osteomyelitis.

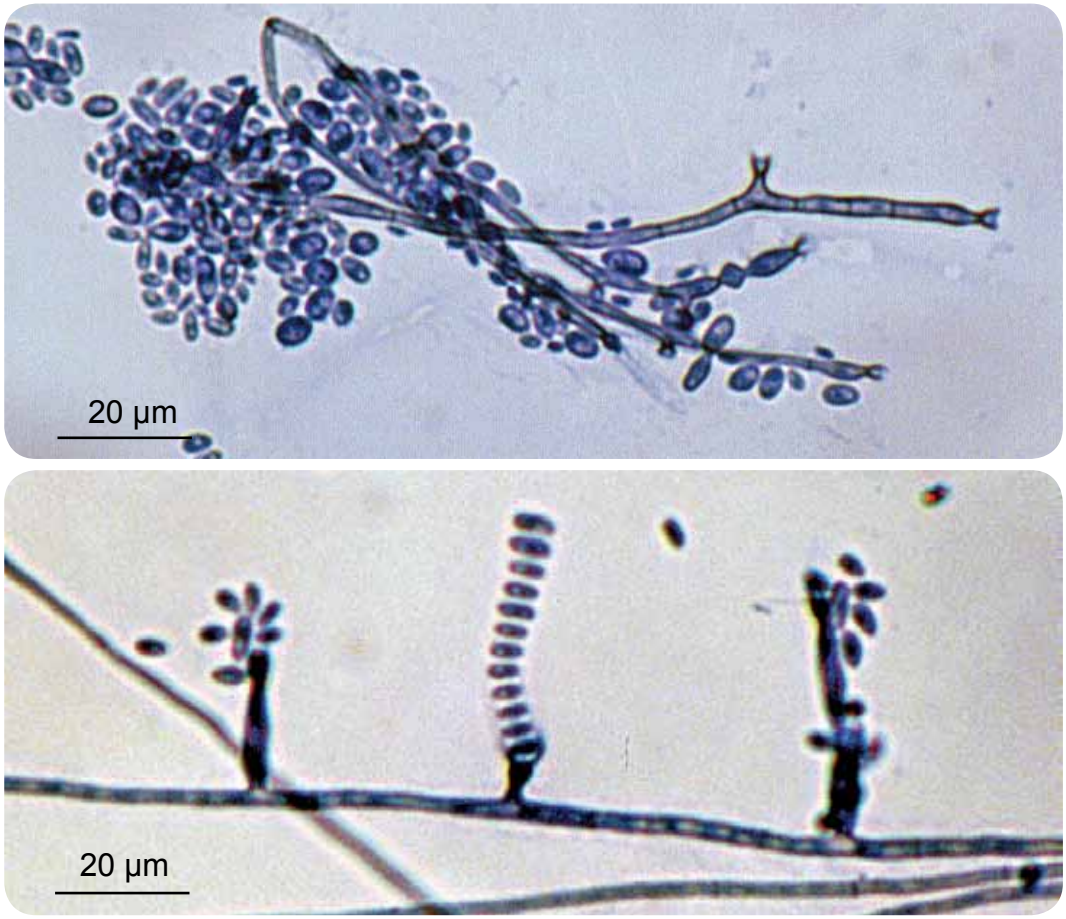
RG-2 organism.

Morphological Description: Colonies (SDA) are slow growing, initially dome-shaped, later becoming flat, suede-like and olivaceous to black in colour. Phialides are flask-shaped or elliptical with distinctive funnel-shaped, darkly pigmented collarettes. Conidia are ellipsoidal, smooth-walled, hyaline, mostly 3.0-5.0 x 1.5-3.0 µm, and aggregate in slimy heads at the apices of the phialide.

Key Features: Characteristic flask-shaped phialides with distinctive funnel-shaped, darkly pigmented collarettes.

Molecular Identification: ITS sequencing recommended (de Hoog *et al*. 1999).

References: Ellis (1971), McGinnis (1978a, 1980), Domsch *et a*l. (1980), de Hoog *et al.* (1999, 2000, 2015), Revankar and Sutton (2010).



Phialophora verrucosa phialides and conidia.

Phoma Saccardo

Members of the genus *Phoma* have a worldwide distribution and are ubiquitous in nature, with over 200 species having been described from soil, as saprophytes on various plants, and as pathogens to plants and humans.

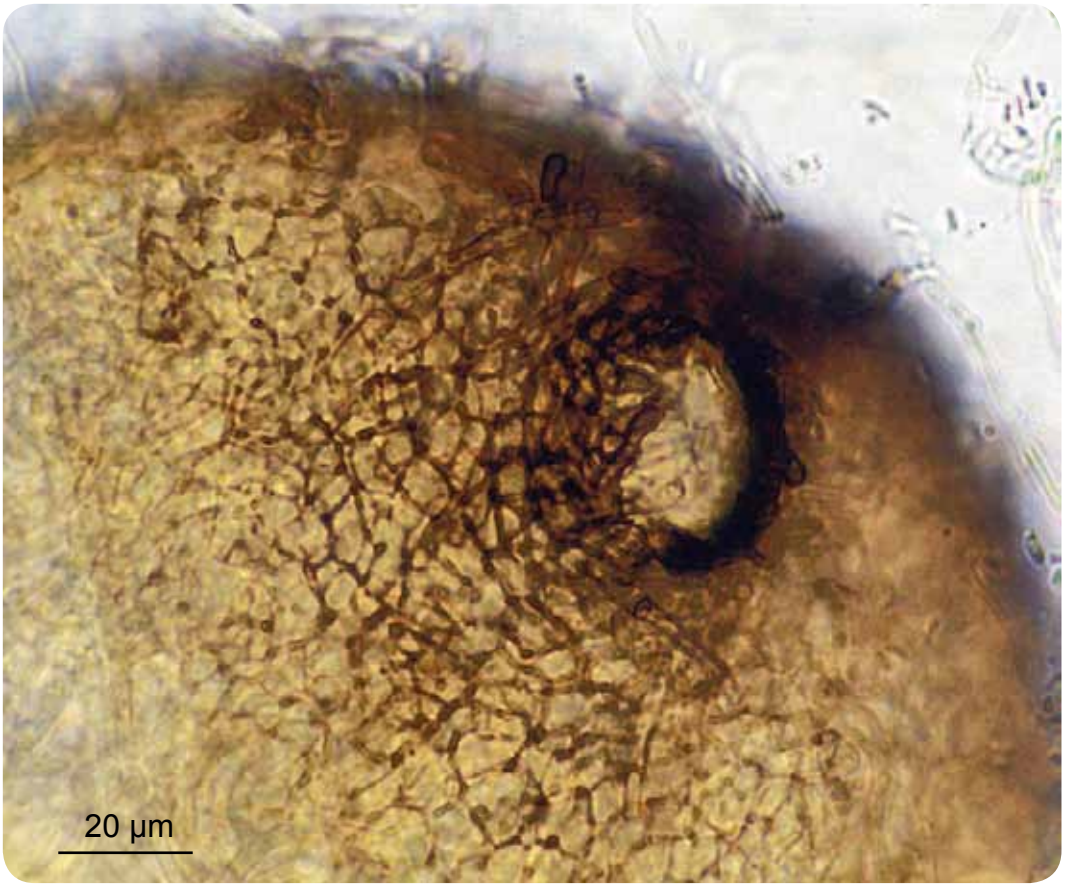
RG-1 organism.

Morphological Description: Colonies are spreading, greyish-brown, powdery or suede-like and produce large, globose, membranous to leathery, darkly pigmented, ostiolate pycnidia. Conidia are produced in abundance within the pycnidia on narrow thread-like phialides, which are hardly differentiated from the inner pycnidial wall cells. Conidia are globose to cylindrical, one-celled, hyaline, and are usually extruded in slimy masses from the apical ostiole.

Molecular Identification: ITS, D1/D2, β-tubulin and 18*S* sequencing has been used to identify *Phoma* species (de Gruyter *et al.* 2009, Aveskamp *et al*. 2010). **Note:** Public sequence databases, particularly GenBank, contain many sequences from incorrectly identified species, making identifications of coelomycetous fungi very difficult, without confirmatory morphological studies.

Key Features: Coelomycete, ostiolate pycnidia producing masses of slimy, hyaline, single-celled conidia.

References: Punithalingam (1979), McGinnis (1980), Sutton (1980), Rippon (1988), Montel *et al.* (1991), Samson *et al.* (1995), de Hoog *et al.* (2000, 2015).



Phoma spp. pycnidia with apical ostiole.

Pichia kudriavzevii Boidin *et al.*

Synonymy: *Candida krusei* (Castellani) Berkhout.
Issatchenkia orientalis Kudryavtesev.

Pichia kudriavzevii is regularly associated with some forms of infant diarrhoea and occasionally with systemic disease. It has also been reported to colonise the gastrointestinal, respiratory and urinary tracts of patients with granulocytopenia. Environmental isolations have been made from beer, milk products, skin, faeces of animals and birds. **RG-2 organism.**

Culture: Colonies (SDA) white to cream-coloured smooth, glabrous, yeast-like.

Microscopy: Predominantly small, elongated to ovoid blastoconidia, 2-5 x 4-5 µm.

India Ink Preparation: Negative - no capsules present.

Dalmau Plate Culture: Abundant long, wavy, branched pseudohyphae with elongated to ovoid blastoconidia, budding off in verticillate branches.

Molecular Identification: ITS sequencing recommended.

MALDI-TOF MS: Able to accurately identify this species.

Physiological Tests: + Positive, - Negative, v Variable, w Weak, s Slow							
Germ Tube	-	L-Sorbose	-	L-Arabinose	-	D-Glucitol	-
Fermentation		Sucrose	-	D-Arabinose	-	α-M-D-glucoside	-
Glucose	+	Maltose	-	D-Ribose	-	D-Gluconate	-
Galactose	-	Cellobiose	-	L-Rhamnose	-	DL-Lactate	+
Sucrose	-	Trehalose	-	D-Glucosamine	+	*myo*-Inositol	-
Maltose	-	Lactose	-	*N*-A-D-glucosamine	+	2-K-D-gluconate	-
Lactose	-	Melibiose	-	Glycerol	+	D-Glucuronate	-
Trehalose	-	Raffinose	-	Erythritol	-	Nitrate	-
Assimilation		Melezitose	-	Ribitol	-	Urease	-
Glucose	+	Soluble Starch	-	Galactitol	-	0.1% Cycloheximide	v
Galactose	-	D-Xylose	-	D-Mannitol	-	Growth at 40°C	+

Key Features: Germ tube negative yeast and sugar assimilation pattern. Colonies pale pink on *Candida* CHROMagar.

Antifungal Susceptibility: ***P. kudriavzevii*** (Australian National data); **MIC µg/mL.** CLSI clinical breakpoints are marked where available (Pfaller and Diekema 2012). **Note:** *P. kudriavzevii* is intrinsically resistant to fluconazole (No CLSI breakpoints).

	No.	≤0.008	0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	≥64
AmB	152		1		2	25	41	61	18	4					
FLU	152											1	9	45	77
VORI	135				5	16	62	40	7	3	2				
POSA	115			1	8	18	49	30	6	1		2			
ITRA	152			1	3	16	73	47	10				2		
ANID	86		5	35	26	19	1								
MICA	86		1	0	32	34	19								
CAS	115				1	15	55	29	13	1			1		
5FC	152				3				2	5	15	92	33	2	

Pichia norvegensis Leask & Yarrow

Synonymy: *Candida norvegensis* Dietrichson ex van Uden & Buckley.

Pichia norvegensis is a very rare clinical isolate that has been reported as a causative agent of peritonitis and disseminated candidiasis in a patient on CAPD.

RG-1 organism.

Culture: Colonies (SDA) white to cream-coloured smooth, glabrous, yeast-like.

Microscopy: Ovoid to ellipsoid, budding blastoconidia, 2.0-4 x 3-10 µm.

India Ink Preparation: Negative - no capsules present.

Dalmau Plate Culture: Spherical to ovoid budding yeast cells only. Abundant pseudohyphae produced.

Molecular Identification: ITS sequencing recommended.

MALDI-TOF MS: Able to accurately identify this species.

Physiological Tests: + Positive, - Negative, v Variable, w Weak, s Slow							
Germ Tube	-	L-Sorbose	-	L-Arabinose	-	D-Glucitol	-
Fermentation		Sucrose	-	D-Arabinose	-	α-M-D-glucoside	-
Glucose	s	Maltose	-	D-Ribose	-	D-Gluconate	-
Galactose	-	Cellobiose	+	L-Rhamnose	-	DL-Lactate	w
Sucrose	-	Trehalose	-	D-Glucosamine	+	*myo*-Inositol	-
Maltose	-	Lactose	-	*N*-A-D-glucosamine	-	2-K-D-gluconate	-
Lactose	-	Melibiose	-	Glycerol	+	D-Glucuronate	-
Trehalose	-	Raffinose	-	Erythritol	-	Nitrate	-
Assimilation		Melezitose	-	Ribitol	-	Urease	-
Glucose	+	Soluble Starch	-	Galactitol	-	0.1% Cycloheximide	-
Galactose	-	D-Xylose	-	D-Mannitol	-	Growth at 37^{O}C	+

Key Features: Germ tube negative yeast and sugar assimilation pattern.

Antifungal Susceptibility: ***P. norvegensis*** very limited data (Guitard *et al.* 2013, Australian National data); **MIC µg/mL.**

	No.	**≤0.008**	**0.016**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**≥64**
AmB	**8**						**1**	**6**	**1**						
FLU	**8**											**1**	**2**	**1**	**4**
VORI	**5**					**2**	**1**	**2**							
POSA	**5**					**2**	**3**								
ITRA	**7**					**1**	**4**	**1**		**1**					
ANID	**1**			**1**											
MICA	**1**			**1**											
CAS	**4**					**2**	**2**								
5FC	**5**								**1**		**1**	**1**	**1**	**1**	

Pithomyces chartarum (Berk. & M.A. Curtis) M.B. Ellis

The genus *Pithomyces* contains about 50 species commonly isolated from a wide range of plant material, also from air, soil, hay, sawn timber and ceiling plaster. *Pithomyces chartarum* has long been reported as causing facial eczema in sheep. However, recent molecular studies have identified at least two additional species *P. sacchari* and *P. maydicus* (de Cunha *et al.* 2014). Most human isolates are recovered from skin, nail, respiratory and sinus specimens.

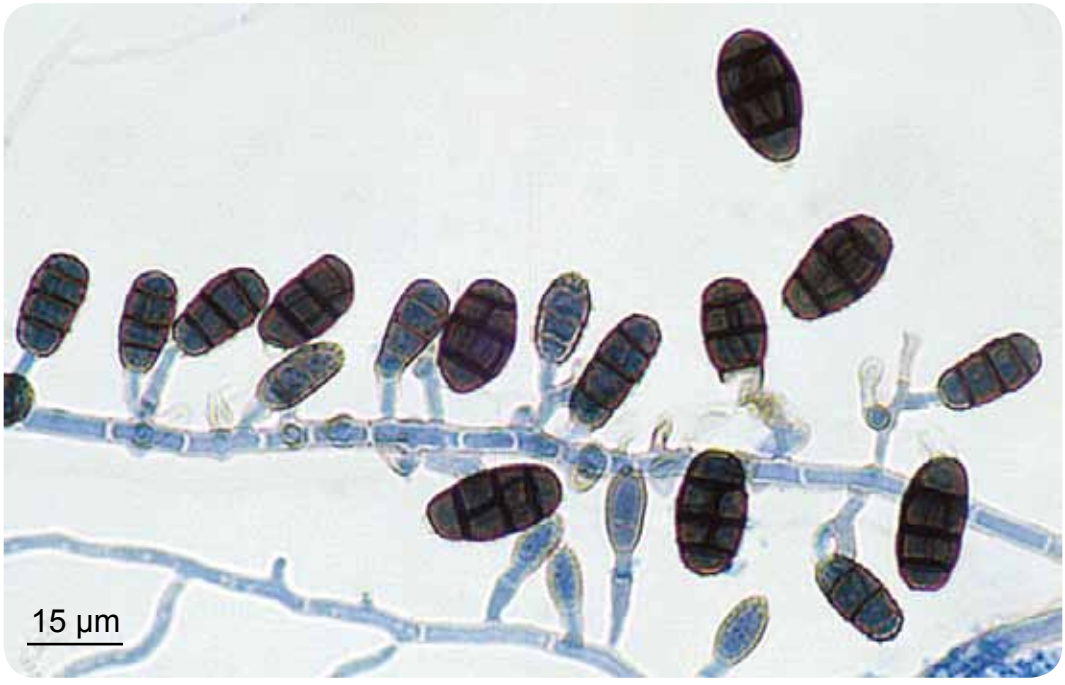
RG-1 organism.

Morphological Description: Colonies are fast growing, suede-like to downy and black. Conidiophores are pale olive, smooth or verrucose, 2.5-10 x 2-3.5 µm. Conidiogenous cells integrated, intercalary or terminal, indeterminate, with one to two loci of similar width in the conidiogenous cells. Conidia are muriform, medium to dark brown, echinulate to verrucose, three-(some up to five)-euseptate, slightly constricted at the septa, with one or both median cells divided by longitudinal septa, thick-walled, broadly ellipsoidal, apex obtuse, base truncate and characteristically with part of the conidiogenous cell remaining attached as a small pedicel, 18-29 x 10-17 µm.

Key Features: Dematiaceous hyphomycete with multicelled conidia produced on small peg-like branches of the vegetative hyphae.

Molecular Identification: ITS and D1/D2 sequencing recommended (de Cunha *et al*. 2014).

References: Ellis (1971, 1976), Domsch *et a*l. (1980), Rippon (1988), de Hoog *et al.* (2000, 2015), de Cunha *et al*. (2014).



Pithomyces chartarum conidiophores and conidia.

Pleurostomophora richardsiae (Nannf.) Mostert *et al*.

Synonymy: *Phialophora richardsiae* (Nannf.) Conant.

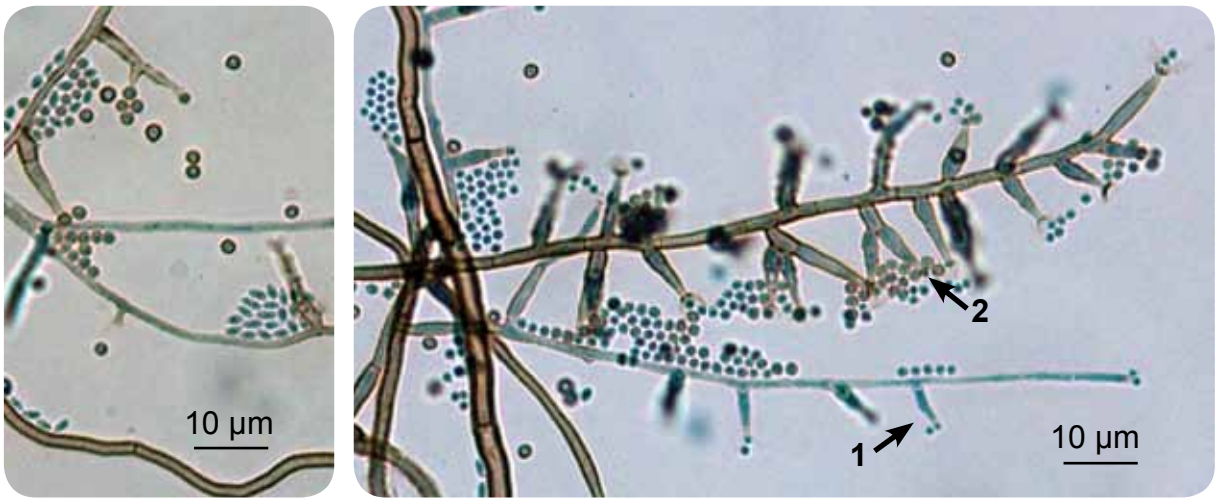
Vijaykrishna *et al.* (2004) separated *Pleurostomophora richardsiae* from *Phialophora* based on molecular data. *P. richardsiae* is a soft rot fungus of wood and is an uncommon cause of human infection, usually through traumatic implantation causing subcutaneous phaeohyphomycosis. **RG-2 organism.**

Morphological Description: Colonies grow rapidly, and are powdery to woolly or tufted, greyish-brown with a grey-brown to olivaceous-black reverse. Two conidial types are produced: (1) hyaline conidia which are allantoid or cylindrical, 3-6 x 1.5-2.5 µm in size, formed on inconspicuous, peg-like phialides on thin-walled hyphae; and (2) brown, thick-walled conidia which are spherical to subspherical, 2.5-3.5 x 2-3 µm, formed on dark brown, slender, tapering phialides with flaring collarettes.

Key Features: *P. richardsiae* is characterised microscopically by phialides with prominent flaring collarettes bearing globose, brown conidia while phialides with indistinct collarettes bear pale allantoid to cylindrical conidia.

Molecular Identification: ITS sequencing is recommended (Vijaykrishna *et al.* 2004).

References: Ellis (1971), McGinnis (1978a, 1980), Domsch *et al*. (1980), de Hoog *et al*. (2000, 2015), Vijaykrishna *et al*. (2004), Revankar and Sutton (2010).



Pleurostomophora richardsiae phialides producing two types of conidia. (1) hyaline conidia, and (2) brown, thick-walled conidia (arrows).

Antifungal Susceptibility: *Pleurostomophora richardsiae* limited data (Australian National data); **MIC µg/mL.**

	No.	≤0.008	0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	≥64
AmB	7						1	1	4	1					
VORI	7					3	4								
POSA	5				1	2		2							
ITRA	7					2	3	2					1		

P. richardsiae data from 11 isolates (McGinnis and Pasarell 1998a); **MIC µg/mL.**

AmB	**Range 0.125-1; Geometric mean = 0.73**
VORI	**Range 0.25-2; Geometric mean = 0.64**
ITRA	**Range 0.03-2; Geometric mean = 0.44**

Prototheca Kruger

Prototheca species are achlorophyllous algae with phylogenetic affinities to the genus *Chlorella.* To date only *P. wickerhamii* and *P. zopfii* have been involved in human or animal infections (Lass-Florl and Mayr 2007).

RG-1 organisms.

Morphological Description: Colonies are smooth, moist, white to cream and yeast-like. Cultures are sensitive to cycloheximide (actidione) and optimal growth occurs at 25-30^{O}C. Mycelium and conidia are absent. Vegetative cells are globose to ovoid, hyaline, varying in size from approximately 3-30 µm, and have a relatively thick and highly refractile wall. No budding cells are present; reproduction is by the development of large sporangia (theca) which contain from 2-20 or more small sporangiospores (endospores or autospores) which are asexually produced by nuclear division and cleavage of the cytoplasm.

Molecular Identification: ITS and D1/D2 sequencing is recommended (Wang *et al*. 2014).

Key Features: Achlorophyllous algae reproducing by sporangia (theca) and sporangiospores (autospores). *Prototheca* species which can be differentiated by assimilation tests and morphological criteria as outlined below. The API 20C yeast identification strip may be used for species identification.

References: Kaplan (1977), McGinnis (1980), Rippon (1988), Pore (1985), Ueno *et al.* (2005), Lass-Florl and Mayr (2007), Wang *et al.* (2014).

	P. wickerhamii	***P. zopfii***	***P. stagnora***
Colony morphology	Hemispheric, with smooth margin	Flat, rough, corrugated margin	Flat, with smooth margin
Cell diameter µm	3-10	7-30	7-14
Growth at 37^{o}C	+	+	-
Glucose	+	+	+
Trehalose	+	-	-
L-propanol	-	+	+/-
Acetate (pH 5)	-	+	+/-
Galactose	+	-	+
Capsule	-	-	+

Prototheca wickerhamii thecae and autospores.

Prototheca Kruger

Antifungal Susceptibility: *P. wickerhamii* (Australian National data); **MIC µg/mL**.													
	No.	**≤0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**≥64**
AmB	**9**			**3**	**3**	**2**	**1**						
VORI	**7**				**2**		**4**	**1**					
POSA	**7**				**1**	**2**	**4**						
ITRA	**9**					**2**	**5**				**2**		

Purpureocillium lilacinum (Thom) Luangsa-ard *et al.*

Synonymy: *Paecilomyces lilacinus* (Thom) Samson.

Purpureocillium lilacinum is commonly isolated from soil, decaying vegetation, insects, nematodes and as a laboratory contaminant. It is also a causative agent of infection in human and other vertebrates (Luangsa-ard *et al*. 2011).

RG-1 organism.

Morphological Description: Colonies are fast growing, suede-like to floccose, vinaceous to violet-coloured. Conidiophores are erect 400-600 µm in length, bearing branches with densely clustered phialides. Conidiophore stipes are 3-4 µm wide, yellow to purple and rough-walled. Phialides are swollen at their bases, gradually tapering into a slender neck. Conidia are ellipsoidal to fusiform, smooth-walled to slightly roughened, hyaline to purple in mass, 2.5-3.0 x 2-2.2 µm, and are produced in divergent chains. Chlamydospores are absent. Growth at 38^{O}C.

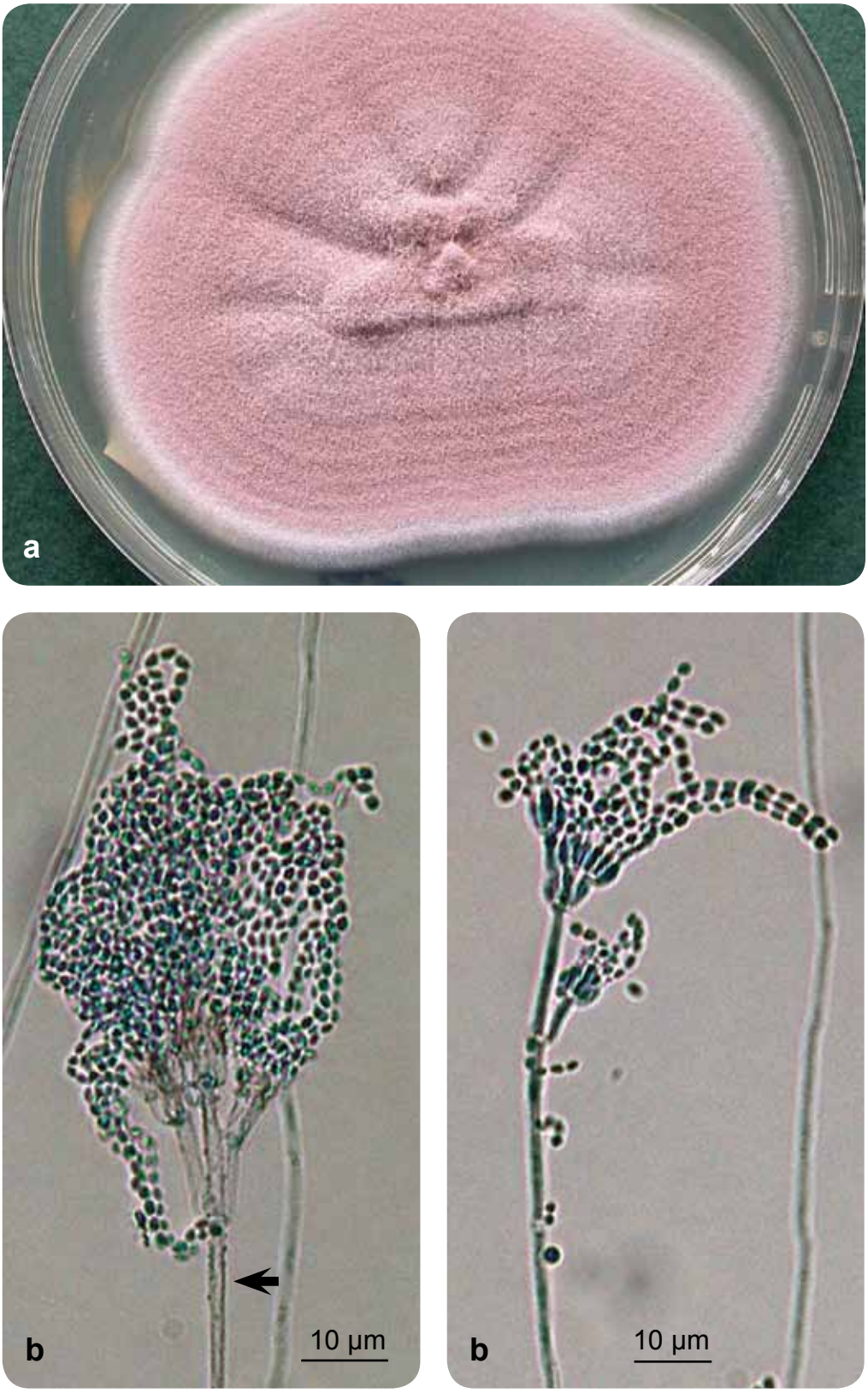
Molecular Identification: ITS sequencing is recommended (Atkins *et al.* 2005, Luangsa-ard *et al*. 2011).

Key Features: Colony pigmentation, phialides with swollen bases, pigmented and rough-walled conidiophore stipes, absence of chlamydospores and growth at 37^{O}C. **Note:** *Paecilomyces marquandii* differs by having a yellow reverse pigment, smooth conidiophore stipes, presence of chlamydospores, and no growth at 37^{O}C.

References: Samson (1974), Domsch *et al.* (1980), McGinnis (1980), Onions *et al*. (1981), Rippon (1988), de Hoog *et al*. (2000, 2015), Perdomo *et al.* (2013).

Antifungal Susceptibility: *P. lilacinum* (Australian National data); **MIC µg/mL.**											
	No.	**≤0.03**	**0.06**	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**≥16**
AmB	**52**			**1**		**1**		**6**	**6**	**24**	**14**
VORI	**50**		**8**	**30**	**9**	**1**			**2**		
POSA	**37**		**2**	**7**	**4**	**23**	**1**				
ITRA	**53**				**5**	**13**	**18**	**2**	**7**	**2**	**6**

Purpureocillium lilacinum (Thom) Luangsa-ard et al.



Purpureocillium lilacinum (a) culture and (b) conidiophores, phialides and conidia. **Note:** Rough-walled conidiophore (arrow).

Quambalaria cyanescens

Synonymy: *Sporothrix cyanescens* de Hoog & de Vries.
Cerinosterus cyanescens (de Hoog & de Vries) R.T. Moore

The genus *Quambalaria* contains five species, including *Q. cyanescens, Q. pitereka, Q. eucalypti, Q. coyrecup* and *Q. simpsonii. Q. cyanescens* is a hyaline basidiomycete isolated from a broad range of ecological niches, including air, soil, and insect larvae as well as in association with diverse plant sources, including *Corymbia* and *Eucalyptus* species from Australia. *Q. cyanescens* appears to be an emerging opportunistic pathogen in immunocompromised or debilitated individuals. It has been isolated from human skin and subcutaneous infections, blood, nosocomial infections in patients with pneumonia, peritonitis and invasive pulmonary infection (Jackson *et al.* 1990, Tambini *et al.* 1996, Schmidt *et al.* 2000, Kuan *et al.* 2015).

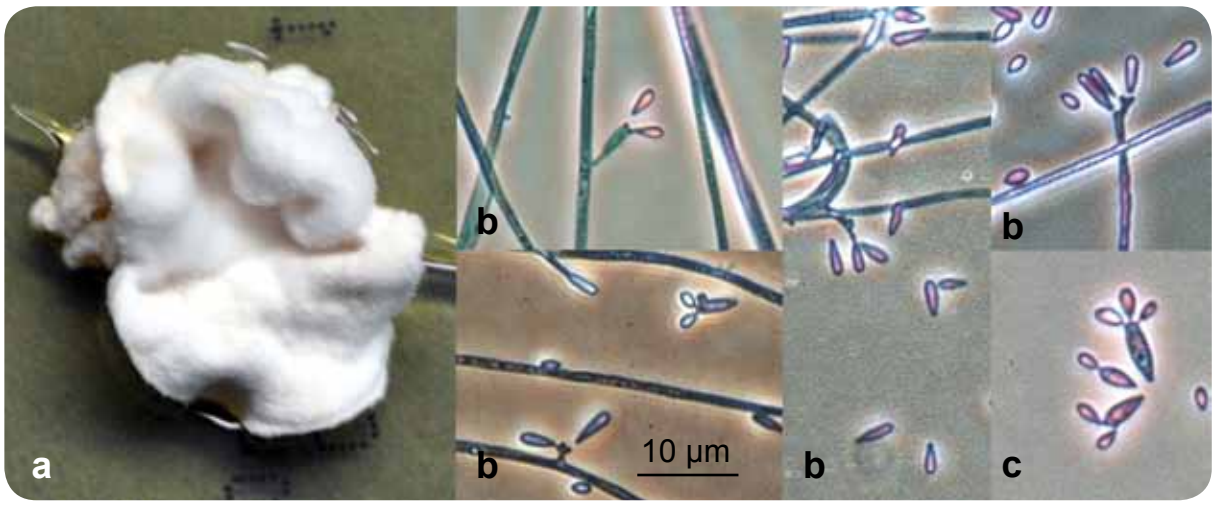
RG-1 organism.

Morphological Description: Colonies are restricted, farinose or velvety, often compact and somewhat cerebriform, snow-white, and later often exuding a pH-dependent, deep blue/violet pigment into the agar. Conidiogenous cells are undifferentiated, cylindrical, of variable size (1.5-3.0 µm wide), apically with a cluster of small denticles, the cluster often repeatedly proliferating and forming similar clusters. Conidia are hyaline, smooth-walled or finely verrucose, obovoidal, 3-4 µm long, somewhat larger (3.5-6.5 µm long) when bearing secondary conidia.

Molecular Identification: ITS and D1/D2 sequencing recommended.

Key Features: *Q. cyanescens* is similar to *Sporothrix*, but the conidial scars are very small and cultures are thin and fragile. In fresh cultures the diffusible pigment is characteristic. *Sporothrix schenckii* forms tough colonies, which finally become blackish-brown.

References: de Hoog and de Vries (1973), de Beer *et al.* (2006), Simpson (2000), de Hoog *et al.* (2015).



Quambalaria cyanescens (a) culture, (b) conidiogenous cells with small denticles and conidia, and (c) mature conidium bearing secondary conidia.

Rhinocladiella Nannfeldt

Rhinocladiella contains six to eight species, with five species of medical interest; *R. aquaspersa, R. atrovirens, R. basitona, R. mackenziei* (formerly *Ramichloridium mackenziei*) and *R. similis. R. mackenziei* is a frequently fatal neurotropic organism and appears to be restricted to individuals residing in, or immigrating from, Middle Eastern countries (Revankar and Sutton 2010).

