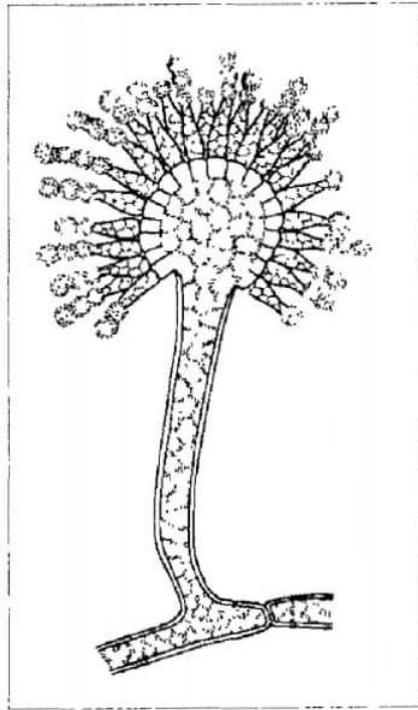




A Text Book of  
**Practical  
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# Introduction to Laboratory

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## **Preamble**

Science is a systematised study based on facts and observations. It involves curiosity, inquisitiveness and unbiased analysis. Most of the scientific work is done in a laboratory. It provides an opportunity to a person with scientific frame of mind to see and study various aspects of an object under observation. Hence, a biology student too is obliged to attend laboratory work-out with utmost sincerity, honesty and inquisitiveness.

## **Laboratory Etiquette**

The study of living things in laboratory requires that facilities provided are properly used.

One is expected to complete the assigned work within a specified time. This requires proper utilization and planning of time. One should, therefore, keep busy with own work and wherever necessary consult the teacher alone.

Laboratory provisions should be handled with utmost care. At the end of the laboratory period, working place should be left clean and in order.

Laboratory exercise to be performed should be read in advance and one is expected to arrive to the class theoretically prepared.

## **Work Plan**

1. Listen and understand the instructions and information given by teacher-in-charge.
2. Work out or observe the materials carefully.
3. Mount to prepare slides as per requirements.
4. Study the preparations or specimen carefully.
5. Draw suitable diagrams in a proper sequence and label them in your practical record.
6. Write down the observations sequentially and watch carefully if variations occur.
7. Get your work checked by teacher-in-charge and make necessary corrections.

## **Necessary Instruments**

The variety of instruments required depends upon the nature of work. It has, however, been found convenient to prepare a small kit in suitable containers such as a pencil box containing

1. a pair of forceps,
2. two fine, long handle, dissecting needles,
3. glass droppers,
4. good and sharp razor,
5. safety blade,
6. a fine hair brush,
7. a pair of sharpened pencils,
8. pencil eraser,
9. a clean and soft handkerchief and
10. practical record with cover file and spare pages, etc.

## **Microscope**

It is the most indispensable instrument in a biology laboratory, so much so that it comes to be called 'The primary instrument of the biologists'. It helps to increase the resolving power (property to distinguish objects lying very close as separate bodies) of human eye which fails to recognise objects lying closer between 0.01 to 0.25 mm.

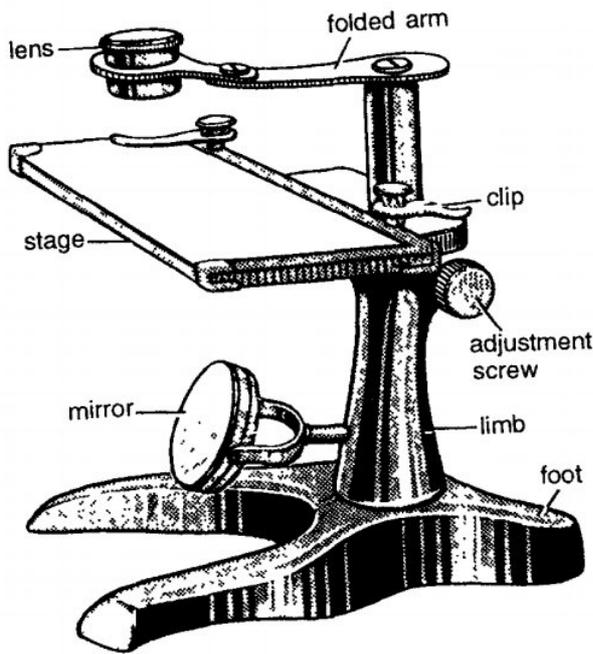


Fig. 1. A dissecting microscope.

Some common types of microscopes are listed below—

1. dissecting microscope,
2. compound microscope,
3. binocular microscope,
4. phase contrast microscope and
5. electron microscope.

Of these, dissecting microscope and compound microscopes are very commonly used by the students.

### [I] Dissecting microscope

It is used for dissection, specially during taxonomic studies, embryo separation, etc.

**Construction.** It consists of basal foot, a vertical limb, stage and a lens. The basal foot is a stand. The limb has an attached stage made of glass plate. A folded arm which can be moved vertically holds the lens. A mirror is attached at the base of the limb.

**Mechanical operation.** 1. Move the lens and adjust it over the object.

2. Illuminate the object suitably by adjusting the mirror.
3. Focus the object by using adjustment screw.

### [II] Compound microscope

It is one of the most commonly used and by far the most suitable microscope in the Botany Laboratory. (B-14)

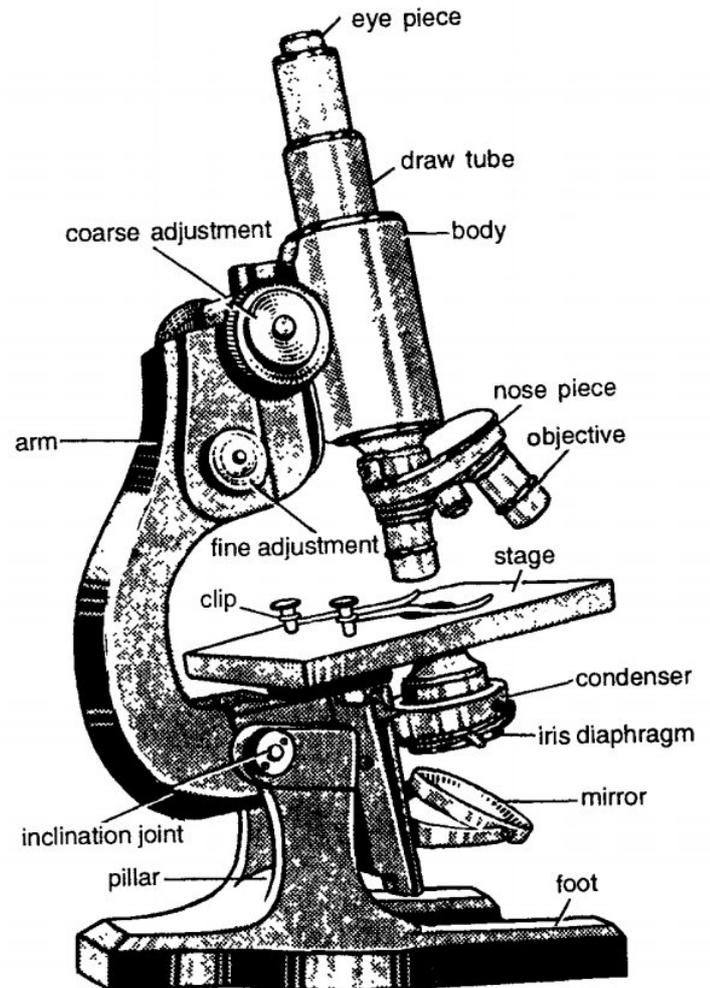


Fig. 2. Compound microscope.

At one time, it employs one ocular (eye piece) and one objective, in working position. As such, it is also known as monocular-mono-objective microscope.

**Construction.** The microscope is built around a strong basal foot and a vertical limb. The foot supports the vertical limb.

A round, rectangular or square stage is fixed to the limb. It is provided with spring clips to hold the slide in position.

A movable or fixed sub-stage is situated directly below the stage. It is provided with an iris diaphragm and condenser lens. Iris diaphragm is a wheel-shaped metal disc to regulate the aperture, through which light rays reach the condenser and are passed to an object. Condenser is a system of two or more lenses under the stage which receives parallel light rays from mirror and converge them at the level of stage.

A movable concave mirror is fixed at the lowermost part of the limb to focus a converging

cone of rays at the level of specimen. Whether day or artificial light is used as a source, concave mirror converges the light if there are no condensing lenses.

Body of the microscope is composed of a tube. At the upper end of the tube, is an ocular (eye piece) which can be changed for lower or higher values of magnifications. At the lower end of this tube is a revolving nose-piece with about three objectives viz. low power, high power and oil immersion. These magnifications range from 3.2x to 100x. The conventional low power objective is 10x.

Tube of the microscope is vertically movable with the help of coarse and fine adjustment screws on the limb, operated by a rack and pinion system. Coarse adjustment moves the tube rapidly, while fine adjustment screw does it gradually.

**Mechanical operation.** 1. Microscope is placed in maximum diffuse light. Direct sunlight is harmful for the eyes. The northern light is most suitable. If light source is artificial, filter (preferably blue coloured) is used.

2. Light is adjusted by turning the mirror towards the source of light and also by moving the sub-stage up and down, as well as with the help of iris diaphragm.
3. A prepared slide is placed on the stage. Object is adjusted just over the stage aperture.
4. The object is located and focussed with a low-power objective using coarse adjustment.
5. If higher magnification is desired, nose-piece is turned to next higher power. Fine adjustment can be used freely at this stage, while the use of coarse adjustment be avoided.
6. High power objective and subsequent higher powers are used only when object is properly mounted under coverslip.
7. The object should always be observed with both eyes open.

**Care.** 1. Before and after the use, all the lenses and metal parts including stage should be cleaned. The lenses are cleaned with tissue paper, muslin cloth or clean and soft handkerchief.

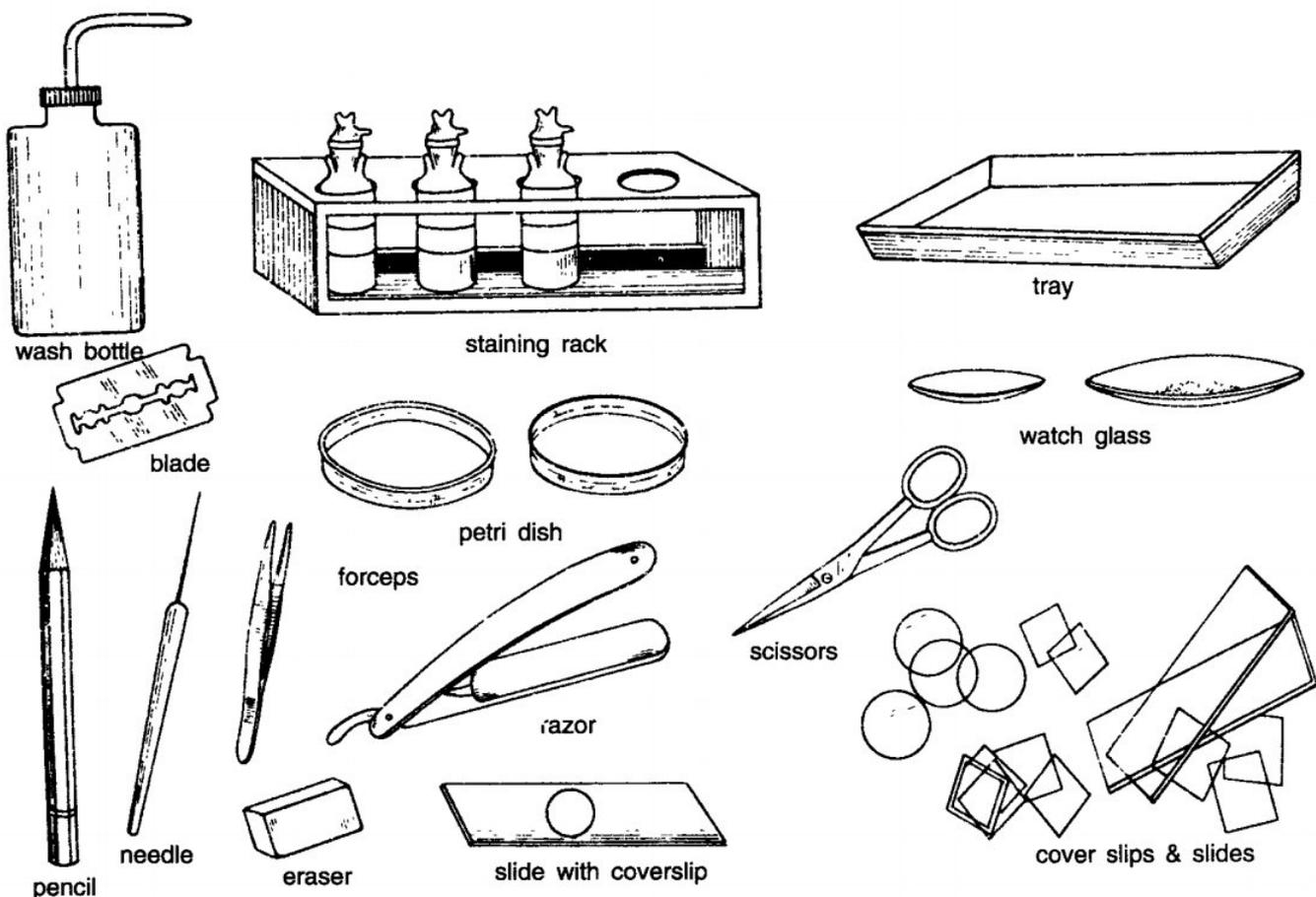


Fig. 3. Some laboratory provisions and necessary instruments.

2. Microscope is kept covered when not in use. Proper wooden box, plastic bags, bell jars or even a clean cloth can be used.
3. Objectives should not be ordinarily removed from the nose-piece.
4. Operating screws, condenser, iris diaphragm, mirror and stage or stage clips should always be handled carefully.

### Other Laboratory Provisions

Some other provisions available in the laboratory include staining rack, dropping bottles, slides, cover glasses, watch glasses, petri dishes, beakers, enamel trays, wash bottles, spirit lamp, hone, strop, dusters, etc. Some of these are described below—

**1. Staining rack.** It is mostly made of wood to hold the dropping bottles. The capacity of number of bottles per rack varies.

**2. Dropping bottle.** The stains, chemicals, mounting media, etc., are stored in these bottles. This glass bottle has a narrow mouth fitted with a slotted cock. Cock is provided with a beak that permits the liquid to flow out in drops.

**3. Slides.** The size of slides is mostly 3" × 1" (25 mm × 75 mm). It is about 1 mm thick. These are used to mount the material under study.

**4. Cover glasses.** The cover glasses are mounted on the object when the preparation is finally

ready. These may be either square or round shaped. The standard thickness of the coverslip is 0.17 mm.

### Fixing Agents and Preservatives

The plants or plant parts, collected fresh need to be immediately killed and subsequently preserved for a long time.

For this purpose, a few chemicals are used which do not cause any structural disturbance or distortion of the material. Carnoy's fluid, Formalin-aceto-alcohol, Formalin-propiono-alcohol, Randolph's modified Navashin fluid and Bouin's fluid are some of the common agents used.

Plants are generally fixed immediately after collection but these can also be fixed after bringing them to laboratory. The collected material must always be kept completely immersed in preservatives.

### Laboratory Techniques

#### [I] Section cutting

Sections of preserved material are cut in suitable planes for histological and ecological studies. Razor is suitable for cutting the sections in laboratory.

**1. Honing and stropping.** Razor should be sharp and free from nicks. Hence, it should be sharpened on a hone (fine-grit stone). Oblique,

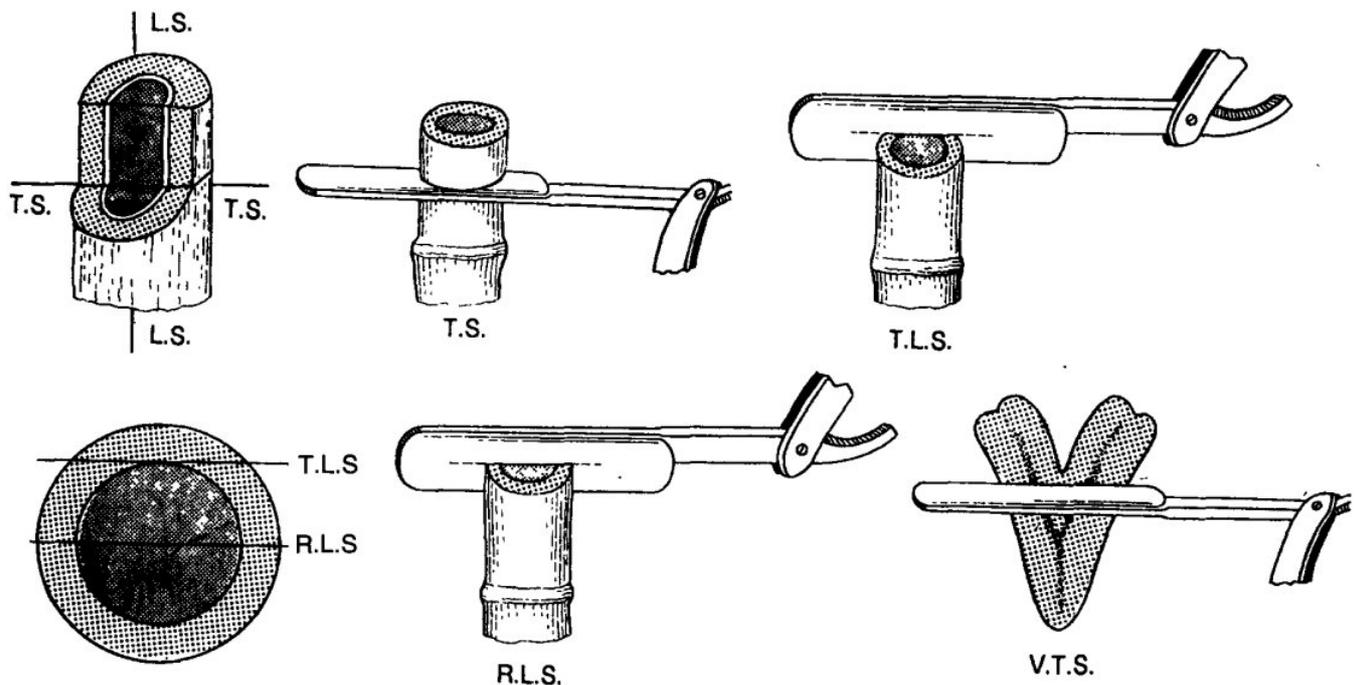


Fig. 4. Planes for section cutting.

uniform and slow strokes are carefully given to the razor with edge foremost on this stone.

After honing, uniform strokes are given on the strop (a smooth leather belt). The leather side of the belt is first slightly oiled and then razor is moved over. This should be done more frequently than honing, to maintain razor edge in good condition.

**2. Planes.** The following are a few commonly needed planes—

In case of cylindrical organs : (e.g., stems, roots, etc.).

**Transverse.** The section is cut by passing razor's edge at right angles to the longitudinal axis.

**Longitudinal.** The section is cut by passing razor's edge at right angles to the transverse axis. Two sections are possible in this plane.

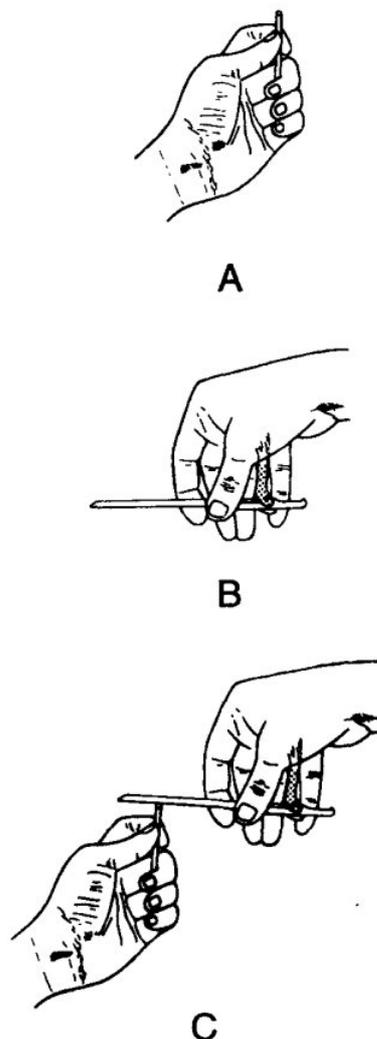
(i) **Radial Longitudinal section (R.L.s.)** if it passes along one of the radii.

(ii) **Tangential Longitudinal section (T.L.s.)** if section is cut along one of its tangents.

In case of dorsiventral organs (e.g. leaf, thallus of liverwort, etc.), transverse section is cut. It is known as vertical transverse section (being cut in vertical plane).

**3. Method.** Following steps would be useful for section cutting.

1. Soft, thin and small materials are placed in pith either by piercing a hole with a needle or by splitting it longitudinally with a blade. The pith used include carrot root and radish root, potato tubers, etc.
2. A razor must be held properly to cut the section. The handle and the blade of the razor should be at right angles to one another. The handle should remain free while the index finger is placed on the hooked end of the razor; 1st, 2nd and 3rd fingers pressed against the thick back edge of the razor and thumb against the milled surface of the thick shank of blade.
3. The material or the pith with embedded material is held between the thumb and the fingers of the left hand.
4. The material in the left hand and the razor's edge should form right angle.
5. The razor is now moved quickly over the material and the stroke is completed in one action only.



**Fig. 5. Method of section cutting.** A. holding the material, B. right way of holding the razor, C. holding the material and stroke of the razor.

6. More and more uniform strokes are used till desired quality and number of sections are obtained. Care is taken to keep the material and the razor flooded with water.
7. Sections float in water on the razor's edge. These are carefully lifted by a fine camel hair brush and then transferred to a watch glass containing water.
8. After the section cutting is over, the razor is tapped dry and cleaned without disturbing the edge. It is honed, stropped and encased.
9. The sections which float on water in the watch glass are considered to be thin.
10. These sections are lifted by a hair brush, placed on a slide in a drop of water and observed through microscope. A thin and uniform section is selected for staining.

### [II] Stains and staining

The selected sections need to be stained. The stains help to distinguish different tissues, cells or inclusions from one another by developing specific colours. Acetocarmine, Aniline blue, Crystal violet, Erythrosine, Hematoxylin, Fast green, Light green and Safranin are some of the commonly used stains.

**1. Specificity.** Most of the stains are specific in reaction and are purposely used so that definite structures or substances are stained. The following are some of the stains used for staining different structures.

<b>Achromatic figure</b>	<b>Cutinised cell wall</b>
Aniline blue	Crystal violet
Erythrosine	Erythrosine
Fast green	Safranin
Light green	<b>Callose</b>
<b>Cellulose cell wall</b>	Aniline blue
Aniline blue	<b>Chitin</b>
Delafield hematoxylin	Safranin
Fast green	<b>Proteins</b>
Light green	Safranin
<b>Lignified cell wall</b>	<b>Mitochondria</b>
Crystal violet	Crystal violet
Safranin	<b>Plastids</b>
<b>Suberised cell wall</b>	Crystal violet
Safranin	Iron hematoxylin
<b>Cytoplasm</b>	<b>Nucleus</b>
Aniline blue	Crystal violet
Erythrosine	Hematoxylin
Fast green	Safranin
Light green	<b>Chromosomes</b>
	Hematoxylin
	Safranin

**2. Single stains.** Safranin or fast green is used alone to stain filaments of algae, fungi, sections of bryophytes, spores of pteridophytes, pollen grains of gymnosperms, etc. Aniline blue or safranin is suitable for algae.

Following is the common method of staining.

1. The material is kept in a watch glass. A few drops of stain are added so that the material is immersed in the stain.
2. The material is allowed to remain so for a few minutes and allowed to take stain. The time required varies with materials.
3. After the stain is taken up, the excess of stain is washed off in water. The washing is repeated till stain stops coming out.
4. In some cases, excess stain is removed by acid water or acid alcohol if water alone fails to do so.

5. The stained material is ready for mounting. Fungi are stained in cotton blue as given below—

1. A drop of cotton blue (prepared in lactophenol) is placed on a slide.
2. Fungal hyphae is now placed in this drop.
3. The slide is run over the flame of the spirit lamp so that the stain is warmed up.
4. The preparation is now ready for mounting.

**3. Combinations.** Commonly two or more stains are employed wherever tissue differentiation is found. Combination of acidic and basic dyes of contrasting colours is of general use. This permits the distinction of woody tissue from non-woody tissue. The following few combinations are commonly recommended—

1. hematoxylin and safranin,
2. safranin and fast green,
3. safranin and aniline blue,
4. safranin and crystal violet and
5. crystal violet and erythrosine.

**4. Staining procedures.** There are two types of preparations—semi-permanent and permanent. The procedures differ in both the cases. These are given below.

*(a) For semi-permanent and temporary preparations.* Certain preparations are made for temporary use. The material is studied and the slide is then discarded. The method for staining them is given below.

1. The selected sections are transferred from watch glass containing water to another watch glass containing principal stain (e.g. hematoxylin, safranin or crystal violet).
2. The sections are allowed to remain in the stain for sometime (for about 4-5 minutes).
3. Excess amount of stain is removed by washing the sections repeatedly with water. (This can be seen under the microscope. The stain should be taken either by lignified or non-lignified tissues. Otherwise the section should be washed till the stain disappears from one type of tissue).
4. If destaining is not achieved, sections are washed with acid alcohol. In this case, further washing with water is necessary till traces of acid are removed.
5. This is followed by transfer of sections to a watch glass containing counter-stain

(e.g., safranin, fast green, erythrosine). This stain acts on the tissue more rapidly than the principal stain. Therefore, section is kept in this stain for short period (about a minute or two).

- Excess of stain is removed by washing stained sections with glycerine (15-20%). The section should distinctly bring out demarcation between tissue system while preserving the colour of the stain.
- The section is now ready for mounting.

**(b) For permanent preparations.** In certain cases preparations need to be stored permanently as a future record. The method of preparation followed is described below.

- The section is first stained with principal stain (aqueous hematoxylin, safranin or crystal violet).
- The section is then washed with water till no more stain dissolves and water remains colourless.
- Section is passed through a graded series of alcohol for dehydration. A watch glass is filled with requisite amount of alcohol, (beginning with 30% alcohol) and the section is transferred to it. This watch glass should always be covered with another larger one. In order not to disturb the section, used alcohol is removed by glass dropper. All the 30% alcohol is replaced with 50% alcohol. This procedure is repeated till 70% of alcohol grade is reached.
- At this stage, counterstain is employed (e.g. safranin, fast green or erythrosine prepared in 80% or 90% alcohol).
- This stain acts quickly and as such section is washed immediately after the requisite time is over.
- Destaining is done by washing sections with 90% or 100% alcohol.
- The section is now transferred to absolute alcohol to complete the dehydration.
- Clearing now begins with 25% of xylol (25 cc of xylol and 75 cc of absolute alcohol). The sections are gradually passed through xylol series of 25%, 50%, 70%, 90% and finally transferred to pure xylol. If dehydration is not complete, pure xylol turns white or turbid. At this stage section should be passed through reverse series.

- Pure xylol is the last stage of clearing. Section is now ready for mounting.
- Mounting is done in Canada balsam.

### Specific Schemes for Staining Combinations

(for temporary and semi-permanent preparations)

1. Hematoxylin & safranin	2. Safranin & fast green or aniline blue
Select a section ↓	Select a section ↓
Stain with hematoxylin ↓	Stain with safranin (for 4-5 minutes) ↓
Wash with water ↓	Wash with water ↓
Wash with ammonia water till stain turns blue (tap water is suitable if alkaline) ↓	Destain with acid alcohol if necessary ↓
Wash with water ↓	Wash repeatedly with water ↓
Stain with safranin ↓	Stain with fast green or aniline blue (for about a minute) ↓
Wash with glycerine ↓	Wash with glycerine ↓
Mount in glycerine	Mount in glycerine

### [III] Mounting an object

Mounting is necessary to properly position an object for clear view. Lactophenol, glycerine and glycerine jelly are used for temporary mounting while Canada balsam is used for permanent mounting.

**1. Mounting media.** Following are some of the common media.

**(a) Canada balsam.** It is a resin obtained from a conifer—*Abies balsamea*, most suitable for permanent slide preparation. The material to be mounted should come through alcohol (dehydration) and xylol (clearing) series.

**(b) Lactophenol.** It is a mixture of equal parts of phenol crystals, lactic acid, glycerine (sometimes two parts) and distilled water. Stains may be mixed with this medium (e.g. cotton blue in lactophenol used to stain fungi) or copper acetate is added to preserve green colour of the pigment.

### Specific Schemes for Staining Combinations (for permanent preparations)

1. Hematoxylin & safranin	2. Safranin & fast green 3. Crystal violet & erythrosine
Select a section (If necessary use mordant)	Aqueous safranin/ crystal violet
↓	↓
Stain in hematoxylin (If necessary destain with mordant)	Water change, until colourless
↓	↓
Wash in ammonia water or tap water	Dehydration with 30% alcohol
↓	↓
Dehydration with 30% alcohol	50% alcohol
↓	↓
50% alcohol	70% alcohol
↓	↓
70% alcohol	90% alcohol
↓	↓
Stain with safranin	Stain with fast green/ erythrosine
↓	↓
Destain with 70% alcohol	Destain with 90% alcohol
↓	↓
90% alcohol	Absolute alcohol
↓	↓
Absolute alcohol	Clearing or de-alcoholizing with 25% xylol
↓	↓
Clear with 25% xylol	50% xylol
↓	↓
50% xylol	70% xylol
↓	↓
70% xylol	90% xylol
↓	↓
90% xylol	Pure xylol
↓	↓
Pure xylol	Mount in Canada balsam
↓	
Mount in Canada balsam	

(c) **Glycerine.** Pure glycerine diluted to 15-25% is widely used. Semi-permanent and temporary preparations are mounted in glycerine.

(d) **Glycerine jelly.** Jelly is also used for mounting. It is made of gelatin 1 : glycerine 7 : water 6.

Warm the gelatin for two hours by adding water. Phenol (1%) is added later. Add crystals of safranin if desired. Allow the solution to cool and settle into jelly.

Many other mounting media like cedar oil, dammar, balsam, venetian turpentines and synthetic resins are also used.

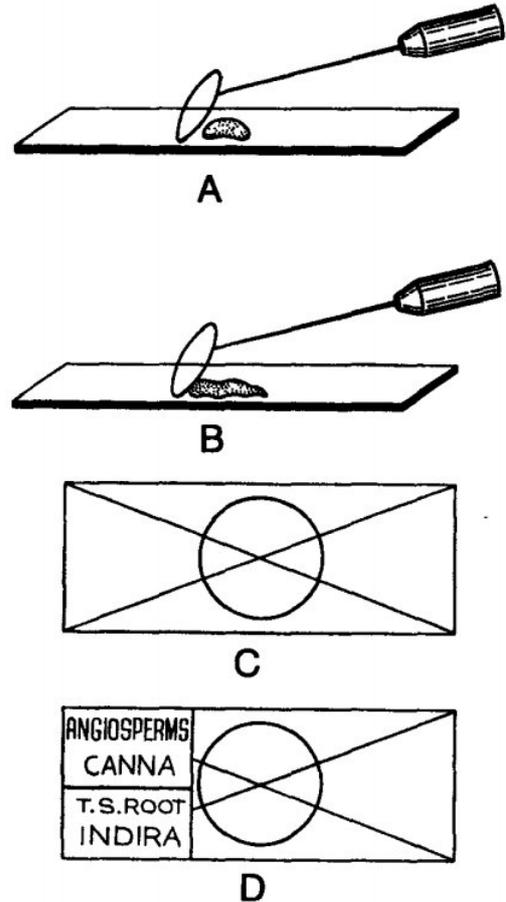


Fig. 6. Method of mounting coverslip.

**2. Care.** Following care should be taken during mounting—

1. Object should be mounted in the centre of the slide. A simple method may prove suitable for this purpose. Take a piece of thick and white cardboard sheet larger than the size of the slide. Place the slide over it. Draw lines along all the four edges. Join all the four corner points diagonally by two lines. The point, where these two lines cross, gives the centre of the slide. While mounting an object, place the slide over this drawn sheet and an object on the central point.
2. No air bubbles should enter the medium while mounting. This results in drying of medium and preparation is spoiled. To avoid air bubbles, touch one side of the coverslip to the drop of mounting medium on the slide. Support the coverslip by needle and lower it gradually before finally removing it.
3. Use the necessary small quantity of mounting medium so that it does not flow on to the

slide. If so, use little lesser quantity for the next preparation. The extra amount can be soaked by touching a piece of blotting paper to the edge of the coverslip.

4. Preparation should be clean, hence the edges of slide and the coverslip alone should be held between the fingers.
5. Labels are pasted uniformly on one side of the prepared slide. It should carry the name of the division or generic and specific names, the part mounted and the section's plane. At the bottom be written, the name of the student who has prepared the slide.

**3. Sealing the coverslip.** Temporary preparations can be sealed with Canada balsam, gum, dammar, nail polish, etc. Such a preparation is called a semi-permanent preparation.

Sealing is done by simply painting the edges of the coverslip with sealing agent in such a way that the space between the slide and the coverslip gets filled with the agent. It will prevent the mounting medium from drying.

Similarly ringing table should be used for sealing the round coverslips. The use of Canada balsam for ringing is more convenient.

#### [IV] Maceration

This is a technique of separating individual cells from a group or tissue by dissolution of pectic middle lamella. There are three common methods.

**1. Jeffery's method.** The following are the steps—

1. Cut the fresh or dried material into small slices thinner than a tooth-pick.
2. Fill the test tube with material. Boil it in water till it settles down at the bottom indicating that it is free from air.
3. Replace water with the following macerating solution— (i) 10% Nitric acid  
(90 cc water + 10 cc nitric acid)  
(ii) 10% Chromic acid  
(90 cc water + 10 cc chromic acid)  
Mix both these acids in equal parts.
4. Heat the test tube filled with macerating fluid.
5. Stop heating as soon as the material becomes soft and pulpy.
6. Transfer the fluid to a watch glass.

7. Drain out all the macerating fluid. Wash the material repeatedly with water till all the traces of acids are removed.
8. The material is now stained with safranin and destained with water.
9. The pulp of the material is crushed with the glass rod and teased by a needle so that it is spread over the slide.
10. The material is mounted in glycerine or glycerine jelly.

**2. Harlow's method.** The following are the steps—

1. Sliced and boiled material is treated with chlorine water for two hours.
2. It is then washed with tap water.
3. The material is now boiled in sodium sulphate for about 15 minutes.
4. The liquid is transferred to a watch glass.
5. The material is now washed repeatedly with water.
6. It is teased with needle or crushed with glass rod.
7. The teased material is evenly spread on the slide, stained in safranin and then mounted in glycerine or glycerine jelly.

**3. Schultze's method.** The following are the steps—

1. Material is sliced and boiled in a test tube filled with water.
2. The tube is now filled with concentrated nitric acid, to which a few crystals of potassium chlorate are added.
3. The test tube is heated slowly and gradually till the material is bleached white.
4. The liquid is then transferred to watch glass and drained out leaving only the material.
5. The material is now washed with water.
6. Later it is teased or crushed, till individual cells appear isolated.

#### [V] Peelings

The removal of leaf epidermis, to study the number, arrangement, distribution and structure of stomata, is called peeling. The method consists of breaking the leaf irregularly with a force. This easily separates a little part of the lower epidermis which remains protruding on the lower surface of the leaf. It is

pulled out so that a long ribbon or strip of lower epidermis gets removed. If lower epidermis does not separate easily, a needle or forceps is inserted, and a small part is first slowly broken. This can now be held in hand and considerably large strip is pulled apart.

The stripped lower epidermis is stained in safranin and washed. It can be mounted in glycerine or glycerine jelly. If permanent preparation is desired, normal procedure of dehydration and clearing is followed before mounting it in mounting medium.

### [VI] Smearing

Smearing is used to study the chromosomes. The method consists of spreading the cells in a single layer. The cells are smeared at a stage when they are in the process of cell division. This permits the study of chromosome structure and various stages of cell division. Pre-requisite for such studies is the killing of dividing tissues at a proper stage of cell division and selection of material where cells are not firmly united with one another by middle lamellae. Microsporocytes of *Trillium* spp., *Lilium* spp. and *Oenothera* spp., as well as anthers of *Tradescantia* spp., *Triticum* spp. and *Nicotiana* spp. and root tips of onion, *Ficus*, etc. fixed at appropriate time are widely used for smear preparations.

**1. Technique.** The following are the steps—

1. Slides should be perfectly clean for preparation of smears. In order to do so these are immersed in sulphuric acid potassium bichromate mixture or concentrated nitric acid for a long time.
2. Slides are thoroughly washed with running water and finally dried with absolutely clean cloth, free from dust and lint.
3. Fresh anthers dissected out from the buds are placed in the centre of slide. The anthers on the slide are crushed with scalpel or another clean slide.
4. Slide is now inverted over a petri dish containing killing fluid (most suitable being Randolph modified Navashin fluid), in a way that smeared surface comes in contact with the fluid. It should be allowed in this position for about 10-15 minutes.

5. Slide is now inverted with smeared side upward. It is now ready for staining. It may also be stained immediately without immersing in killing fluid.

**2. Staining procedure.** The method described below is called Belling's iron acetocarmine method. The slides are stained in the following way.

1. A few drops of acetocarmine are placed on the smeared material or unsmeared anthers are kept on slide in a drop of acetocarmine. After a few minutes, stain is replaced with a fresh drop of stain.
2. At this stage, anthers are crushed and large pieces and debris are removed.
3. Slide is gently heated over a flame, cover glass is placed on the material and uniform pressure is applied on the material by placing blotting paper on the cover glass and then pressing it.
4. Slide is immediately sealed with melted wax.

Another simple method is followed where anthers are smeared on the cover glass. It is then inverted on the slide with a drop of acetocarmine. Cover glass is sealed with slide by melted wax.

### [VII] Squash

This technique is also useful in the study of cell division especially mitosis and the chromosome structure. Root tips give the best results. For this purpose allow the onion bulbs to grow in bottle filled with water. If the lower root portion of the bulb touches the water, it quickly sends forth large number of roots. Cut the root tips and fix them.

1. Place the fixed root tip in a drop of 45% acetic acid.
2. Place a cover glass over the tip and diffuse acetocarmine.
3. Tap and apply uniform pressure over the cover glass.
4. The squash preparation is ready.

### [VIII] Micrometry

(Measurement by means of microscope)

This is the procedure used to measure the size of microscopic objects like cell, spore, pollen grain, etc. The method consists of using a calibrated ocular micrometer (a glass disc with engraved scale). The calibration is done by comparing ocular with stage

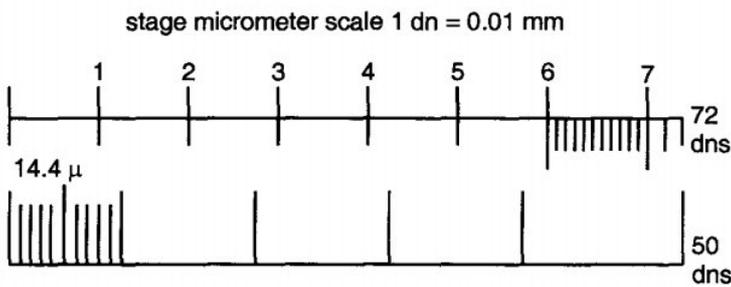


Fig. 7. Matching ocular micrometer with stage micrometer.

micrometer (a slide bearing an engraved scale of known values). The stage micrometer is usually ruled into tenths and hundredths of a millimeter (scales in hundredths of an inch are also obtainable). Each of the 100 parts of stage micrometer scale represents 0.01 mm or  $10\mu$  ( $1\text{ mm} = 1000\text{ microns or } \mu$ ).

**1. Calibration of ocular micrometer.** The calibration is done as follows.

1. Place the ocular micrometer inside the eye piece by unscrewing the upper lens.
2. The stage micrometer slide is now placed on the stage of the microscope and focussed to observe the scale.
3. The stage micrometer scale is moved in such a way that it lies by the side of the scale of ocular micrometer when focussed.
4. Now compare and count the divisions on both micrometers to find out the number of divisions where both scales are equally opposite.
5. For example when  $45\times$  objective and  $10\times$  eye piece are used, divisions of ocular micrometer are found equal to 72 divisions of stage micrometer.

6. Calibrate the ocular micrometer as given below.

Stage micrometer scale :

$100\text{ dns} = 1\text{ mm} (=100\mu\text{ or microns}^*)$

$1\text{ dn} = 0.01\text{ mm} (=10\mu\text{ or microns})$

If,  $50\text{ dns}$  (ocular micrometer)

$= 72\text{ dns}$  (stage micrometer)

then,  $50\text{ dns}$  (ocular micrometer)

$= 0.72\text{ mm} (=720\mu\text{ or microns})$

therefore,  $1\text{ dn}$  (ocular micrometer)

$= 0.14\text{ mm} (=14.4\mu\text{ or microns})$

**2. Measurement of objects.** The following method is useful in actually determining the size of objects. An example is given below.

\*One millimeter =  $1,000\mu$ .

$\mu$ , this Greek letter is an abbreviation for micron.

1. Thus as in above example (when objective  $45\times$  and eye piece  $10\times$  are used), each division of ocular (micrometer) would measure the distance of  $14.4\mu$  or microns.
2. Now remove the stage micrometer and place a slide with object to be measured.
3. Use oculometer (micrometer) to measure the width of a bacillus or diameter of a pollen grain or a fungal spore. For example a fungal spore measures 2 divisions.
4. The diameter of a fungal spore would be  $(2 \times 14.4\mu)$   $28.8\mu$ .

The length, breadth, diameter, etc. of different structures can be measured in this way.

## Record of Work

After the preparations are ready, these should be carefully observed, salient features noted and drawn on a practical record sheet. The following suggestions would prove useful.

1. Always use a sharp and pointed pencil for thin and uniform lines.
2. Punched holes should be on the left hand side of the drawing sheet.
3. Diagrams of the entire plant or its various aspects are drawn on the same page. The diagrams of unrelated specimens should in no case be drawn on the same page.
4. The sequence of the diagrams should always be—external features, anatomy and then reproduction.
5. For anatomical studies an outline diagram followed by a cellular sketch of its suitable sector are drawn one above the other on the same page.
6. All the parts of the diagram must be labelled. Capital letters are used for labelling. The labels are arranged one below the other in a row.
7. Labelling lines should never cross one another. Beautification and shading are not required until specific effects are to be produced.
8. Every diagram must have caption at its bottom (e.g. T.s. stem).
9. Date is written in the left hand corner of the page.
10. Classification and name of the plant are given in the right hand corner of the sheet.

11. The description is written either on the reverse side of the drawing sheet or on a new facing page.
12. During description only technical terms are used. The points of identification are added in the end.
13. Anatomical studies are described as others. A section should be described starting from epidermis to the central region; give thickness of layer (how many cells deep), shape and size of the cells constituting it. Also give in details of the structure of stele and vascular bundle.

### Collection

Field work is one of the most essential part in the Botanical study. It permits to come across many types of plants, otherwise not seen and available in the laboratory. It is, therefore, advisable to go round many localities and explore their vegetation. Organised excursions or outings, led by experienced persons, add to the knowledge of common plants in nature.

