

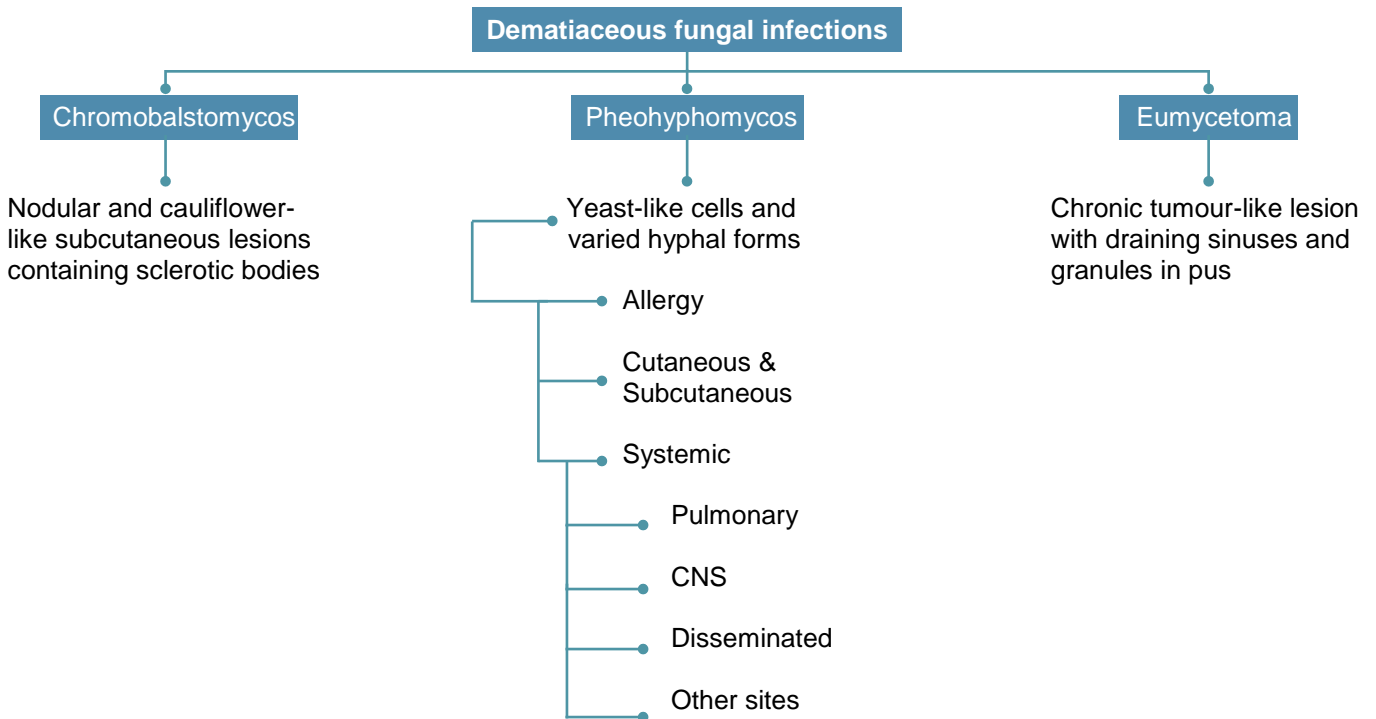
# Dematiaceous Molds and Melanised Fungi

Joveria Farooqi and Kauser Jabeen

Dematiaceous fungi are a group of fungi with melanin in their cell walls. Being environmental fungi, their sources are plant materials and soil. Most lesions arise due to traumatic implantation or inhalation and deep mycoses result either from haematogenous dissemination or contiguous invasion.

Lesions may be divided into three main categories on the basis of morphology and histopathological features:

- **Chromoblastomycoses** are pathognomically characterised by the presence of rounded copper-coloured structures with cross-walls called sclerotic bodies and cause nodular and cauliflower-like subcutaneous lesions.
- **Pheohyphomycoses** are characterised by moniliform and toruloid (yeast-like) hyphae which may be cutaneous, subcutaneous or deep lesions e.g. cerebral, pulmonary, bone or spinal abscesses.
- **Eumycetomas** usually involve exposed areas of the body and are distorted nodular lesions giving rise to draining sinuses with granules. Granules are frequently dark coloured, and microscopically show hyphal forms.



## Clinical Presentation

Chromoblastomycosis

Fig. 3.6-1

Chromoblastomycosis

Fig. 3.6-2



Nodular subcutaneous lesions with cauliflower-like outgrowths on exposed areas of the body (hands, feet and face).



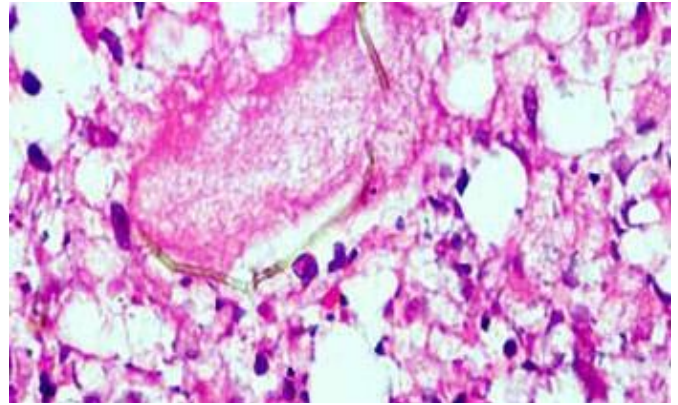
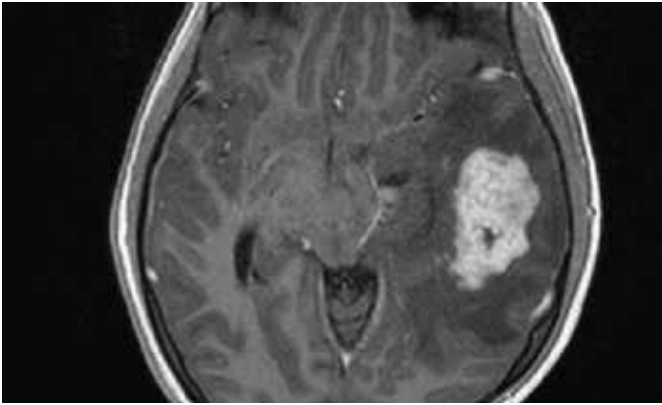
Nodular subcutaneous lesions with cauliflower-like outgrowths on exposed areas of the body (hands, feet and face).

