

## Safety Procedures for the Microbiology Laboratory

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### General Laboratory Safety Practices and Procedures

1. If you are **taking immune-suppressants, are pregnant,** or have a known medical condition that would prevent full participation in the laboratory, please contact the course instructor before the first day of lab.
2. Read and understand each laboratory exercise **before** you come to class.
3. Do not eat, drink, smoke, or chew pens in the laboratory.
4. You must wear close-toed shoes while in the laboratory and long pants.
5. No hats of any kind will be allowed in lab, unless allowed by University policy and cleared with the instructor.
6. Long hair should be pulled back to keep it away from bacterial cultures, bacticinerator or open flames.
7. Follow precautionary statements given in each exercise.
8. Personal electronic devices will be turned off and stored while in this laboratory. \*The unauthorized use of any electronic device (phone, tablet, computer) in lab will result in a loss of course points.
9. Know where specific safety equipment is located in the laboratory, such as the fire extinguisher, safety shower, and the eyewash station.
10. Recognize the international symbol for biohazards, and know where and how to dispose of all waste materials, particularly biohazard waste. Note that all biohazard waste must be sterilized by autoclave before it can be included in the waste stream.



**Figure 1:** Biohazard Symbol

11. Keep everything other than the cultures and tools you need OFF the lab bench. Only necessary work material should be at or on the laboratory bench. Coats, backpacks, and other personal belongings will not be allowed on the laboratory bench top. Store them in a place designated by your instructor. This is to prevent cluttering of the workspace and to avoid exposing them to permanent stains, caustic chemicals, and microorganisms used in the exercises.
12. Leave all laboratory facilities and equipment in good order at the end of each class. Before leaving the laboratory, check to make sure the bacticinerator heat sterilizer is turned off.
13. Never, under any circumstances, remove equipment, media, or microbial cultures from the laboratory.

14. No pets are allowed in the laboratory.

### Microbiology Specific Laboratory Safety Practices

During the course of the semester in the laboratory you will be taught the methods used in the proper handling of microorganisms. Although you will not be working with any that are human pathogens, exercise caution in handling all material coming in contact with live microbial cultures. All cultures should be handled with respect and proper aseptic technique *as if they were potential pathogens*. This is called "**universal precaution**". Specific instructions that should be followed:

1. Remember that all bacteria are potential pathogens that may cause harm under unexpected or unusual circumstances. If you as a student have a compromised immune system or a recent extended illness, you should share those personal circumstances with your lab instructor.
2. Wear gloves when working with cultures, and when your work is completed, dispose of the gloves in the biohazard garbage. Lab coats, safety glasses or goggles are also required. These will be stored in the laboratory each week in a ziplock bag.
3. Disinfect your work area both BEFORE and AFTER working with bacterial cultures.
4. Cultures of live microorganisms and any material coming in contact with live cultures must be properly sterilized after use in the laboratory. Your instructor will inform you of specific procedures. Follow the general rules outlined below.
  - a. Glassware such as test tubes, bottles, and flasks may be reused and washed after sterilization. These are normally placed on a cart at the front of the laboratory after you have finished an experiment or exercise. BE SURE TO REMOVE LABELS before placing any glassware on the cart. Your instructor will sterilize and then wash these items.
  - b. Some materials, such as plastic petri dishes, plastic pipettes, microscope slides, and swabs, are considered disposable. These are used once and if they become contaminated by contact with live microorganisms are sterilized and discarded. All of these disposable contaminated materials should be placed in the designated waste container containing a BIOHAZARD autoclave bag.
5. Never place contaminated pipette tips (or pipettes), inoculating loop, or any other contaminated material on the bench top. Sterilize loops before and after each use. Place contaminated pipette tips in the orange biohazard buckets on your bench. Place all other contaminated materials in their designated waste containers. Do not place or put anything containing live microorganisms in the sink.
6. Aerosols should be avoided by the use of proper technique for sterilizing the inoculating loops and by performing any mixing of cultures and reagents in such a way as to avoid splashing.

## Soil Fungi

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7. Cultures or reagents should always be transferred with an automatic pipettor that will be provided. In no case should one employ mouth pipetting.
8. Always keep cultures capped and in proper storage racks when not being used during an exercise.
9. In the event of an accidental spill involving a bacterial culture, completely saturate the spill area with disinfectant, then cover with paper towels and allow the spill to sit for 10 minutes. Then carefully remove the saturated paper towels, dispose of them in the biohazard waste, and clean the area again with disinfectant. Notify your instructor about the spill. If the chemical is marked "danger" or "caustic" you should notify the instructor who will handle this type of spill.
10. Immediately report all accidents such as spills, cuts, burns, or other injuries to the instructor
11. Make sure that lab benches are completely cleared (everything either thrown away or returned to storage area) before you leave the lab.
12. Clothing worn in the microbiology laboratory should be washed before being subsequently worn in a facility such as a hospital, clinic or nursing home, or in an area of public food preparation.
13. In the event of a fire alarm, follow the directions of your instructor, and meet at the place designated by your instructor.

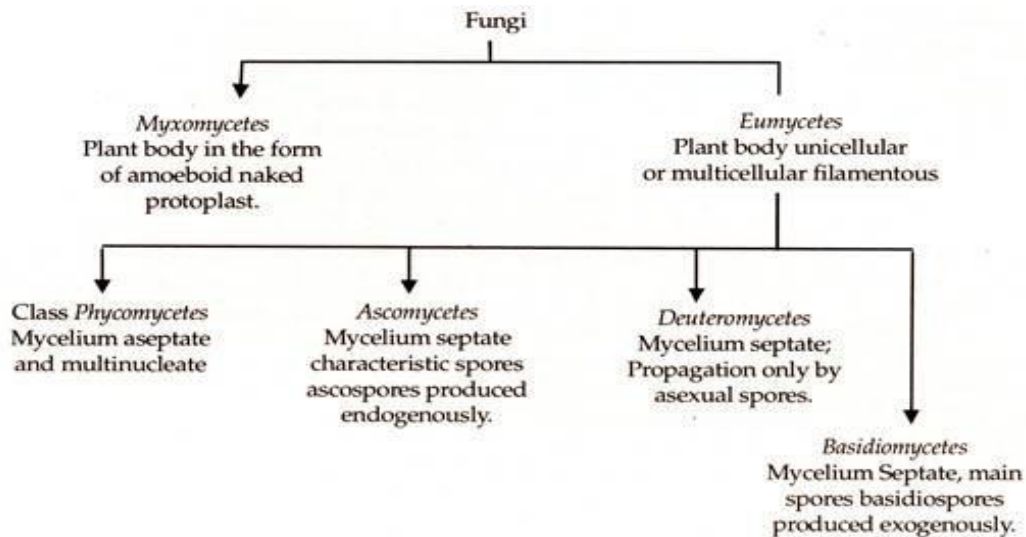
# Soil Fungi

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## Classifications

Alexopoulos (1956) places all fungi in the division Mycota. The division Mycota is divided into two subdivisions (1) Myxomycotina (2) Eumycotina (true fungi). Myxomycotina has only one class – Myxomycetes.

