

HMB436H – Human & Veterinary Mycology
 Lecture 9 – Opportunistic mycoses I:
 Aspergillosis, Sporotrichosis, Zygomycosis &
 Rhinosporidiosis

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Opportunistic systemic mycoses

- Various mould infections
- Example: aspergillosis caused by *Aspergillus fumigatus*
 - fungi usually widely distributed “table top mould” (inoculum very likely to be detected on any table top, floor, etc.)
 - patients usually strongly immunocompromised: few weeks’ vulnerability during chemotherapy, transplant
 - barrier breaks
 - ear canal infestations: childhood, objects in ear
 - chronic mycotic sinusitis: may follow long-term allergy

Predisposing Factors for Opportunistic Fungal Infections

Underlying Condition

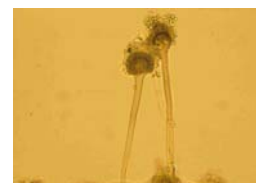
Leukemia
 Lymphoma
 Bone marrow transplant
 Organ transplant
 AIDS
 Primary immune disorders
 GI disease
 Diabetes
 Severe burns
 Premature birth
 IV drug abuse

Immune Defect

Granulocytopenia
 T-cell-mediated immunity

Iatrogenic Factors

Chemotherapy
 Immunosuppressive drugs
 Broad-spectrum antibiotics
 Breaks in skin or mucosa
 Indwelling catheters
 Peritoneal dialysis
 Prolonged hospitalization
 Antifungal prophylaxis (?)



Aspergillus spp.

ASPERGILLOSIS



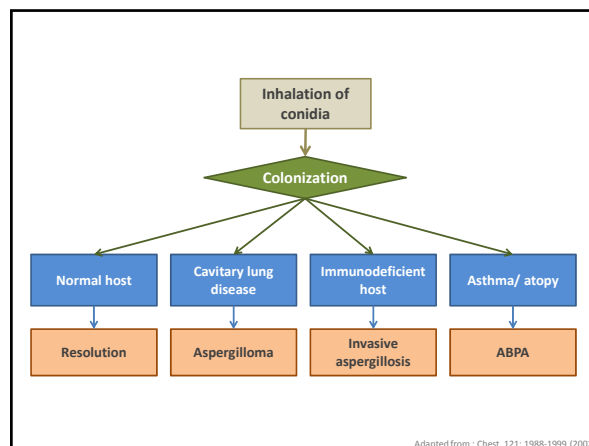
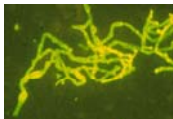
Aspergillus fumigatus infection of the air sacs of a jackdaw was reported by Mayer in 1815



Aspergillus infection of the air sacs of a great bustard was reported by Fresenius in 1863

Mycoses caused by *Aspergillus*

- wide diversity of clinical mycotic diseases
 - aspergilloma
 - aspergillosis
 - invasive
 - allergic
- etiologic agents
 - *Aspergillus* spp.
 - Trichocomaceae | Eurotiales | Ascomycota



History

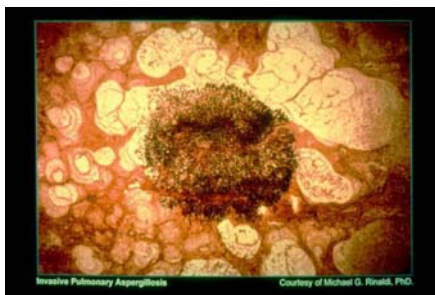
- Genus *Aspergillus* was named by Micheli in 1729
 - one of the oldest named genera of microfungi
 - named for its resemblance to the liturgical aspergillum



Primary disease symptoms

- Invasive aspergillosis
 - pulmonary infiltrates seen on xray in a patient with granulocytopenia (low granulocyte count)
 - xray shows 'halo sign' – low density area around an area of infiltration, or air crescent lesion, or cavitary lesion, or a consolidation combined with pneumothorax (collapsed lung)
- Non-invasive sinusitis
 - persistent allergic rhinitis or intractable sinusitis, not responsive to antibacterials; sometimes calcifications seen on CT scan
- Invasive sinusitis
 - fever, headache, epistaxis (nosebleed), cough → in a patient with neutropenia, leukemia, poorly controlled diabetes, etc.

Invasive pulmonary aspergillosis



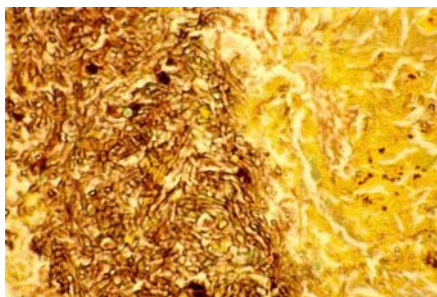
Aspergillus fumigatus

Courtesy of The Geraldine Kaminski Medical Mycology Library
Produced by: David Ellis and Roland Harmanis
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<http://www.doctorfungus.org>

• Genus/Species: <i>Aspergillus fumigatus</i>	• Slide Reference #: GK 038
• Image Type: Histopathology	• Disease(s): Aspergillosis

Sinusitis histopathology



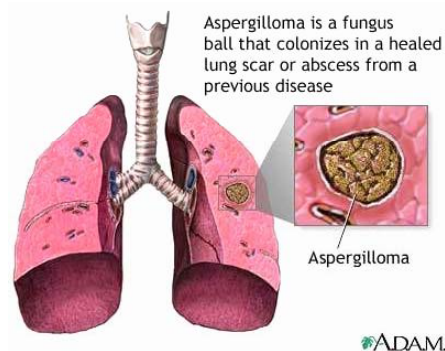
Primary disease symptoms (cont'd)

- Allergic bronchopulmonary aspergillosis (ABPA)
 - episodic bronchial obstruction
 - prolonged bronchiectasies
 - infiltrates on chest xray
 - immediate cutaneous reactivity to *Aspergillus fumigatus*
 - total serum IgE over 150 IU/ml
 - peripheral blood eosinophilia coinciding with chest infiltrates
 - elevated serum IgG against *A. fumigatus*.

