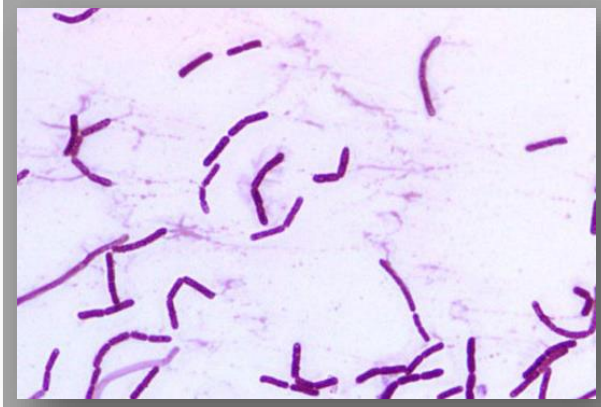
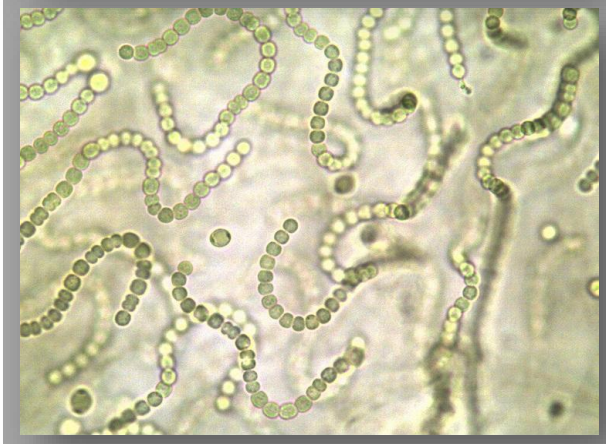
Manual for Botany Laboratory
School of Sciences, NSOU





Contents

❖ What is Laboratory Manual ?

- Laboratory Manual is an exercise book containing various topics as allotted in the syllabus. This provides a prospective to students for practical work in the classrooms. By studying such manual a student becomes aware of the brief theoretical information, preparation of the sample material for study and observation, thereafter description and ultimately derivation of conclusion. The manuals are also provided with necessary diagrammes and sketches of the prepared samples as per observations made by the students.

❖ Introduction :

- The early botanical knowledge leads to the development and evolution of multidisciplinary subject as modern botany in recent years. The inputs from various sources of science and technology have tremendously enriched the subject Botany. Today this subject is a fusion of traditional and classical components with that of modern disciplines resulting in to a splendid natural science. We have today the various branches of Botany which have been enriched through inputs from extensive studies and researches, e.g. Taxonomy/Syatematics, Paleobotany, Angiosperms Morphology, Ecology, Biodiversity and Conservation, Palynology, Cytology and Cell Biology, Genetics, Plant Physiology and Biochemistry, Molecular Biology, Biotechnology, Microbiology, Phycology, Mycology and Plant Pathology, Lichenology, Bryology, Pteridology, Gymnosperms, Economic Botany, Pharmacognosy or Medical Botany, Ethnobotany etc.

❖ Instruments usually used in Botanical Laboratory :

1. Simple Microscope :

- It consists of only one lens unit. This lens unit may even be an ordinary magnifying glass. Dissecting microscope is used either for dissecting the material or for less magnifications, i.e., only 5X, 10X or rarely 20X. It is mainly used for taxonomic studies, embryo separation etc.

- It consists of a basal foot and a limb. The 'stage', made up of a simple glass plate, is attached to the limb. For the light adjustment purposes, a mirror is attached to the limb under the stage. Mirror can be moved vertically with the help of an adjustment screw.

- At the tip of the limb is present a folded arm, on which a lens of definite magnification (5X, 10X etc.) is fitted. Folded arm is moved to keep the lens in the desired position on the stage.