Eumycotina has the four classes as shown in the figure.



# Soil Fungi

K. Mycophyta

D. Myxomycophyta

C. Myxomycetes

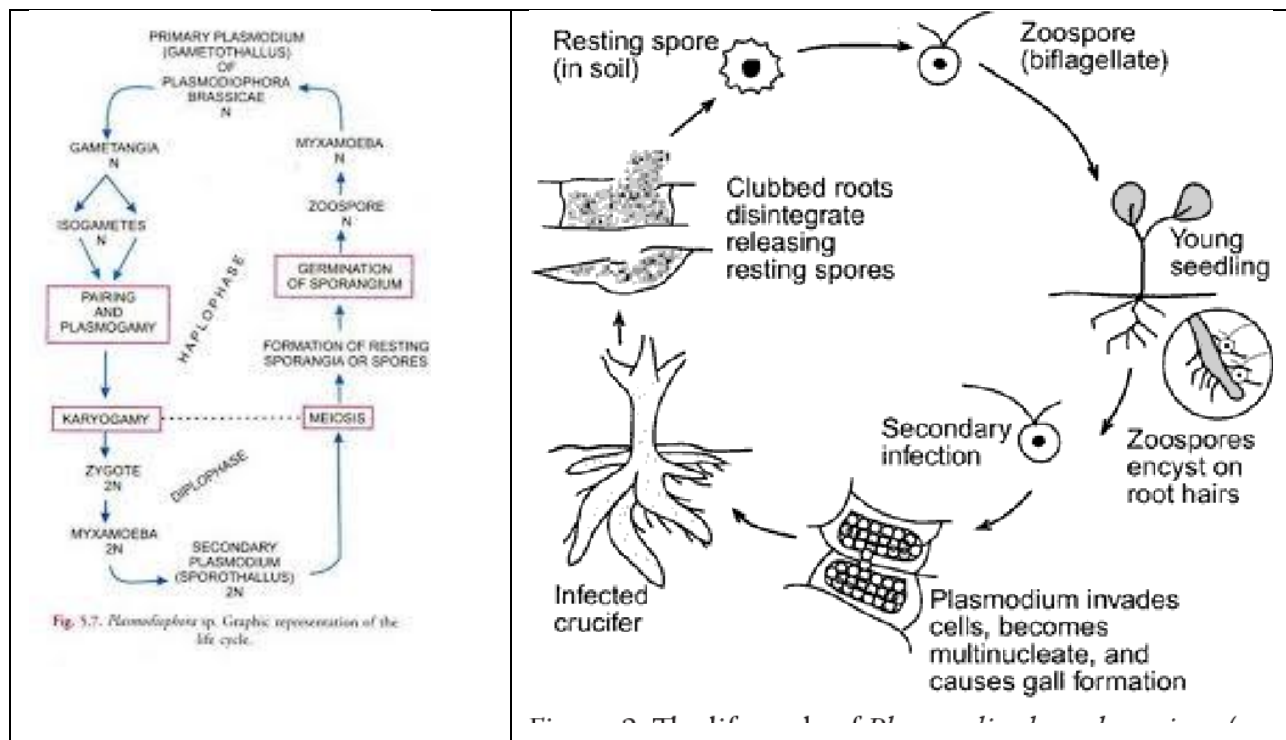
O: *Plasmodiophorales*

F: *Plasmodiophoraceae*

Ex. *Plasmodiophora brassicae*

Please check images in the following link

<https://www.shutterstock.com/search/plasmodiophora-brassicae>



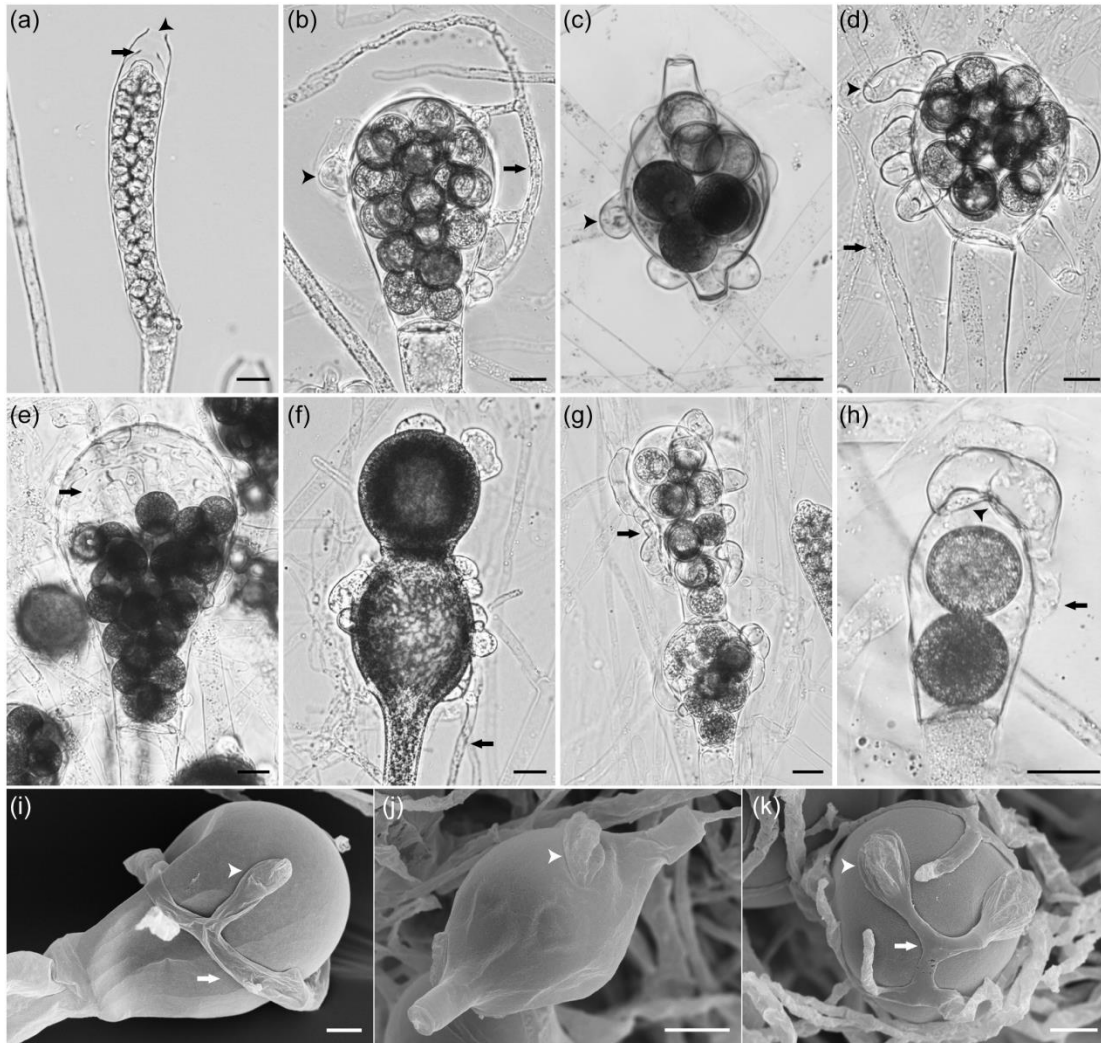
# Soil Fungi

C: Phycomycetes

O: Saprolegniales

F: Saprolegniaceae

Ex.: *Saprolegnia* sp.



# Soil Fungi

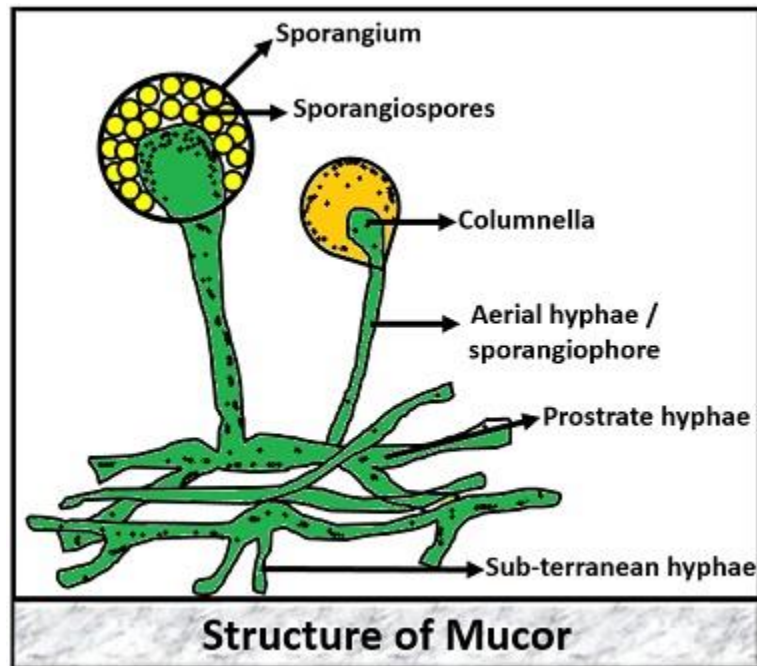