Primary disease symptoms (cont'd)

- Aspergilloma
 - long-lasting pulmonary cavitation (a predisposition)
 - cavitary mass surrounded by air
 - massive hemoptysis (spitting up blood)
 - fever, malaise, weight loss
 - detectable IgG antibodies to *Aspergillus*

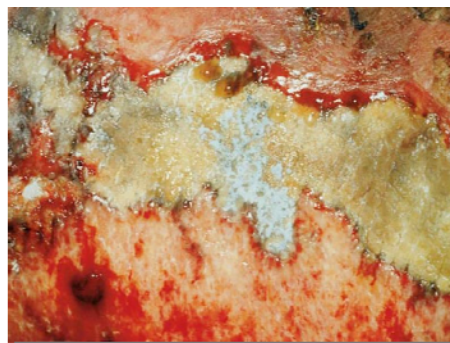
Aspergillus fungus ball



- aspergilloma characterised by uniform opacity with peripheral radiolucent crescent
- "Monod's sign"

AJ Chandrasekhar

Invasive cutaneous aspergillosis

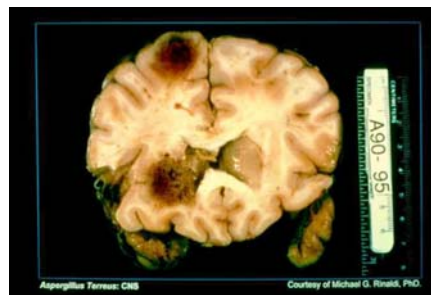


David Denning, Wythenshawe Hospital

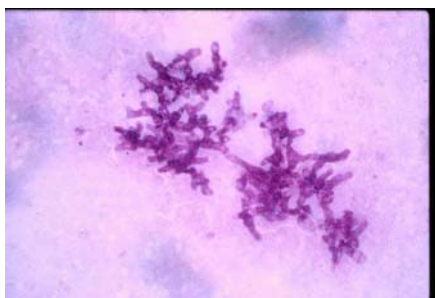
Disseminated disease

- infection foci seen in any internal organ in scans in a sufficiently immunocompromised patient
- includes endophthalmitis (inner regions of the eye) and endocarditis (heart valve infection)
- black, necrotizing skin lesions may be seen
- blood culture is usually negative, biopsy culture positive, biopsies with branching, septate (cross-walled) hyphae

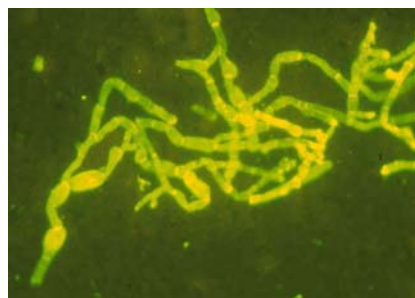
Aspergillus terreus brain infection



Aspergillus filaments in tissue (scapula)



Aspergillus fumigatus filaments in brain tissue (fluorescent-stained)



Differential diagnosis

- *Candida* disseminated infections show yeast cells in biopsied tissue; infection often yielding positive blood culture. Drug reactions different from aspergillosis (susceptible to fluconazole).
- *Fusarium* disseminated infections often yield positive blood culture. Drug reactions different from aspergillosis (resistant or semi-tolerant to older conventional antifungals such as amphotericin B).
- *Pseudallescheria* infections may appear very similar; important to distinguish in culture because drug reactions are different (tendency to be resistant to amphotericin B)

Risk factors for serious disease

- prolonged profound granulocytopenia (< 500,000/ml)
- high-dose corticosteroid therapy
- broad spectrum antibiotic therapy
- chronic granulomatous disease
- AIDS with a CD4+ lymphocyte count below 50,000/ml combined with treatment with cytotoxic drugs such as ganciclovir
- cytotoxic therapies for leukemia

Non-human infections

- progressive/ disseminated/ fatal disease
 - birds (especially chicken, turkey, penguin)
 - accounts for up to 10% of mortality losses in young chicks
 - high mortality in captive penguins, require frequent replacement
 - horse, sheep, pig
 - guttural pouch mycosis in horses
- sinusitis
 - e.g. German Sheppard dogs
- mycotic abortion
 - cattle and sheep



Treatment

- 'traditional' – amphotericin B desoxycholate → bind with sterols in the fungal cell membrane, principally ergosterol. This changes the transition temperature (T_g) of the cell membrane, thereby placing the membrane in a less fluid, more crystalline state
 - tends to be toxic to kidneys; causes 'rigours' (uncontrollable muscular tension and shaking)
- itraconazole – long history of use. Azoles inhibit the enzyme lanosterol 14 α -demethylase, needed to produce ergosterol in fungal membrane. Depletion disrupts the structure and many functions of membranes.
- Modern therapies and promising new drugs
 - voriconazole – relatively new, widely used therapy
 - posaconazole
 - echinocandins → inhibit the synthesis of glucan in the cell wall via the enzyme 1,3- β glucan synthase
 - micafungin
 - caspofungin
 - liposomal amphotericin B: amphotericin 'packaged' in lipid membrane beads, targeting capillary beds more accurately than amphotericin in solution
- recovery from neutropenia remains the most important factor influencing outcome.

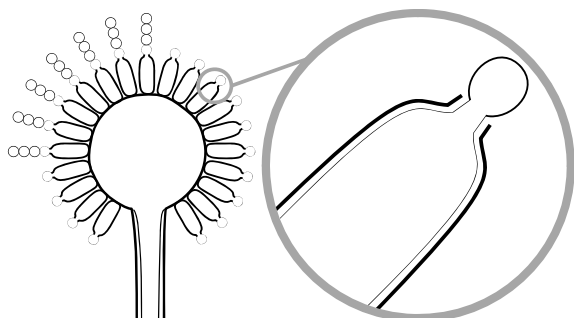
Immunology

- alveolar macrophages are first line of defense
- if conidia in the lungs escape this defense and grow hyphae, granulocytes will adhere to and kill the fungus
- corticosteroids inhibit phagocytosis of conidia
- IgE antibody response gives a type I hypersensitivity reaction (allergy-like)
- IgG antibodies form but appear not to be significant in host defense

Mycology

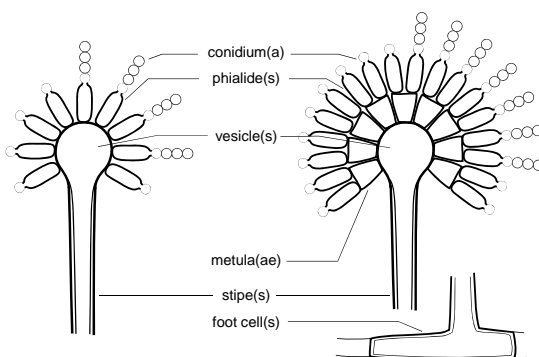
- most commonly occurring agents
 1. *Aspergillus fumigatus*
 2. *Aspergillus flavus*
 3. *Aspergillus niger*
- other species frequently involved
 - *A. amstelodami*, *A. tritici*, *A. carneus*, *A. nidulans*, *A. restrictus*, *A. sydowii*, *A. terreus*, *A. calidoustus*, *A. versicolor*