Phaeohyphomycosis

Fig. 3.6-3

Phaeohyphomycosis, H&E stain

Fig. 3.6-4



Deep phaeohyphomycoses present as abscesses involving one or several organs. This is an image of a large brain abscess due to *Neoscytalidium* species.

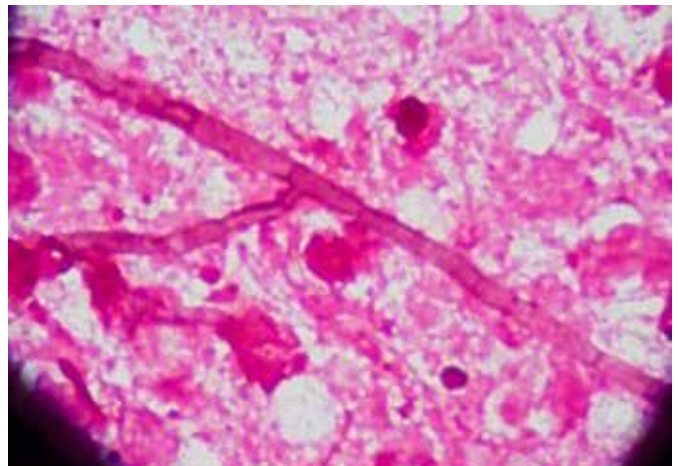
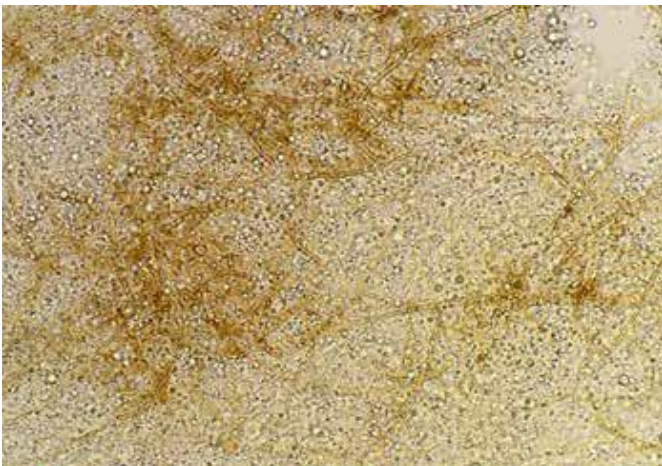
Biopsy of a brain abscess shows golden brown septate hyphae. (x40)

Phaeohyphomycosis, 10% KOH wet mount

Fig. 3.6-5

Phaeohyphomycosis, Gram stain

Fig. 3.6-6



Smear from patient with brain abscess shows numerous pigmented hyphae. (x10)

Stain sample from a patient with brain abscess shows pigmented septate hyphae. (x100)

Eumycetoma

Fig. 3.6-7

Eumycetoma, 10% KOH wet mount smear

Fig. 3.6-8



The lesions are characteristically situated in exposed body parts. They are deeply penetrating infections resulting in sinus formation and hardening of surrounding

Mount of a eumycetoma granule showing distorted septate hyphae which may themselves be pigmented or secreting a dark pigment into the surrounding

tissue. The pus draining from the sinuses shows granules tissue. (x40)  
which may be pigmented (melanised fungi), whitish  
(aerobic *Actinomyces* such as *Nocardia*) or  
yellow (*Actinomyces*).

Fig. 3.5.2-3 Copyright © American Society for Microbiology, [J Clin Microbiol 51, 2013,3167, doi: 10.1128/JCM.01604-12]



Madura (mycetoma) foot

Fig. 3.6-9

A close-up view of mycetoma foot

Fig. 3.6-10



Patient presented with a 1-year history of slowly enlarging painless nodules on foot after minor trauma. Later these papules and pustules formed draining sinuses discharging grains.

The infection can permanently scar and damage skin, subcutaneous tissues and bones. This patient was treated with debulking and reconstructive surgery by a plastic surgeon combined with itraconazole and rifampicin therapy for 2 years.