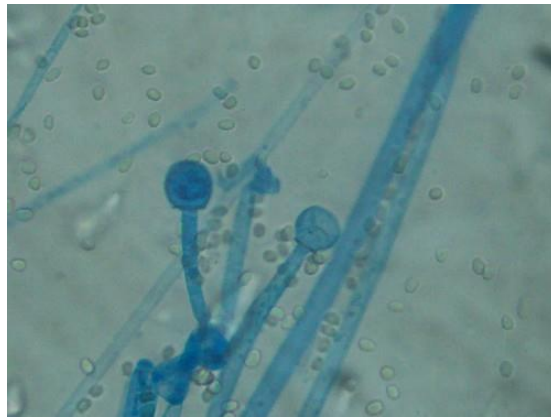
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**C:** *Phycomycetes*

**O:** *Mucorales*

**F:** *Mucoraceae*

**Ex.1.** *Mucor*

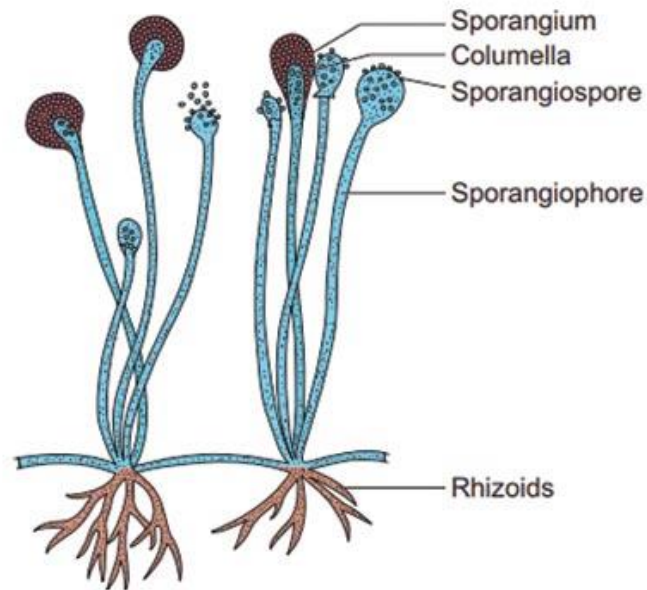




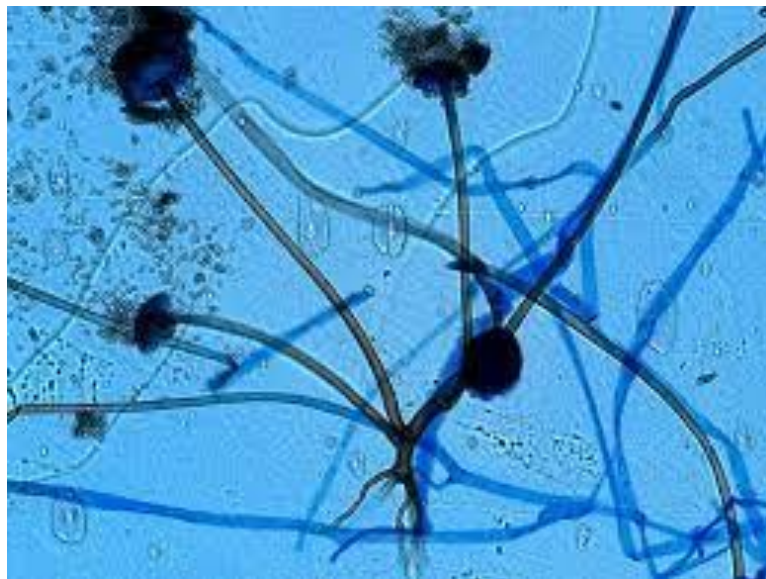
## Soil Fungi

### Ex. 2 *Rhizopus* sp.

*Rhizopus* is a **genus of saprophytic and parasitic fungi**. They are found in moist or damp places. They are found on organic substances like vegetables, fruits, bread, jellies, etc. The vegetative structure is made up of coenocytic (multinucleated) and branched hyphae.