Aspergillus

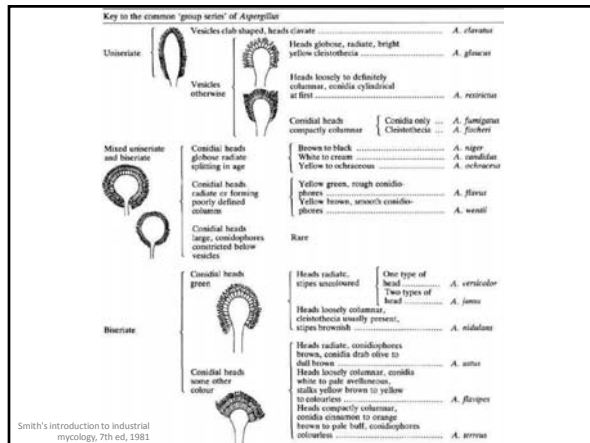
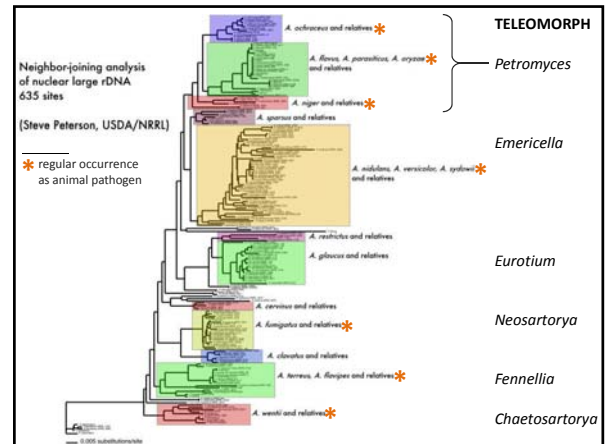
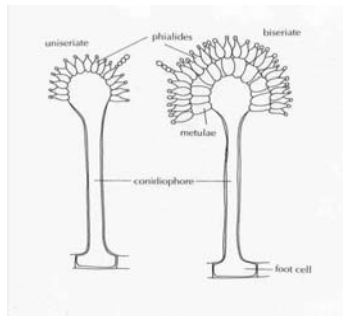


Uniseriate

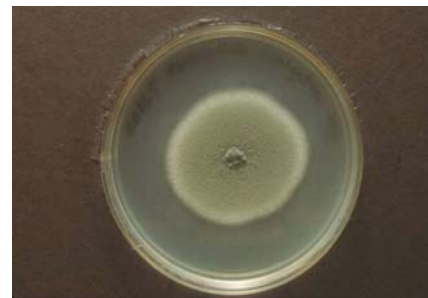
Biseriate



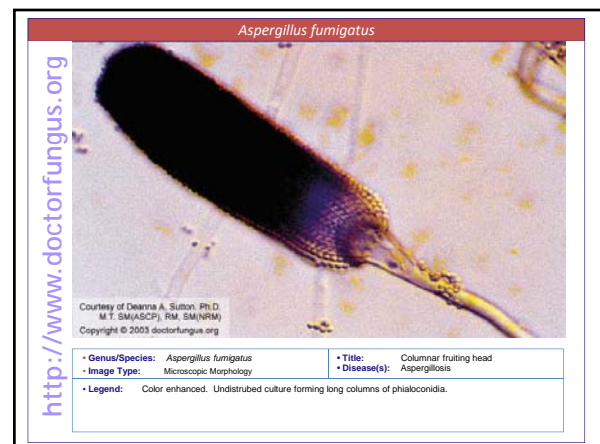
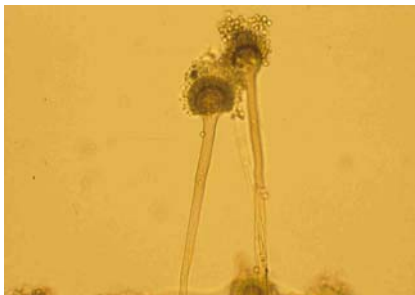
Aspergilli



Aspergillus fumigatus



Aspergillus fumigatus conidium production on long fertile stalks



Dysgonic *A. fumigatus*

- this is the most medically important “atypical” *Aspergillus* in the lab
- conidiation can be absent or a wide variety of small, deformed heads can be found scattered here and there on the colony surface
- growth rate can be typical or atypical, but **most importantly, thermotolerance** (best seen as growth at 42°) **remains**.
- conidiation sometimes stimulated by 37° growth on sporulation media
- the recently described *Aspergillus lentulus* is similar but doesn't grow at 45 C, whereas *A. fumigatus* does.

Dysgonic *Aspergillus fumigatus*



Dysgonic *A. fumigatus*

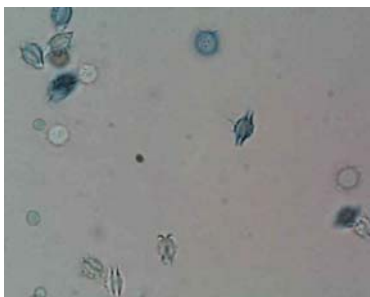


Neosartorya

the clade that includes *Aspergillus fumigatus*

- *Neosartorya* species occasionally cause opportunistic infection, e.g., endocarditis, osteomyelitis, keratitis, ABPA
- Though *Neosartorya* isolates produce conidiophores and bluegreenish conidia similar to those of *A. fumigatus* – though heads are usually a bit smaller – they mostly do this at 37°.
- At room temperature colonies are whitish and are mostly covered with ascospores. Morphological species identification is purely by ascospore morphology – SEM helps a lot but is not absolutely needed.
- Actual cases almost always caused by *N. pseudofischeri* or *N. udagawae* (which may be asexual)