Fungal osteomyelitis and arthritis

Fig. 3.6-11

Fungal osteomyelitis and arthritis, 10% KOH wet mount smear

Fig. 3.6-12



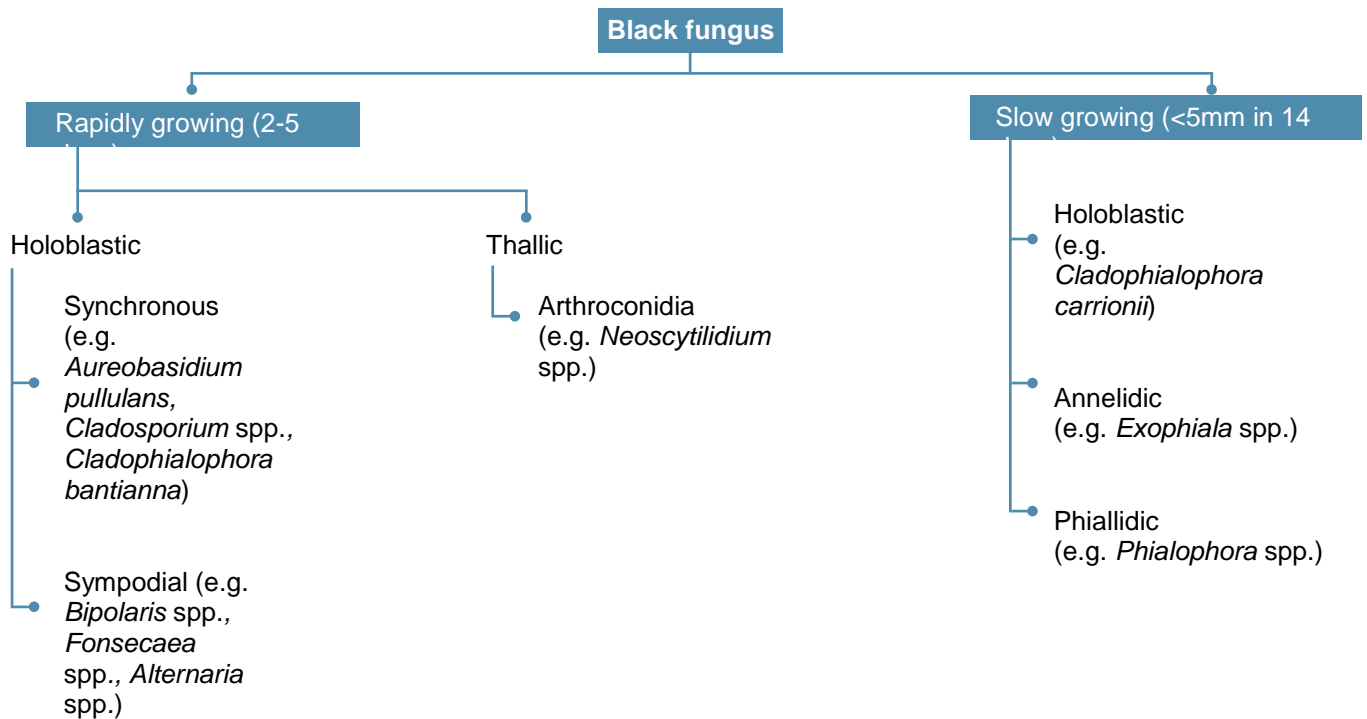
A 20-year-old patient presented with a swollen and discharging sinus over the medial side of right foot. Radiograph shows a soft tissue swelling overlying the big toe along with sclerosis and destruction of the proximal phalanx as well as the metatarsophalangeal joint (arrow). The appearances are consistent with fungal osteomyelitis and arthritis.

Septate hyphae in tissue from the patient with mycetoma. (x40)

### Identification

- Dematiaceous molds are differentiated based on the rates of growth (slow growing versus rapidly growing) and then further divided based on the origin of conidia:
  - Holoblastic: conidia arise with all layers of cell wall from conidiophores.
  - Enteroblastic: conidia arise from internal layer of conidiophore by bursting out of the outer cell wall.

- Thallic: conidia form by breaking off from the main hyphae after lysis of the separating cells.
- Common dematiaceous fungi can be identified using the simple schema shown.



### Alternaria species

*Alternaria* is one of the most common rapidly growing black molds found in the environment and soil. It is frequently isolated from fungal polyps, sputum samples, nails of patients with onychomycosis and other infections due to traumatic implantation e.g. keratitis, soft tissue infections and even brain abscesses.

Culture on SDA leads to rapidly growing colonies (>5 mm in 5 days) that are olivaceous green at the front and black on reverse side (not shown). Initially, colonies are flat and then become fluffy with a white downy border.

*Alternaria* spp., LPCB wet mount preparation

Fig. 3.6-13

Muriform elongated conidia of *Alternaria* spp. in chains (using objective lens x10)

Fig. 3.6-14



Microscopically, the tapering conidia arise in chains and have both transverse and vertical septae giving a muriform appearance. The conidiation is considered sympodial as conidia arising from the same conidiophore

are seen in various stages of development. (x40), (x10) (see arrow)



### *Bipolaris* and *Drechslera* species

Rapidly growing black molds found in the environment. Similar to *Alternaria* species in clinical relevance, they are commonly isolated from nasal sinuses, respiratory specimens and wounds contaminated with environmental debris. To differentiate between *Bipolaris* and *Drechslera*, perform the germ tube test and observe germination of the hyphae from the hilum:

- *Bipolaris* – germ tubes arise along the long axis of the conidia.
- *Drechslera* – germ tubes arise at right angle to the long axis of the conidia.

Both show fluffy colonies with olivaceous black surface on the front and a dark brown tinge on reverse side (not shown).

*Bipolaris* spp., LPCB wet mount preparation

Fig. 3.6-15



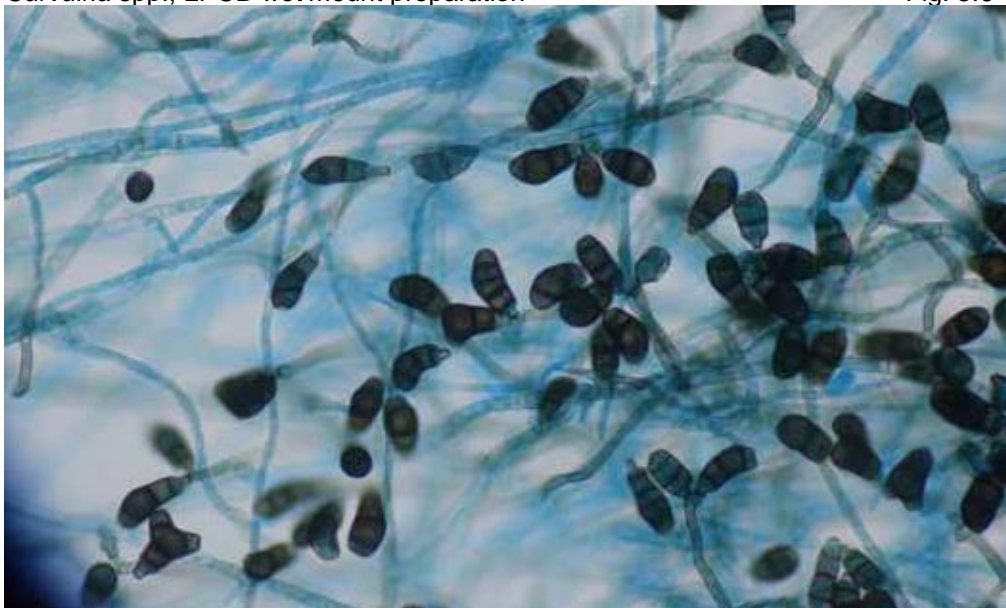
Microscopically, they both have elongated conidia with transverse septae with 3-6 cells. Conidia are holoblastic and sympodial. *Bipolaris* species have more abundant conidia arising at the tips of conidiophores along their long axis while *Drechslera* species have fewer conidia arising at right angles to the conidiophores. (x40)

### *Curvularia* species

*Curvularia* is a saprophytic rapidly-growing black mold with similar distribution to *Alternaria*, *Drechslera* and *Bipolaris* spp. Human infections with *Curvularia* spp. are rare, but can lead to fatal cerebral disease. Colonies are fluffy in texture and appear a deep black both on front and reverse, giving off a bluish hue as they age (not shown).