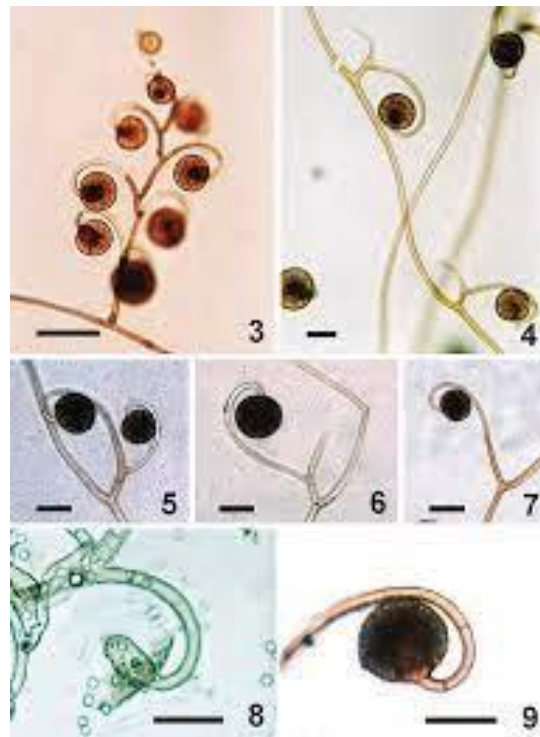
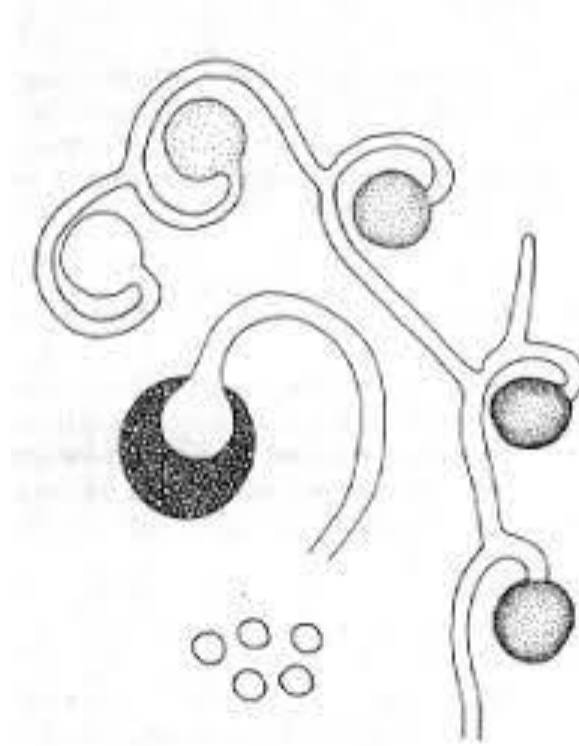


### *Rhizopus* Morphology



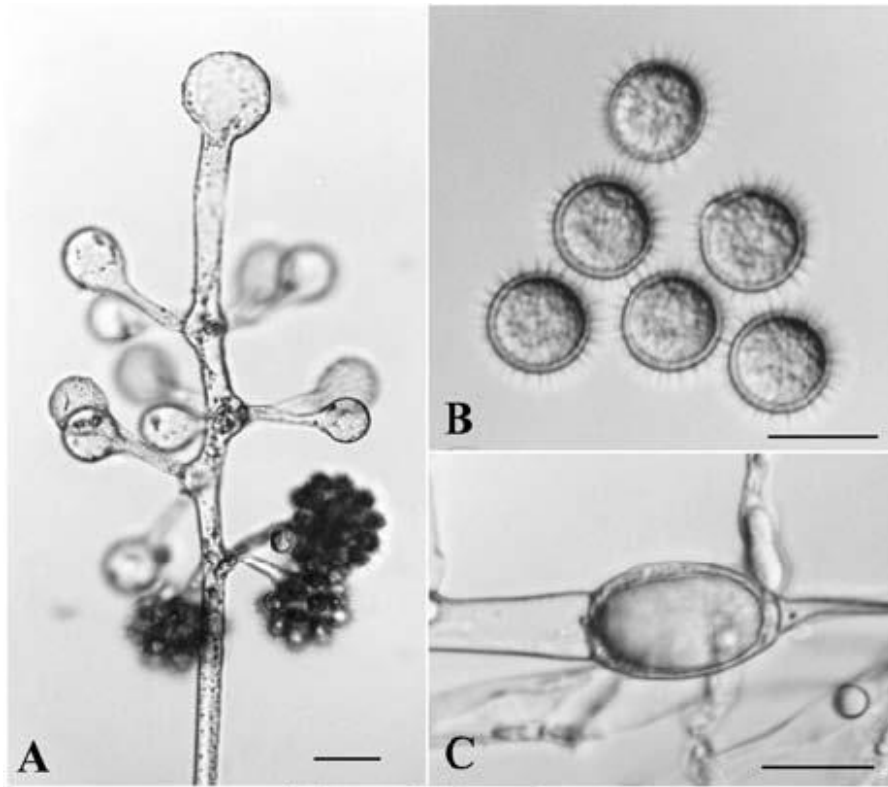
# Soil Fungi

Ex. 3 *Circinella* sp.