Neosartorya pseudofischeri



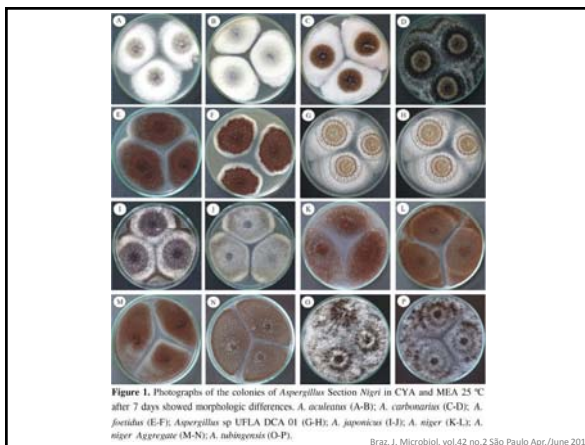
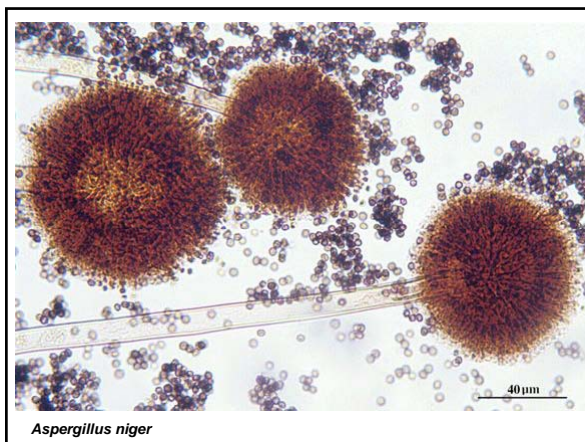
Petromyces

the *Aspergillus flavus* clade



Aspergillus niger

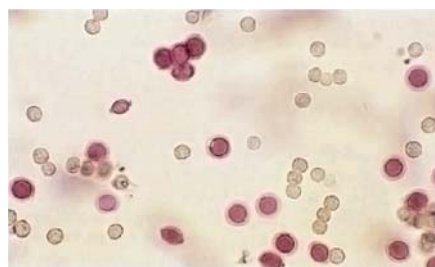
- associated mostly with colonizing pulmonary infections (remaining on mucous membrane surfaces, not invading underlying tissue), sinus infections and outer ear canal colonizations (otomycosis)
- often distinguished in pulmonary situations by oxalosis (large numbers of oxalate crystals in areas of fungal colonization)

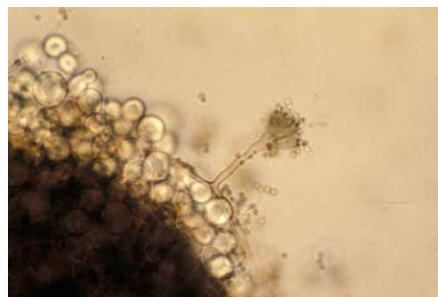


Emmericella

- *E. nidulans*, anamorph *Aspergillus nidulans*, is a well known opportunist and easily recognized by its purple, winged ascospores with smooth valves (also green colonies, biserial heads, brown stalks, and hülle cells)
- Various other *Emmericella* species cause some opportunistic infections or are likely to, and they are usually distinguished by ascospore colour and shape

Emmericella nidulans / *Aspergillus nidulans*

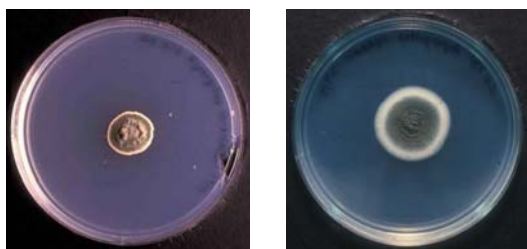


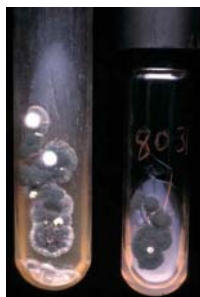
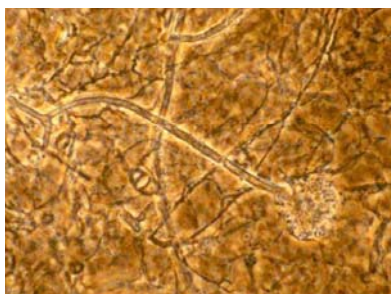
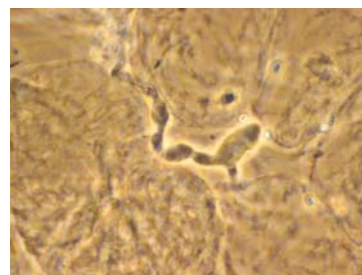
Aspergillus nidulans***Emericella nidulans******Aspergillus versicolor* and *A. sydowii***

- very common contaminants, distinguished morphologically only by colour.
- Nonetheless, molecular study shows the two species are really separate
- They do act differently. *A. sydowii* appears to be much more likely to cause onychomycosis.
- Well evidenced records of infection other than for onychomycosis are very rare

Aspergillus versicolor* and *A. sydowii

- *Aspergillus versicolor*: possible intracranial lesion, ocular keratitis, subcutaneous granuloma, but identification not adequately attested or seemingly undermined by discrepant photos or descriptive text
- *A. sydowii*: one definite case of ocular keratitis

Aspergillus versicolor* and *A. sydowii***Aspergillus versicolor***

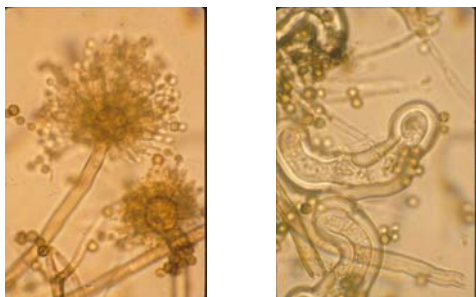
A. sydowii from nail*Aspergillus versicolor* and *A. sydowii**Aspergillus sydowii* conidiophore growing out from infected nail tissue*Aspergillus sydowii* filaments in nail tissue (vesiculate swellings)*Aspergillus calidoustus*

- a well demonstrated opportunist
- pulmonary, disseminated, and primary cutaneous invasion in severely immunocompromised patients
- grey-brown colonies, biserial heads with brown stalks, sometimes “squashed banana” Hülle cells
- was called *A. ustus* until recently, but the true *A. ustus* doesn't grow at 37 C and isn't known to cause human infection.