*Curvularia* spp., LPCB wet mount preparation

Fig. 3.6-16



The thallus consists of dark septate hyphae with conidia arising sympodially from the conidiophore. Conidia have transverse septae with 3-5 cells. Identifying characteristic is a large and darker central cell due to which the conidia appear curved or geniculate (like a bent knee). (x40)



### *Exserohilum* species

A rapidly growing black mold, *Exserohilum* gained fame during the 2012 iatrogenic fungal meningitis outbreak when a batch of epidural steroid injections was found to be contaminated with its spores. Clinical niche otherwise is similar to other saprophytic melanised fungi with sinusitis as the main manifestation. Colonies have fluffy grey surface on front and a dark reverse side (not shown).

*Exserohilum* spp., LPCB wet mount preparation

Fig. 3.6-17



Hyphae are septate and pigmented. Holoblastic conidiation gives rise to elongated conidia with 8-12 cells divided by transverse walls and ending in a prominent hilum. (x40)

### *Cladosporium* species

*Cladosporium* is an environmental contaminant that can cause serious disseminated haematogenous infections. Colonies grow slowly and mature within 3 weeks. Colour of colonies varies from dark grey, grey green to dark green on front, and appears black on reverse surface (not shown). A distinctive feature is the species inability to grow at temperatures above 37°C.

*Cladosporium* spp., LPCB wet mount preparation

Fig. 3.6-18



On microscopy hyphae are dark and septate, conidiophore have medium to long chains of conidia. Conidia are brown, smooth, oval with scar of attachment. Maximum growth temperature of the colonies is 35-37°C. Conidia are holoblastic and can be dislodged easily and resemble shields. (x40)

### *Cladophialophora* species

*Cladophialophora* is a mold that is an environmental contaminant and can cause serious disseminated hematogenous infections.

#### ***Cladophialophora bantiana***

*C. bantiana* has a tropism for central nervous system and infection results in brain abscess. Colonies growth rate is slow and mature in 2 weeks. Colour of colonies varies from olive green to brown to black; reverse is black (not shown). The distinct feature of this species is thermotolerance and ability to grow at 42°C. On microscopy dark septate hyphae are seen. Conidiophores are long with unbranched chains of pale brown, oval conidia (not shown). No scar of attachment is seen in conidia.

**CAUTION:** *C. bantiana* is a Hazard Group 3 pathogen and can be transmitted to health care workers through respiratory route. Colonies should be handled in a biosafety cabinet and slide cultures should not be performed.

***Cladophialophora carrionii***

*C. carrionii* mainly causes chromoblastomycosis. Colonies grow slowly and mature within 3 weeks. Colour of colonies varies from dark grey, grey green to dark green with a black reverse (not shown). Maximum growth temperature of the colonies is 35-37°C.

*C. carrionii*, LPCB wet mount preparation

Fig. 3.6-19



Dark septate hyphae are seen. Conidiophores have medium length chains branching after 10-15 conidia. Conidia are brown, smooth, and oval with scar of attachment. (x40)

***Sporothrix schenckii***

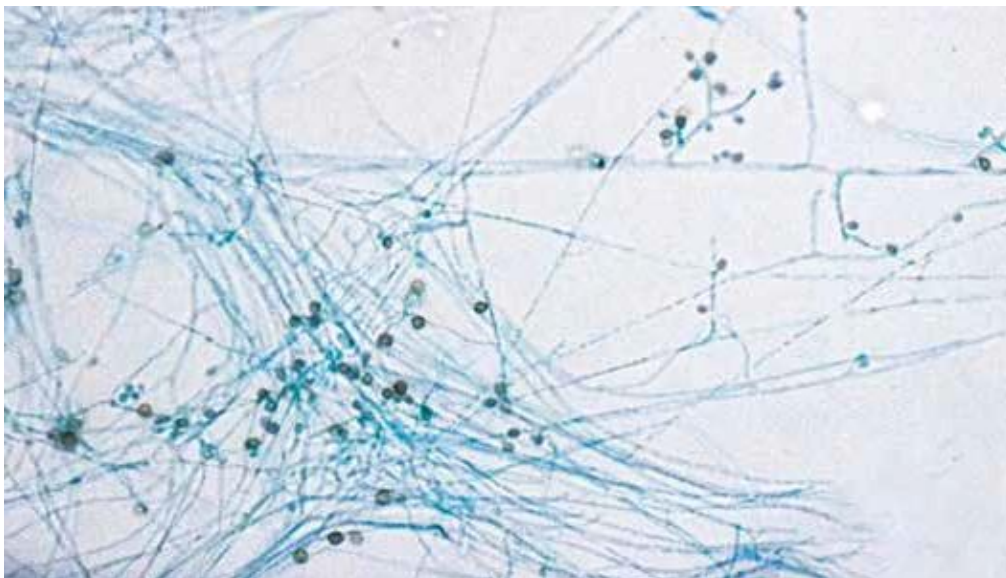
*S. schenckii* is a dimorphic fungus with a global distribution as an environmental contaminant. It is a known agent of sporotrichosis, a subcutaneous fungal infection that may involve lymphatic vessels, lymph nodes, bones and joints, as well as disseminated infection in immunosuppressed patients.