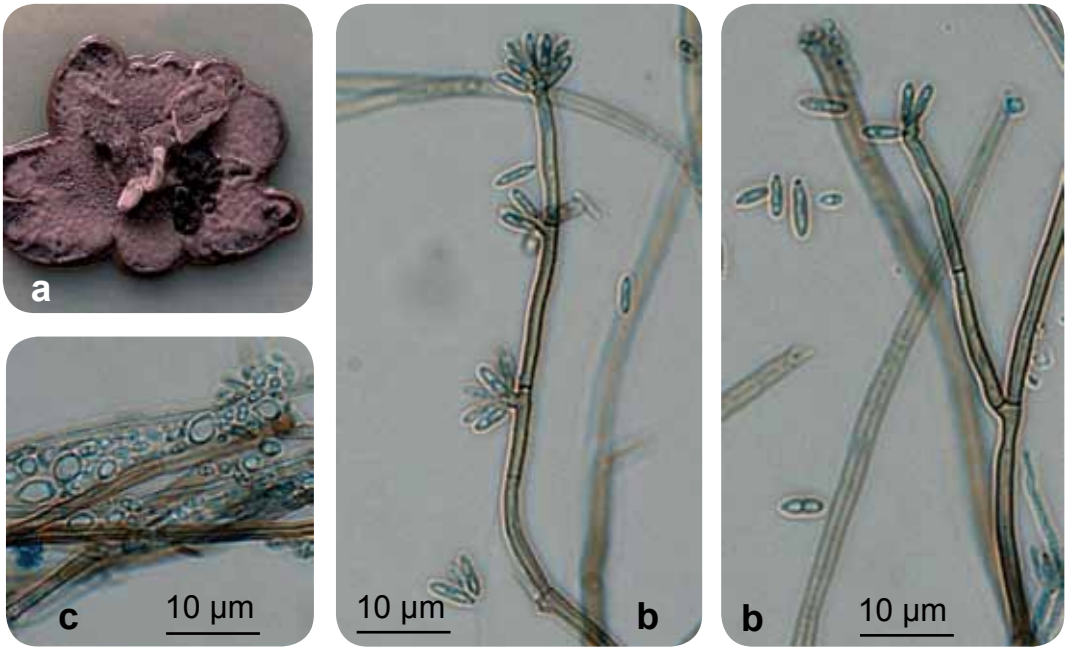
Rhinocladiella atrovirens Nannfeldt

RG-1 organism.

Morphological Description: Colonies are restricted, velvety or lanose, olivaceous, often slightly mucoid at the centre; reverse dark olivaceous green to blackish. Conidiophores are short, brown, thick-walled. Conidiogenous cells are cylindrical, intercalary or free, 9-19 x 1.6-2.2 µm; denticulate rachis up to 15 µm long, with crowded, flat or butt-shaped, unpigmented conidial denticles. Conidia are hyaline, thin- and smooth-walled, short-cylindrical, with truncate basal scars, 3.7-5.5 x 1.2-1.8 µm. Budding cells, if present, are hyaline, thin-walled, broadly ellipsoidal, 3.0-4.3 x 1.7-2.5 µm. Germinating cells are inflated, spherical to subspherical, 4.5-6.0 µm. An annellidic *Exophiala* synanamorph may be present.

Molecular Identification: ITS sequencing is recommended for accurate species identification (Taj-Aldeen *et al*. 2010).

References: de Hoog (1977, 1983), Schell *et al.* (1983), de Hoog *et al.* (2000, 2015).



Rhinocladiella atrovirens (a) culture, (b) conidiophores showing a terminal denticulate rachis with conidia, and (c) budding yeast cells.

Rhinocladiella Nannfeldt

Antifungal Susceptibility: *Rhinocladiella atrovirens* very limited data (McGinnis and Pasarell 1998a); **MIC µg/mL.**					
Antifungal	**Range**	**Antifungal**	**Range**	**Antifungal**	**Range**
AmB	**0.03-0.25**	**ITRA**	**0.03-0.06**	**VORI**	**0.03-0.5**

Rhinocladiella mackenziei (Campbell & Al-Hedaithy) Arzanlou & Crous

WARNING: RG-3 organism. Cultures of *R. mackenziei* represent a potential biohazard to laboratory personnel and must be handled with extreme caution in a Class II Biological Safety Cabinet (BSCII).

R. mackenziei is an extremely rare, neurotropic organism that causes fatal brain lesions, mostly in patients who are immunocompromised or suffered from underlying metabolic diseases. The species is typically restricted to the Middle East, in an arid zone between Israel and Pakistan (Kanj *et al.* 2001, Khan *et al.* 2002, Taj-Aldeen *et al.* 2010), with a single autochtonous case in India (Badali *et al*. 2010). Cases in the USA and Europe invariably were found in patients originating from the Middle East (Sutton *et al.* 1998, Revankar and Sutton 2010, de Hoog *et al.* 2015).

Morphological Description: Colonies growing moderately rapidly, velvety, olivaceous-brown. Conidiophores arising at right angles from creeping hyphae, stout, thick-walled, brown, 3.0-4.5 µm wide, 10-25 µm long, apically with short-cylindrical denticles. Conidia brown, ellipsoidal, 8.5-12.0 × 4-5 µm, with a prominent, wide basal scar.

Molecular Identification: ITS and D1/D2 sequencing is recommended for accurate species identification (Taj-Aldeen *et al*. 2010).

References: de Hoog (1977, 1983), Schell *et al*. (1983), de Hoog *et al.* (2000, 2015), Taj-Aldeen *et al.* (2010), Revankar and Sutton (2010).

Antifungal Susceptibility: *R. mackenziei* very limited data compiled from six case reports (Taj-Aldeen *et al*. 2010); **MIC µg/mL.**													
	No.	**≤0.016**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**≥32**
AmB	**7**		**1**					**3**				**2**	**1**
VORI	**3**	**1**	**1**				**1**						
POSA	**5**	**2**	**2**			**1**							
ITRA	**7**	**5**	**1**		**1**								

Rhizomucor Lucet & Costantin

The genus *Rhizomucor* is distinguished from *Mucor* by the presence of stolons and poorly developed rhizoids at the base of the sporangiophores and by the thermophilic nature of its two species: *R. miehei and R. pusillus*. Both of these species are potential human and animal pathogens and were originally classified in the genus *Mucor. Rhizomucor pusillus* is cosmopolitan and both *R. miehei* and *R. pusillus* have been reported as pathogens to humans and animals, the latter to a greater extent.

References: Cooney and Emerson (1964), Schipper (1978), Domsch *et al. (*1980), McGinnis (1980), Ellis and Keane (1981), Scholer *et al*. (1983), de Hoog *et al.* (2000, 2015), Schipper and Stalpers (2003) and Ellis (2005b).

Identification of most Mucorales is based primarily on the morphology of the sporangia; i.e. arrangement and number of sporangiospores, shape, colour, presence or absence of columellae and apophyses, as well as the arrangement of the sporangiophores and the presence or absence of rhizoids. Growth temperature tests can also be especially helpful in identifying and differentiating members of the genera *Rhizomucor, Rhizopus* and *Lichtheimia*.

Rhizomucor miehei (Cooney and Emerson) Schipper

Synonymy: *Mucor miehei* Lindt.

This species has been reported as a rare cause of bovine mastitis (Scholer *et al.* 1983) and is similar in many respects to *R. pusillus*.

RG-1 organism.

Morphological Description: All strains are homothallic forming numerous zygospores, which are reddish-brown to blackish-brown, globose to slightly compressed, up to 50 µm in diameter, with stellate warts and equal suspensor cells. Colony colour is a dirty grey rather than brown, and sporangia have spiny walls, are up to 50-60 µm in diameter, with columellae rarely larger than 30 µm in diameter. Growth is stimulated by thiamine, with no assimilation of sucrose and maximum growth temperature is 54-58^{O}C.

Key Features: Growth at 45^{O}C, the formation of numerous zygospores, a dirty grey culture colour and a partial growth requirement for thiamine.

Rhizomucor pusillus (Lindt) Schipper

Synonymy: *Mucor pusillus* Lindt.

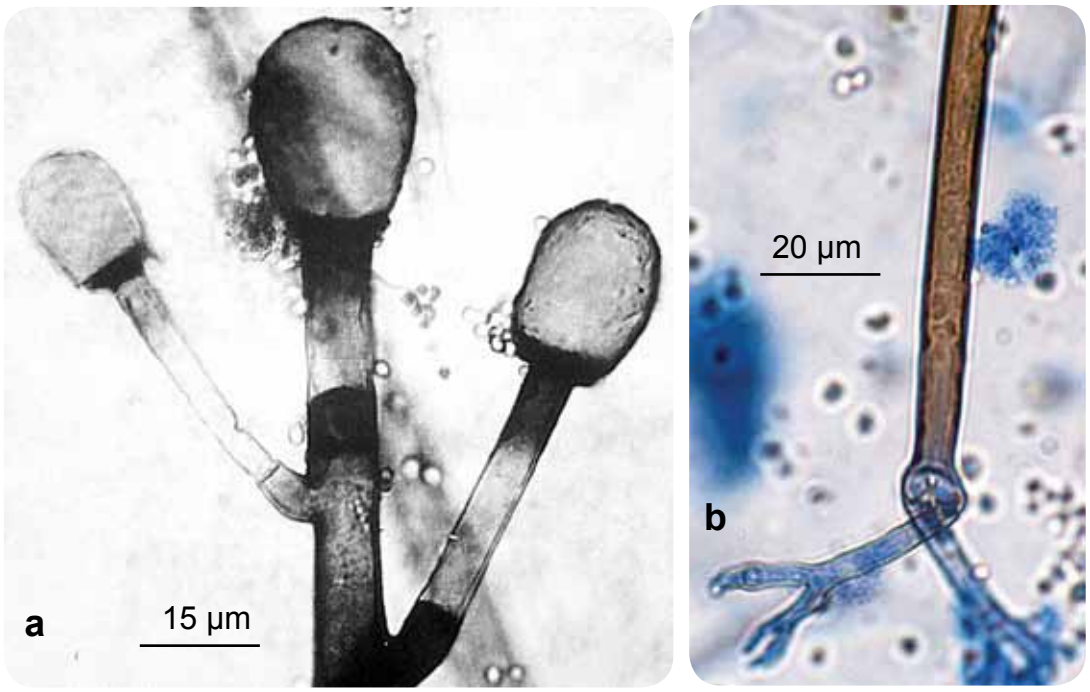
This species is a rare human pathogen. It has been reported from cases of pulmonary, disseminated and cutaneous types of infection. It is more often associated with animal disease, especially bovine abortion. *Rhizomucor pusillus* has a worldwide distribution and is commonly associated with compost heaps.

Rhizomucor pusillus (Lindt) Schipper

RG-2 organism.

Morphological Description: Cultures are characterised by compact, low growing (2-3 mm high), grey to greyish brown-coloured mycelium and by the development of typical sympodially branched, hyaline to yellow-brown sporangiophores (8-15 µm in diameter), always with a septum below the sporangium. Sporangia are globose (40-60 µm in diameter), each possessing an oval or pear-shaped columella (20-30 µm), often with a collarette. Sporangiospores are hyaline, smooth-walled, globose to subglobose, occasionally oval (3-5 µm), and are often mixed with crystalline remnants of the sporangial wall. Chlamydospores are absent. Zygospores are rough-walled, reddish brown to black, 45-65 µm in diameter and may be produced throughout the aerial hyphae in matings between compatible isolates. Temperature growth range: minimum 20-27^{O}C; optimum 35-55^{O}C; maximum 55^{O}C. There is positive assimilation of sucrose and no thiamine dependence.

Key Features: Mucorales, growth at 45^{O}C (thermophilic), poorly developed stolons and rhizoids, branching sporangiophores with a septum below the sporangium, dark-coloured sporangia without apophyses and smooth-walled globose to subglobose sporangiospores.



Rhizomucor pusillus (a) sporangiophores, collumellae and (b) primitive rhizoids.

Antifungal Susceptibility: ***R. pusillus*** (Espinel-Ingroff *et al*. 2015a, Australian National data); **MIC µg/mL.**

	No.	≤0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	≥32
AmB	**36**		**3**	**8**	**9**	**12**	**1**	**2**		**1**		
POSA	**36**		**1**	**5**	**11**	**11**	**7**	**1**				
ITRA	**18**			**3**	**6**	**4**	**3**	**1**				**1**

Rhizopus Ehrenberg ex Corda.

A molecular phylogenetic study of the genus *Rhizopus* by Abe *et al*. (2010) recognised eight species; *R. caespitosus, R. delemar, R. homothallicus, R. microsporus, R. arrhizus* (*R. oryzae*)*, R. reflexus, R. schipperae* and *R. stolonifer*. Based on these results and confirmed by Dolatabadi *et al*. (2014) the previous varieties of *Rhizopus microsporus* (*R. microsporus var. oligosporus* and *R. microsporus var. rhizopodiformis*) have been reduced to synonyms. In addition, *R. azygosporus* has been reduced to a synonym of *R. microsporus*. Finally, the controversy surrounding which species name to use for *R. oryzae - R. arrhizus* has been resolved in favour of the latter (Ellis 1985, de Hoog *et al.* 2015). Thus the important medical pathogens have now been reduced to just *R. arrhizus and R. microsporus*. These two species are the most common causative agents of mucormycosis, accounting for some 60% of the reported cases.

Morphological Description: The genus *Rhizopus* is characterised by the presence of stolons and pigmented rhizoids, the formation of sporangiophores, singly or in groups from nodes directly above the rhizoids, and apophysate, columellate, multispored, generally globose sporangia. After spore release the apophyses and columella often collapse to form an umbrella-like structure. Sporangiospores are globose to ovoid, one-celled, hyaline to brown and striate in many species. Colonies are fast growing and cover an agar surface with a dense cottony growth that is at first white becoming grey or yellowish brown with sporulation.

Molecular Identification: ITS sequencing is recommended but sequences must be compared to those of quality controlled reference strains with updated species names. (Alvarez *et al*. 2009 and Abe *et al.* 2010).

References: Domsch *et al*. (1980), McGinnis (1980), Onions *et al*. (1981), Scholer *et al*. (1983), Schipper (1984), Schipper and Stalpers (1984, 2003), Yuan and Jong (1984), Ellis (1985, 1986), Rippon (1988), Kwon-Chung and Bennett (1992), Samson *et al*. (1995), Schipper *et al*. (1996), de Hoog *et al.* (2000, 2015), Ellis (2005b), Alvarez *et al*. (2009), Abe *et al*. (2010), Dolatabadi *et al.* (2014).

Antifungal Susceptibility: *R. arrhizus* (Espinel-Ingroff *et al*. 2015a, Australian National data); **MIC µg/mL.**

	No.	≤0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	≥32
AmB	280	1	7	21	30	67	112	39	3			
POSA	370	1	5	14	84	161	65	29	4		4	2
ITRA	238		5	9	42	93	41	34	2	4	9	

Antifungal Susceptibility: *R. microsporus* (Espinel-Ingroff *et al*. 2015a, Australian National data); **MIC µg/mL.**

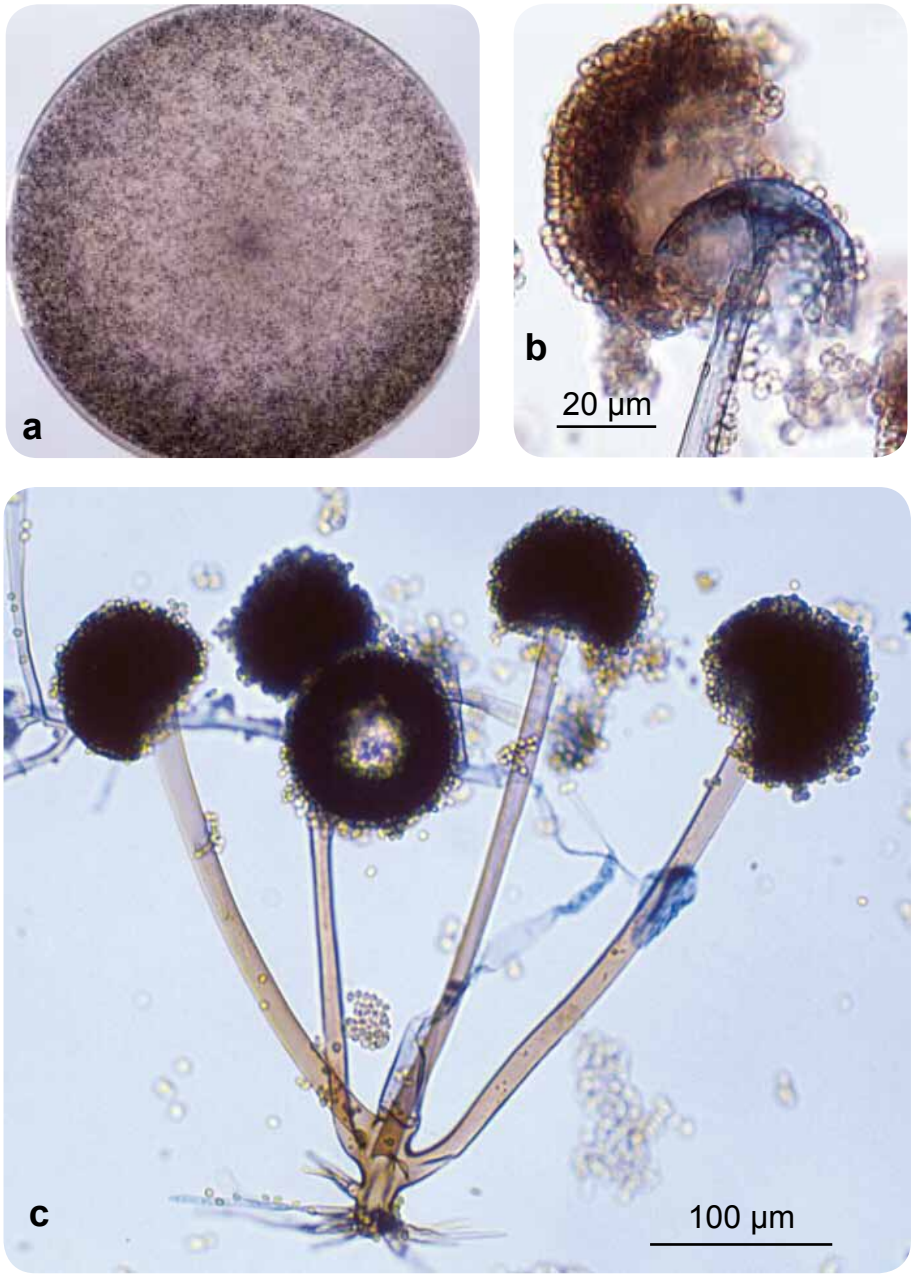
	No.	≤0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	≥32
AmB	189		2	23	22	95	72	22	5			
POSA	180		3	12	34	60	21	4	1		2	
ITRA	117		1	1	8	27	39	24	3	1	13	

Rhizopus arrhizus Fischer

Synonymy: *Rhizopus oryzae* Went & Prinsen Geerligs.

RG-2 organism.

Morphological Description: Colonies are very fast growing, about 5-8 mm high, with some tendency to collapse, white cottony at first becoming brownish grey to blackish-grey depending on the amount of sporulation. Sporangiophores up to 1500 µm in length and 18 µm in width, smooth-walled, non-septate, simple or branched, arising from stolons opposite rhizoids usually in groups of three or more. Sporangia are globose, often with a flattened base, greyish black, powdery in appearance, up to 175 µm in diameter and many spored. Columellae and apophysis together are globose, subglobose or oval, up to 130 µm in height collapsing to an umbrella-like form after spore release. Sporangiospores are angular, subglobose to ellipsoidal, with striations on the surface, and up to 8 µm in length. No growth at 45^{O}C; good growth at 40^{O}C.



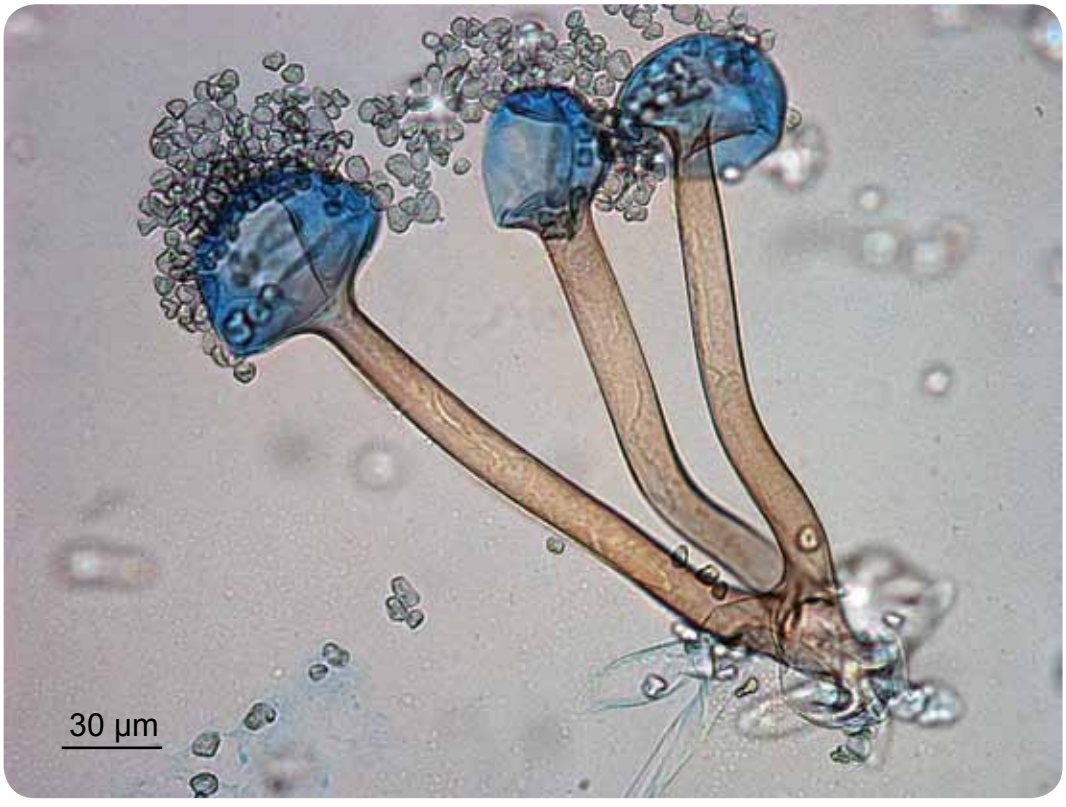
Rhizopus arrhizus (a) culture, (b) columellae and (c) sporangia showing sporangiospores, sporangiophores and rhizoids.

Rhizopus microsporus v. Tiegh

Synonymy: *Rhizopus azygosporus* Yuan & Jong.
Rhizopus microsporus var. *microsporus* Tiegh.
Rhizopus microsporus var. *oligosporus* (Saito) Schipper & Stalpers.
Rhizopus microsporus var. *rhizopodiformis* (Cohn) Schipper & Stalpes.
Rhizopus microsporus var. chinensis (Saito) Schipper & Stalpers.

RG-2 organism.

Morphological Description: Colonies are dark greyish-brown, up to 10 mm high producing simple rhizoids. Sporangiophores are brownish, up to 400 µm high and 10 µm wide, and may be produced in groups of one to four, usually in pairs. Sporangia are greyish-black, spherical, up to 100 µm in diameter. Columellae are subglobose to globose to conical comprising 80% of the sporangium. Sporangiospores are angular to broadly ellipsoidal or subglobose, up to 5-9 µm in length and are distinctly striate. Chlamydospores may be present. Zygospores are dark red–brown, spherical, up to 100 µm in diameter, with stellate projections and unequal suspensor cells. Some strains may be homothallic and produce azygospores. There is good growth at 45^{O}C, with a maximum of 50-52^{O}C.



Rhizopus microsporus sporangia showing sporangiospores, columellae, sporangiophores and rhizoids.

Rhodotorula Harrison

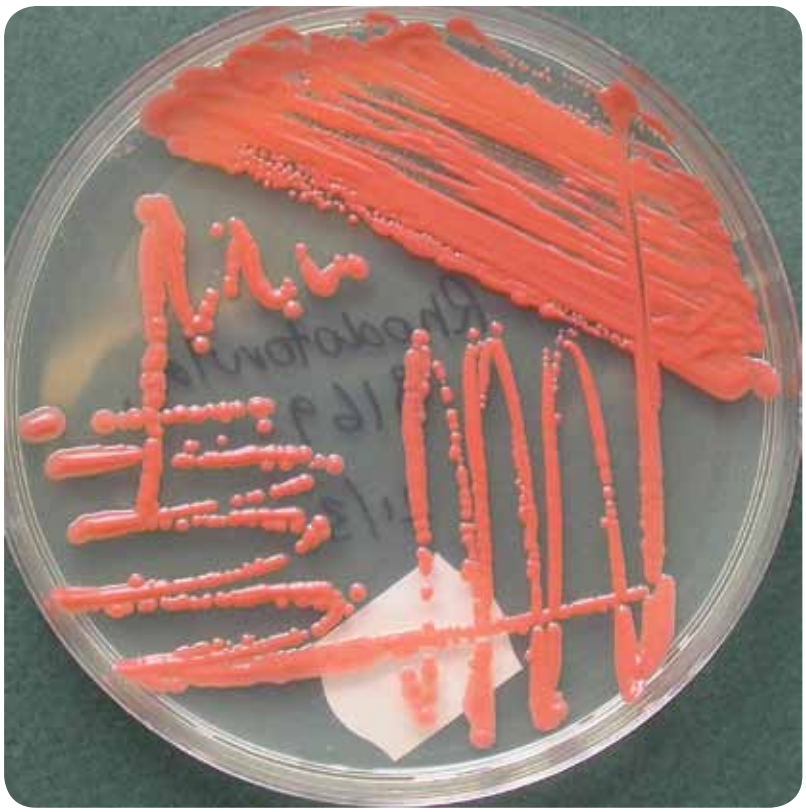
Rhodotorula species are common environmental basidiomycetous yeasts, which can be found in soil, ocean and lake water, fruit juice and milk, and on shower curtains and toothbrushes. Today, the genus contains 46 species of which three have been described as rare human pathogens: *R. mucilaginosa* (formerly known as *R. rubra*), *R. glutinis* and *R. minuta* (Arendrup *et al.* 2014).

Rhodotorula mucilaginosa is a common airborne contaminant of skin, lungs, urine and faeces. *R. mucilaginosa* is a known cause of fungal peritonitis in patients on continuous ambulatory peritoneal dialysis (CAPD). This is usually due to saprophytic colonisation of catheters or dialysis machinery and removal of the source of contamination usually leads to clearing of the symptoms. This species accounts for the majority of the infections (74–79%) followed by *R. glutinis* (7.7%) (Tuon and Costa 2008, Arendrup *et al.* 2014).

Molecular Identification: In many clinical cases species identification requires ITS and/or D1/D2 sequencing (Duboc de Almeida *et al.* 2008, Tuon and Costa 2008, Arendrup *et al*. 2014).

MALDI-TOF MS: reliably identifies clinically relevant *Rhodotorula* spp.

References: McGinnis (1980), Barnett *et al.* (1983), Kreger-Van Rij (1984), Rippon (1988), Kurtzman and Fell (1988), de Hoog *et al.* (2000, 2015), Spiliopoulou *et al.* (2012), Duboc de Almeida *et al.* (2008), Tuon and Costa (2008), Arendrup *et al*. (2014).



Rhodotorula mucilaginosa culture.

Rhodotorula glutinis (Fresenius) Harrison

RG-1 organism.

Morphological Description: Colonies are coral red to salmon-coloured or slightly orange, smooth to wrinkled, highly glossy to semi-glossy. Mucoid to pasty to slightly tough, yeast-like colonies. Ovoidal to globose or more elongate budding yeast-like cells or blastoconidia, 2.3-5.0 x 4.0-10 µm.

India Ink Preparation: Small capsules present.

Molecular Identification: Requires sequencing of the ITS and/or D1/D2 regions.

Key Features: Germ tube negative yeast and sugar assimilation pattern. Common saprophyte however cases of fungaemia have been reported.

Physiological Tests: + Positive, - Negative, v Variable, w Weak, s Slow, nd No data							
Germ Tube	-	L-Sorbose	v	L-Arabinose	v	D-Glucitol	v
Fermentation		Sucrose	+	D-Arabinose	v	α-M-D-glucoside	
Glucose	-	Maltose	+	D-Ribose	v	D-Gluconate	+
Galactose	-	Cellobiose	v	L-Rhamnose	v	DL-Lactate	v
Sucrose	-	Trehalose	+	D-Glucosamine	-	*myo*-Inositol	-
Maltose	-	Lactose	-	*N*-A-D-glucosamine	-	2-K-D-gluconate	nd
Lactose	-	Melibiose	-	Glycerol	v	D-Glucuronate	nd
Trehalose	-	Raffinose	v	Erythritol	-	Nitrate	-
Assimilation		Melezitose	+	Ribitol	v	Urease	+
Glucose	+	Soluble Starch	-	Galactitol	v	0.1% Cycloheximide	v
Galactose	v	D-Xylose	v	D-Mannitol	v	Growth at 37^{O}C	v

Antifungal Susceptibility: ***R. glutinis*** (Diekema *et al.* 2005, Australian National data); **MIC µg/mL**. **Note:** *Rhodotorula* species are intrinsically resistant to azoles and echinocandins (Arendrup *et al*. 2014).

	No.	**≤0.03**	**0.06**	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**≥64**
AmB	**38**		**1**	**2**	**4**	**3**	**28**						
FLU	**38**												**38**
VORI	**36**				**1**	**1**	**12**	**12**	**9**	**1**			
POSA	**34**					**2**	**9**	**18**	**4**	**1**			
ITRA	**38**		**1**		**1**	**3**	**9**	**11**	**2**	**3**	**8**		
5FC	**38**		**17**	**12**	**8**		**1**						

Rhodotorula mucilaginosa (Jorgensen) Harrison

Synonymy: *Rhodotorula rubra* (Demme) Lodder.

RG-1 organism.

Morphological Description: Colonies are coral pink, usually smooth, sometimes reticulate, rugose or corrugated, moist to mucoid, yeast-like colonies. Spherical to elongate budding yeast-like cells or blastoconidia, 2.5-6.5 x 6.5-14.0 µm.

India Ink Preparation: Small capsules present.

Molecular Identification: Requires sequencing of the ITS and/or D1/D2 regions.

Key Features: Germ tube negative yeast and sugar assimilation pattern. Common saprophyte however cases of peritonitis and fungaemia have been reported.

Physiological Tests: + Positive, - Negative, v Variable, w Weak, s Slow, nd No Data							
Germ Tube	-	L-Sorbose	v	L-Arabinose	v	D-Glucitol	v
Fermentation		Sucrose	+	D-Arabinose	v	α-M-D-glucoside	
Glucose	-	Maltose	v	D-Ribose	v	D-Gluconate	+
Galactose	-	Cellobiose	v	L-Rhamnose	v	DL-Lactate	v
Sucrose	-	Trehalose	+	D-Glucosamine	v	*myo*-Inositol	-
Maltose	-	Lactose	-	*N*-A-D-glucosamine	-	2-K-D-gluconate	nd
Lactose	-	Melibiose	-	Glycerol	v	D-Glucuronate	nd
Trehalose	-	Raffinose	+	Erythritol	v	Nitrate	-
Assimilation		Melezitose	v	Ribitol	v	Urease	+
Glucose	+	Soluble Starch	-	Galactitol	v	0.1% Cycloheximide	-
Galactose	v	D-Xylose	+	D-Mannitol	v	Growth at 40^{O}C	+

Antifungal Susceptibility: ***R. mucilaginosa*** (Diekema *et al.* 2005, Australian National data); **MIC µg/mL. Note:** *Rhodotorula* species are intrinsically resistant to azoles and echinocandins (Arendrup *et al*. 2014).

	No.	**≤0.03**	**0.06**	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**≥64**
AmB	**39**			**4**	**4**	**5**	**25**	**1**					
FLU	**39**												**39**
VORI	**37**				**2**	**3**	**6**	**11**	**10**	**4**	**1**		
POSA	**37**				**1**	**3**	**8**	**17**	**2**	**2**	**4**		
ITRA	**39**				**3**	**8**	**15**	**4**	**1**	**2**	**6**		
5FC	**39**	**1**	**14**	**12**	**11**	**1**							

Saccharomyces cerevisiae Meyen ex Hansen

Synonomy*: Candida robusta* Diddens & Lodder

Saccharomyces cerevisiae, commonly known as Baker's yeast, may be found as a harmless and transient digestive commensal and coloniser of mucosal surfaces of normal individuals. The anamorphic state of *S. cerevisiae* is sometimes referred to as *Candida robusta*. This species is phylogenetically closely related to *Candida glabrata* and shares many clinical and microbiological characteristics to this species (Arendrup *et al.* 2014). *S. cerevisiae* may be involved in mucosal infections like vaginitis, and in bloodstream infections, particularly in fluconazole-exposed patients. **Note:** *Saccharomyces boulardii,* a genetically similar subtype that is used as a probiotic for prevention and treatment of various sorts of diarrhoea and recurrent *Clostridium difficile*-associated diarrhoea should be avoided in immunocompromised hosts (Enache-Angoulvant and Hennequin 2005, Arendrup *et al.* 2014). **RG-1 organism.**

Morphological Description: Colonies are white to cream, smooth, glabrous and yeast-like. Large globose to ellipsoidal budding yeast-like cells or blastoconidia, 3.0-10.0 x 4.5-21.0 µm. No capsules present on India Ink preparation.

Physiological Tests: + Positive, - Negative, v Variable, w Weak, s Slow, nd No data							
Germ Tube	-	L-Sorbose	-	L-Arabinose	-	D-Glucitol	-
Fermentation		Sucrose	+	D-Arabinose	-	M-D-glucoside	
Glucose	+	Maltose	+	D-Ribose	-	D-Gluconate	-
Galactose	v	Cellobiose	-	L-Rhamnose	-	DL-Lactate	v
Sucrose	+	Trehalose	+	D-Glucosamine	-	*myo*-Inositol	-
Maltose	v	Lactose	-	*N*-A-D-glucosamine	-	2-K-D-gluconate	nd
Lactose	-	Melibiose	v	Glycerol	-	D-Glucuronate	nd
Trehalose	-	Raffinose	+	Erythritol	-	Nitrate	-
Assimilation		Melezitose	v	Ribitol	-	Urease	-
Glucose	+	Soluble Starch	-	Galactitol	-	0.1% Cycloheximide	-
Galactose	v	D-Xylose	-	D-Mannitol	-	Growth at 37^{O}C	v

Molecular Identification: ITS and/or D1/D2 sequencing is recommended (McCullough *et al.* 1988).

References: McGinnis (1980), Barnett *et al.* (1983), Kreger-Van Rij (1984), Rippon (1988), Kurtzman and Fell (1988), de Hoog *et al.* (2000, 2015).

Antifungal Susceptibility: *S. cerevisiae* (Australian National data); **MIC µg/mL.**													
	No.	**≤0.03**	**0.06**	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**≥64**
AmB	**41**	**1**	**1**	**8**	**14**	**11**	**6**						
FLU	**42**					**3**	**2**	**4**	**6**	**5**	**12**	**10**	
VORI	**38**	**5**	**8**	**7**	**8**	**8**	**2**						
POSA	**35**		**1**	**2**	**4**	**10**	**11**	**5**		**2**			
ITRA	**42**		**2**	**6**	**9**	**10**	**10**	**1**	**1**		**3**		
ANID	**32**	**3**	**3**	**17**	**6**	**3**							
MICA	**32**		**1**	**21**	**9**	**1**							
CAS	**36**		**2**	**3**	**11**	**14**	**5**	**1**					
5FC	**42**	**7**	**32**	**1**					**1**	**1**			

Saksenaea vasiformis Complex

The genus *Saksenaea* is characterised by the formation of flask-shaped sporangia with columellae and simple, darkly pigmented rhizoids. It is an emerging human pathogen (Holland, 1997) that is most often associated with cutaneous or subcutaneous lesions after trauma. Until recently, *Saksenaea vasiformis* was the only known species with a worldwide distribution in association with soil. However *S. vasiformis* has recently been split into three species; *S. vasiformis, S erythrospora* and S. *oblongispora* (Alvarez *et al.* 2010b). All three species have been isolated from clinical samples, but as yet no proven case reports have been published on the new species (de Hoog *et al.* 2015).