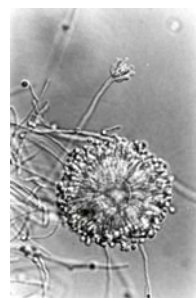
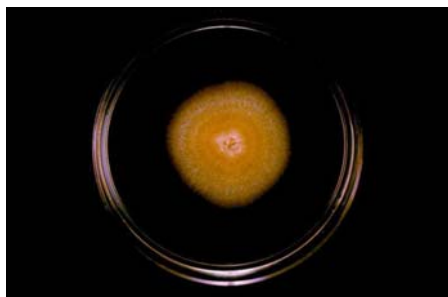
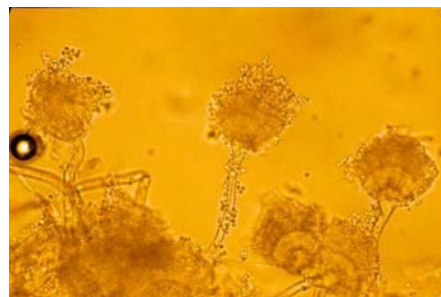
Aspergillus calidoustus

note: the pink margin is a film artifact

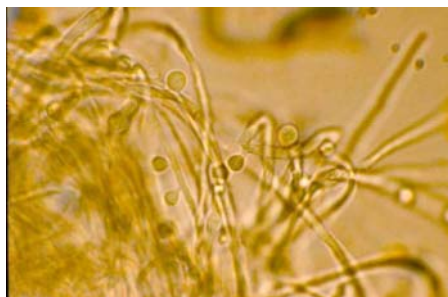


Aspergillus calidoustusWhitish Aspergilli related to *Fennellia*

- all are biserial
- *A. tritici*, formerly considered conspecific with *A. candidus*, is most common. It has pale stalks, and has metulae all around the vesicle. There are often some aberrant small heads mixed with the large ones.
- *F. flavipes* has yellow-brown stalks and this can give the colony an orangey cast even though conidia are white
- *F. nivea* has pale stalks but its vesicles have metulae only on the upper portion. Thus conidia are produced in columns

Aspergillus candidus complex*Aspergillus candidus* complex*Aspergillus terreus**Aspergillus terreus* conidiophores

Aspergillus terreus aleurioconidia



Ecology

- Aspergilli are associated with:
 - tropical soils: RVOS (relatively virulent opportunistic species, *A. fumigatus*, *A. flavus*, *A. terreus*)
 - composts, potted plants, dirty humidifiers: RVOS
 - water-damaged indoor drywall (*A. versicolor* complex)
 - skin scale depositions in carpets (*A. glaucus* complex)
 - peanuts and corn (*A. flavus*)
 - materials containing hydrocarbons (e.g. matchsticks soaked in paraffin, creosoted wood) - *A. flavus*

Rhizopus, Mucor, Absidia, etc.

ZYGOMYCOSIS

Systemic zygomycosis (mucormycosis)

- rare infection
- two clinical courses
 - fulminant (e.g., rhinocerebral zygomycosis in diabetics)
 - chronic with multiple remissions and exacerbations
- affect rhino-facial-cranial area, lungs, gastrointestinal tract, skin or other organ systems
 - affected tissue depends on portal of entry
- predilection for **vascular invasion**
 - embolization and necrosis of surrounding tissue
 - seen with Mucorales but usually not Entomophthorales

- focal infections may be nodular or have extensive necrosis and hemorrhage to adjacent tissue
 - abscess with central necrosis and peripheral tissue invasion is common
 - acute inflammatory exudate (**suppuration**) often accompanies infections in non-neutropenic patients
 - granuloma formation is uncommon

History

- first recorded case 1885 in a German cancer patient
 - probably due to *Absidia corymbifera* (recently transferred to *Lichtheimia corymbifera*)
- most early cases were simply called "mucormycosis", and the agent was not identified
 - historically most members of the Zygomycota were placed in the genus *Mucor*

Etiologic agents

- Ubiquitous members of the Mucormycotina
 - species of *Rhizopus* (most common), *Mucor*, *Rhizomucor*, *Absidia* (= *Lichtheimia*), *Cunninghamella*, *Saksenaea* and *Mortierella*
 - all cause similar clinical disease
 - several are known to undergo conversion to yeast-like phase at 37 C (e.g., *Mucor* spp., *Cokeromyces recurvatus*)
- A few species in Entomophthoromycotina
 - Conidiobolus* spp.

Rhinocerebral zygomycosis

- initial symptoms of sinusitis
 - pain, drainage, and soft tissue swelling
- may rapidly invade adjacent tissues
 - suppuration, thrombosis and necrosis
- periorbital region and orbit
 - swelling, ptosis & tearing with pain and blurring / loss of vision
- hard palate
 - necrotic ulcerations
- brain
 - altered consciousness
 - often terminating in death < 1 wk



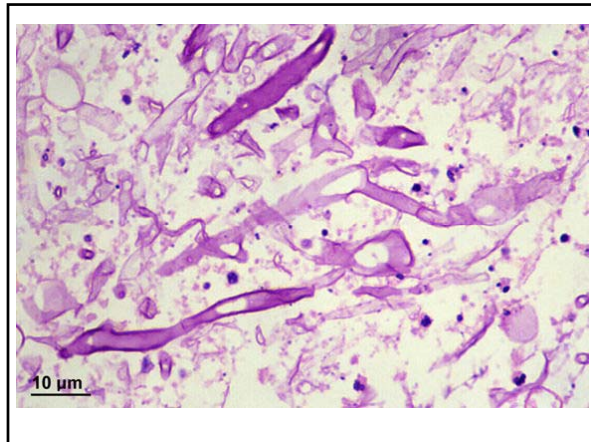
Rhinocerebral zygomycosis caused by *Rhizopus oryzae*



Rhinocerebral
zygomycosis caused
by *Rhizopus oryzae*



Rhinocerebral zygomycosis (palate) caused by *Apophysomyces elegans*

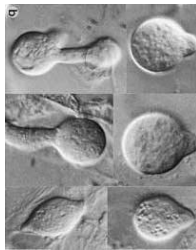


Other mucoralean zygomycoses

- inhalation of contaminated dust may produce **allergic interstitial pneumonitis** or **alveolitis syndrome** seen in malt and lumberworkers
- outbreaks of rhinocerebral or pulmonary zygomycosis linked to excavation, construction, or contaminated air conditioning filters
- contaminated tongue depressors in a hematology/ oncology clinic
- contaminated herbal / homeopathic remedies have been linked to gastrointestinal disease (*Mucor*)