Colonies grow rapidly and mature in 5-7 days. Characteristics vary depending on incubation temperature:

- At 25-30°C initial colonies are white to grey that become black with age with a grey to black reverse. Colonies can be flat, leathery or wrinkled (not shown).
- At 35-37°C colonies are cream and yeast-like and may require repeated subcultures to obtain good yeast phase (not shown). Sometimes conversion to yeast form is incomplete and therefore an inhibited and slower growth similar to that at 25-30°C is seen.

*S. schenckii*, LPCB wet mount preparation

Fig. 3.6-20



On microscopy hyaline, septate hyphae with delicate conidiophore are seen. Conidia are oval and appear on the apex of conidiophore in a rosette-like or star-like pattern. Later developed conidia are dark and develop singly along the hyphae. Microscopy of yeast form exhibits round, oval or cigar-shaped yeast cells. (x10)



## *Exophiala* species

*Exophiala* is a slow-growing anamorphic fungus found in decaying wood and soil. Common cause of subcutaneous infections and cerebral mycosis.

*Exophiala* spp., front view of culture on SDA agar

Fig. 3.6-21



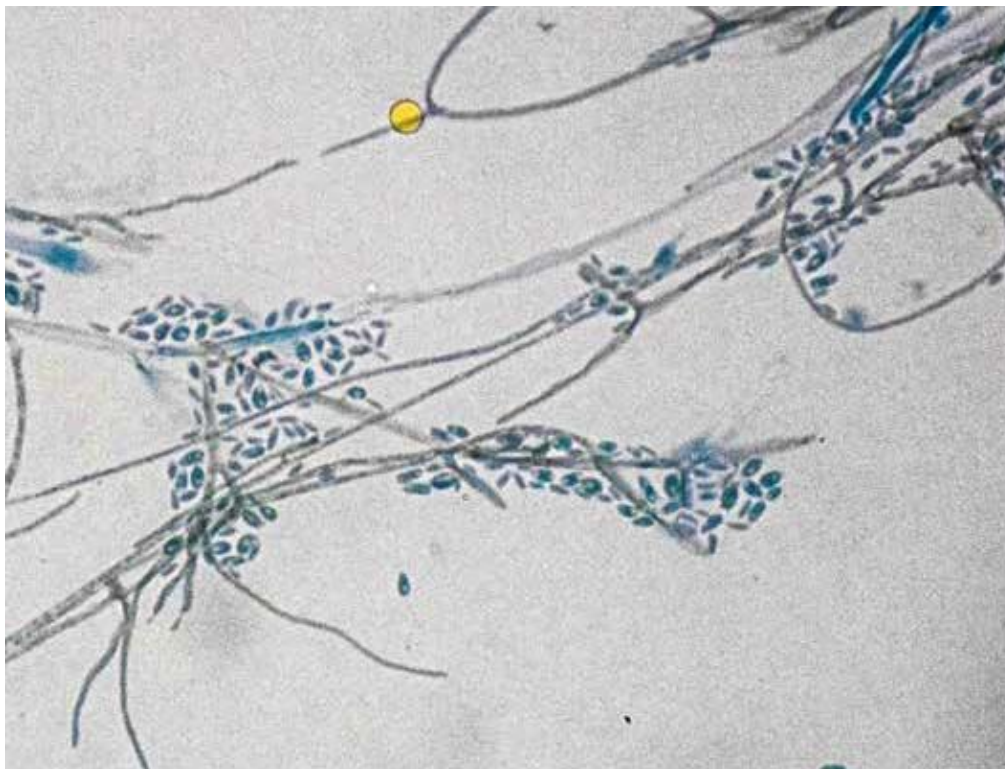
Young cultures show yeast-like appearance that convert to mold form with time.

## *Exophiala jeanselmei*

Colonies are olive green to greenish black to black with a black reverse (not shown). Maximum growth temperature is 37°C. Does not grow at higher temperature. Matures in 2 weeks.

*E. jeanselmei*, LPCB wet mount preparation

Fig. 3.6-22



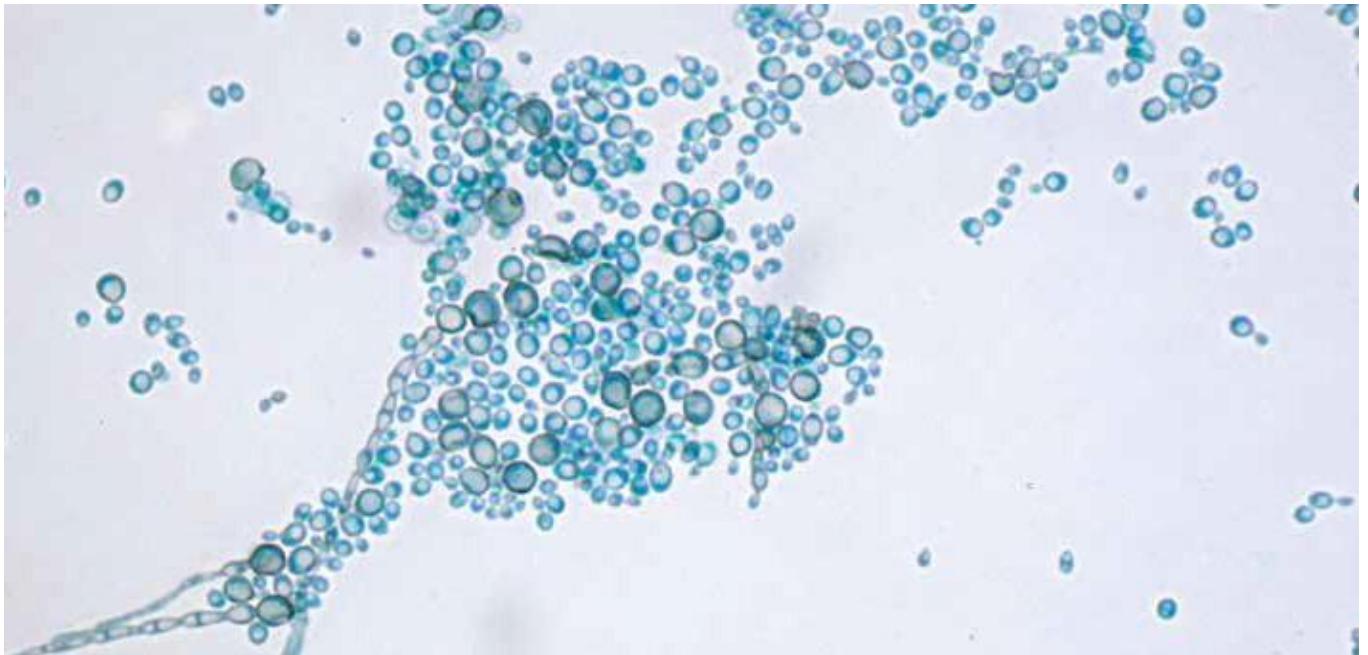
Septate hyphae with conidiophores, which are cylindrical with needle-like projections. Numerous brownish conidia that accumulate in groups at the tips of conidiophores or along the hyphae. (x10)