Fig 1: Simple Microscope

2. Light Microscope (Compound) :

- It is so named because it consists of two or more lens systems.
- At the top is present the ocular lens. It can be turned around or may be removed. At the top of ocular lens is written 5X or 10X signifying the 5 times or 10 times magnification, respectively.
- Just below the ocular is a body tube, the bottom end of which contains a circular piece called nose piece. It contains three lenses called objective lenses. Nose piece can be rotated to change the position of objectives as per desire.
- The flat platform present below the objectives is called **stage**.
- On the arm of the microscope are present two knobs called **coarse adjustment knob** and **fine adjustment knob**.
- Out of the three objectives, the shortest is the low power objective. It has the largest lens but its magnifying power is least of the objective lenses. On the objective may also be written 10X similar to ocular lens. It means if a 10X ocular lens is used the magnification is 10 x 10 = 100 times.
- The other objective is high power objective. Its magnification is equal to the number written on it multiplied by the power of ocular, i.e., 5X or 10X (objective X ocular).
- The third objective is called oil immersion. Generally, it contains a black band around the lower end. Use a drop of oil on the slide at the time of studying with the oil immersion objective. Its magnification can be estimated as ocular x objective.
- The use of oil (e.g., cedar-wood oil) is essential in order to keep the light rays properly aligned with the small objective.
- Just below the stage is the condenser. Its function is to gather light from the mirror and to direct it to the objective lens. Condenser may be lowered or raised by a knob present on one side of the microscope beneath the stage.
- Condenser contains a shutter called Iris diaphragm.
- Just below the condenser is present a mirror having its one surface flat and other concave. Use the concave surface in the day light. Flat surface of the mirror is used when electric lamp is used.

Fig 2: Compound Microscope

Precautions for handling:

1. Clean the ocular and objective lenses with lens paper/tissue paper, and do not remove them.
2. While studying an object, learn to keep one hand on the fine focus knob and focus continuously up and down.
3. While observing any kind of preparation, do not tilt the microscope.
4. Leave the low power objective in place after finishing all the observations.
5. To examine an object, always first use the low power and then the other objectives.
6. Never allow an objective lens to strike either the stage or a slide while focusing.
7. Use always the fine adjustment with high power objective.
8. All wet-mount preparations should be pre-covered by a cover slip.
9. Avoid the habit to remove the parts of the microscope.
10. Do not use oil immersion objective without oil.
11. Diaphragm should be wide open while using oil immersion objective.

3. Weighing Balance:

- Laboratory balances are defined by high levels of accuracy and precision in analytical testing and quantitative analysis.

- Used in a variety of applications, they are divided into five subcategories according to their readability:

  - Precision balances (>= 0.001 g)
  - Analytical balances (0.0001 g)
  - Semi-micro balances (0.00001 g)
  - Micro balances (0.000001 g)
  - Ultra-micro balances (0.0000001 g)

Fig 3: Analytical Balance

Precautions :

- The instrument should be kept on a vibration free support.
- Balance should not be located next to doors or windows as opening and closing gives rise to air drafts.
- Always weigh samples after closing the weighing chamber doors.
- Every time use a clean spatula of appropriate size.
- Properly clean the surface area after use.

4. Single Glass Distillation Unit :

- Distilled water (DH_2O) is needed for different laboratory experiments such as culture media preparation, staining solutions preparation etc. Single glass distillation unit provides pyrogen free distilled water.

Fig 4: Single Glass Distillation Unit

Precautions :

- Donot operate the machine without water.
- Always maintain the water level.

5. Autoclave :

- It is a pressure chamber used to carry out scientific processes requiring elevated temperature and pressure in relation to ambient.
- Used to sterilize equipment and culture media by subjecting them to pressurized saturated steam at 121 °C (250 °F) for around 15–20 minutes.

Fig 5: Autoclave

Precautions :

- Donot operate the machine without water.
- Use only distilled water.
- Always maintain the water level above the heater.
- Never sealing containers; under pressure they pose an explosion risk.
- Never opening the door to the autoclave if there is water running out the bottom. Clogged steam lines, equipment malfunction, or plugged drains may cause a buildup of scalding water.
- Waiting for the pressure to reach zero and the temperature is at or below 121°C before opening the door at the end of a cycle to avoid steam burns and shattered glassware. Do not stand directly in front of the door.

6. pH Meter :

- It is a scientific instrument that measures the hydrogen-ion activity in water-based solutions, indicating its acidity or alkalinity expressed as pH.