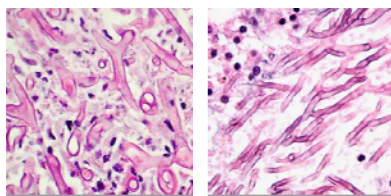
Conidiobolus (Entomophthoromycotina)

- first cases of infection caused by *Conidiobolus* spp. described in 1960s
 - nasal granulomatous disease in a horse
 - "Entomophthoromycosis"
- **human nasal granuloma & sinusitis**
 - first case reported in Jamaica in 1965
 - several hundred cases known to date
- **subcutaneous nodular disease**
 - outdoor workers in tropical West Africa
 - also in patients with Burkitt's lymphoma
 - fungus is normally found in soil and rotten plant material



Zycomycosis

- hyphal elements stain more poorly than most fungi



Characteristic	Zygomycota	<i>Aspergillus</i> spp.
Hyphal type	rarely septate	regularly septate
Hyphal width	variable and wide, 6-16 μm wide	consistently thin, 2-3 μm wide
Sporulation/ conidiation	rare in tissue	often present, especially in airways

Adapted from : Clin Microbiol Rev 13(2): 236-301, 2000
photos: W McDonald 2001 (L); zh.wikipedia.org/wiki/File:Pulmonary_aspergillosis.jpg Nephron 2010 (R)

Characteristic	Mucorales	Entomophthorales
Geographic distribution of organisms	Most but not all species distributed worldwide	Worldwide distribution, but endemic in tropical climates
Geographic distribution of cases	Most species cause infections worldwide	Predominantly seen in tropical and subtropical regions
Mode of transmission	Majority of infections result from inhalation of spores or traumatic implantation	Majority of infections result from inhalation of spores, traumatic implantation, bug bites, or other percutaneous mechanisms
Host immune status	Predominantly immunocompromised, but some competent hosts also seen	Predominantly immunocompetent, only a few compromised hosts
Most common disease manifestations	Pulmonary disease most common; rhinocerebral, cutaneous/subcutaneous, gastric, and other forms also seen	Sinusitis disease predominates for <i>Conidiobolus coronatus</i> , while subcutaneous mycosis predominates for <i>Blastobolus ramosus</i>
Invasive qualities	Primarily angioinvasive	Most infections are localized, demonstrating no angioinvasion
Organism colony morphology	Floccose aerial mycelium; often seen as "lid lifters"	Waxy, folded, and compact mycelium
Organism mycelium morphology	Coccytic hyphae, predominantly aseptate; Splendore-Hoeppli phenomenon rarely seen	Coccytic hyphae, becoming moderately septate with age; Splendore-Hoeppli phenomenon characteristically seen in tissue sections

Adapted from : Clin Microbiol Rev 13(2): 236-301, 2000

Risk factors - rhinocerebral zygomycosis

- immune abrogation
 - especially **neutropenia** / neutrophil dysfunction
- **diabetic ketoacidosis**
 - neutrophil & macrophage dysfunction predisposes most cases of **rhinocerebral zygomycosis**
 - insulin deficiency causes a shift to fatty acid metabolism
 - generates free organic acids, decreases serum pH (acidosis)
 - acidotic environment impairs neutrophil function

Risk factors - general zygomycosis

- bone marrow suppression during **chemotherapy** and post-transplantation **immunosuppressive therapy** are responsible for increased prevalence
- barrier break may lead to disease
 - e.g., particularly in **burn patients**, **IV drug users**
 - also in healthy people

Treatment

- **Amphotericin B** is the first-line drug of choice
 - modifies fungal cell wall, binds ergosterol, increases wall permeability causing ion leak & membrane depolarization
 - secondarily stimulates oxidative immune responses
 - hyperbaric oxygen therapy may accentuate effectiveness
- not curative in all cases, e.g., late in the disease course with inoperable or disseminated disease

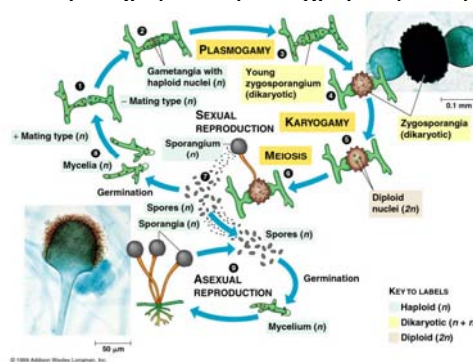
Mycology

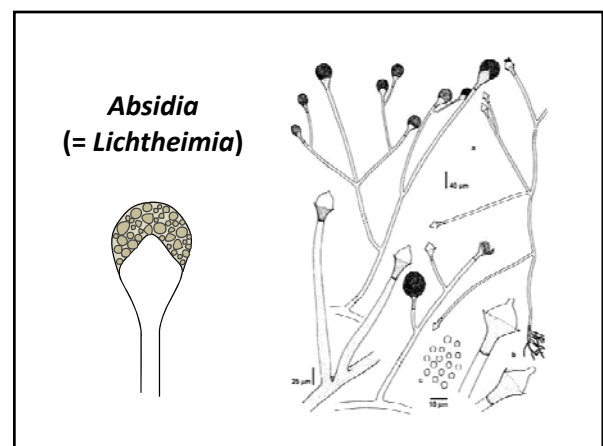
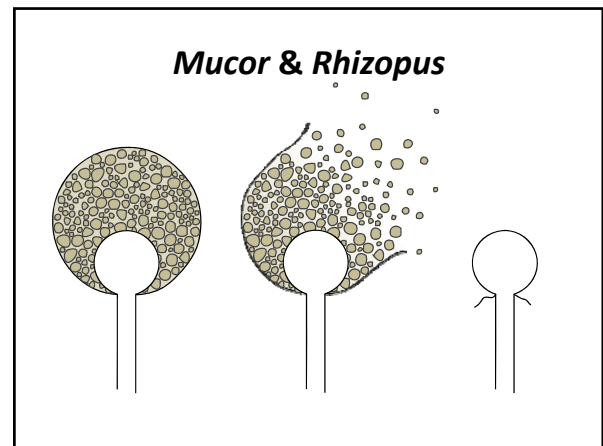
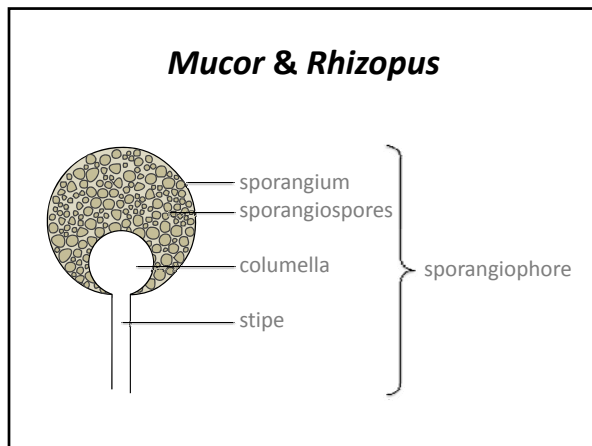
- Cosmopolitan members of the Mucorales
 - species of ***Rhizopus***, ***Mucor***, ***Rhizomucor***, ***Absidia***, ***Cunninghamella***, ***Saksenaea***, ***Syncephalastrum*** and ***Mortierella***