While on a collection trip, local or outstation, following things are to be carried along.

**1. Containers.** For packing the collected material, preferably carry plastic unbreakable containers or polyethylene bags.

**2. Preservatives.** Formalin-Acetic-Alcohol (FAA) or Alcohol 70% or Alcohol 90%, and/or Formalin 6%-10%.

**3. Other requirements.** Scalpel, knife, blade, forceps, pencil, paper, a hand lens, a bag or vasculum for keeping plants or plant press with many newspapers or blotting papers.

After collecting the plant, it should be immediately killed and preserved or pressed to avoid its rotting and dehydration. Plants are either sprinkled or immersed with a little of the killing agent at the spot. On return to the laboratory collected material should be transferred to new and suitable containers with fresh preservative. The plants should be completely immersed in the preservative.

A few plants e.g. filamentous algae, fungi, reproductive parts of bryophytes, fertile parts of pteridophytes and different parts of gymnosperms, if collected in large quantities, are preserved in

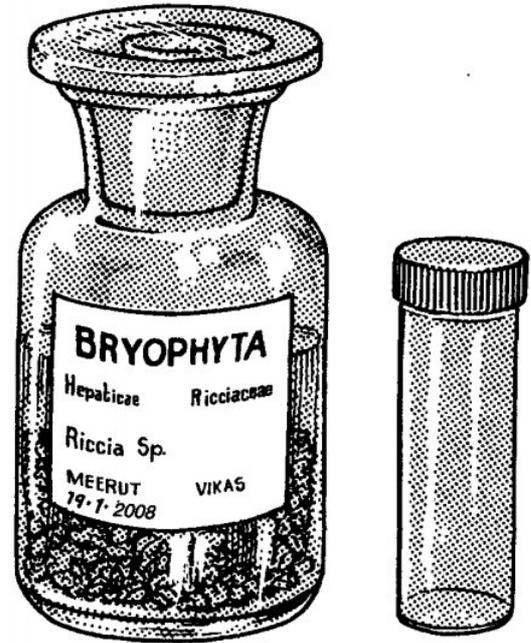


Fig. 8. Collection bottles.

containers. But if material (except a few algae and fungi) are collected in lesser quantities a herbarium sheet is prepared. Even if large quantity of such plants is available, one plant with fertile parts be preserved in the form of a herbarium sheet, while others should be packed in a container.

Every tube should be labelled. It is desired to write the name of the specimen, place and date of collection. The place of collection and date should also be written on a small piece of white card with a pencil, on the spot and inserted in the container. On return to laboratory, material is identified with the help of standard books. A label bearing name of the division and class to which the material belongs, the name of the material, date and place of collection and also the name of student is pasted on the container. All the containers should be of uniform size as far as possible.

### Herbarium

A collection of dried plant specimen, mounted on sheets is known as herbarium. Freshly-picked specimen are dried and pasted on mounting paper of regulation-sized herbarium sheets. The purpose of such a collection is to study the vegetation of a locality and maintain its record.

# Pteridophyta

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## Preamble

The pteridophyta include those plants which are sometimes called “**Vascular cryptogams**”, because of the presence of true xylem and hidden type of sexual reproduction. This group includes not only a large number of present day genera, but also a great many fossil types. Many of the genera which at the present day are quite small plants, have descended from ancient groups which in their days, formed large trees. Unlike bryophytes, the pteridophytes are found in great majority of habitats, ranging from aquatic to xerophytic. Generally they are land inhabiting but *Salvinia* and *Azolla* grow in aquatic habitat.

The members of the group vary greatly in form. These show two main evolutionary tendencies. One resulted in the production of large leaves and relatively small stems, and is known as the *megaphyllous* types. It is represented by ferns. The second tendency in which the leaves are small in relation to the stem and moreover the leaf trace leaves no gap in the stele, are known as the *microphyllous* types. This tendency is represented by club mosses and horse tails. The anatomy of these plants is to some extent dependent upon the type of the leaf they bear, but basically in all of them, the stem is divided into an outer cortex, and a central conducting system, the stele. A similar, though generally simpler structure is found in roots. The leaves in megaphyllous types consist of a petiole and a leaf blade or lamina with many veins. Microphyllous leaves are much simpler, with no petiole and usually only one vein.

The reproductive organs are generally borne either on the leaves or in the axils between the leaves and the stem. They are made up of little capsules called sporangia, in which are developed the spores. All the spores may be of the same size, when the plant is said to be the homosporous, or they may be of the two different sizes and the plant is called as heterosporous. In heterosporous types, the smaller spores are termed as microspores, and are developed in microsporangia, while in larger spores, which are generally produced in smaller numbers, are termed as megaspores, and are found in megasporangia.

Since the plant body of the members belonging to this group, produces spores, they represent the sporophytes, being comparable to the sporogonium of bryophyta. The gametophytes are, however, small and insignificant and bear sex organs. The fern plant and the moss plant, thus cannot be compared, as they belong to different generations of life cycle.

## Distinguishing Characters of Taxa

Various classifications of Pteridophytes have been proposed, time and again. Of these, a few classifications have been adopted by different workers. The classification followed in this book is a modified version of Riemers (1954). Below are given the characters of certain taxa, described in the book.

### DIVISION PTERIDOPHYTA

- (1) True roots generally present
- (2) Plant body differentiated into stem, roots and leaves

- (3) True vascular strand present

### SUB-DIVISION 1. PSILOPHYTOPSIDA

- (1) True roots absent
- (2) Sporangia borne at the tips of erect branches either singly or in pairs
- (3) Plants homosporous
- (4) Plants found only as fossils

### Order **Psilophytales**

- (1) Sporophyte dichotomously branched
- (2) Sporangia generally borne singly

### Family **Rhyniaceae**

- (1) Rhizoids unicellular, on rhizomes
- (2) Aerial portion leafless

## Classification of Pteridophyta

### Division—PTERIDOPHYTA

Sub-division	Class	Order	Family	Examples
1. Psilophytosida		Psilophytales	Rhyniaceae	<i>Rhynia</i> * <i>Horneophyton</i> *
2. Psilotopsida		Psilotales	Psilotaceae	<i>Psilotum</i>
3. Lycoposida		1. Lycopodiales	Lycopodiaceae	<i>Lycopodium</i>
		2. Lepidodendrales	Lepidodendraceae	<i>Lepidodendron</i> * <i>Lepidocarpon</i> *
		3. Selaginellales	Selaginellaceae	<i>Selaginella</i>
		4. Isoetales	Isoetaceae	<i>Isoetes</i>
4. Sphenopsida		1. Equisetales	Equisetaceae	<i>Equisetum</i>
		2. Calamitales	Calamitaceae	<i>Calamites</i> *
5. Pteropsida	1. Eusporangiatae	1. Ophioglossales	Ophioglossaceae	<i>Ophioglossum</i>
	2. Leptosporangiatae	2. Marattiales	Angiopteridaceae	<i>Angiopteris</i>
		1. Filicales	1. Adiantaceae	<i>Adiantum</i>
			2. Polypodiaceae	<i>Dryopteris</i> <i>Lastraea</i> <i>Nephrolepis</i> <i>Polypodium</i> <i>Pteridium</i>
		2. Marsileales	Marsileaceae	<i>Marsilea</i>
		3. Salviniales	1. Salviniaceae	<i>Salvinia</i>
			2. Azollaceae	<i>Azolla</i>

\* Fossils members

Examples *Rhynia*\*, *Horneophyton*\*

#### SUB-DIVISION 2. PSILOTOPSIDA

- (1) Sporangia are borne in the axils of scaly appendages or foliage leaves
- (2) Plants living, true roots absent and homosporous

##### Order Psilotales

- (1) Sporophyte dichotomously branched
- (2) Sporangia generally borne singly
- (3) Stele protostele to actinostele
- (4) Eusporangiate and homosporous

##### Family Psilotaceae

- (1) Axis branched
- (2) Scale leaves small and minute

Example *Psilotum*

#### SUB-DIVISION 3. LYCOPSIDA

- (1) Leaves microphyllous
- (2) Sporangia borne singly on adaxial face of the sporophyll or in its axil
- (3) Sporophytes homosporous

##### Family Lycopodiaceae

- (1) Leaves without ligules

- (2) Sporophylls and foliage leaves may be similar or dissimilar in shape

Example *Lycopodium*

##### Order 2. Lepidodendrales

- (1) Plants tree-like
- (2) Secondary tissues formed due to cambium
- (3) Leaves microphyllous and ligulate
- (4) Strobili heterosporous.

##### Family Lepidodendraceae

- (1) Aerial portion freely branched
- (2) Strobili at the tips of branches
- (3) Trunk and branches with spirally arranged leaf scars

Examples *Lepidodendron*\*, *Lepidocarpon*\*

##### Order 3. Selaginellales

- (1) Each foliage leaf with a ligule at the base on adaxial side
- (2) Sporophytes heterosporous

##### Family Selaginellaceae

- (1) Stem herbaceous and dorsiventral or erect
- (2) Gametophytes extremely reduced

Example *Selaginella*

**Order 4. Isoetales**

- (1) Herbaceous sporophytes with a massive rhizomorph at the base of the stem
- (2) Leaves microphyllous and ligulate
- (3) Sporophytes heterosporous
- (4) Sporophylls may or may not be grouped in strobili
- (5) Antherozoids multiflagellate

**Family Isoetaceae**

- (1) Stem corm-like
- (2) Sporophylls bearing sporangia on adaxial face, not grouped in strobili

Example *Isoetes***SUB-DIVISION 4. SPHENOPSISIDA**

- (1) Stem branched, articulated, ridged and furrowed with distinct nodes and internodes
- (2) Leaves microphyllous, small, scaly and arranged in whorls at the nodes

**Order 1. Equisetales**

- (1) Stem branched, branches in transverse whorls
- (2) Internodes alternate with one another
- (3) Vascular cylinder siphonostele, endarch

**Family Equisetaceae**

- (1) No secondary growth
- (2) Monosporous
- (3) Sporangia borne on sporangiophores which form a compact cone

Example *Equisetum***Order 2. Calamitales**

- (1) Tree-like sporophytes with considerable secondary thickening of stem and branches

**Family Calamitaceae**

- (1) Stem branched, branches in whorls at nodes
- (2) Stem shows endarch siphonostele

Example *Calamites\****SUB-DIVISION 5. PTEROPSISIDA**

- (1) Vascular cylinder siphonostelic, with leaf gaps
- (2) Leaves megaphyllous, compound with rachis
- (3) Leaves bear sporangia in sori
- (4) Gametophytes small, green and free-living

**CLASS 1. EUSPORANGIATAE**

- (1) Sporangium develops from a group of initials (eusporangiate development)
- (2) Sporangial jacket more than one cell in thickness
- (3) Large number of spores within sporangium

- (4) Sporangia borne in spike or sori situated on the abaxial surface of the leaf.

**Order 1. Ophioglossales**

- (1) Sporangia borne on a special structure called spike. It projects adaxially from a leaf and near the junction of blade and petiole.

**Family Ophioglossaceae**

- (1) Single family, characters same as the order

Example *Ophioglossum***Order 2. Marattiales**

- (1) Young leaves with circinate vernation
- (2) Leaves with fleshy stipules

**Family Angiopteridaceae**

- (1) Sporangia almost free
- (2) Sporangia linear in two rows on both the sides of the veins

Example *Angiopteris***CLASS 2. LEPTOSPORANGIATAE**

- (1) Sporangium develops from a single initial cell (leptosporangiate development)
- (2) Sporangial jacket one cell in thickness
- (3) Definite number of spores

**Order 1. Filicales**

- (1) Homosporous

**Family 1. Adiantaceae**

- (1) Sori apparently marginal but superficial in origin
- (2) Indusia oblong or linear, usually many and distinct
- (3) Leaflet margins bearing sori sharply reflexed

Example *Adiantum***Family 2. Polypodiaceae**

- (1) Annulus vertical
- (2) Each sporangium with 32 to 64 spores

Examples *Dryopteris*, *Lastraea*, *Nephrolepis*, *Polypodium*, *Pteridium***Order 2. Marsileales**

- (1) Members heterosporous
- (2) sporangia formed within sporocarps

**Family Marsileaceae**

- (1) Members aquatic
- (2) Sorus of gradate type, each producing both the types of sporangia
- (3) Leaf circinate coiled in bud condition

Example *Marsilea***Order 3. Salviniiales**

- (1) Members heterosporous

- (2) Sporangia produced within sporocarps
- (3) Sporocarp contains either a single megasporangium or numerous microsporangia
- (4) The wall of the sporocarp is a modification of an idusium

Family 1. **Salviniaceae**

- (1) Sporocarps globose or ovoid, all of them of the same size

Example *Salvinia*

Family 2. **Azollaceae**

- (1) Sporocarps of two types, one is larger and male-microsporocarp and the other smaller and female-megasporocarp.

Example *Azolla*

***Psilotum***

**Classification**

Division	—	<b>Pteridophyta</b>
Sub-division	—	<b>Psilotopsida</b>
Order	—	<b>Psilotales</b>
Family	—	<b>Psilotaceae</b>
Genus	—	<b><i>Psilotum</i></b>

**Exercise 1**

**Object :** Study of external features of the plant.

**Work procedure**

Study the plant specimen.

**Comments**

1. The plant body may be pendent or erect and dwarfed (about 8 cm high) or may reach a height of 75-100 cm.
2. It is differentiated into (i) a basal rhizomatous system and (ii) aerial branches.
3. Basal rhizomatous system is subterranean, brown and rootless. The rhizome is repeatedly dichotomously branched and remains covered by small scales. The rhizome bears aerial branches.
4. Aerial shoots may be pendent (epiphytic species) or erect (terrestrial species). The slender and green aerial system is freely and dichotomously



Fig. 1. *Psilotum*. External features.

branched. The basal part of the shoot is cylindrical with longitudinal ribs. The distal green portion is radially cylindrical with three longitudinal ribs.

5. Aerial shoots bear many, small and scale-like, irregularly distributed scale leaves.
6. Sporangia are borne in triads (synangium) on very short stalks in axil of leaves (bifid), mostly towards the tip of the aerial branches.

**Exercise 2**

**Object :** Study of anatomy of the rhizome.

**Work procedure**

Cut a T.s. of the rhizome, stain in safranin and fast green combination, mount in glycerine and study.

**Comments**

1. The transverse section is almost circular in outline.

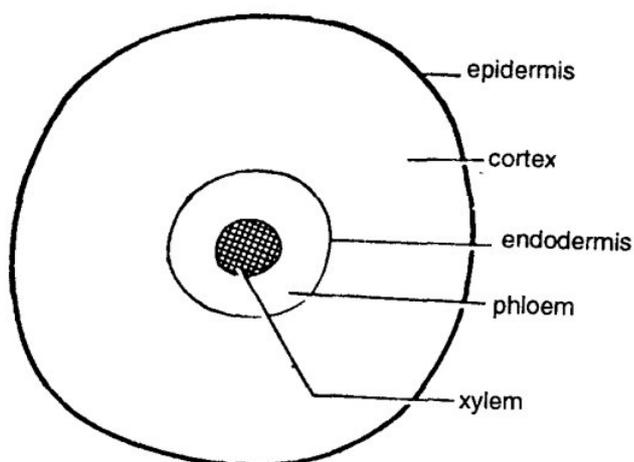


Fig. 2. *Psilotum*. T. s. rhizome (outlines)

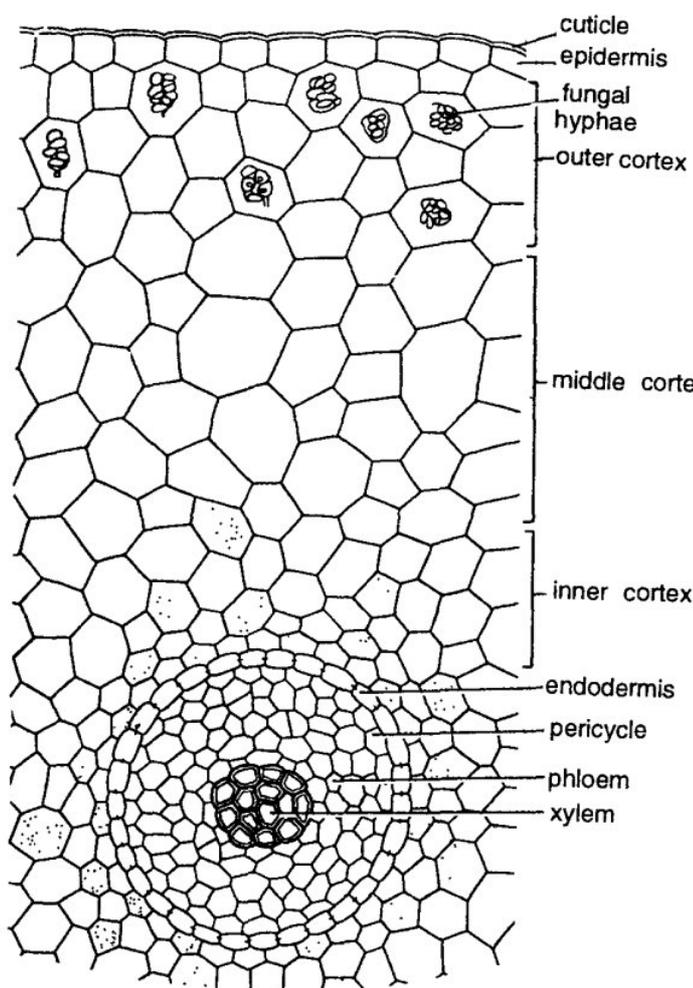


Fig. 3. *Psilotum*. T.s. rhizome (cellular).

2. It shows three distinct regions—epidermis, cortex and stele.
3. The cuticularised epidermis consists of rectangular or square cells, not much different from the underlying cells. The cells are slightly thick on their outer tangential faces.

(B-14)

4. The cortex is divisible into three regions—
  - (i) Outer: The cells are thin walled and parenchymatous containing the hyphae of endophytic mycorrhiza.
  - (ii) Middle : The cells are thin walled and parenchymatous with abundant starch grains.
  - (iii) Inner : The cells are small, thin walled and parenchymatous. These are coloured brown due to the presence of tannins.
5. Endodermis separates cortex from the stele. The cells are radially elongated and bear distinct casparian strips.
6. The centrally located stele is a protostele. It remains enclosed by a pericycle situated next to the endodermis. Pericycle is single layered and parenchymatous.
7. In the centre of the stele is a solid xylem strand, consisting of a few tracheids (the number and lobes of xylem vary with the age of rhizome. In young condition only 2-3 tracheids and a solid strand is present while in mature rhizome, number of tracheids increases and core becomes progressively lobed). The distinction between metaxylem and protoxylem elements is not clear.
8. The phloem extends from the xylem strand upto the pericycle thus completely surrounding the xylem strand.

### Exercise 3

**Object :** To study the anatomy of aerial shoot.

### Work procedure

Cut a T.s. of the aerial shoot, stain in safranin-fast green combination, mount in glycerine and study.

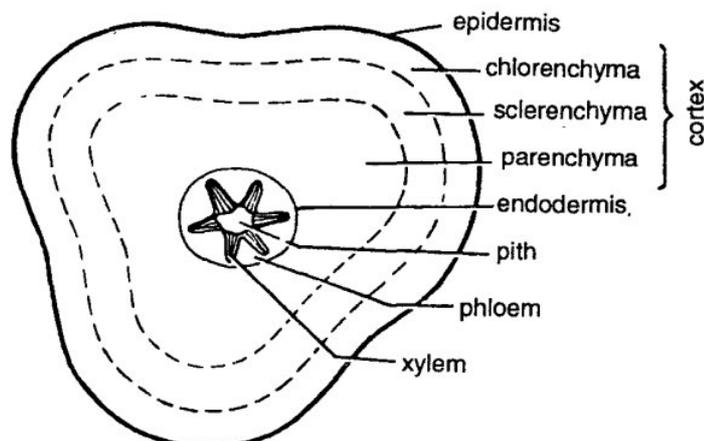


Fig. 4. *Psilotum*. T.s. aerial branch (outlines).

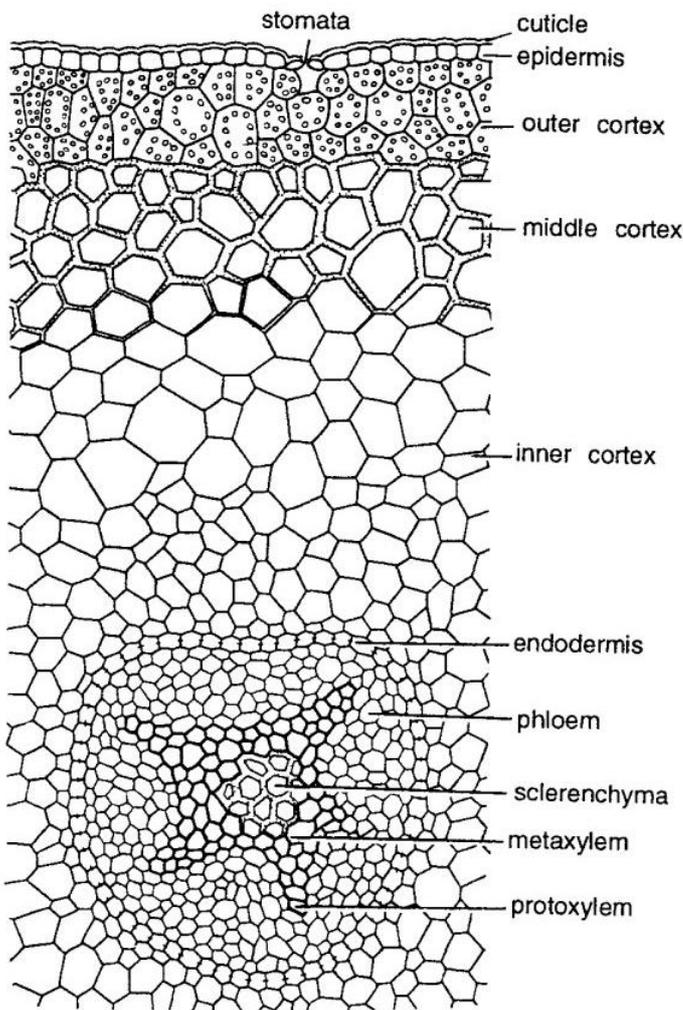


Fig. 5. *Psilotum*. T.s. aerial branch (a part cellular).

### Comments

1. The transverse section appears slightly triangular in outline.
2. It shows three distinct regions—epidermis, cortex and stele.
3. **The epidermis** consists of single layered, rectangular cells. The outer tangential walls are heavily cutinised and covered by a definite cuticle. Stomata are slightly sunken and are situated mainly in areas between the longitudinal ribs.
4. Internal to the epidermis is a broad cortex distinguishable into three zones—outer, middle and inner.
5. **Outer cortex**—the photosynthetic region, about 2-5 cells broad, is composed of vertically elongated cells and with small intercellular spaces. These cells contain numerous small chloroplasts.

6. **Middle cortex.** This zone is made of thick walled cells. The cells are sclerenchymatous and become progressively thinner towards inner zone.
7. **Inner cortex** is many celled broad, parenchymatous and contain numerous starch grains progressively towards centre.
8. **Endodermis** separates stele and cortex. The cells of the endodermis are tangentially elongated and exhibit distinct casparian strips on the radial end walls.
9. **The stele** is actinostelic, generally with six lobes. Each lobe has a few protoxylem elements at the tip while metaxylem is situated at its base.
10. The lobes of xylem are surrounded by phloem which extends upto the endodermis. (Typical sieve tubes are said to be absent).
11. Centre of the xylem is occupied by thick walled sclerenchymatous fibres with simple pits on their walls.

### Exercise 4

**Object :** To study the anatomy of leaf.

### Work procedure

Study a slide of T.s. of leaf.

### Comments

1. The leaf is divisible into (i) epidermis, (ii) cortical tissue and (iii) a small leaf trace if present.
2. **The epidermis** consists of thin walled cells while the rest of the foliar appendage is filled by photosynthetic tissue.
3. **A leaf trace** ends into the base of foliar appendage (e.g. *P. flaccidium*), however, in *P. nudum* there is no vascular bundle.

### Exercise 5

**Object :** To study the spore producing organ—the syngonium.

### Work procedure

Study the external features of syngonium, slide of T.s. and also a single spore.

(B-14)

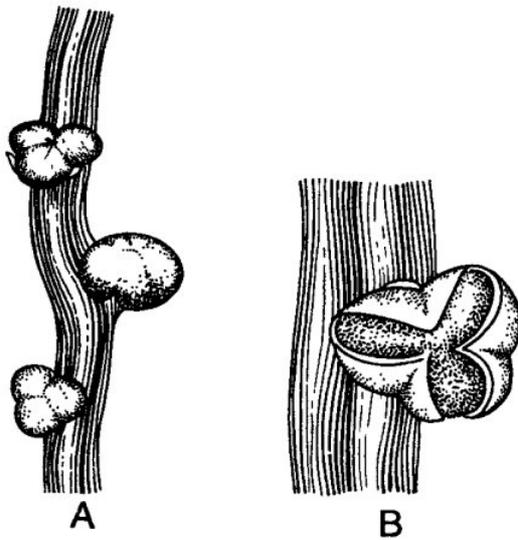


Fig. 6. *Psilotum*. A. Aerial branch with synangia, B. A single synangium.

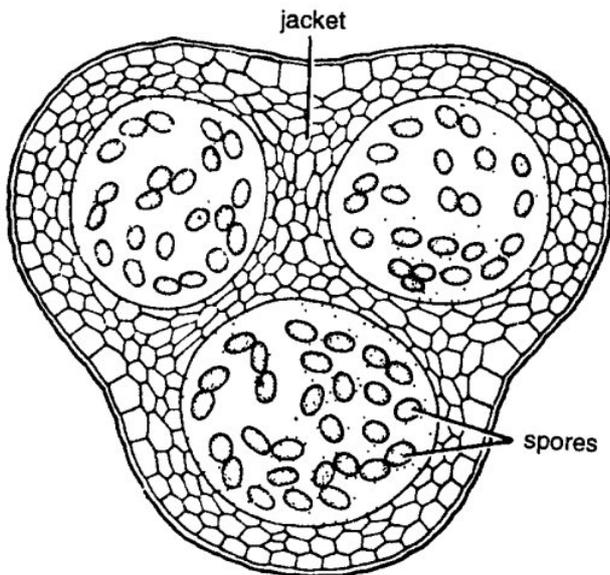


Fig. 7. *Psilotum*. T. s. synangium.

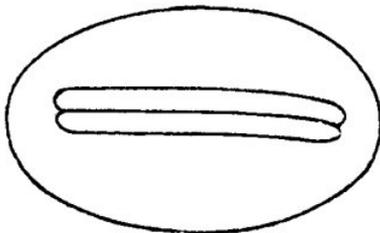


Fig. 8. *Psilotum*. A single spore.

### Comments

1. Sporangia, the spore producing organs, are produced on the aerial branches.
2. These are borne in triads on minute appendages subtended by a bract. (Since the sporangia are fused with one another, the structure is called as synangium).
3. In a transverse section, synangium reveals 4-5 layered jacket, outer of which is made of thick walled cells. The loculi are filled with numerous spores. Interspersed among the spores are

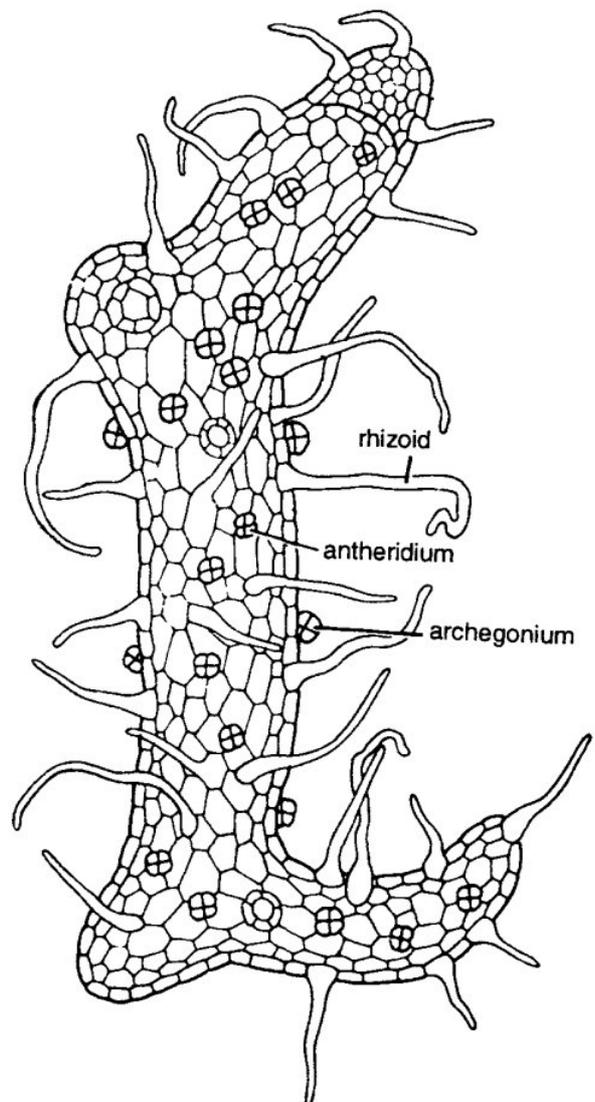


Fig. 9. *Psilotum*. A gametophyte.

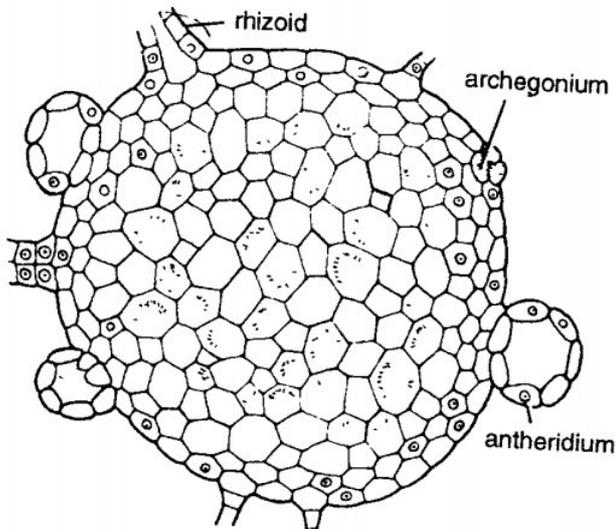


Fig. 10. *Psilotum*. T.s. gametophyte.

disintegrated sporocytes which serve as nutritional fluid.

- Individual spores are bean-shaped or bilaterally symmetrical. The wall pattern is reticulate. There is a narrow slit with a median ridge.

### Exercise 6

**Object : Study of gametophyte.**

### Work procedure

Study the slide of prothallus.

### Comments

- Gametophyte is subterranean and colourless. It is saprophytic and lives through the medium of symbiotic phycomycetous fungi.
- Gametophytes are irregularly cylindrical and once or twice dichotomously forked. Rhizoids are also given out.
- Gametophytes are homothallic. Sex organs are scattered over the entire surface. Archegonia are more in number than antheridia (or otherwise).

### Identification

**Division—Pteridophyta.** (1) True roots generally present (except in Psilopsida), (2) True vascular strand present.

**Sub-division—Psilotopsida.** (1) True roots absent, (2) Shoot differentiated into subterranean rhizome and aerial portion, (3) Sporangia borne terminally.

**Order—Psilotales.** (1) Sporophyte dichotomously branched, (2) Sporangia generally borne singly, (3) Stele protostele, generally actinostele, (4) Eusporangiate and homosporous.

**Family—Psilotaceae.** (1) Axis branched, (2) Scale leaves small and minute

**Genus—Psilotum.** Sporangia borne in triads (synangium).

### Hints for Collection

Only two species of *Psilotum* viz. *P. nudum* (= *P. triquetrum*) and *P. flaccidum* (= *P. complanatum*) are known. These are widespread in tropical and subtropical regions of both the hemispheres. *P. nudum* is of widespread occurrence and is known to occur at Pachmarhi in M. P. and Darjelling, West Bengal. It is primarily terrestrial and is collected from crevices of rocks and occasionally as epiphyte on tree ferns and palms.

## *Lycopodium* (Club Moss)

### Classification

<i>Division</i>	—	<b>Pteridophyta</b>
<i>Sub-division</i>	—	<b>Lycopsida</b>
<i>Order</i>	—	<b>Lycopodiales</b>
<i>Family</i>	—	<b>Lycopodiaceae</b>
<i>Genus</i>	—	<b><i>Lycopodium</i></b>

The genus *Lycopodium* is divided into 2 sub-genera—Urostachya and the Rhopalostachya. The following are the differences between the two.

Urostachya	Rhopalostachya
1. Plant body erect or pendent.	1. Plant generally trailing or creeping.
2. Branching rare; if present always dichotomous.	2. Branching is dichotomous at the base but in the upper region only one of the branches grows more prominently.
3. The roots take their origin only from the basal part of the stem.	3. Adventitious roots may arise from any part of the stem.
4. Organized strobili are rarely found.	4. Strobili are always well organized and borne on long stalks.
5. Sporophylls are almost similar to the foliage leaves, the only difference is in their size.	5. Sporophylls are different from foliage leaves. They are pale yellowish and chaffy.
6. The margins of sporophylls are entire.	6. Margins of sporophylls are toothed.
7. The spores possess a pitted surface without any external outgrowth.	7. Spores possess reticulate surface

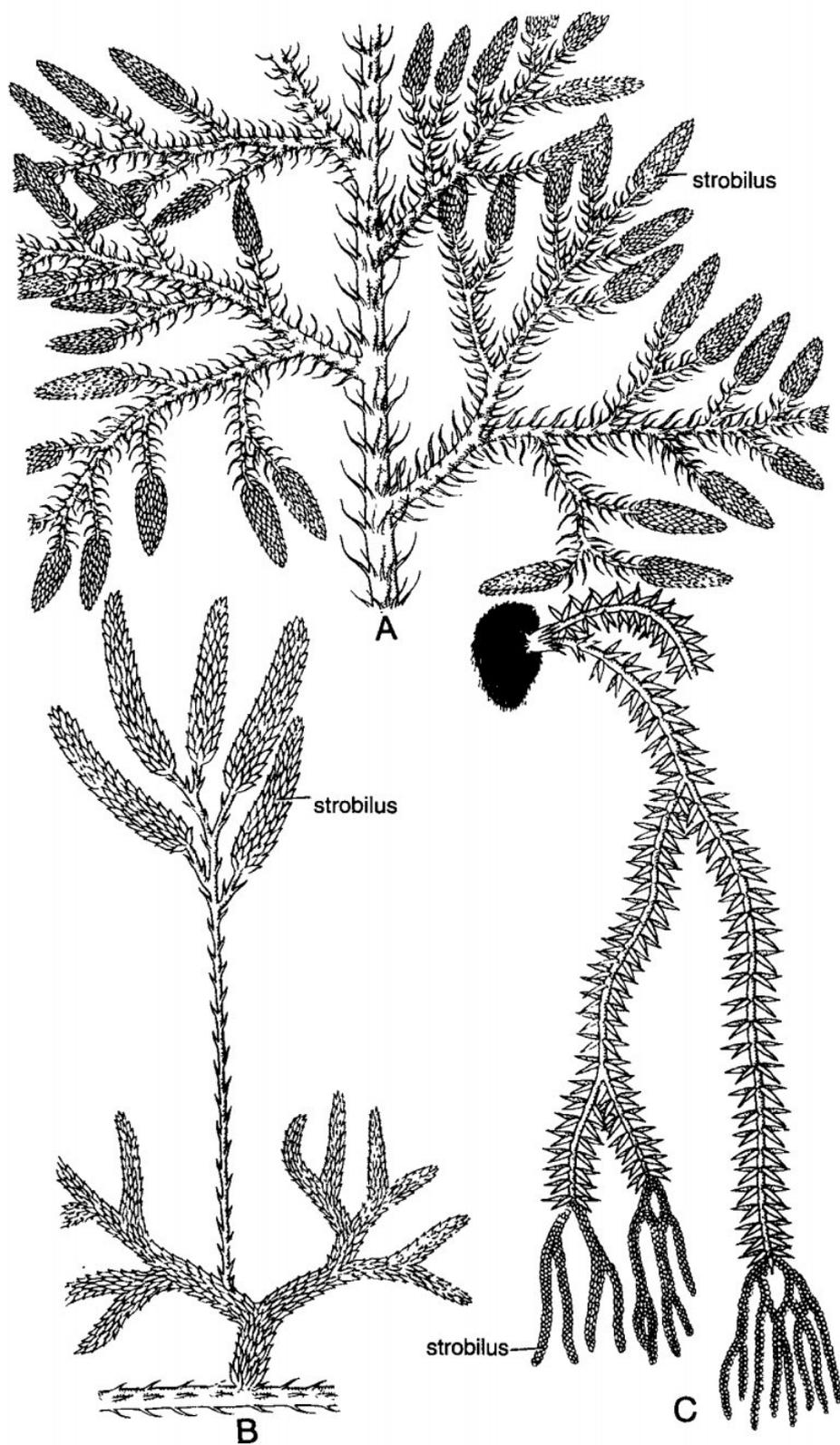


Fig. 1. *Lycopodium*. Sporophytes with strobili. A. *L. cernum* (terrestrial), B. *L. clavatum* (terrestrial), C. *L. phlegmaria* (epiphytic).

**Exercise 1****Object : Study the external morphology.****Work procedure**

Study the specimen. Observe the differentiation of plant body into root, stem and leaves. Study the leaves and the stomata.

**Comments**

1. **The plant body** consists of creeping rhizome which gives off slender, elongated aerial branches from the upper side and adventitious roots from the lower. The aerial branches vary from 3-8 inches in length. *L. cernum* is exceptional in attaining a height of 2 feet or more.
2. **Habitat.** Most of the species are terrestrial and the sporophyte may either have an upright stem or a horizontally creeping stem. Some species grow as epiphytes on higher plants which show pendent habit e.g. *L. phlegmaria*, *L. squarrosum*.
3. **The branching** is mostly dichotomous but in some species it may be monopodial also.
4. **The stem** and its branches are densely covered with small leaves present in close spirals or whorls.
5. **The leaves** are entire, small and membranous, rarely, exceeding 1 cm in length. Each leaf is supplied by a single mid-vein which runs almost unbranched right upto the apex.
6. **Epidermis.** The walls of the epidermal cells of the leaf are sinuous.
7. **Stomata.** Stomata are more or less parallel to the midrib. These are equally distributed on both of the leaf surfaces.

**Exercise 2****Object : Study of anatomy of root.****Work procedure**

Cut a T.s. of the root, stain in safranin and fast green combination, mount in glycerine and study.

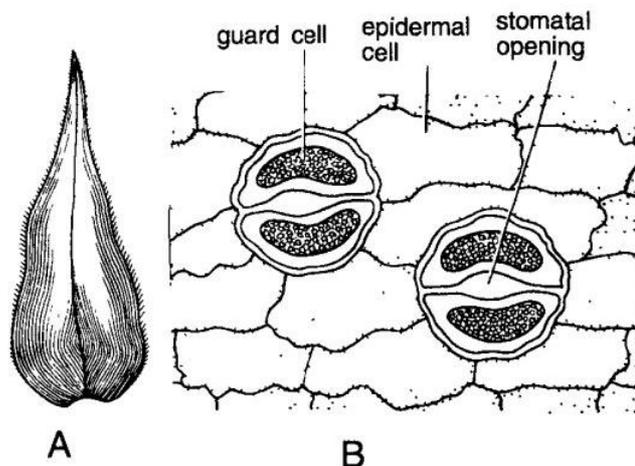


Fig. 2. *Lycopodium*. A. Entire leaf, B. A part of leaf epidermis showing stomata.

**Comments**

1. The root is differentiated into an epidermis, cortex and the stele.
2. **The epidermis** is single layered and gives rise to root hairs, the latter occur in pairs.
3. **The cortex** is several layered and in older roots a few of the outer layers become sclerified. The inner cells are thin walled and parenchymatous without any intercellular spaces.
4. **The stele** ranges from monarch to tetrarch but generally it is diarch with two protoxylem masses.

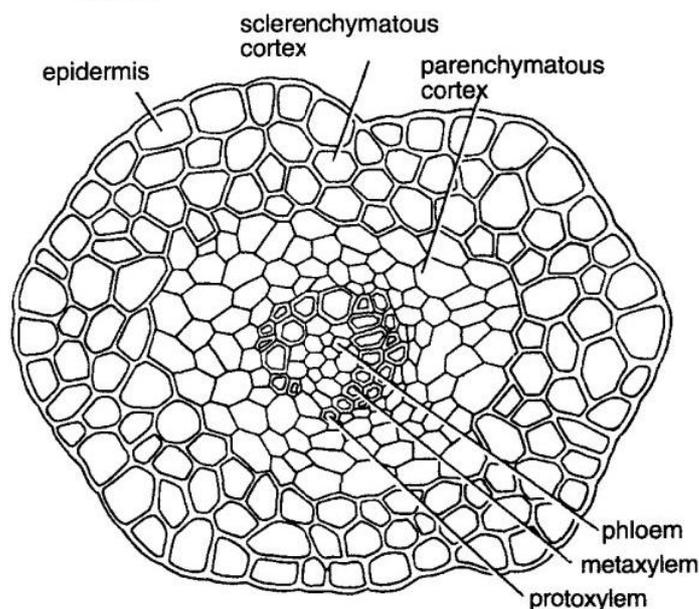


Fig. 3. *Lycopodium*. T.s. root.

5. The xylem is C or U shaped and is so oriented that the opening of C or U faces away from the stem.
6. The protoxylem is present at the tips of C or U and the intervening portion consists of metaxylem.
7. The phloem is present in between the arms of C or U.

### Exercise 3

**Object : Study of anatomy of stem.**

#### Work procedure

Cut T.s. of the stem, stain in safranin-fast green combination, mount in glycerine and study.

#### Comments

1. A transverse section of the stem shows an epidermis, a wide cortex and a stele.
2. **Epidermis** is single layered and is provided with stomata.
3. **The stem** has certain ridges and grooves. Chloroenchyma is present in the ridges.
4. **Cortex.** The structure varies from species to species. In some species, it is parenchymatous throughout, in others the inner and other portions are thick walled, and in still others the entire cortex is thick walled.
5. **Endodermis.** It is single layered and lies inner to cortex. In older stems, however, the endodermis may not be well defined.
6. **Pericycle.** The endodermis is followed by this layer which is composed of 3 to 6 cells.
7. **Stele.** The centre is occupied by a protosteles. Three different types of steles are found in species of *Lycopodium*.
  - (i) In some species viz. *L. clavatum* and *L. complanatum*, it is definitely organised into a plectosteles i.e. xylem and phloem occur in alternating bands that are more or less parallel.
  - (ii) In other species viz. *L. seratum* and *L. phlegmaria*, it is star-shaped with 4 arms in which grooves are occupied by phloem. This is known as actinosteles.
  - (iii) In still other species, as exemplified by *L. cernuum*, it is a haplosteles in which the xylem strands lie scattered in phloem.