Family 2: *Choanephoraceae*

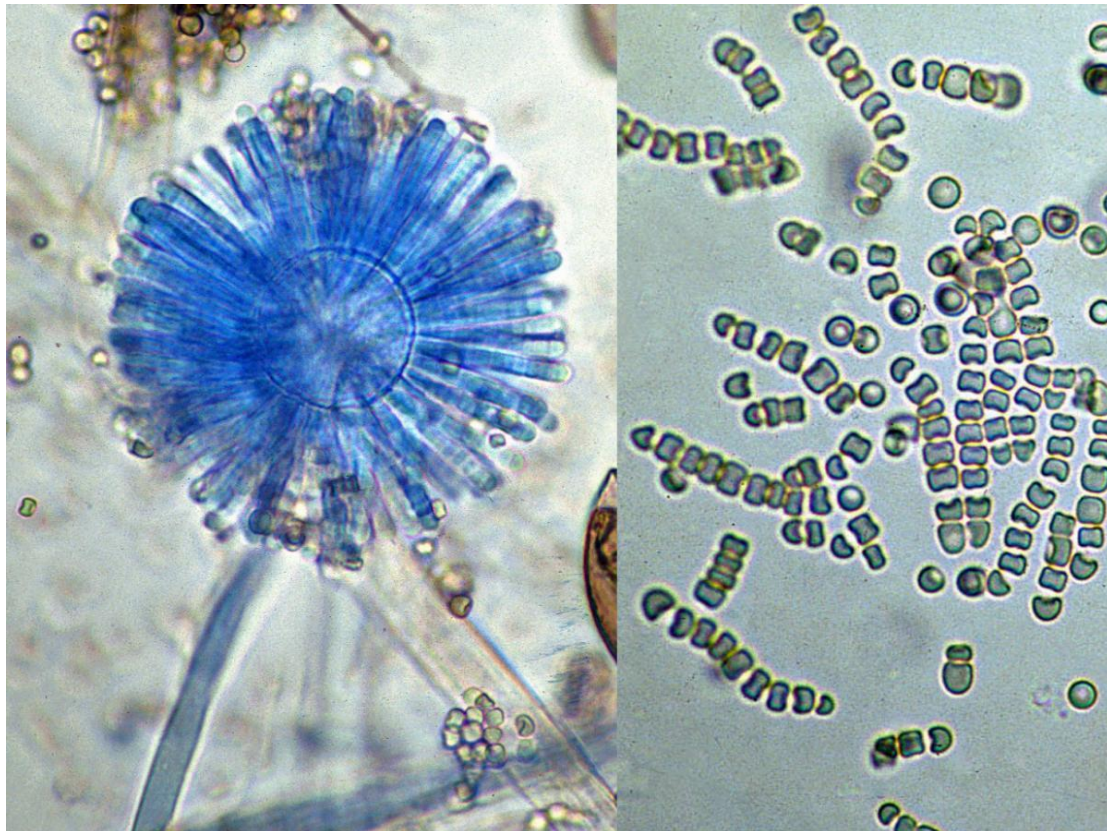
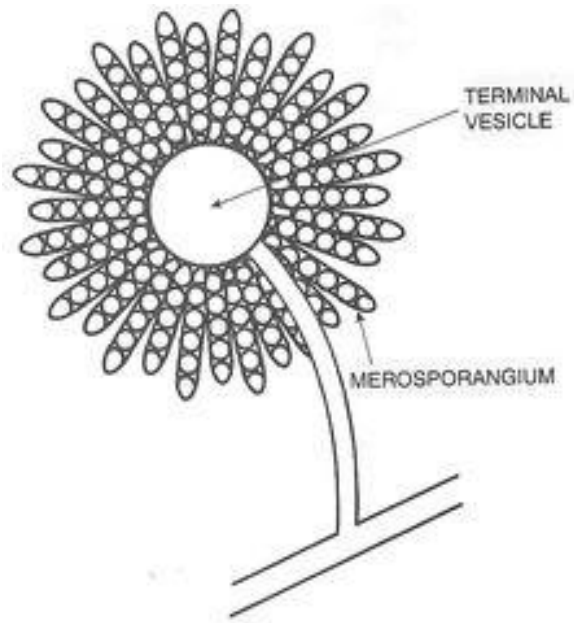
Ex. *Cunninghamella echinulata*



# Soil Fungi

Family 3 : *Cephalidaceae*

Ex. *Syncephalastrum* sp.



# Soil Fungi

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Class 2: *Ascomycetes*

Subclass: *Euascomycetes*

Series: *Plectomycetes*

Order: *Aspergillales*

Family : *Aspergillaceae*

Ex.1 *Aspergillus*

## **General characteristics**

- 1- Colony colour
- 2- Colony reverse
- 3- Sterigmata: Biserial – uniserial
- 4- Conidia: globose – subglobose – elliptical – ovate – rough – smooth – hyaline – pigment.
- 5- Vesicle : globose – subglobose – clavate.
- 6- Conidial head: radiate – columnar – clavate.
- 7- Conidiophore: long – short – branched – unbranched – smooth – rough – hyaline – pigment – straight – sinuate.
- 8- Ascospore
- 9- Hull cell
- 10- Sclerotia

## **Different *Aspergillus* sp.**

1	<i>Aspergillus clavatus</i>
2	<i>Aspergillus chevalieri</i>
3	<i>Aspergillus fumigatus</i>
4	<i>Aspergillus candidus</i>
5	<i>Aspergillus flavus</i>
6	<i>Aspergillus ochraceus</i>
7	<i>Aspergillus niger</i>
8	<i>Aspergillus versicolor</i>
9	<i>Aspergillus nidulans</i>
10	<i>Aspergillus ustus</i>
11	<i>Aspergillus flavipes</i>
12	<i>Aspergillus terreus</i>

## Ex. 2 *Penicillium*

### General characteristics

- 1- Colony colour
- 2- Colony reverse
- 3- Metulae: Present – Absent
- 4- Penicillin: Monoverticillata – biverticillata – symmetrica – asymmetrica – divaricate – nondivaricata (velutina – lanata – fasciculata).
- 5- Conidia: globose – subglobose – elliptical – ovate – rough – smooth – hyaline – pigment.
- 6- Conidiophore: long – short – branched – unbranched – smooth – rough – hyaline – pigment – straight – sinuate.
- 7- Ascospore
- 8- Hull cell
- 9- Sclerotia

Different *Penicillium* sp.

1	<i>Penicillium corylophilum</i>
2	<i>Penicillium duclauxi</i>
3	<i>Penicillium funiculosum</i>
4	<i>Penicillium chrysogenum</i>
5	<i>Penicillium steckii</i>
6	<i>Penicillium waksmani</i>
7	<i>Penicillium purpurogenum</i>
8	<i>Penicillium corylophilum</i>
9	<i>Penicillium duclauxi</i>
10	<i>Penicillium funiculosum</i>
11	<i>Penicillium chrysogenum</i>
12	<i>Penicillium steckii</i>
13	<i>Penicillium waksmani</i>
14	<i>Penicillium purpurogenum</i>