***Exophiala dermatitidis***

On culture, colonies are olive grey to greyish black with a dark reverse. May have a diffusible brown pigment. Maximum growth temperature is 42°C and maturity takes 3-4 weeks (not shown).

*E. dermatitidis*, LPCB wet mount preparation

Fig. 3.6-23



Abundant budding yeast like cells with few septate hyphae with conidiophores that are flask-shaped and lack a collarette. Few brownish conidia are seen that accumulate in groups at the apex or down the sides of conidiophores or along the hyphae. (x10)

***Fonsecaea pedrosoi***

*F. pedrosoi* is the most common cause of chromoblastomycosis worldwide. Single isolate may have two to three types of conidiation.

Conidiation types includes *Fonsecaea* type (conidiophore: erect, septate and sympodial with distal end swollen; conidia: single-celled; long chains: not formed); *Rhinocladiella* type (conidiophore: erect, septate and sympodial with distal end swollen; conidia: single-celled either on the distal end of conidiophore or along the side); *Cladosporium* type (conidiophore: erect; conidia: scarred and in short chains; shield cells seen); and *Phialophora* type (vase-shaped phialides with terminal collarettes having oval conidia).

*F. pedrosoi*, front view of culture on SDA

Fig. 3.6-24

*F. pedrosoi*, front view of culture on SDA

Fig. 3.6-25



Day 5. Colonies are dark green, grey or black, flat and velvety. Reverse view is black. Culture on SDA shows colonies that grow slowly and mature in around 14 days.

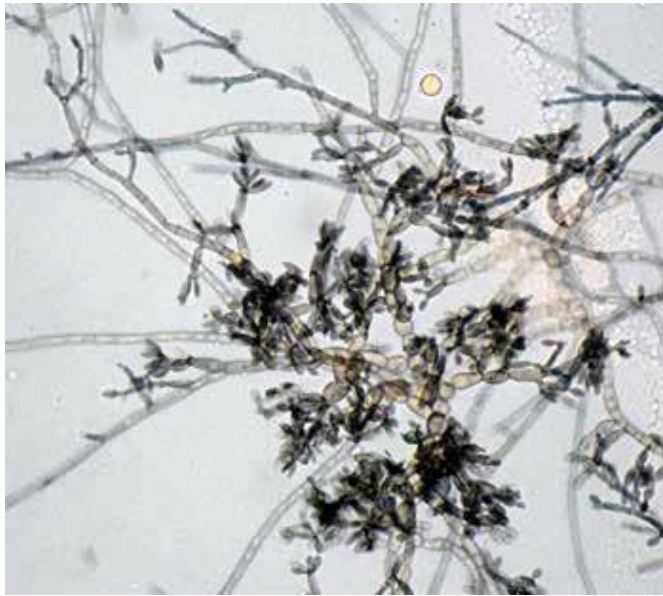


Day 15. Colonies are slow growing.



*F. pedrosoi*, LPCB wet mount preparation

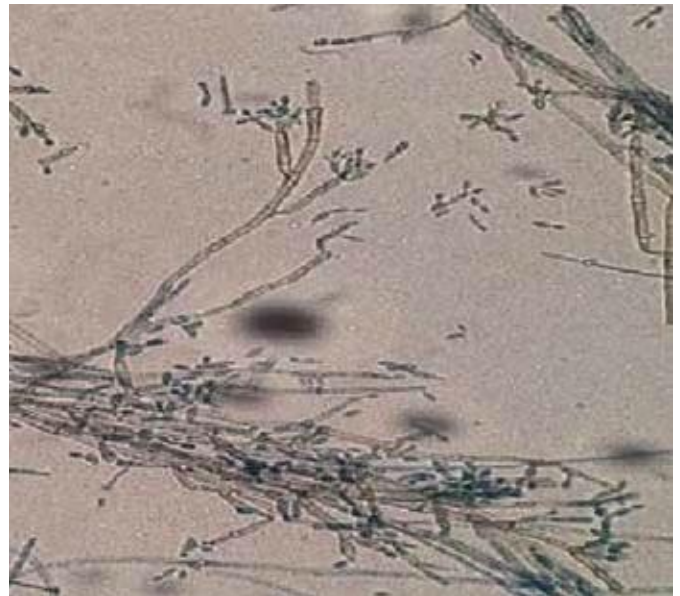
Fig. 3.6-26



Pigmented hyphae with dark conidia. Single isolate may have two to three types of conidiation. In this slide, fonsecaea, rhinocladiaella and *Cladosporium* conidiation are seen. (x40)

*F. pedrosoi*, LPCB wet mount preparation

Fig. 3.6-27



Close-up view shows fonsecae conidia. (x40)

### *Rhinocladia mackenziei*

*R. mackenziei* is a mold commonly found in arid regions of Middle East, Afghanistan, Pakistan and India. The majority of infections occur in immunocompetent individuals. In Pakistan, *R. mackenziei* has been as an agent of cerebral phaeohyphomycosis, which has an extremely low survival rate.