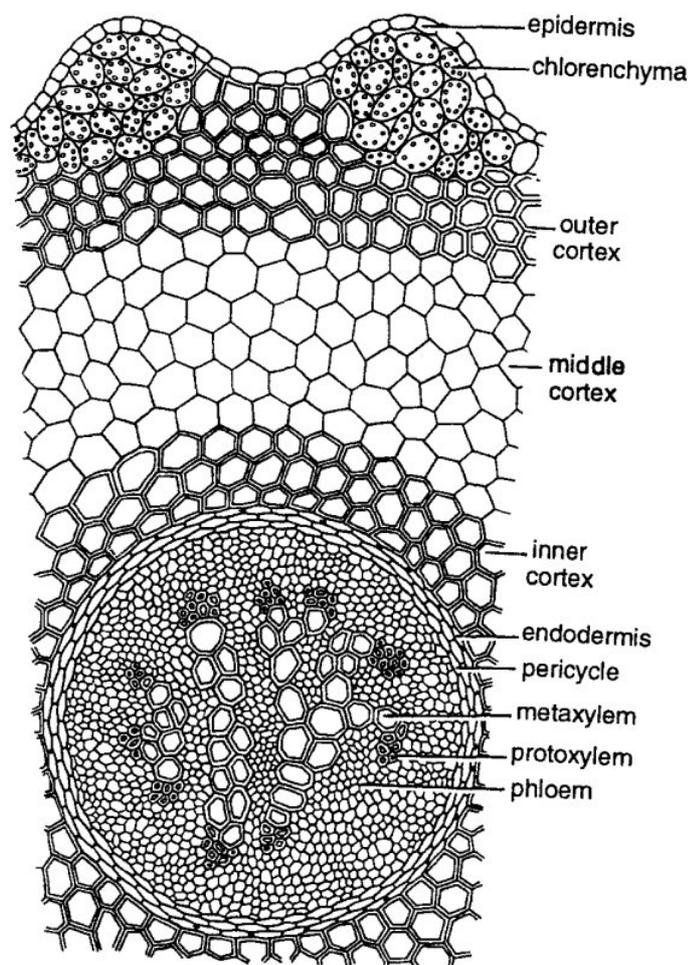


Fig. 4. *Lycopodium*. T.s. stem (a part cellular).

8. **The xylem** is exarch with protoxylem facing towards the periphery and metaxylem towards the centre.
9. **The tracheids** in metaxylem are scalariform while in protoxylem they are spiral or annular.
10. **The phloem** consists of unicellular sieve tubes, with numerous sieve plates, and phloem parenchyma. The companion cells are absent.
11. **Leaf traces** are seen to traverse the cortex.

### Exercise 4

**Object : Study the anatomy of leaf.**

#### Work procedure

Place the leaf in pith, cut T.s., stain in safranin-fast green combination, mount in glycerine and study.

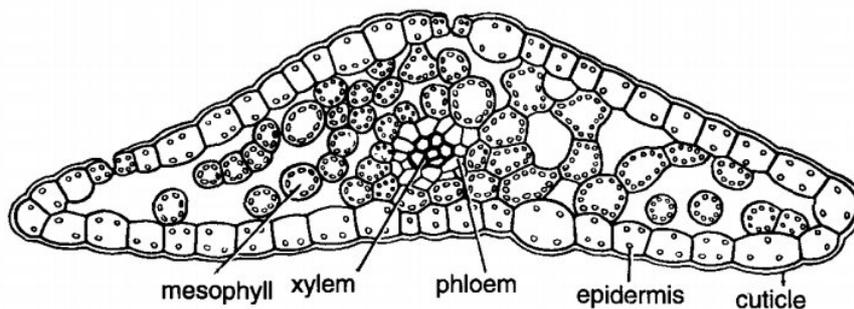


Fig. 5. *Lycopodium*. T.s. leaf.

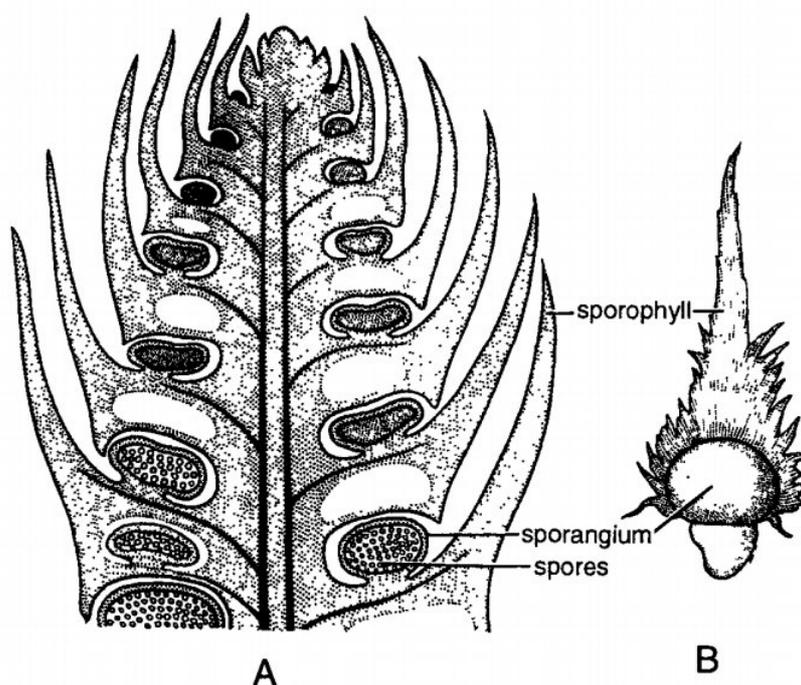


Fig. 6. *Lycopodium*. A. L.s. apical portion of strobilus. B. Dorsal view of sporophyll and a sporangium.

### Comments

1. The epidermis is single layered. The stomata are equally distributed on both the sides.
2. The leaf has a single vascular strand which is concentric with xylem in the centre.
3. The cells between the epidermis and the vascular strand form spongy parenchyma.

### Exercise 5

**Object : Study of spore producing organ.**

### Work procedure

Study the strobili, L.s. of strobilus and the spores; by observing a prepared slide.

### Comments

1. **External features of strobilus.** Sporangia are the spore producing organs. These are grouped to form strobili which are situated at the apices of branches. (In *L. selago* distinct strobilus is lacking and the vegetative and reproductive regions alternate each other.)
2. **L.s. of the strobilus** shows a central strobilar axis with spirally arranged sporophylls.
3. Each sporophyll bears a sporangium near its base on the adaxial side.
4. A sporangium is a black, kidney shaped structure, with a long or short massive stalk.
5. Sporangium consists of a wall and the cavity with spore.

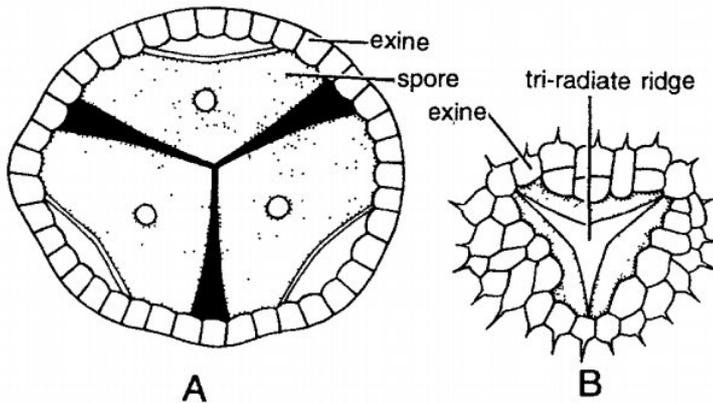


Fig. 7. *Lycopodium*. A. Spore tetrad, B. A spore.

6. The wall of sporangium is several layered thick. Tapetum forms the innermost layer.
7. The cavity has many spores, arranged in tetrahedral tetrads. Since all the spores are of one type, the plant is called homosporous.
8. Each spore is a minute structure with a tri-radiate ridge. It has a thick and spiny exine and a thin and membranous intine.
9. The spores germinate to form the prothallus.

### Exercise 6

**Object :** To study the gametophyte.

### Work procedure

Study the slide showing gametophyte. Observe the structure of sex organs.

### Comments

1. The gametophytes may be subterranean or sub-aerial.
2. The sub-aerial type is green, about 2 or 3 mm in length and bears the sex organs.
3. The subterranean type is non-green and is bigger as compared to the sub-aerial type. It generally consists of a tuberous body with a lobed crown that bears the sex organs.
4. The prothallus is monoecious and the sex organs lie almost wholly embedded in the tissue of the prothallus except the uppermost portion.
5. The antheridium is spherical with a single layer of jacket, containing within, a number of antherozoids or antherozoid mother cells. The antherozoids are fusiform and biciliate.

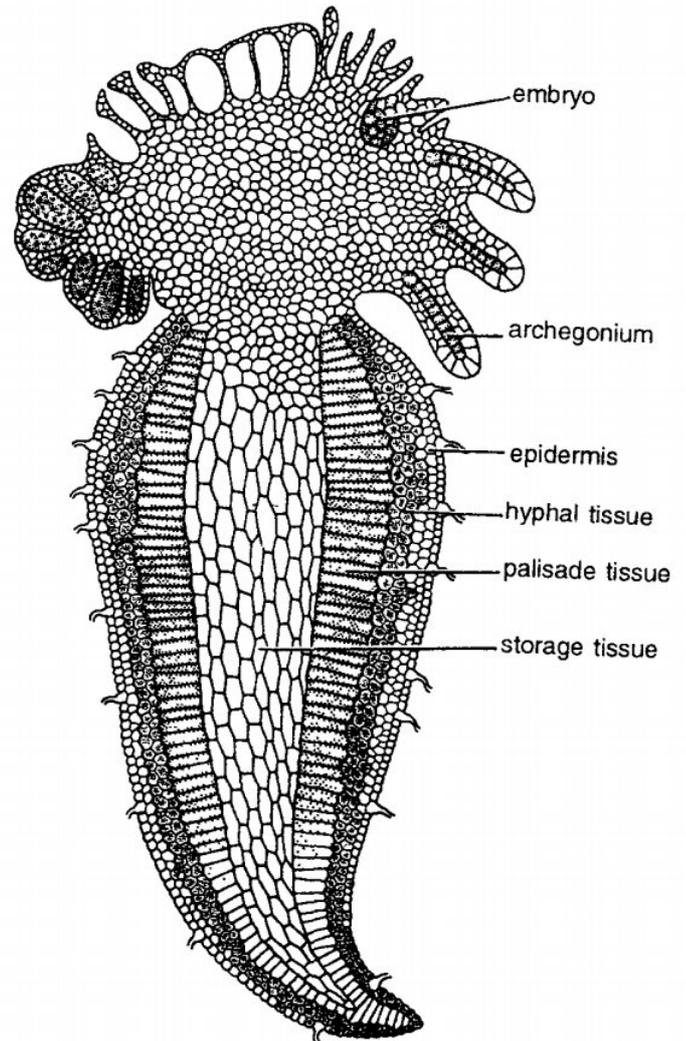


Fig. 8. *Lycopodium*. V.s. of mature gametophyte.

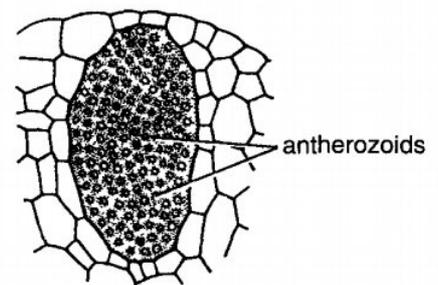


Fig. 9. *Lycopodium*. Mature antheridium.  
cover cells

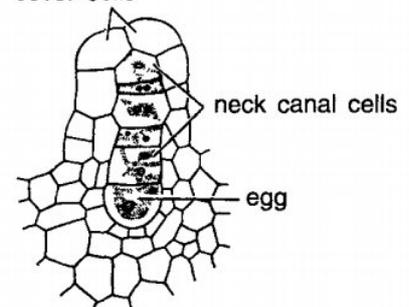


Fig. 10. *Lycopodium*. Nearly mature archegonium.  
neck canal cells  
egg

6. The archegonium is a narrow elongated structure. It has a narrow venter with an egg and venter canal cell and a long neck generally containing 4-6 neck canal cells.

### Identification

**Division—Pteridophyta.** (1) Plant body differentiated into stem, roots and leaves, (2) A definite vascular strand present.

**Sub-division—Lycopsida.** (1) Laves microphyllous, (2) Sporangia borne singly on adaxial face of the sporophyll or in its axil, (3) Sporophylls borne in strobili.

**Order—Lycopodiales.** (1) Stem has protosteles, (2) Sporophytes homosporous.

**Family—Lycopodiaceae.** (1) Leaves without ligules, (2) Sporophylls and foliage leaves may be similar or dissimilar in shape.

**Genus—Lycopodium.** (1) The sporophyte is long and always more than 2 inches, (2) Sporangia kidney-shaped, (3) Stele either a plectosteles or actinosteles or haplosteles.

### Hints for Collection

Out of the 100 species of *Lycopodium* about 18 occur in India. These are found in Himalayan and sub-Himalayan tracts, Garhwal, Assam, Bengal and also in Nilgiris.

## Selaginella (Small Club Moss)

### Classification

Division	—	<b>Pteridophyta</b>
Sub-division	—	<b>Lycopsida</b>
Order	—	<b>Selaginellales</b>
Family	—	<b>Selaginellaceae</b>
Genus	—	<b>Selaginella</b>

### Exercise 1

**Object :** Study the external features of the plant.

### Work procedure

Study the plant, specimen observe the differentiation of plant body into root, stem and leaves. Study the two types of leaves, their arrangement and structure. Also observe the structure of a ligule. Note the presence of rhizophore.

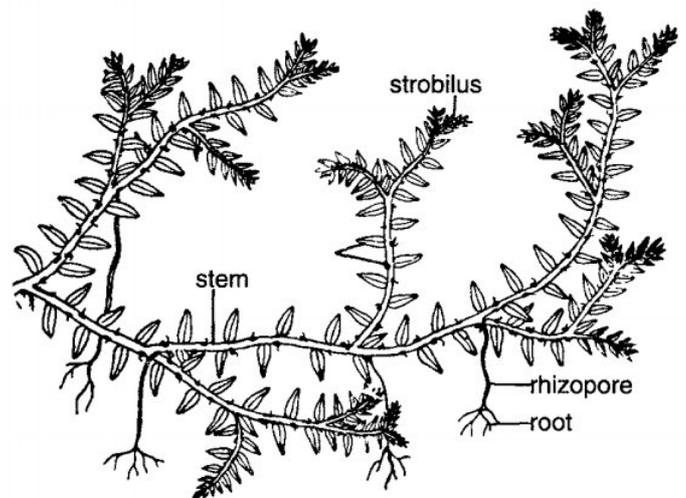


Fig. 1. *Selaginella*. External features.

### Comments

1. Many species are prostrate, creeping on the ground e.g. *S. kraussiana*, others are sub-erect e.g. *S. trachyphylla* or erect e.g. *S. erythropus*. A few species climb with the help of rhizophores e.g. *S. alligans*.
2. The plant body is divided into root, stem and leaves.
3. The primary root is short lived and all other roots are adventitious.
4. On the basis of nature of stem and form of the leaves, the genus is sub-divided into two

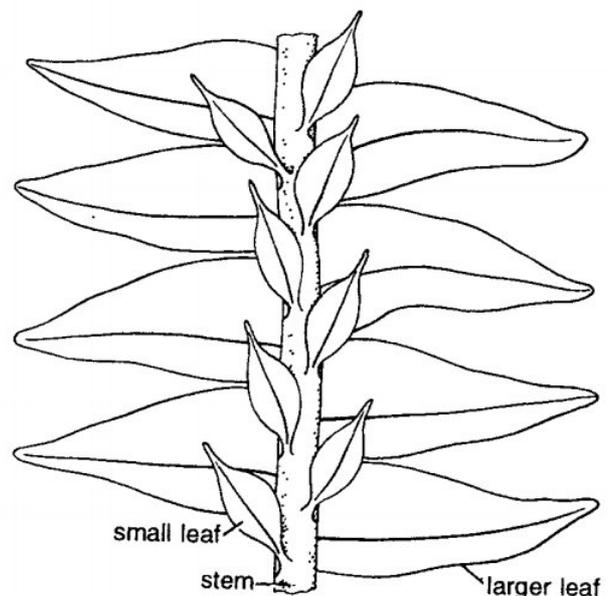


Fig. 2. *Selaginella*. A part of stem showing arrangement of leaves.

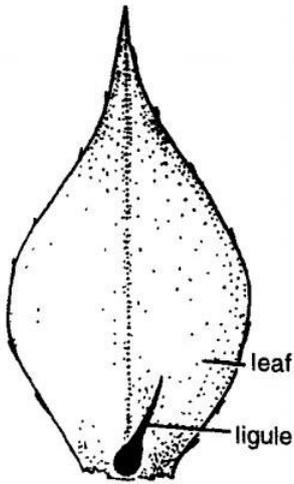


Fig. 3. *Selaginella*.  
Leaf (adaxial face  
showing ligule).

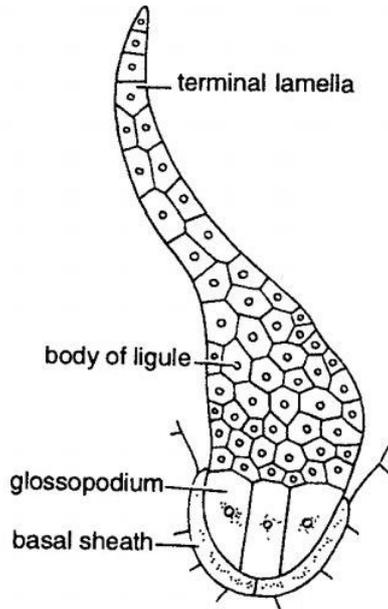


Fig. 4. *Selaginella*. A ligule.

sub-genera—the homoeophyllum and the heterophyllum.

5. In homoeophyllum species (*S. selaginoides*, *S. rupestris*, etc.) the stem is upright and all leaves are alike, while in heterophyllum species (majority of the species), the stem is prostrate and dorsiventral; and leaves are dimorphic (small and large).
6. In homoeophyllum, all the leaves are alike, spirally arranged, small and simple.
7. In heterophyllous species, they are dimorphic and are borne in pairs on dorsiventral stem. The two leaves are markedly different in size (one is larger and other smaller).
8. The smaller leaf of each pair is inserted on the dorsal side of the stem while the larger leaf is inserted on the ventral side.
9. The successive pairs of leaves are so arranged, that large leaf always alternates with the large leaf, and small leaf with the small leaf.
10. Each leaf is sessile, generally obovate with acute apex, and has a distinct midrib.
11. At the base of each young leaf, on the adaxial face, there is a small tongue-like outgrowth, the ligule.
12. It is differentiated into basal sheath, glossopodium and the body of the ligule.
13. Whereas the cells of the sheath are tubular in shape and are dead, those of the glossopodium are vertically elongated.
14. The body of the ligule has parenchymatous cells with dense protoplasm.
15. From the point where stem branches, a cylindrical leafless organ is seen growing downward. This is known as rhizophore.
16. On reaching the ground, rhizophore terminates into roots (The morphological nature of the rhizophore is still open to question).
17. Certain vertical branches from the stem are reproductive in nature and bear strobili.

### Features of special interest

1. Presence of rhizophore.
2. Dimorphic leaves (in heterophyllous species).
3. Presence of ligule.

### Exercise 2

**Object :** To study anatomy of the root.

### Work procedure

Cut a T.s. of the root, stain in safranin-fast green combination, mount in glycerine and study.

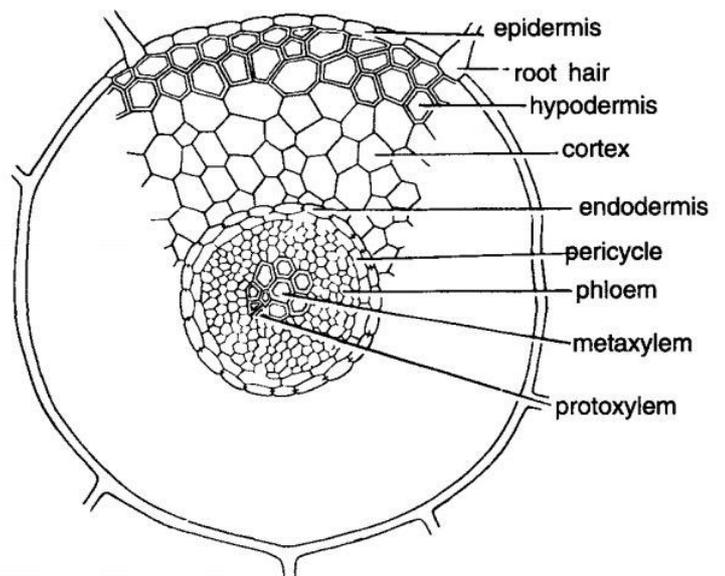


Fig. 5. *Selaginella*. T.s. root (a part cellular).

**Comments**

1. The section is almost circular in outline.
2. The tissues are differentiated into epidermis, cortex and stele.
3. **The epidermis** is single layered and its cells are tangentially elongated. Few of these cells give rise to the root hairs.
4. **Cortex** may either be made of parenchymatous (thin walled) cells or the outer layer of cells may form a sclerenchymatous (thick walled) hypodermis.
5. **The stele** lies in the centre. It is protostelic, monarch and exarch.
6. **Endodermis** is one layered and generally indistinct.
7. **Pericycle** is one to three layered.
8. **Xylem** forms only one group. Protoxylem is situated towards the periphery.
9. **Phloem** surrounds the centrally located xylem.

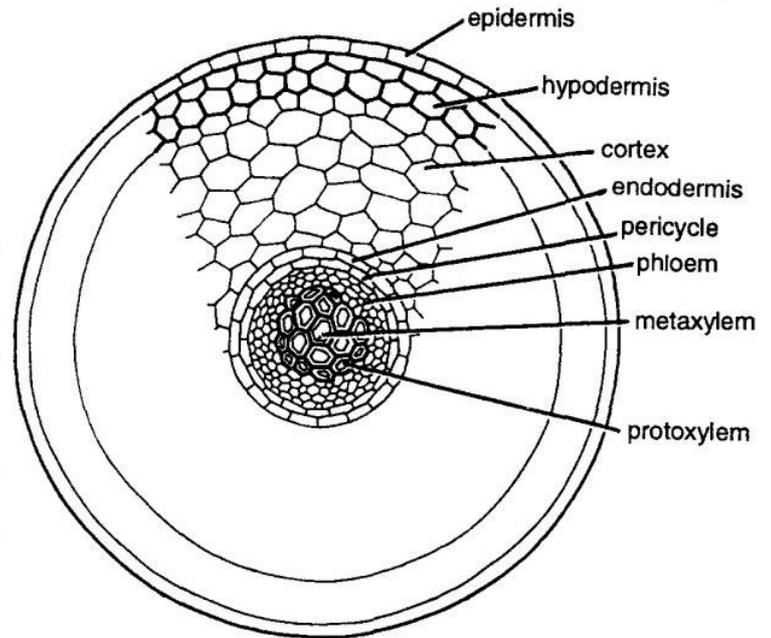


Fig. 6. *Selaginella*. T.s. rhizophore (a part cellular).

**Exercise 3**

**Object :** Study of anatomy of rhizophore.

**Work procedure**

Cut a T.s. of the rhizophore, stain in safranin-fast green combination, mount in glycerine and study. Anatomically, the structure of the rhizophore is similar to that of root, with some minor differences, which occur on account of its environment.

**Comments**

1. The outline of the section is almost circular.
2. The section shows epidermis, hypodermis, cortex, endodermis and stele.
3. **The epidermis** is cuticularised.
4. **Hypodermis** that follows is 2-3 celled thick.
5. **Cortex** is few celled and parenchymatous. It occupies most of the part of section.
6. **Endodermis** is present between the stele and the cortex. It is followed by a single layered parenchymatous pericycle.
7. **The stele** is a protostele. It shows monarch and exarch condition. In some species (e.g. *S. atroviridis*) the metaxylem is lunar shaped and many protoxylem groups are situated on the concave adaxial side.

**Exercise 4**

**Object :** Study of anatomy of the stem.

**Work procedure**

Cut a T.s. of the stem, stain in safranin-fast green combination, mount in glycerine and study.

**Comments**

1. **The outline** of the section appears slightly wavy.
2. **The section** shows epidermis, cortex and the stele.
3. **Epidermis** is the outermost layer. It is cuticularised and lack stomata.
4. **The cortex** consists of parenchymatous cells, without any intercellular spaces. All the cells of the cortex are thin walled.
5. **Hypodermis** occurs close to epidermis. It develops from cells of outer cortex which become thick walled. In xerophytic, species (e.g. *S. rupestris*, *S. lepidophylla*) hypodermis is more thickened.
6. **The stele** is generally a protostele.
7. **Endodermis** separates vascular tissue from the cortical region, by radially elongated endodermal cells, called as trabeculae, with

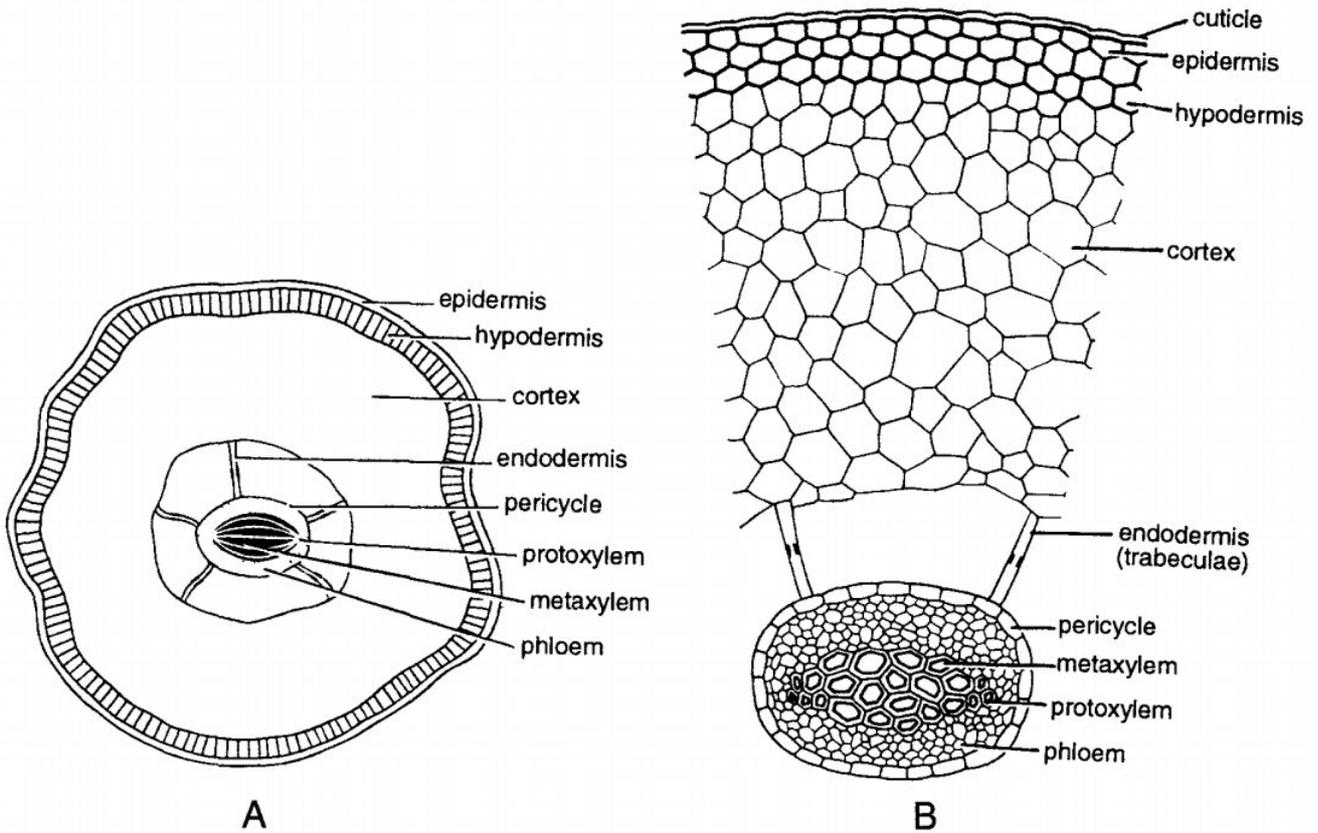


Fig. 7. *Selaginella*. T.s. stem A. Outline B. A part cellular.

conspicuous intercellular spaces between two trabeculae.

In spite of their great elongation, trabeculae still retain the transverse thickenings, the casparian strips, on their radial walls, characteristic of endodermal cells. Xerophytic species lack trabeculae (e.g. *S. lepidophylla* and *S. rupestris*).

8. **Pericycle** is a single layer surrounding the xylem and phloem and follows endodermis.
9. **Stele**. The number of steles in a stem varies from 1-16 thus exhibiting a polystelic condition.
10. Single stele, when present is generally diarch and exarch.
11. In *S. kraussiana*, the commonest species, there are two steles, each with a single exarch mass of protoxylem.
12. **The protoxylem** masses of the two steles point in opposite directions.
13. **The phloem** consists of smaller cells with dense protoplasm and completely surrounds the central core of xylem, in each stele.

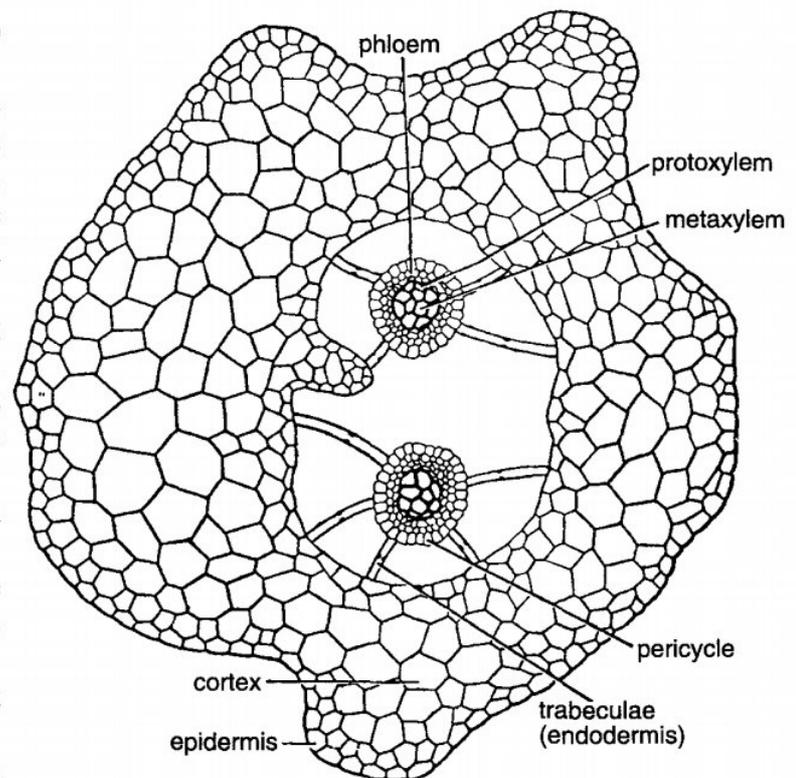


Fig. 8. *Selaginella*. T.s. stem—*S. kraussiana* (cellular).

### Features of special interest

1. Presence of modified endodermis in the form of trabeculae.
2. Presence of more than one stele i.e. polystelic condition.

### Exercise 5

**Object : Study of anatomy of leaf.**

### Work procedure

Cut a T.s. of leaf, stain in safranin-fast green combination, mount in glycerine and study.

### Comments

1. The section shows a slightly bulged midrib in the centre and the wings.
2. It shows definite upper and lower epidermis, usually undifferentiated mesophyll and a central vascular bundle.
3. **The epidermis** is one layered. The stomata are generally present on the abaxial surface (lower) but may also be present on the adaxial surface (upper), or on both the surfaces.
4. **The mesophyll** is usually not differentiated into palisade and spongy parenchyma. It shows many conspicuous intercellular spaces.
5. The cells of mesophyll contain chloroplasts, each of which has several pyrenoid-like bodies.
6. **The vascular bundle** is concentric with xylem surrounded by phloem and is bounded by a bundle sheath.

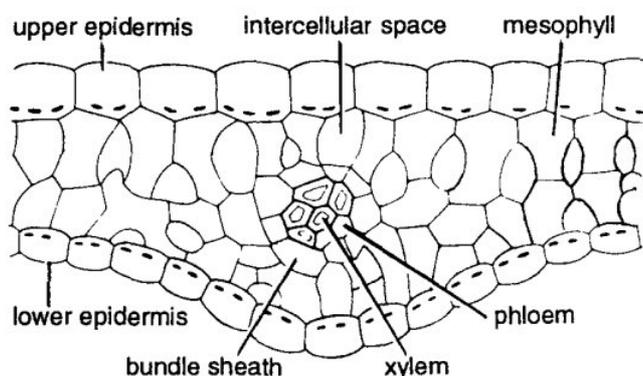


Fig. 9. *Selaginella*. T.s. leaf (a part cellular).

### Exercise 6

**Object : Study of spore producing organs.**

### Work procedure

Study the external features of the strobilus. Cut L.s. of the strobilus, stain in safranin-fast green combination, mount in glycerine and study. (Alternatively study the slide of L.s. of the strobilus).

### Comments

1. The spore producing organs are sporangia, aggregated in strobili which are generally present at the apices.
2. In some cases (as exemplified by *S. patula*) the axis may grow beyond the strobilus, terminating into a vegetative shoot or even in a second strobilus.
3. L.s. of the strobilus shows a strobilar axis, around which sporophylls are spirally arranged. Each sporophyll is ligulate and similar to a foliage leaf.
4. The sporangia are of two types, borne in the axils of the sporophylls, attached either strictly to the axils or to the axis just above.
5. *Selaginella* is heterosporous, with megaspores (large) and microspores (small), borne in their respective sporangia, known as megasporangia and microsporangia.

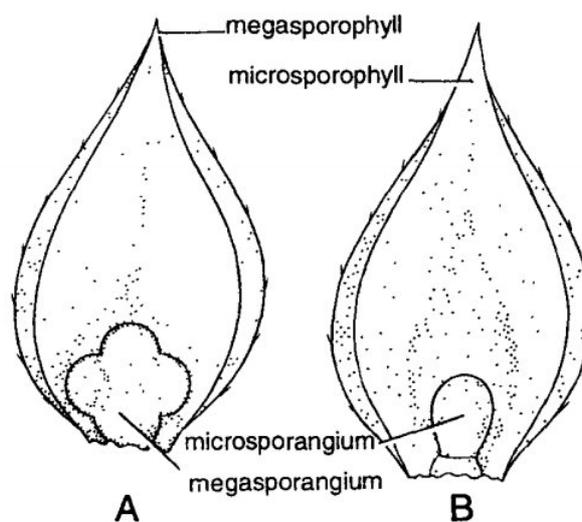


Fig. 10. *Selaginella*. Adaxial views of sporophylls showing sporangia; A. Megasporophyll, B. Microsporophyll.

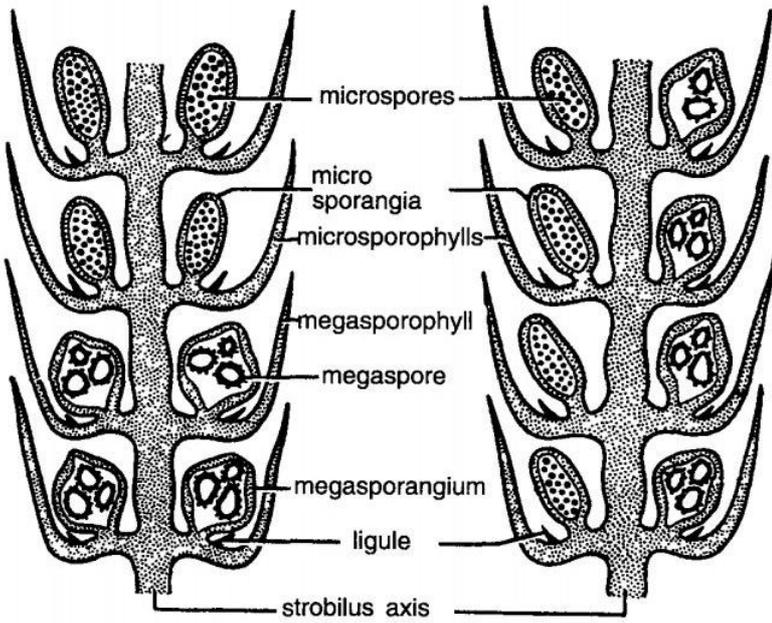


Fig. 11. *Selaginella*. L.s. strobilus showing different positions in which megasporangia and microsporangia occur.

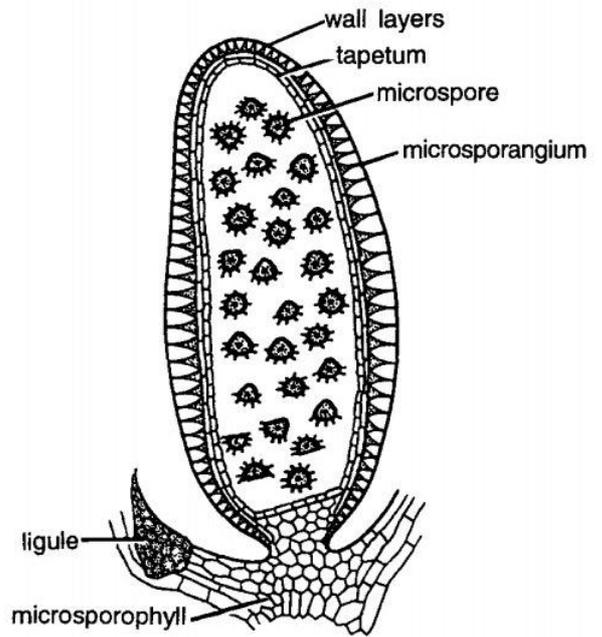


Fig. 13. *Selaginella*. L.s. microsporangium.

6. If a microsporangium is borne in the axil of the sporophyll, it is known as a microsporophyll but if it is a megasporangium, the sporophyll is termed as a megasporophyll.
7. Generally strobilus bears both types of sporangia but in *S. gracilis*, there are only one type of sporangia (either mega-or micro sporangia).

8. When both kinds of sporangia occur in one and the same strobilus, their arrangement differs from species to species:
  - (i) In some species (e.g. *S. oregana*) there are only megasporangia on one side and only microsporangia on the other.

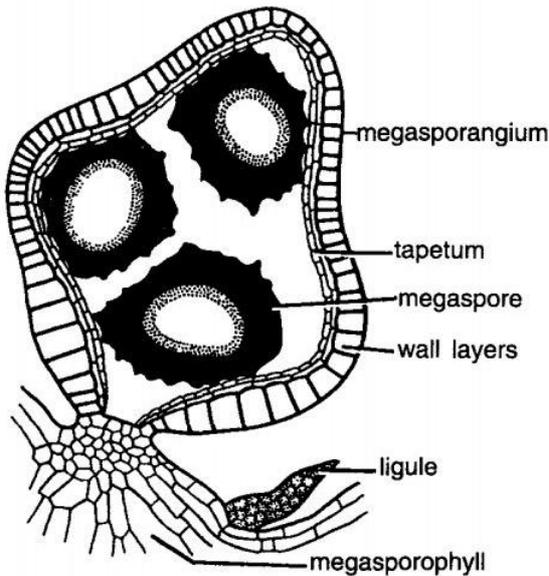


Fig. 12. *Selaginella*. L.s. megasporangium.

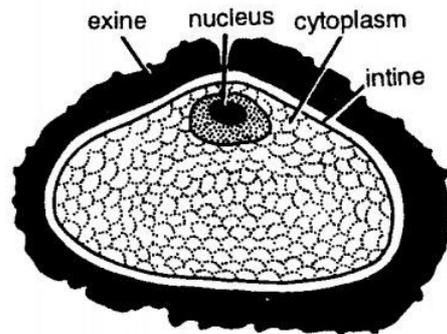


Fig. 14. *Selaginella*. A megaspore

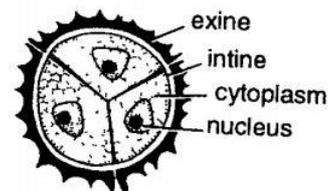


Fig. 15. *Selaginella*. Microspore tetrad.

- (ii) In most of the species (e.g. *S. kraussiana*) there are only one or two megasporangia at the base and the rest are microsporangia.
9. Both types of sporangia are stalked and have two layered jackets. The outer layer of the jacket is chlorophyllous and has columnar cells, whereas the inner layer has tangentially elongated cells. It may form tapetum.
  10. The cells of the outer jacket are thickened, except at the apex.
  11. The two types of sporangia when ripe, differ in their size, form, structure and colour.
  12. The megasporangium is much larger, four lobed, pale green or orange in colour and has only four megaspores.
  13. The microsporangium is smaller with uniform outline. It is dark brown or red in colour and has many spores.
  14. The megaspores are large in size and possess a triadate ridge at its apex. It has thick sculptured exine and thin uniform intine.
  15. The microspores are pyramidal in shape, and have thick, ornamented exine and a thin, uniform intine.
  16. Both types of spores have a nucleus suspended in a rich cytoplasm.

### Identification

- Division—Pteridophyta.** (1) Plant body differentiated into stem, roots and leaves, (2) A definite vascular strand present.
- Sub-division—Lycopsidea.** (1) Leaves microphyllous, (2) Sporangia borne singly on the adaxial face of the sporophyll or in its axil, (3) Sporophylls borne in strobili.
- Order—Selaginellales.** (1) Each foliage leaf with a ligule at the base on adaxial side, (2) Sporophytes heterosporous.
- Family—Selaginellaceae.** (1) Stem herbaceous and dorsiventral or erect, (2) Gametophytes extremely reduced.
- Genus—Selaginella.** (1) Roots arise from rhizophore, (2) Trabeculae present, (3) Stele generally a protostele, sometimes siphonostele.

### Hints for Collection

About 58 species of *Selaginella* have so far been reported from India. Many Indian species are found growing in western and eastern Himalayas and the hills of South India on damp shady sides. A few species are xerophytic. *S. oregana* is epiphyte on trunks and branches of moss covered trees in the forests.

## *Equisetum* (Horse Tails)

### Classification

<i>Division</i>	—	<b>Pteridophyta</b>
<i>Sub-division</i>	—	<b>Sphenopsida</b>
<i>Order</i>	—	<b>Equisetales</b>
<i>Family</i>	—	<b>Equisetaceae</b>
<i>Genus</i>	—	<b><i>Equisetum</i></b>

### Exercise 1

**Object : Study of external morphology.**

#### Work procedure

Study the external features of the plant. Observe the differentiation of plant body into roots, rhizome, aerial branches and leaves. Note the ribbed nature of the stem, its branching and the scaly leaves. Also see the difference between sterile and fertile branches.

#### Comments

1. The plants are erect and bushy.
2. The plant is differentiated into roots, rhizome, aerial branches and leaves.
3. **The underground rhizome** has distinct nodes and internodes. The nodes bear aerial branches and roots.
4. **The roots** are produced on the lower side of the node. These are slender and fibrous.
5. **The aerial stems** are less than a metre in height with characteristic joints. Stem is rough due to the deposition of silica.
6. **The aerial branches** fall into two general categories—(i) typical sterile branches which are green and branched, and (ii) typical fertile branches which are non-green, unbranched and terminate in a cone. Such branches die after the spores are shed.
7. Some species have green, branched fertile shoots, with a cone at the apex of each lateral branch. Such branches do not die after the spores are shed.
8. Organization of the rhizome and aerial branch is the same, but is best seen in aerial branches.