Fig 6: pH Meter

Precautions :

- pH electrode is fragile and should not be tempted to use it as a stirring glass rod when adjusting pH.
- It needs to be calibrated daily with the help of standard buffer solutions.
- The glass electrode should not be left out of the storage solution for prolonged intervals as the glass membrane gets dehydrated resulting in slower response and can eventually lead to complete response failure.
- Glass is rapidly attacked by hydrofluoric acid so never keep the electrode in HF solution as it can lead to irreparable damage of the electrode.
- Avoid vibrations and shock.
- Adopt the suggested cleaning procedures after use:
  - General cleaning – if the solutions are fairly clean soak in 0.1M HCl or 0.1M HNO_3 for about 30 minutes
  - Bacteria or deposits – soak in 1: 10 dilution of laundry bleach for 10 min
  - Protein deposits – soak in 1% pepsin in 0.1 M HCl for 5 min.
  - Oil or grease – soak in detergent or methanol
- After every soaking the electrode should be rinsed with distilled water.

- Droplets adhering to electrode should be gently dried with tissue paper and not rubbed or wiped.

7. Incubator :

- This instrument used to grow and maintain microbiological cultures or cell cultures at desired temperature.

Fig 7: Incubator

Precautions:-

- Place your unit properly.
- Clean the incubator regularly.
- Calibrate on a regular basis.

8. Laminar Flow Cabinet:

- A laminar flow cabinet is a carefully enclosed bench designed to prevent contamination of semiconductor wafers, biological samples, or any particle sensitive materials.
- Air is drawn through a High-efficiency particulate air (HEPA) filter and blown in a very smooth, laminar flow towards the user.
- Laminar flow cabinets have a UV-C germicidal lamp to sterilize the interior and contents before usage to prevent contamination of experiment.

Fig 8: Laminar Flow Cabinet

Precautions:-

- Germicidal lamps are usually kept on for 15 minutes to sterilize the interior.
- No contact is to be made with a laminar flow hood during work.
- switch off UV lamp during use.

❖ Maintenance of discipline and cleanliness in the laboratory :

- A list of do's and don'ts can be a helpful reminder of laboratory safety issues. These are as follows-
  - **Observe to :**
    - Become familiar with experiments before coming to the laboratory.
    - Follow all laboratory instructions carefully.
    - Know the emergency and fire procedures.
    - Ask for clarification on any laboratory procedures that are not understood.
    - Wear safety glasses during practical where glassware, heat or chemicals are used.
    - Wear laboratory aprons.
    - Wear protective gloves when instructed to do so.
    - Assume all chemicals are toxic and act accordingly.
    - Read the labels on reagent containers for safety precautions and understand the chemicals being used. Have Material Safety Data Sheets available for all chemicals.
    - Stopper or cap all reagent bottles when not in use.
    - Wash any chemical contact areas immediately with water and inform your instructor.
    - Keep your work area neat, clean, organized.
    - Know the location of emergency equipment (first aid kit, eyewash, fire extinguisher, spill containers and emergency exits).
    - Exercise great caution whenever using heat.
    - Keep hair and loose clothing restricted and well away from flames.
    - Turn off the gas source when a Bunsen burner is not in use.
    - Use proper ventilation and hoods when instructed to do so.
    - Handle hot glassware with appropriate clamps.
    - Use extra caution when working with sharp objects (e.g. scalpels, knives, glass tubing etc.).
    - Ask for instructor assistance in cleaning up broken glass or chemical spills.
    - Discard sharp items (e.g. needles, razor blades, scalpel blades etc.) in a "sharps container."
    - Discard cracked or broken glass in a "broken glass container."
    - Report all accidents to your instructor immediately.
    - Report to your instructor any condition that seems unsafe.
    - Keep all laboratory exits clear of debris.
    - Use special care when carrying heavy and expensive equipment (e.g.- Microscopes, Stage Micrometer, Ocular Micrometer etc.).
    - Entry in Lab record book before using an instruments.
    - Seek first aid assistance for all cuts or other minor injuries.
    - Always add acid slowly to water. Never add water to acid.
    - Notify your instructor of any symptoms of illness or allergic reactions during laboratory work.
    - Remove cultural spills and clean the area with 70% ethanol.

- Place culture materials in biohazard bags and dispose after autoclaved as directed by your instructor.
- Turn off all gas nozzles and water faucets when leaving the laboratory.
- Properly clean your lab table, work area and return all cleaned equipment to its proper place.
- Wash hands before leaving the laboratory.