Saksenaea vasiformis Saksena

Morphological Description: Colonies are fast growing, downy, white with no reverse pigment, and made up of broad, non-septate hyphae typical of a mucormycetous fungus. Sporangia are typically flask-shaped with a distinct spherical venter and long-neck, arising singly or in pairs from dichotomously branched, darkly pigmented rhizoids. Collumellae are prominent and dome-shaped. Sporangiospores are small, oblong, 1-2 x 3-4 µm, and are discharged through the neck following the dissolution of an apical mucilaginous plug**. RG-2 organism.**

Key Features: Mucorales, unique flask-shaped sporangia, failure to sporulate on primary isolation media.

Molecular Identification: ITS sequencing is required for differentiation of species within the complex, and may be necessary to achieve identification in a timely manner (Alvarez *et al*. 2010b, Walther *et al*. 2012, Halliday *et al*. 2015).

Comment: Laboratory identification of this fungus may be difficult or delayed because of the mould's failure to sporulate on primary isolation media or on subsequent subculture onto potato dextrose agar. Sporulation may be stimulated by using the agar block method described by Ellis and Ajello (1982), Ellis and Kaminski (1985) and Padhye and Ajello (1988), although this may still take a period of days to weeks. Failure to sporulate prohibits antifungal susceptibility testing.

The agar block method to induce sporulation of S*aksenaea* spp*.* and *Apophysomyces spp*.

A small block of agar is cut from a well established culture grown on PDA and is placed in the centre of petri dish containing 1% agar in distilled water. After 21 days at 26^{O}C sporangia might be formed at the periphery of the petri dish.

Antifungal Susceptibility: *S. vasiformis* very limited data, due to poor sporulation (Sun *et al*. 2002 and Australian national data); **MIC µg/mL**.

Antifungal	**Range**	**MIC_{90}**	**Antifungal**	**Range**	**MIC_{90}**
AmB	0.125-2	2	**POSA**	0.016-0.25	0.25
ITRA	0.016-0.03	0.03	**VORI**	0.5-4	4

***Saksenaea vasiformis* Saksena**

Saksenaea vasiformis showing a typically flask-shaped sporangium.

References: Saksena (1953), Ellis and Hesseltine (1966), Ajello *et al*. (1976), Ellis and Ajello (1982), Ellis and Kaminski (1985), Pritchard *et al.* (1986), Padhye *et al.* (1988), Padhye and Ajello (1988), Goldschmied-Reouven *et al*. (1989), de Hoog *et al.* (2000) and Ellis (2005b).

Saprochaete clavata (de Hoog et al.) de Hoog & M.Th. Smith

Synonmy: *Geotrichum clavatum* de Hoog, M.Th. Smith & Guého.

Saprochaete clavata (formerly known as *Geotrichum clavatum*), which is also closely related to *Magnusiomyces capitatus* (formerly known as *Geotrichum capitatum* or *Saprochaeta capitata*), has only very infrequently been described as involved in invasive human infection. However, an outbreak of invasive infections caused by *Saprochaete clavata* in haematology patients has been reported (Vaux *et al.* 2013).

RG-1 organism.

Morphological Description: Colonies are moderately fast growing, flat, whitish and butyrous. True hyphae are abundant, soon breaking up into rectangular arthroconidia of variable size, 2.8-4 3 x 6-20 µm. Sympodial conidiogenesis is occasionally present. Terminal parts of hyphae may swell and become thick-walled.

Note: *Saprochaete clavata and Magnusiomyces capitatus* are human pathogens that are closely related and are frequently mistaken for each other.

Molecular Identification: ITS sequencing is recommended for accurate species identification.

MALDI-TOF MS: reliably identifies *S. clavata, M. capitatus* and *Geotrichum candidum* to the species level (Desnos-Ollivier *et al*. 2014).

Physiological Tests: + Positive, - Negative, v Variable, w Weak, s Slow, nd No Data							
Germ Tube	-	L-Sorbose	+	L-Arabinose	-	D-Glucitol	-
Fermentation		Sucrose	-	D-Arabinose	-	M-D-glucoside	-
Glucose	-	Maltose	-	D-Ribose	-	D-Gluconate	-
Galactose	-	Cellobiose	+	L-Rhamnose	-	DL-Lactate	+,w
Sucrose	-	Trehalose	-	D-Glucosamine	-	*myo*-Inositol	-
Maltose	-	Lactose	-	*N*-A-D-glucosamine	nd	2-K-D-gluconate	-
Lactose	-	Melibiose	-	Glycerol	+	D-Glucuronate	nd
Trehalose	-	Raffinose	-	Erythritol	-	Nitrate	-
Assimilation		Melezitose	-	Ribitol	-	Urease	-
Glucose	+	Soluble Starch	-	Galactitol	-	0.1% Cycloheximide	-
Galactose	+	D-Xylose	-	D-Mannitol	-	Growth at 37°C	+

Antifungal Susceptibility: ***Saprochaete clavata*** (Vaux *et al*. 2013); **MIC µg/mL**. **Note:** *S. clavata* is intrinsically resistant to echinocandins.

Antifungal	**Range**	**Median**	**Antifungal**	**Range**	**Median**
AmB	0.125-1	0.5	**POSA**	0.125-1	0.5
5FC	≤0.125-1	0.25	**VORI**	0.06-2	1

References: de Hoog and Smith (2004, 2011b), de Hoog *et al.* (2015), Desnos-Ollivier *et al.* (2014), Vaux *et al*. (2013), Arendrup *et al.* (2014).

Sarocladium W. Gams & D. Hawksw.

Based on a recent molecular phylogenetic study the taxonomy of *Acremonium* was reviewed and some medically important species have been transferred to *Sarocladium*; i.e. *S. kiliense* (formerly *A. kiliense*) and *S. strictum* (formerly *A. strictum)*. Although these genera are morphologically similar they are phylogenetically distant. *Sarocladium* can be morphologically differentiated from *Acremonium* by its elongated phialides rising solitary on vegetative hyphae or on conidiophores that are sparsely or repeatedly branched, the production of abundant adelophialides and elongated conidia. (Glenn *et al.* 1996, Summerbell *et al.* 2011, Giraldo *et al.* 2015).

Sarocladium strictum (W. Gams) Summerbell

Sarocladium strictum is commonly found in soil and plant debris. Cutaneous, CAPD-related peritonitis and invasive infections in immunosuppressed patients have been reported.

RG-1 organism.

Morphological Description: Colonies growing rapidly, moist to slimy, pink or orange; reverse remaining colourless or turning pink to orange. Conidiophores simple, occasionally branched. Phialides slender, arising from submerged or slightly fasciculate aerial hyphae, 20-65 × 1.4-2.5 µm. Submerged sporulation frequent from reduced phialides. Conidia grouped in slimy heads, cylindrical or ellipsoidal, 3.3-5.5 × 0.9-1.8 µm, hyaline.

Molecular Identification: Summerbell *et al.* (2011) revised the genus and recommends using D1/D2 sequences for phylogenetic analysis and sequence-based identification.

References: Glenn *et al*. (1996), Summerbell *et al*. (2011), Giraldo *et al.* (2015), de Hoog *et al.* (2015).

Sarocladium strictum (a) colony and (b) slender phialides with conidia in slimy heads.

Antifungal Susceptibility: *S. strictum* very limited data (Australian National data); **MIC µg/mL.**													
	No.	**≤0.016**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**≥32**
AmB	**5**						**1**		**2**		**2**		
VORI	**5**					**1**	**1**	**1**	**2**				
POSA	**5**						**1**	**2**	**1**		**1**		
ITRA	**5**								**1**	**1**	**3**		

Scedosporium Sacc. ex Castell. & Chalm.

The taxonomy of this genus has been subject to change on the basis of sequence data; *Scedosporium apiospermum* and *Scedosporium boydii* (formerly *Pseudallescheria boydii*) are now recognised as separate species and along with *S. aurantiacum* are the principal human pathogens (Lackner *et al.* 2014a). The majority of infections are mycetomas, the remainder include infections of the eye, ear, central nervous system, internal organs and more commonly the lungs. *Scedosporium dehoogii* and *S. minutispora* are mainly isolated from environmental samples and have been rarely reported from clinical cases (Gilgado *et al*. 2005, Rainer and de Hoog 2006, Cortez *et al*. 2008, Kaltseis *et al.* 2009).

Scedosporium prolificans has been transferred to the genus *Lomentospora*. *L. prolificans* is phylogenetically and morphologically distinct from the remaining *Scedosporium* species (Lennon *et al*. 1994, Lackner *et al*. 2014a).

Morphological identification of *Scedosporium* species has become increasingly unreliable and molecular identification methods are now recommended. The conidial states of *S. apiospermum* and *S. boydii* are morphologically indistinguishable; although the latter is homothallic and produces ascocarps. *S. aurantiacum* also exhibits similar conidial morphology but most strains produce a pale to bright yellow diffusible pigment on potato dextrose agar.

Molecular Identification: Recommended genetic markers are ITS and β-tubulin (Lackner *et al.* 2012a).

MALDI-TOF MS: A comprehensive 'in-house' database of reference spectra allows accurate identification of *Scedosporium* and *Lomentospora species* (Lau *et al*. 2013, Sitterlé *et al*. 2014).

References: McGinnis (1980), Domsch *et al.* (1980), McGinnis *et al*. (1982), Campbell and Smith (1982), Rippon (1988), de Hoog *et al.* (2000, 2015), Gilgado *et al*. (2005), Rainer and de Hoog (2006), Guarro *et al.* (2006), Lackner *et al*. (2014a).

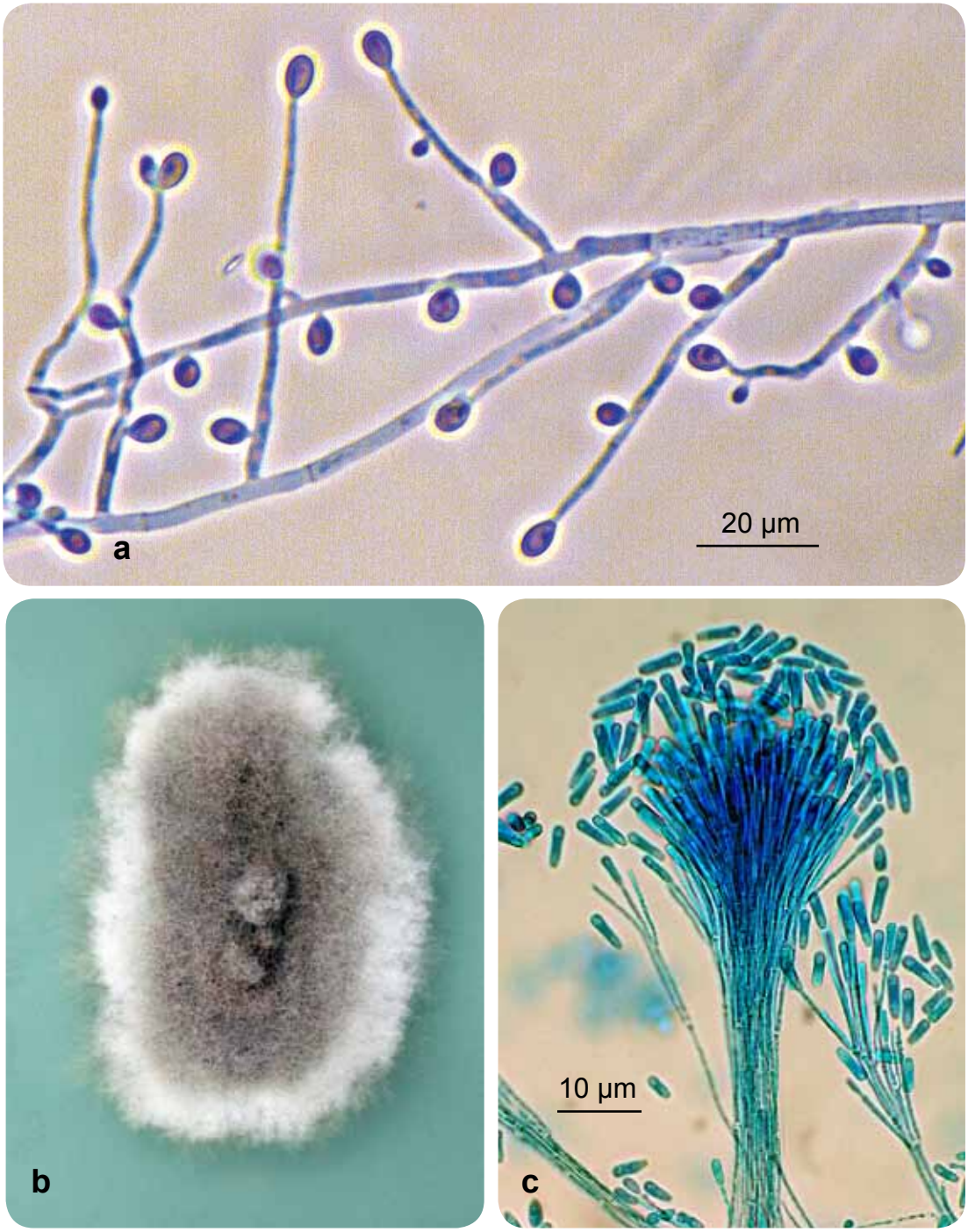
Scedosporium apiospermum (Saccardo) Castellani and Chalmers

Synonymy: *Pseudallescheria apiosperma* Gilgado, Gené, Cano & Guarro

RG-2 organism.

Morphological Description: Colonies are fast growing, greyish-white, suede-like to downy with a greyish-black reverse. Numerous single-celled, pale-brown, broadly clavate to ovoid conidia, 4-9 x 6-10 µm, rounded above with truncate bases are observed. Conidia are borne singly or in small groups on elongate, simple or branched conidiophores or laterally on hyphae. Conidial development can be described as annellidic, although the annellations (ring-like scars left at the apex of an annellide after conidial secession) are extremely difficult to see. Erect synnemata may be present in some isolates. Optimum temperature for growth is 30-37^{O}C.

Scedosporium apiospermum (Saccardo) Castellani and Chalmers

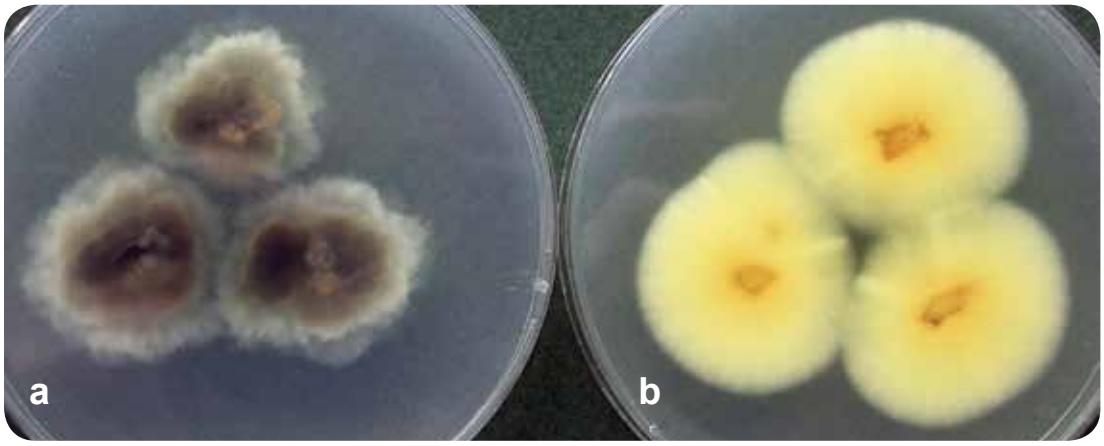


Scedosporium apiospermum (a) conidiophores and conidia, (b) culture and (c) synnemata.

Antifungal Susceptibility: *S. apiospermum* (Australian National data); **MIC µg/mL.**													
	No.	**≤0.03**	**0.06**	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**≥64**
AmB	**167**				**2**	**2**	**10**	**45**	**51**	**40**	**17**		
VORI	**163**	**5**	**27**	**57**	**47**	**23**			**1**	**3**			
POSA	**112**		**2**	**6**	**22**	**49**	**27**	**3**	**1**	**2**			
ITRA	**167**		**2**	**5**	**21**	**86**	**38**	**7**			**8**		

Scedosporium aurantiacum Gilgado *et al.*

Morphological Description: Most isolates produce a light yellow diffusible pigment on potato dextrose agar after a few days incubation. Conidiogenous cells and conidia are similar in shape and size to *S. apiospermum*, and the two can best be distinguished by genetic analysis. Conidiogenous cells arising from undifferentiated hyphae are cylindrical to slightly flask-shaped, producing slimy heads of one-celled , smooth-walled, subhyaline, obovoid or sub-cylindrical conidia. 5-14 x 2-5 um. Erect synnemata may be present in some isolates, but the teleomorph is unknown. Optimum temperature for growth 37-40^{O}C, max 45^{O}C. **RG-2 organism.**



Culture reverse (PDA) of (a) *S. apiospermum* and (b) *S. aurantiacum* showing production of a light yellow diffusible pigment that is typical of *S. aurantiacum.*

Scedosporium aurantiacum conidiophores (annellides) and conidia.

Antifungal Susceptibility: *S. aurantiacum* (Australian National data); **MIC µg/mL**.													
	No.	**≤0.03**	**0.06**	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**≥64**
AmB	**11**							**1**	**7**	**3**			
VORI	**11**			**5**	**5**				**1**				
POSA	**11**					**4**	**4**	**2**		**1**			
ITRA	**11**					**4**	**4**				**3**		

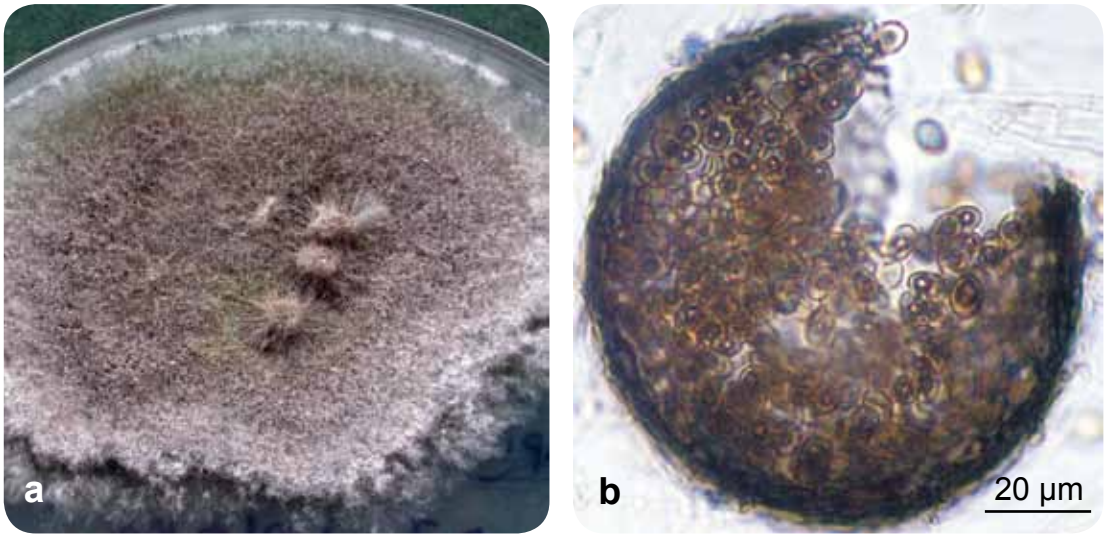
Scedosporium boydii (Shear) Gilgado *et al.*

Synonymy: *Pseudallescheria boydii* (Shear) McGinnis, A.A. Padhye & Ajello.

RG-2 organism.

Morphological Description: Colonies are fast growing, greyish-white, suede-like to downy with a greyish-black reverse. Numerous single-celled, pale-brown, broadly clavate to ovoid conidia, 4-9 x 6-10 µm, rounded above with truncate bases are observed. Conidia are borne singly or in small groups on elongate, simple or branched conidiophores or laterally on hyphae. Cleistothecia (non-ostiolate ascocarps) are yellow-brown to black, spherical, 50-200 µm in diameter, and are mostly submerged in the agar and are composed of irregularly interwoven brown hyphae. When crushed cleistothecia release numerous, faintly brown, ellipsoidal ascospores, 4-5 x 7- 9 µm in size. Erect synnemata may be present in some isolates. Optimum temperature for growth is 30-37^{O}C.

Note: *S. boydii* is homothallic and is recognised by smaller cleistothecia (50-200 µm) whereas *S. apiospermum* is heterothallic (requires mating of two strains) and has larger cleistothecia, 140-480 µm (Gilgado *et al*. 2010).



Scedosporium boydii (a) culture and a (b) cleistothecium.

Antifungal Susceptibility: *S. boydii* vs *S. apiospermum* (Lackner *et al.* 2014b).				
Antifungal (MIC µg/mL)	***S. boydii***		***S. apiospermum***	
	Range	**MIC_{90}**	**Range**	**MIC_{90}**
AmB	**0.5->16**	**≥16**	**0.5->16**	**≥16**
VORI	**0.125-2**	**2**	**0.25->8**	**2**
POSA	**0.125->16**	**≥16**	**0.25->16**	**≥16**
ITRA	**0.125->16**	**≥16**	**0.25->16**	**≥16**

Schizophyllum commune Fries

Schizophyllum commune is a common basidiomycete bracket fungus found on rotten wood, and is an occasional human pathogen, principally associated with sinusitis, allergic bronchopulmonary mycosis and as a contaminant from respiratory specimens. However the introduction of DNA sequencing and/or MALDI-TOF MS identification in the clinical laboratory has seen many more cases of *S. commune* fungal rhinosinusitis identified (Michel *et al.* 2012, Chowdhary *et al.* 2013a, 2014a,b).

RG-1 organism.

Morphological Description: Colonies on 2% malt extract agar are spreading, woolly, whitish to pale greyish-brown, soon forming macroscopically visible fruiting bodies. Some isolates may take up to 12 weeks to form fruiting bodies. Fruit bodies are sessile, kidney-shaped, lobed with split gills on the lower side. Hyphae are hyaline, wide and have clamp connections. Basidia bear four basidiospores on erect sterigmata. Basidiospores hyaline, smooth-walled, elongate with lateral scar at lower end, 6-7 x 2-3 µm.

Note: Many clinical isolates of *S. commune* are monokaryotic and do not show clamp connections, therefore any white, rapidly growing, sterile isolate showing good growth at 37^{O}C with tolerance to benomyl, susceptibility to cycloheximide, and a pronounced odour should be suspected of being *S. commune* (Sigler *et al*. 1995).

Molecular Identification: Sequencing of the ITS and D1/D2 regions is recommended (Buzina *et al.* 2001, Won *et al*. 2012, Chowdhary *et al*. 2013b, Michel *et al*. 2012), however the number of well identified nucleotide sequences of these fungi in the GenBank database remains limited.

MALDI-TOF MS: Michel *et al.* (2012), Chowdhary *et al.* (2014b), Huguenin *et al*. (2015) provide identification procedures, however the number of mass spectral profiles to be found in MALDI-TOF libraries remains limited.

References: McGinnis (1980), Rippon (1988), Sigler *et al.* (1995), de Hoog *et al.* (2015).

Schizophyllum commune basidiocarps growing on malt extract agar.

Antifungal Susceptibility: *S. commune* (Chowdhary *et al.* 2013b); **MIC µg/mL**.					
Antifungal	**Range**	**MIC_{90}**	**Antifungal**	**Range**	**MIC_{90}**
AmB	**0.03-2**	**1**	**FLU**	**2-64**	**64**
ITRA	**0.03-8**	**1**	**VORI**	**0.06-2**	**0.5**

Scopulariopsis Bain

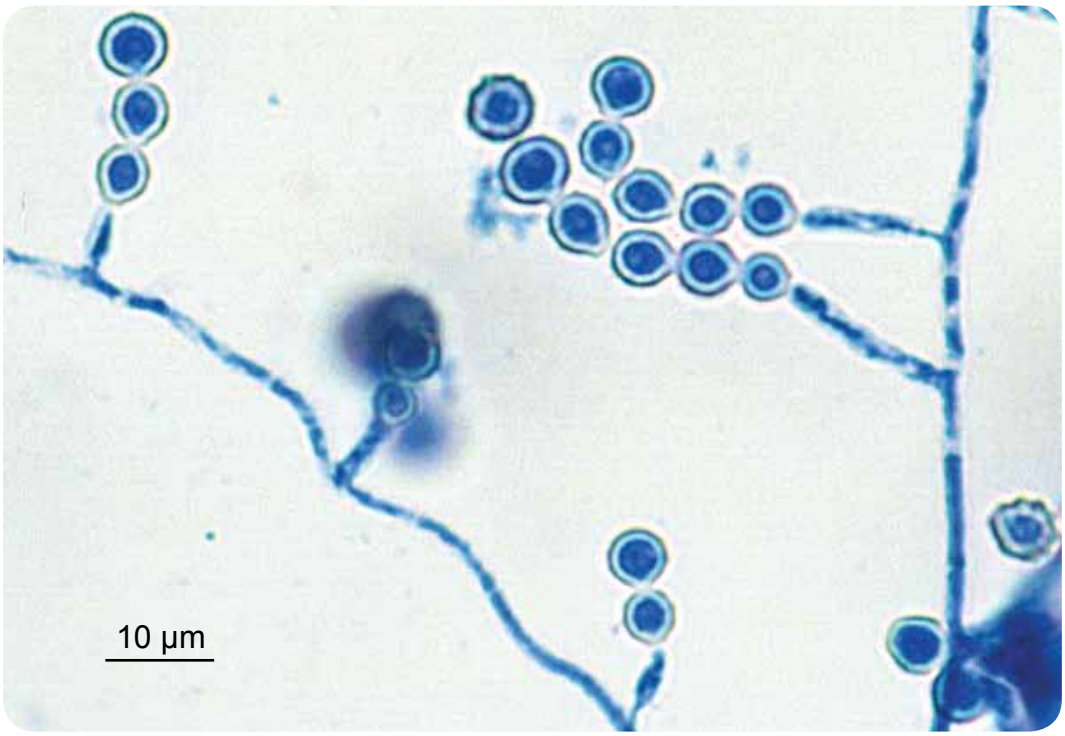
Most members of the genus *Scopulariopsis* are soil fungi, which are frequently isolated from food, paper and other materials. They also occur as laboratory contaminants. Several species have been reported as causative agents of onychomycosis and hyalohyphomycosis (Sandoval-Denis *et al*. 2013). The most common species seen in the clinical laboratory is *S. brevicaulis*, followed by *S. gracilis S. brumptii, Microascus cinereus, S. candida* complex, and *M. cirrosus* (Sandoval-Denis *et al*. 2013).

Morphological Description: Colonies are fast growing, varying in colour from white, cream, grey, buff to brown and black, but are predominantly light brown. Microscopic morphology shows chains of single-celled conidia produced in basipetal succession from a specialised conidiogenous cell called an annellide. Once again, the term basocatenate can be used to describe such chains of conidia where the youngest conidium is at the basal end of the chain. In *Scopulariopsis*, annellides may be solitary, in groups, or organised into a distinct penicillus. Conidia are globose to pyriform, usually truncate, with a rounded distal portion, smooth to rough, and hyaline to brown in colour. **RG-2 for species isolated from humans.**

Key Features: Hyphomycete, conidia often shaped like light globes, basocatenate arising from annellides.

Molecular Identification: D1/D2 and *EF-1α* sequence analysis can be useful for the identification of the most common clinically relevant species (Sandoval-Denis *et al.* 2013).

References: Morton and Smith (1963), McGinnis (1980), Rippon (1988), Samson *et al*. (1995), Domsch *et al.* (2007), de Hoog *et al.* (2000, 2015).



Scopulariopsis brevicaulis conidiophores (annellides) and conidia.

Antifungal Susceptibility: *S. brevicaulis* (Skora *et al.* 2014); **MIC µg/mL**.					
Antifungal	**Range**	**MIC_{90}**	**Antifungal**	**Range**	**MIC_{90}**
AmB	**4-16**	**≥16**	**VORI**	**8->16**	**≥16**
ITRA	**≥16**	**≥16**	**TERB**	**0.5-16**	**4**

Sepedonium Link ex Greville

Sepedonium species are common soil fungi and parasites of fleshy fungi, however Yogo *et al.* (2014) reported an intra-abdominal infection in an immunosuppressed patient. *Sepedonium* species closely resemble *Histoplasma capsulatum*. **Note:** For laboratory safety, culture identification to exclude *Histoplasma capsulatum* should be performed by either exoantigen test or DNA sequencing.

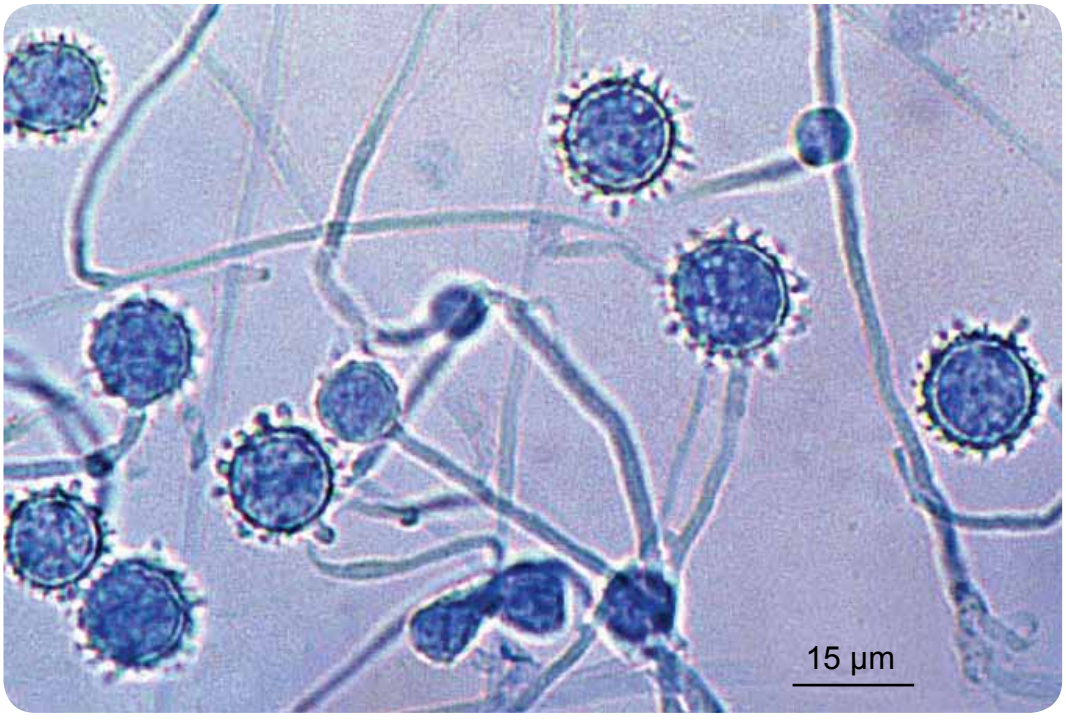
RG-1 organism.

Morphological Description: Colonies are moderately fast growing, usually white to golden yellow, suede-like to downy, becoming fluffy with age. Conidiophores are hyaline and non-specialised, resembling short branches of the vegetative hyphae. Conidia are terminal, solitary, or in clusters, one-celled, globose to ovoid, 7-17 µm, hyaline to amber, smooth to verrucose and usually with a thick wall.

Key Features: Hyphomycete, producing large, thick-walled, one-celled, verrucose, globose, terminal conidia from non-specialised conidiophores, resembling the macroconidia seen in *Histoplasma capsulatum.*

Molecular Identification: ITS and D1/D2 sequencing may be used for accurate species identification (Halliday *et al*. 2015).

References: McGinnis (1980), Rippon (1988), Yogo *et al.* (2014).



Sepedonium spp. showing large, globose, thick-walled, one-celled, verrucose, terminal conidia.

Sporothrix schenckii complex

It is now recognised that *Sporothrix schenckii* is a species complex of five distinct species: *S. schenckii sensu strictu, S. brasiliensis, S. globosa, S. mexicana* and *S. luriei* (Marimon *et al*. 2007, Romeo *et al*. 2011, Barros *et al*. 2011, Oliveira *et al*. 2014, Zhang *et al*. 2015b). **RG-2 organism.**

S*porothrix schenckii* complex is a dimorphic fungus and has a worldwide distribution, particularly in tropical and temperate regions. It is commonly found in soil and on decaying vegetation and is a well-known pathogen of humans and animals. Sporotrichosis is primarily a chronic mycotic infection of the cutaneous or subcutaneous tissues and adjacent lymphatics characterised by nodular lesions which may suppurate and ulcerate. Infections are caused by the traumatic implantation of the fungus into the skin, or very rarely, by inhalation into the lungs. Secondary spread to articular surfaces, bone and muscle is not infrequent, and the infection may also occasionally involve the central nervous system, lungs or genitourinary tract.

Sporothrix schenckii Hektoen & Perkins

Morphological Description: Colonies at 25^{O}C, are slow growing, moist and glabrous, with a wrinkled and folded surface. Some strains may produce short aerial hyphae and pigmentation may vary from white to cream to black. Conidiophores arise at right angles from thin septate hyphae and are usually solitary, erect and tapered toward the apex. Conidia are formed in clusters on tiny denticles by sympodial proliferation at the apex of the conidiophore, their arrangement often suggestive of a flower. As the culture ages, conidia are subsequently formed singly along the sides of both conidiophores and undifferentiated hyphae. Conidia are ovoid or elongated, 3-6 x 2-3 µm, hyaline, one-celled and smooth-walled. In some isolates, solitary, darkly-pigmented, thick-walled, one-celled, obovate to angular conidia may be observed along the hyphae. On brain heart infusion (BHI) agar containing blood at 37^{O}C, colonies are glabrous, white to greyish-yellow and yeast-like consisting of spherical or oval budding yeast cells.

Molecular Identification: DNA sequencing using ITS, D1/D2, β-tubulin, calmodulin and chalcone synthase genes is recommended for species identification (Marimon *et al.* 2007, Romeo *et al*. 2011, Barros *et al.* 2011, Oliveira *et al*. 2014, Zhang *et al.* 2015b).

MALDI-TOF MS: Oliverira *et al.* (2015) established a MALDI-TOF protocol and reference database for the identification of *Sporothrix* species.