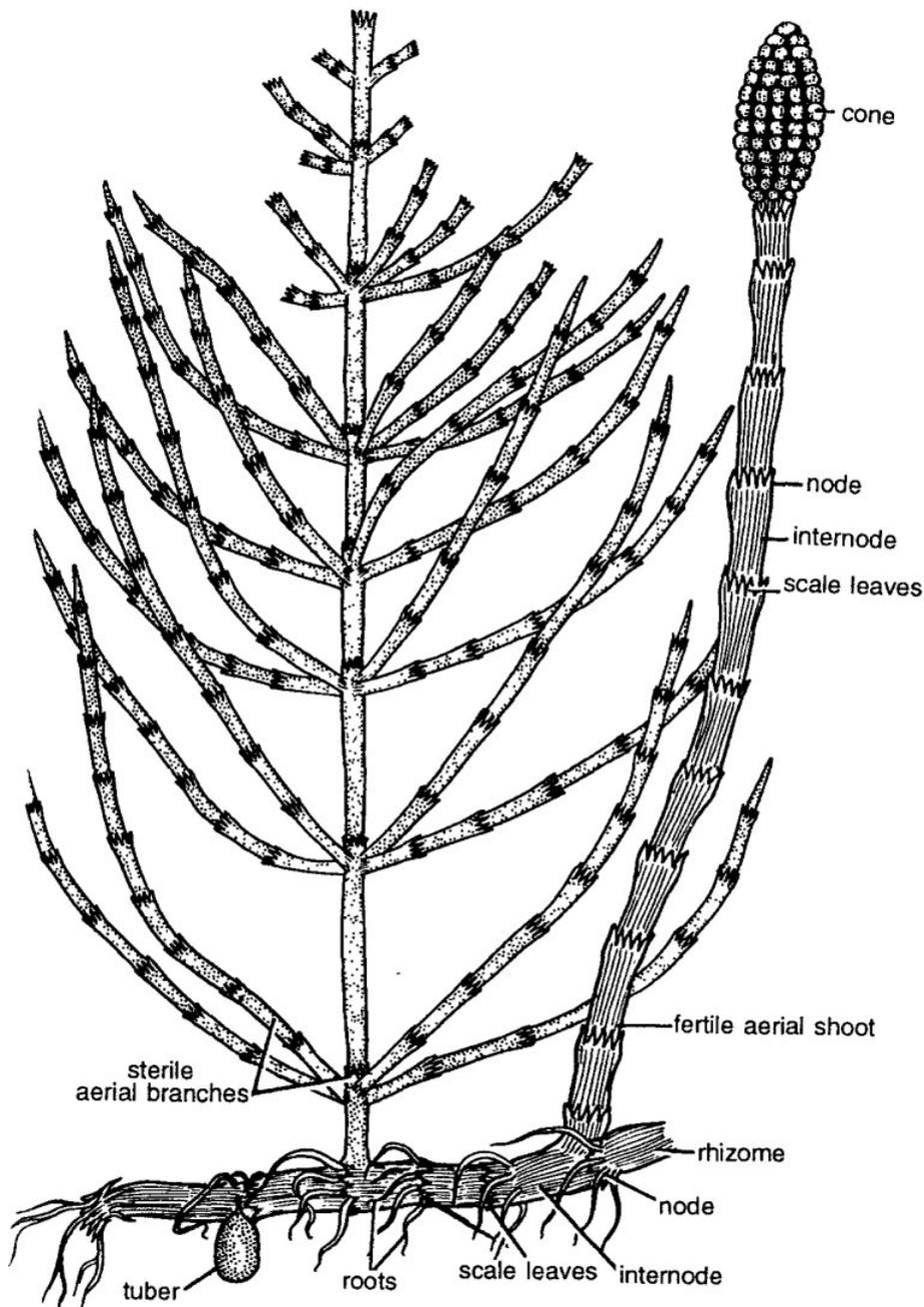


Fig. 1. *Equisetum*. External features.

9. **Each internode** of an aerial branch is longitudinally ribbed. The number of ridges is same as the number of leaves, and each leaf stands directly above a ridge present in the internodes below.
10. The ridges on the stem of successive internodes alternate, as also the leaves of the successive nodes.
11. **Leaves** are simple, small, scaly, whorled and fused laterally and possess longer or shorter free tips.
12. Leaves are present at nodes in whorls. Each whorl forms a sheath closely appressed to the node. The number of leaves in a whorl varies with the species and the size of the stem.
13. The leaves are non-chlorophyllous and scaly. These alternate at the successive nodes.
14. **The branches** develop at the node in between each two leaves. Therefore, the branches are equal in number to the leaves and appear to arise in a whorl.

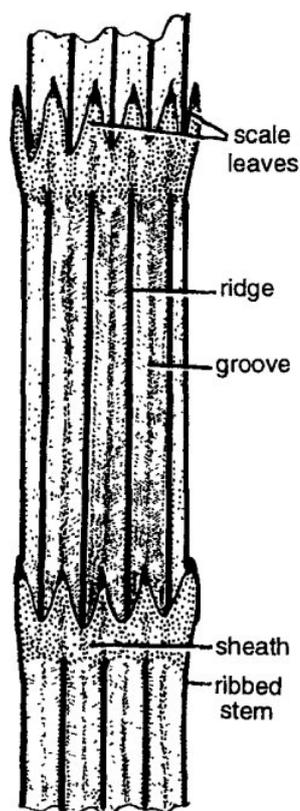


Fig. 2. *Equisetum*. A part of stem showing alternation of ridges and grooves and scale leaves.

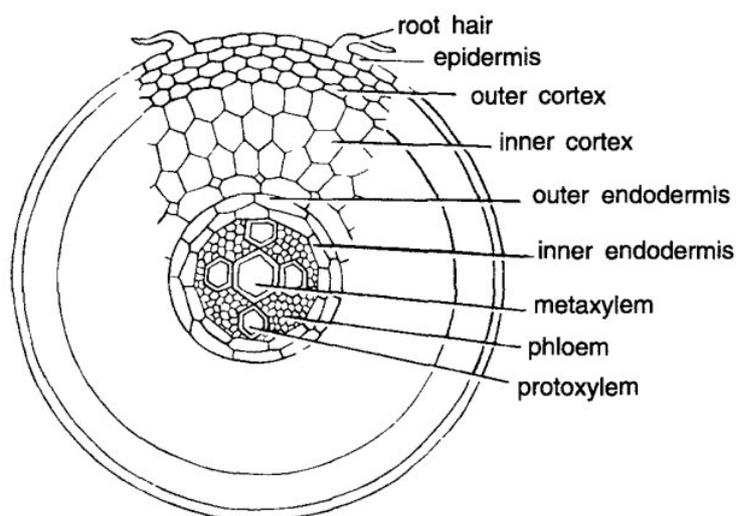


Fig. 3. *Equisetum*. T.s. root (a part cellular).

7. **The vascular bundle** shows a single, large metaxylem element in the centre with 3 to 4 protoxylem, triarch to tetrarch elements surrounding it. The number of protoxylem groups increases with increase in the diameter of the root.
8. The angles between the protoxylem are occupied by phloem.

### Exercise 2

**Object :** Study of anatomy of root.

#### Work procedure

Cut a T.s. of the root, stain in safranin-fast green combination, mount in glycerine and study.

#### Comments

1. The section appears almost circular in outline.
2. **Epidermis** is single layered and possesses a few root hairs.
3. **The cortex** is often divided into an outer cortex and an inner cortex.
4. **The outer cortex** is a few layered deep. It is made of thick walled cells.
5. **The inner cortex** is also a few layered deep. The cells are large sized and parenchymatous with intercellular spaces.
6. **Endodermis** separates from the vascular tissues. It is two layered—outer and inner endodermis. The pericycle is absent.

### Exercise 3

**Object :** Study of anatomy of internode of aerial shoot.

#### Work procedure

Cut a T.s. of the aerial shoot passing through the internode, stain in safranin-fast green combination, mount in glycerine and study.

#### Comments

1. The outline is wavy with ridges and grooves.
2. The tissues are organised into epidermis, cortex, stele and a pith cavity.
3. **The epidermis** is cuticularized with tangentially elongated and silicified cells.
4. **The stomata** are mostly found in the grooves. The guard cells are surrounded by two subsidiary cells, one on either side.
5. **Cortex** follows the epidermis and is highly differentiated. It is divided into outer and inner cortex.

6. **Outer cortex**, below the ridges has a group of sclerenchyma. Small patches of sclerenchyma may also occur, below the grooves.
7. Beneath the ridges radially elongated chlorenchymatous cells (palisade tissue) are present. The amount of palisade beneath the grooves is lesser.
8. **The inner cortex** is composed of large and thin-walled, parenchymatous cells.
9. **Vallecular canals** are present in the cortex. These are situated below the grooves.
10. **The stele** is an ectophloic siphonostele that consists of ring of vascular bundles.
11. **Endodermis** occurs at different positions in different species.
  - (i) Most commonly, the endodermis forms a simple sheath, outside the ring of bundles.
  - (ii) In some cases, in addition, there is also an internal endodermis and outer endodermis dips in between the bundles.
  - (iii) In third condition, each bundle is surrounded by an individual endodermis.
12. **Pericycle** lies below the endodermis.
13. **The vascular bundles** are collateral and endarch, arranged in a ring and each bundle lies below each ridge.
14. Each bundle has one inner strand of protoxylem and two outer of metaxylem.

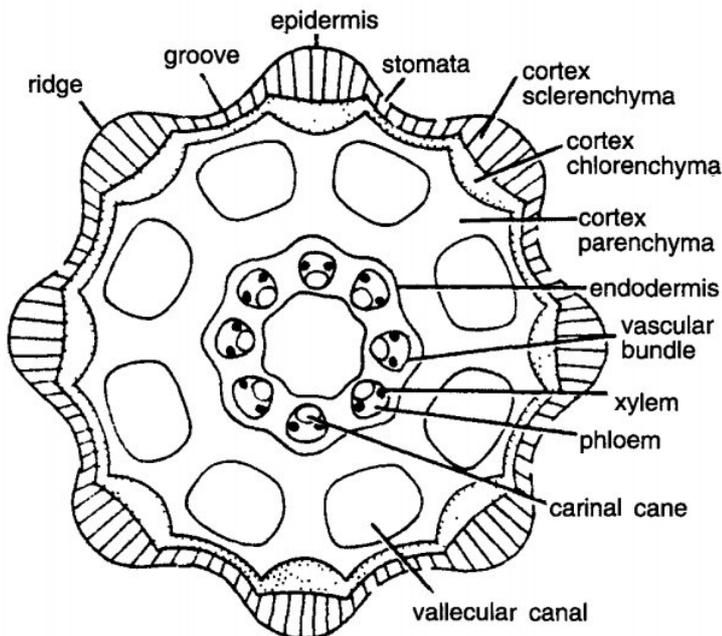


Fig. 4. *Equisetum*. T.s. aerial shoot : internode (diagrammatic).

(B-14)

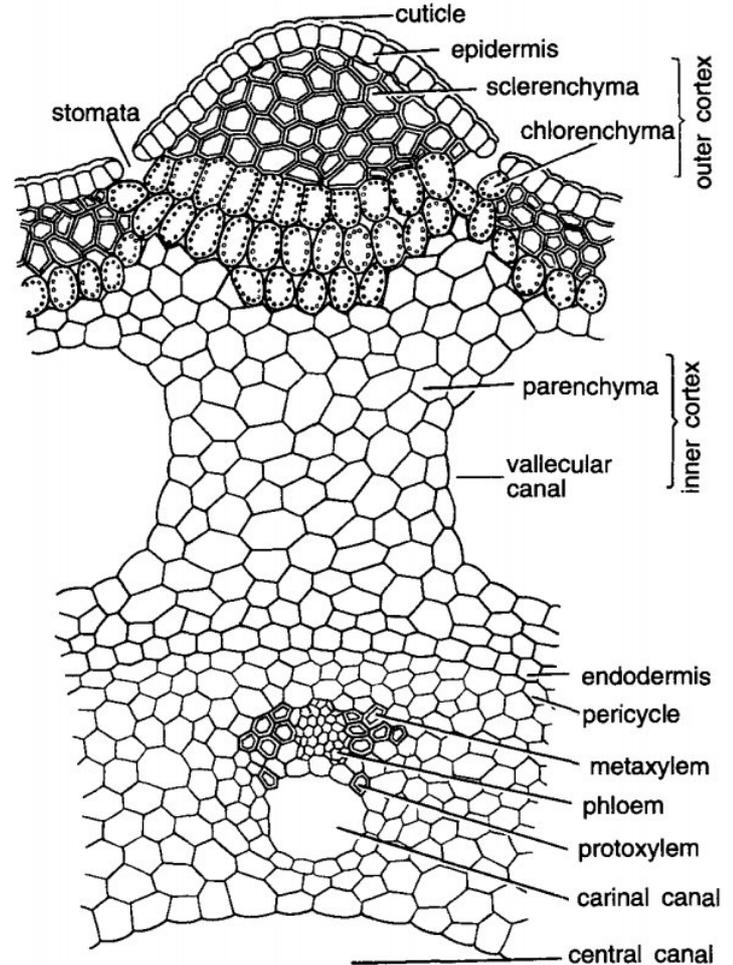


Fig. 5. *Equisetum*. T.s. aerial shoot : internode (a part cellular).

15. **The protoxylem** elements lie on the sides of a protoxylem lacuna, the carinal canal, formed by the disintegration of protoxylem elements.
16. **The two metaxylem** groups lie on two lateral sides of carinal canal (i.e. on the shoulders of the bundle).
17. The rest of the tissue of the vascular strands is parenchymatous.
18. Pith cavity known as central canal lies in centre.

#### Features of special interest

Anatomy shows both xerophytic as well as hydrophytic characters.

##### *Xerophytic characters*

- (1) Presence of ridges and grooves.
- (2) Position of stomata in grooves.
- (3) Thick cuticle over epidermis.
- (4) Well developed sclerenchyma below the ridges.
- (5) Presence of palisade.

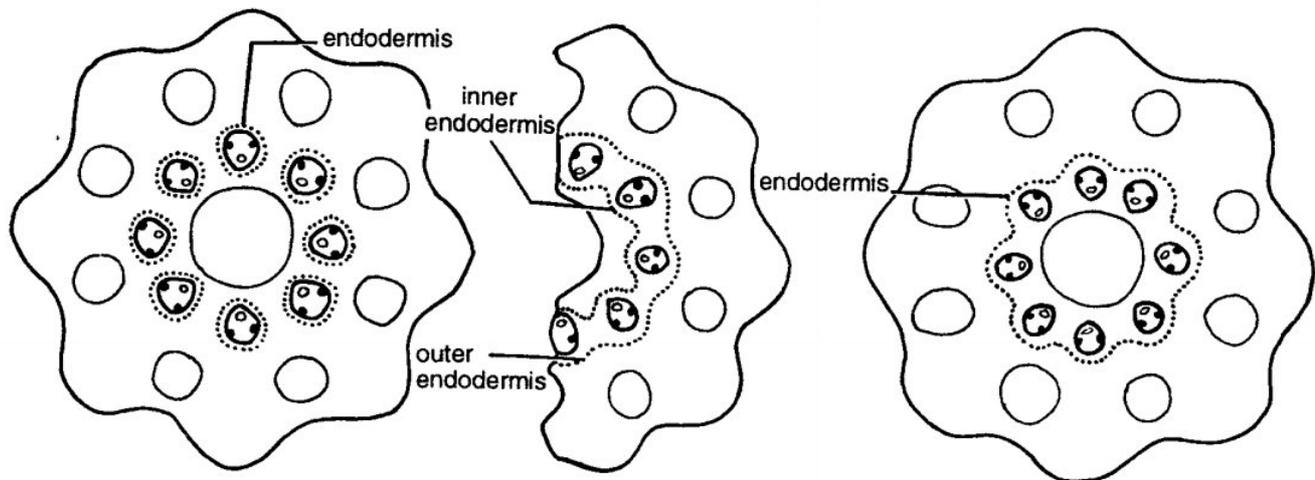


Fig. 6. *Equisetum*. T.s. aerial shoot : internode showing different conditions of endodermis.

### Hydrophytic characters

- (1) Presence of vallecular, carinal and central canals.

### Exercise 4

**Object :** Study the anatomy of node of aerial shoot.

### Work procedure

Cut a T.s. of the aerial shoot passing through the node, stain in safranin-fast green combination, mount in glycerine and study.

### Comments

1. The section shows distinct ridges and grooves.
2. The anatomy is almost similar to that of internode except for a few differences.
3. The section shows epidermis, cortex, stele and nodal diaphragm instead of pith cavity in internode.
4. **The epidermis** is the outermost thickly cuticularised layer.
5. **The cortex** is divisible into outer, middle and inner cortex.
6. **The outer cortex** is sclerenchymatous. It is followed by middle cortex made of palisade (chlorenchyma) tissues.
7. **The inner cortex** is parenchymatous and occupies most part of the section.
8. **Vallecular canals** are absent. Many leaf traces and branch traces are found scattered all over the inner cortex.

9. **Vascular bundles.** Instead of ring there is a complete vascular cylinder with outer ring of phloem enclosing a ring of xylem.
10. **Leaf and branch traces** are given off from the vascular cylinder. Leaf traces arise beneath the ridges and do not produce leaf gaps in the vascular cylinder. Branch traces arise beneath the grooves.
11. **Nodal diaphragm.** In the centre there is parenchymatous or sclerenchymatous tissue. It is known as nodal diaphragm. In L.s. it appears like an arc. The internodes easily break and separate at these places.

### Exercise 5

**Object :** Study of anatomy of rhizome.

### Work procedure

Cut a T.s. of rhizome, stain in safranin-fast green combination, mount in glycerine and study.

### Comments

1. The outline is wavy with ridges and grooves.
2. **Epidermis.** This is the outermost thickly cuticularised layer. Stomata are absent.
3. **The cortex** consists of a few layers of sclerenchyma just below the epidermis and a large zone of parenchyma spread upto the ring of vascular bundles.
4. **Large vallecular canals** are present in the parenchymatous cortex below the grooves.

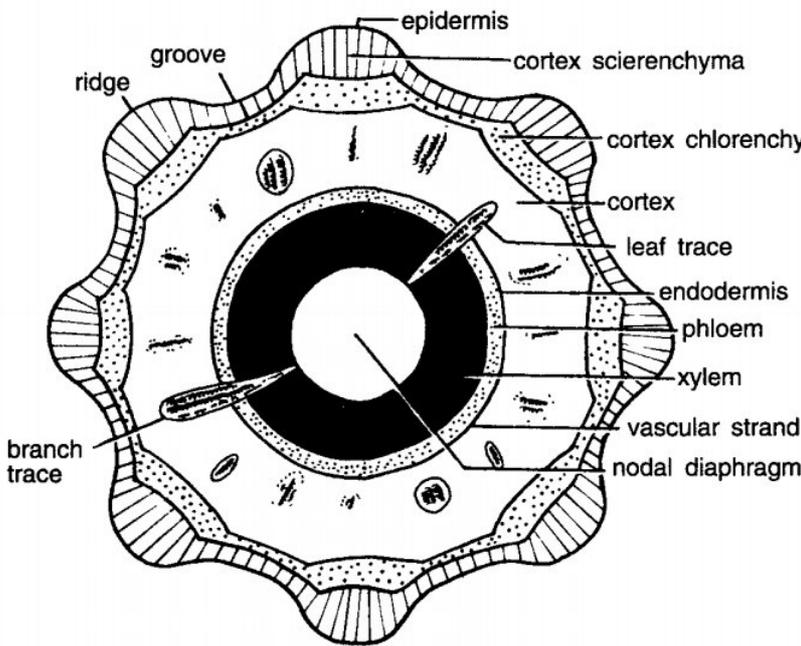


Fig. 7. *Equisetum*. T.s. aerial shoot : node (diagrammatic).

5. **Endodermis** is single layered and encloses a ring of vascular bundles.
6. **Each bundle** is located below the ridge.
7. The bundle is conjoint, collateral and endarch.
8. The bundle has a large protoxylem lacuna, carinal canal.
9. **Pith cavity**. The centre has a large cavity, called pith cavity.

### Exercise 6

**Object :** Study of spore producing organs :  
L.s. cone.

### Work procedure

The spore producing organs are sporangia borne in cones, generally terminating the main axis and sometimes the lateral branches. The structure is best studied by observing L.s. of cone, single sporangiophore and spores. Study the features shown by respective slides.

### Comments

1. L.s. of the cone shows cone axis and attached sporangiophores.
2. Cone axis is centrally located.
3. It bears sporangiophores in whorls which are mostly alternate though not regularly.

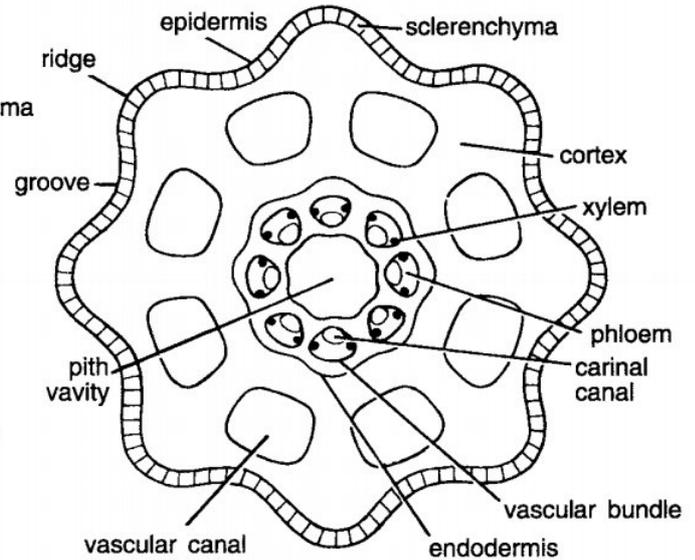


Fig. 8. *Equisetum*. T.s. rhizome : internode (diagrammatic).

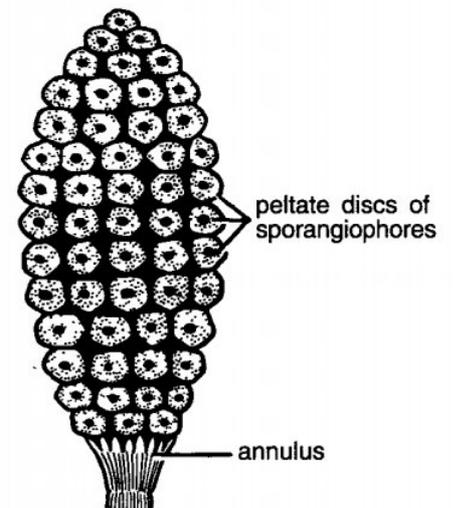


Fig. 9. *Equisetum*. A cone.

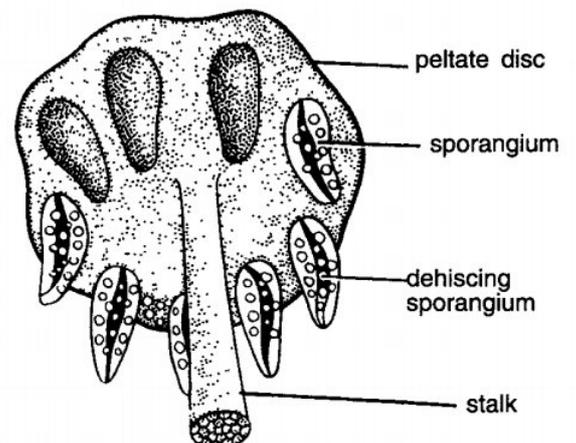


Fig. 10. *Equisetum*. Sporangiphore from ventral side.

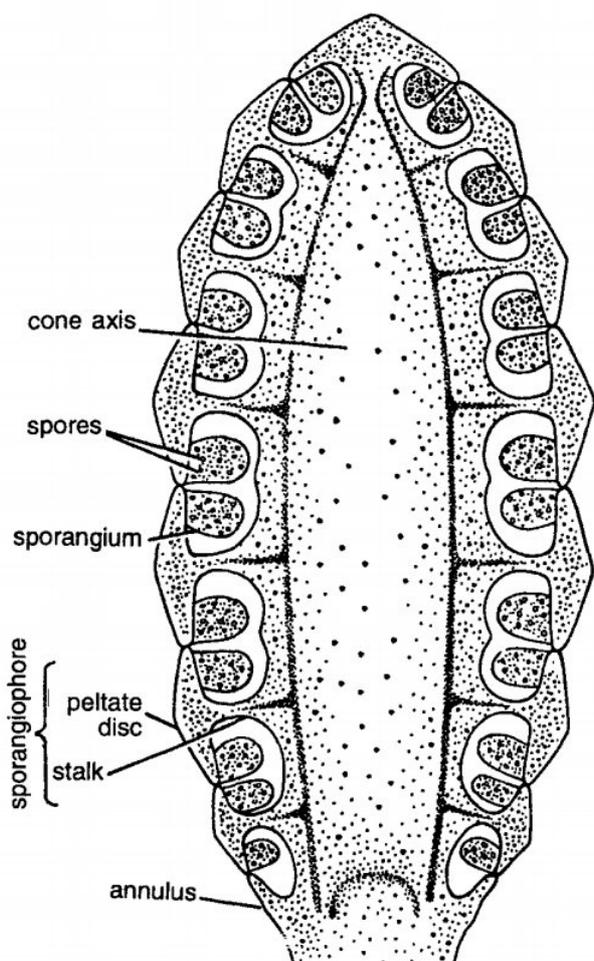


Fig. 11. *Equisetum*. L.S. cone

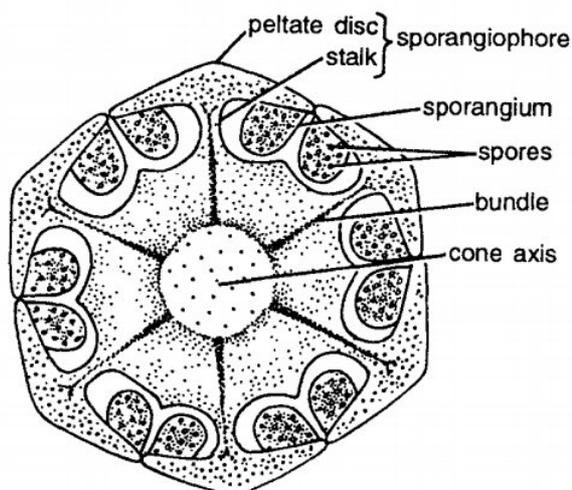


Fig. 12. *Equisetum*. T.S. cone.

6. The stalk holds a polygonal peltate disc at right angles to it. The peltate discs of sporangiophores fit closely to form a protective cover for the sporangia below.
7. Sporangia appear attached on the lower side of the disc.
8. Each sporangium is elongated and sac-like. It has one-layered jacket that encloses numerous spores.

### Exercise 7

**Object** : Study of spore producing organs :  
**T.s. cone.**

### Work procedure

Study the characters of the cone by observing various features as shown by the slide of T.s. cone.

### Comments

1. T.s. of cone shows a cone axis and sporangiophores attached to it.
  2. Centrally located part is called cone axis.
  3. Sporangiohores are attached in a whorl.
  4. Each sporangiophore consists of a stalk and a disc.
  5. Stalk keeps the disc attached to cone axis.
  6. The peltate disc bears sporangia on the underside, with one layered jacket which enclose the spores.
  7. Each sporangium appears elongated and cylindrical.
  8. Sporangiohores are one of the units, of which cone is made of.
  9. These are attached to the central cone axis in successive whorls.
  10. Each sporangiophore consists of a stalk and a polygonal peltate disc.
  11. The stalk is attached to the cone axis on one side and to the peltate disc on the other.
  12. About 5-10 cylindrical sporangia are arranged in a ring near the margins on the lower side of the disc.
  13. Sporangium has a one layered jacket with helical thickenings.
  14. Numerous spores, all similar (homosporous condition) are present in the sporangial cavity.
  15. A longitudinal line of dehiscence is also clearly seen.
4. At the base of the cone is a calyx-like whorl, the annulus (which most probably represents a modified leaf whorl).
  5. The sporangiophores are attached to the cone axis at right angles with its stalk.

6. Leaves are often dichotomously branched into many leaflets.
7. The blade of the leaflets may be entire, and either simply or repeatedly branched. The leaflets are deltoid in shape. When fertile, the leaflet margin remains folded toward the lower side forming a false indusium which encloses many sori.
8. **Leaflet** is traversed by dichotomously branched veins which generally do not unite to form a reticulum. The venation is, therefore, of open dichotomous type.

### Exercise 2

**Object : Study of anatomy of root.**

#### Work procedure

Cut a T.s. of the root, stain with safranin-fast green combination, mount in glycerine and study.

#### Comments

1. The root is almost circular in outline.
2. It is differentiated into epidermis, cortex and stele.
3. **The epidermis** is single layered, cells are thin walled and tangentially elongated. It bears a few unicellular root hairs.
4. **Cortex** is inner to the epidermis. It is multilayered and parenchymatous.

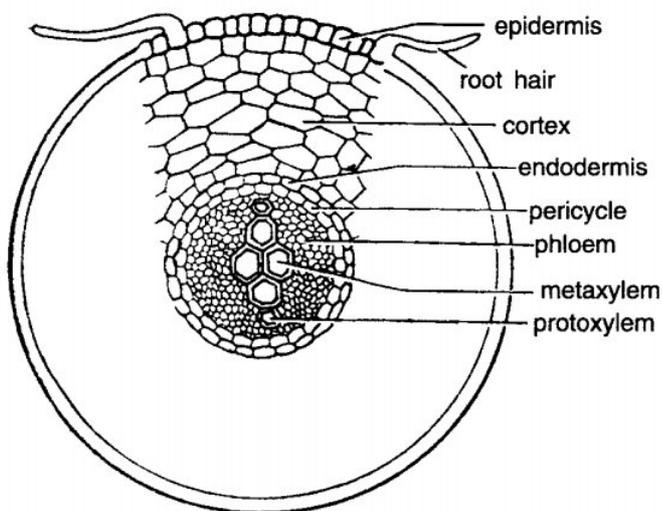


Fig. 2. *Adiantum*. T.s. root (a part cellular).

5. **Endodermis** is single layered and is followed by a single layered pericycle.
6. A **protostele** is present in the centre. It is diarch and exarch.
7. **Xylem** elements occur in the centre. Of these, metaxylem elements are present in the centre. Protoxylem groups are situated on the two opposite sides of the metaxylem (Thus xylem groups are exarch and diarch).
8. **Phloem** surrounds the xylem on all the sides.

### Exercise 3

**Object : Study of anatomy of rhizome.**

#### Work procedure

Cut a T.s. of rhizome, stain in safranin-fast green combination, mount in glycerine and study.

#### Comments

1. Rhizome appears almost circular or gutter-shaped in a transection.
2. It shows differentiation into epidermis, hypodermis, ground tissue and stele.
3. **Epidermis** is single layered and bears numerous multicellular hairs.
4. **Hypodermis** that follows epidermis is a 2-3 layered deep and sclerenchymatous.
5. **Ground tissue** occupies major part of rhizome. It is parenchymatous and many layered deep.

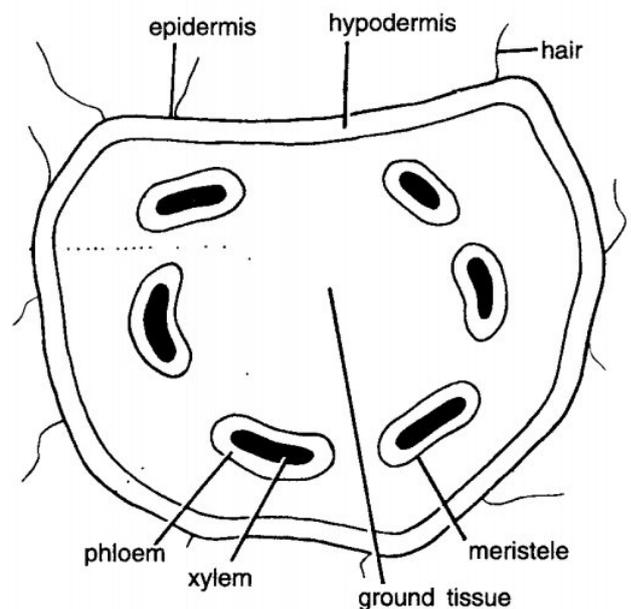


Fig. 3. *Adiantum*. T.s. rhizome (outlines : diagrammatic).

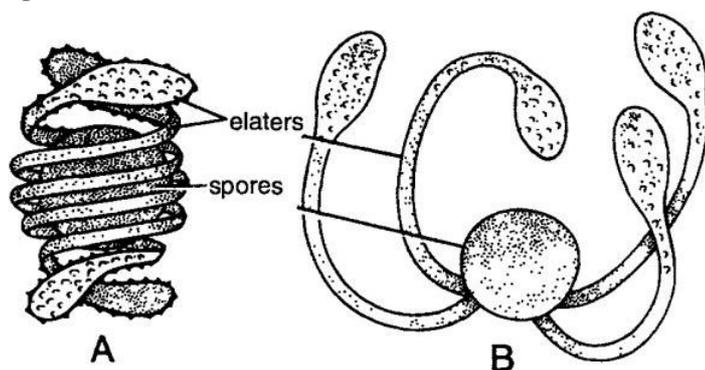


Fig. 13. *Equisetum*. Spores. A. Spore with elaters coiled, B. Spore with elaters uncoiled.

### Exercise 8

**Object :** Study of spores.

#### Work procedure

Tease a sporangium with needle. Mount spores in water. These can also be stained with safranin and then studied.

#### Comments

1. The spores consist of a four layered wall.
2. Surrounding the two usual wall layers, there is a third cuticular layer known as the middle layer and a fourth, thick, outermost layer known as perispore.
3. The perispore of each spore is differentiated into four narrow spirally wound bands, with flat-spoon tips, all attached at the common point.
4. These projecting bands are called as "elaters", but are very different from the elaters of bryophyta.
5. The elaters are hygroscopic and with the changes in the atmospheric humidity, they coil and uncoil. (this can be observed under the microscope by allowing the wet spores to dry on a slide).
6. Each spore in a section shows a single nucleus with rich cytoplasm and all the four wall layers.

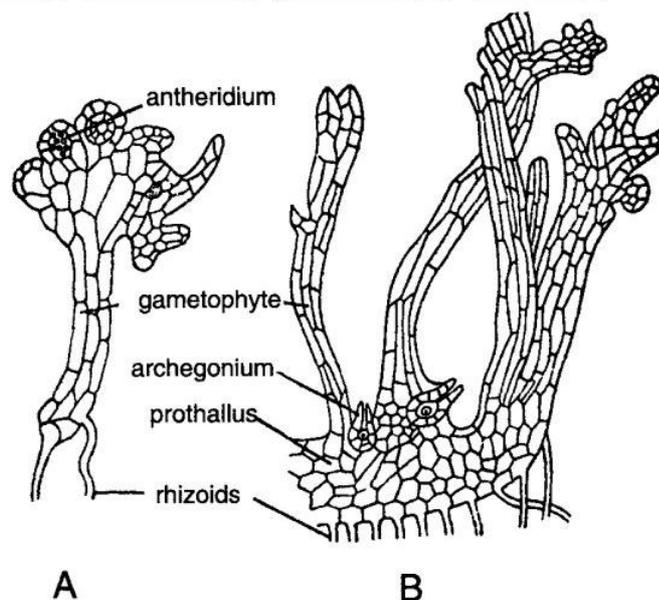


Fig. 14. *Equisetum*. Prothalli. A. Prothallus with antheridia, B. Prothallus with archegonia.

### Exercise 9

**Object :** Study of prothallus.

#### Work procedure

Study a prepared slide of prothallus.

#### Comments

1. Both male (antheridia) and female (archegonia) sex organs are borne on the same prothallus. Thus it is monoecious.
2. In younger stages only antheridia are developed. Therefore, small and younger prothalli show antheridia only. In older prothalli, however, archegonia are found. Hence it is protandrous.
3. The multicellular central part gives out many flat branches. These branches further get irregularly dissected in uniseriate filaments.
4. From the central region, long, brown and unbranched rhizoids are also given off.
5. Archegonia remain embedded in the tissue of the prothallus, at the place where branches are given out.

6. Prothallus that bears antheridia, is less branched and smaller. Antheridia may arise at the base of the branch or on the branch itself.

### Identification

**Division—Pteridophyta.** (1) Plant body differentiated into stem, roots and leaves, (2) A definite vascular strand present.

**Sub-division—Sphenopsida.** (1) Stem branched, articulated, ridged and furrowed with distinct nodes and internodes, (2) Leaves microphyllous, small, scaly and in whorls at nodes.

**Order—Equisetales.** (1) Stem branched. Branches borne in transverse whorls, (2) Internodes alternate with one another, (3) Vascular cylinder endarch, siphonostele.

**Family—Equisetaceae.** (1) Homosporous, (2) Sporangia borne on sporangiophores which form a compact cone, (3) No secondary growth.

**Genus—Equisetum.** (1) Leaves scaly and colourless, (2) Sunken stomata in grooves, (3) Presence of palisade in the stem, (4) Presence of valecular, carinal and central canals.

### Hints for Collection

*Equisetum debile* which is common in India grows abundantly along the banks of rivers, in sandy soil and on the woods along the river. Another common species, *E. arvense*, grows in grasslands.

## Adiantum (Maiden Hair Fern)

### Classification

Division	—	Pteridophyta
Sub-division	—	Pteropsida
Class	—	Leptosporangiatae
Order	—	Filicales
Family	—	Adiantaceae
Genus	—	<i>Adiantum</i>

### Exercise 1

**Object :** Study of external features of the plant.

#### Work procedure

Study a fresh plant or a preserved specimen for external morphology. Observe the differentiation of plant body into roots, rhizome and leaves. Note the circinate vernation of young leaf, compound

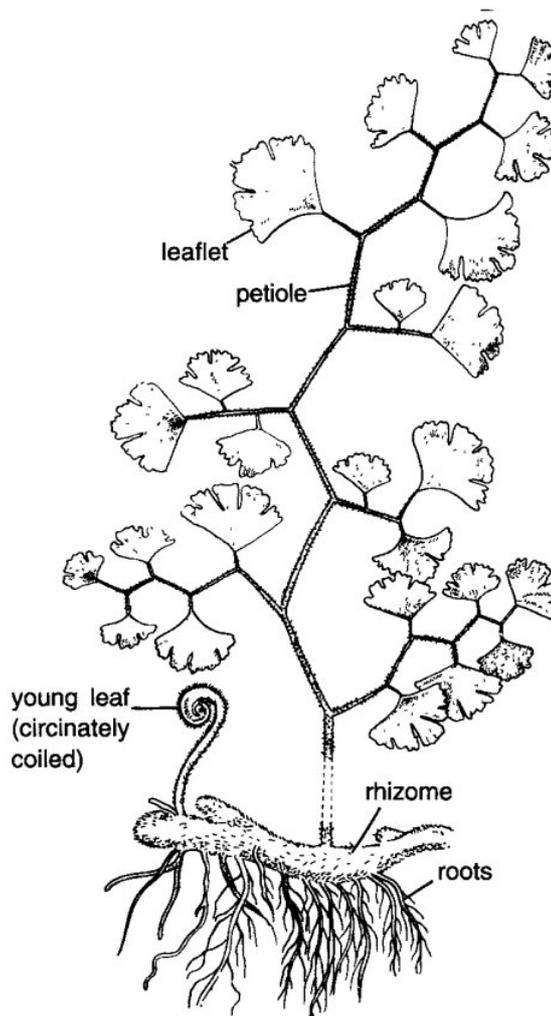


Fig. 1. *Adiantum*. External features.

nature of mature leaf and reflexed margins of the leaflets.

#### Comments

1. **Plant body** is a sporophyte. It is differentiated into roots, rhizome and leaves.
2. **Roots** are produced on the lower side of the rhizome. Primary root is short-lived. Secondary roots are adventitious and branched.
3. **Rhizome** may either be creeping or erect. It is scaly, covered with hairs and bears adventitious roots and leaves.
4. **Leaves** with a long petiole, are spirally or alternately arranged on the rhizome. Young leaves are circinately coiled. Young rhizome, petiole, rachis and circinately coiled leaves are covered with hairs known as ramenta.
5. The leaves are compound and are borne on shining black and brittle petiole.

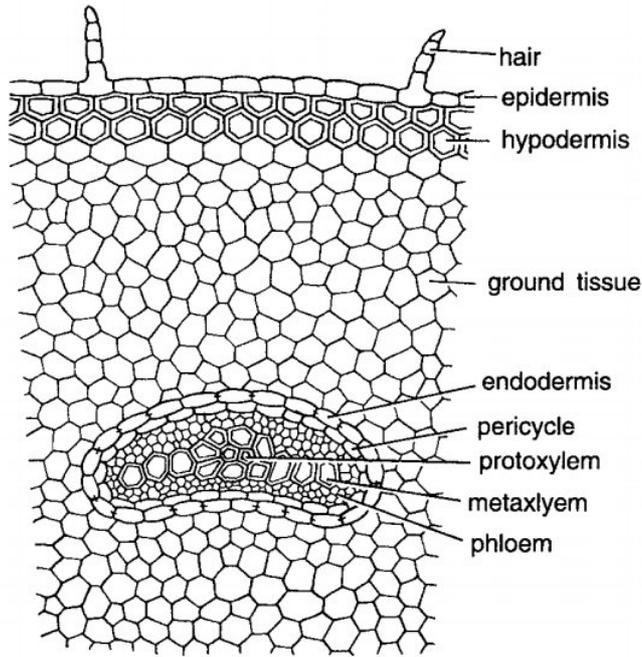


Fig. 4. *Adiantum*. T.s. rhizome (part shown by dotted lines in Fig. 3 : cellular).

6. **Stele** is variable in nature, differing from one region to another. Species with elongated rhizome (*A. pedatum* and *A. hispidulum*) show actual solenostele (amphiphloic siphonostele). But commonly the stele is gutter shaped and appears as several meristeles arranged in a ring, due to numerous leaf gaps (dictyostele).
7. **Meristeles** varying in number, but more often 5-7, lie arranged in ground tissue in a gutter-shaped ring, thus exhibiting dictyostelic condition. The spaces between neighbouring meristeles are leaf gaps.
8. Each of the meristeles is surrounded by a distinct, single-layered endodermis subsequently followed by a single layered pericycle.
9. **Xylem** elements occupy the central part. Metaxylem and protoxylem are arranged in a way to form mostly mesarch condition.
10. **Phloem** surrounds xylem.

#### Exercise 4

**Object : Study of anatomy of rachis.**

#### Work procedure

Cut a T.s. of rachis, stain with safranin-fast green combination, mount in glycerine and study.

#### Comments

1. Tissues of the rachis are differentiated into epidermis, hypodermis, cortex and stele.
2. **Epidermis** is a single layer of cells, covered by a cuticle.
3. **Hypodermis** follows the epidermis. It is few layered deep. The cells are sclerenchymatous.
4. **Cortex** forms larger part of the rachis. It is made of many layers of parenchyma.