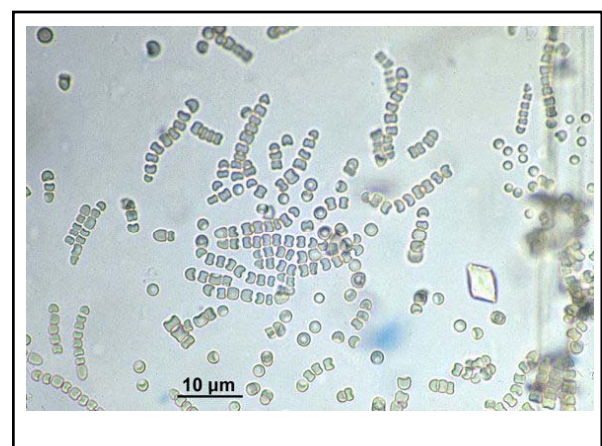
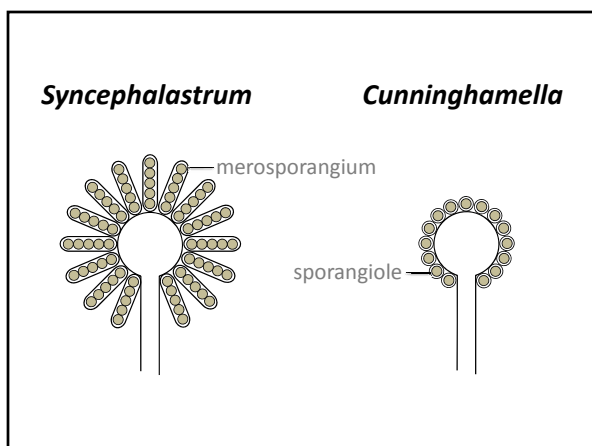
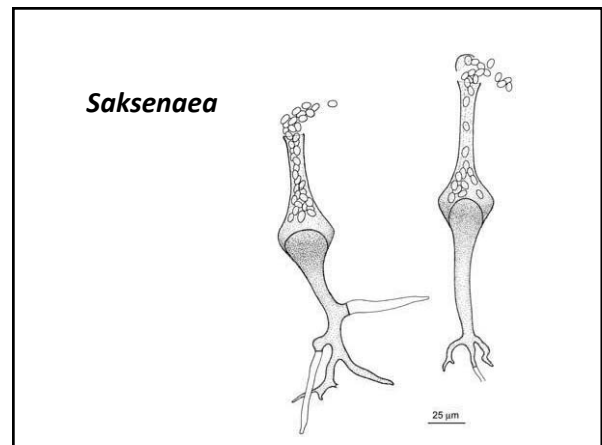
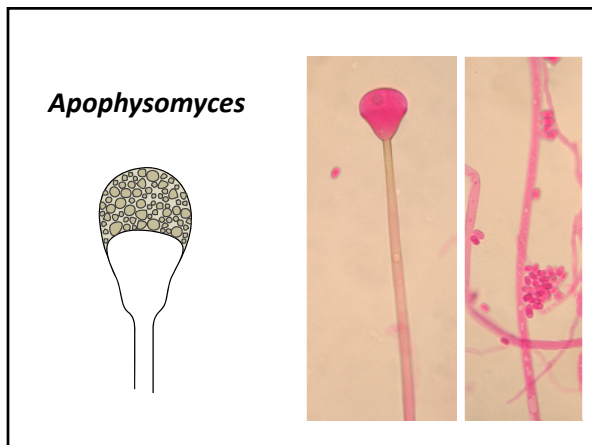
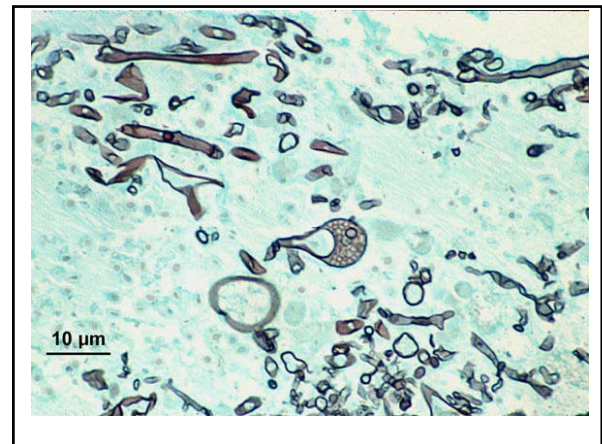
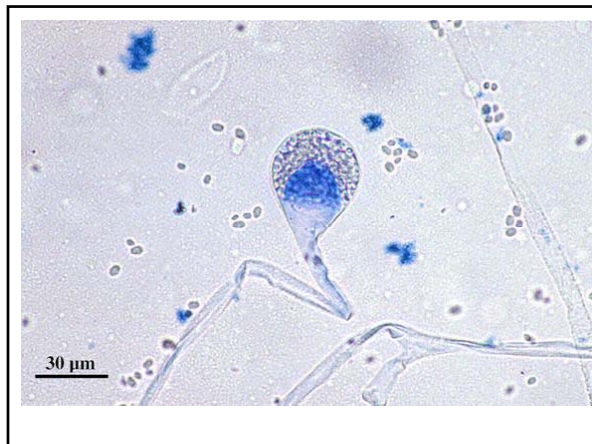
Zygomycota (Conjugation Fungi)

- Saprotrophic molds with coenocytic hyphae (lack septa).
- Also known as bread molds.
- Asexual Reproduction: Used most of the time.
- Sporangiospore: Asexual spore enclosed within a sporangium or sac at the end on an aerial hypha.
- Sexual Reproduction: Occurs through conjugation, the joining of hypha of two different strains (plus and minus).
- Zygospores: Sexual spores which are enclosed in a thick, resistant wall.
- Normally nonpathogenic
- *Rhizopus nigricans*: Common black bread mold. Related species may cause opportunistic infections in immunocompromised patients.