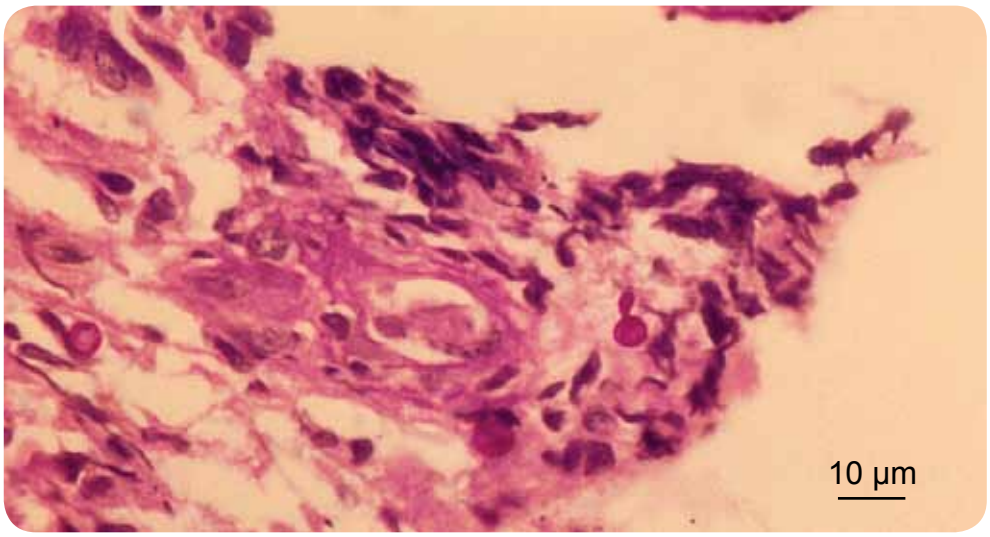
Key Features: Hyphomycete characterised by thermal dimorphism and clusters of ovoid, denticulate conidia produced sympodially on short conidiophores.

References: McGinnis (1980), Domsch *et al.* (1980), Rippon (1988), de Hoog *et al.* (1985, 2000, 2015).

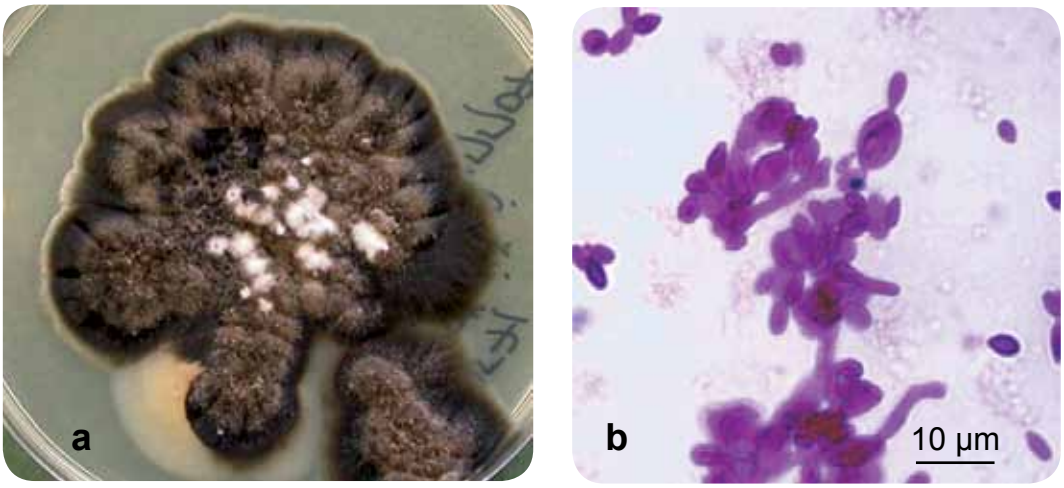
Antifungal Susceptibility: *S. schenckii* variable data for amphotericin B and azoles; testing of individual strains recommended (Alvarado-Ramirez and Torres-Rodriguez 2007, Marimon *et al.* 2008, Silveira *et al*. 2009, Oliveira *et al*. 2011, Ottonelli Stopiglia *et al*. 2014, Rodrigues *et al.* 2014, Australian National data); **MIC µg/mL.**

Antifungal	Range	MIC_{90}	Antifungal	Range	MIC_{90}
AmB	**0.03->16**	**≥16**	**POSA**	**0.03->16**	**8**
ITRA	**0.03->16**	**≥16**	**VORI**	**0.125->16**	**≥16**
TERB	**0.03-1**	**0.5**			

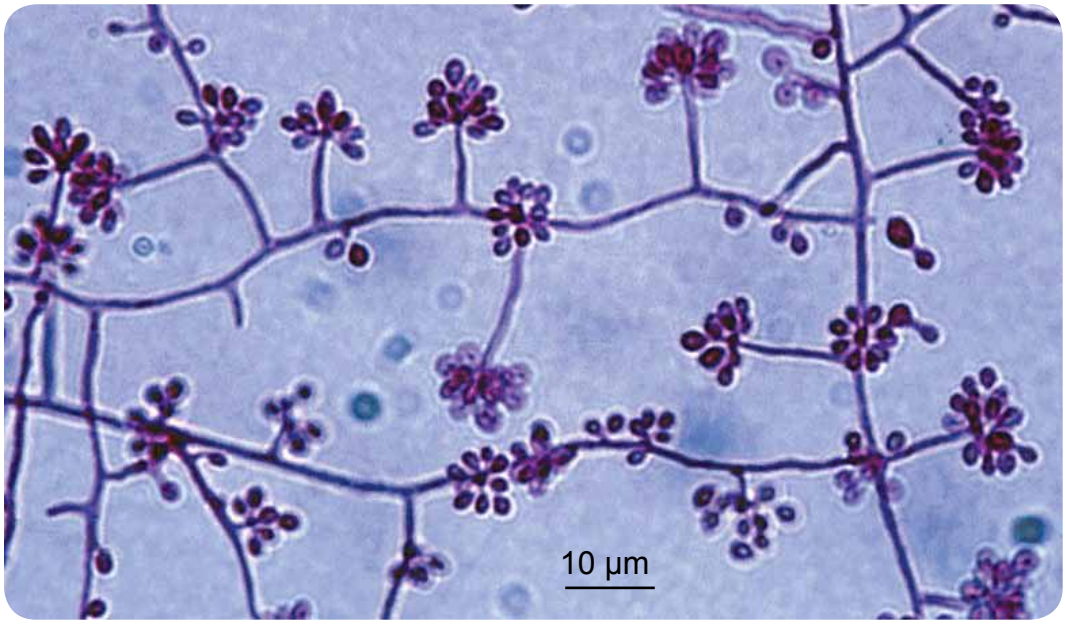
Sporothrix schenckii complex



Sporothrix schenckii PAS stained tissue section showing budding yeast-like cells.



Sporothrix schenckii (a) culture at 25°C and (b) budding yeast cells in BHI at 37°C.



Sporothrix schenckii conidiophores and conidia at 25°C.

Stemphylium Wallroth

Most species of *Stemphylium* are plant pathogens with occasional isolates from soil, they are rarely seen in the clinical laboratory.

RG-1 organism.

Morphological Description: Colonies are rapid growing, brown to olivaceous-black or greyish and suede-like to floccose. Microscopically, solitary, darkly pigmented, terminal, multicellular conidia (dictyoconidia) are formed on a distinctive conidiophore with a darker terminal swelling. **Note:** The conidiophore proliferates percurrently through the scar where the terminal conidium (poroconidium) was formed. Conidia are pale to mid-brown, oblong, rounded at the ends, ellipsoidal, obclavate or subspherical and are smooth or in part verrucose. *Stemphylium* should not be confused with *Ulocladium* which produces similar dictyoconidia from a sympodial conidiophore, not from a percurrent conidiogenous cell as in *Stemphylium*.

Molecular Identification: ITS sequencing (Woudenberg *et al.* 2013).

Key Features: Dematiaceous hyphomycete producing darkly pigmented, dictyoconidia from the swollen end of a percurrent conidiophore.

References: Ellis (1971, 1976), Rippon (1988), de Hoog *et al*. (2000).

Stemphylium spp. conidiophores and conidia.

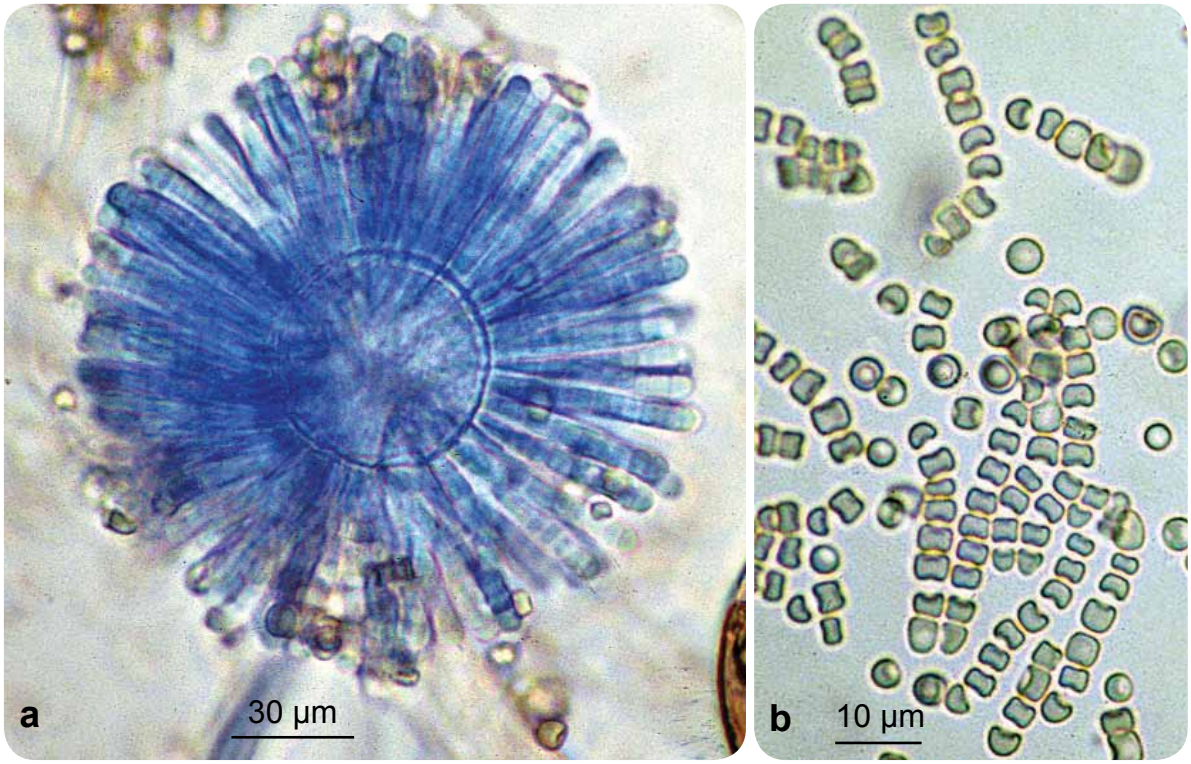
Syncephalastrum racemosum Cohn

The genus *Syncephalastrum* is characterised by the formation of cylindrical merosporangia on a terminal swelling of the sporangiophore. Sporangiospores are arranged in a single row within the merosporangia. *Syncephalastrum racemosum* is the type species of the genus and a potential human pathogen; however, well-documented cases are lacking. It is found mainly from soil and dung in tropical and subtropical regions. It can also be a laboratory aerial contaminant. The sporangiophore and merosporangia of *Syncephalastrum* species may also be mistaken for an *Aspergillus* species, if the isolate is not examined carefully. **RG-2 organism.**

Morphological Description: Colonies are very fast growing, cottony to fluffy, white to light grey, becoming dark grey with the development of sporangia. Sporangiophores are erect, stolon-like, often producing adventitious rhizoids, and show sympodial branching (racemose branching) producing curved lateral branches. The main stalk and branches form terminal, globose to ovoid vesicles which bear finger-like merosporangia directly over their entire surface. At maturity, merosporangia are thin-walled, evanescent and contain five to ten (up to 18) globose to ovoid, smooth-walled sporangiospores (merospores). Maximum growth temperature 40^{O}C.

Key Features: Mucorales, producing sympodially branching sporangiophores with terminal vesicles bearing merosporangia.

References: Domsch *et al*. (1980), McGinnis (1980), Onions *et al.* (1981), Rippon (1988), Samson *et al.* (1995), de Hoog *et al.* (2000, 2015), Ellis (2005b).



Syncephalastrum racemosum (a) merosporangia and (b) merospores.

Antifungal Susceptibility: *S. racemosum* (Espinel-Ingroff *et al*. 2015a); **MIC µg/mL**.											
	No.	**≤0.03**	**0.06**	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**≥16**
AmB	**35**	**8**	**16**	**3**	**6**	**2**					
POSA	**36**	**1**	**2**	**4**	**10**	**11**	**5**	**1**	**2**		
ITRA	**26**	**4**	**3**	**5**	**7**	**4**		**1**			**2**

Talaromyces marneffei (Segretain *et al.*) Samson *et al.*

Synonymy: *Penicillium marneffei* Segretain *et al.*

WARNING: RG-3 organism. Cultures of *Talaromyces marneffei* may represent a biohazard to laboratory personnel and should be handled with caution in a class II Biological Safety Cabinet (BSCII). *T. marneffei* exhibits thermal dimorphism and is endemic in Southeast Asia and the southern region of China.

Samson *et al*. (2011b) redefined *Talaromyces* by combining *Penicillium* subgenus *Biverticillium* into *Talaromyces* based upon phylogenetic analysis of the ITS and *RPB1* loci. The genus contains 88 species that were placed into seven sections based on a multigene phylogeny of the ITS, β-tubulin and *RPB2* regions (Yilmaz *et al.* 2014). *T. marneffei* is the only known dimorphic species in the genus, producing filamentous growth at 25^{O}C and a yeast phase at 37^{O}C (Andrianopoulos 2002).

Molecular Identification: ITS sequencing is recommended, as well as β-tubulin as a secondary molecular marker for identification (Yilmaz *et al*. 2014).

Morphological Description: Colonies at 25^{O}C are fast growing, suede-like to downy, white with yellowish-green conidial heads. Colonies become greyish-pink to brown with age and produce a diffusible brownish-red to wine-red pigment. Conidiophores generally biverticillate and sometimes monoverticillate; hyaline, smooth-walled and bear terminal verticils of three to five metulae, each bearing three to seven phialides. Phialides are acerose to flask-shaped. Conidia are globose to subglobose, 2-3 µm in diameter, smooth-walled and are produced in basipetal succession from the phialides.

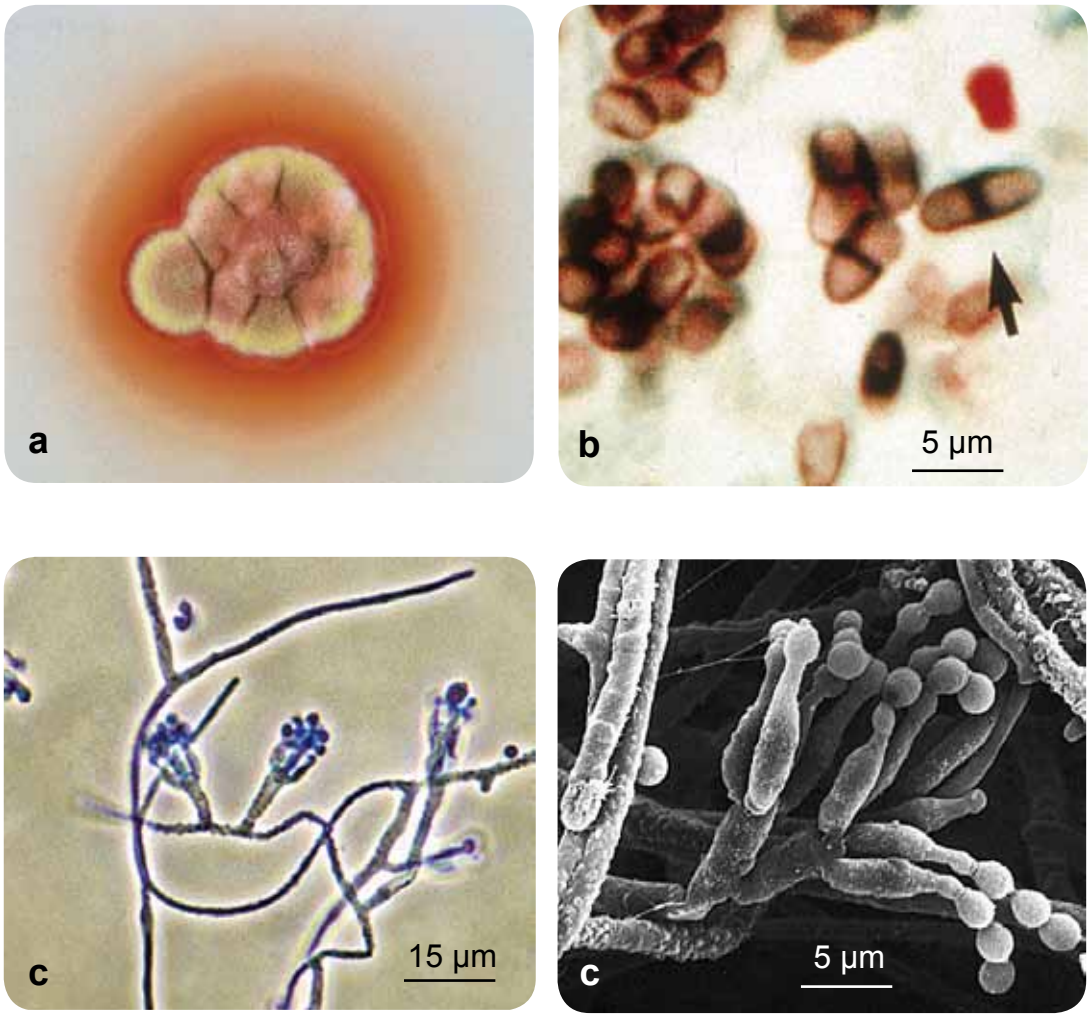
On brain heart infusion (BHI) agar containing blood incubated at 37^{O}C, colonies are rough, glabrous, tan-coloured and yeast-like. Microscopically, yeast cells are spherical to ellipsoidal, 2-6 µm in diameter, and divide by fission rather than budding. Numerous short hyphal elements are also present.

Histopathology: Tissue sections show small, oval to ellipsoidal yeast-like cells, 3 µm in diameter, either packed within histiocytes or scattered through the tissue. Occasional, large, elongated sausage-shaped cells, up to 8 µm long, with distinctive septa may be present.

Key Features: *Talaromyces marneffei* is the only dimorphic species of *Talaromyces*, which grows as a yeast at 37^{O}C. It produces a red soluble pigment on general media and conidiophores have flask-shaped to acerose phialides.

References: Pitt (1979), Ramirez (1982), de Hoog *et al.* (2000, 2015), Andrianopoulos (2002), Lyratzopoulos *et al.* (2002), Samson *et al*. (2011b), Visagie *et al*. (2014), Yilmaz *et al.* (2014).

Talaromyces marneffei (Segretain *et al.*) Samson *et al.*



Talaromyces marneffei (a) colony, (b) a giemsa stained touch smear showing typical septate yeast-like cells (arrow), (c) phialides and conidia.

Antifungal Susceptibility: ***T. marneffei*** very limited data (Australian National data); **MIC µg/mL.**

	No.	≤0.008	0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	≥8
AmB	**5**					**2**	**2**	**1**				
VORI	**5**	**3**	**2**									
POSA	**4**	**3**	**1**									
ITRA	**5**	**1**	**4**									

Torulaspora delbrueckii (Lindner) Lindner

Synonymy: *Candida colliculosa* (Hartmann) S.A. Meyer & Yarrow.

Torulaspora delbrueckii is a rare cause of candidaemia.

RG-1 organism.

Culture: Colonies (SDA) white to cream-coloured smooth, glabrous, yeast-like.

Microscopy: Spherical to ellipsoidal budding blastoconidia, 2-6 x 3-7 µm in size. Ascospores may be produced on 5% malt extract or cornmeal agar after 5-30 days at 25^{O}C.

India Ink Preparation: Negative - no capsules present.

Dalmau Plate Culture: Budding yeast cells only. No pseudohyphae or true hyphae produced.

Molecular Identification: ITS sequencing recommended.

MALDI-TOF MS: Able to accurately identify this species.

Physiological Tests: + Positive, - Negative, v Variable, w Weak, s Slow							
Germ Tube	-	L-Sorbose	v	L-Arabinose	-	D-Glucitol	v
Fermentation		Sucrose	v	D-Arabinose	-	α-M-D-glucoside	v
Glucose	+	Maltose	v	D-Ribose	-	D-Gluconate	v
Galactose	v	Cellobiose	-	L-Rhamnose	-	DL-Lactate	v
Sucrose	v	Trehalose	-,s	D-Glucosamine	-	*myo*-Inositol	-
Maltose	v	Lactose	-	*N*-A-D-glucosamine	-	2-K-D-gluconate	+
Lactose	-	Melibiose	-	Glycerol	v	D-Glucuronate	v
Trehalose	v	Raffinose	v	Erythritol	-	Nitrate	-
Assimilation		Melezitose	v	Ribitol	v	Urease	-
Glucose	+	Soluble Starch	-	Galactitol	-	0.1% Cycloheximide	-
Galactose	v	D-Xylose	v	D-Mannitol	+	Growth at 37^{O}C	v

Key Features: asci containing one to four spheroidal ascospores, strong growth at 37^{O}C and a variable sugar assimilation profile.

Antifungal Susceptibility: ***T. delbrueckii*** very limited data (Australian National data); **MIC µg/mL.**

	No.	**≤0.008**	**0.016**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**≥64**
AmB	**2**						**2**								
FLU	**2**											**2**			
VORI	**2**				**1**		**1**								
POSA	**2**				**1**		**1**								
ITRA	**2**						**2**								
CAS	**1**				**1**										
5FC	**2**			**2**											

Trichoderma Persoon ex Grey

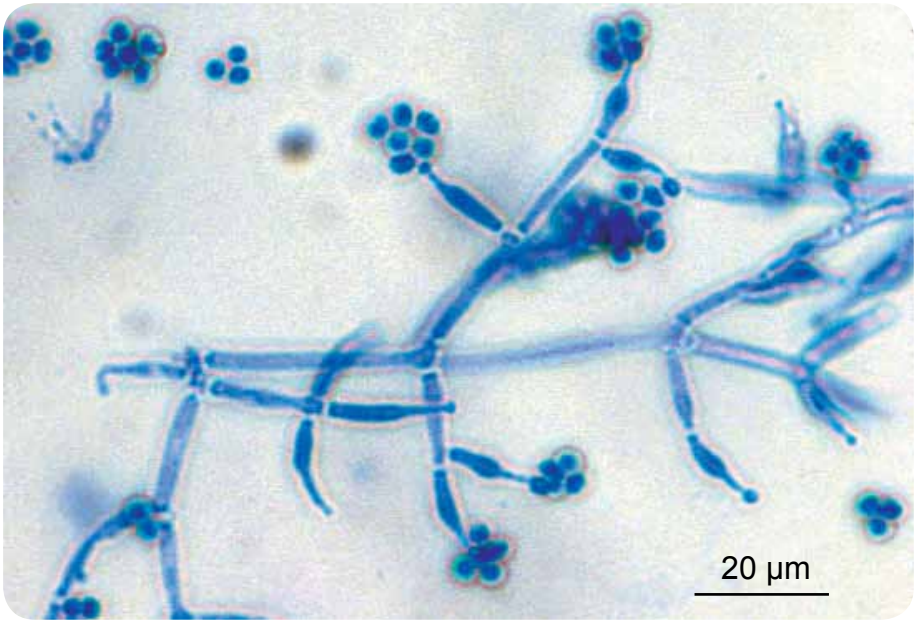
Trichoderma is a very common genus especially in soil and decaying wood. *Gliocladium* (with strongly convergent phialides) and *Verticillium* (with straight and moderately divergent phialides) are closely related genera. *Trichoderma* infections in humans have been associated mostly with peritoneal dialysis, organ transplantation, and haematologic disorders (Sandoval-Denis *et al*. 2014b).

Morphological Description: Colonies are fast growing, at first white and downy, later developing yellowish-green to deep green compact tufts, often only in small areas or in concentric ring-like zones on the agar surface. Conidiophores are repeatedly branched, irregularly verticillate, bearing clusters of divergent, often irregularly bent, flask-shaped phialides. Conidia are mostly green, sometimes hyaline, with smooth or rough walls and are formed in slimy conidial heads (gloiospora) clustered at the tips of the phialides. **RG-1 organism**.

Key Features: Hyphomycete with repeatedly branched conidiophores bearing clusters of divergent, flask-shaped phialides.

Molecular Identification: Species identification is based on multilocus sequence data using ITS, *EF-1α, Chi18-5*, and actin genes (Sandoval-Denis *et al.* 2014b).

References: Domsch *et al.* (1980), McGinnis (1980), Rippon (1988), Samson *et al*. (1995), de Hoog *et al*. (2000, 2015), Sandoval-Denis *et al.* (2014b).



Trichoderma harzianum species complex phialides and conidia.

Antifungal Susceptibility: *Trichoderma* spp.(Australian National data); **MIC µg/mL.**															
	No.	**≤0.008**	**0.016**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**≥64**
AmB	**3**							**2**	**1**						
VORI	**3**					**1**	**1**		**1**						
POSA	**2**								**2**						
ITRA	**3**								**2**				**1**		
***Trichoderma* spp.** data from 73 isolates (Sandoval-Denis *et al*. 2014b); **MIC µg/mL**.															
AmB	**Range 0.03-8; MIC_{90}= 2**						**VORI**	**Range 0.125-32; MIC_{90}= 4**							
ITRA	**Range 1-32; MIC_{90}= 32**						**POSA**	**Range 1-32; MIC_{90}= 32**							

Trichophyton Malmsten

Rippon (1988) accepted 22 species and four varieties in the genus *Trichophyton* based on morphology. DNA sequences now play a prominent role in delineating phylogenetic relationships, and as such species concepts in *Trichophyton* have changed. Sixteen species are now recognised in the genus. The descriptions and species concepts provided in this publication are based upon a combination of traditional morphological criteria and the current (2016) recognised phylogenetic species (de Hoog *et al*. 2016).

The genus *Trichophyton* is characterised morphologically by the development of both smooth-walled macro- and microconidia. Macroconidia are mostly borne laterally directly on the hyphae or on short pedicels, and are thin- or thick-walled, clavate to fusiform, and range from 4-8 x 8-50 µm in size. Macroconidia are few or absent in many species. Microconidia are spherical, pyriform to clavate or of irregular shape and range from 2-3 x 2-4 µm in size. The presence of microconidia differentiates this genus from *Epidermophyton,* and the smooth-walled, mostly sessile macroconidia differentiates it from *Lophophyton, Microsporum and Nannizzia*.

In practice, two groups may be recognised on direct microscopy:

1. **Those species that usually produce microconidia**; macroconidia may or may not be present i.e. *T. rubrum, T. interdigitale*, *T. mentagrophytes, T. equinum, T. eriotrephon, T. tonsurans,* and to a lesser extent *T. verrucosum*, which may produce conidia on some media. In these species the shape, size and arrangement of the microconidia is the most important character. Culture characteristics are also useful.
2. **Those species that usually do not produce conidia.** Chlamydospores or other hyphal structures may be present, but microscopy is generally non-diagnostic; i.e. *T. verrucosum, T. violaceum, T. concentricum, T. schoenleinii* and *T. soudanense*. Culture characteristics and clinical information such as the site, appearance of the lesion, geographic location, travel history, animal contacts and even occupation are most important.

Many laboratories have used growth on additional media and/or confirmatory tests to help differentiate between species of *Trichophyton*, especially isolates of *T. rubrum, T. interdigitale, T. mentagrophytes* and *T. tonsurans*. These include growth characteristics on media such as Littman oxgall agar, lactritmel agar, potato dextrose agar, Sabouraud's agar with 5% Salt, 1% peptone agar, bromocresol purple-milk solids glucose agar (BCP), *Trichophyton* agars No. 1-5, hydrolysis of urea and hair perforation tests.

Molecular Identification: ITS and *EF-1α* sequencing is recommended for accurate species identification (Gräser *et al*. 1998, 1999b, 2000a, 2008; Irinyi *et al*. 2015; Mirhendi *et al*. 2015).

MALDI-TOF MS: Methods reported by Erhard *et al.* (2008), Nenoff *et al*. (2011), Cassange *et al*. (2011), l'Ollivier *et al*. (2013), Calderaro *et al.* (2014), Packeu *et al.* (2013, 2014).

References: Rebell and Taplin (1970), Ajello (1972), Vanbreusegham *et al*. (1978), Rippon (1988), McGinnis (1980), Domsch *et al.* (1980), Kane *et al.* (1997), Chen *et al.* (2011), de Hoog *et al.* (2000, 2015, 2016).

Trichophyton concentricum Blanchard

Trichophyton concentricum is an anthropophilic fungus which causes chronic widespread non-inflammatory tinea corporis known as tinea imbricata because of the concentric rings of scaling it produces. It is not known to invade hair. Infections among Europeans are rare. Distribution is restricted to the Pacific Islands of Oceania, South East Asia and Central and South America.

RG-2 organism.

Morphological Description: Colonies are slow growing, raised and folded, glabrous becoming suede-like, mostly white to cream-coloured, but sometimes orange-brown coloured, often deeply folded into the agar which may produce splitting of the medium in some cultures. Reverse is buff to yellow-brown to brown in colour. Cultures consist of broad, much-branched, irregular, often segmented, septate hyphae which may have “antler” tips resembling *T. schoenleinii*. Chlamydospores are often present in older cultures. Microconidia and macroconidia are not usually produced, although some isolates will produce occasional clavate to pyriform microconidia. **Note:** Hyphal segments may artificially resemble macroconidia.

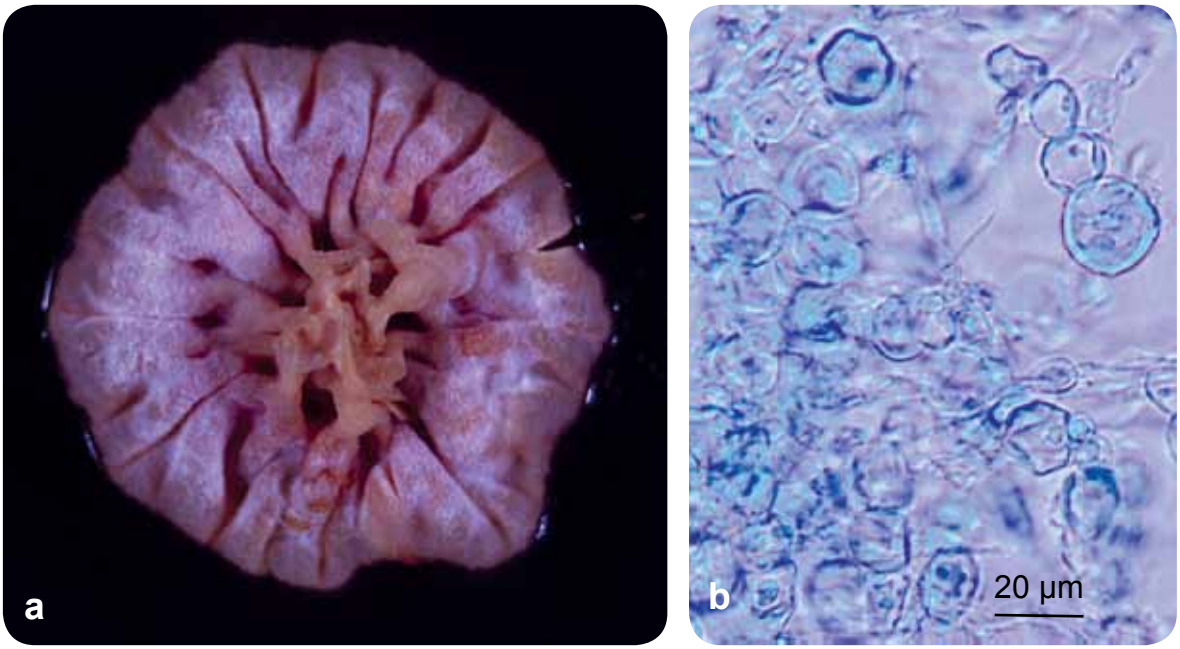
Confirmatory Tests:

Hydrolysis of Urea: Negative after 7 days.

Vitamin Free Agar (Trichophyton Agar No.1): Growth occurs on vitamin free agar (T1) but is usually slightly better on media containing thiamine i.e. T3 = T1 + thiamine and inositol, and T4 = T1 + thiamine. The slight enhancement of growth in the presence of thiamine helps to distinguish *T. concentricum* from *T. schoenleinii*, although this does not occur in all strains.

Hair Perforation Test: Negative at 28 days.

Key Features: Clinical disease, geographical distribution and culture characteristics.



Trichophyton concentricum (a) culture showing a typical slow growing, heaped and folded, glabrous to suede like colony, and (b) the formation of typical “balloon-shaped” chlamydospores. **Note:** Microconidia and macroconidia are usually not produced.

Trichophyton equinum (Matruchot & Dassonville) Gedoelst

Synonomy: *Trichophyton equinum* var. *autotrophicum* J.M.B. Smith *et al.*

Trichophyton equinum is a zoophilic fungus causing ringworm in horses and rare infections in humans. It has a worldwide distribution except for the *autotrophicum* strain which is restricted to Australia and New Zealand. Invaded hairs show an ectothrix infection but do not fluoresce under Wood's ultra-violet light.

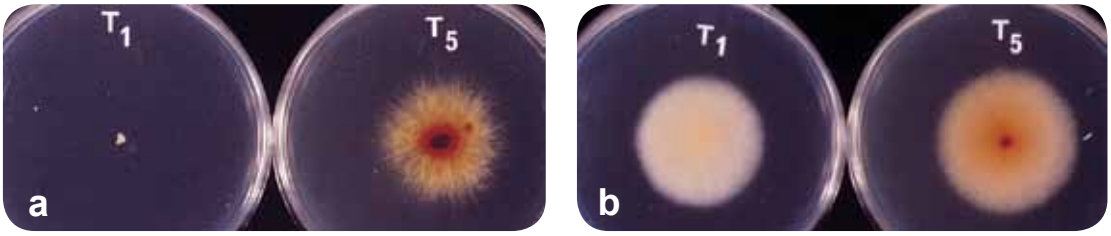
RG-2 organism.

Morphological Description: Colonies are usually flat, but some may develop gentle folds or radial grooves, white to buff in colour, suede-like to downy in texture, and are similar to *T. mentagrophytes*. Cultures usually have a deep-yellow submerged fringe and reverse which later becomes dark red in the centre. Microscopically: abundant microconidia which may be clavate to pyriform and sessile or spherical and stalked are formed laterally along the hyphae. Macroconidia are only rarely produced, but when present are clavate, smooth, thin-walled and of variable size. Occasional nodular organs may be present and the microconidia often undergo a transformation to produce abundant chlamydospores in old cultures.

Confirmatory Tests:

Lactritmel Agar: Flat spreading, white to cream-coloured, powdery to granular surface with a central downy papilla, and deep brownish-red reverse. Microscopic morphology as described above.

Hydrolysis of Urea: Positive in 4-5 days.