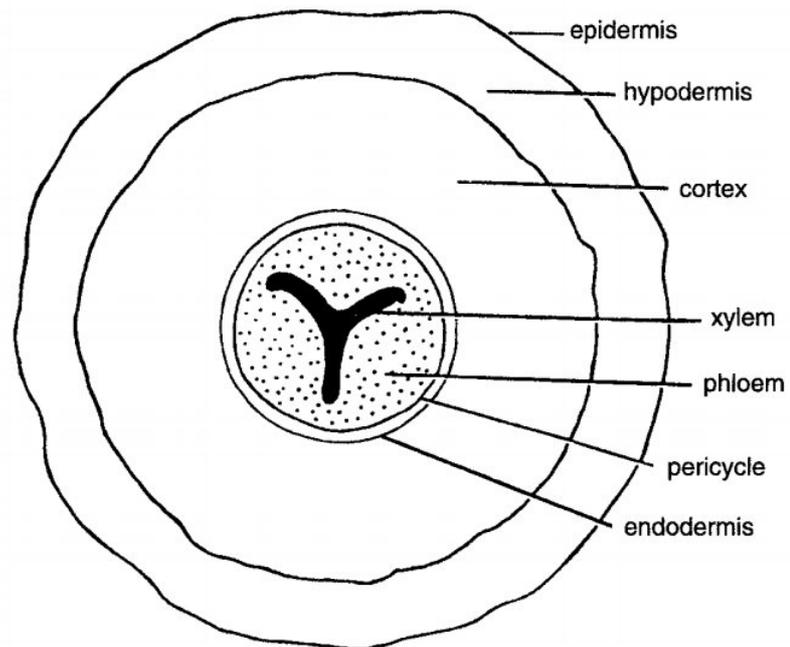


Fig. 5. *Adiantum*. T.s. rachis (outlines : diagrammatic).

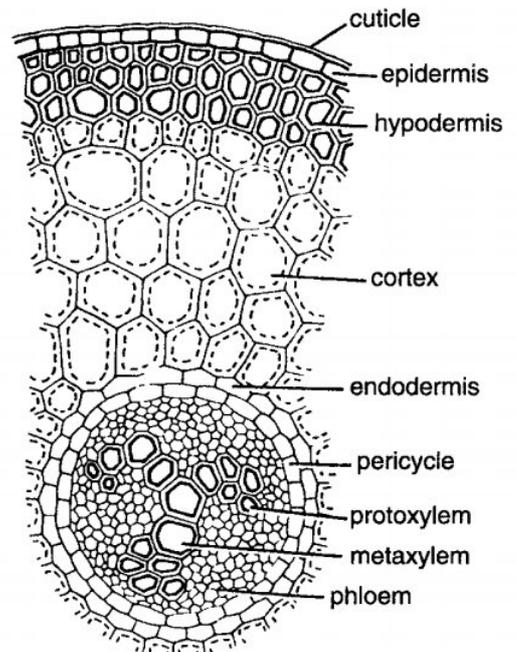


Fig. 6. *Adiantum*. T.s. rachis (a part cellular).

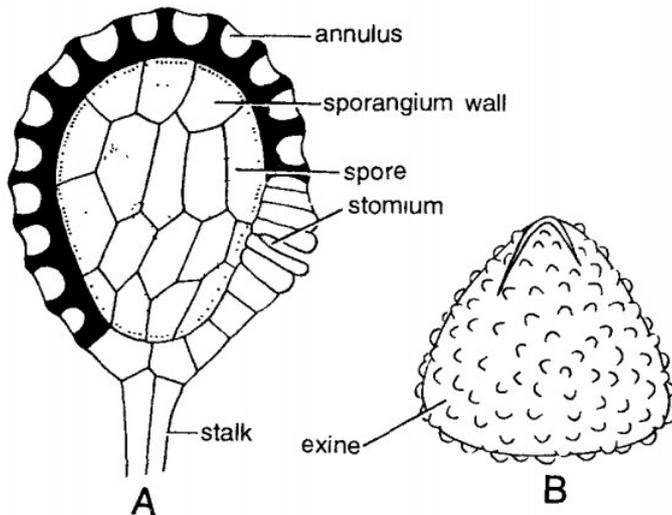


Fig. 9. *Adiantum*. A. A sporangium. B. A single spore.

8. It has a single big nucleus, surrounded by cytoplasm.

### Exercise 8

**Object :** Study of structure of prothallus.

#### Work procedure

Mount young prothallus, stain in fast green, mount in glycerine and study.

#### Comments

1. It is formed after the germination of a spore and is thus a gametophytic structure.
2. It is leafy and heart-shaped.
3. It consists of a single layer of cells, one cell in thickness, except in the central region where apical notch is situated.
4. Many unicellular rhizoids are given out from the ventral surface.
5. Antheridia are located in the posterior part of prothallus away from the apical notch.
6. Archegonia lie near the apical notch, on the thickened, central apical cushion.
7. Parts of antheridia and necks of archegonia protrude outside the general surface of the prothallus.
8. All the cells of the prothallus are thin walled and bear many discoid chloroplasts.

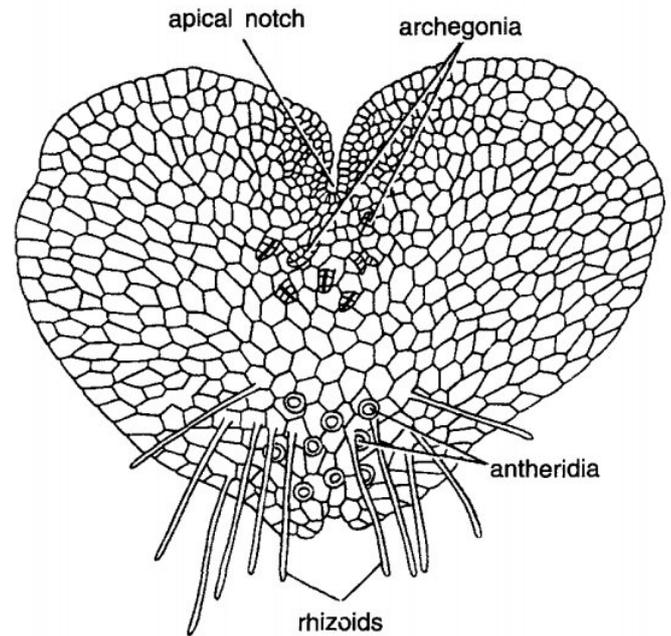


Fig. 10. *Adiantum*. Prothallus bearing young leafy sporophyte.

### Exercise 9

**Object :** Study of old prothallus with sporophyte.

#### Work procedure

Mount an old prothallus that bears a sporophyte stain in fast green and mount in glycerine.

#### Comments

1. Sporophyte is formed as result of fertilization.
2. Gametophyte (prothallus) still persists.
3. Young sporophyte is differentiated into young leaves, primary root and secondary roots.
4. Leaves stand erect and appear near the apical notch. They are simpler than the mature leaves. They may also be circinate coiled.
5. Primary root grows on the lower side and gives out secondary roots.

### Identification

**Division—Pteridophyta.** (1) Plant body differentiated into stem, roots and leaves, (2) A definite vascular strand present.

**Sub-division—Pteropsida.** (1) Vascular cylinder siphonostelic, with leaf gaps, (2) Plants macrophyllous, leaves compound, with rachis, (3) Leaves bear sporangia in sori, (4) Gametophytes small, green and free living.

5. **Stele** is a protostele.
6. **Endodermis** is single layered and is followed by a pericycle.
7. **The xylem** group is almost Y-shaped. Protoxylem elements are situated at the tips of three free ends; while the rest of the part is occupied by metaxylem.
8. **Phloem** surrounds the xylem on all the sides.

### Exercise 5

**Object : Study of anatomy of leaflet.**

#### Work procedure

Cut a T.s. of leaflet by keeping a leaf in pith. Stain in safranin-fast green combination, mount in glycerine and study.

#### Comments

1. Leaflet is differentiated into upper and lower epidermis, mesophyll, sclerenchyma and vascular bundle.
2. **Epidermis.** The cells of the upper and lower epidermis possess chloroplast. The lower epidermis is frequently interrupted by stomata.
3. **Chlorenchyma.** Just above the lower epidermis lies a single layer of compactly arranged cells containing numerous chloroplasts.
4. **Mesophyll.** Following this compact layer, mesophyll tissue extends up to the upper epidermis. It is undifferentiated into palisade and spongy parenchyma but is composed of loosely arranged spongy parenchyma only.
5. **Vascular bundle.** Each vascular bundle is

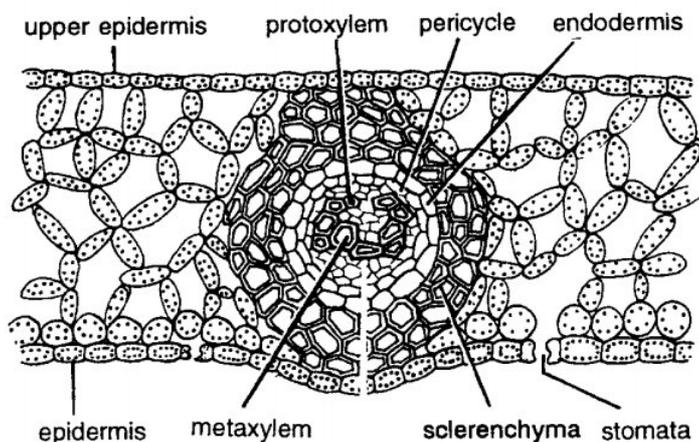


Fig. 7. *Adiantum*. T.s. leaflet (part cellular).

6. **Endodermis.** Surrounding the vascular bundle is endodermis followed by a pericycle.
7. **Xylem.** Centrally located xylem has protoxylem groups, facing towards the adaxial surface of the leaf.
8. **Phloem** surrounds xylem.

### Exercise 6

**Object : Study of structure of sorus.**

#### Work procedure

Since the sori are present on the lower reflexed side of the leaflet, sections of this part are cut to study the arrangement and structure of sorus. The sections are stained in safranin-fast green combination, mounted in glycerine and studied.

#### Comments

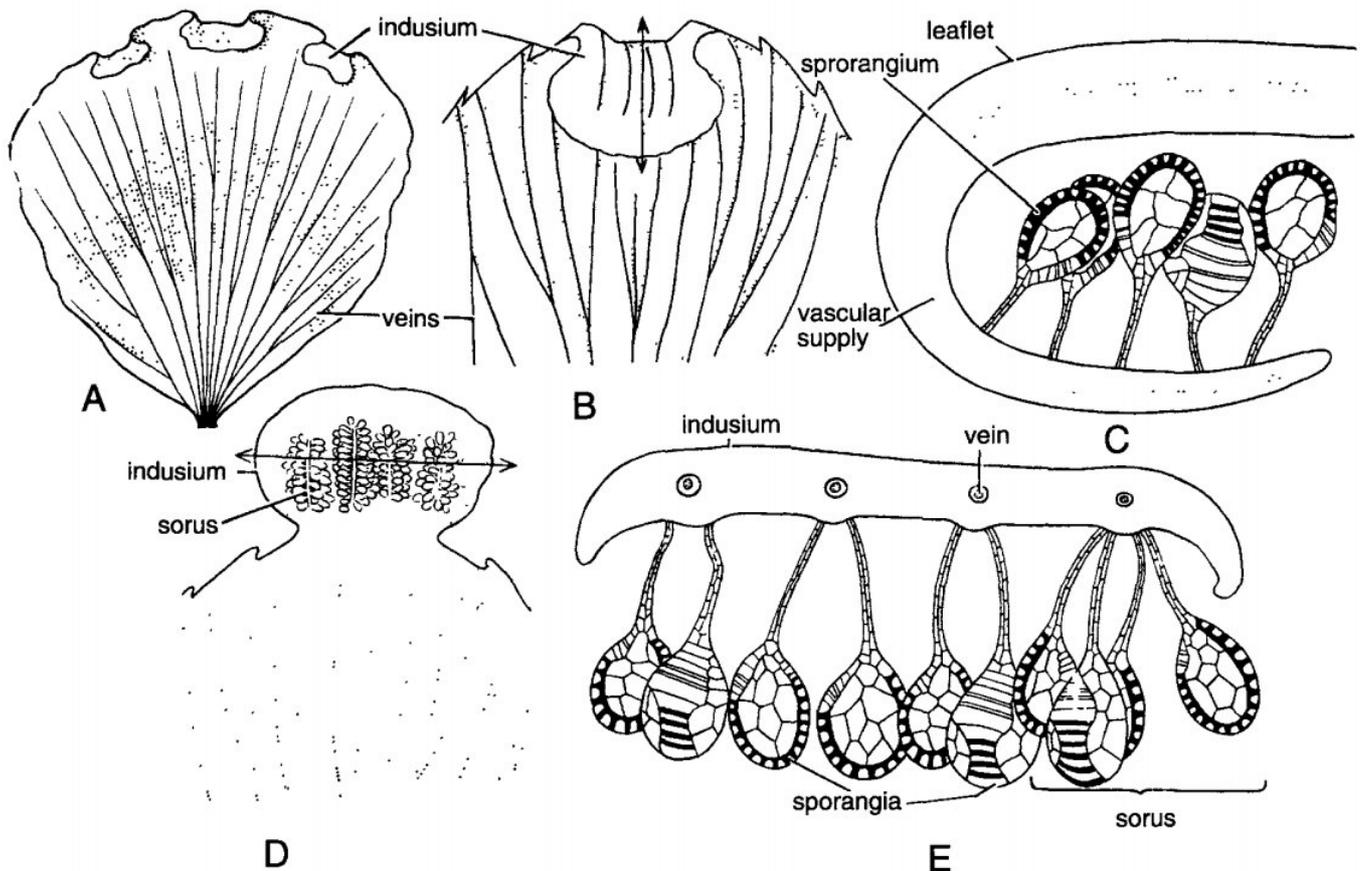
1. The spore producing organs are the sporangia grouped in sori. Each sorus is mixed in nature and shows sporangia of different ages.
2. Sori are present, along both the sides of the veins, on the dorsal side of the marginal reflexed lobe. This part remains folded towards the lower side and acts as a false indusium which is membranous and brown coloured.
3. Lower part of each leaflet shows many such reflexed lobes along its margins. The reflexed part is traversed by veins in continuation with those in the unfolded part.

*To study the relation of the folded part with the unfolded, cut a L.s. of leaflet in folded condition.*

1. It shows the upper part of the leaflet possessing vascular bundles cut longitudinally and the reflexed or folded margin on its lower side.
2. This reflexed part is a portion of the leaflet and bears sporangia in sori, thus forming a false indusium.
3. Many sporangia are seen arranged in groups.
4. Sporangia are attached to the indusium by their long slender stalks.

*To study the relation between veins, sori and sporangia with indusium, unfold the reflexed lobe and cut its T.s.*

1. Reflexed lobe of leaflet covers sori and is called false indusium.



**Fig. 8. *Adiantum*.** A. Surface view of leaflet showing reflexed margin (indusium) and venation. B. Magnified portion of leaflet showing single reflexed lobe (indusium). C. L.s. leaflet through indusium (plane shown by arrow in Fig. B) D. Unfolded reflexed lobe (indusium) showing sori on both the sides of vein. E. T.s. through indusium (plane shown by arrow in Fig. D).

2. Veins traverse the indusium but do not form reticulum (open dichotomous venation).
3. Along both the sides of each vein are many sporangia, attached by their long and slender stalks. This group of sporangia is called a sorus and many such sori are situated along each of the veins.

### Exercise 7

**Object : Study of structure of a sporangium and a spore.**

#### Work procedure

Study the preparation of section of leaflet showing sori or unfold the reflexed lobe of a leaflet, tease out a few sporangia, also crush open a few of these sporangia to release spores. Stain with safranin, mount in glycerine and study.

#### Comments

1. Each sporangium is attached to the indusium by a slender, long and multicellular stalk.
2. The sporangium (capsule) is oblong in shape and borne at the tip of the stalk.
3. The wall of the sporangium is made of thin-walled cells.
4. Some cells of this wall lying in a vertical row are characteristically thickened on their radial and inner tangential walls. These together are called as annulus.
5. On one side of the annulus are a few (2-3) thin walled cells forming a stomium wherefrom dehiscence of the sporangium takes place.
6. The wall encloses many spores inside.
7. Each spore is a double walled structure. The outer layer is exine which is thick and ornamented. The inner layer, called intine, is thin and smooth.

**Class—Leptosporangiatae.** (1) Sporangium with a jacket layer one cell in thickness, (2) Definite number of spores.

**Order—Filicales.** (1) Sori are mixed, (2) Homosporous.

**Family—Adiantaceae.** (1) Sori marginal, (2) Indusium oblong or linear, formed of the more or less changed and reflexed margin of the frond. opening inwardly.

**Genus—Adiantum.** (1) Sori apparently marginal, but superficial in origin, (2) Indusia globose to linear, usually many and distinct, (3) Leaflet margins bearing sori are sharply reflexed, (4) Open dichotomous venation of the leaflet.

### Hints for Collection

*Adiantum* is very common in hills, lower slopes of the hills and in the plains. In hills it is commonly seen in dense evergreen forests and also on limestone rocks. In plains, it can easily be collected from moist places such as banks of rivers, etc. It is also seen growing on the inner walls of the wells.

### *Nephrolepis* (Sword Fern)

#### Classification

Division	—	Pteridophyta
Sub-division	—	Pteropsida
Class	—	Leptosporangiatae
Order	—	Filicales
Family	—	Polypodiaceae
Genus	—	<i>Nephrolepis</i>

#### Exercise 1

**Object : Study of external morphology.**

#### Work procedure

Study the characters of roots, rhizome and leaves of a potted plant or a museum specimen.

#### Comments

1. The plant body is a sporophyte. It is differentiated into roots, rhizome and leaves.
2. The rhizome gives out adventitious roots from its underside. These adventitious roots are small and branched.
3. The stem is modified to rhizome. It is subterranean, short and erect. The rhizome

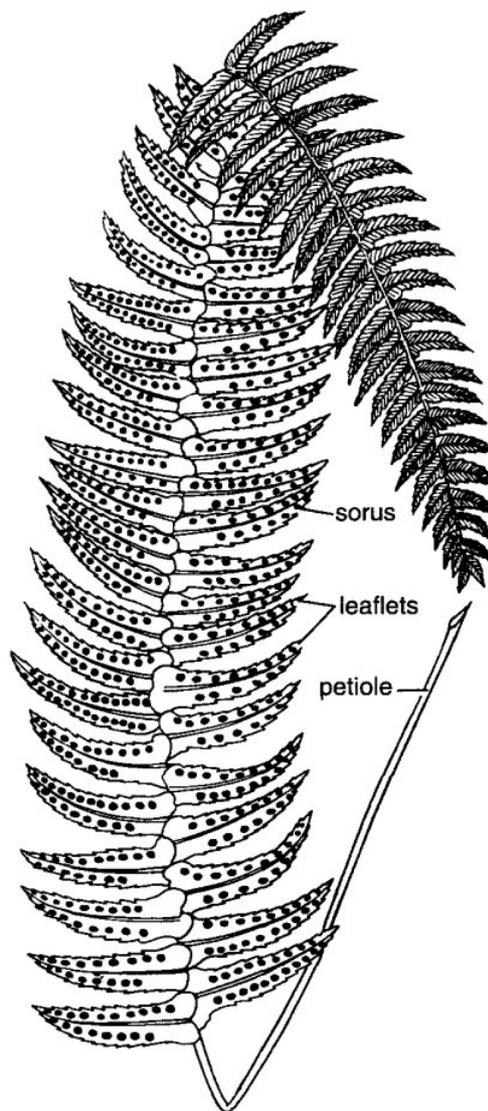


Fig. 1. *Nephrolepis*. External morphology.

produces elongated slender stolons. Peltate scales cover the rhizome.

4. In *N. tuberosa*, rhizome bears tubers. These are reservoirs of carbohydrates and water.
5. The leaves are long, narrow, sub-coriaceous and unipinnate.
6. The pinnae are sessile or shortly petioled. They have a usually rounded or cordate base. Each pinna has articulation with a pouch-like structure at the base.
7. The veins are prominent and the veinlets are branched with open ends. The tips of veinlets are gland dotted and they extend up to the margins.

**Exercise 2****Object : Study of anatomy of the root.****Work procedure**

Cut a thin transverse section of the root. Stain with safranin-fast green combination. Mount in glycerine and study.

**Comments**

1. **The outline** of the section is almost circular.
2. The tissues are differentiated into—epiblema, cortex and the vascular cylinder.
3. **Epiblema** is the outermost single layer of cells. The cells are unicellular and thin walled. A few root hairs are produced by this layer.
4. **Cortex** forms the major part of the section. It shows outer parenchymatous region and the inner small sclerenchymatous region.
5. **Endodermis** follows the cortex and separates it from the vascular tissues. The cells of endodermis show casparian strips.
6. **Pericycle.** Endodermis is followed by 1 or 2 layered parenchymatous pericycle.
7. **Vascular cylinder** is represented by a radial, diarch and exarch vascular bundle.

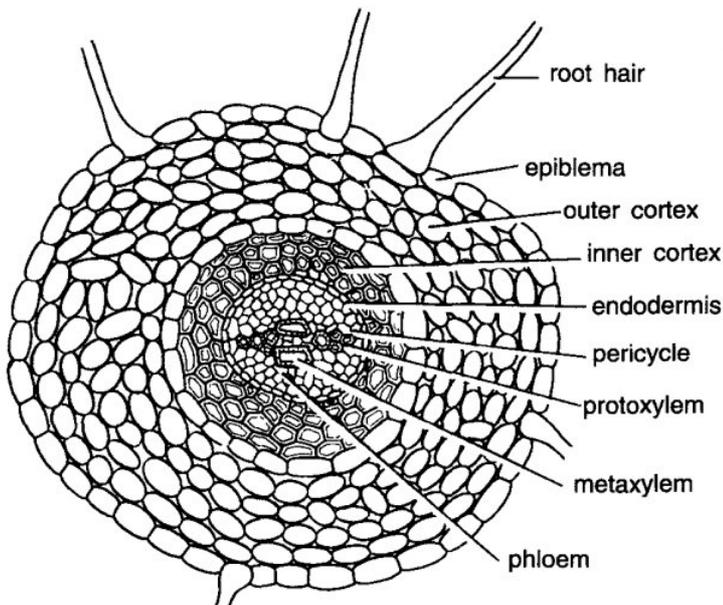


Fig. 2. *Nephrolepis*. T.s. root (diagrammatic).

**Exercise 3****Object : Study of anatomy of rhizome.****Work procedure**

Cut a thin transverse section of rhizome, stain in safranin-fast green combination, mount in glycerine and study.

**Comments**

1. **The outline** of the section is almost biconvex.
2. The section can be divided into epidermis, hypodermis, ground tissue and the stele.
3. **Epidermis** is the outermost single layer of thickly cuticularised cells.
4. **Hypodermis** that follows epidermis is made of a few sclerenchymatous layers.
5. **Ground tissue.** Rest of the tissue is called ground tissue. It is parenchymatous with numerous starch grains in the cells.
6. **Stele.** The structure of the stele varies with the age of the rhizome.
  - (i) In youngest part of rhizome, it is protostele.
  - (ii) In a few weeks old plant with a few leaves, the rhizome shows ectophloic siphonostele.
  - (iii) The old part of rhizome shows a dictyostele.
7. **Dictyostele** is made of two rings of meristele, separated by two sclerenchymatous bands.
8. **Meristele** has its own endodermis and pericycle. The centre is occupied with xylem which is completely surrounded by phloem on all its sides.

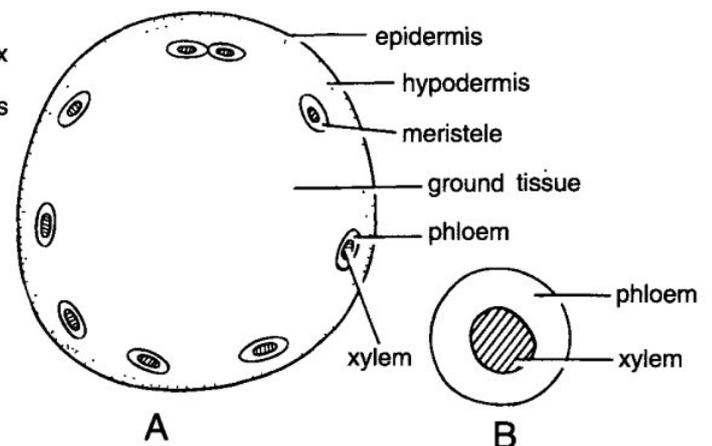


Fig. 3. *Nephrolepis*. T.s. rhizome (diagrammatic).

**Exercise 4****Object : Study of anatomy of rachis.****Work procedure**

Cut a thin and uniform transverse section of the rachis, stain with safranin-fast green combination, mount in glycerine and study.

**Comments**

1. The section appears horse-shoe shaped.
2. It shows epidermis, hypodermis, ground tissue and the stele.
3. **Epidermis** is made of single layer of thickly cuticularised cells.
4. **Hypodermis** lies below the epidermis. The cells are sclerenchymatous.
5. **Ground tissue.** The rest of the parenchymatous region extending throughout the section is called ground tissue.
6. **Stele.** In the ground tissue is situated U-shaped or horse-shoe shaped stele.
7. **Endodermis.** The stele is surrounded by a single layered endodermis followed by a few layered pericycle.

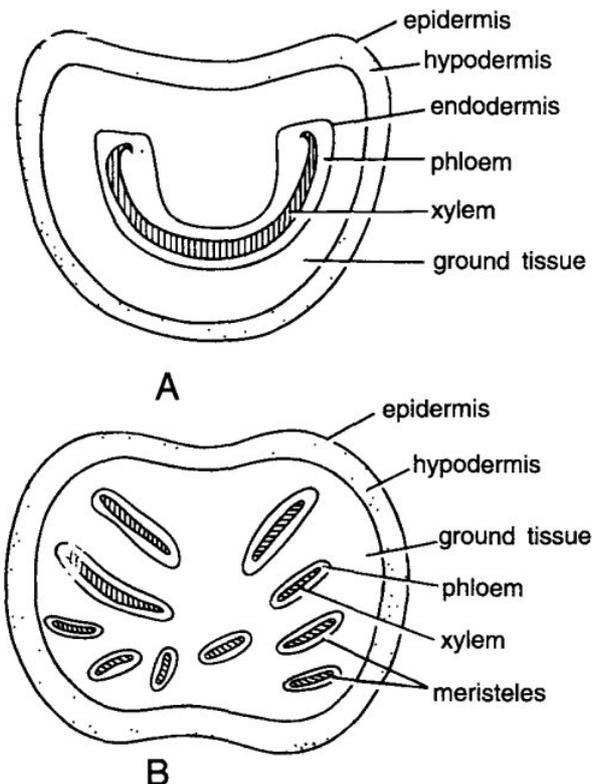


Fig. 4. *Nephrolepis*. T.s. rachis (diagrammatic).

8. **Xylem and phloem.** Centrally located xylem is surrounded by phloem on all sides.
9. **The structure of the stele** differs at various levels of rachis—
  - (i) In younger parts, there is a single U-shaped stele.
  - (ii) Little above the base, the U-breaks at the bottom, thereby producing two steles.
  - (iii) In mature parts, dissection of the stele results in many meristeles.

**Exercise 5****Object : Study of structure of the sporophyll.****Work procedure**

Observe the underside of the sporophyll, cut a transverse section of pinnae passing through a sorus. Stain in safranin-fast green combination, mount in glycerine and study.

**Comments**

1. The leaf bearing sori is called sporophyll.
2. The sporangia are present on the lower side of

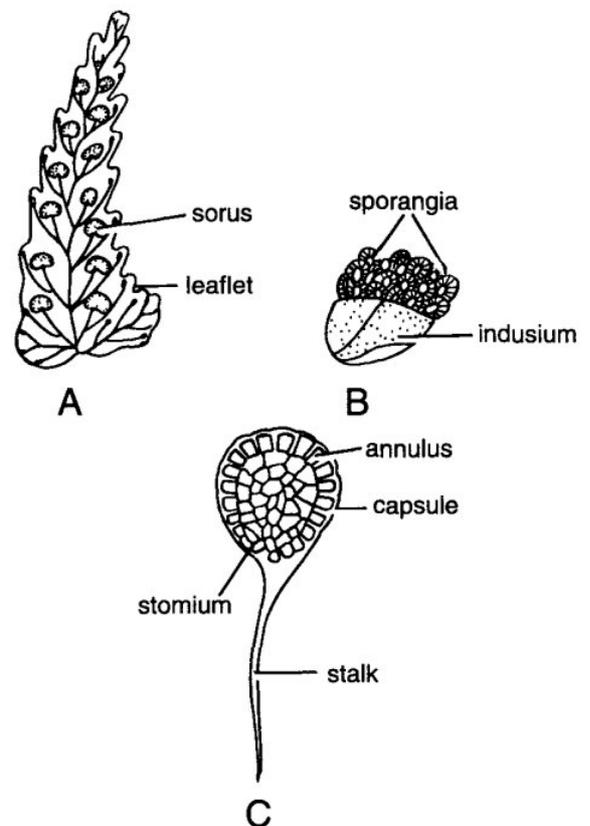


Fig. 5. *Nephrolepis*. A. Sporophyll. B. Sorus. C. A sporangium. (B-14)

mature pinnae. These occur in groups called sori.

- Sori are superficial and form definite rows, one on either side of the vein.
- The sorus appears semi-rounded, and arises at the tip of the veinlet.
- The sori are indusiate. The indusium is reniform (kidney-shaped), roundish or sub-orbicular.
- Each sporangium has a stalk and a capsule.
- The stalk is long, slender and multicellular.
- The wall of sporangial capsule is one celled thick. A ring of thick walled cells called annulus is present. A few thin walled cells forming stomium are situated in the ring.
- The capsule wall encloses 32 or 64 spores. All the spores are similar, the fern being homosporous.

### Identification

**Division—Pteridophyta.** (1) Plant body differentiated into stem, root and leaves, (2) A definite vascular strand present.

**Sub-division—Pteropsida.** (1) Vascular cylinder siphonostele/dictyostele, (2) Plants macrophyllous with large leaf gaps, (3) Leaves bear sporangia in sori, (4) Gametophytes small, green and free-living.

**Class—Leptosporangiatae.** (1) Sporangial wall one-celled thick, (2) Number of spores per sporangium definite.

**Order—Filicales.** Mixed sori.

**Family—Polypodiaceae.** (1) Annulus of sporangium vertical, (2) Each sporangium with 32-64 spores.

**Genus—Nephrolepis.** (1) Leaves unipinnate with articulate or pouch like base, (2) Sori distinct and enclosed by individual indusium, (3) Indusium true.

### Hints for Collection

It is commonly found in tropics, but a few species like *Nephrolepis acuta*, *N. tuberosa*, etc. are also grown as ornamentals.

## Pteridium (Bracken Fern)

### Classification

Division	—	<b>Pteridophyta</b>
Sub-division	—	<b>Pteropsida</b>
Class	—	<b>Leptosporangiatae</b>
Order	—	<b>Filicales</b>
Family	—	<b>Polypodiaceae</b>
Genus	—	<b>Pteridium</b>

### Exercise 1

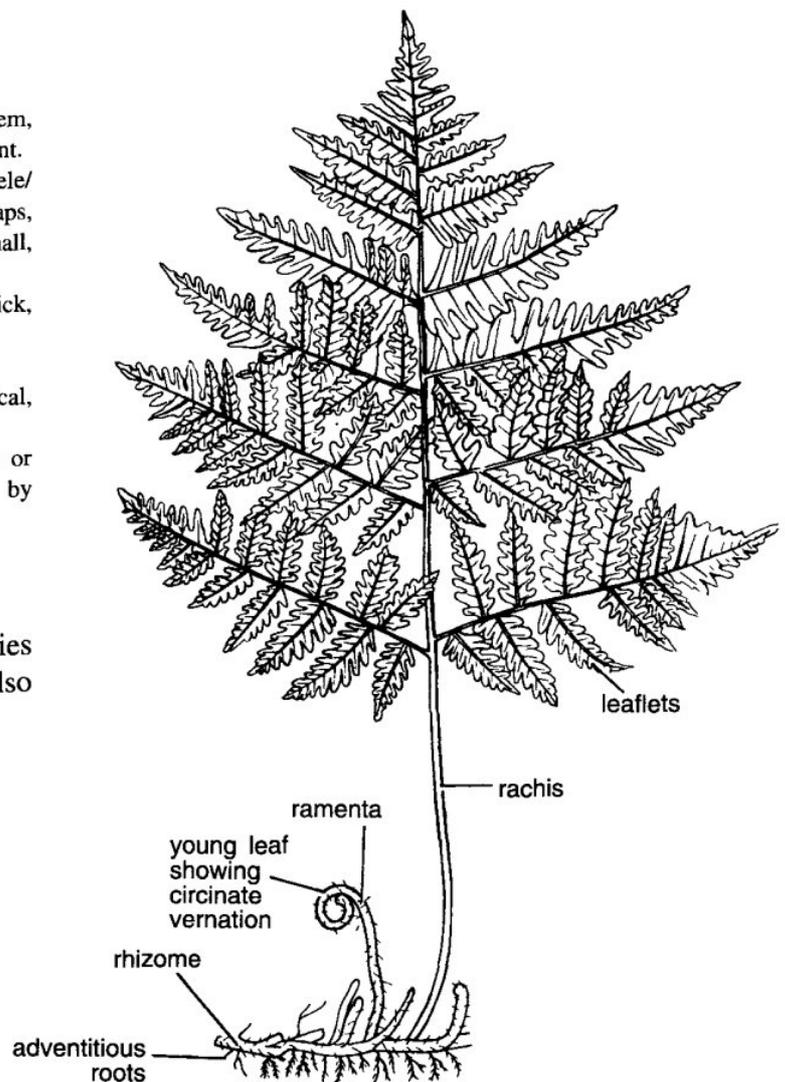
**Object :** Study of external morphology of the plant.

### Work procedure

Study a fresh plant or a preserved specimen, observe the differentiation of plant body into roots, rhizome and leaves.

### Comments

- The plant body** is a sporophyte. It is differentiated into roots, rhizome and leaves.
- Stem** is modified to rhizome. It is subterranean. The rhizome is long, slender and dichotomously



**Fig. 1. Pteridium.** External morphology : Plant showing young and mature leaves, rhizome and roots.

- branched. It is covered with brown and multicellular hairs called ramenta.
- Roots.** The rhizome gives out adventitious roots on its underside. These are small and branched.
  - The leaves** are borne alternately on the upper side of the rhizome at the nodes.
  - The young leaves** are circinately coiled. The rachis is covered with ramenta.
  - Each leaf is tripinnately compound. Each pinna is sessile. It has a distinct midrib that gives out lateral branches.

### Exercise 2

**Object : Study of anatomy of the root.**

#### Work procedure

Cut a thin transverse section of the root. Stain with safranin-fast green combination. Mount in glycerine and study.

#### Comments

- The outline** of the section is almost circular.
- It shows three regions—epiblema, cortex and the vascular cylinder.
- Epiblema** is the outermost single layer of cells. The cells are thin walled and produce unicellular root hairs.

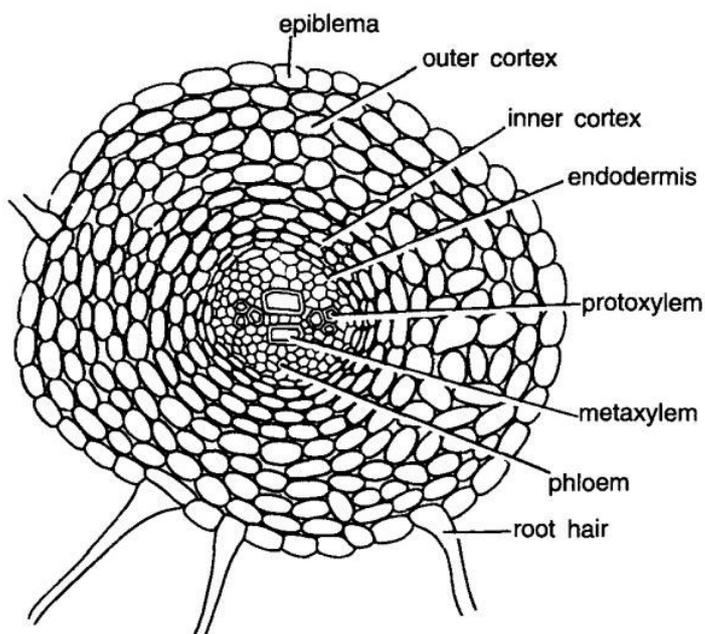


Fig. 2. *Pteridium*. T.s. of root (cellular).

- Cortex** occupies most part of the section. It is differentiated into outer and inner regions.
- The outer region is parenchymatous while the inner few layers are sclerenchymatous.
- Endodermis** follows the cortex. The radial walls of endodermal cells are characterised by casparian thickenings.
- Pericycle** is situated inner endodermis. It is 1 or 2 layered and parenchymatous.
- Vascular cylinder** shows radial, diarch and exarch conditions.
- The xylem** consists of two central metaxylem tracheids with groups of small protoxylem elements on their both sides.
- Phloem** is present on both the sides of xylem plate.

### Exercise 3

**Object : Study of anatomy of rhizome.**

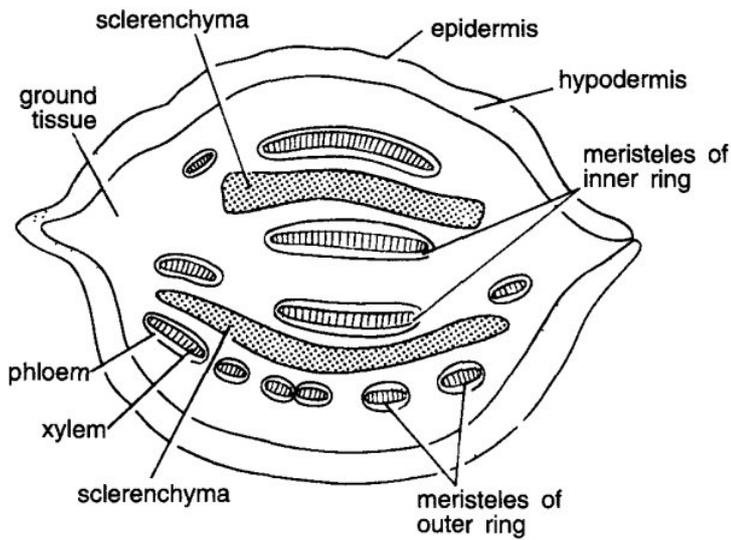
#### Work procedure

Cut a transverse section of rhizome, stain in safranin-fast green combination, mount in glycerine and study.

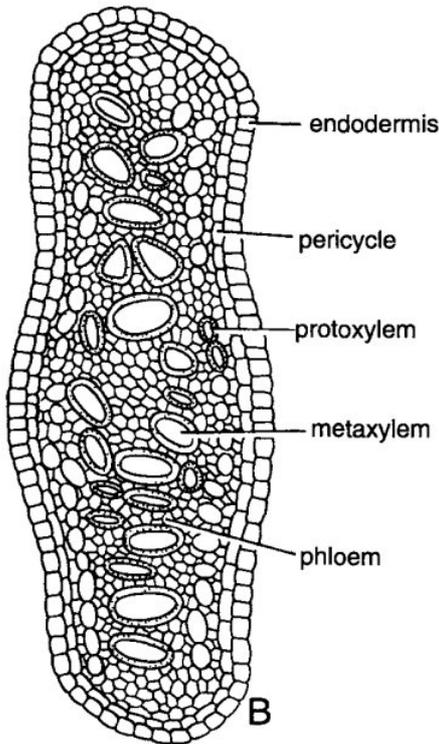
#### Comments

- The outline** of the section appears almost like a biconvex lens.
- The tissues are differentiated into epidermis, hypodermis, ground tissue and the stele.
- Epidermis** is the outermost single layer of cells. The cells are thickly cuticularised.
- Hypodermis** lies below the epidermis. The cells are sclerenchymatous which often show pitted walls. It is generally interrupted on the lateral sides by parenchyma.
- Ground tissue** follows the hypodermis. It is parenchymatous and is spread up to the centre of the section. The cells are filled with starch grains.
- Stele.** The structure of the stele varies with the age of the rhizome.
  - In just formed rhizome, condition is protostelic.
  - In a few weeks old plant with 2-3 leaves, the rhizome shows ectophloic siphonostele.

(B-14)



A



B

Fig. 3. *Pteridium*. T.s. rhizome; A. Diagrammatic, B. A meristele enlarged.

- (iii) In mature plant, the old part of rhizome shows a dictyostele.
- Dictyostele** is made of meristeles arranged in two rings, separated by two sclerenchymatous bands.
  - Meristele** is surrounded by its own endodermis, which is followed by one or two layers of parenchymatous pericycle.

(B-14)

9. The centre of the meristele is occupied by xylem which is completely surrounded by phloem on all sides.

#### Exercise 4

**Object : To study the anatomy of rachis.**

#### Work procedure

Rachis is thin and wiry, hence a sharp blade or razor would be required to cut a section. Cut transverse section, stain in safranin-fast green combination, mount in glycerine and study.

#### Comments

- The outline** of the section appears horse-shoe shaped or hemispherical.
- The tissues of the section are differentiated into epidermis, hypodermis, ground tissue and the stele.
- Epidermis** which is the outermost single layer of cells is thickly cuticularised.
- Hypodermis** is present below the epidermis. It is 2 to 3 layered thick. The cells are sclerenchymatous.
- Ground tissue.** Following the hypodermis is a large region of parenchyma called ground tissue.
- Stele.** In the ground tissue is situated U-shaped or horse-shoe shaped stele.
- Endodermis and pericycle.** Stele is surrounded by a single layered endodermis followed by a few layered parenchymatous pericycle.
- Xylem.** The centre of the stele is occupied by massive xylem. Metaxylem is present in the centre with protoxylem located at two of its ends.
- Phloem.** The region between xylem and the pericycle is filled by phloem.
- The nature of the stele varies with the maturity of the rachis.
  - In younger parts stele is U-shaped.
  - Little above the base, it gets dissected into two large meristeles.
  - In mature parts, many meristeles are present as a result of further dissection of the original stele.

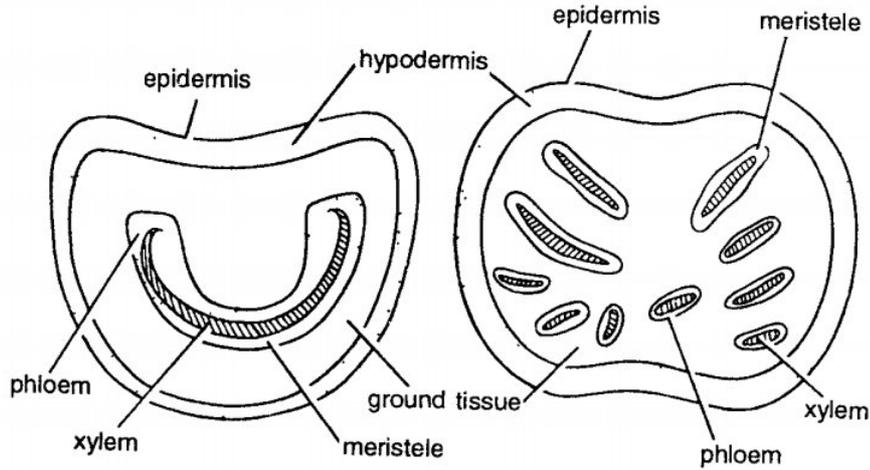


Fig. 4. *Pteridium*. Anatomy of rachis; A. T.s. of young rachis, B. T.s. of old rachis (both diagrammatic).