- **Avoid to:**
  - Begin laboratory work until instructed to do so.
  - Do anything in the laboratory that is not understood.
  - Engage in any horseplay in the laboratory.
  - Perform unauthorized experiments.
  - Operate any equipment until you have been instructed in its proper use.
  - Work in isolation—a second person should always be nearby.
  - Remove any materials from the laboratory unless instructed to do so.
  - Speak during work in Laminar Air Flow/Culture room.
  - Touch your face when working with cultures.
  - Let potential hazards make you afraid to participate in laboratory activities.
  - Leave a Bunsen burner or other flame source unattended.
  - Light a flame source near flammable materials.
  - Move a lit Bunsen burner.
  - Taste or ingest any chemicals or plant material.
  - Handle or consume food or drink in the laboratory.
  - Pipet anything by mouth.
  - Put chemicals in a sink or in the trash unless instructed to do so.
  - Pour chemicals back into dispensing containers.
  - Leave a test tube stopper in place when heating test tubes.
  - Drink from laboratory glassware.

❖ **Examples :**

- Some exercises based on selected topics of syllabus have been provided here as samples.

EXCERCISE 1

Gram staining technique

- **Requirements:**
  - 24 hours cultures of *Bacillus* sp. and *Escherichia coli*.
  - Gram staining reagent:
    - Gram Crystal violet solution.
    - Gram's iodine solution.
    - Safranin
  - Ethanol (95%)
  - Staining tray
  - Wash bottle of distilled water
  - Inoculating loop
  - Glass slides
  - Blotting paper
  - Bunsen burner/spirit lamp
  - Compound Microscope

- **Procedure:**
  - Make thin smears of one loop full inoculum of Bacterial samples on separate glass slides by inoculating needle.
  - Let the smears air dry.
  - Heat fix the smears.
  - Cover each smear with crystal violet for 30 seconds.
  - Wash each slide with distilled water for a few seconds, using wash bottle.
  - Cover each smear with Gram's iodine solution for 60 seconds.
  - Wash off the iodine solution with distilled water.
  - Add Ethanol (95%) drop by drop, until no more colour flows from the smear. (the gram-positive bacteria are not affected while all gram- neagative bacteria are completely decolorized).
  - Wash the slides with distilled water and drain.
  - Apply safranin to smears for 30 seconds (Counter-staining)
  - Wash with distilled water and blot dry with absorbent paper.
  - Let the stained slides air dry.

- **Observations:**
  - Examine the slides microscopically using oli-immersion objective.
  - Identity the gram reaction of both the cultures and classify them.
  - Make sketches for morphology of the cultures.
  - Describe the morphology and arrangement of the cells.

- **Results:**
  - Those bacteria that appear purple are referred to as Gram-positive, those appearing pink are described Gram-negative. In *B.* sp. the appear dark purple or blue in colour, thus it is a gram-positive bacterium whereas in *E. coli* the rods appear pink and is thus a gram-negative bacterium. To get reliable results, one should use cultures that are 18 to 24 hours old.

Fig 1.1: Gram staining of *Bacillus* sp (Microscopic view).

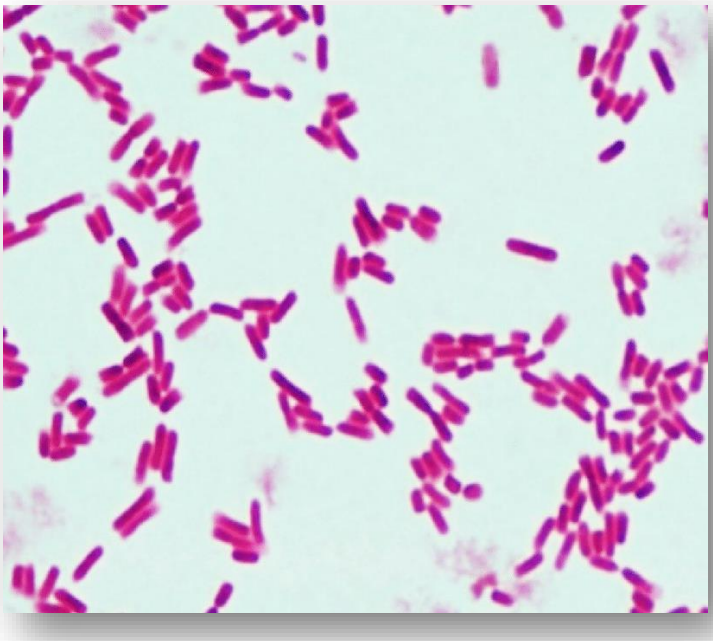


Fig 1.2: Gram staining of *Escherichia coli* (Microscopic view).