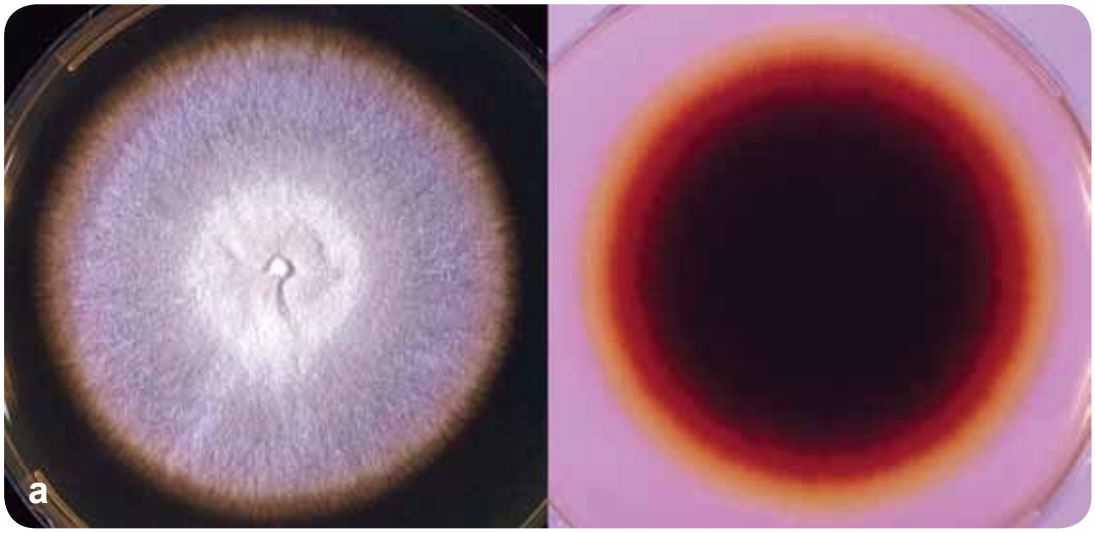
Nutritional Tests on Trichophyton Agars: (a) Most strains require nicotinic acid for growth except those from Australia and New Zealand, which are autotrophic (b). T1 = vitamin free agar, T5 = vitamin free + nicotinic acid agar.

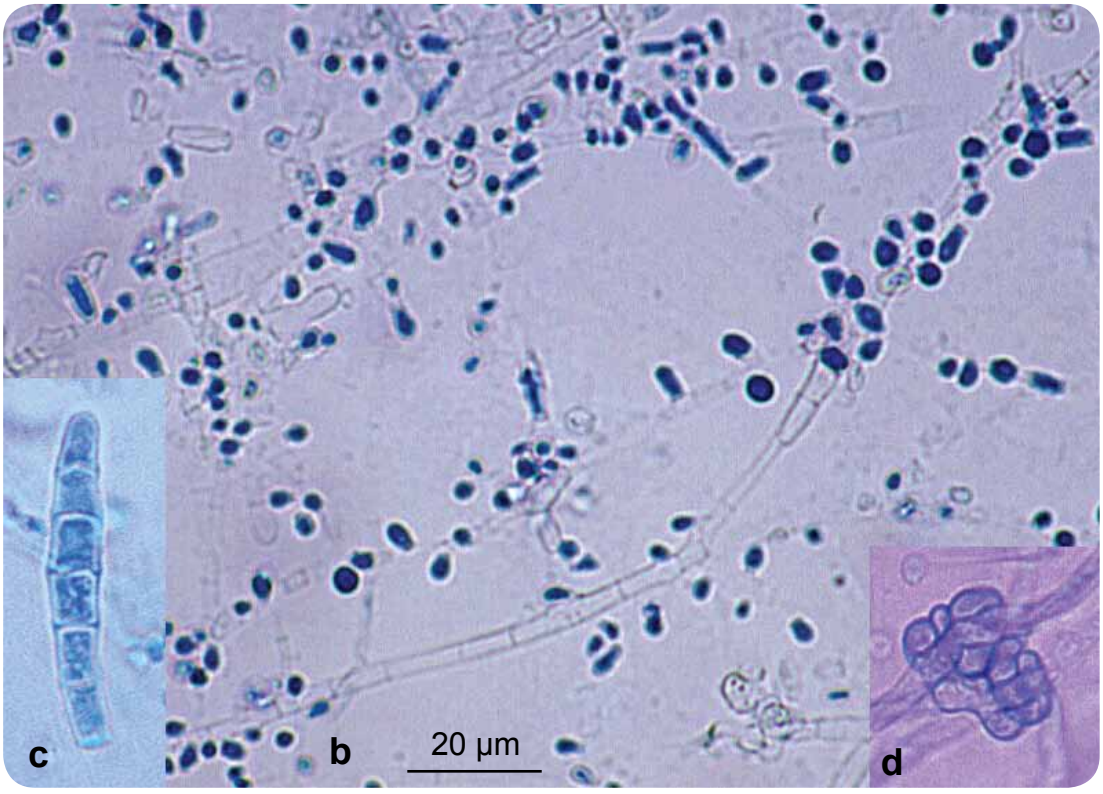
Hair Perforation Test: Negative; but positive for the *autotrophicum* strains.

Molecular Diagnostics: Species identification supported by ITS sequencing (Gräser *et al*. 1999a; Chen *et al*. 2011).

Key Features: Microscopic morphology, culture characteristics, nicotinic acid requirement and clinical lesions in horses. Most strains require nicotinic acid for growth except autotrophic strains.

Trichophyton equinum (Matruchot & Dassonville) Gedoelst





Trichophyton equinum (a) culture, (b) microconidia, (c) macroconidia and (d) nodular organs.

Trichophyton eriotrephon Papegaaij, Nederl. Tijdschr. Geneesk

Synonymy: *Trichophyton mentagrophytes* var. *erinacei* J.M.B. Smith & Marples.
Trichophyton erinacei (Smith & Marples) Quaife.

Trichophyton eriotrephon is a zoophilic fungus associated with hedgehogs and the epidermal mites, harboured by hedgehogs. Human infections occur most frequently on the exposed parts of the body, but tinea of the scalp and nails can also occur. Invaded hairs show an ectothrix infection but do not fluoresce under Wood's ultra-violet light. The distribution of this fungus is New Zealand and Europe.

RG-2 organism.

Morphological Description: Colonies are white, flat, powdery, sometimes downy to fluffy with a brilliant lemon-yellow reverse. Numerous large clavate microconidia are borne on the sides of hyphae. Macroconidia are smooth-walled, two to six-celled, clavate, variable in size, and may have terminal appendages. Macroconidia are much shorter than those seen in *T. mentagrophytes*.

Confirmatory Tests:

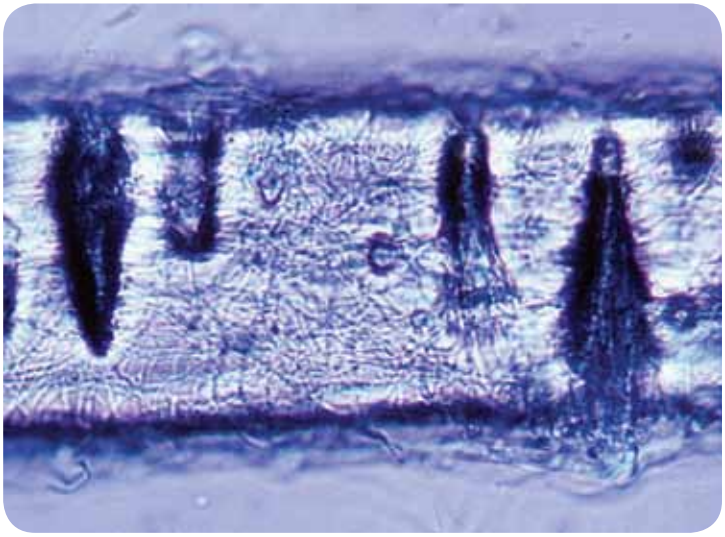
Lactritmel Agar: White suede-like to powdery colony with brilliant yellow reverse. Numerous large slender clavate microconidia.

Hydrolysis of Urea: Negative at 7 days.

Vitamin Free Agar (Trichophyton Agar No.1): Good growth indicating no special nutritional requirements. Colonies are white suede-like to powdery with no reverse pigment.

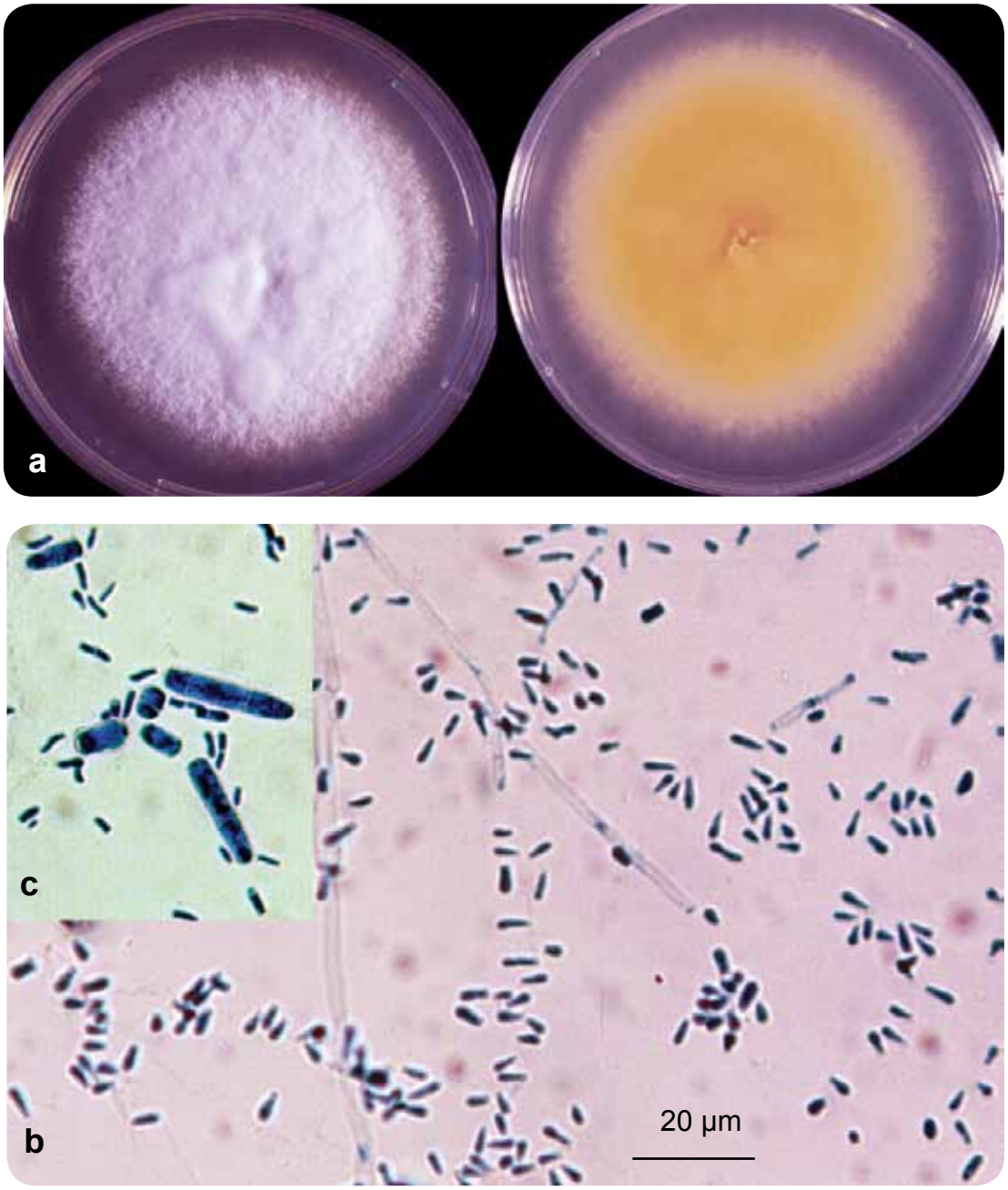
Hair Perforation Test: Positive.

Key Features: Include microscopic morphology and culture characteristics; slender clavate microconidia and brilliant lemon-yellow reverse pigment on SDA; and negative hydrolysis of urea.



Positive "*in vitro*" hair perforation test.

Trichophyton eriotrephon Papegaaij, Nederl. Tijdschr. Geneesk



Trichophyton eriotrephon (a) culture, (b) microconidia and (c) macroconidia.

Trichophyton eriotrephon is generally distinguished from *T. mentagrophytes* by: (a) its microscopic morphology showing numerous large slender clavate microconidia borne on the sides of hyphae and its smooth, thin-walled clavate macroconidia; (b) its brilliant lemon yellow reverse pigment on SDA and lactritmel agar; (c) its lack of reverse pigment on Sabouraud's salt agar; and (d) its negative hydrolysis of urea.

Trichophyton interdigitale Priestley

Synonymy: *T. mentagrophytes* var. *interdigitale* (Priestley) Moraes, Anais Bras.

Trichophyton interdigitale is an anthropophilic fungus which is a common cause of tinea pedis, particularly the vesicular type, tinea corporis, and sometimes superficial nail plate invasion in humans. It is not known to invade hair *in vivo* but produces hair perforations *in vitro*. Distribution is worldwide. This species may be regarded as a clonal offshoot of the zoophilic *T. mentagrophytes* (de Hoog *et al*. 2016).

RG-2 organism.

Morphological Description: Colonies are usually flat, white to cream in colour with a powdery to suede-like surface and yellowish to pinkish brown reverse pigment, often becoming a darker red-brown with age. Numerous subspherical to pyriform microconidia, occasional spiral hyphae and spherical chlamydospores are present, the latter being more abundant in older cultures. Occasional slender, clavate, smooth-walled, multiseptate macroconidia are also present in some cultures.

Confirmatory Tests:

Littman Oxgall Agar: Raised white downy colony with no reverse pigment.

Lactritmel Agar: Macroscopic and microscopic features as described above.

Sabouraud's Dextrose Agar with 5% Salt: Heaped and folded, buff-coloured, suede-like surface with a dark reddish-brown submerged fringe and brown reverse.

1% Peptone Agar: Flat, white to cream, suede-like surface with raised white downy centre. No reverse pigment.

Vitamin Free Agar (Trichophyton Agar No.1): Good growth indicating no special nutritional requirements, flat cream powdery surface with central downy tuft. Reverse pale pinkish-brown.

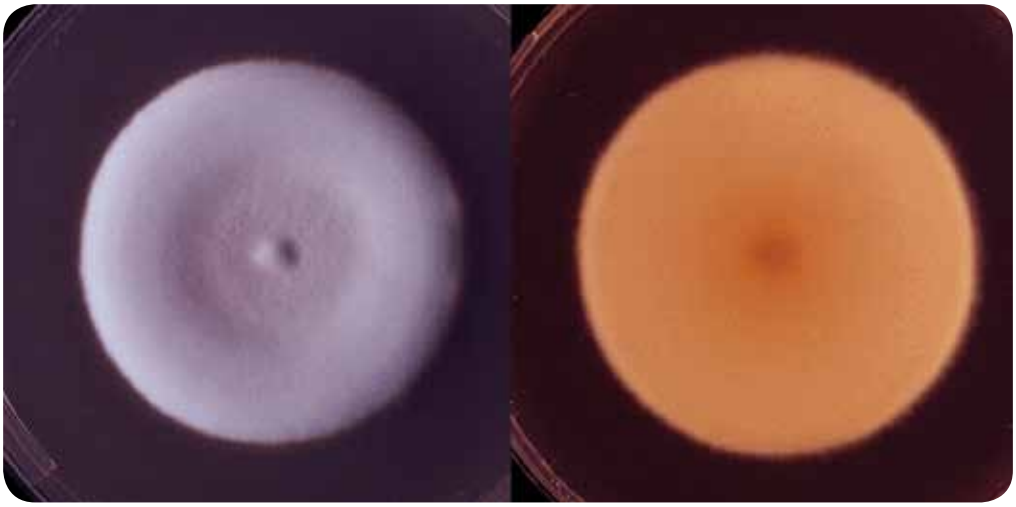
Hydrolysis of Urea: Positive within 7 days (usually 3 to 5 days).

Hair Perforation Test: Positive.

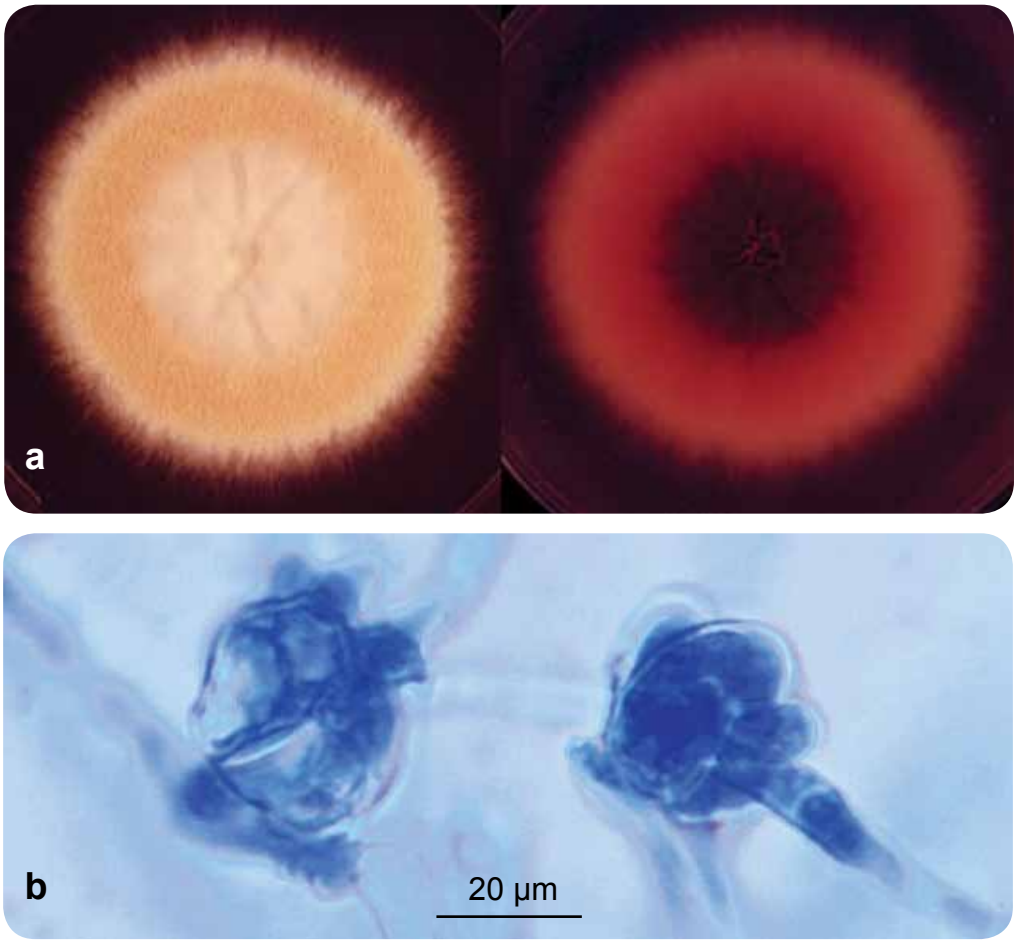
Key Features: Culture characteristics, microscopic morphology and *in vitro* perforation of human hair.

Trichophyton interdigitale can be distinguished from *T. rubrum* and *T. mentagrophytes* by: (a) its culture characteristics and microscopic morphology on SDA and/or lactritmel agar; (b) its growth and colony morphology on Sabouraud's salt agar (colonies of *T. interdigitale* and *T. mentagrophytes* unlike *T. rubrum*, grow very well on this medium and usually produce a distinctive dark reddish-brown reverse pigment); (c) a positive urease test (within 7 days), a positive hair perforation test and the production of a yellow-brown to pinkish-brown reverse pigment on pigment stimulation media like lactritmel and Trichophyton No.1 agars; (d) *T. interdigitale* demonstrates profuse growth and alkalinity on BCP milk solids agar; (e) on 1% peptone agar *T. interdigitale* has a suede-like to downy surface whereas *T. mentagrophytes* has a characteristic granular appearance.

Trichophyton interdigitale Priestley

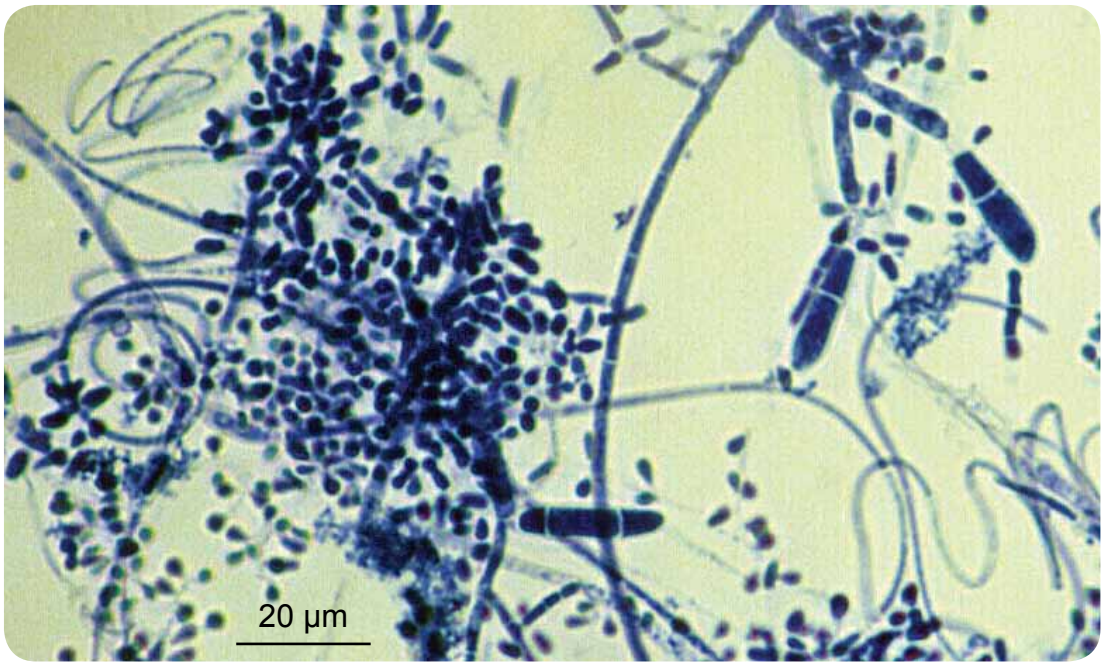


Trichophyton interdigitale culture.

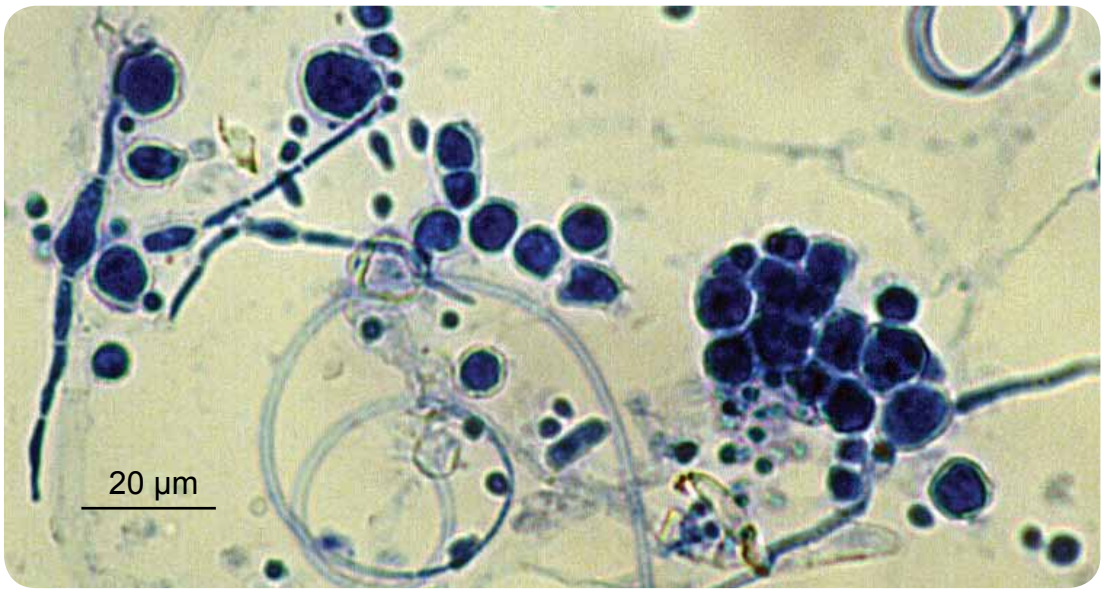


A dysgonic variant of *Trichophyton interdigitale* (formerly var. *nodulare*); (a) culture showing distinctive bright yellow to apricot-coloured colonies with a suede-like to powdery surface and a bright yellow-brown to orange reverse; and (b) microscopically characteristic “nodular organs” are observed in the vegetative hyphae. Usually, no conidia are seen but some isolates, especially with subculture may produce subspherical to pyriform microconidia similar to those of *T. interdigitale.*

Trichophyton interdigitale Priestley



Trichophyton interdigitale microconidia, macroconidia and spiral hyphae.



Trichophyton interdigitale microconidia, chlamydospores and spiral hyphae.

Trichophyton mentagrophytes (Robin) Blanchard

Synonymy: *T. mentagrophytes* var. *mentagrophytes* (Robin) Sabour.

Trichophyton mentagrophytes is a zoophilic fungus with a worldwide distribution and a wide range of animal hosts including mice, guinea-pigs, kangaroos, cats, horses, sheep and rabbits. Produces inflammatory skin or scalp lesions in humans, particularly in rural workers. Kerion of the scalp and beard may occur. Invaded hairs show an ectothrix infection but do not fluoresce under Wood's ultra-violet light. Distribution is worldwide.

RG-2 organism.

Morphological Description: Colonies are generally flat, white to cream in colour, with a powdery to granular surface. Some cultures show central folding or develop raised central tufts or pleomorphic suede-like to downy areas. Reverse pigmentation is usually a yellow-brown to reddish-brown colour. Numerous single-celled microconidia are formed, often in dense clusters. Microconidia are hyaline, smooth-walled, and are predominantly spherical to subspherical in shape, however occasional clavate to pyriform forms may occur. Varying numbers of spherical chlamydospores, spiral hyphae and smooth, thin-walled, clavate-shaped, multicelled macroconidia may also be present.

Confirmatory Tests:

Littman Oxgall Agar: Raised greyish-white, suede-like to downy colony. Some cultures may show a diffusible yellow to brown pigment.

Lactritmel Agar: Cultures are flat, white to cream in colour, with a powdery to granular surface. Some cultures develop raised central tufts or pleomorphic downy areas. Reverse pigmentation is yellow-brown to pinkish-brown to red-brown. Microscopic morphology similar to that described above, with predominantly spherical microconidia, often formed in dense clusters, and varying numbers of spherical chlamydospores, spiral hyphae and smooth, thin-walled, clavate, multiseptate macroconidia.

Sabouraud's Dextrose Agar with 5% Salt: Cultures are heaped and folded, buff to brown in colour, with a suede-like surface texture and characteristically have a very dark reddish-brown submerged peripheral fringe and reverse pigmentation.

1% Peptone Agar: Flat, cream-coloured, powdery to granular colony with no reverse pigment.

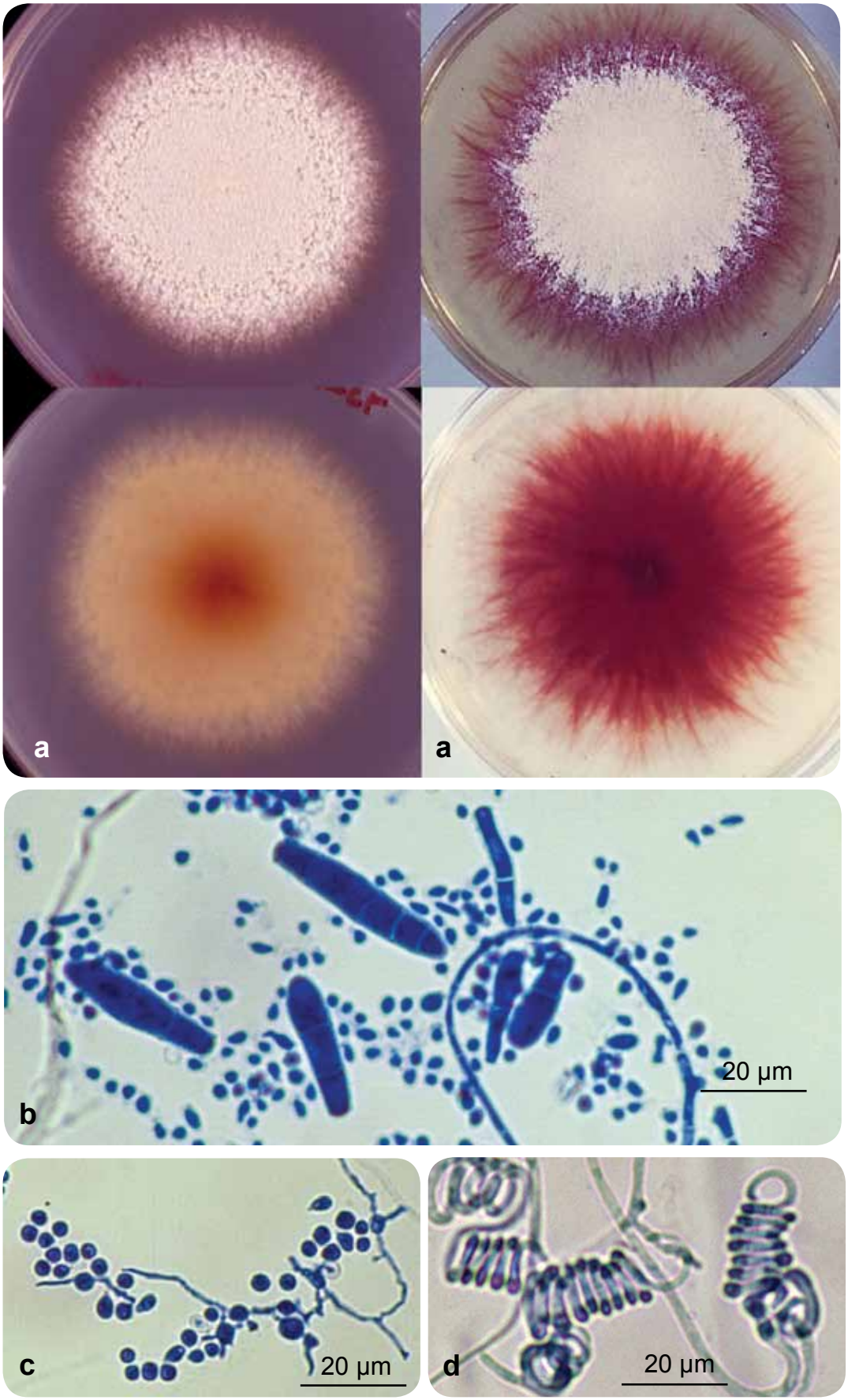
Hydrolysis of Urea: Positive within 7 days (usually 3 to 5 days).

Vitamin Free Agar (Trichophyton Agar No.1): Good growth indicating no special nutritional requirements. Cultures are flat, cream-coloured, with a powdery to suede-like surface, and have a reddish-brown reverse pigmentation.

Hair Perforation Test: Positive within 14 days.

Key Features: Culture characteristics, microscopic morphology and clinical disease with known animal contacts. *T. mentagrophytes* can be distinguished from *T. interdigitale* by: (a) its granular appearance on the 1% Peptone agar; (b) its microscopic morphology of more spherical microconidia and generally greater numbers of macroconidia; and (c) a yellow to brown diffusible pigment is often seen on the Littman oxgall agar. Both *T. interdigitale* and *T. mentagrophytes* demonstrate profuse growth and alkalinity on BCP milk solids agar.

Trichophyton mentagrophytes (Robin) Blanchard



Trichophyton mentagrophytes (a) cultures, (b) macroconidia, (c) microconidia and (d) hyphal spirals.

Trichophyton quinckeanum (Zopf) MacLeod & Münde

Synonymy: *T. mentagrophytes* var. *quinckeanum* (Zopf) J.M.B. Smith & Austwick.

Trichophyton quinckeanum causes “mouse favus” on mice, seen as thick, yellow, saucer-shaped crusted lesions up to 1 cm in diameter called scutula. Invaded hairs are rarely seen but they may show either ectothrix or endothrix infection. Infected human hairs do not fluoresce under Wood’s ultra-violet light, but very occasional hairs from experimental lesions in guinea pigs have shown a pale yellow fluorescence. The geographical distribution of this dermatophyte is difficult to establish, but is probably worldwide. It is often associated with mice plagues in the Australian Wheat Belt.

RG-2 organism.

Morphological Description: Colonies are generally flat, white to cream in colour, with a powdery to granular surface. Some cultures show central folding or develop raised central tufts or pleomorphic suede-like to downy areas. Reverse pigmentation is usually a yellow-brown to reddish-brown colour. Numerous microconidia are borne laterally along the sides of hyphae, and are predominantly slender clavate when young. With age the microconidia become broader and pyriform to spherical in shape. Occasional to moderate numbers of smooth, thin-walled, multiseptate, clavate to cigar-shaped macroconidia may be present. Varying numbers of spherical chlamydospores and spiral hyphae may also be present.

Confirmatory Tests:

Littman Oxgall Agar: Raised, dome-like bluish-grey, suede-like colony with a narrow flat, greyish-white, suede-like border. No diffusible or reverse pigment is present.

Lactritmel Agar: Flat, white to cream, suede-like to powdery colony with either no reverse pigment or a pale yellow-brown to pinkish-brown reverse. Numerous slender clavate to pyriform (depending on age of subculture) microconidia and occasional to moderate numbers of smooth, thin-walled, clavate macroconidia are present.

Sabouraud’s Dextrose Agar with 5% salt: Heaped and much folded white suede-like colony with very pale yellow-brown reverse. No submerged fringe.

1% Peptone Agar: Raised white suede-like to downy colony with no reverse pigment.

Hydrolysis of Urea: Positive within 7 days (usually very rapid 2-3 days).