### Exercise 5

**Object :** Study of anatomy of pinnule.

#### Work procedure

Cut vertical transverse section of the pinnule. Stain with safranin-fast green combination, mount in glycerine and study.

#### Comments

1. The section shows the 'midrib' region and the wings.
2. **The midrib region** consists of compact parenchyma in which a single concentric vascular bundle is situated. It shows centrally located xylem surrounded by phloem. A distinct parenchymatous bundle sheath surrounds the bundle.

3. **The upper and the lower epidermis** are single layered. The stomata are present only on the lower surface.
4. **Mesophyll** that lies between the two epidermal layers is differentiated into palisade and spongy parenchyma.
5. **The spongy tissue** is situated close to the lower epidermis. The cells are loosely arranged and contain many chloroplasts. The intercellular spaces open into stomata.

### Exercise 6

**Object :** Study of structure of the sporophyll.

#### Work procedure

Cut a vertical transverse section of the pinnule that has sori on the lower side. Stain in safranin and mount in glycerine. Study the characters of sorus and the sporangium.

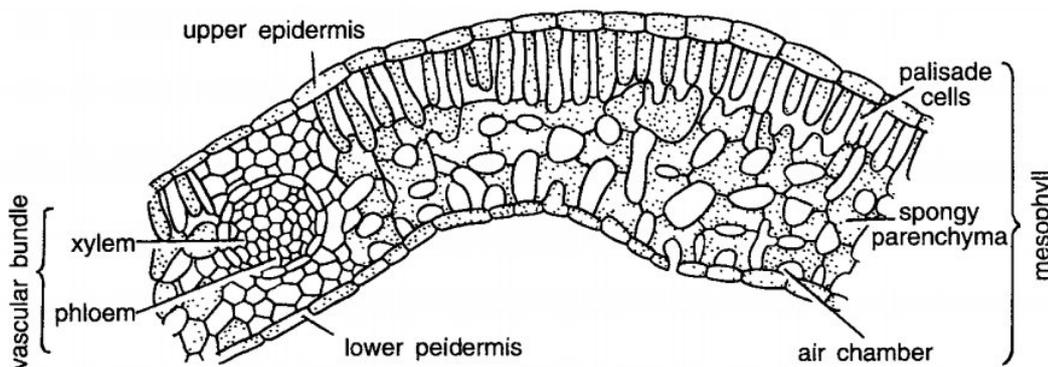


Fig. 5. *Pteridium*. T.s. pinnule.

### Comments

1. The leaf bearing sori is called sporophyll.
2. The sporangia occur in groups called sori on the lower or abaxial side of pinnules. The sporangia form a continuous linear sorus along the margins. Such a confluent sorus is called coenosorus. The identity of sorus is thus lost and only one long sorus appears along the two lateral margins of the fertile pinnules.
3. The sorus is protected by indusium. It is made of upper indusial flap formed by the incurved margins of the pinnule and the lower true indusial flap that is poorly developed.
4. The sporangia in the sorus occur mixed. The development is leptosporangiate.
5. Each mature sporangium is differentiated into a stalk and a capsule.
6. The stalk of the capsule is made of three rows of cells. It is long and slender.
7. The capsule is ovate or biconvex. The sporangial jacket is single layered thick. A ring of thick walled cells forms the annulus. A few thin walled cells of the ring form the stomium. The capsule wall encloses 32 or 64 spores.
8. All the spores being similar, the fern is homosporous. Spores are haploid and uninucleate. The wall is two layered. The outer thick layer is called exine and the inner thin layer is called intine.

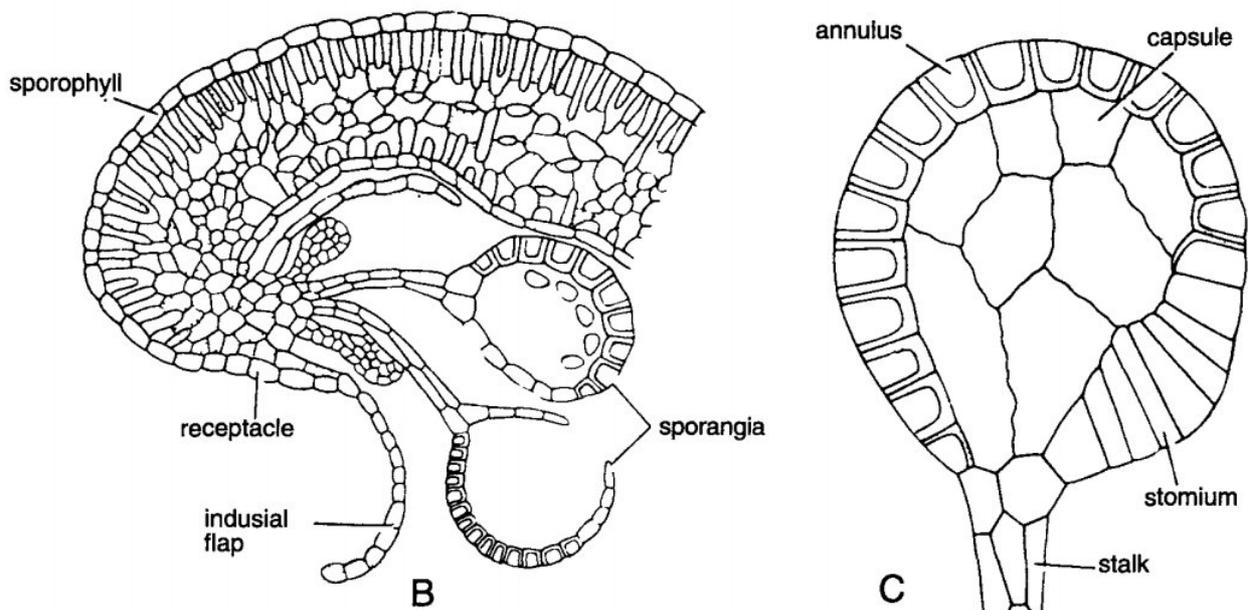


Fig. 5. *Pteridium*. A. sporophyll, B. Vertical section of a leaflet, C. A sporangium.

### Exercise 7

**Object : Study of structure of prothallus.**

### Work procedure

Study a slide of prothallus showing sex organs. Note the positions of sex organs and also the young sporophyte.

### Comments

1. The prothallus is a gametophyte formed as a result of spore germination.
2. It is dark green, heart-shaped and single layered sheet of cells. The midrib region becomes a

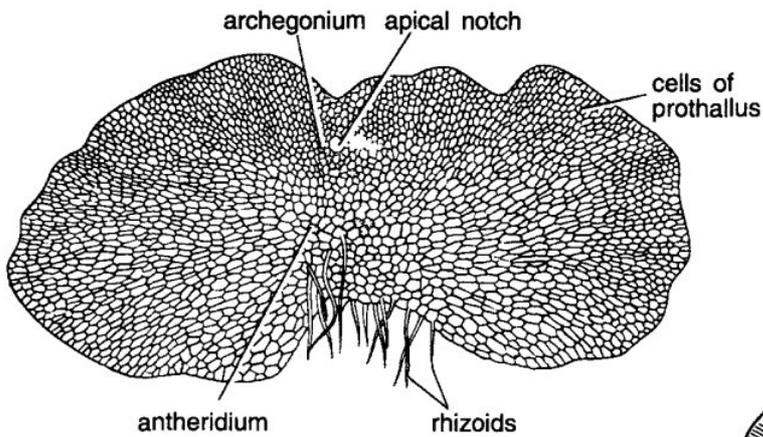


Fig. 7. *Pteridium*. Young prothallus with sex organs.

cushion of several cells. It remains attached to the substratum by rhizoids produced on the lower side in the central region.

3. The antheridium is surrounded by the cells of the prothallus. Each antheridium consists of wall of three rings of cells. It encloses 30-40 multiflagellate antherozoids at maturity.
4. Archegonia develop near the apical notch. Each is made of neck and ventre. The neck is 5-7 celled high with a single binucleate neck canal cell. The ventre has a small ventre canal cell and a large egg.

### Exercise 8

**Object :** Study of prothallus with young a sporophyte.

#### Work procedure

Study a slide of old prothallus. If prothallus is collected from the pot or natural habitat, stain in fast green, mount in glycerine and study.

#### Comments

1. Sporophyte is formed as a result of fertilization. The zygote grows into a sporophyte that still remains attached to the prothallus.
2. Young sporophyte is differentiated into young leaves, primary and secondary roots.
3. The leaves are petiolate and erect. These emerge through the apical notch. The leaves are simpler than the mature leaves. Sometimes these even show circinate vernation.

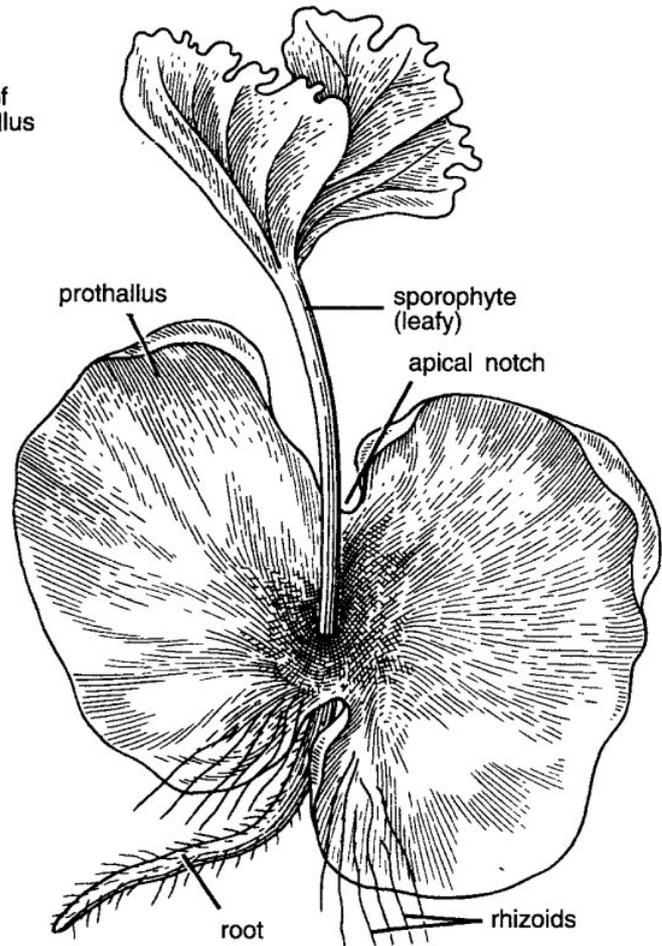


Fig. 8. *Pteridium*. Prothallus with a young sporophyte.

4. Primary root grows on the lower side and gives out secondary roots.
5. The sporophyte is dependent on the gametophyte till first leaf is formed. It absorbs its food through the foot of the young embryo.

### Identification

**Division—Pteridophyta.** (1) Plant body differentiated into stem, root and leaves, (2) A definite vascular strand present.

**Sub-division—Pteropsida.** (1) Vascular cylinder siphonostele/dictyostele, (2) Plants macrophyllous with large leaf gaps, (3) Leaves bear sporangia in sori, (4) Gametophytes small, green and free-living.

**Class—Leptosporangiatae.** (1) Sporangial wall one-celled thick, (2) Number of spores per sporangium is definite.

**Order—Filicales.** Mixed sori.

**Family—Polypodiaceae.** (1) Annulus of sporangium vertical, (2) Each sporangium with 32-64 spores.

**Genus—Pteridium.** (1) Leaves tripinnately divided, (2) Presence of coenosorus, (3) Sorus enclosed between indusial flaps.

## Hints for Collection

*Pteridium* is cosmopolitan. It is widely distributed along the entire Himalayan tract. It grows particularly well at altitudes between 1,000 to 3,000 meters. *P. aquilinum* is found on forest floors, mountain slopes, open grasslands, etc.

### *Marsilea*

#### Classification

Division	—	Pteridophyta
Sub-division	—	Pteropsida
Class	—	Leptosporangiateae
Order	—	Marsileales
Family	—	Marsileaceae
Genus	—	<i>Marsilea</i>

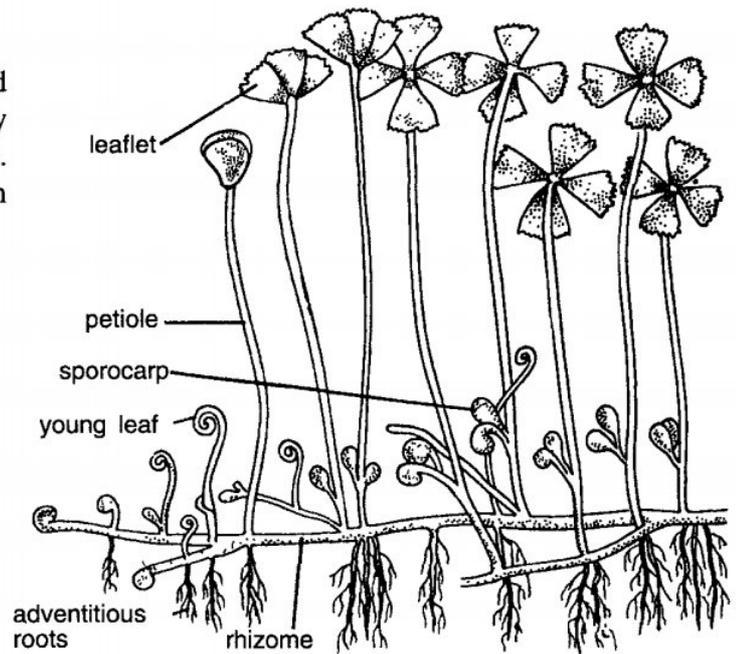


Fig. 1. *Marsilea*. External features.

#### Exercise 1

**Object :** Study of external morphology.

#### Work procedure

Study external characters of the plant. Observe various features of root, rhizome and leaves. Note the circinate vernation of young leaves and the characteristic leaf venation.

#### Comments

1. **The plant body** is differentiated into a rhizome, roots and leaves.
2. **The rhizome** is slender, creeping branched. It may either grow in water or attached by roots in the damp soil.
3. It bears nodes and internodes. The leaves and roots occur in acropetal succession (youngest towards the apex of rhizome) on the nodes. The adventitious roots grow downwards and the leaves grow upwards. Young leaves are circinate, a characteristic of most ferns.
4. **The leaves** present at the nodes occur in two rows, (two ranked) one on either side of the mid-line of the rhizome.
5. Each leaf consists of a long petiole, bearing at its top generally four leaflets or pinnae, apparently arising from one common point. In *M. quadrifolia*, a common Indian species, six

6. The division of lamina into four pinnae is the result of three dichotomies, close to each other. Therefore, out of the four leaflets, two form a distal pair while the lower two are alternate. The leaflets, thus give a false impression of arising from one common point.
7. **Each leaflet** is obovate. The venation is dichotomous with several cross connections. The free veinlets at the apex of the leaflet are tied up with marginal loops.
8. Leaflets fold up in the night or early morning, thus showing sleeping movements.
9. The plant when grows in water, has long, flexible petioles and the leaflets float on the surface of the water but when it grows on

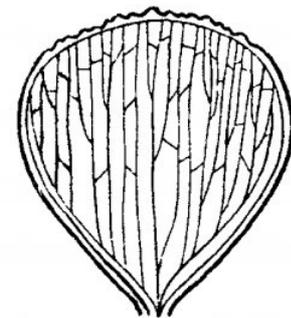


Fig. 2. *Marsilea*. Leaflet showing dichotomous venation.

mud or damp soil, the petioles become short and rigid.

(It is interesting to note that, when in a pond in which *Marsilea* is growing, water level rises, the petioles are also seen to increase in length. Contrary to it when level goes down, the petioles are found to coil, as such in both the conditions, the leaflets float on water surface).

10. The spore bearing structures known as sporocarps are commonly borne laterally near the base, on the petiole, but sometimes higher up. The two common Indian species, *M. minuta* and *M. quadrifolia* show variation in the number of sporocarps from one to four.

### Exercise 2

**Object : Study of anatomy of the root.**

#### Work procedure

Cut a T.s. of the root, stain in safranin-fast green combination, mount in glycerine and study.

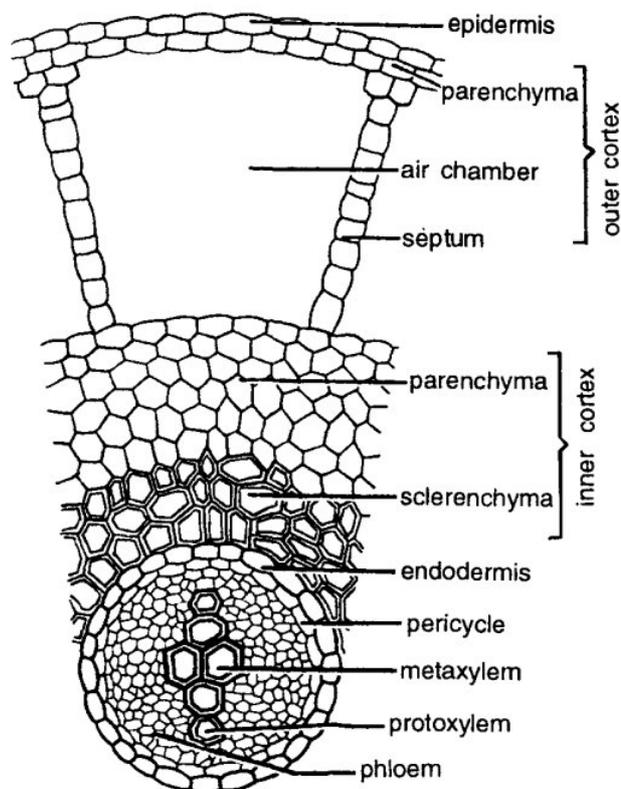


Fig. 3. *Marsilea*. T.s. root (a part cellular).

#### Comments

1. **The outline** of the section appears almost circular.
2. **The epidermis** is single layered with tangentially elongated cells.
3. **The cortex** is differentiated into an outer and an inner cortex.
4. **The outer cortex** has many air chambers separated by radial septa.
5. **The inner cortex** has either all the parenchymatous cells or some of the cells towards the inner side may become thick walled and sclerenchymatous.
6. **Endodermis** is single layered. It is followed by one layered pericycle. These surround vascular bundle.
7. **Xylem** is diarch and exarch xylem. It is situated in the centre. The protoxylem elements are situated opposite one another.
8. **The phloem** has smaller cells and forms two bands, one on either side of the xylem mass.

#### Features of special interest

The root shows aerenchyma in the outer cortex (hydrophytic character).

### Exercise 3

**Object : Study of anatomy of rhizome.**

#### Work procedure

Cut a T.s. of rhizome, stain in safranin-fast green combination, mount in glycerine and study.

#### Comments

1. **The outline** of the section appears almost circular.
2. **The section** shows three regions—epidermis, cortex and stele.
3. **The epidermis** is single layered without stomata. The epidermis of aquatic plants lacks cuticle but that of terrestrial individuals has a distinct cuticle.
4. **The cortex** is differentiated into three regions—the outer, the middle and the inner.
5. **The outer cortex** has well-developed air

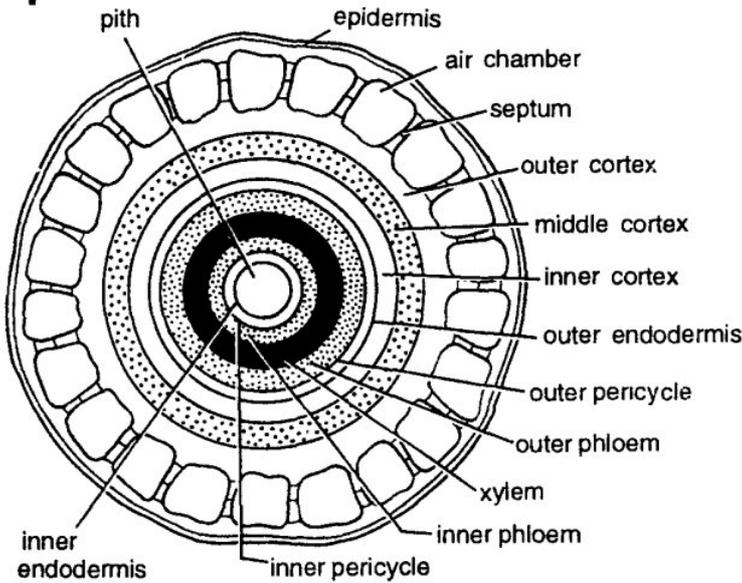


Fig. 4. *Marsilea*. T.s. rhizome (diagrammatic).

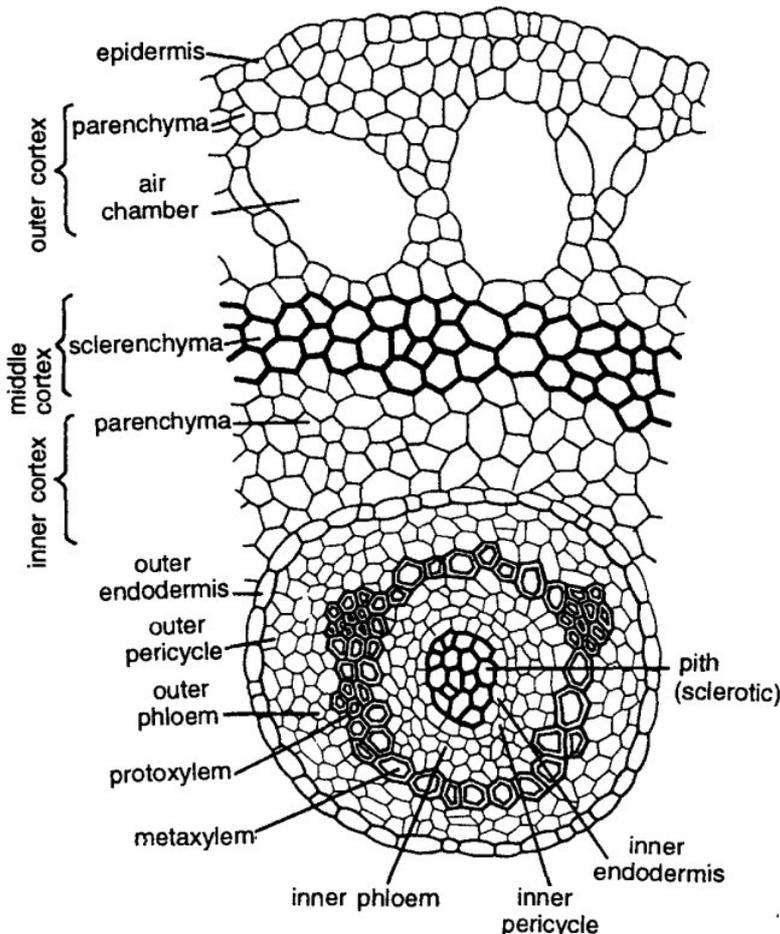


Fig. 5. *Marsilea*. T.s. rhizome (a part cellular).

spaces, separated by radially arranged parenchymatous cells (aerenchyma). The outermost cells of the cortex contain chloroplasts.

6. **The middle cortex** is thick walled, made up of sclerenchymatous cells and is only a few celled thick.
7. **The inner cortex** is composed of thin-walled parenchymatous cells containing starch.
8. **The stele** is an amphiphloic solenostele.
9. **Stele** shows a central xylem ring. On its outer side is outer phloem ring, outer pericycle and outer endodermis. On the inner side i.e. towards the pith are present inner phloem ring, inner pericycle and inner endodermis.
10. **Protoxylem** groups may or may not be distinct. They are generally exarch, but in some cases mesarch too.
11. **Pith** lies in the center. In aquatic plants it is parenchymatous and in terrestrial plants it is sclerotic.

#### Features of special interest

1. It shows hydrophytic character viz. presence of aerenchyma in the cortex, as well as some xerophytic characters viz.
  - (i) thick walled middle cortex and
  - (ii) sclerotic pith
2. Presence of amphiphloic solenostele.

#### Exercise 4

**Object** : Study of anatomy of petiole.

#### Work procedure

Cut a T.s. of petiole, stain in safranin-fast green combination, mount in glycerine and study.

#### Comments

1. **The outline** of the section is circular.
2. **Epidermis** is the outermost layer with rectangular cells.
3. **Hypodermis** is sometimes present below the epidermis. It is one or two layered.
4. **The cortex** is differentiated into an outer and an inner zone.
5. **The outer cortex** has many air chambers, separated by narrow radially arranged parenchymatous cells (aerenchyma).
6. **The inner cortex** has parenchymatous cells containing starch. A few cells contain tannins also.

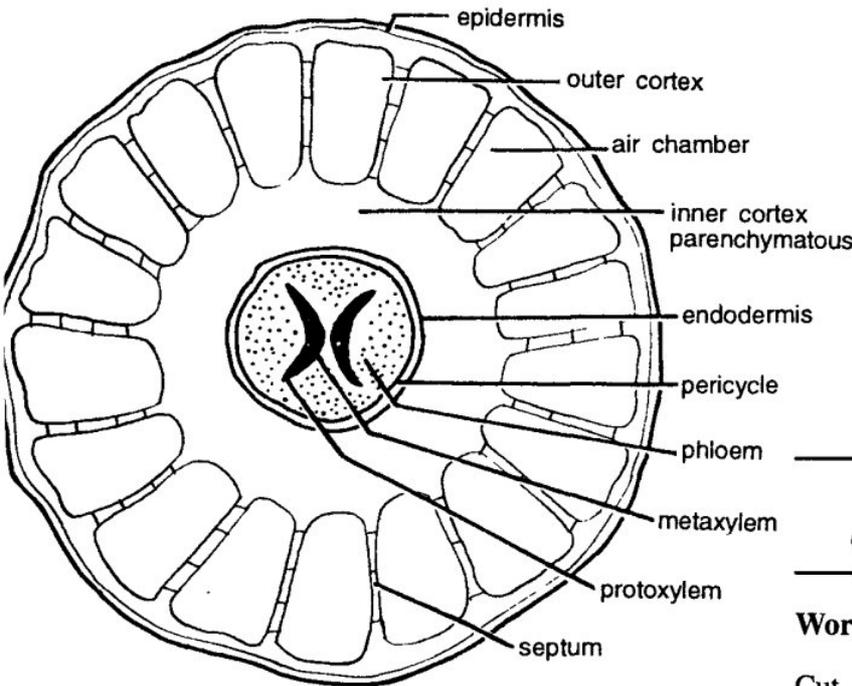


Fig. 6. *Marsilea*. T.s. petiole (outlines).

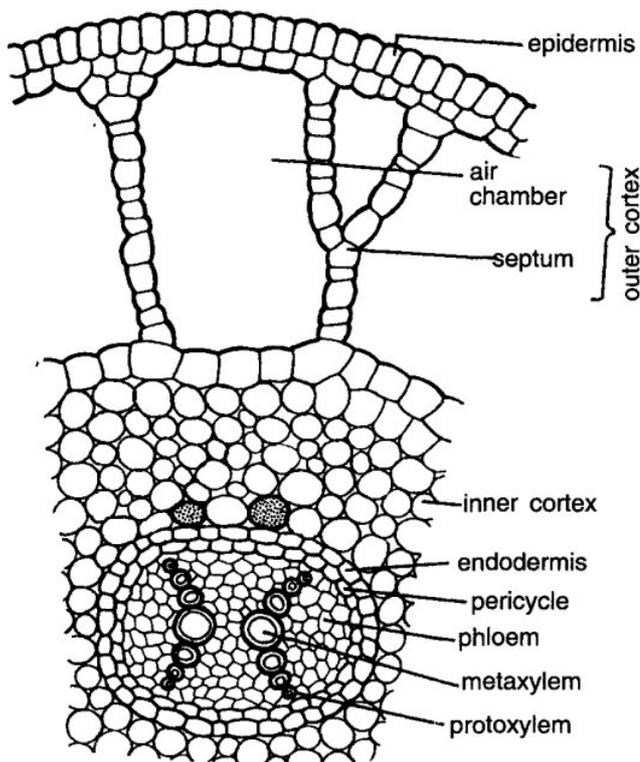


Fig. 7. *Marsilea*. T.s. petiole (a part cellular).

7. The stele is a protostele.
8. Endodermis is single layered. It is followed by a single layer of pericycle.
9. The xylem is 'V shaped' with exarch protoxylem. The two arms of 'V' are slightly curved and separate. Each arm has generally

one or two large tracheids in the middle and smaller tracheids towards both the ends. The open end of 'V' always points towards the adaxial side of the petiole (towards the axis).

10. Phloem surrounds the xylem.

**Features of special interest**

1. Shows hydrophytic character viz. presence of aerenchyma in the outer cortex.
2. Presence of V-shaped xylem.

**Exercise 5**

**Object : Study of anatomy of leaflet.**

**Work procedure**

Cut a T.s. of the leaflet, stain with safranin-fast green combination, mount in glycerine and study.

**Comments**

1. The section shows an upper and lower epidermis, mesophyll and a vascular bundle.
2. The stomata are found on both upper and lower epidermis if the plant is terrestrial but they are found only on upper epidermis if leaves float on water surface.
3. Mesophyll is differentiated into palisade and spongy parenchyma.
4. Palisade is arranged in one layer near the upper epidermis. Spongy parenchyma is located near

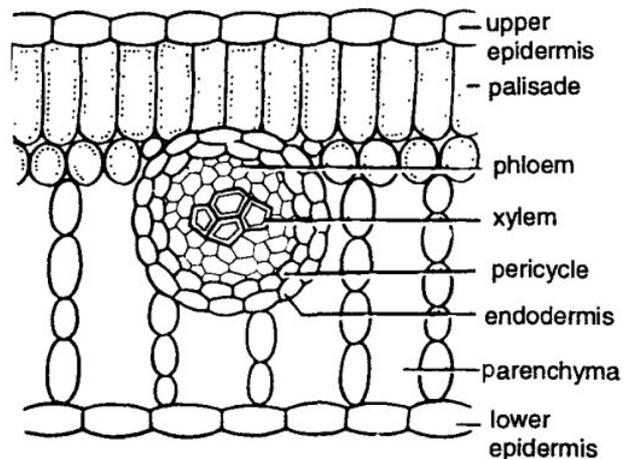


Fig. 8. *Marsilea*. T.s. leaflet (a part cellular).

the lower epidermis. It is loosely arranged to form large air spaces and is called aerenchyma.

5. There are many vascular bundles. Each bundle is concentric with centrally located xylem surrounded by phloem.
6. The distinct endodermis is present just outside the vascular bundle.

**Exercise 6**

**Object : Study of external features of sporocarp.**

**Work procedure**

Study the external characters of a sporocarp.

**Comments**

1. The spore-bearing organs are the sporocarps which are borne laterally on the adaxial side of the petiole. Their number and positions vary from species to species.
2. Sporocarp is stalked, bean-shaped or ovoid structure.
3. The place of attachment of the body of the sporocarp to the peduncle (stalk) is known as raphe.
4. Beyond the raphe, there are two projections known as teeth or tubercles, one tooth being lower than the other.
5. The lower tooth is usually stouter and more prominent while the upper tooth, which lies a short distance above is usually more slender and delicate.
6. The side on which the raphe is present is the basal side and the side opposite to it is the

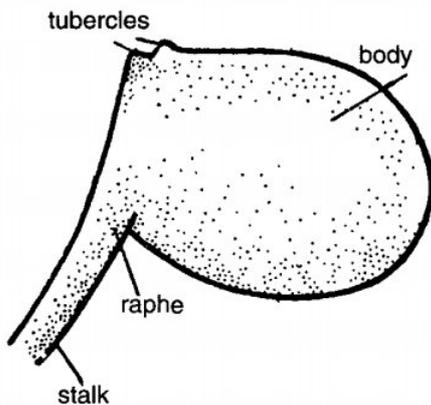


Fig. 9. *Marsilea*. A single sporocarp.

apical side. The side on which the tubercles are present is the dorsal side and the side opposite to it is ventral side.

**Exercise 7**

**Object : Study of V.T.s. of sporocarp.**

**Work procedure**

Cut a section of the sporocarp in a plane almost parallel to the stalk as shown in reference diagram. Stain the section in safranin, mount in glycerine and study.

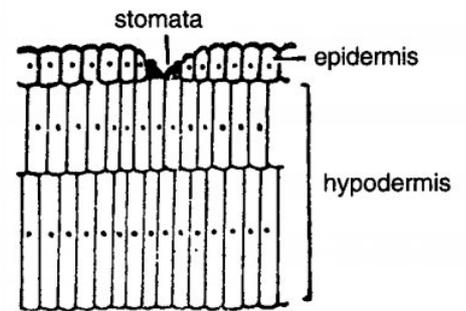


Fig. 10. *Marsilea*. A part of the sporocarp wall (cellular).

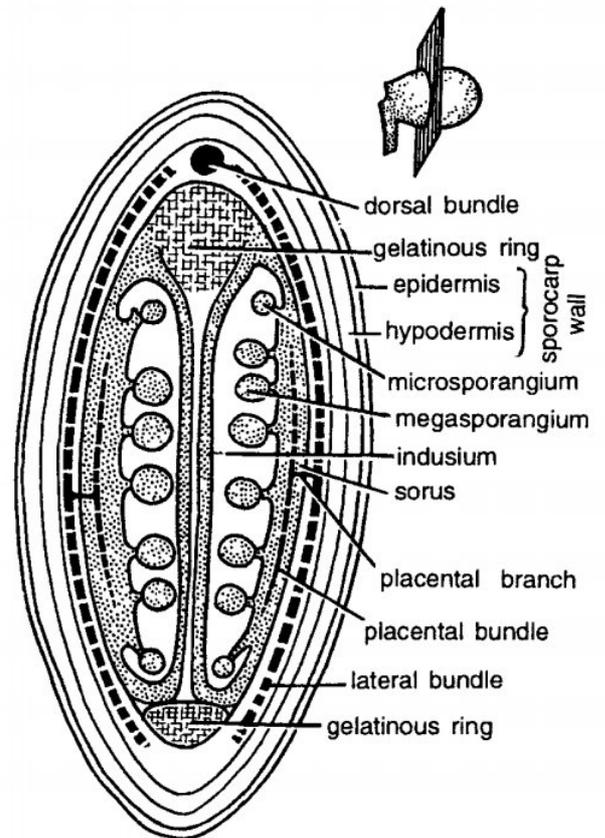


Fig. 11. *Marsilea*. V.T.s. sporocarp.

### Comments

1. **The section** shows wall of the sporocarp which encloses sori.
2. **The wall** is made of outer epidermis followed by hypodermis.
3. **Epidermis** consists of thick walled cells. Numerous stomata are present in the epidermis.
4. **Hypodermis** consists of two layers of radially elongated cells. The cells of the inner layer are double in length as compared to the cells of the outer layer.
5. All the cells of both the layers have their nuclei arranged in one row.
6. **Receptacles** are cut longitudinally. Only two sori are seen, each of which is covered by its own 2 layered indusium. The receptacle of the sorus bears microsporangia at the corners and megasporangia all along the receptacular ridge.
7. On the upper and lower sides of the receptacles, two masses of gelatinous ring, cut transversely, are present. The upper one is bigger in size than the lower.
8. The dorsal bundle, lateral bundles, placental branches and placental bundles are seen.

### Exercise 8

**Object : Study of V.L.s. of the sporocarp.**

### Work procedure

Hold the sporocarp with tubercles pointing upwards. Split the sporocarp by a sharp blade in two halves. Study the section under dissecting microscope, section being thick.

### Comments

1. The section shows wall of the sporocarp enclosing sori embedded in a gelatinous wall.
2. The outermost is the sporocarp wall. It is made of an epidermis with stomata and two layered hypodermis.
3. Below the sporocarp wall is a gelatinous ring which surrounds sori. It is relatively more prominent on the dorsal side than on the ventral.
4. The sori are cut longitudinally and appear in a row.

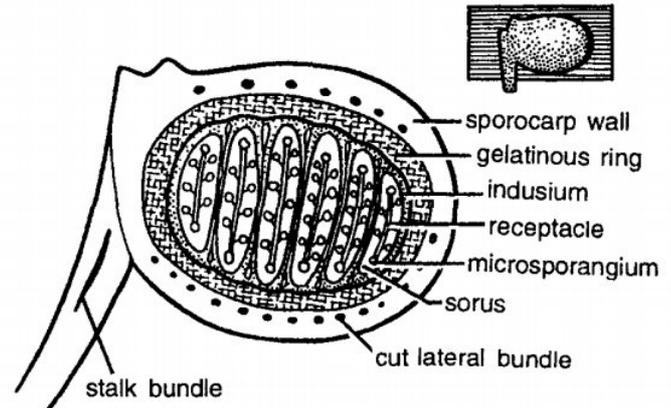


Fig. 12. *Marsilea*. V.L.s. sporocarp showing microsporangia.

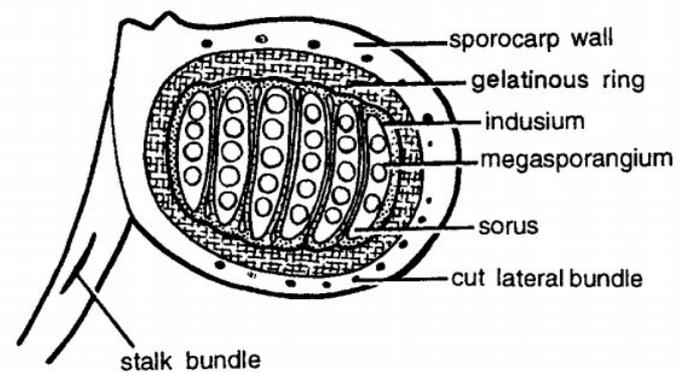


Fig. 13. *Marsilea*. V.L.s. sporocarp showing megasporangia.

5. Each sorus is surrounded by its own indusium.
6. If the section passes through the centre, then megasporangia are seen in all the sori. Since the megasporangia are present at the apex of the receptacle, no receptacle is seen.
7. If the section is not perfectly median, then microsporangia are seen attached on either sides of the receptacle in each sorus.
8. In this section the stalk bundle and cut lateral bundles are seen.

### Exercise 9

**Object : Study of H.L.s. of the sporocarp.**

### Work procedure

Hold the stalk between the thumb and the index finger. Cut a section by passing a blade at right angles to the stalk axis (see reference diagram). Stain in safranin, mount in glycerine and study.

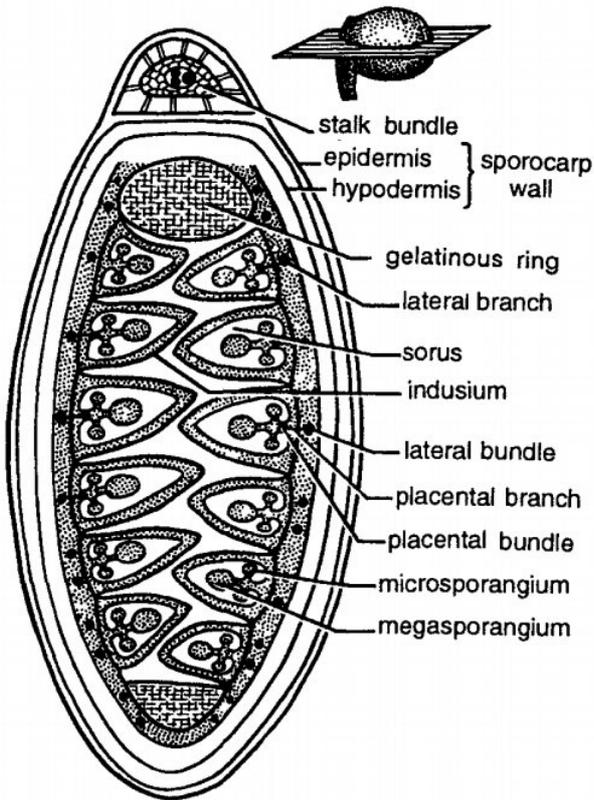


Fig. 14. *Marsilea*. H.L.S. sporocarp.

### Comments

1. The section shows transversely cut stalk, wall of the sporocarp and two rows of sori.
2. Transversely cut stalk appears on one side. It shows the stalk bundle.
3. The wall of the sporocarp is made of epidermis with stomata and two layered hypodermis.
4. Gelatinous ring shows two patches, heavier on the dorsal side than on the ventral.
5. There are two rows of sori, one row alternating with the other.
6. Each sorus is covered by its indusium.

7. A sorus consists of a receptacle. Megasporangium is present at the apex of receptacle while microsporangia are present on the sides.
8. The lateral bundles are cut transversely and each is seen to supply its own receptacle by a receptacular or placental branch.
9. Thus, in this section dorsal bundle, many lateral bundles and receptacular branches (placental branches) are seen.

### Exercise 10

**Object : Study of dispersal of spores.**

### Work procedure

Cut open the body of the sporocarp on ventral side by a sharp blade or scalpel. Place in water for some time. Gelatinous ring with sporangia attached to it comes out.

### Comments

1. The sporocarp is hard and resistant to unfavourable conditions.
2. It opens through its ventral margins.
3. It imbibes water and the gelatinous ring inside swells up.
4. This ring ultimately comes out of the sporocarp wall.
5. Gelatinous ring bears two rows of sporangia, one on each side, alternating with one another.

### Identification

**Division—Pteridophyta.** (1) Plant body differentiated into stem, roots and leaves, (2) A definite vascular strand present.  
**Sub-division—Pteropsida.** (1) Plants are always megaphyllous

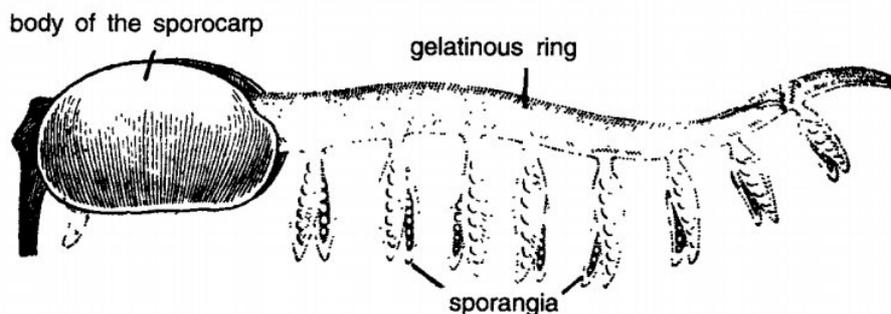


Fig. 15. *Marsilea*. Sporocarp showing extension of gelatinous ring during germination.

excluding a few exceptions, (2) Leaves differentiated into a petiole and dissected blade, (3) The sporangia are borne on abaxial surface of leaves.

**Class—Leptosporangiatae.** (1) Development of sporangium is of leptosporangiate type, (2) Jacket one cells in thickness, (3) Spores definite within a sporangium.

**Order—Marsileales.** (1) Members heterosporous, (2) Sporangia formed within sporocarps.

**Family—Marsileaceae.** (1) Members aquatic, (2) Sorus gradate type and each produces both types of sporangia, (3) Leaf circinately coiled in bud condition.

**Genus—Marsilea.** (1) Leaflet with dichotomous venation and cross connections, (2) Presence of aerenchyma in vegetative organs of the sporophyte, (3) Presence of amphiphloic solenostele in the rhizome, (4) Presence of V-shaped xylem in petiole.