# Soil Fungi

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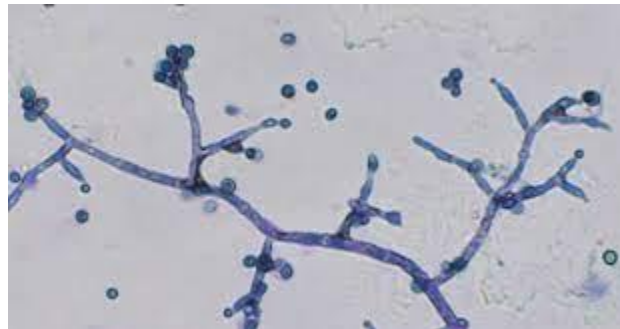
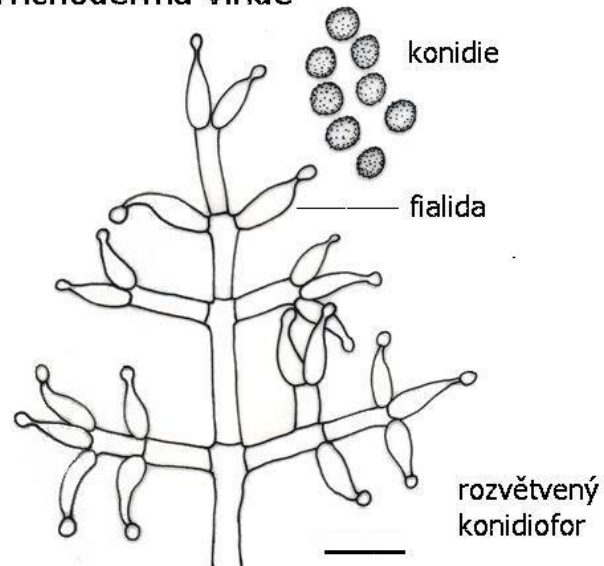
Class 3 : *Deuteromycetes*