EXCERCISE 2

Algae

- From Algae one specimen, ***Nostoc*** is considered here for practical work.
- **Objectives**:-
  - Preparation of Algal Slide.
  - Familiarity with its vegetative structures under Cyanophyceae.
- **Requirements:**
  - Compound Microscope.
  - Slide, Cover slip
  - Needle and Brush
  - 10% Glycerine
  - Cotton blue solution (1%)
  - Vegetative part of ***Nostoc*** **sp.**
  - Blotting paper
  - Bunsen burner/spirit lamp
  - Wax

- **Method of sample preparation:**
  - Place on the slide a little part of ***Nostoc*** by using 10% glycerin solution.
  - Spread the material with the help of needle and add a single drop of cotton blue (1%).
  - Wait for a while.
  - Take another slide, add few drops of lactophenol and place the spreaded material.
  - Place cover slip over the material carefully.
  - Care should be taken to absorb the excess amount of lactophenol without specimen by blotting paper.
  - Place the prepared slide on turn table and seal the margin of cover slip with melted wax with the help of a brush.

- **Description and Identifying Characters:**
  - Place the prepared slide under a compound microscope, draw the characteristics features and observe the following features as identifying characters
    - Thalli are present in the form of colony.
    - Ball like colony is enveloped by a gelatinous sheath.
    - Each colony contains thousands of straight or twisted filaments or Trichomes.
    - Each trichome is surrounded by its individual sheath and called the filament.
    - A trichome is contorted and consists of many cells arranged in a beaded manner.
    - Each cell is somewhat cylindrical or spherical in shape.
    - There are present some large, spherical or cylindrical, colourless empty cells present in the filaments, called heterocysts.
    - Some thick-walled cells of the filament become enlarged. These are called Akinetes.

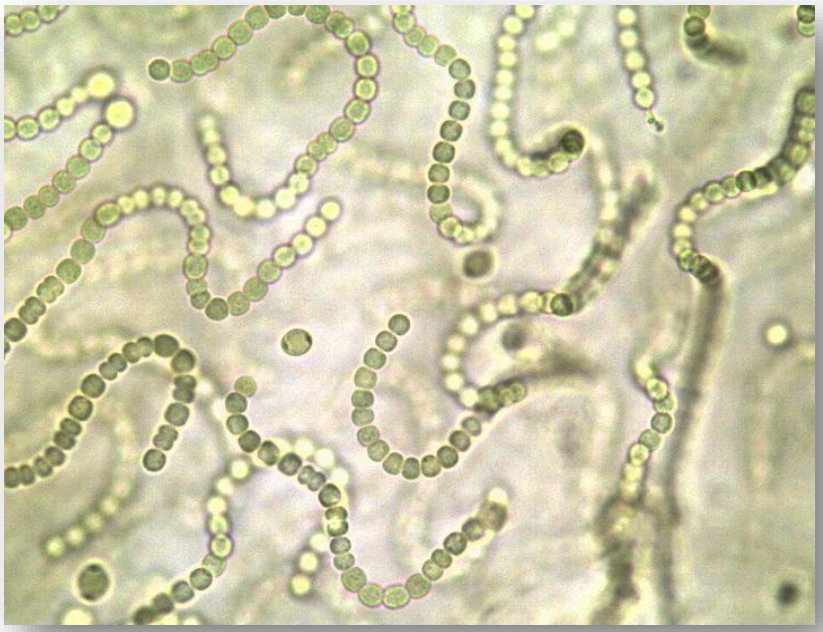


Fig 2.1: *Nostoc* sp (Microscopic view).

EXCERCISE 3

Fungi

- From Fungi one specimen, ***Ascobolus*** is considered here for practical work.
- **Objectives**:-
  - Preparation of Fungal Slide.
  - Familiarity with its fruit body structures containing Ascus and Ascospore under Ascomycetes.
- **Requirements:**
  - Compound Microscope.
  - Fruit body of ***Ascobolus* sp.**
  - Slide, Cover slip
  - Blade, Needle and Brush
  - Cotton blue solution (1%)
  - Lactophenol (1%)
  - Blotting paper
  - Bunsen burner/spirit lamp
  - Wax
- **Method of sample preparation:**
  - Make a thin Vertical Longitudinal Section (VLS) of fruit body of the specimen with a sharp blade.
  - Transfer the section into the slide and add 1% cotton blue solution.
  - Wait for a while.