### Hints for Collection

The two Indian species, *Marsilea minuta* and *M. quadrifolia*, are commonly found growing either in shallow water or on moist banks of ponds and ditches. They grow either completely submerged or partially or entirely out of water in damp and wet places.

## Azolla

### Classification

Division	—	Pteridophyta
Sub-division	—	Pteropsida
Class	—	Leptosporangiatae
Order	—	Salviniales
Family	—	Salviniaceae
Genus	—	<i>Azolla</i>

### Exercise 1

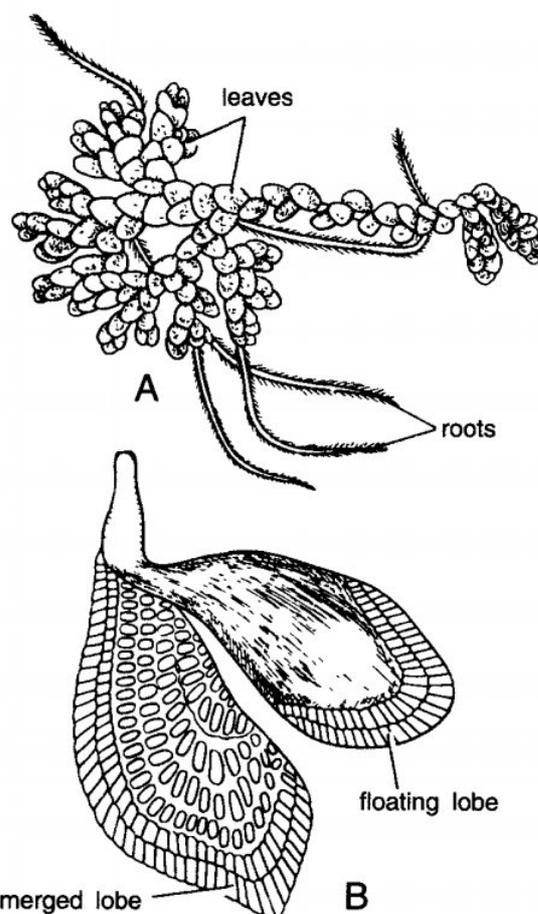
**Object :** Study of external morphology of the sporophyte.

#### Work procedure

Collect a fresh specimen or study a preserved plant.

#### Comments

1. The plant is a sporophyte. It grows free floating in ditches and ponds.



**Fig. 1.** *Azolla*. External features of *A. filiculoides*; **A.** Plant showing roots and leaves, **B.** A single leaf.

2. **The plant body** is differentiated into stem, roots and leaves.
3. **The stem** is pinnately branched. It is horizontally floating. The branches are extra-axillary.
4. **Roots** are produced from the lower side of the stem. These remain submerged in water.
5. **Leaves** cover stem and its branches. These are present in two alternate and overlapping rows.
6. **Each leaf** is divided into two lobes of approximately equal size.
7. The upper or aerial lobe is thick and green. It is somewhat obliquely placed and only one of the edges touches the water.
8. The thin lower or submerged lobe is nearly colourless. The absorption of water is believed to take place through this lobe.

**Exercise 2****Object : Study of anatomy of root****Work procedure**

Cut a T.s. of the root, stain in safranin-fast green combination, mount in glycerine and study.

**Comments**

1. **The outline** of the section is almost circular.
2. It shows epidermis, cortex and the stele.
3. **Epidermis** is the outermost single layer of cells.
4. **Cortex** consists of 2-8 layers of parenchymatous cells.
5. **Endodermis** lies inner to cortex. It is made of a single layer consisting of 6 cells.
6. **Pericycle** that follows is also made of a single layer consisting of 6 cells.
7. **Xylem** lies in the centre. It is represented by two centrally placed metaxylem tracheids. These are surrounded by four small outer groups of protoxylem elements.
8. **Phloem** consists of only a few elements. These are placed on either sides of the metaxylem elements.

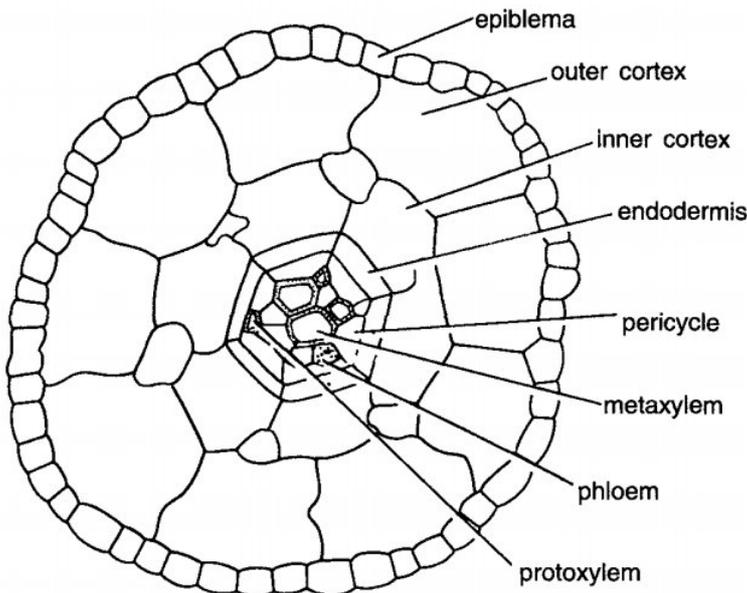


Fig. 2. *Azolla*. T.s. root (cellular).

**Exercise 3****Object : Study of anatomy of stem.****Work procedure**

Cut a transverse section of the stem, stain with safranin-fast green combination, mount in glycerine and study.

**Comments**

1. **Outline.** Transverse section of the stem is almost circular in outline.
2. It shows epidermis, cortex and stele.
3. **Epidermis** is the outermost single layer of cells.
4. **Cortex** is five to eight cells in thickness. The cells are thin walled and parenchymatous without intercellular spaces.
5. **Stele** is centrally located. It is surrounded by single layer of endodermis followed by a single layer of parenchymatous pericycle.
6. The central cylinder is protostelic. The vasculature of the stem is greatly reduced in response to aquatic habitat.
7. **Vascular tissues** are represented by about six xylem elements and twice as many phloem elements, in a stele.

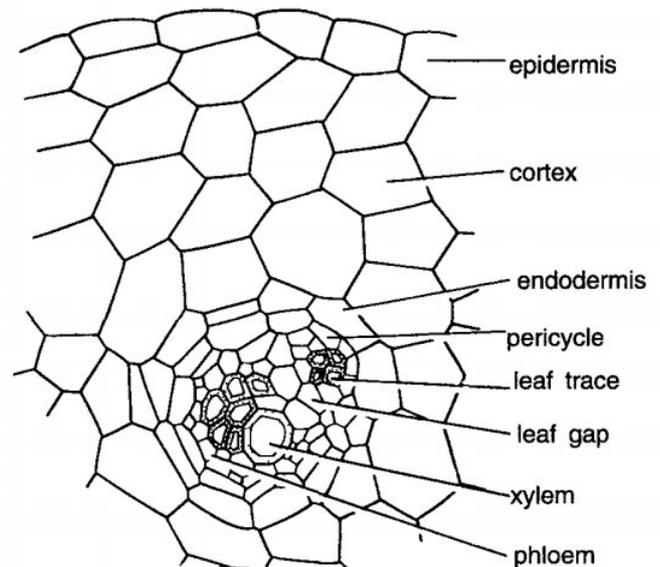


Fig. 3. *Azolla*. T.s. stem (cellular).

**Exercise 4****Object : Study of anatomy of leaf.****Work procedure**

Cut a vertical transverse section of the upper lobe by keeping it in suitable sized pith. Stain in safranin-fast green combination, mount in glycerine and study.

**Comments**

1. The upper lobe of leaf is bound on both the sides by upper and lower epidermal cells.
2. Both the layers possess stomata.
3. The upper epidermis has many unicellular or bicelled hairs.
4. Major portion of the leaf between both epidermal layers is made of palisade-like photosynthetic cells. Large intercellular spaces are present between them.
5. The upper lobe has a large cavity at its base. It opens to the outside through a circular pore.
6. The cavity is filled with the filaments of blue green alga—*Anabaena azollae*. The alga has a symbiotic relationship with the fern. It fixes atmospheric nitrogen.
7. The pore is later closed by outgrowths of the tissue of the margin. It becomes filled with mucilage.

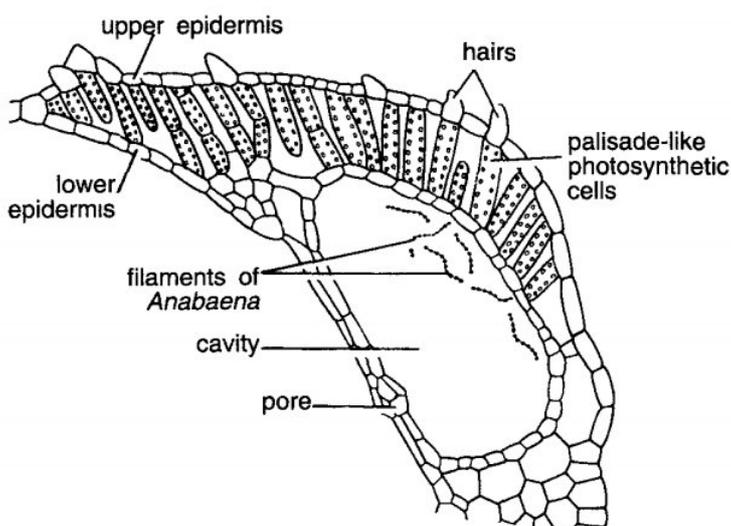


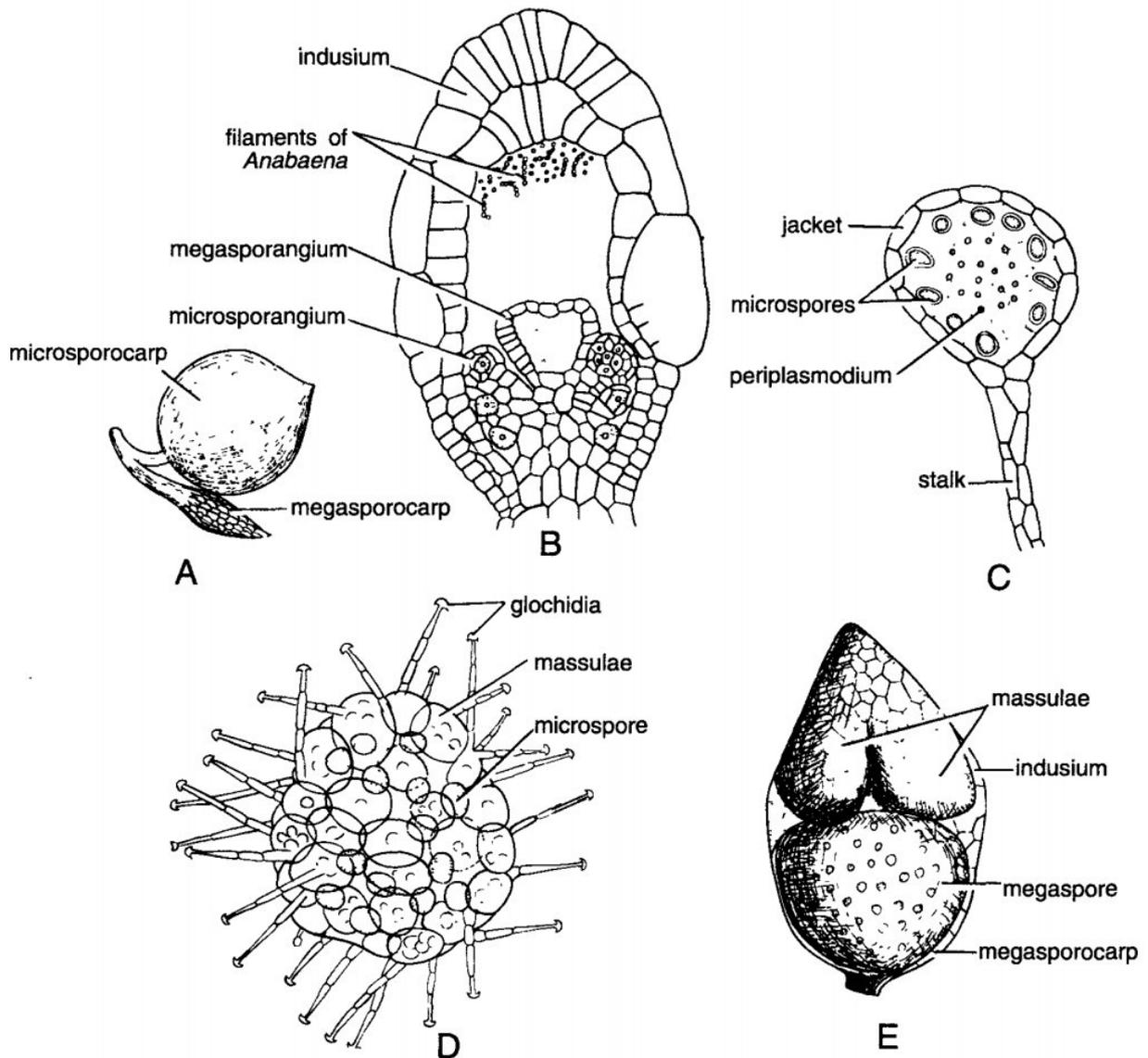
Fig. 4. *Azolla*. T.s. through dorsal (floating) lobe of leaf.

**Exercise 5****Object : Study of structure of sporocarp.****Work procedure**

Look for the sporocarp on the lower side of the plant. Identify microsporocarp and megasporocarp. Tease them. Stain with safranin, and study the internal structure.

**Comments**

1. Sporocarps are borne only on the lowermost leaf of a lateral branch at the end of annual season.
2. Submerged lobe of the leaf bears 2-4 sporocarps.
3. The upper lobe of the fertile leaf forms a hood-like covering around the sporocarp.
4. The sporocarps are dimorphic i.e. these are of two types: microsporocarps and megasporocarps.
5. Larger sized is a microsporocarp and the smaller sized is a megasporocarps.
6. Each sporocarp is a sorus covered by indusium.
7. Microsporocarp shows a central raised cushion on which sporangia develop basipetally. Each microsporangium has one layered jacket. It is followed by tapetum. The cavity encloses 64 microspores.
8. Microsporangium has a multinucleate periplasmodium formed as a result of breakdown of tapetum. Periplasmodium forms four or more quadrately arranged massulae in which spores remain embedded at periphery.
9. The surface of massulae has many anchor-shaped barbed hairs called glochidia which help the attachment of massulae to the microspore.
10. Megasporocarp shows a single large megasporangium. It is surrounded by a flask-shaped indusium. It envelops the sporangium completely except for a narrow slit at the apex.
11. Megasporangium is covered by a single layered wall. It encloses a single megaspore.
12. Megaspore is surrounded by a hardened vacuolate layer—the perispore. The megaspore wall is hard and ornamented. It is called episore.



**Fig. 5. *Azolla*.** A. The fertile submerged lobe with one large microsporocarp and one small megasporocarp. B. L.s. of nearly mature microsporocarp. C. Nearly mature microsporangium. D. Massulae inside microsporangium. E. Massulae inside megasporangium.

13. At the distal end of the megaspore, four quadrately arranged massulae are present. These are formed by the remaining aborted spores and the tapetal cells.

### Identification

**Division—Pteridophyta.** (1) Plant body differentiated into stem, root and leaves, (2) A definite vascular strand present.

**Sub-division—Pteropsida.** (1) Vascular cylinder siphonostele/dictyostele, (2) Plants macrophyllous with large leaf gaps, (3) Leaves bear sporangia in sori, (4) Gametophytes small, green and free living.

**Class—Leptosporangiatae.** (1) Sporangial wall one celled thick, (2) Number of spores per sporangium is definite.

(B-14)

**Order—Salviniales.** (1) Sporocarp is a single sorus enclosing either megasporangia or microsporangia, (2) Sporocarp walls formed by the indusia.

**Family—Salviniaceae.** Single family.

**Genus—*Azolla*.** (1) Presence of endophytic blue green algae *Anabaena* in the leaf, (2) Each leaf divided into two lobes, (2) Megasporocarp with only one megasporangium.

### Hints for Collection

*Azolla* forms red coloured bloom in ditches and ponds. It is found floating freely on the surface of water. Common Indian species is *A. pinnata*. Another species *A. filiculoides* is also known to occur frequently while the third species *A. imbricata* is found mostly in Eastern Himalayas.

# Gymnosperms

# 9

## Chapter

### Preamble

Gymnosperms form a large group of evergreen, slow growing plants. Though true seeds are formed, the group differs from other group of seed-bearing plants the angiosperms, firstly in possessing naked ovules; secondly, in the lodging of pollen grains directly on the micropyle and thirdly, in the absence of true vessels and companion cells. This group is more ancient than angiosperms, claiming fossils as well as living members and form a bridge between the pteridophyta on one hand and the angiosperms on the other.

The gymnosperms vary in size from small plants to very large gigantic plants. *Sequoia sempervirens* grows up to a height of about 150 meters (California) and *Taxodium maxicanum* has a trunk with the enormous diameter of about 17 meters. (Contrary to this, *Zamia pygma* is the smallest gymnosperm with and underground tuberous stem. In gymnosperms, there are two main structural types, the leaves. Most of the members of this group grow in relatively dry and poor soils, the plants thus exhibit thermographic features.

The fructifications (cones) are made up of an aggregation of sporophylls bearing sporangia, in which the spores are produced. The cones are generally unisexual. The male and female cones differ in shape and size. Whereas the male cones are usually smaller and short lived, the female cones are quite larger and long lived. Considerably the gametophytic generation is even more reduced than it is in any of the pteridophyta.

The gymnosperms are also important from economic point of view. Some conifers as *Cedrus deodara* (vern.deodar) form valuable timber. Canada balsam, a chief familiar mounting medium used in biological laboratories, is the resin of *Abies balsamea*. Turpentine oil which is chiefly used as medicine is also extracted from a conifer tree. Last, but not the least gymnosperms have also proved themselves for their food value viz. sago palm (*Cycas revoluta*) yield sago or sabudana (of course the chief commercial supply now comes from *Metroxylon rumphii*- an angiosperm) and the very familiar fruit of chilgoza is the seed of *Pinus gerardiana*.

## Classification of Gymnosperms

### Division. GYMNOSPERMS

Class	Order	Family	Examples
Cycadopsida	Pteridospemales	Lyginopteridaceae	<i>Heterangium*</i> , <i>Lyginopteris*</i>
		Glossopteridaceae	<i>Glossopteris*</i>
	Bennettitales	Williamsoniaceae	<i>Williamsonia*</i>
		Cycadeoidaceae	<i>Cycadeoidea*</i>
Coniferopsida	Cycadales	Cycadaceae	<i>Cycas</i>
	Coniferales	Pinaceae	<i>Pinus</i>
		Taxales	Taxaceae
Gnetopsida	Gnetales	Gnetaceae	<i>Gnetum</i>
		Ephedraceae	<i>Ephedra</i>

\*Fossil members

(B-14)

## Distinguishing Characters of Taxa

### DIVISION. GYMNOSPERMS

- (1) Ovules naked
- (2) Seeds attached to a scale
- (3) Scales forming a strobilus

### CLASS 1. CYCADOPSIDA

- (1) Wood manoxlic
- (2) Large frond-like leaves
- (3) Seeds with radial symmetry

#### Order 1. Pteridospermales

- (1) Leaves large, frond-like, pinnately compound
- (2) Large leaf traces with one or more strands
- (3) Spores formed in sporangia, aggregated in synangia

#### Family 1. Lyginopteridaceae

- (1) Stem monostelic
- (2) Petioles with a strong midrib
- (3) Seeds small

Examples. *Heterangium*\*, *Lyginopteris*\*

#### Family 2. Glossopteridaceae

- (1) Leaves with a strong midrib
- (2) Stellar structure unusual, showing several plates of vascular tissues
- (3) Reproductive structure cupulate and bisexual

Example. *Glossopteris*\*

#### Order 2. Bennettitales

- (1) Tree trunk covered by a mantle of persistent leaf-bases
- (2) Microsporophylls in groups at the tip of frond-like leaves
- (3) Megasporophylls in cone-like organization

#### Family 1. Williamsoniaceae

- (1) Stem delicate, branched
- (2) Inflorescence stalked or sessile, not sunk in the scales of persistent leaf bases

Example. *Williamsonia*\*

#### Family 2. Cycadeoidaceae

- (1) Trunk columnar
- (2) Trunk covered by a mantle of leaf bases
- (3) Flowers' sunk in the distal part of the trunk

Example. *Cycadeoidea*\*

#### Order 3. Cycadales

- (1) Plants woody, stem unbranched
- (2) Wood manoxylic
- (3) Presence of mucilage canals
- (4) Leaf trace diploxylic

- (5) Dioecious plants
- (6) Ovules orthotropous
- (7) Sperm with spiral band of flagella

Example. *Cycas*

### CLASS 2. CONIFEROPSIDA

- (1) Wood pycnoxylic
- (2) Leaves needle-shaped, or fan-shaped
- (3) Seeds with bilateral symmetry

#### Order 1. Coniferales

- (1) Plants branched, leaves needle shaped
- (2) Resin canals present
- (3) Male and female cones compact
- (4) Male gametes non-flagellate

#### Family 1. Pinaceae

- (1) Wood resinous
- (2) Plants monoecious
- (3) Sporophylls spirally arranged
- (4) Microsporophylls with two microsporangia
- (5) Pollen grains winged
- (6) Female cone woody
- (7) Polyembryony present
- (8) Seeds dry and winged

Example. *Pinus*

#### Order 2. Taxales

- (1) Profusely branched trees or shrubs
- (2) Leaves simple, solitary, flat and spirally arranged
- (3) Wood pycnoxlic without parenchyma
- (4) Plants mostly dioecious
- (5) Female strobilus represented by a single terminal ovule, enclosed in aril

#### Family. Taxaceae

- (1) Typical of order

Example. *Taxus*

### CLASS 3. GNETOPSIDA

- (1) Wood with vessels,
- (2) Flowers in compound strobili or inflorescence, unisexual, usually dioecious,
- (3) Ovule surrounded by several envelopes.

#### Order 1. Gentales

- (1) Plants woody trees, shrubs or lianas
- (2) Leaves simple, arrangement opposite or whorled
- (3) Male flowers with perianth

#### Family 1. Gnetaceae

- (1) Ovules cauline
- (2) Leaves - scaly and foliage; foliage leaves oblong-lanceolate

\* Fossil members

- (3) Plants dioecious
- (4) Male and female cones in panicles
- (5) Cones with 'cupules' or collars'
- (6) Seeds with protective inner envelope

Example. *Gnetum*

#### Family 2. Ephedraceae

- (1) Plants shrubs or woody climbers
- (2) Leaves- scaly and foliage
- (3) Presence of nodal diaphragm
- (4) Stamens enclosed by bract
- (5) Seeds covered with fleshy bracts

Example. *Ephedra*

### Cycas

#### Classification

Division	–	Gymnosperm
Class	–	Cycadopsida
Order	–	Cycadales
Family	–	Cycadaceae
Genus	–	<i>Cycas</i>

#### Exercise 1

**Object :** Study of external features of the plant.

#### Work procedure

Study the external features of the plant. Observe the armour of leaf bases on the stem, absence of branching, crown of leaves, two types of roots, etc.

#### Comments

1. **Plant body** is differentiated into an underground root system, that is distinguished into an erect stem and a crown of leaves.
2. **Roots** are of two types : (i) primary or normal root and (ii) secondary of coralloid root.
3. **Normal root** is a tap root, growing deep into the soil (positively geotropic). It is sparsely branched and sometimes grows as thick as aerial stem.
4. **Secondary roots** are negatively geotropic projecting above the soil surface, repeatedly dichotomously branched and appear as coralloid clusters.

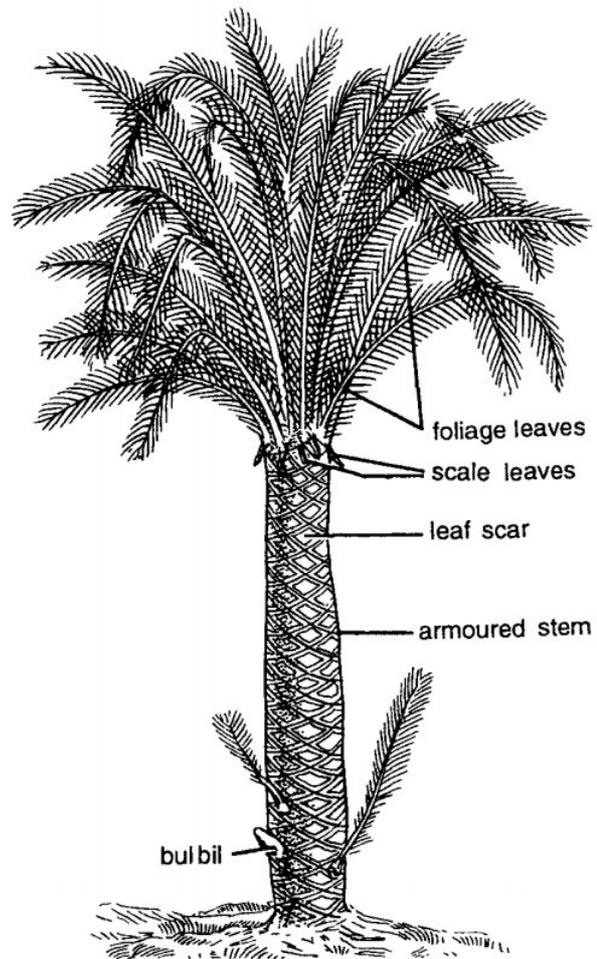


Fig. 1. *Cycas*. External features.

5. **The young stem** is almost tuberous but when grows old, it becomes thick, columnar and unbranched (Branching is rare and is caused due to injury, etc.). The trunk is covered by persistent leaf bases.
6. **Leaves.** The stem bears a terminal group of leaves which are dimorphic (i.e. of two types) (i) foliage leaves (green assimilatory fronds) and (ii) scale leaves (brown and hairy). These leaves alternate with one another.
7. Young foliage leaves are circinate and are covered with ramenta (hairs).
8. **Mature leaves** are spirally arranged and pinnately compound. Each leaf has about 80-100 pairs of pinnae that are closely arranged, opposite one another on the rachis with a decurrent base. Each pinna is tough, leathery and entire with a definite midrib but no lateral veins.

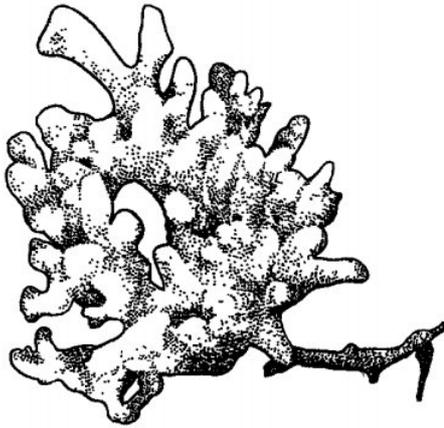


Fig. 2. *Cycas*. Coralloid root-external features.

9. **Scale leaves** are small, simple, brown with aborted lamina and covered with hairs. These leaves cover the apex and young developing foliage leaves. Scales are also persistent, like leaf bases.
10. **Reproductive organs.** *Cycas* is dioecious and, as such, bears terminally, either male cone or female reproductive structures.
11. **The male cone** is borne terminally at the apex of the stem and the further growth of the stem continues by axillary bud (developed at the base of the cone) which pushes the male cone on one side. The branching in *Cycas* stem is thus referred to as sympodial.
12. **The female reproductive structures** are the sporophylls developing in place of foliage leaves. The vegetative apex continues to grow as usual.
13. **The sporophylls** are smaller than the foliage leaves. They are brown or light brown in colour and are densely covered with woolly hairs.

### Exercise 2

**Object :** Study of anatomy of normal young root.

#### Work procedure

Cut a T.s. of the young part of primary root, stain in safranin-fast green combination, mount in glycerine and study.

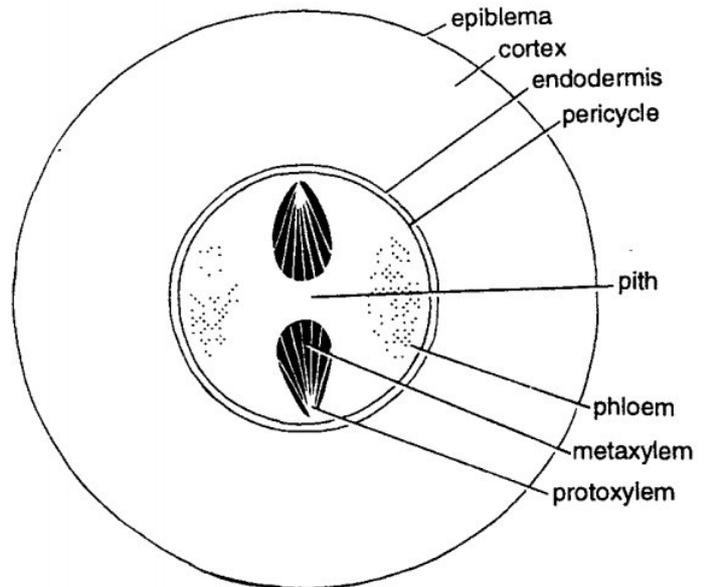


Fig. 3. *Cycas*. T. s. of normal young root (diagrammatic).

#### Comments

1. **The section** is circular in outline. It shows an outer layer or epiblema, cortex and centrally located stele.
2. **Epiblema** is made of single layer of thin walled cells. Some of these cells bear unicellular root hairs.
3. **Cortex** is multilayered with starch filled parenchymatous cells. A few tannin filled cells are also scattered in this region.
4. **Endodermis** is single layered and indistinguishable. Many-layered pericycle separates the cortex from vascular tissues.
5. **The central stele** is made of radial and exarch vascular bundles. There are two protoxylem groups and thus condition is diarch.

### Exercise 3

**Object :** Study of anatomy of older part of normal root.

#### Work procedure

Cut a T.s. of the older part of normal or primary root, stain in safranin-fast green combination, mount in glycerine and study.

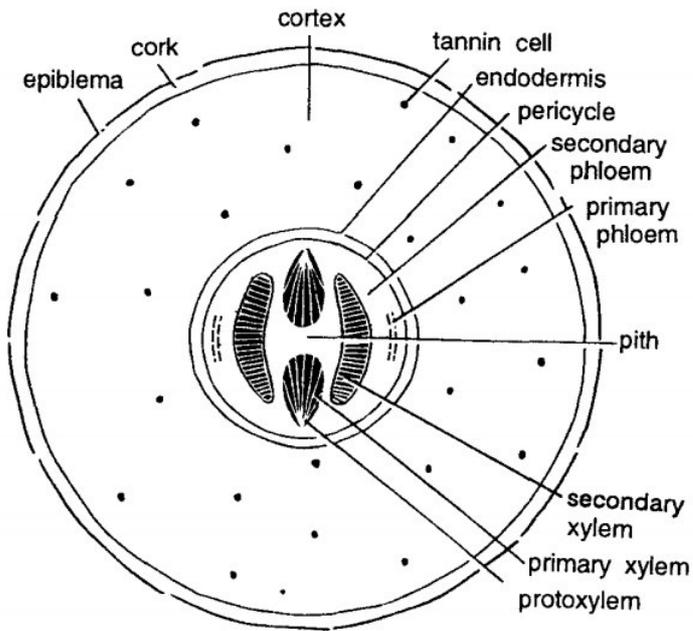


Fig. 4. *Cycas*. T. s. of normal old root (diagrammatic).

2. **The epiblema** is ruptured due to the thick walled cork cells formed below it. Cork cells are a few layered deep and are arranged in brick-like fashion.
3. **Cortex** is large, parenchymatous and multilayered. It is present below the cork. A few tannin filled cells occur scattered in the cortex.
4. **Endodermis** is single layered. It is followed by many layered pericycle.
5. **Primary phloem** is the outermost (near the pericycle) and is crushed during secondary growth. Secondary phloem follows this layer, the cells of which are intact.
6. **Cambium** arcs are formed along the inner edges of phloem in the vascular region.
7. **Secondary xylem** is situated towards pith. The primary xylem is situated in the same region as it was before the secondary growth.
8. **Medullary rays** are formed.
9. **In the centre** is a small parenchymatous pith.

#### Exercise 4

**Object : Study of anatomy of coralloid root.**

#### Work procedure

Cut a T.s. of the root, stain in safranin- fast green combination mount in glycerine and study.

#### Comments

1. The structure is almost similar to that of a normal root. It consists of epiblema, differentiated cortex and vascular tissues.
2. **Epiblema** is outermost and single layered.
3. **The cortex** is divisible into three regions -outer, middle and inner. These are similar in size. Cortex parenchymatous.
4. **The middle** cortex is also called algal zone. The cells are radially elongated. A blue-green alga *Anabaena cycadae* occurs endophytically in these cells. It is believed to be symbiotic and helps in nitrogen fixation.
5. **Endodermis** separates cortex and vascular tissues . It is single layered and followed by many layered pericycle.
6. **Vascular bundles** are radial and xylem is triarch and exarch.

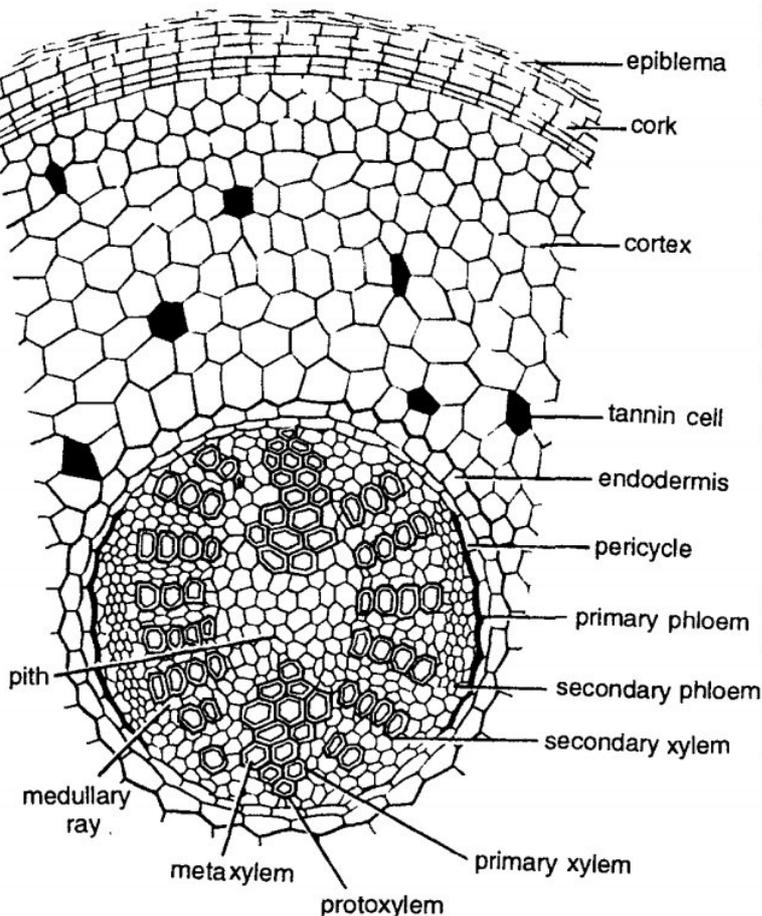


Fig. 5. *Cycas*. T. s. of old root (a part cellular).

#### Comments

1. It shows secondary growth, rest of the structures being similar to that of a young root.

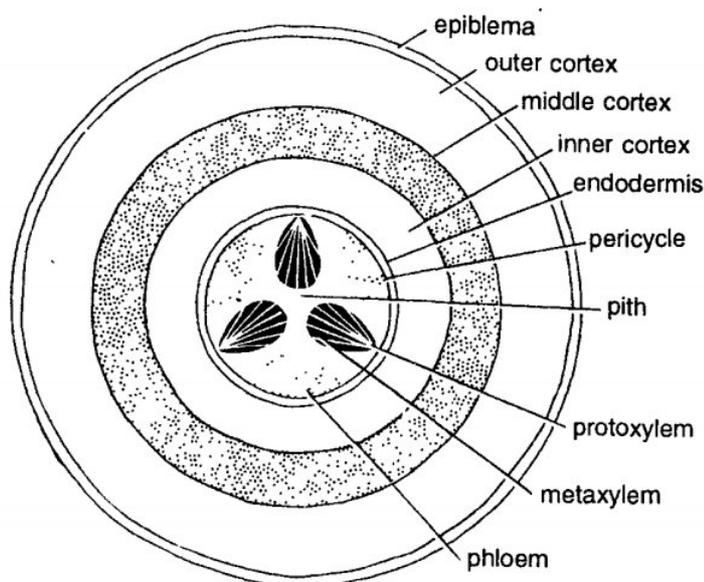


Fig. 6. *Cycas*. T.s. of coralloid root (diagrammatic).

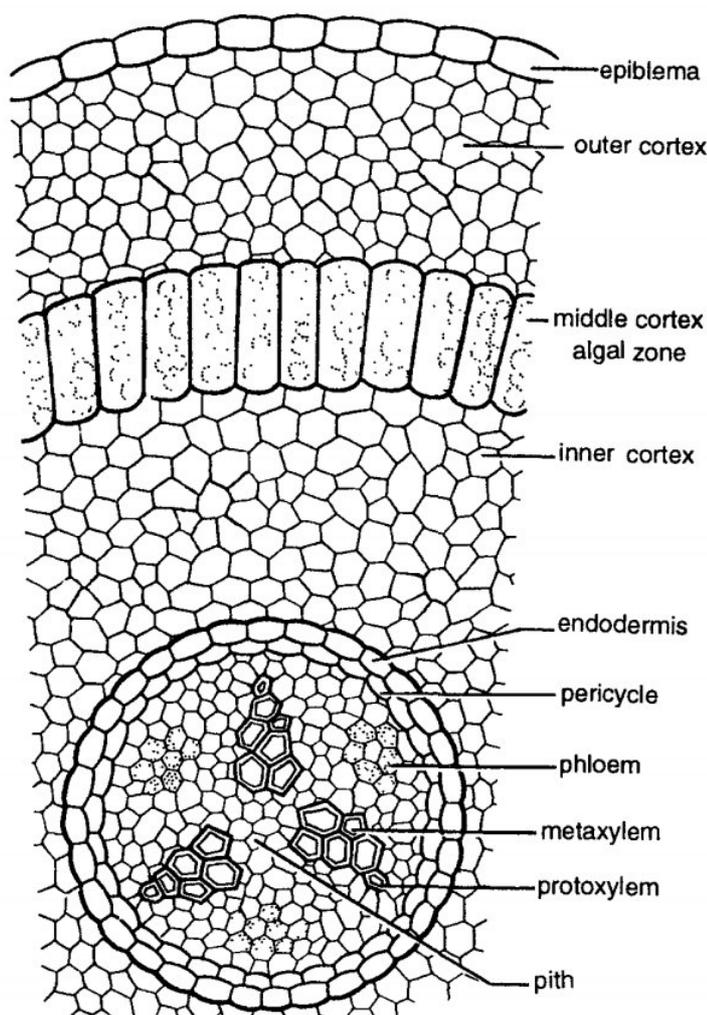


Fig. 7. *Cycas*. T.s. of coralloid root (a part cellular).

7. **Secondary** growth is generally absent; if present, it is very less.

### Exercise 5

**Object :** Study the anatomy of young part of stem.

### Work procedure

Since the stem is very thick, unbranched and very slow growing, sections are not cut, instead a slice of stem cut transversely can be preserved as specimen. It shows some important anatomical characters.

### Comments

1. **Outline** of the section is irregular due to the presence of numerous persistent leaf bases.
2. **The structure** is divisible into cortex, vascular tissue and pith.
3. **Cortex.** Greater part of the stem is made of starch filled parenchymatous cortex. It is traversed by many cut, girdle-shaped leaf traces, supplying the leaves. Many mucilage ducts are irregularly scattered in this region. (In *Cycas*, a leaf is supplied by two large girdle traces, two direct traces and numerous smaller radial traces. The two girdle traces arise from the side of the stele, opposite the leaf. These unite, bifurcate and take a circular route through the cortex before entering the leaf. The radial traces arise from other points of vascular ring but contrary to girdle traces, they adopt a straight radial course in the cortex. They bifurcate producing anastomosing branches which get attached to the girdle traces. In a transverse section large number of girdling leaf traces are cut. This is one of the most conspicuous features of the stem anatomy).
4. **Stele** is an ectophloic siphonostele.
5. **Endoermis** surrounds the stele. It is single layered while underlying pericycle is few celled thick
6. **Vascular cylinder** is composed of many vascular bundles arranged in a ring. Ring of vascular bundles lies near the centre and is very small in comparison to the massive cortex.
7. **The vascular bundles** are conjoint, collateral, endarch and open.

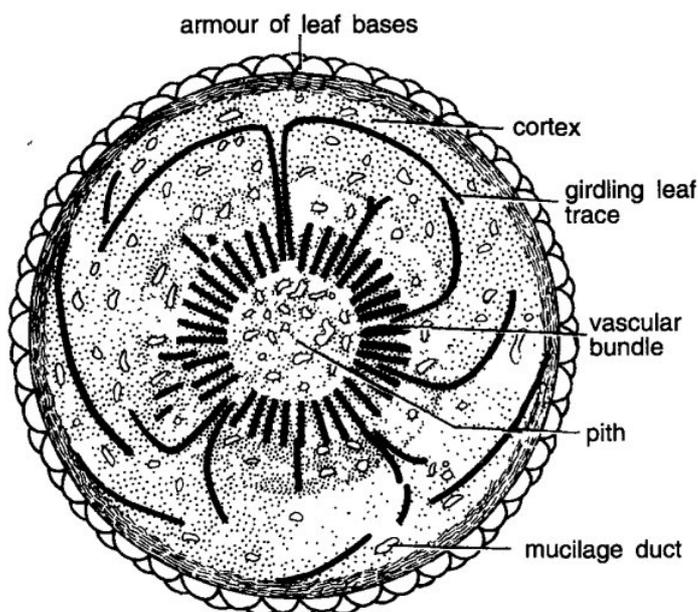


Fig. 8. *Cycas*. T.s. of young stem (diagrammatic).

8. **Xylem** is made of tracheids only and xylem parenchyma. Vessels are absent.
9. **Phloem** consists of sieve tubes, phloem parenchyma and phloem fibres.
10. The young stem is **monoxyletic** (i.e. with one ring of vascular bundles only).
11. **Pith**. There is parenchymatous pith in the centre, with scattered mucilage canals.

#### Exercise 6

**Object : Study of anatomy of the old stem.**

#### Work procedure

A thick slice of an old stem is generally preserved as specimen. Only prominent features could be observed.

#### Comments

1. It shows almost the same structures as those in young stem, except those formed after secondary growth.
2. A **periderm** is present on the outer side. It is composed of thick walled cells.
3. **Cortex** is large and parenchymatous. It forms most part of the section. A few mucilage canals are also present.