Order: *Moniliales*

Family: *Moniliaceae*

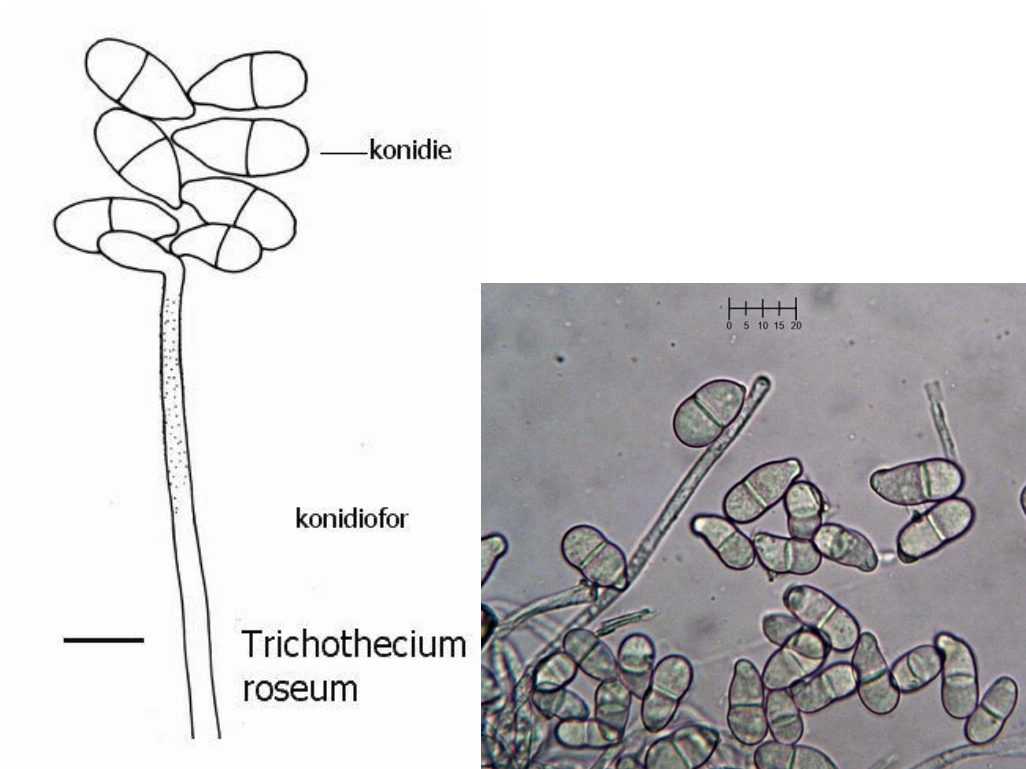
Ex. 1: *Trichoderma*

*Trichoderma viride*

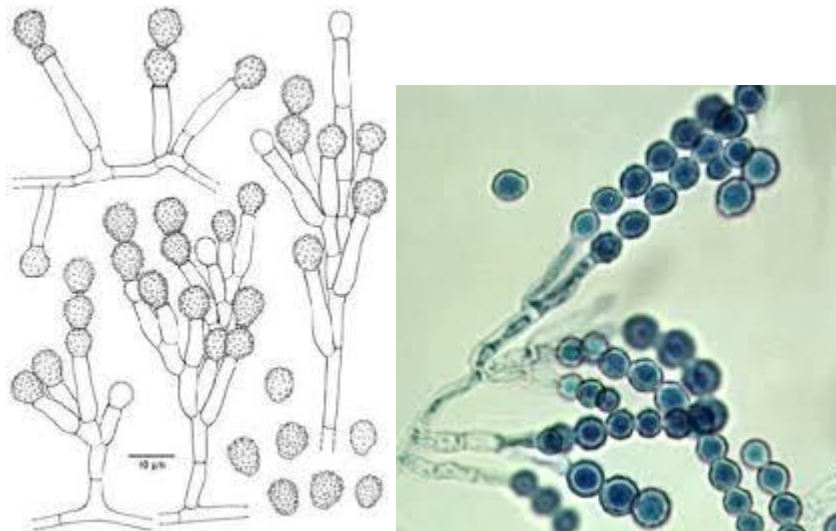


# Soil Fungi

Ex. 2: *Trichothecium roseum*



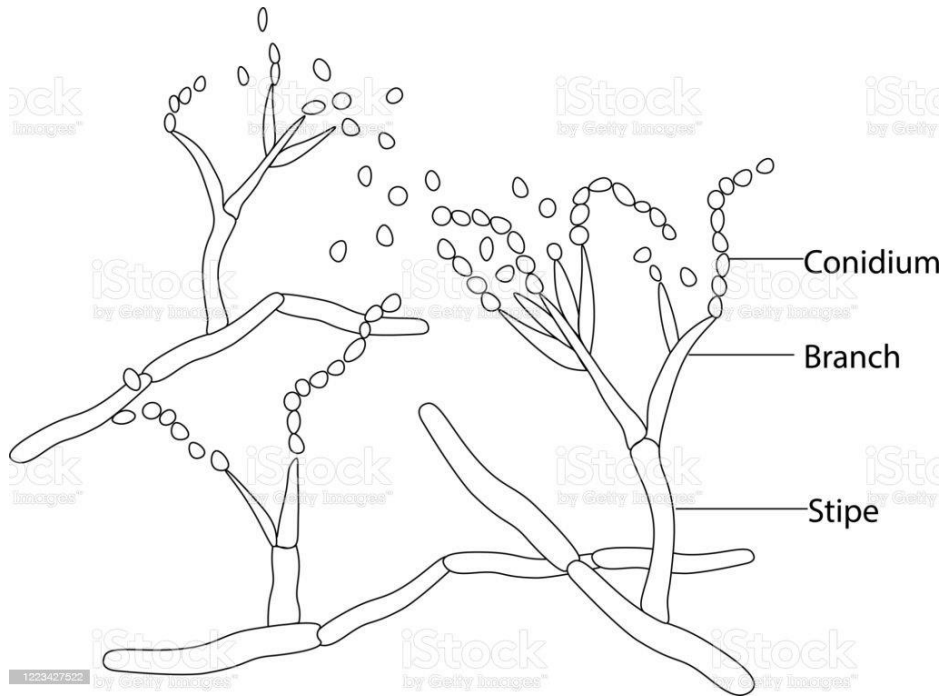
Ex. 3: *Scopulariopsis*





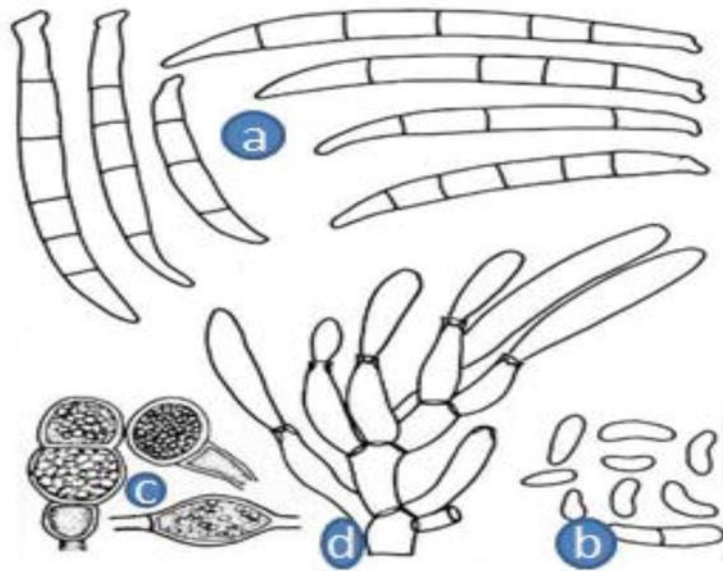
# Soil Fungi

Ex. 4: *Paelelomyces*

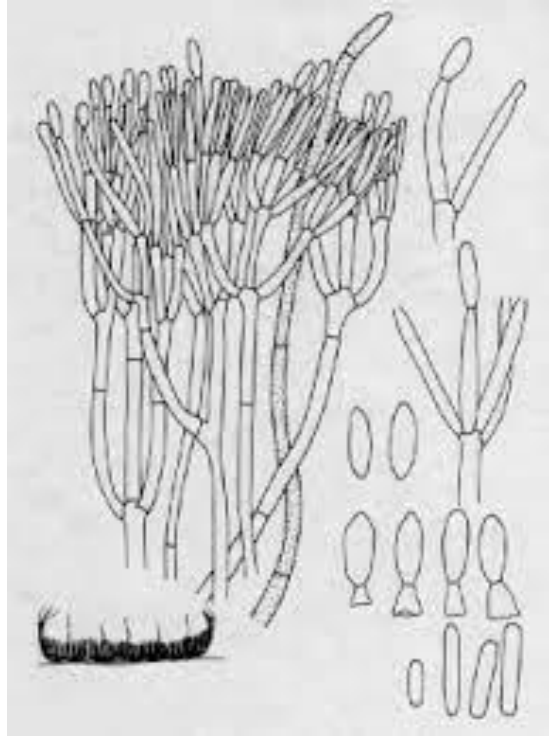


Family 2: *Tuberculariaceae*

Ex. 1: *Fusarium*

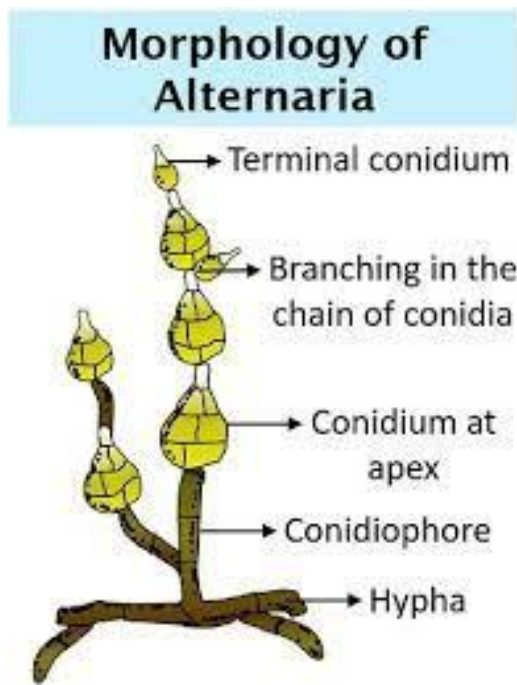


Ex. 2: *Myrothecium*

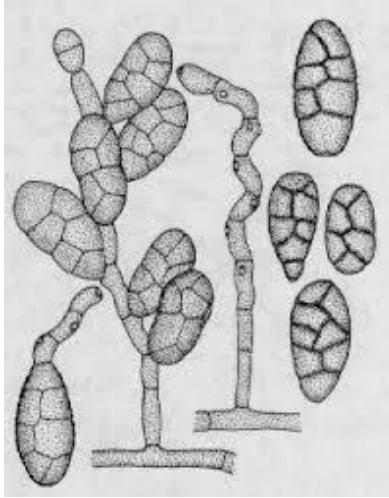


Family 3: *Dematiaceae*

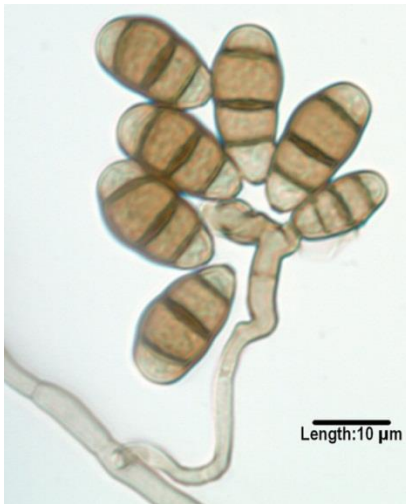
Ex. 1: *Alternaria*