*R. mackenziei*, front view of culture on SDA

Fig. 3.6-28



Growth is dark grey-brown to black. Colonies are slow growing and mature in 3-4 weeks. Some isolates do not grow up to 4 weeks and grow initially as black discolouration of media. Reverse is black.

*R. mackenziei*, LPCB wet mount preparation

Fig. 3.6-29



On microscopy, hyphae are pigmented, septate with dark brown oval conidia. Conidiation is poor on first isolation and develops later after subsequent subcultures. The characteristic diagnostic feature is the *Rhinocladia* type sporulation that gives the "mickey mouse appearance": multiple budding with thick-walled cells. This is not seen with any other type of sporulation even after prolonged incubation. (x40)



## *Neoscytalidium dimidiatum*

Mold commonly found in tropical and subtropical region environments, causing infection secondary to trauma. Results in dermatomycosis, onychomycosis and, very rarely, systemic infections.

*N. dimidiatum*, front view of culture on SDA

Fig. 3.6-30

*N. dimidiatum*, LPCB wet mount preparation

Fig. 3.6-31



Colonies grow fast and mature in 3 days. Surface is initially white but rapidly changes to dark brown or black. Reverse is black. Woolly in texture and may fill the air space of the culture plate.



Septate hyphae without conidiophore are seen. Dark brown wider and hyaline narrow arthrospores are seen in abundance. These arthroconidia are either one-celled or two-celled separated by a thick septum. Shape of arthrospores varies from rectangle, square, oval, or barrel shaped. There are no empty cells between consecutive arthrospores. (x40)

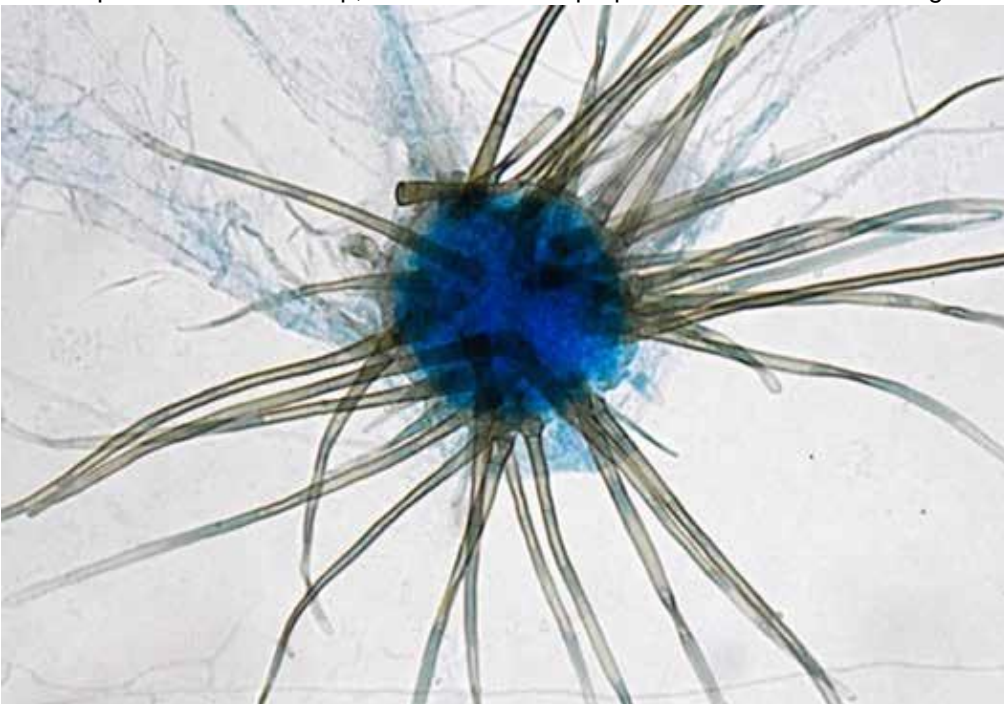
## *Chaetomium* species

*Chaetomium* is a mold found in environment particularly water-damaged buildings and can result in serious life-threatening systemic infections.

On culture, colonies grow fast and mature within 3-5 days. On front, initially colonies are white but with aging turn pale buff, olive green and then greyish green. Reverse is buff (may be brown to black) with sometimes a green, yellow or red diffusible pigment (not shown).

Close-up view of an ascocarp, LPCB wet mount preparation

Fig. 3.6-32



Ascocarp is a large brown oval structure with unbranched wavy and dark-coloured hyphae radiating from it. They contain asci that are club-shaped and contain 4-8 spores. Ascospores are lemon-shaped, brown and single-celled. (x40)

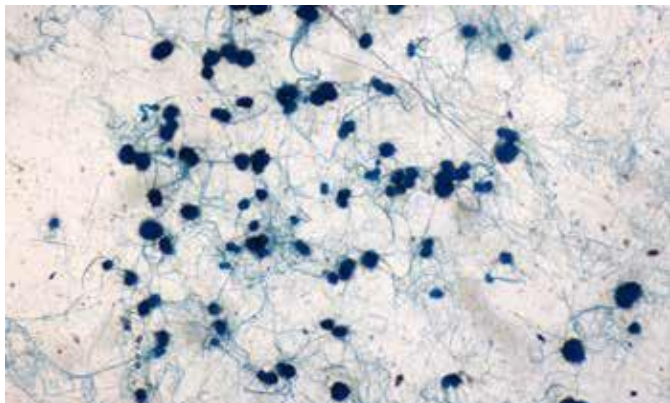


## Phoma species

*Phoma* species is typically an environmental contaminant causing infections in plants but can cause rare fatal disseminated fungal infections in humans. On culture on SDA, colonies are fast-growing and mature within 3-5 days. On gross examination, colonies are reddish to greyish brown on front and brown to black on reverse (not shown). Sometimes a reddish brown diffusible pigment is seen.