- Place cover slip over the material carefully.
- Care should be taken to absorb the excess amount of stain by blotting paper.
- Place the prepared slide on turn table and seal the margin of cover slip with melted wax with the help of a brush.

- **Description and Identifying Characters:**
  - Place the prepared slide under a compound microscope, draw the characteristics features and observe the following features as identifying characters
    - Fruit body is Apothecium in nature.
    - Ascus and Paraphyses are arranged alternately paralleled.
    - Each Ascus contain eight oval shaped Ascospores.

Fig 3.1: *Ascobolus* sp.

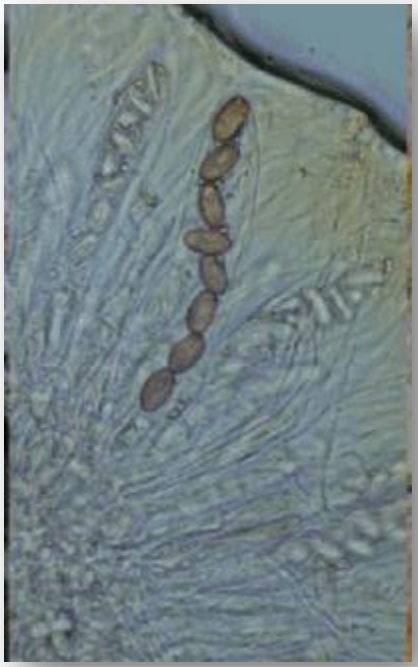


Fig 3.2: VLS of a portion of fruit body of *Ascobolus* sp. showing ascus containing eight ascospores (Microscopic view)

EXCERCISE 4

Bryophyte

- From Bryophyte one specimen, ***Marchantia*** is considered here for practical work.
- **Objectives**:
  - Familiarity with gametophytic plant body of ***Marchantia*** sp. under Hepaticopsida.
- **Requirements:**
  - Compound Microscope.
  - Permanent slide of antheridiophore of ***Marchantia*** sp**.**
- **Description and Identifying Characters:**
  - Place the permanent slide under a compound microscope, draw the characteristics features and observe the following features as identifying characters
    - Antheridiophore consists of a long stalk and a lobed disc at the apex.
    - The disc is usually eight lobed.
    - The disc consists of air chambers alternating with heridial cavities.
    - Air chambers are more or less triangular and open on upper surface by a pore called ostiole.
    - Antheridia arise in acropetal succession i.e., the older near the center and youngest at the margins.

Fig 4.1: *Marchantia* sp. showing gemma cup.

Fig 4.2: Gametophytic plant body of *Marchantia* sp. bearing archegoniophore (Left) and antheridiophore (Right).

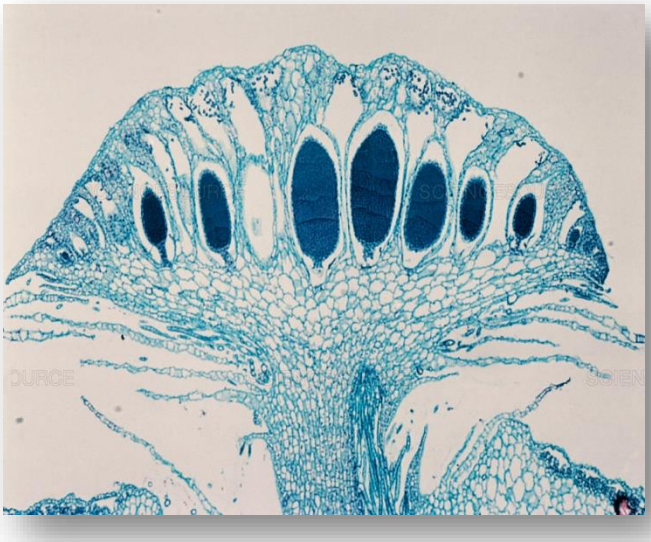


Fig 4.3: LS through Antheridiophore of *M.* sp. (Microscopic view)

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