Reproductive Structures of Zygomycete (*Rhizopus*) Sporangia (asexual) and Zygospore (sexual)







Ecology

- typically early decay fungi of well hydrated vegetative material, including material in soils
- thermotolerant species often inhabit composts
- may also grow in protein-rich materials such as cadavers, carnivore faeces

Sporothrix schenckii

SPOROTRICHOSIS

Sporotrichosis

- Four kinds:
 - Lymphocutaneous (most common kind)
 - Acquired by inoculation, usually otherwise-healthy patient
 - Fixed cutaneous
 - Acquired by inoculation, usually patient from endemic tropical area who has been repeatedly exposed and has a prior antibody level
 - Systemic
 - Acquired by inhalation or rarely via otomycosis; patient usually chronic alcoholic. HIV patients also vulnerable.
 - Barrier-break-related (miscellaneous opportunism)
 - Ocular keratitis, CAPD peritonitis, etc.

Lymphocutaneous sporotrichosis



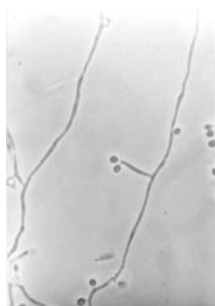
Sporothrix schenckii colonies
– outgrowth from lesion



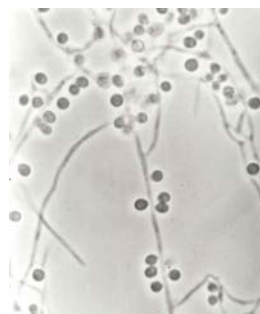
Sporothrix schenckii mould phase in culture with conidia



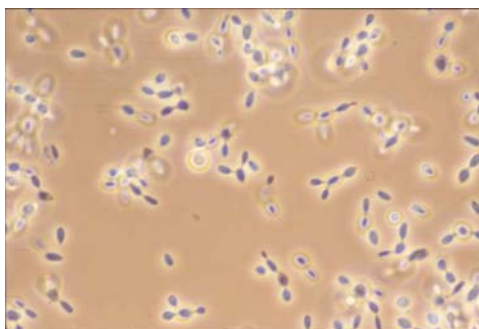
S. schenckii primary conidia (rosette)



S. schenckii
Secondary conidia



Sporothrix schenckii converted to yeast phase in culture at 37°C



Sporotrichosis

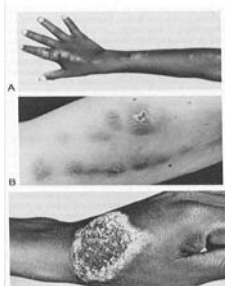


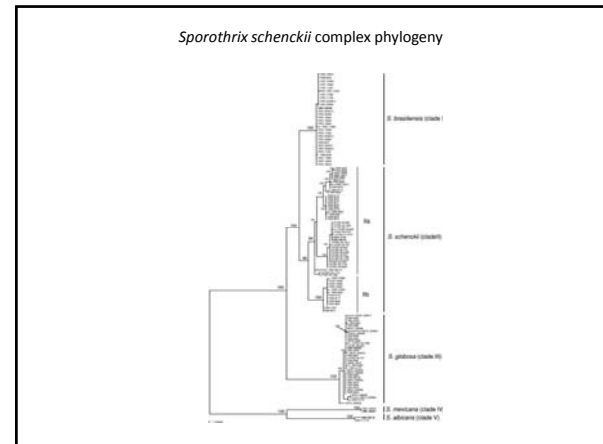
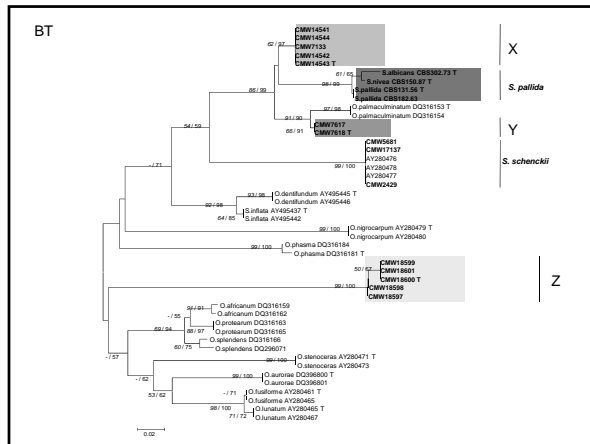
Fig. 26.1. Sporotrichosis. A, Lymphangitic spread from a primary digital lesion up the dorsal surface of the forearm. B, Forearm lesions as a consequence of lymphangitic spread from a digital lesion. C, Large crusted primary lesion of the hand. From Lurie, H.L.: Histopathology of sporotrichosis. Arch. Pathol. Lab. Med., 75:421-427, 1965.

Recognizing *Sporothrix schenckii*

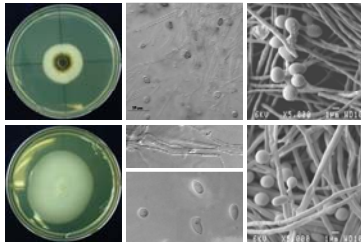
- Primary sympodial conidia egg-shaped, in “rosettes”
- Dark secondary conidia often “sheaths” around the substrate hyphae
- Converts to yeast on BHI + 10 drops 10% yeast extract slant at 35° and/or 37° C
- Yeast has “cigar shaped buds” and “rabbit ears”

Ecology

- typically grows on moist, finely-divided cellulosic plant materials
 - peat moss (unventilated)
 - dead grass in tropical Americas
 - very roughly cut mine timbers



Sporothrix schenckii vs. *S. pallida*



Rhinosporidium seeberi

RHINOSPORIDIOSIS

Rhinosporidiosis

- granulomatous disease
 - nose/ nasopharynx
 - mucosa of the eyelid
- endemicity
 - South India, Sri Lanka, South America and Africa
- etiologic agent
 - *Rhinosporidium seeberi*
 - affiliation was unclear for most nearly a century, long thought to be fungal because a yeast-like form is present
 - now thought to be a **protist** in the Mesomycetozoea (Opisthokonta - recall this group from the first lecture)