*Phoma* spp., LPCB wet mount preparation

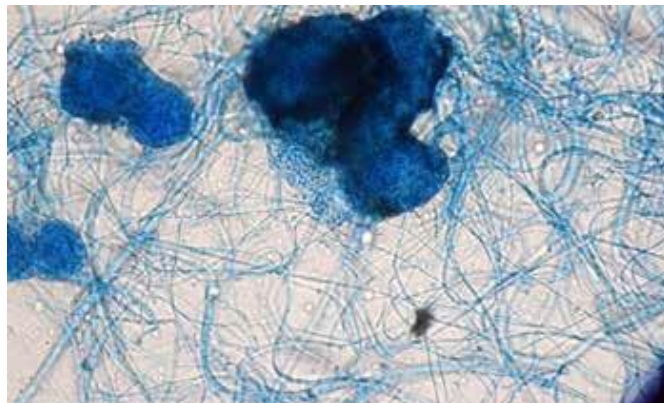
Fig. 3.6-33



On microscopy, septate hyphae are seen. A prominent feature is the presence of large pycnidia or fruiting bodies that are dark and oval to round with an opening called ostiole. (x40)

Close-up view of ostiole, LPCB wet mount preparation

Fig. 3.6-34



Through this opening, colourless ovoid single-celled conidia are released. (x100)

## Hormonema dematioides

*H. dematioides* is a yeast-like fungus found in environment that causes rare but clinically-significant infection in immunosuppressed patients.

On culture on SDA, colonies mature rapidly in 3-5 days. Initial colonies are buff and smooth with a pink tinge (not shown). On extended incubation, colonies are brownish black on front and black on reverse.

*H. dematioides*, LPCB wet mount preparation

Fig. 3.6-35



On microscopy young colonies have abundant yeast-like cells. Microscopy of older colonies exhibit two types of hyphae:

a. hyaline and thin-walled with conidia arising directly, and

b. dark and thick-walled that break into arthroconidia. The pigmented hyphae also bear conidia. (x40)



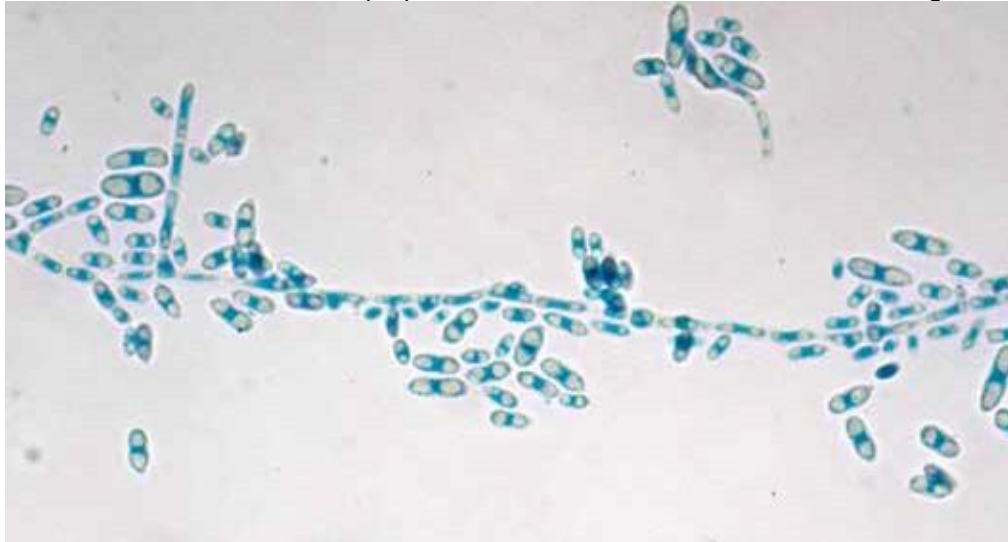
### *Phaeoannellomyces werneckii*

Also known as *Hortaea werneckii*, this fungus is found in soil, compost and wood, and is a causative agent of tinea nigra.

On culture, colonies are slow growing and can take up to 3 weeks to mature. Initial colonies are yeast-like. Growth on front is olive-black and black on reverse (not shown).

*P. werneckii*, LPCB wetmount preparation

Fig. 3.6-36



Microscopy of young colonies reveals pale or dark brown yeast-like cells with a central septum. These cells are pointed at one end and rounded at the other. Older colonies develop septate hyphae with thick brown walls. (x10)

### *Madurella* species

*Madurella* is a common soil fungus found in tropical regions and causes infections secondary to trauma. Infection results in mycetomas localised to cutaneous and subcutaneous tissues, fascia and bone. On culture, colonies are slow growing and may take up to 14 days to mature.

#### *Madurella mycetomatis*

Culture on SDA produces colonies that are cream, yellow to olive brown in front, and on reverse brown with a diffusible pigment (not shown).

On microscopic examination, septate hyphae are visible that are usually sterile. At times, culture on CMT agar produces phialides that bear small oval to round conidia (not shown).

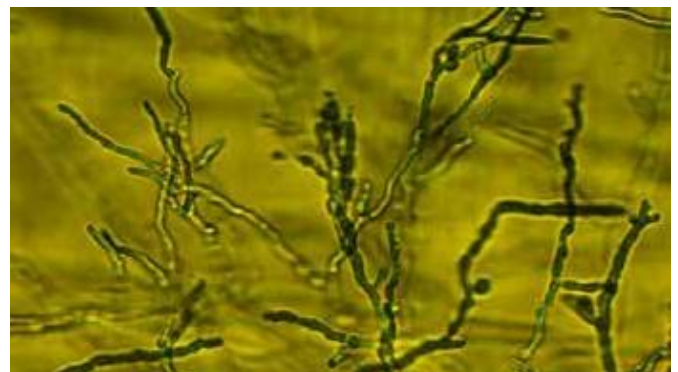
#### *Madurella grisea*

*M. grisea*, front view of culture on SDA

Fig. 3.6-37

*M. grisea*, microscopic examination

Fig. 3.6-38



Colonies are folded and dark brown to black. On reverse, colonies are dark. A diffusible pigment may be seen.

Colonies do not grow at 37°C.

Dark and wide septate hyphae. The hyphae are made of chains of round cells. Apart from these hyphae, thinner septate hyphae are also seen. These hyphae are usually sterile.

Chlamydoconidia are rarely produced. (x40)