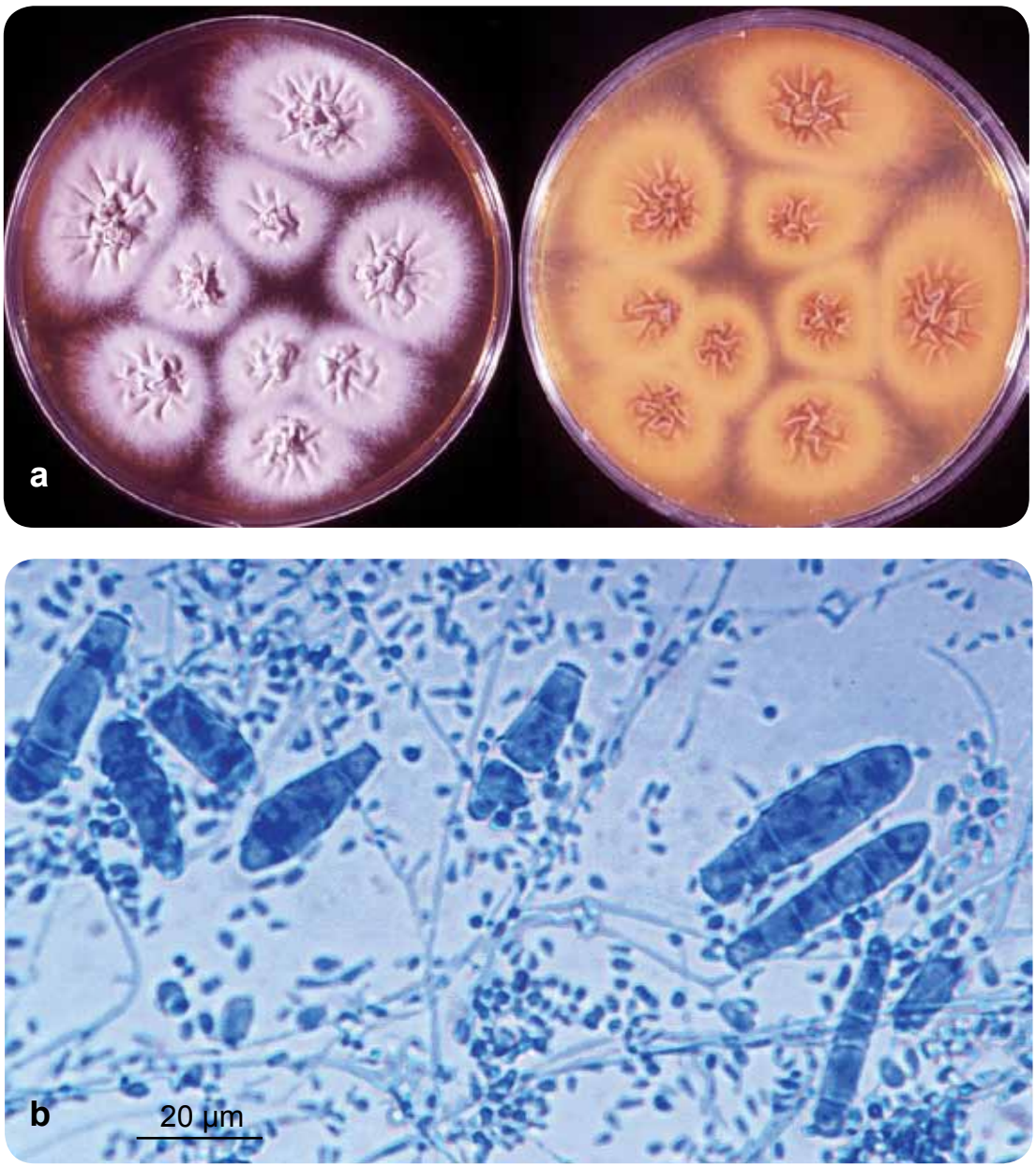
Vitamin Free Agar (Trichophyton Agar No.1): Flat, white to cream, suede-like colony with either no reverse pigment or a pale yellow-brown reverse, i.e. no special nutritional requirements.

Hair Perforation Test: Positive in 7 to 10 days.

Key Features: Culture characteristics, microscopic morphology, and rapid urease test.

Trichophyton quinckeanum (Zopf) MacLeod & Münde

Trichophyton quinckeanum may be distinguished from *T. mentagrophytes* by: (a) its characteristic culture appearance on Littman oxgall agar (i.e. raised, dome-like, bluish-grey suede-like colony with a narrow flat, greyish-white, suede-like border and no diffusible or reverse pigment) and on Sabouraud's 5% salt agar (typically heaped and folded white suede-like colony, but with no distinctive dark reddish-brown submerged fringe and reverse pigment as seen on *T. mentagrophytes*); (b) microscopic morphology showing numerous slender clavate with some pyriform microconidia and moderate numbers of smooth thin-walled, clavate macroconidia; and (c) a rapid urease test, usually positive within 2 to 3 days.



Trichophyton quinckeanum (a) culture and (b) microconidia and macroconidia.

Trichophyton rubrum (Castellani) Semon

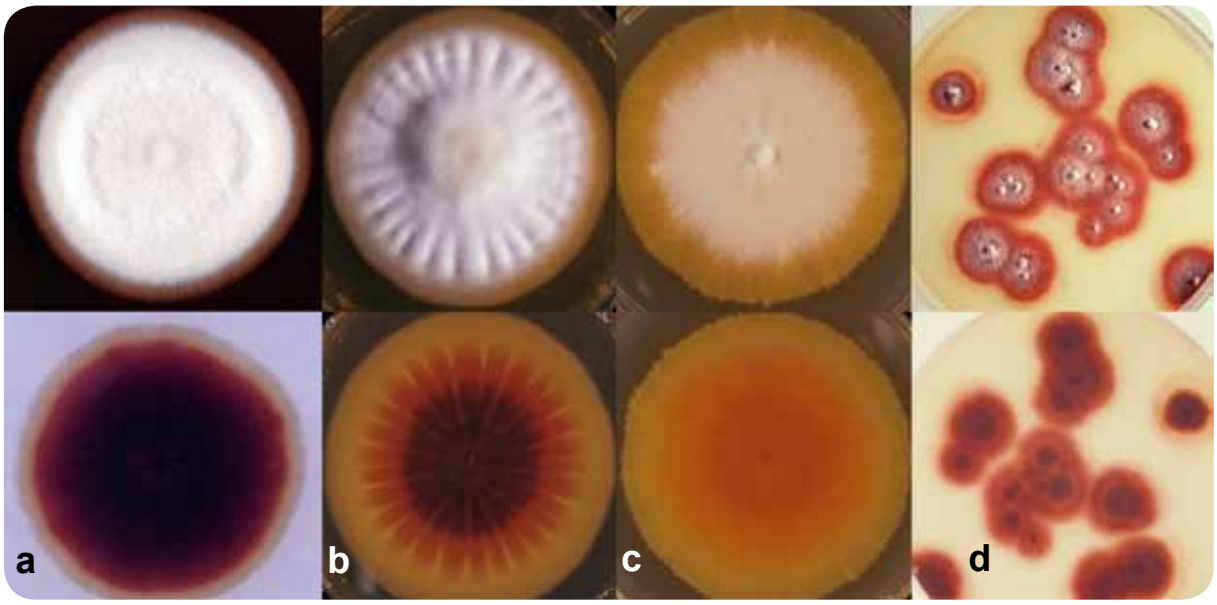
Synonymy: *Trichophyton fischeri* Kane.
Trichophyton raubitschekii Kane, Salkin, Weitzman & Smitka.
Trichophyton kanei Summerbell.
All varieties of *T. rubrum.*

Trichophyton rubrum is an anthropophilic fungus that has become the most widely distributed dermatophyte of humans. It frequently causes chronic infections of skin, nails and rarely scalp. Granulomatous lesions may sometimes occur. Infected hairs do not fluoresce under Wood's ultraviolet light, and microscopically may show endothrix or ectothrix type of invasion.

Morphologically *T. rubrum* exhibits a spectrum of overlapping characters; for example culture surface texture may vary from downy to suede-like; culture surface pigmentation may vary from white to cream to deep red; culture reverse pigmentation may vary from colourless to yellowish to yellow-brown to wine red; numbers of microconidia range from none to scanty to many; shape of microconidia vary from slender clavate to pyriform; numbers of macroconidia range from none to scanty to many and may or may not have terminal projections. This is why so many varieties or synonyms have been described in the past. However, molecular evidence by Gräser *et al.* (1999b) reveals little variation between strains of *T. rubrum* and determined that the species is largely clonal.

The majority of isolates, especially those causing tinea pedis and onychomycosis, are characterised by the production of scanty to moderate numbers of slender clavate microconidia and no macroconidia (formerly the "downy strain"). Some isolates, usually from cases of tinea corporis, are characterised by the production of moderate to abundant numbers of clavate to pyriform microconidia and moderate to abundant numbers of thin-walled, cigar-shaped macroconidia (formerly the "granular strain").

It should be stressed that intermediate strains do occur as many culture and morphological characteristics overlap.



Trichophyton rubrum culture morphology; (a) "downy strain" with typical wine-red reverse; (b) "Y variety" with both yellow and red pigmentation; (c) "var. *flava*" with yellow pigmentation; (d) "granular strain" with red surface and reverse pigmentation.

Trichophyton rubrum (Castellani) Semon

RG-2 organism.

Morphological Description: Colonies are mostly flat to slightly raised, white to cream, suede-like to downy, with either no reverse pigment or a yellow-brown to wine-red reverse. Most cultures show scanty to moderate numbers of slender clavate to pyriform microconidia. Macroconidia are usually absent, but when present are smooth, thin-walled multiseptate, slender and cylindrical to cigar-shaped. Older cultures may show numerous chlamydospores with a few clavate to pyriform microconidia.

Note: On primary isolation some cultures may lack reverse pigmentation and fail to produce microconidia. These need to be subcultured onto media like lactritmel agar or potato dextrose agar, which stimulate pigmentation and sporulation. If sporulation still fails subculture the fungus onto Trichophyton agar No.1.

Confirmatory Tests:

Littman Oxgall Agar: Raised, greyish-white, suede-like to downy colony with no reverse pigment. Some cultures may show a greenish-yellow diffusible pigment.

Lactritmel Agar: Flat, white to rose pink, downy to granular colonies with a deep wine-red reverse pigment.

Sabouraud's Dextrose Agar with 5% salt: Very stunted, white to cream, downy to glabrous colony with a pale yellow-brown reverse pigment.

1% Peptone Agar: Flat, white to cream, downy to glabrous colony with no reverse pigment.

BCP Agar: restricted colony growth and neutral (unchanged) pH. Colonies typically demonstrate red pigment on reverse

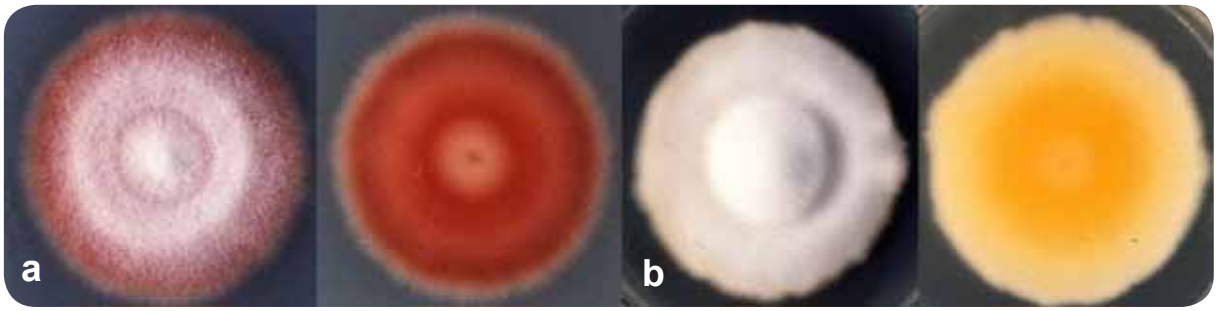
Hydrolysis of Urea: Typically, negative at 7 days (some may be positive).

Vitamin Free Agar (Trichophyton Agar No.1): Good growth indicating no special vitamin requirements. Colonies are flat, white to cream, suede-like to downy with a deep wine-red reverse pigment.

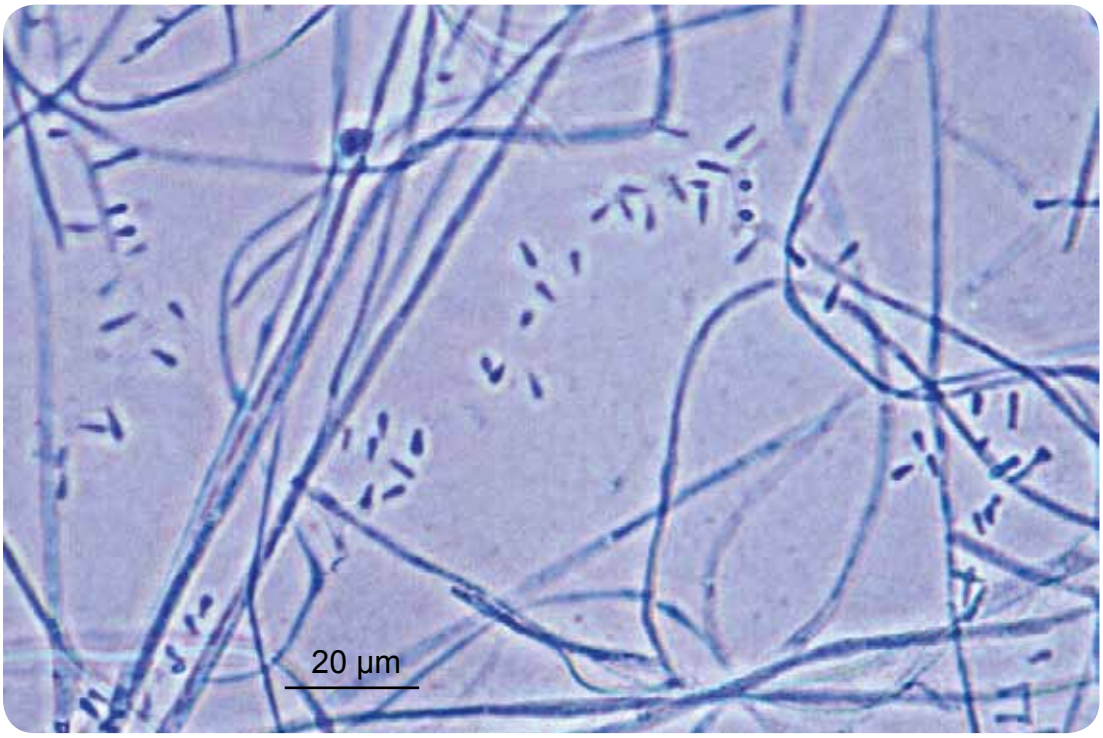
Hair Perforation Test: Negative at 28 days.

Key Features: Include clinical history, culture characteristics, microscopic morphology and failure to perforate hair *in vitro*.

Trichophyton rubrum (Castellani) Semon

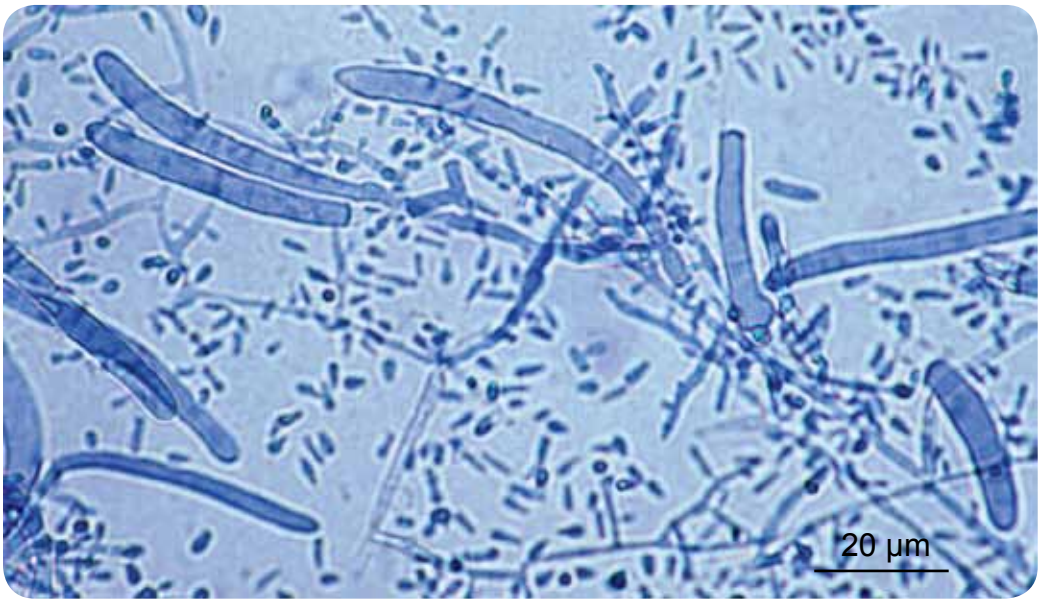


Note: *Trichophyton rubrum* produces both red and yellow pigments. Culture colours may range from none to dark red to dark yellow, with all combinations in between. The images above show the same strain (a) grown on lactritmel agar that promotes red pigmentation and (b) on mycobiotic agar that shows an underlying yellow pigmentation.



Trichophyton rubrum showing typical slender clavate microconidia.

Trichophyton rubrum (Castellani) Semon



Trichophyton rubrum showing slender clavate microconidia and cigar-shaped macroconidia, some with terminal appendages.

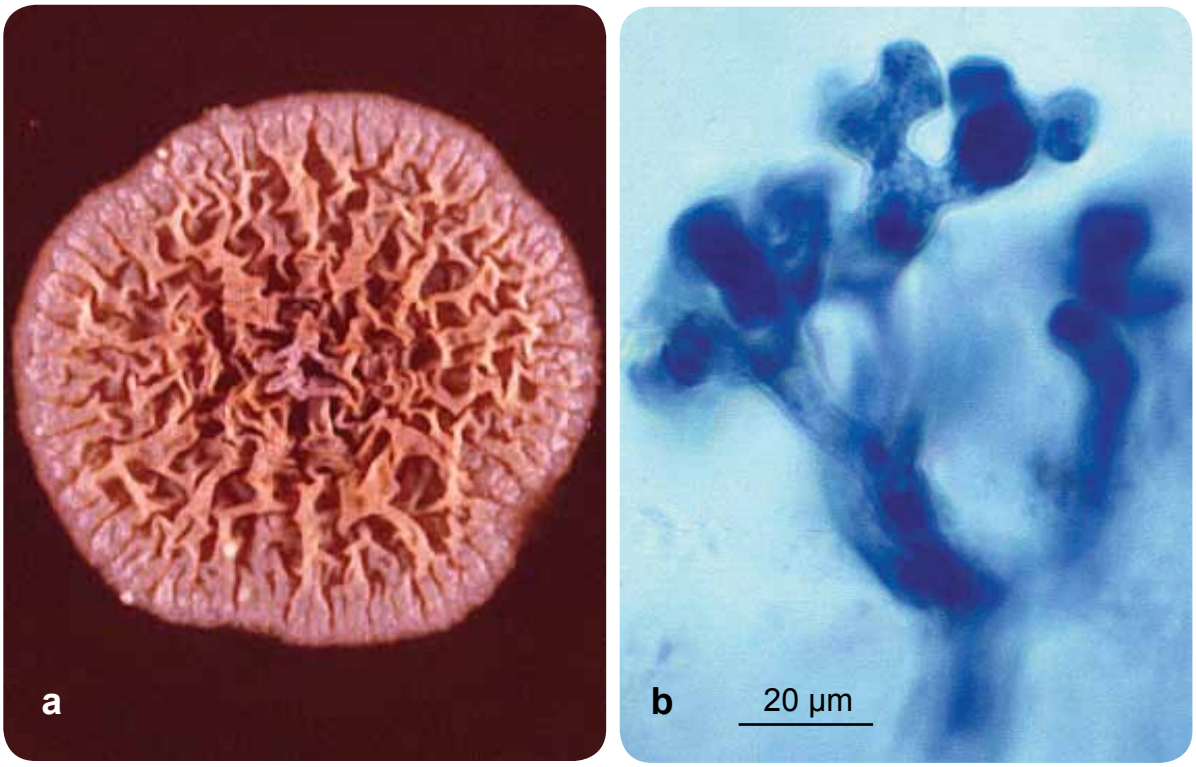
Trichophyton schoenleinii (Lebert) Langeron & Milochevitch

Trichophyton schoenleinii is an anthropophilic fungus causing favus in humans. Favus is a chronic, scarring form of tinea capitis characterised by saucer-shaped crusted lesions or scutula and permanent hair loss. Invaded hairs remain intact and fluoresce a pale greenish-yellow under Wood's ultra-violet light. Favus was once common in Eurasia and North Africa, however its incidence is now in decline.

RG-2 organism.

Morphological Description: Colonies are slow growing, waxy or suede-like with a deeply folded honey-comb-like thallus and some subsurface growth. The thallus is cream-coloured to yellow to orange brown. Cultures are difficult to maintain in their typical convoluted form, and rapidly become flat and downy. No reverse pigmentation is present. No macroconidia and microconidia are seen in routine cultures, and numerous chlamydospores may be present in older cultures. Characteristic antler "nail head" hyphae, also known as "favic chandeliers", may be observed. A few distorted clavate microconidia may be formed by some isolates when grown on polished rice grains.

Key Features: Clinical history, culture characteristics and microscopic morphology showing "favic chandeliers".



Trichophyton schoenleinii (a) culture and (b) "favic chandeliers".

Trichophyton soudanense Joyeux.

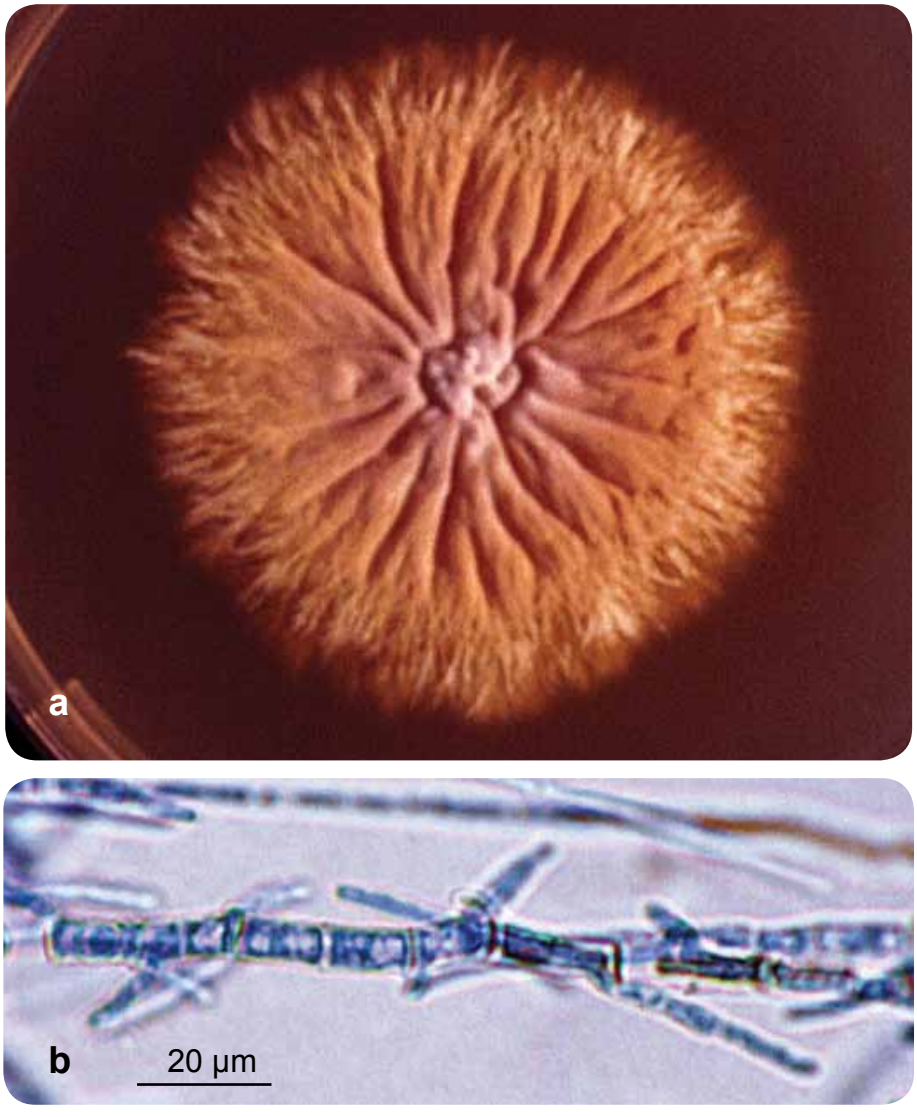
Trichophyton soudanense is an anthropophilic fungus that is a frequent cause of tinea capitis in Africa. Invaded hairs show an endothrix infection but do not fluoresce under Wood's ultra-violet light. Distribution is mainly in Africa with imported cases now reported from Europe, Brazil, Australia and USA.

RG-2 organism.

Morphological Description: Colonies are slow-growing with a flat to folded, suede-like surface. Often there is a broad fringe of submerged growth. Surface mycelium and reverse pigment are characteristically a deep apricot-orange in colour. Microscopically, the hyphae often show reflexive or right-angle branching. Pyriform microconidia may occasionally be present and numerous chlamydospores are often found in older cultures.

Note: *T. soudanense* demonstrates profuse growth and alkalinity on BCP milk solids agar. A thin halo of clearing usually appears in the milk solids around the colony edge at 7-10 days.

Key Features: clinical history, culture characteristics and microscopic morphology showing reflexive hyphal branching and endothrix invasion of hair.



Trichophyton soudanense (a) culture and (b) "reflex" hyphal branching.

Trichophyton tonsurans Malmsten

Trichophyton tonsurans is an anthropophilic fungus with a worldwide distribution which causes inflammatory or chronic non-inflammatory finely scaling lesions of skin, nails and scalp. It is a common cause of tinea capitis in Australian Aborigines and African Americans. Invaded hairs show an endothrix infection and do not fluoresce under Wood's ultra-violet light.

RG-2 organism.

Morphological Description: Colonies show considerable variation in texture and colour. They may be suede-like to powdery, flat with a raised centre or folded, often with radial grooves. The colour may vary from pale-buff to yellow, (the sulfureum form which resembles *Epidermophyton floccosum*), to dark-brown. The reverse colour varies from yellow-brown to reddish-brown to deep mahogany. Hyphae are relatively broad, irregular, much branched with numerous septa. Numerous characteristic microconidia varying in size and shape from long clavate to broad pyriform, are borne at right angles to the hyphae, which often remain unstained by lactophenol cotton blue. Very occasional smooth, thin-walled, irregular, clavate macroconidia may be present on some cultures. Numerous swollen giant forms of microconidia and chlamydospores are produced in older cultures.

Confirmatory Tests:

Mycosel Agar: Chlamydospore-like structures produced by 5 days is characteristic of *T. tonsurans* (Mochizuki *et al.* 2013).

Hydrolysis of Urea: positive at 5 days.

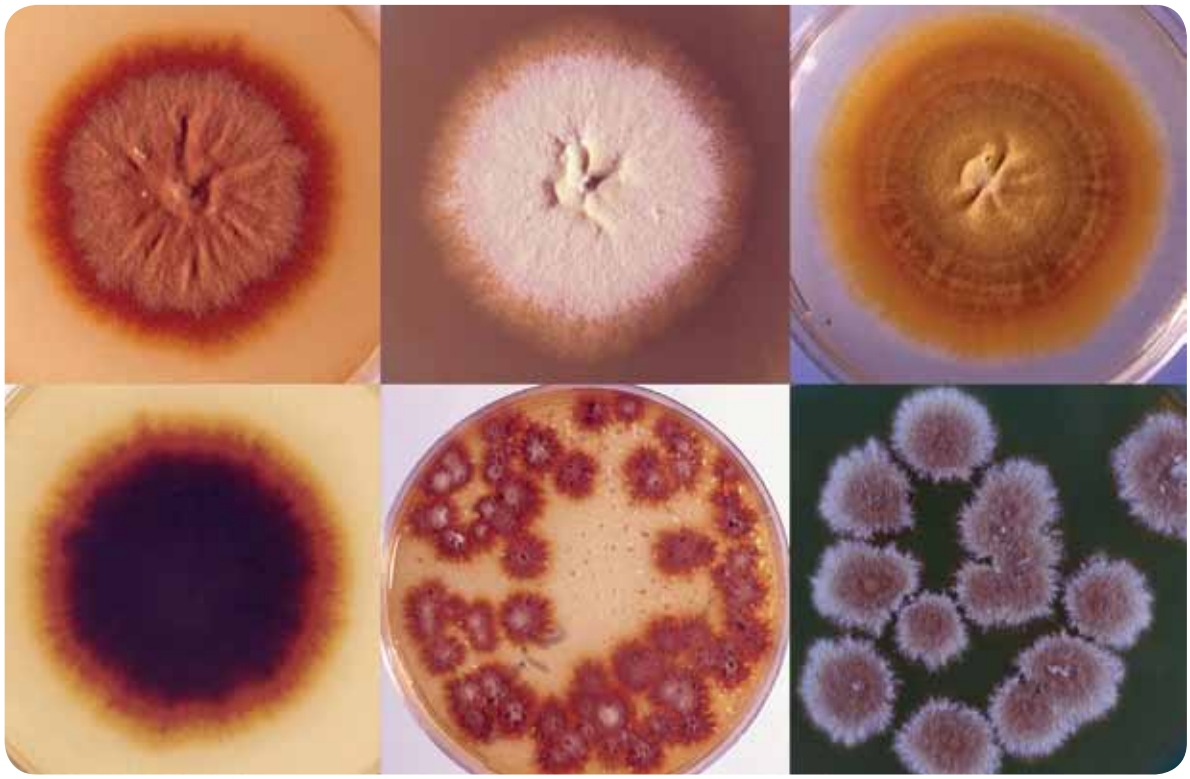
Nutritional Tests on Trichophyton Agars: results demonstrate a partial requirement for (a) thiamine. T1 = vitamin free agar, (b) T4 = vitamin free + thiamine agar.

Hair Perforation Test: Positive within 14 days.

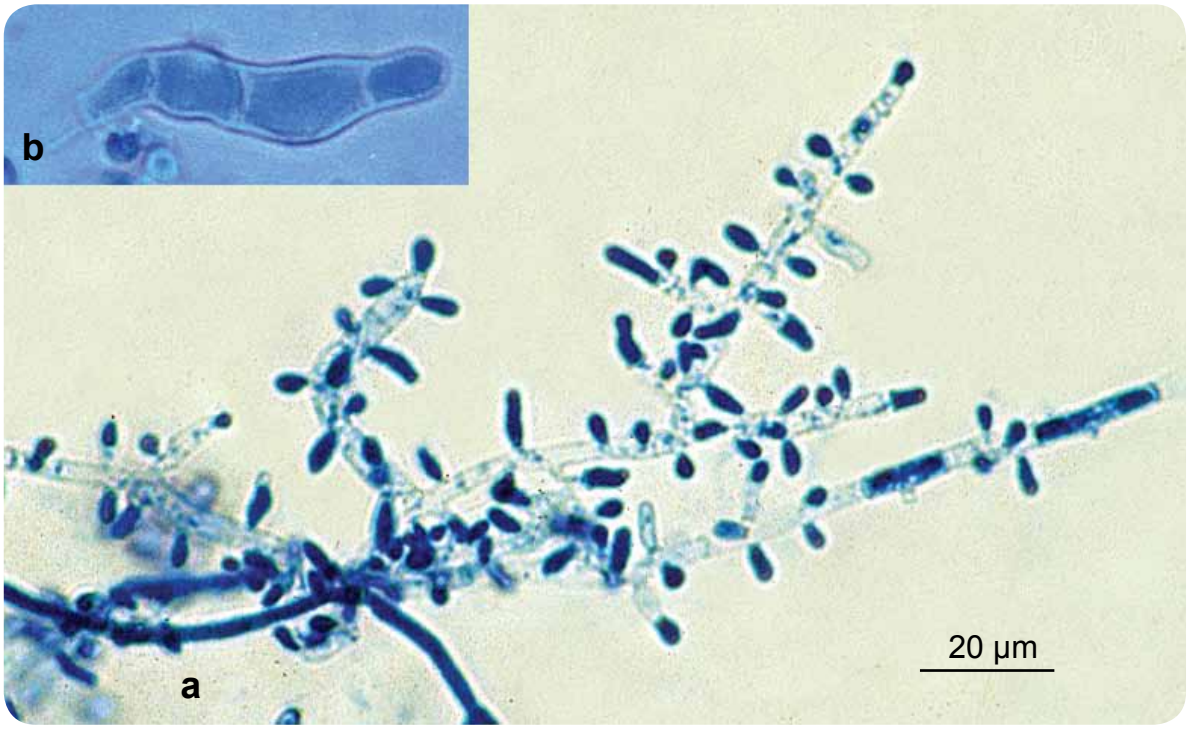
Key Features: microscopic morphology, culture characteristics, endothrix invasion of hairs and partial thiamine requirement.

References: Rebell and Taplin (1970), Ajello (1972), Vanbreusegham *et al.* (1978), Rippon (1988), McGinnis (1980), Domsch *et al.* (1980), Kane *et al.* (1997) and de Hoog *et al.* (2000, 2015).

Trichophyton tonsurans Malmsten



Trichophyton tonsurans cultures show considerable variation in texture and colour. The colour may vary from pale-buff to yellow to dark-brown. The reverse colour varies from yellow-brown to reddish-brown to deep mahogany.



Trichophyton tonsurans (a) hyphae, microconidia and (b) macroconidia.

Trichophyton verrucosum Bodin

Trichophyton verrucosum is a zoophilic fungus causing ringworm in cattle. Infections in humans result from direct contact with cattle or infected fomites and are usually highly inflammatory involving the scalp, beard or exposed areas of the body. Invaded hairs show an ectothrix infection and fluorescence under Wood's ultra-violet light has been noted in cattle but not in humans. Distribution is worldwide. **RG-2 organism.**

Morphological Description: Colonies are slow growing, small, button or disk-shaped, white to cream-coloured, with a suede-like to velvety surface, a raised centre, and flat periphery with some submerged growth. Reverse pigment may vary from non-pigmented to yellow. Broad, irregular hyphae with many terminal and intercalary chlamydospores. Chlamydospores are often in chains. The tips of some hyphae are broad and club-shaped, and occasionally divided, giving the so-called "antler" effect. When grown on thiamine-enriched media, occasional strains produce clavate to pyriform microconidia borne singly along the hyphae. Macroconidia are only rarely produced, but when present have a characteristic tail or string bean shape.

Confirmatory Tests:

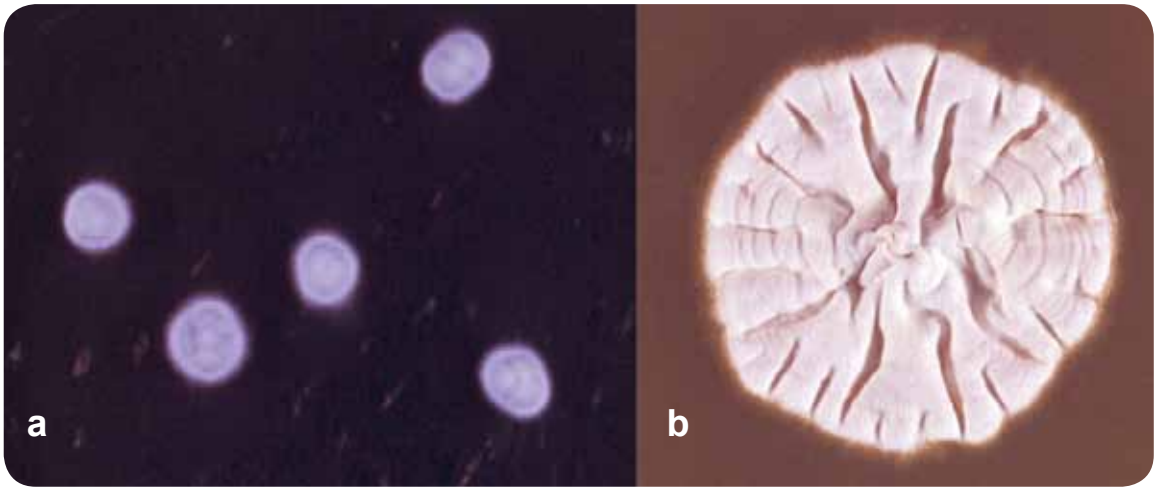
Growth at 37°C: Unlike other dermatophytes, growth is enhanced at 37°C.