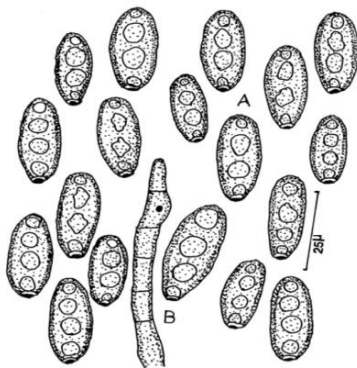
Ex. 2: *Ulocladium*



Ex. 3: *Curvularia*

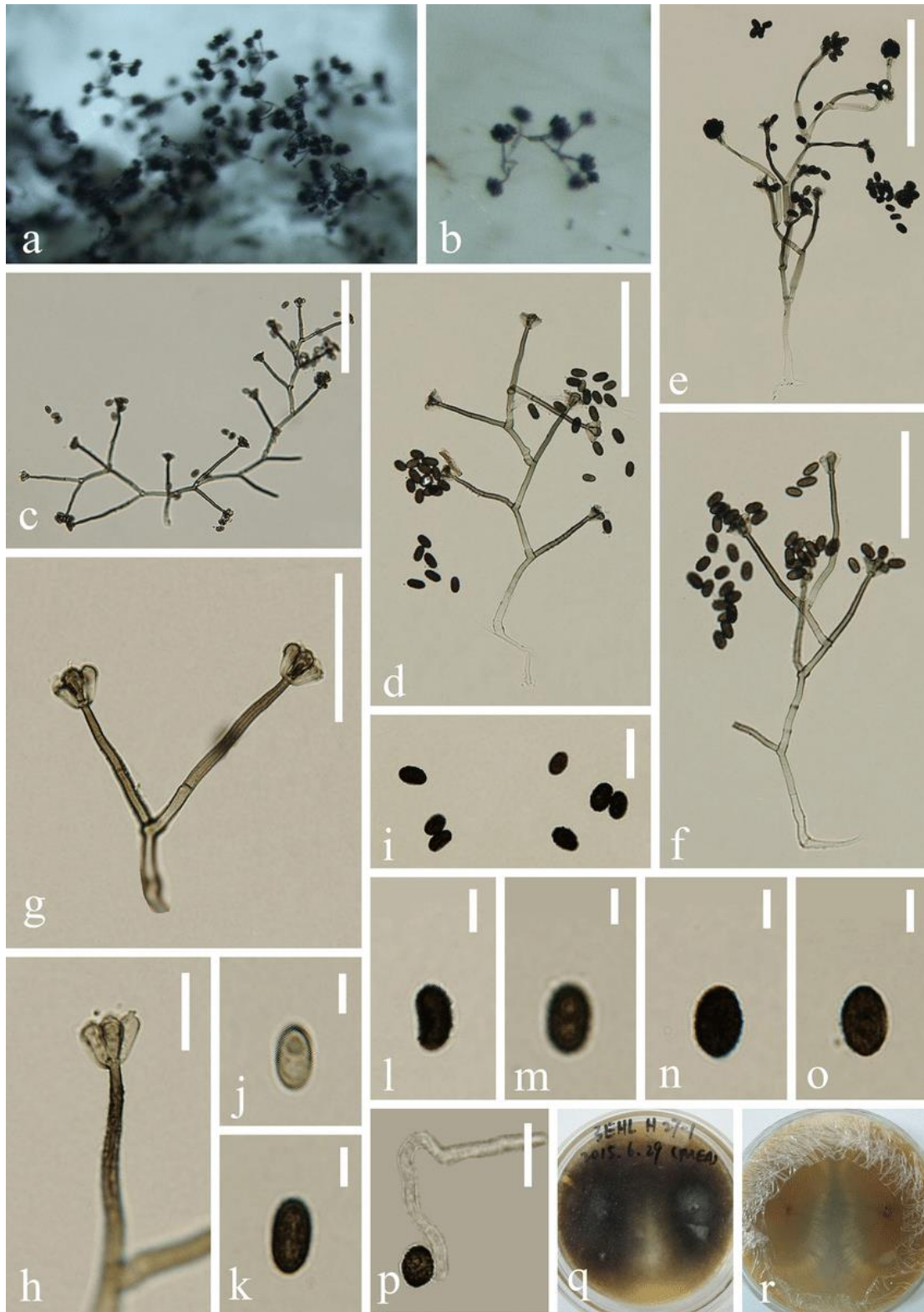


Ex. 4: *Drechslera*

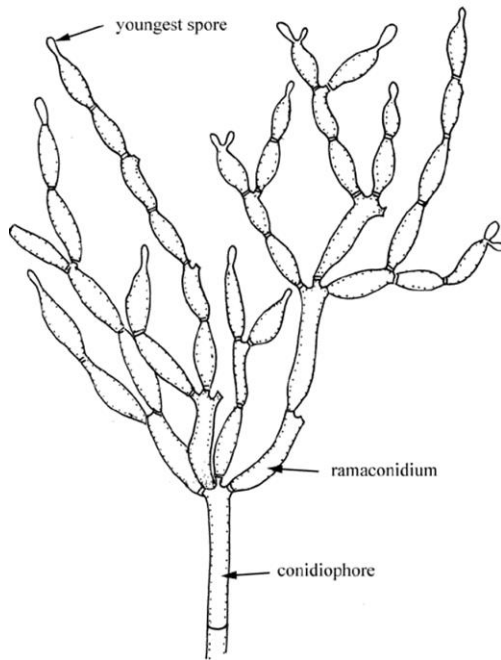


# Soil Fungi

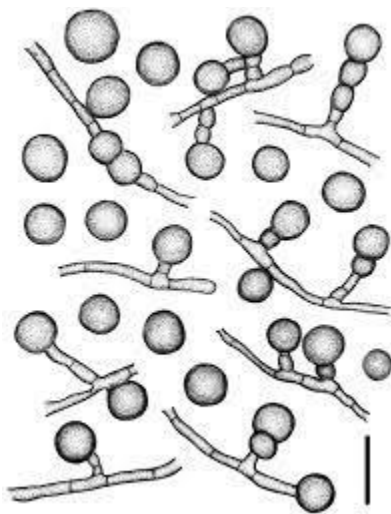
Ex. 5: *Stachybotrys*



Ex. 6: *Cladosporium*



Ex. 7: *Humicola*



## Soil Fungi

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Family 3: *Stilbaceae*

Ex. *Trichorus spirales*

## References

- Introduction to Modern Mycology. Second edition. J. W. Deacon (1988).
- An Introduction to fungi. Third edition. H. C. Dube (2005).
- Introduction to Fungi. First edition. S. S. Rajan (2001).