Nutritional Requirements: all strains require thiamine and approximately 80% require thiamine and inositol. There is no growth on casein vitamin free agar (T1), minimal submerged growth on T1 + inositol (T2), good growth on T1 + inositol and thiamine (T3) and good growth on T1 + thiamine only (T4).

All strains produce typical chains of chlamydospores, often referred to as "chains of pearls", especially when grown on BCP milk solids glucose agar at 37°C. When grown at 25°C on milk solids glucose agar a "halo"-like zone of peripheral clearing of milk solids occurs within 7 days.

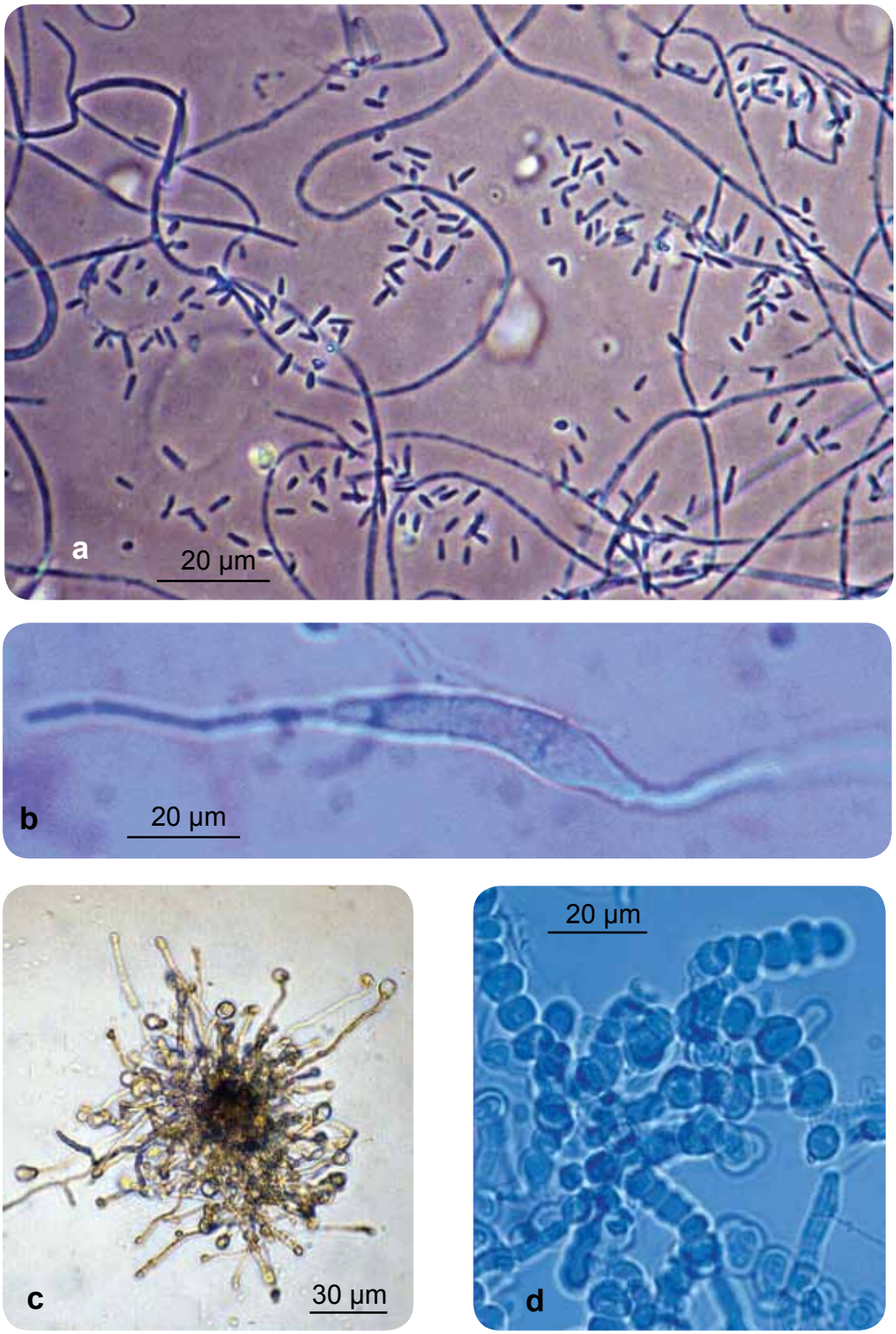
Microscopic examination of young colonies (4 to 5 day old), grown from a very small inoculum, on Sabouraud's dextrose agar containing 0.5% yeast extract and incubated at 30°C, show characteristic terminal vesicles (not chlamydospores) at the tips of hyphae. The number of vesicles produced is greater from primary inoculations of skin scrapings or hairs than from subcultures.

Key Features: Culture characteristics and requirements for thiamine and inositol, large ectothrix invasion of hair, clinical lesions and history.



Trichophyton verrucosum (a) young button-shaped colonies and (b) mature culture.

Trichophyton verrucosum Bodin



Trichophyton verrucosum showing (a) clavate to pyriform microconidia, (b) characteristic rat tail or string bean-shaped macroconidia, (c) terminal vesicles at the tips of hyphae in young colonies and (d) chains of chlamydospores.

Trichophyton violaceum Sabouraud apud Bodin

Synonymy: *Trichophyton yaoundei* Cochet & Doby-Dubois.
All varieties of *T. violaceum.*

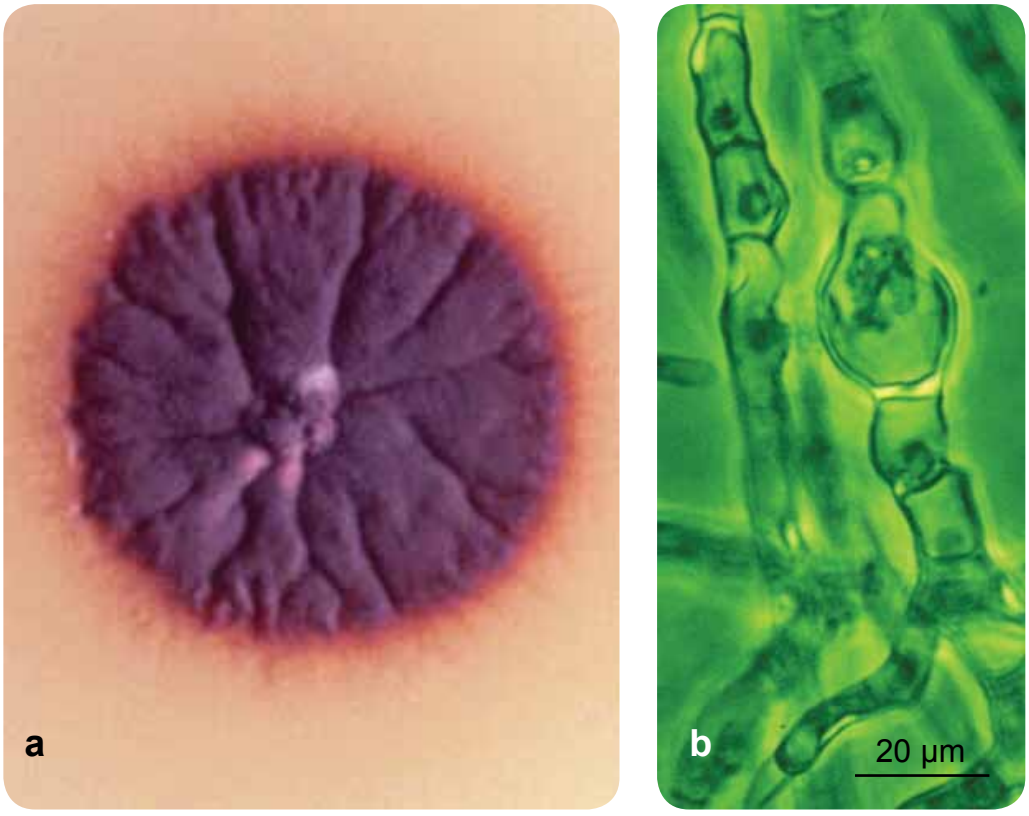
Trichophyton violaceum is an anthropophilic fungus causing inflammatory or chronic non-inflammatory finely scaling lesions of skin, nails, beard and scalp, producing the so-called “black dot” tinea capitis. Distribution is worldwide, particularly in the Near East, Eastern Europe, Russia and North Africa. Invaded hairs show an endothrix infection and do not fluoresce under Wood’s ultra-violet light.

RG-2 organism.

Morphological Description: Colonies are very slow growing, glabrous or waxy, heaped and folded and deep violet in colour. Cultures often become pleomorphic, forming white sectors. Occasional non-pigmented strains may occur. Hyphae are relatively broad, tortuous, much branched and distorted. Young hyphae usually stain well in lactophenol cotton blue, whereas older hyphae stain poorly and show small central fat globules and granules. Typically, no conidia are present, although occasional pyriform microconidia have been observed on enriched media. Numerous chlamydospores are usually present, especially in older cultures.

Nutritional Requirements: *T. violaceum* has a partial nutrient requirement for thiamine. There is minimal growth on casein vitamin-free agar (T1), and slightly better growth on vitamin-free agar plus thiamine (T4). The partial requirement for thiamine separates this organism from *T. rubrum,* and other species that may produce purple pigmented colonies.

Key Features: Culture characteristics, partial thiamine requirement and endothrix hair invasion.



Trichophyton violaceum (a) culture and (b) chlamydospores.

Trichosporon Behrend

Trichosporon species are urease-positive, non-encapsulated basidiomycetous yeasts characterised by the development of hyaline, septate hyphae that fragment into oval or rectangular arthroconidia. Some blastoconidia are also seen. The colonies are usually raised and have a waxy appearance, which develop radial furrows and irregular folds. They are widely distributed in the environment and many have different habitats, usually occupying narrow ecological niches. Some are soil borne and others are associated with humans and animals (Colombo *et al.* 2011, Sugita 2011, Arendrup *et al.* 2014).

The genus has undergone major taxonomic revision (Gueho *et al.* 1992, de Hoog *et al*. 2000, Rodriguez-Tudela *et al*. 2005). In particular, the name *Trichosporon beigelii* is now obsolete, and previously described infections reported in the literature under this name could in fact be due to any one of the species listed below.

Six species are of clinical significance: *T. asahii, T. asteroides, T. cutaneum, T. inkin, T. mucoides* and *T. ovoides*. Other species reported from human and animal infections include *T. dermatis, T. domesticum, T. faecale, T. jirovecii, T. loubieri* and *T. mycotoxinovorans* (Rodriguez-Tudela *et al*. 2005, Chagas-Neto *et al.* 2008, Colombo *et al.* 2011).

Trichosporon species are a minor component of normal skin flora, and are widely distributed in nature. They are regularly associated with the soft nodules of white piedra, and have been involved in a variety of opportunistic infections in the immunosuppressed patient. Disseminated infections are most frequently (75%) caused by *T. asahii* (Arendrup *et al.* 2014) and have been associated with leukaemia, organ transplantation, multiple myeloma, aplastic anaemia, lymphoma, solid tumours and AIDS. Disseminated infections are often fulminate and widespread, with lesions occurring in the liver, spleen, lungs and gastrointestinal tract. Infections in non-immunosuppressed patients include endophthalmitis after surgical extraction of cataracts, endocarditis usually following insertion of prosthetic cardiac valves, peritonitis in patients on continuous ambulatory peritoneal dialysis (CAPD), and intravenous drug abuse.

Note: Genus identification is mandatory for clinical management and should be performed and provided in a timely manner. Species identification remains difficult and requires molecular analysis or MALDI-TOF MS (with an extensive database) (Arendrup *et al.* 2014).

Molecular Identification: ITS and D1/D2 sequencing is required for accurate species identification (Arendrup *et al.* 2014).

MALDI-TOF MS: A promising identification tool to accurately identify species (with an extensive database) (Kolecka *et al.* 2013).

Comment: The API 20C yeast identification system is recommended for sugar assimilation tests.

References: Kurtzman and Fell (1988), Gueho *et al*. (1992), de Hoog *et al.* (2000, 2015), Rodriguez-Tudela *et al.* (2005), Chagas-Neto *et al*. (2008), Guo *et al.* (2011), Xiao *et al*. (2013).

Trichosporon Behrend

Key to medically important species (de Hoog *et al*. 2000).

1.	Growth with melibiose	2
	No growth with melibiose	3
2.	Tolerant to cycloheximide	*T. mucoides*
	Not tolerant to cycloheximide	*T. cutaneum*
3.	Growth with *myo*-inositol, no growth with L-arabinose	*T. inkin*
	No growth with myo-inositol, growth with L-arabinose	4
4.	Colony with very slow growth; thallus consisting of clumps of meristematic cells	*T. asteroides*
	Colonies and microscopy otherwise	5
5.	Appressoria present in slide cultures	*T. ovoides*
	Appressoria absent in slide cultures	6
6.	Arthroconidia barrel-shaped; thallus not meristematic	*T. asahii*
	Arthroconidia elongate, or thallus meristematic	*T. asteroides*

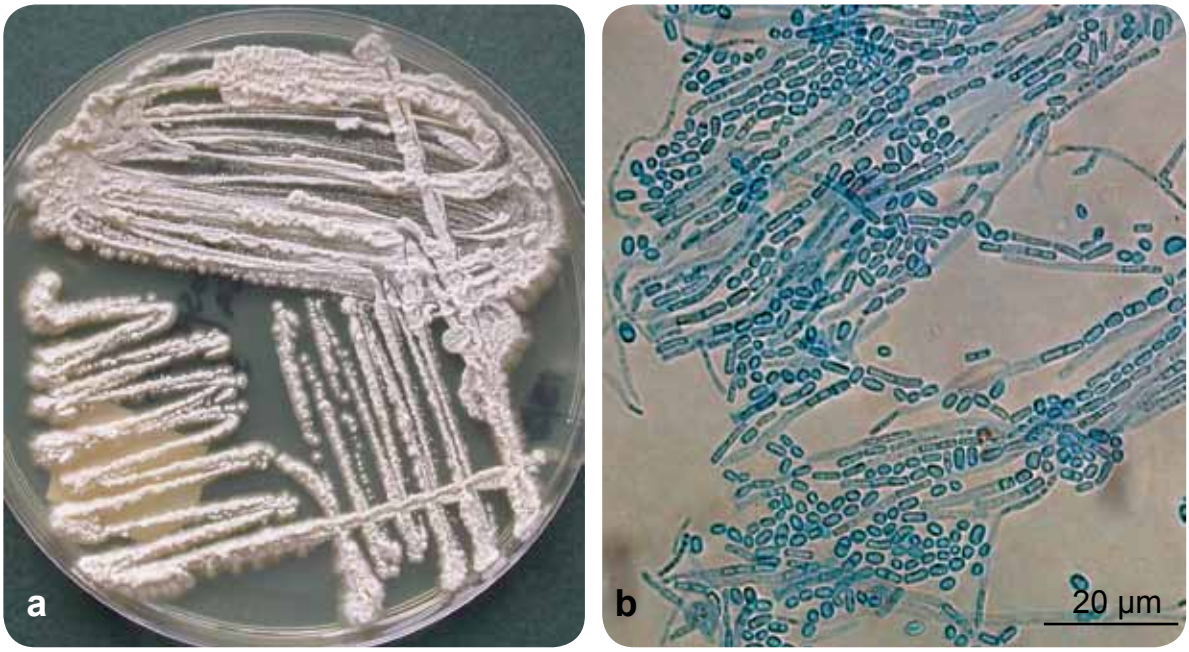
Trichosporon asahii Akagi ex Sugita *et al.*

RG-2 organism.

Morphological Description: Colonies are white to cream-coloured, powdery, suede-like to farinose with radial furrows and irregular folds. Budding cells and lateral conidia are absent. Arthroconidia are barrel-shaped. Appressoria absent. This species assimilates L-arabinose but not melibiose. Growth at 37^{O}C. Most common species, especially from invasive infections.

Assimilation Tests: + Positive, - Negative, v Variable, w Weak, s Slow							
Glucose	+	Melibiose	-	L-Rhamnose	+	D-Glucitol	v
Galactose	+	Raffinose	-	D-Glucosamine	+	α-M-D-glucoside	+
L-Sorbose	v	Melezitose	v	*N*-A-D-glucosamine	+	D-Gluconate	+
Sucrose	v	Soluble Starch	v	Glycerol	v	DL-Lactate	v
Maltose	+	D-Xylose	v	Erythritol	+	*myo*-Inositol	v
Cellobiose	+	L-Arabinose	+	Ribitol	v	Nitrate	-
Trehalose	+	D-Arabinose	+	Galactitol	-	2-K-D-gluconate	+
Lactose	+	D-Ribose	+	D-Mannitol	v	D-Glucuronate	+

Trichosporon asahii Akagi ex Sugita *et al.*



Trichosporon asahii (a) culture and (b) hyphae and arthroconidia.

Antifungal Susceptibility*: T. asahii* (Australian National data); **MIC µg/mL**.

	No.	≤0.008	0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	≥64
AmB	**22**					**4**	**4**	**6**	**4**	**3**	**1**				
FLU	**22**								**1**	**3**	**11**	**7**			
VORI	**20**	**1**	**1**		**7**	**10**	**1**								
POSA	**19**				**1**	**1**	**11**	**4**	**2**						
ITRA	**22**			**3**	**15**	**3**	**1**								

Note: Non-*T. asahii* isolates appear to be more susceptible than *T. asahii* isolates to AmB, FLU, and ITRA, while the new triazoles are active against both *T. asahii* and non-*T. asahii* isolates (Rodriguez-Tudela *et al.* 2005).

Trichosporon inkin. Colonies are restricted, white, finely cerebriform with a granular covering, without marginal zone, often cracking the media.

Trichosporon asteroides (Rischin) Ota

Morphological Description: Colonies are restricted, dry, cream-coloured, cerebriform, with radial furrows and irregular folds. The meristematic form is punctiform, brownish and consists of hyphae which swell and become multiseptate which may fall apart into smaller packets. Budding cells and lateral conidia are absent. Arthroconidia are elongate and hyphae are often present. Appressoria absent. This species assimilates L-arabinose but not myo-inositol. Growth at 37^{O}C is variable. Uncommon species usually associated with superficial infections. **RG-2 organism**.

Assimilation Tests: + Positive, - Negative, v Variable, w Weak, s Slow							
Glucose	+	Melibiose	-	L-Rhamnose	+	D-Glucitol	v
Galactose	+	Raffinose	-	D-Glucosamine	v	α-M-D-glucoside	+
L-Sorbose	v	Melezitose	+	*N*-A-D-glucosamine	+	D-Gluconate	+
Sucrose	+	Soluble Starch	+	Glycerol	+	DL-Lactate	+
Maltose	+	D-Xylose	+	Erythritol	+	*myo*-Inositol	-
Cellobiose	+	L-Arabinose	+	Ribitol	v	Nitrate	-
Trehalose	+	D-Arabinose	+	Galactitol	-	2-K-D-gluconate	+
Lactose	+	D-Ribose	+	D-Mannitol	v	D-Glucuronate	+

Trichosporon cutaneum (de Beurmann *et al.*) Ota

Morphological Description: Colonies are cream-coloured, cerebriform, glabrous, with radial furrows and irregular folds. Budding cells abundant in primary cultures; hyphae developing in older cultures. Arthroconidia are cylindrical to ellipsoidal. Appressoria absent. This species assimilates melibiose; not tolerant to 0.1% (variable tolerance to 0.01%) cycloheximide. No growth at 37^{O}C. Uncommon species usually associated with superficial infections. **RG-2 organism.**

Assimilation Tests: + Positive, - Negative, v Variable, w Weak, s Slow							
Glucose	+	Melibiose	+	L-Rhamnose	+	D-Glucitol	+
Galactose	+	Raffinose	+	D-Glucosamine	v	α-M-D-glucoside	+
L-Sorbose	v	Melezitose	+	*N*-A-D-glucosamine	+	D-Gluconate	+
Sucrose	+	Soluble Starch	+	Glycerol	+	DL-Lactate	+
Maltose	+	D-Xylose	+	Erythritol	+	*myo*-Inositol	+
Cellobiose	+	L-Arabinose	+	Ribitol	+	Nitrate	-
Trehalose	+	D-Arabinose	v	Galactitol	-	2-K-D-gluconate	+
Lactose	+	D-Ribose	+	D-Mannitol	+	D-Glucuronate	+

Trichosporon inkin (Oho ex Ota) do Carmo-Sousa & van Uden

Morphological Description: Colonies are restricted, white, finely cerebriform with a granular covering, without marginal zone, often cracking the media. Budding cells and lateral conidia absent. Arthroconidia are long cylindrical. Appressoria present in slide cultures. Sarcinae present on media with high sugar-content. This species assimilates myo-inositol but not melibiose and is tolerant to 0.01% (variable tolerance to 0.1%) cycloheximide. Growth at 37^{O}C. Usually associated with white piedra on pubic hairs. **RG-2 organism**.

Trichosporon inkin (Oho ex Ota) do Carmo-Sousa & van Uden

Assimilation Tests: + Positive, - Negative, v Variable, w Weak, s Slow							
Glucose	+	Melibiose	-	L-Rhamnose	-	D-Glucitol	-
Galactose	v	Raffinose	-	D-Glucosamine	v	α-M-D-glucoside	+
L-Sorbose	v	Melezitose	+	*N*-A-D-glucosamine	+	D-Gluconate	+
Sucrose	+	Soluble Starch	+	Glycerol	v	DL-Lactate	+
Maltose	+	D-Xylose	+	Erythritol	+	*myo*-Inositol	+
Cellobiose	+	L-Arabinose	v	Ribitol	-	Nitrate	-
Trehalose	+	D-Arabinose	v	Galactitol	-	2-K-D-gluconate	+
Lactose	+	D-Ribose	+	D-Mannitol	v	D-Glucuronate	+

Trichosporon mucoides Gueho & M.Th. Smith

Morphological Description: Colonies are moist and glabrous, white, cerebriform, heaped and folded. Budding cells present in primary cultures. Broadly clavate, terminal or lateral blastoconidia often present, becoming thick-walled with age. Arthroconidia are barrel-shaped. Appressoria absent. This species assimilates melibiose and is tolerant to 0.01% (variable tolerance to 0.1%) cycloheximide. Growth at 37^{O}C. Common species associated with superficial infections, white piedra and onychomycosis. **RG-2 organism**.

Assimilation Tests: + Positive, - Negative, v Variable, w Weak, s Slow							
Glucose	+	Melibiose	+	L-Rhamnose	+	D-Glucitol	+
Galactose	+	Raffinose	+	D-Glucosamine	+	α-M-D-glucoside	+
L-Sorbose	+	Melezitose	+	*N*-A-D-glucosamine	+	D-Gluconate	+
Sucrose	+	Soluble Starch	+	Glycerol	+	DL-Lactate	+
Maltose	+	D-Xylose	+	Erythritol	+	*myo*-Inositol	+
Cellobiose	+	L-Arabinose	+	Ribitol	+	Nitrate	-
Trehalose	+	D-Arabinose	+	Galactitol	+	2-K-D-gluconate	+
Lactose	+	D-Ribose	+	D-Mannitol	+	D-Glucuronate	+

Trichosporon ovoides Behrend

Morphological Description: Colonies are restricted, white, granular, folded at the centre, with a flat marginal zone. Budding cells and lateral conidia absent. Arthroconidia are cylindrical. Appressoria present in slide cultures. This species does not assimilate melibiose, but tolerates 0.01% cycloheximide. Growth at 37^{O}C is variable. Uncommon species usually associated with superficial infections, like white piedra. **RG-2 organism**.

Assimilation Tests: + Positive, - Negative, v Variable, w Weak, s Slow							
Glucose	+	Melibiose	-	L-Rhamnose	+	D-Glucitol	v
Galactose	+	Raffinose	v	D-Glucosamine	v	α-M-D-glucoside	+
L-Sorbose	v	Melezitose	v	*N*-A-D-glucosamine	+	D-Gluconate	+
Sucrose	+	Soluble Starch	+	Glycerol	v	DL-Lactate	+
Maltose	+	D-Xylose	+	Erythritol	+	*myo*-Inositol	+
Cellobiose	+	L-Arabinose	v	Ribitol	-	Nitrate	-
Trehalose	v	D-Arabinose	v	Galactitol	-	2-K-D-gluconate	+
Lactose	+	D-Ribose	+	D-Mannitol	+	D-Glucuronate	+

Trichothecium roseum (Persoon) Link

Trichothecium roseum has a worldwide distribution and is often isolated from decaying plant substrates, soil, seeds of corn, and food-stuffs (especially flour products). It is occasionally isolated as a saprophyte in the clinical laboratory.

RG-1 organism.

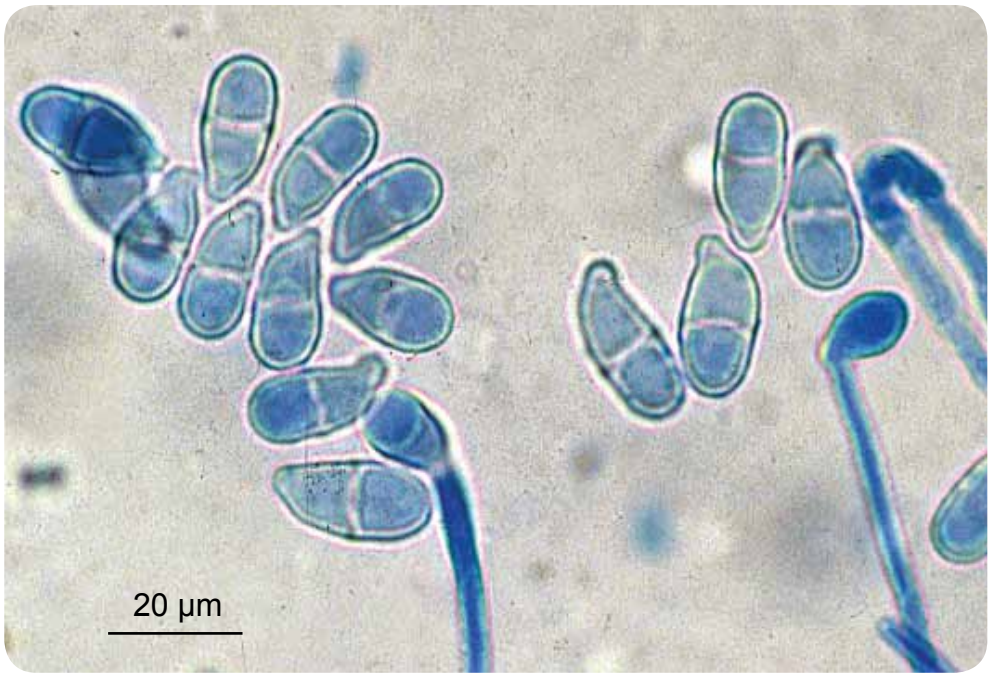
Morphological Description: Colonies are moderately fast growing, flat, suede-like to powdery, initially white but becoming rosy, pink or orange with age. The conidiophores are indistinguishable from the vegetative hyphae until the first conidium is produced. They are erect, unbranched, often septate near the base, more or less rough-walled, bearing basipetal zig-zag (alternating) chains of conidia at the apex. **Note:** The conidiophore is progressively shortened with the formation of each conidium i.e. retrogressive conidial development. Conidia are two-celled ellipsoidal to pyriform, with an obliquely truncate basal scar, hyaline, smooth to delicately roughened and thick-walled.

Comment: *T. roseum* should not be confused with *Nannizzia nanum*. Colonies of the latter may be pinkish-buff in colour and also produce ovoid to pear-shaped, mostly two-celled macroconidia with thin, verrucose walls. However, *N. nanum* usually produces a red-brown reverse pigment and the two-celled macroconidia are sessile and formed singly, they are not produced in basipetal chains as in *T. roseum*.

Molecular Identification: Summerbell *et al*. (2011) revised the genus using D1/D2 sequences for phylogenetic analysis and sequence based identification.

Key Features: Hyphomycete, basipetal zig-zag chains of two-celled conidia showing retrogressive development where the conidiophore becomes progressively shorter.

References: McGinnis (1980), Domsch *et al.* (2007), Rippon (1988), Samson *et al.* (1995), Summerbell *et al*. (2011).



Trichothecium roseum conidiophores showing retrogressive conidial development.

Ulocladium Preuss

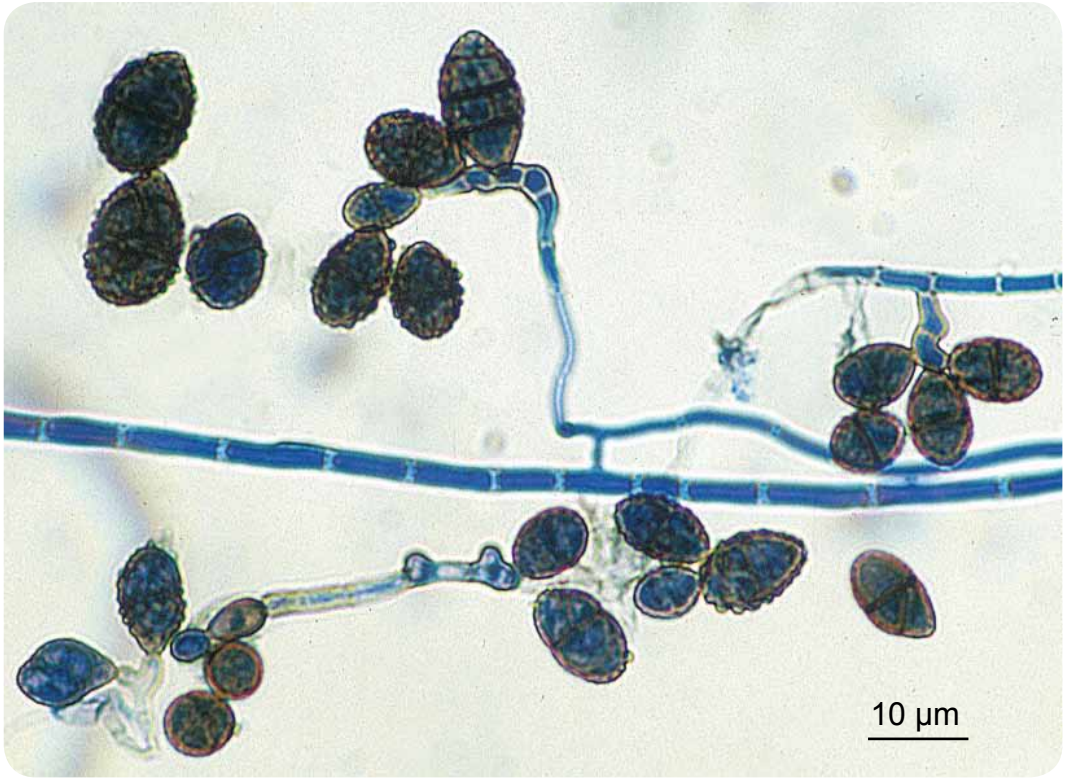
Species of *Ulocladium* should not be confused with other poroconidial genera such as *Stemphylium, Alternaria, Bipolaris, Exserohilum, Dreschlera* and *Curvularia*. A human case of keratitis has been reported (Badenoch *et al*. 2006).

RG-1 organism.

Morphological Description: Colonies are rapid growing, brown to olivaceous-black or greyish and suede-like to floccose. Microscopically, numerous, usually solitary, multicelled conidia (dictyoconidia) are formed through a pore (poroconidia) by a sympodially elongating geniculate conidiophore. Conidia are typically obovoid (narrowest at the base), dark brown and often rough-walled. Seven species have been described, all being saprophytes.

Molecular Identification: ITS sequencing is sufficient for genus identification (Badenoch *et al*. 2006) and Woudenberg *et al.* 2013).

References: Ellis (1970, 1976), Domsch *et al.* (1980), Rippon (1988), Samson *et al.* (1995), de Hoog *et al.* (2000).



Ulocladium spp. conidiophores and conidia.

Antifungal Susceptibility: *Ulocladium sp.* very limited data (Pujol *et al.* 2000, Australian National data); **MIC µg/mL.**					
Antifungal	**Range**	**Antifungal**	**Range**	**Antifungal**	**Range**
AmB	**1->16**	**VORI**	**0.25**	**ITRA**	**0.06->16**

Veronaea botryosa Ciferri & Montemartini

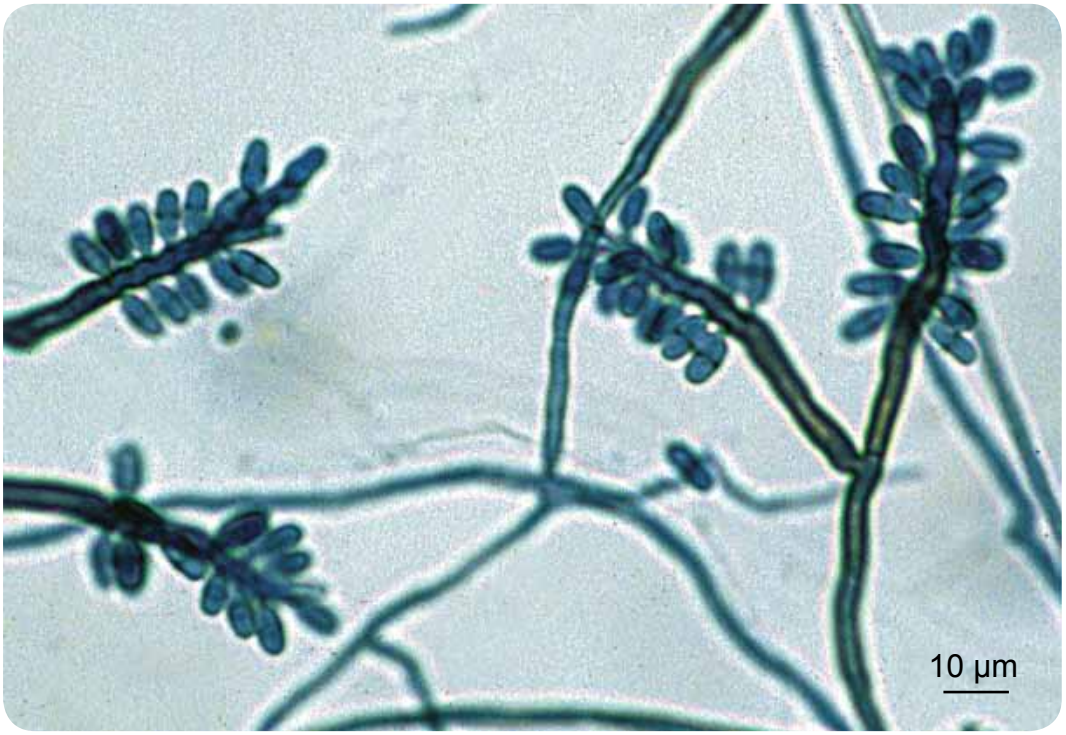
This genus is very similar to *Rhinocladiella*, however the conidia are typically two-celled. Occasional skin infections have been reported from humans (Revankar and Sutton 2010).

RG-2 organism.

Morphological Description: Colonies grow rapidly and are suede-like to downy, greyish-brown to blackish-brown. Conidiophores are erect, straight or flexuose, occasionally branched and are usually geniculate, due to the sympodial development of the conidia. They are smooth-walled, pale to medium olivaceous-brown, up to 250 µm long and 2-4 µm wide. Conidia are pale brown, two-celled, cylindrical with a truncated base, smooth-walled or slightly verrucose, 5-12 x 3-4 µm.

Molecular Identification: Arzanlou *et al*. (2007) used D1/D2 and ITS sequence data in a phylogenetic revision.

References: Ellis (1971), de Hoog *et al*. (2000, 2015), Revankar and Sutton (2010).



Veronaea botryosa conidiophores and conidia.

Antifungal Susceptibility: *V. botryosa* limited data (Badali *et al.* 2013); **MIC µg/mL**.					
Antifungal	**Range**	**MIC_{90}**	**Antifungal**	**Range**	**MIC_{90}**
AmB	**8-16**	**16**	**POSA**	**0.03-0.25**	**0.25**
ITRA	**0.25-1**	**1**	**VORI**	**1-8**	**4**

Verruconis gallopava (W.B. Cooke) Samerpitak & de Hoog

Synonymy: *Ochroconis gallopava* (W.B. Cooke) de Hoog.

Verruconis species are thermophilic, with *Verruconis gallopava* occurring in hot environments, such as thermal soils, broiler house litter, hot springs, and self-heated waste (Samerpitak *et al*. 2014). *V. gallopava* is neurotropic and is a recognised agent of human brain infections and is responsible for encephalitis in poultry and wild birds dogs and cats (Seyedmousavi *et al.* 2014). Occasional human pulmonary infections in immunocompetent hosts have also been reported (Samerpitak *et al*. 2014, Seyedmousavi *et al*. 2014, Giraldo *et al.* 2014).

RG-2 organism.

Morphological Description: Colonies are smooth to suede-like, dry, flat, tobacco-brown to brownish-black with a dark brown diffusible pigment. Hyphae are brown with relatively thick walls. Conidiophores are mostly cylindrical to acicular, sometimes poorly differentiated, bearing a few conidia at the tip. Conidia are two-celled, subhyaline to pale brown, smooth-walled to verrucose, cylindrical to clavate, constricted at the septum, 11-18 x 2.5-4.5 µm in size, with the apical cell wider than the basal cell. A remnant of a denticle may also be seen at the conidial base. Optimum growth at 35^{O}C, tolerant to 40^{O}C.

Molecular Identification: ITS sequencing can identify species. Additional genes include β-tubulin, actin, and the D1/D2 region (Giraldo *et al.* 2014, Seyedmousavi *et al*. 2014).

References: Domsch *et al.* (1980), McGinnis (1980), de Hoog *et al.* (2000, 2015), Samerpitak *et al.* (2014), Seyedmousavi *et al*. (2014) and Giraldo *et al*. (2014).

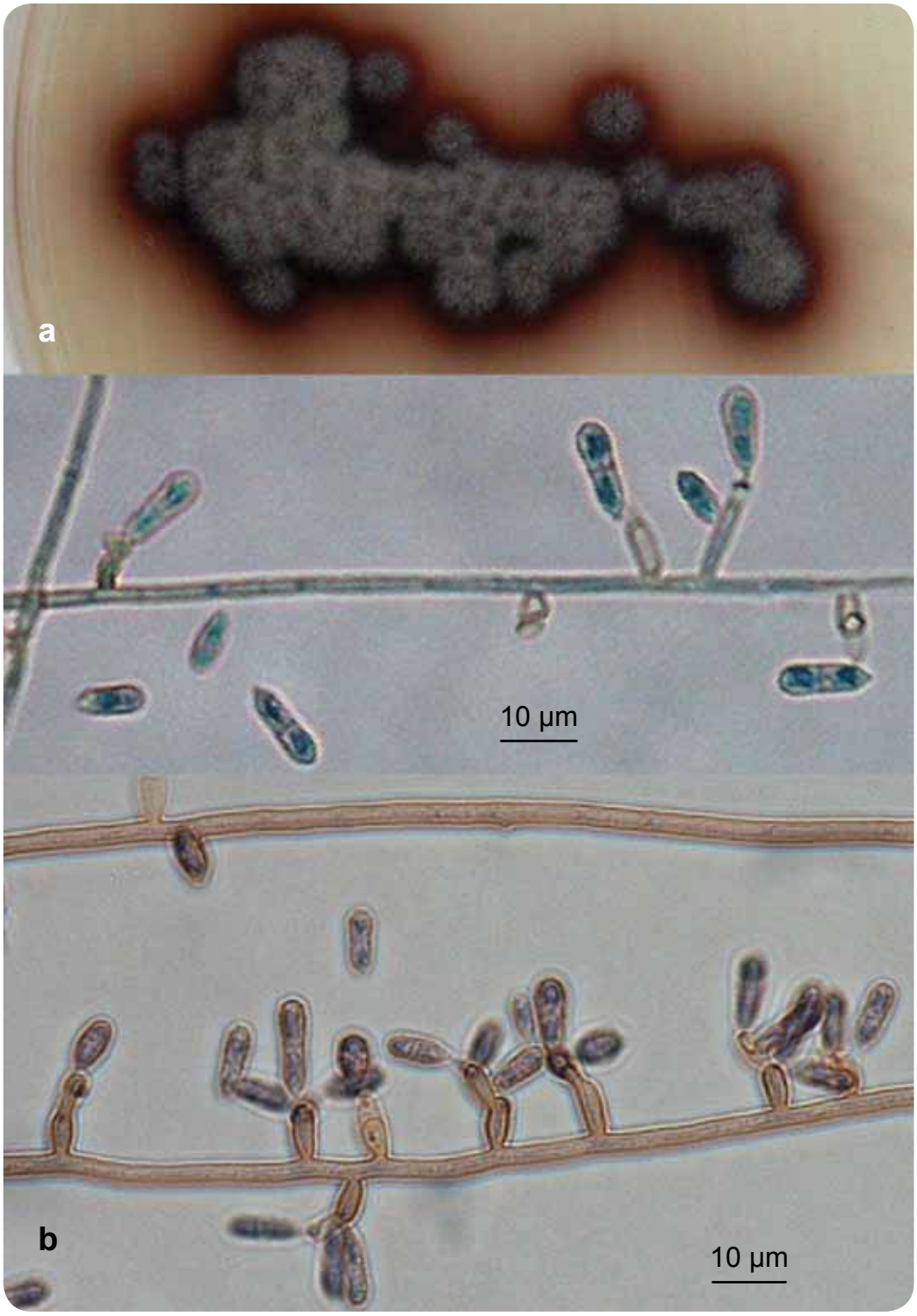
Antifungal Susceptibility: ***V. gallopava*** limited data (Australian National data); **MIC µg/mL.**

	No.	≤0.008	0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	≥64
AmB	**8**					**1**	**2**	**2**	**1**	**2**					
VORI	**8**					**1**	**3**	**3**	**1**						
POSA	**7**	**1**	**2**	**1**		**1**	**1**		**1**						
ITRA	**8**			**3**		**1**	**3**	**1**							

V. gallopava data for 11 isolates (Seyedmousavi *et al.* 2014); **MIC µg/mL**.

AmB	**Range 0.125-4; MIC_{90}= 0.5**	**VORI**	**Range 0.5-2; MIC_{90}= 2**
ITRA	**Range 0.016-4; MIC_{90}= 0.5**	**POSA**	**Range <0.016-4; MIC_{90}= 0.125**

Verruconis gallopava (W.B. Cooke) Samerpitak & de Hoog



Verruconis gallopava (a) culture and (b) hyphae, conidiophores and conidia.

Verticillium Nees ex Link

Members of this genus are often isolated from the environment. It has been reported as a rare agent of mycotic keratitis.

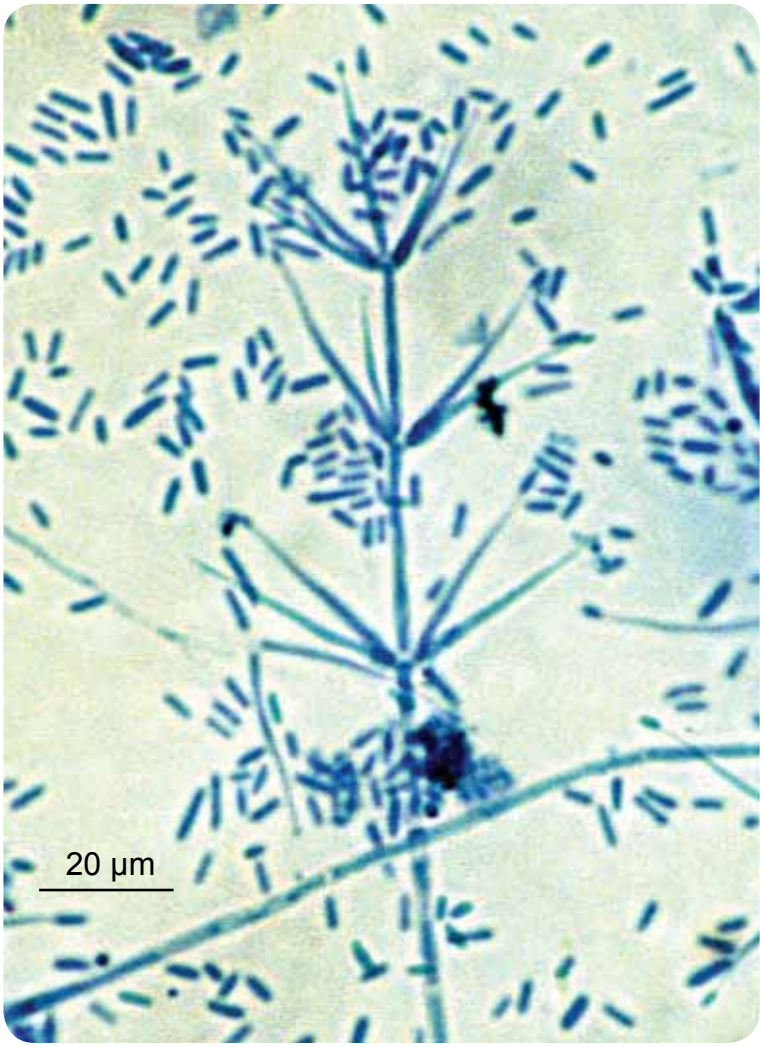
RG-1 organism.

Morphological Description: Colonies are fast growing, suede-like to downy, white to pale yellow in colour, becoming pinkish-brown, red, green or yellow with a colourless, yellow or reddish-brown reverse. Conidiophores are usually well differentiated and erect, verticillately branched over most of their length, bearing whorls of slender awl-shaped divergent phialides. Conidia are hyaline or brightly-coloured, mostly one-celled, and are usually borne in slimy heads (glioconidia).

Molecular Identification: ITS, actin, *EF-1α*, *GPDH* and tryptophan synthase genes have been used to identify all recognised *Verticillium* species (Inderbitzin *et al.* 2013).

Key Features: Hyphomycete, verticillate branched conidiophores bearing whorls of awl-shaped, divergent phialides.

References: Domsch *et al.* (1980), McGinnis (1980), Rippon (1988), Samson *et al.* (1995), de Hoog *et al*. (2000, 2015).



Verticillium spp conidiophores, phialides and conidia.

Wickerhamomyces anomalus (E.C. Hansen) Kurtzman *et al.*

Synonymy: *Candida pelliculosa* Redaelli.

Wickerhamomyces anomalus has been reported from cases of candidaemia and catheter related infections in humans and has been isolated from soil, grains, fruit and warm-blooded animals.

RG-1 organism.

Culture: Colonies (SDA) white to cream-coloured smooth, glabrous, yeast-like.

Microscopy: Spherical to ellipsoidal budding blastoconidia, 2-4 x 2-6 µm. Pseudohyphae may be present. Asci when present, containing one to four hat-shaped ascospores.

India Ink Preparation: Negative - no capsules present.

Dalmau Plate Culture: Spherical to ellipsoidal budding yeast cells and abundant pseudohyphae in most strains.

Molecular Identification: ITS sequencing recommended.

MALDI-TOF MS: Able to accurately identify this species.

Physiological Tests: + Positive, - Negative, v Variable, w Weak, s Slow, nd No Data							
Germ Tube	-	L-Sorbose	-	L-Arabinose	v	D-Glucitol	+
Fermentation		Sucrose	+	D-Arabinose	-	α-M-D-glucoside	+
Glucose	+	Maltose	+	D-Ribose	v	D-Gluconate	v
Galactose	v	Cellobiose	+	L-Rhamnose	-	DL-Lactate	+
Sucrose	+	Trehalose	+	D-Glucosamine	-	*myo*-Inositol	-
Maltose	v	Lactose	-	*N*-A-D-glucosamine	-	2-K-D-gluconate	-
Lactose	-	Melibiose	-	Glycerol	+	D-Glucuronate	nd
Trehalose	-	Raffinose	+	Erythritol	+	Nitrate	+
Assimilation		Melezitose	+	Ribitol	v	Urease	-
Glucose	+	Soluble Starch	+	Galactitol	-	0.1% Cycloheximide	-
Galactose	v	D-Xylose	v	D-Mannitol	+	Growth at 37°C	v

Key Features: Germ tube negative yeast and sugar assimilation pattern

Antifungal Susceptibility: ***W. anomalus*** (Diekema *et al.* 2009, Australian National data); **MIC µg/mL.**

	No.	≤0.008	0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	≥64
AmB	**42**				**1**	**5**	**14**	**21**	**2**						
FLU	**43**								**2**	**8**	**25**	**8**			
VORI	**42**		**1**	**2**	**1**	**22**	**13**	**3**							
POSA	**42**				**1**	**1**	**7**	**4**	**15**	**12**	**2**				
ITRA	**3**						**3**								
ANID	**16**	**2**	**10**	**3**	**1**										
MICA	**16**		**5**	**9**	**2**										
CAS	**39**	**1**	**16**	**17**	**4**	**1**									
5FC	**3**			**1**	**2**										

Yarrowia lipolytica (Wickerham *et al*.) van der Walt & von Arx.

Synonymy: *Candida lipolytica* (F.C. Harrison) Diddens & Lodder.

Yarrowia lipolytica is a rare cause of candidaemia.

RG-1 organism.

Culture: Colonies (SDA) white to cream-coloured smooth, glabrous, yeast-like.

Microscopy: Spherical, ellipsoidal to elongate budding blastoconidia, 3-5 x 3-15 µm.

India Ink Preparation: Negative - no capsules present.

Dalmau Plate Culture: Pseudohyphae and true hyphae are produced.

Molecular Identification: ITS sequencing recommended.

MALDI-TOF MS: Able to accurately identify this species.

Physiological Tests: + Positive, - Negative, v Variable, w Weak, s Slow, nd No Data							
Germ Tube	-	L-Sorbose	v	L-Arabinose	-	D-Glucitol	+
Fermentation		Sucrose	-	D-Arabinose	-	α-M-D-glucoside	-
Glucose	-	Maltose	-	D-Ribose	v	D-Gluconate	v
Galactose	-	Cellobiose	w,-	L-Rhamnose	-	DL-Lactate	+
Sucrose	-	Trehalose	-	D-Glucosamine	-	*myo*-Inositol	-
Maltose	-	Lactose	-	*N*-A-D-glucosamine	+	2-K-D-gluconate	-
Lactose	-	Melibiose	-	Glycerol	+	D-Glucuronate	nd
Trehalose	-	Raffinose	-	Erythritol	+	Nitrate	-
Assimilation		Melezitose	-	Ribitol	v	Urease	-
Glucose	+	Soluble Starch	-	Galactitol	-	0.1% Cycloheximide	-
Galactose	v	D-Xylose	-	D-Mannitol	+	Growth at 37^{O}C	v

Key Features: Germ tube negative yeast and sugar assimilation pattern.

Antifungal Susceptibility: ***Y. lipolytica*** limited data (Diekema *et al*. 2009, Australian National data); **MIC µg/mL.**

	No.	≤0.008	0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	≥64
AmB	19				1	1		1	6	5	4	1			
FLU	19							1	1	8	6	2			1
VORI	19		1	6	9	2			1						
POSA	19					2	2	9	5		1				
ITRA	3					1	1	1							
ANID	12					1	3	5	2	1					
MICA	12						7	3	2						
CAS	17					6	10		1						
5FC	3					1				1		1			

MICROSCOPY STAINS & TECHNIQUES

KOH with Calcofluor White.

For the direct microscopic examination of skin scrapings, hairs, nails and other clinical specimens for fungal elements. This is a very sensitive method, however, a fluorescence microscope with ultraviolet filters is required (Hageage and Harrington, 1984; Hollander *et al.* 1984; Monheit *et al*. 1984; Harrington and Hageage 2003).

Solution A: Potassium hydroxide reagent

Potassium hydroxide	10 g
Glycerine	10 mL
Distilled water	80 mL

Solution B: Calcofluor white reagent

Calcofluor white	0.5 g
Evans blue	0.02 g
Distilled water	50 mL
Mix one drop of each solution on the centre of a clean microscope slide. Place the specimen in the solution and cover with a coverslip.	

KOH with Chlorazol Black.

For the direct microscopic examination of skin scrapings, hairs, nails and other clinical specimens for fungal elements. **Note:** Parker Quink ink is no longer available.

Polysciences list this product as “Chlorazol black E” product number 02730-25.

Potassium hydroxide	10 g
Chlorazol Black E (0.1%)	10 mL
Glycerol	10 mL
Distilled water	80 mL
Using sterile technique, remove a small portion of the specimen with an inoculation needle and mount in a drop of KOH on a clean microscope slide. Cover with a coverslip, squash the preparation with the butt of the inoculation needle and then blot off the excess fluid.	

India Ink Mounts.

For the direct microscopic examination of CSF for *Cryptococcus* species. Place a drop of India Ink on the specimen, mix well with a sterile loop, and cover with a coverslip. Best brands to use are “Pelikan” or “Talons” India Ink.

MICROSCOPY STAINS & TECHNIQUES

Lactophenol Cotton Blue (LPCB)

For the staining and microscopic identification of fungi.

Cotton Blue (Aniline Blue)	0.05 g
Phenol Crystals	20 g
Glycerol	40 mL
Lactic acid	20 mL
Distilled water	20 mL

This stain is prepared over two days.

1. On the first day, dissolve the cotton blue in the distilled water. Leave overnight to eliminate insoluble dye.
2. On the second day, wearing gloves, add the phenol crystals to the lactic acid in a glass beaker. Place on magnetic stirrer until the phenol is dissolved.
3. Add the glycerol.
4. Filter the cotton blue and distilled water solution into the phenol/glycerol/lactic acid solution. Mix and store at room temperature.

Direct Microscopic Mounts or Squash Preparations

Using sterile technique, remove a small portion of the colony with an inoculation needle and mount in a drop of lactophenol cotton blue on a clean microscope slide. Cover with a coverslip, squash the preparation with the butt of the inoculation needle and then blot off the excess fluid.

Cellotape Flag Preparations

An excellent technique for the rapid mounting of sporulating fungi because it keeps more of the reproductive structures intact.

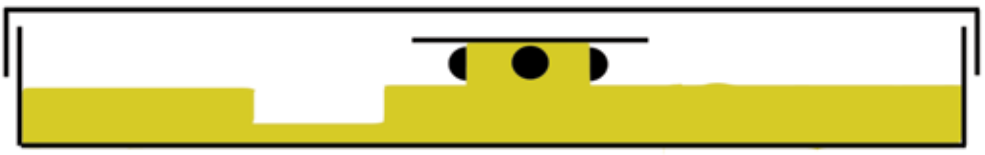
1. Using clear 2 cm wide cellotape and a wooden applicator stick (orange stick) make a small cellotape flag (2 x 2 cm).
2. Using sterile technique, gently press the sticky side of the flag onto the surface of the culture.
3. Remove and apply a drop of 95% alcohol to the flag, this acts as a wetting agent and also dissolves the adhesive glue holding the flag to the applicator stick.
4. Place the flag onto a small drop of lactophenol cotton blue on a clean glass slide, remove the applicator stick and discard, add another drop of stain, cover with a coverslip, gently press and mop up any excess stain.

MICROSCOPY STAINS & TECHNIQUES

Slide Culture Preparations

In order to accurately identify many fungi it is essential to observe the precise arrangement of the conidiophores and the way in which spores are produced (conidial ontogeny). Riddel's simple method of slide culturing (Mycologia 1950; 42:265-270) permits fungi to be studied virtually *in situ* with as little disturbance as possible. A simple modification of this method using a single agar plate is described below.

One plate of nutrient agar; potato dextrose is recommended, however, some fastidious fungi may require harsher media to induce sporulation like cornmeal agar or Czapek Dox agar.
1. Using a sterile blade cut out an agar block (7 x 7 mm) small enough to fit under a coverslip. 2. Flip the block up onto the surface of the agar plate. 3. Inoculate the four sides of the agar block with spores or mycelial fragments of the fungus to be grown. 4. Place a flamed coverslip centrally upon the agar block. 5. Incubate the plate at 26^{O}C until growth and sporulation have occurred. 6. Remove the cover slip from the agar block. 7. Apply a drop of 95% alcohol as a wetting agent. 8. Gently lower the coverslip onto a small drop of lactophenol cotton blue on a clean glass slide. 9. The slide can be left overnight to dry and later sealed with fingernail polish. 10. When sealing with nail polish use a coat of clear polish followed by one coat of red-coloured polish.



Simple agar block method, inoculated on four sides with cover slip on top. Make at least two slides per culture.

SPECIALISED CULTURE MEDIA

Bird Seed Agar

For selective isolation of *Cryptococcus neoformans* and *C. gattii*.

Guizotia abyssinica (niger seed)	50 g	Glucose	1 g
KH_2PO_4 (potassium dihydrogen orthophosphate)	1 g	Creatinine	1 g
Bacto agar (BD 214010)	15 g	Distilled water	1000 mL
Penicillin G (20 units/mL)	1 mL	Gentamicin (40 mg/mL)	1 mL

1. Grind seeds of *Guizotia abyssinica* as finely as possible with an electric grinder and add to 1000 mL distilled water in a stainless steel jug.
2. Boil for 30 minutes, then pass through filter paper and adjust volume to 1000 mL.
3. Add remaining ingredients (except Bacto agar) to filtrate and dissolve.
   If required: Cool to room temperature and adjust pH to 5.5.
   Dispense into 500 mL bottles.
4. Add 7.5 g Bacto agar to each 500 mL bottle.
5. Autoclave 121^{O}C for 20 minutes.
6. Cool to 48^{O}C and add 0.5 mL Penicillin G and 0.5 mL Gentamicin to each 500 mL bottle of bird seed agar.
7. Mix gently and pour into 90 mm plastic petri dishes.

Bromocresol Purple Milk Solids Glucose Agar (BCP)

For the differentiation of *Trichophyton* species (Fischer and Kane 1971; Summerbell *et al*. 1988).

Solution A:

Distilled water	1000 mL
Skim milk powder (Carnation Brand)	80 g
Bromcresol (or bromocresol) purple (1.6% solution in alcohol)	2 mL
Dissolve in 2 litre flask and autoclave 121^{O}C for 10 minutes.	

Solution B:

Glucose	40 g	Distilled water	200 mL
Dissolve and autoclave at 121^{O}C for 10 minutes.			

Solution C:

Bacto agar (BD 214010)	30 g	Distilled water	800 mL
Soak for 15 minutes in 3 litre flask; autoclave at 121^{O}C for 10 minutes.			

To make media add solution A and B to solution C. Adjust final pH to 6.6.
Aseptically dispense for slopes (7 mL amounts into bottles).

SPECIALISED CULTURE MEDIA

Creatinine Dextrose Bromothymol Blue Thymine (CDBT) Agar

For differentiation of *C. neoformans* var. *neoformans* and *C. neoformans* var. *grubii* (Irokanulo *et al.* 1994).

Solution A:

Creatinine	1 g	Dextrose	0.5 g
KH_2PO_4	1 g	$MgSO_4.7H_2O$	0.5 g
Thymine	0.1 g	Distilled water	980 mL
1. Dissolve ingredients in small beaker and adjust pH to 5.6 2. Store in refrigerator.			

Solution B (Aqueous Bromothymol Blue):

Bromothymol Blue	0.4 g	0.01N NaOH	64 mL
Distilled water	36 mL		
1. Dissolve the Bromothymol Blue in the NaOH 2. Add to the water.			

To prepare medium (1 litre for plates):

Solution A	980 mL	Solution B	20 mL
Bacto agar (BD 214010)	20 g		
Autoclave to 121^{O}C for 15 minutes, cool to 48^{O}C and dispense as plates.			

L-Canavanine Glycine Bromothymol Blue (CGB) Agar

For differentiation of *C. neoformans* and *C. gattii* (Kwon-Chung *et al.* 1982).

Solution A:

Glycine Univar	10 g	KH_2PO_4	1 g
$MgSO_4$	1 g	Thiamine HCl	1 mg
L-canavanine sulphate	30 mg	Distilled water	100 mL
1. Dissolve ingredients in small beaker and adjust pH to 5.6 2. Filter sterilise solution using 0.22 µm filter. 3. Store in refrigerator.			

Solution B (Aqueous Bromothymol Blue):

Bromothymol Blue	0.4 g	0.01N NaOH	64 mL
Distilled water	36 mL		
1. Dissolve the Bromothymol Blue in the NaOH 2. Add to the water.			

To prepare medium (1 litre for plates):

Distilled water	880 mL	Solution B	20 mL
Bacto agar (BD 214010)	20 g		
1. Autoclave to 121^{O}C for 15 minutes, cool to 48^{O}C. 2. For plates add 100 mL of the filtered solution A and mix. Dispense as plates.			

SPECIALISED CULTURE MEDIA

Cornmeal Agar

For routine cultivation and identification of fungi.

Cornmeal agar (Oxoid CM0103)	8.5 g
Distilled water	500 mL

1. Mix dry ingredients into 100 mL water, boil remaining water.
2. Add boiling water to mixture and bring to boil.
3. Autoclave for 10 minutes at 121^{O}C, then slope on racks.

Cornmeal Glucose Sucrose Yeast Extract Agar

For mucormycete sporulation.

Cornmeal agar (Oxoid CM0103)	17 g
Dextrose (Glucose)	2 g
Sucrose	3 g
Yeast extract	1 g
Distilled water	1000 mL

1. Soak dry ingredients in 100 mL water, then boil remaining water.
2. Add boiling water to mixture and bring to boil.
3. Dispense for slopes.
4. Autoclave for 10 minutes at 121^{O}C, remove and then angle to form slopes on racks.

Czapek Dox Agar

For routine cultivation of fungi, especially *Aspergillus, Penicillium*, and non-sporulating moulds.

Czapek Dox agar (Oxoid CM97)	45.4 g
Distilled water	1000 mL

1. Soak the ingredients in small amount of water.
2. Bring remaining water to boil, add to soaking ingredients and bring to the boil again, stirring continuously.
3. Dispense for slopes as required.
4. Autoclave at 121^{O}C for 10 minutes, remove and slope or pour for plates as required.

Modified Dixon's Agar

For primary isolation and cultivation of *Malassezia* species.

Malt extract (Oxoid L39)	9 g	Bacto Tryptone	1.5 g
Ox-bile Desiccated (Oxoid L50)	5 g	Tween 40	2.5 mL
Oleic acid	0.5 g	Glycerol	0.5 mL
Bacto agar	3 g	Distilled water	250 mL

1. Soak ingredients in a little of the water.
2. Bring remaining water to boil, add to the soaking ingredients and bring to the boil again constantly stirring.
3. Dispense for slopes (7 mL) into 30 mL bottles.
4. Autoclave at 121^{O}C for 10 minutes and then angle to form slopes on racks.

SPECIALISED CULTURE MEDIA

Hair Perforation Test

For the differentiation of *Trichophyton* species.

Blonde pre-pubital hair cut into short pieces (1 cm)	10-20 hairs
Distilled water	5 mL

1. Autoclave hair at 121^{O}C for 10 minutes and store in sterile container.
2. Place 10-20 short pieces of hair in 5 mL water in vial.
3. Inoculate with small fragments of the test fungus.
4. Incubate at room temperature.
5. Individual hairs are removed at intervals up to 4 weeks and examined microscopically in lactophenol cotton blue. Isolates of *T. mentagrophytes* produce marked localised areas of pitting and marked erosion whereas those of *T. rubrum* do not.

Lactritmel Agar

For the production of pigment by *Trichophyton* species.

Skimmed milk powder (use only Dutch Jug skimmed milk powder)	7 g
Honey	10 g
Cornmeal agar (Oxoid CM0103)	17 g
Chloramphenicol	0.05 g
Distilled water	1000 mL

1. Weigh skimmed milk into stainless steel jug. Slowly add some water, mixing milk into smooth paste. Continue adding small quantities of water until powder is dissolved (about 150 mL).
2. Weigh other ingredients into skimmed milk and allow to soak.
3. Boil remaining water, and with it wash out honey from beaker.
4. Add to other ingredients and boil.
5. Dispense for slopes (7 mL).
6. Autoclave for 10 minutes at 121^{O}C.
7. On removal from autoclave allow to stand 5 minutes then shake and then angle to form slopes on racks.

Note: Do not filter or adjust pH.

Littman Oxgall Agar

For the differentiation of *Trichophyton* species.

Littman oxgall agar (US Biological L3025)	27.5 g
Distilled water	500 mL

1. Soak agar in 100 mL of water. Boil remaining 400mL in a separate container.
2. When water has boiled add to soaking agar and bring back to the boil, stirring constantly.
3. Dispense for slopes.
4. Autoclave for 10 minutes at 121^{O}C, remove and then angle to form slopes on racks.

SPECIALISED CULTURE MEDIA

Malt Extract Agar

For routine cultivation and identification of fungi.

Oxoid Malt Extract (L39)	20 g
Bacto agar (BD 214010)	20 g
Distilled water	1000 mL

1. Dissolve malt extract in a beaker and adjust the solution to pH 6.5 with NaOH.
2. Soak agar in small quantity of solution. Bring malt extract solution to the boil, stirring constantly.
3. Add to soaking agar. Bring to boil, stirring constantly.
4. Dispense for slopes as required.
5. Autoclave at 121^{O}C for 10 minutes, remove and then angle to form slopes on racks or pour for plates as required.

1% Peptone Agar

For the differentiation of *Trichophyton* species.

Tryptone Peptone (BD 211705)	5 g
Bacto agar (BD 214010)	10 g
Distilled water	500 mL

1. Soak agar and peptone in about 50 mL of water.
2. Boil remaining water, add to the soaking ingredients and bring back to the boil again.
3. Dispense for slopes (7 mL).
4. Autoclave for 10 minutes at 121^{O}C, then angle to form slopes.

Potato Dextrose Agar.

For routine cultivation and identification of fungi.

Potato dextrose agar (Oxoid CM139)	39 g
Distilled water	1000 mL

1. Soak potato dextrose agar in 100 mL of the water.
2. Boil remaining water, add to soaking potato dextrose agar, bring back to the boil, stirring constantly.
3. Dispense for slopes as required.
4. Autoclave at 121^{O}C for 15 minutes. Remove and slope or pour for plates as required.

Rice Grain Slopes.

To induce sporulation and for differentiation of *M. audouinii* and *M. canis.*

Polished rice grains	Distilled water

1. Place ~ 0.5 teaspoon rice grains into wide neck 20 mL glass vials.
2. Add 8 mL distilled water to each vial.
3. Loosely cap the vials, then angle to form slopes on racks ensuring rice grains are evenly distributed.
4. Autoclave racks at 121^{O}C for 15 minutes.

SPECIALISED CULTURE MEDIA

Sabouraud's Dextrose Agar (SDA) with Cycloheximide (0.05%), Chloramphenicol and Gentamicin

For the primary isolation and cultivation of dermatophytes.

Sabouraud's dextrose agar (Oxoid CM41)	65 g
Cycloheximide (Actidione)	0.5 g
Chloramphenicol	0.05 g
Gentamicin (40mg/mL)	0.56 mL
Yeast extract	5 g
Distilled water	1000 mL

1. Soak all ingredients, except gentamicin, in 100 mL water.
2. Boil remaining water, add to soaking ingredients, and bring to boil to dissolve, stirring well to prevent from burning.
3. Add the gentamicin. Mix well.
4. Dispense for slopes as required.
5. Autoclave at 121^{O}C for 10 minutes. Remove and slope, or pour for plates as required.

Sabouraud's Dextrose Agar with Chloramphenicol and Gentamicin.

For primary isolation and routine culture of yeasts and moulds.

Sabouraud's dextrose agar (Oxoid CM41)	65 g
Chloramphenicol	0.05 g
Gentamicin (40mg/mL)	0.56 mL
Distilled water	1000 mL

See above method for Sabouraud dextrose agar with cycloheximide, chloramphenicol and gentamicin.

Sabouraud's Dextrose Agar with 5% Salt.

For the differentiation of *Trichophyton* species.

Sabouraud dextrose agar (Oxoid CM41)	32.5 g
Sodium Chloride NaCl (Univar 465)	25 g
Distilled water	500 mL

1. Soak dry ingredients in approximately 100 mL water.
2. Bring remaining water to boil, add to soaking ingredients.
3. Dispense into McCartney bottles for slopes (7 mL).
4. Autoclave at 121^{O}C for 10 minutes, then slope on racks.

SPECIALISED CULTURE MEDIA

Tap Water Agar

For the stimulation of sporulation in *Apophysomyces* and *Saksenaea* isolates.

Bacto agar (BD 214010)	15 g
Distilled water	1000 mL

1. Add agar to water in stainless steel jug, allow to soak.
2. Dispense for slopes.
3. Autoclave at 121^{O}C for 10 minutes, remove and slope.

Urea Agar Slopes with 0.5% Glucose.

For the differentiation of urease producing organisms.

Urea glucose broth base:

Urea, broth base (Oxoid CM71)	0.9 g
Glucose	5 g
Distilled water	450 mL

1. Add the Urea broth base and glucose to the distilled water in a 500mL beaker.
2. Dispense in 5 x 90 mL amounts.
3. Autoclave at 121^{O}C for 20 mins.
4. When cool, label and store in the fridge.

Method to make slopes:

40% Urea Solution (Oxoid SR 20)	10 mL
Bacto agar (BD 214010)	3 g
Distilled water	100 mL

1. Add agar to 100 mL of distilled water in a 250 mL pyrex bottle.
2. Autoclave at 121^{O}C for 15 minutes and place in 50^{O}C water bath.
3. When cool add 90 mL of the Urea broth with glucose and the 10 mL of 40% urea solution to agar and dispense in 3 mL aliquots and angle bottles to form slopes on racks.

Vitamin Free Agar (Trichophyton Agar No.1)

For the differentiation of *Trichophyton* species.

Trichophyton agar No. 1 (BD 287710)	11.8 g
Distilled water	200 mL

1. Add agar to water in stainless steel jug, allow to soak.
2. Bring to boil to dissolve, stirring constantly.
3. Once boiled remove immediately to avoid discolouration.
4. Dispense for slopes.
5. Autoclave at 121^{O}C for 10 minutes, remove and slope.

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