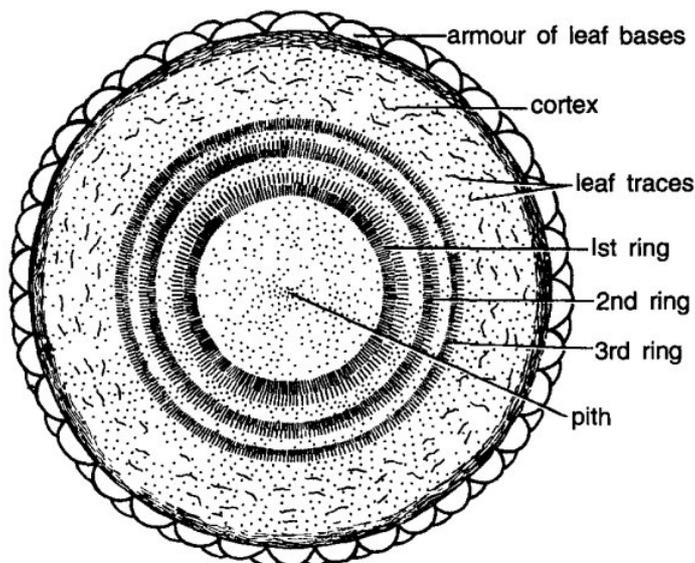


Fig. 9. *Cycas*. T.s. of old stem (diagrammatic).

4. **Vascular bundles** are formed, due to successive development of cambium rings. Thus, the old stem is **polyxylic** (with more than one ring of vascular bundles). The number of vascular bundles as well as the thickness of the successive vascular rings, thus formed, is lesser than the first formed ring.
5. **Secondary vascular tissues**. The successive rings of secondary vascular tissue are separated by parenchymatous zone. This loose, soft and scanty wood is called **manoxyletic**.
6. **Medullary rays** are present.
7. **Pith**. A large pith lies in the centre. Cells are parenchymatous and starch filled. Many mucilage canals are also present.

#### Exercise 7

**Object : Study of anatomy of rachis.**

#### Work procedure

Cut a T.s. of rachis from its middle region, stain in sarfanin-fast green combination, mount in glycerine and study.

#### Comments

1. **Outline**. It is cylindrical. It shows insertion of pinnae on the adaxial side (upper side).

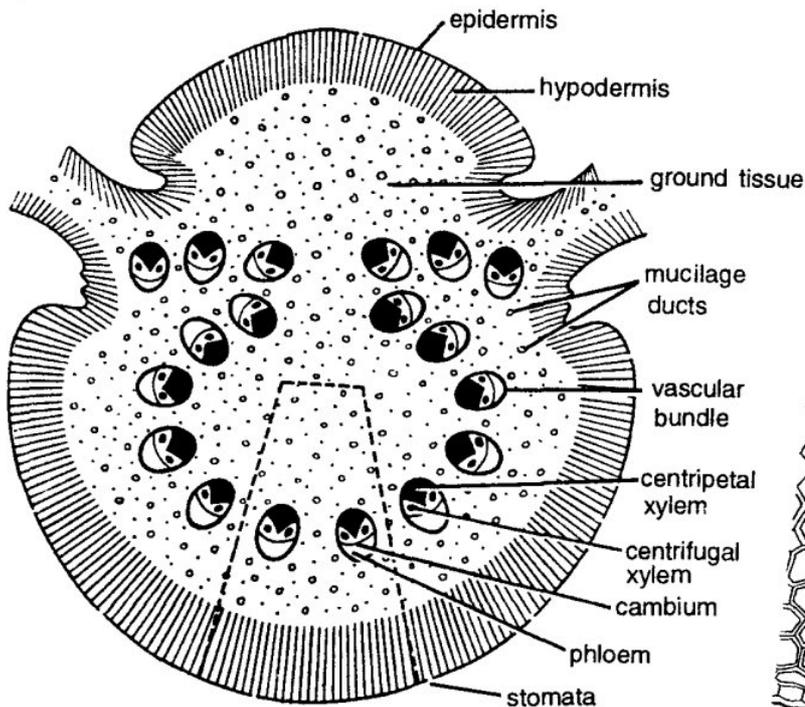


Fig. 10. *Cycas*. T.s. of rachis (diagrammatic).

2. **The rachis** is differentiated into epidermis, hypodermis, ground tissue and a ring of vascular bundles.
3. **Epidermis** is single layered, thickly cuticularized and is interrupted by stomata throughout its surface. The condition is known as amphistomatic.
4. **Hypodermis** is mainly composed of thick-walled cells (sclerenchyma). Intermixed with these cells are a few cells having chloroplasts-chlorenchyma.
5. This sclerenchymatous hypodermis is 2-3 layered toward adaxial side and many layered toward abaxial side.
6. **Ground tissue.** The rest of the tissue that forms most part of the section is called ground tissue. It is parenchymatous.
7. **Mucilage ducts** are scattered throughout the ground tissue. Mucilage ducts are double layered, the inner layer being composed of epithelial cells and the outer of tangentially elongated sclerenchymatous cells.
8. **The vascular bundles** are arranged in an inverted omega ( $\Omega$ ) shaped arc. Each vascular bundle is surrounded by a thick walled, single-layered bundle sheath. It is conjoint, collateral and open.

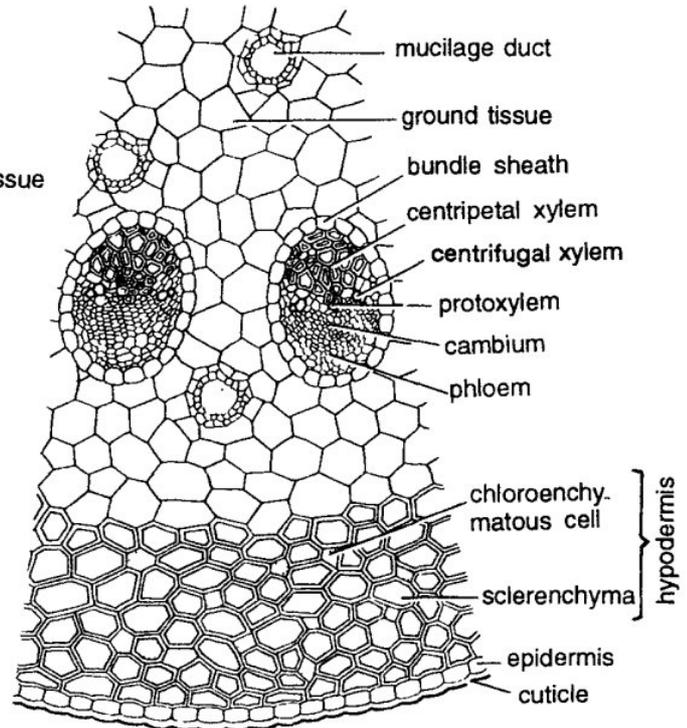


Fig. 11. *Cycas*. T.s. of rachis (a part shown by dotted lines in Fig. 11 in details).

9. **The arrangement of xylem and phloem** differs in vascular bundles at the base, middle and upper region of the rachis.
  - (i) Higher up and for most part of the rachis, bundles are **diploxylic** i.e. two types of xylem elements are present - centripetal and centrifugal xylem. The centrifugal xylem occurs in two small groups, present on both the sides of large triangular and centrally located centripetal xylem. The phloem is situated on the abaxial side of the rachis.
  - (ii) At the very base of the rachis, vascular bundles show only centrifugal xylem which is endarch. Phloem occupies the abaxial side of the rachis.
  - (iii) Little higher up the base of rachis, vascular bundles show centrifugal xylem on abaxial side and centripetal xylem on adaxial side. In the centre of these two xylem groups, lies the protoxylem. This condition is said to be mesarch.

#### Features of special interest

1. Presence of chlorenchyma, dispersed among the thick walled sclerenchymatous hypodermis.

2. Presence of sunken stomata all over the surface. (xerophytic characters).
3. Vascular bundles arranged in inverted omega ( $\Omega$ ) shaped arc.
4. Diploxylic nature of the vascular bundles.
5. Mucilage ducts scattered throughout.

### Exercise 8

**Object : Study of anatomy of leaflet (pinna).**

### Work procedure

Cut a T.s. of leaflet, stain in safranin-fast green combination, mount in glycerine and study.

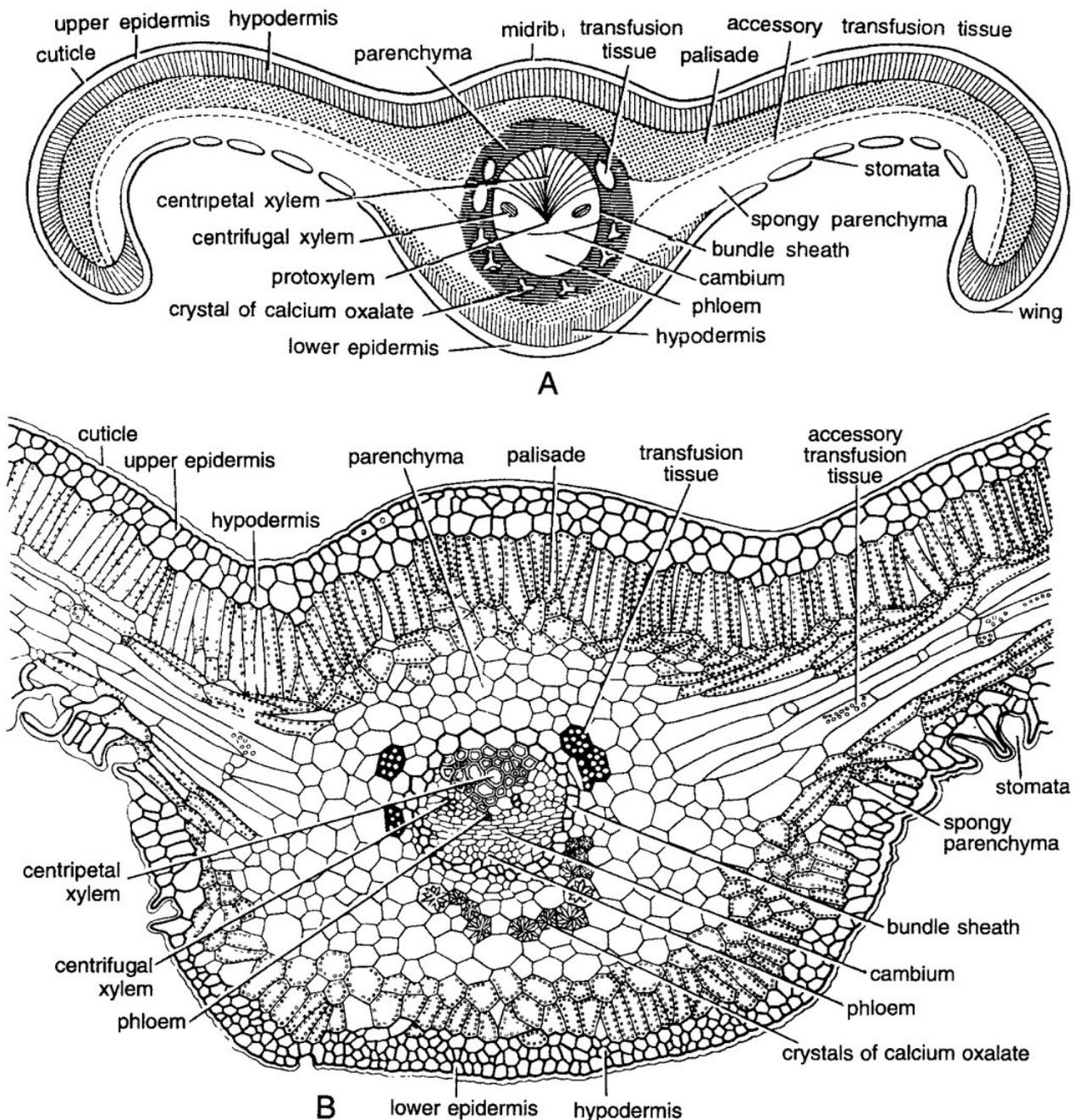


Fig. 12. *Cycas*. A. and B. T.s. of leaflet.

**Comments**

1. **The leaflet shows** a distinct midrib and the wings.
2. **The midrib** is swollen, while wings on the lateral sides are narrower and flattened.
  - (i) In *C. revoluta* midrib is less projected than in *C. circinalis*, where it is much projected on the upper side.
  - (ii) Margins of wings are revolute in *C. revoluta*, and *C. beddomei* while they are straight in *C. circinalis*, *C. rumphii*, *C. pectinata* and *C. siamensis*.
3. **Upper epidermis** is present on the upper side. It is thickly cuticularized and single-layered.
4. **Hypodermis** is present below the epidermis. It is sclerenchymatous.
  - (i) In *C. revoluta*, hypodermis is present in the midrib (near both upper and lower epidermis) and wings (below the upper epidermis).
  - (ii) In *C. circinalis*, hypodermis in the midrib region is present on both the sides (upper and lower) while in the wings, it occupies only the corners, being absent from rest of wings.
5. **Mesophyll** lies below the hypodermis and is well developed. It is differentiated into upper palisade layers and lower of spongy parenchyma.
  - (i) In *C. revoluta*, palisade is present beneath the hypodermis, both in the midrib and the wings.
  - (ii) In *C. circinalis* palisade is absent from the midrib region
6. **Spongy parenchyma** with many intercellular spaces lies immediately above the lower epidermis.
7. **Transfusion tissue.** On either side of the centripetal metaxylem of mid rib bundle and somewhat connected with it, are present two tracheid-like cells-transfusion tissue.
8. **Accessory transfusion tissue.** Between the palisade and spongy parenchyma cells, there are 3 or 4 layers of tracheid-like, long colourless cells which run transversely from the midrib to near the margin of the lamina. This is known as accessory transfusion tissue. It is connected with the xylem of the vascular

bundle of midrib through the transfusion tissue.

9. **Lower epidermis** bounds the leaflet from lower side. It is thickly cuticularized and single layered. Sunken stomata are found in the lower epidermis in the midrib region.
10. **Stomata** are very much sunken in the lower epidermis in *C. revoluta*, while they are not so much sunken in *C. circinalis*.
11. **Midrib bundle.** In middle of the swollen portion representing the midrib lies a single vascular bundle surrounded by parenchymatous tissue (with calcium oxalate crystals). Vascular bundle has a definite and thickened, parenchymatous bundle sheath.
12. **The vascular bundle** is similar in all respects to that found in the upper region of the rachis. It is conjoint, collateral, open and diploxylic.
13. **Phloem** lies towards the abaxial (lower) side. In between xylem and phloem, cambium is present.
14. **Xylem.** It shows a large, triangular patch of centripetal xylem and two small groups of centripetal protoxylem.

**Features of special interest**

1. Lateral veins are absent.
2. Thickly cuticularized upper and lower epidermis.
3. Sunken stomata in the lower epidermis.
4. Presence of transfusion tissue.
5. Diploxylic nature of vascular bundle.

**Exercise 9**

**Object : Study of bulbil.**

**Work procedure**

Study the position on the lower part of the stem and observe external characters.

**Comments**

1. Bulbils are produced adventitiously, on basal part of the plant, in the crevices between the persistent leaf bases.
2. The decurrent base of the bulbil remains covered with scale leaves.
3. A few foliage leaves are given out from the central part.

- It germinates under favourable conditions and produces new plant or else develops into a branch (rarely), giving an appearance of dichotomous branching.
- This is the commonest method of reproduction in *C. revoluta* in north india, male plants being rare in this region.

### Exercise 10

**Object :** Study of external features of male cone.

#### Work procedure

Study the male cone attached to the plant if possible.

#### Comments

- The male cone is terminal, shortly stalked, compact, large and oval or conical in shape and consists of a central cone axis around which numerous microsporophylls are spirally arranged. (Since the male cone terminates the growth of the apex of male plant, a lateral bud later grows and takes over the continuation of growth of apex. The male plant thus shows sympodial growth).
- The outer covering of the male cone is formed by closely set sterile ends of the microsporophylls usually possessing upcurved apices, apophysis.

### Exercise 11

**Object :** Study of L.s. of the male cone.

#### Work procedure

Since the male cone is about 60-80 cms in height, it can be split into two and specimen is studied.

#### Comments

- The L.s. shows stalk and the cone.
- Male cone is attached at the apex of the plant by a stout and broad stalk.
- The cone itself consists of a central cone axis with many microsporophylls.
- Each microsporophyll is attached to the cone axis. The part of microsporophylls away from the axis is upcurved and is called apophysis.

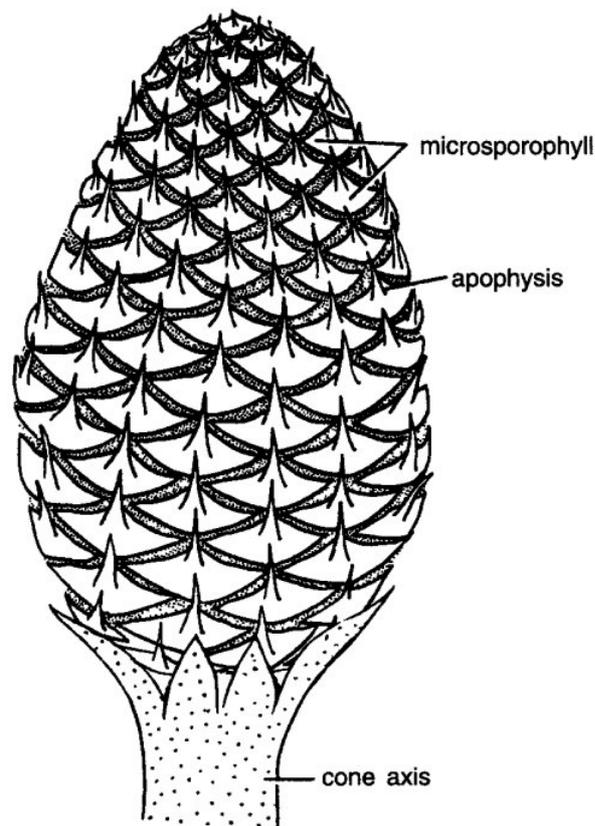


Fig. 13. *Cycas*. A male cone (external features).

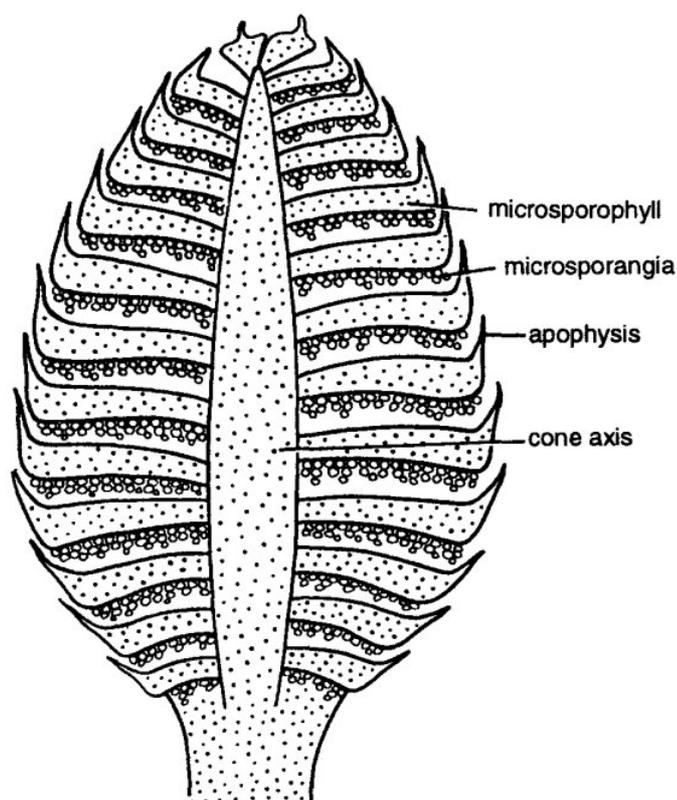


Fig. 14. *Cycas*. L.s. of male cone.

5. The upper surface of the microsporophyll is sterile.
6. The lower surface of the microsporophyll is fertile and bears many microsporangia in groups (sori).
7. Microsporophylls in the middle part of the cone are largest and get gradually smaller towards the base and the apex.

### Exercise 12

**Object : Study of microsporophyll and microsporangia.**

#### Work procedure

Take out a microsporophyll from the male cone. Study both- upper and lower surfaces. Observe the sporangia on the lower surface with a magnifying lens.

#### Comments

1. A single microsporophyll is woody, more or less horizontally flattened and triangular structure.
2. It is differentiated into a fertile and sterile parts. Fertile part is wedge-shaped and is expanded distally from a narrow point of attachment. Sterile part is the distal part of the microsporophyll which tapers into an upcurved apophysis.
3. Lower (abaxial) surface of the fertile part of

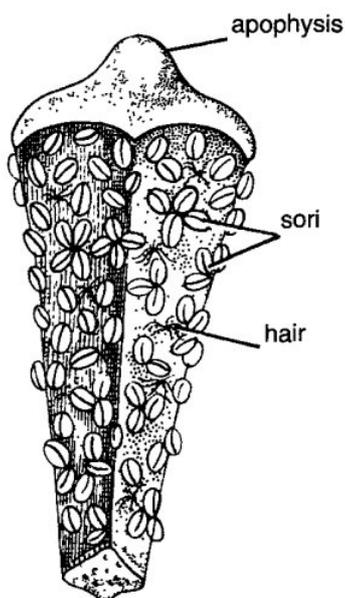


Fig. 15. *Cycas*. A microsporophyll from lower side.

- the microsporophyll bears microsporangia in groups of 3-4, forming definite sori.
4. Microsporangia are arranged in sori around central papilla. Sporangia show radial lines of dehiscence. Many hairs are distributed on this surface mixed with sporangia.

### Exercise 13

**Object : Study of T.s. of microsporophyll.**

#### Work procedure

Study the characters observed in sliok of T.s. of microsporophyll.

#### Comments

1. The section shows microsporangia attached to the abaxial (lower) surface by their short stalks.
2. A mature microsporangium has three layered wall. The outermost layer is thick and cutinized, termed as exothecium. The remaining inner layers are thin and are collectively known as endothecium and enclose a tapetum.
3. Numerous microspores remain enclosed inside the wall of the microsporangium.
4. In the microsporophyll are present many mucilage ducts, regularly scattered, among the rounded mesophyll-like cells forming the tissue of the sporophyll.

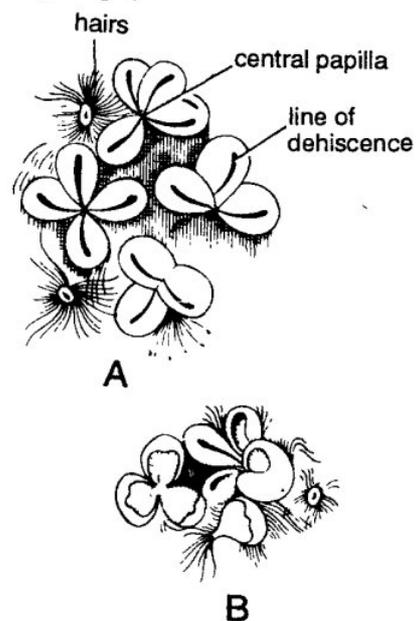


Fig. 16. *Cycas*. Microsporangia in sori. A. Before dehiscence, B. After dehiscence.

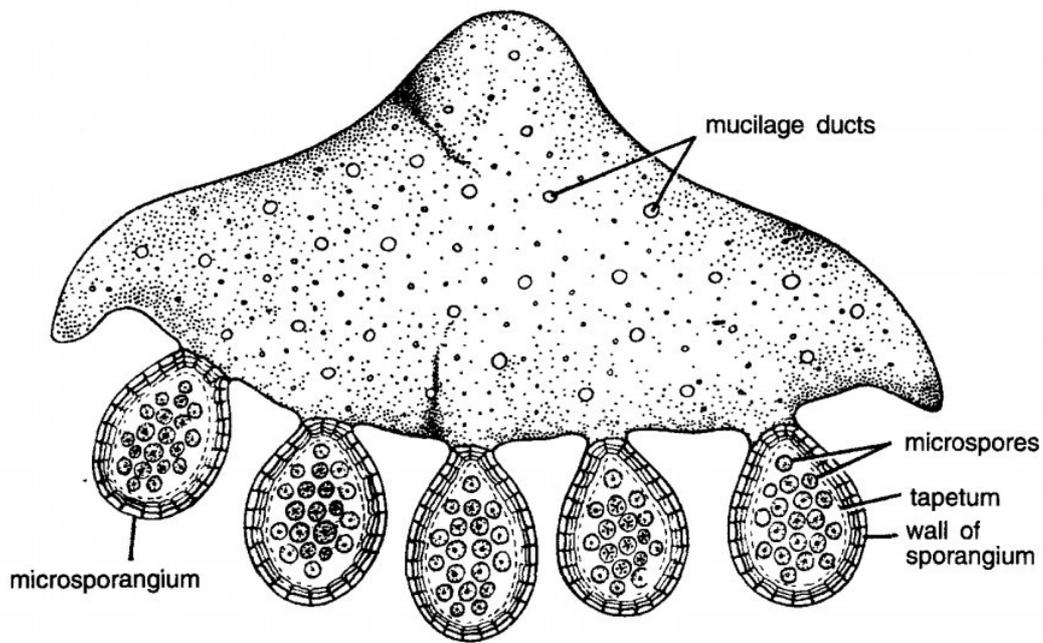


Fig. 17. *Cycas*. T.s. of microsporophyll.

#### Exercise 14

**Object : Study of megasporophyll.**

#### Work procedure

Since there is no female cone, megasporophylls form a crown at the apex like foliage leaves. Only a megasporophyll can be studied as a specimen.

#### Comments

1. Female reproductive body consists of megasporophylls arranged spirally and arising in acropetal succession on the stem.
2. Megasporophylls appear as a rosette or a crown, leaving the apical meristem unaffected to grow further. A crown of megasporophyll is formed each year. Numerically they are more than the leaves.

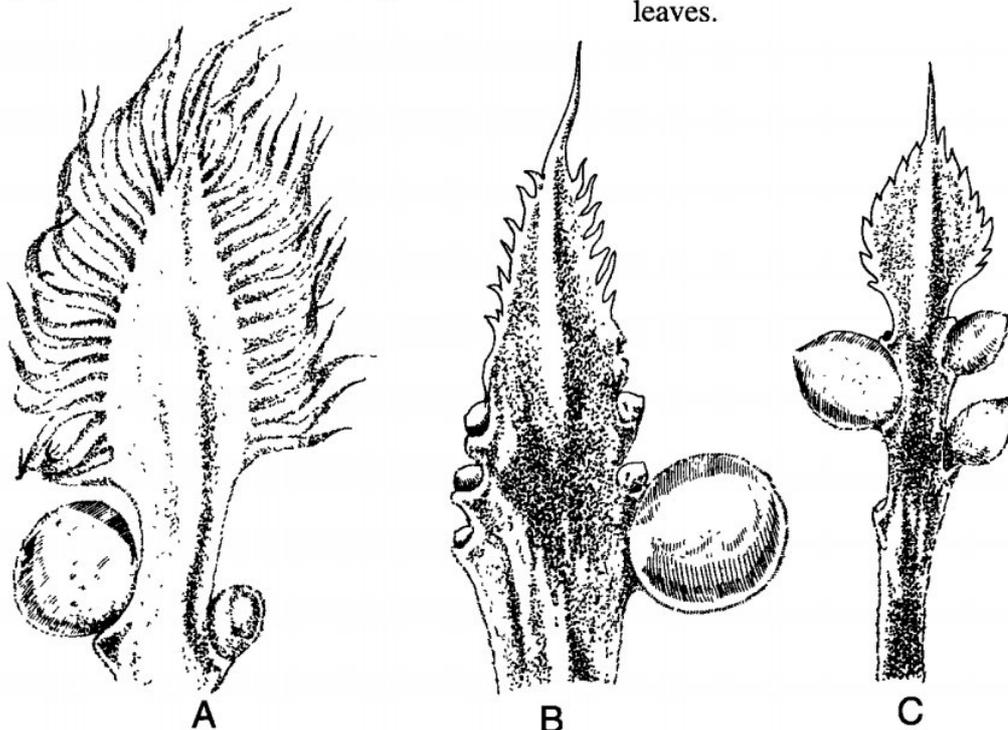


Fig. 18. *Cycas*. Megasporophylls of different species with ovules. A. *C. revoluta*, B. *C. circinalis*, C. *C. rumphii*.

3. They leave their persistent bases on the stem.
4. Each megasporophyll is leaf-like and densely covered with brown hairs. It varies in size from 6 to 12 inches.
5. Each megasporophyll is distinguished into a proximal (lower) petiole, a middle ovule bearing portion and a distal (upper) pinnately dissected sterile part.
6. The nature of upper sterile part varies with species.
  - (i) In *C. revoluta*, the upper part is very much dissected, forming many pinnae.
  - (ii) In *C. rumphii*, the upper part bears only short spines which represent reduced pinnae.
  - (iii) In *C. circinalis*, the pinnate character is altogether absent and upper part shows only dentate or serrate margins.
7. The middle portion of sporophyll bears ovules which are borne in two rows, one on either side. The ovules of the two rows may be opposite or alternate.
8. Ovules are generally yellow or orange or dark green coloured, shortly stalked, oval and smooth. Number and size of the ovules differ from species to species.
  - (i) In *C. revoluta*, ovules are many and orange coloured,
  - (ii) In *C. circinalis* also, they are numerous, but these are dark green and attain a large size,
  - (iii) In *C. siamensis*, the number of ovules is reduced to only two and
  - (iv) In *C. thourarsii*, the ovules become still larger and may be that they are the largest ovules in the plant kingdom.
9. All the ovules do not develop fully. Some of those which remain unpollinated and small, finally abort.

### Exercise 15

**Object : Study of L.s. of mature ovule.**

#### Work procedure

Study the slide showing L. s. of mature ovule.

#### Comments

1. The section shows that the ovule is orthotropous.
2. It is unitegmic (possesses a single integument). The integument is very thick. It remains fused with the nucellus except for the nucellar beak leaving a small and narrow micropyle.

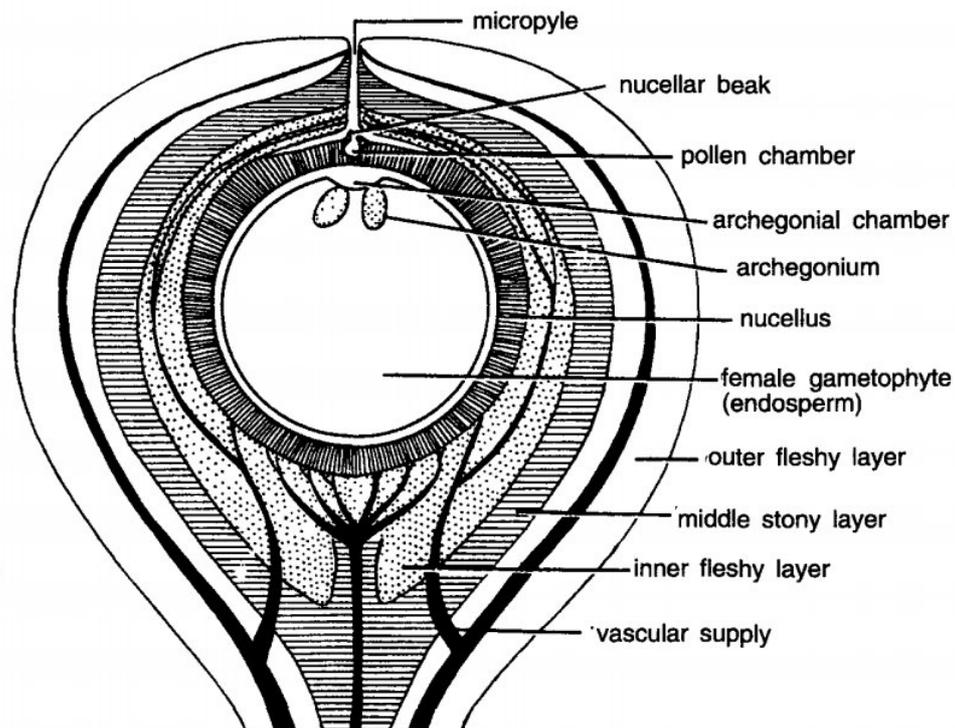


Fig. 19. *Cycas*. L.s. of ovule.

3. **The integument** consists of three distinct layers—an outer fleshy layer, middle stony layer and an inner fleshy layer. The outer and inner fleshy layers are supplied with vascular strands but the middle stony layer receives no vascular supply.
4. **The nucellus** lies just below the integument and forms a nucellar beak in the region of the micropyle.
5. A few cells of this nucellar beak dissolve themselves and form a pollen chamber that lies in the tissue in the central region of the beak.
6. **Female gametophyte.** The innermost region of the ovule is filled with the tissue of female gametophyte, wherein lie two archegonia, situated opposite the pollen chamber.
7. **Archegonial chamber.** Just above the archegonia is the archegonial chamber.
8. **Micropyle.** The orange coloured, fleshy ovules are oval in shape and each shows a small point at the distal end which represents the remnant of the micropyle.

### Exercise 16

**Object : Study of L.s. of seed.**

#### Work procedure

Study a double-stained preparation of the L.s. of seed.

#### Comments

1. It shows seed coat, nucellus, embryo and the female gametophyte.
2. **Seed coat** consists of sarcotesta (from outer fleshy layer of integument), and middle sclerotesta (from middle stony layer). The inner fleshy layer of the integument appears as thin and papery structure in the seed.
3. **Nucellus** is papery and is situated inside the seed coat.
4. **Endosperm** and female gametophyte form the inner part of seed.
5. A **straight embryo** remains embedded in the endosperm. It has two unequal cotyledons.

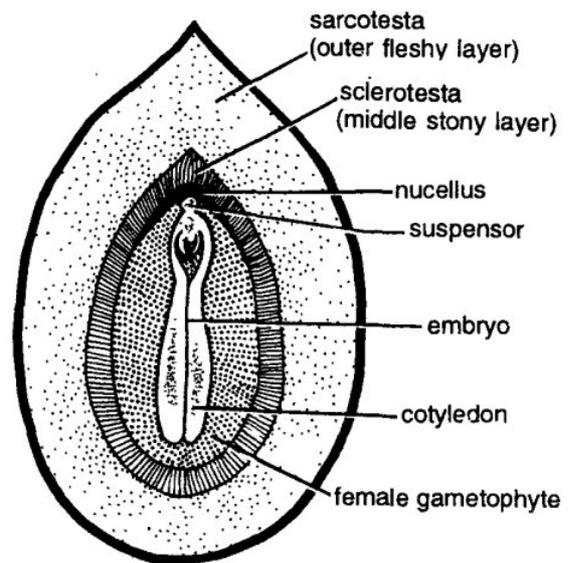


Fig. 20. *Cycas*. L.s. of seed.

### Identification

**Division—Gymnosperms.** (1) Absence of vessels, (2) Ovules naked, (3) Seeds attached with woody acales, (4) Scales generally form a cone.

**Class—Cycadopsida.** (1) Wood manoxylic, (2) Large frond-like leaves (3) Seeds with radial symmetry.

**Order—Cycadales.** (1) Plants woody, stem unbranched, (2) Wood manoxylic, (3) Presence of mucilage canals, (4) Leaf trace diploxylic, (5) Dioecious, (6) Ovules orthotropous, (7) Sperm with band of flagella.

**Family—Cycadaceae.** (1) Leaves with circinate vernation, (2) Presence of coralloid roots and endophytic blue green algae, (3) Megasporophylls foliar.

**Genus—Cycas.** (1) Two types of leaves, (2) Foliage leaves pinnately compound, circinately coiled when young, (3) Presence of transfusion-tissue and diploxylic pascular bundle in leaf, (4) Secondary xylem in stem manoxlic, (5) Two types of roots, (6) Vascular bundles arranged in an inverted omega-shaped manner in the rachis, (7) Male cone large and single.

### Hints for Collection

In India the genus grows naturally only in the north-east (East Nepal, Champaran-Bihar, Sikkim, Assam and East Bengal) and South (Orissa, Andhra Pradesh and Tamil Nadu). Six species are found in India of which *C. circinalis*, *C. pectinata*, *C. rumphii*, and *C. beddomei* grow wild but *C. revoluta*, a native of Japan and *C. siamensis* found in Burma and Siam are cultivated.

***Pinus***  
**(Pine)**

**Classification**

Division	-	Gymnosperms
Class	-	Coniferopsida
Order	-	Coniferales
Family	-	Pinaceae
Genus	-	<i>Pinus</i>

**Exercise 1**

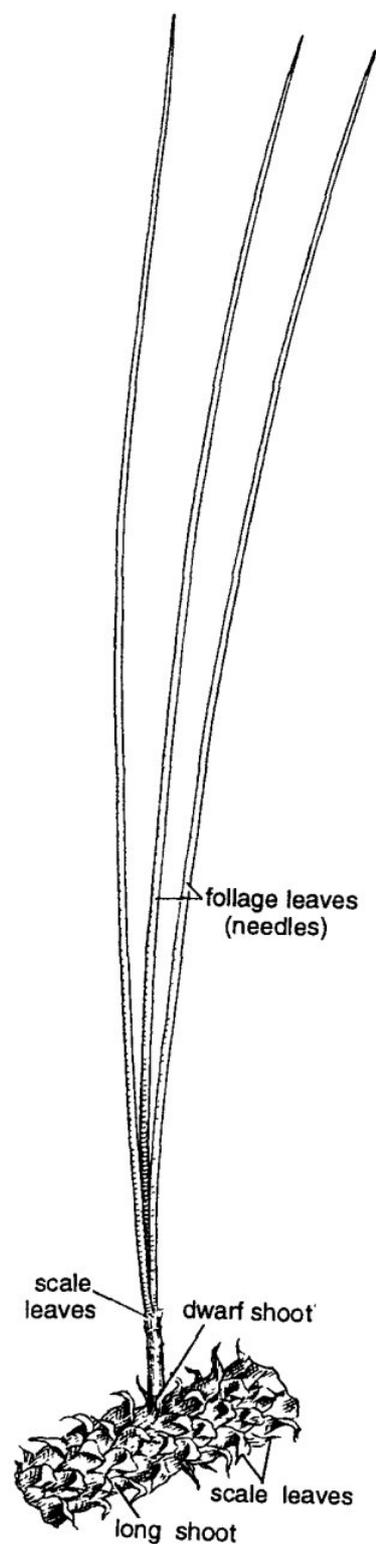
**Object : Study of external morphology.**

**Work procedure**

Note the pattern of branching, the two types of branches, two types of leaves, and male and female cones.

**Comments**

1. It is a tall conical tree and, therefore, commonly grouped under conifers.
2. **The plant body** is differentiated into root, stem and leaves.
3. **Underground root system** is formed by tap roots which disappear early and only lateral roots persist later on.
4. **The younger roots** are generally surrounded by fungal hyphae- the ectotrophic mycorrhizae.
5. **Aerial branch system** consists of cylindrical, rough (being covered with scaly bark) and branched stem.
6. **The branching** is monopodial and the branches are arranged in whorls.
7. **The branches** are dimorphic (of two types)- branches of unlimited growth or long shoots and branches of limited growth or dwarf shoots.
8. **Branches of unlimited growth** or long shoots are present on the main trunk. These are produced at regular intervals.
9. **Branches of limited growth** or dwarf shoots are borne on the main stem and on long shoots in the axils of scale leaves. Dwarf shoots also possess many scale leaves and bear group of foliage leaves at the apex.



**Fig. 1. *Pinus*.** A part of stem showing two types of branches.

10. **The leaves** are also dimorphic (of two types)- scale leaves and foliage leaves.
11. **Scale leaves** are brown, membranous and small. They are present on both the types of branches (i.e. long and dwarf shoots).

12. **Foliage leaves** are green, acicular and needle-like. They are borne only by the dwarf shoots.
13. A **dwarf shoot** with a group of needle-like foliage leaves is known as a foliar spur. The number of needles in a group varies from species to species. *P. monophylla* has a single leaf and spur is known as monofoliar, while in *P. sylvestris*, two leaves are present and spur is called as bifoliar. In *P. longifolia* and *P. gerardiana*, they are three in number, the spur being called as trifoliar. Quadrifoliar spur occurs in *P. quadrifolia* and pentafoliar in *P. excelsa*.
14. **The shape of the needle** varies, according to their number in a spur. In *P. sylvestris* (with bifoliar spur), single needle is semi-circular in T.s. while in *P. longifolia* (with trifoliar spur), single needle is almost triangular in shape.
15. ***Pinus* is monoecious.** Plant bears male and female reproductive parts in cones on the same plant.
16. **The male cones** are borne on lateral branches of unlimited growth. They are produced in clusters and replace the dwarf shoots. Also, they are formed earlier in the season than the female cones.
17. **The female cones** are borne terminally on branches of unlimited growth. They are produced singly and replace the long shoot. The female cone appears after every three years.
18. Generally male and female cones are not formed on one and same branch.

### Exercise 2

**Object : Study of anatomy of young root.**

#### Work procedure

Study a double stained prepared slide of T.s. of young part of root.

#### Comments

1. **The section** is almost circular in outline.
2. The tissues are differentiated into epiblema cortex and vascular tissues.
3. **Epiblema** is outermost single layer. It gives out many thin and unicellular root hairs.

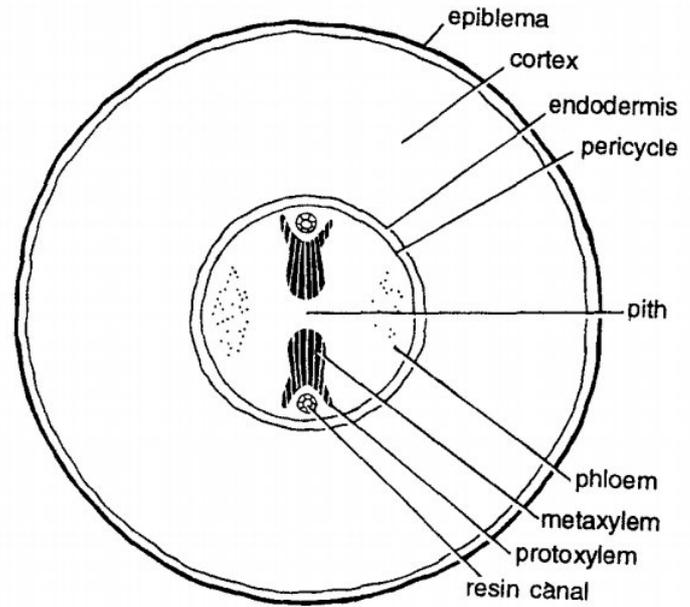


Fig. 2. *Pinus*. T.s. of young root (diagrammatic).

4. **Cortex** is multilayered and parenchymatous.
5. **Endodermis** separates outer cortex and central vascular cylinder. It is single layered and cells are radially thickened.
6. **Pericycle** follows endodermis. It is multilayered.
7. **Vascular bundles** are radial, exarch and diarch to hexarch.
8. **Protoxylem** is generally Y-shaped and a resin canal is present in between the arms of Y.
9. **Pith** is very small and lies between the groups of xylem.

### Exercise 3

**Object : Study of anatomy of the old root.**

#### Work procedure

Study a double stained prepared slide of T.s. of old part of root.

#### Comments

1. **The section** shows cork, cortex, primary and secondary vascular tissues and a small pith.
2. **Cork** forms the outermost several layers. (developed from pericycle and hence primary cortex is completely peeled off).
3. **Stone cells** occur in many groups scattered just below the zone of cork.

(B-14)

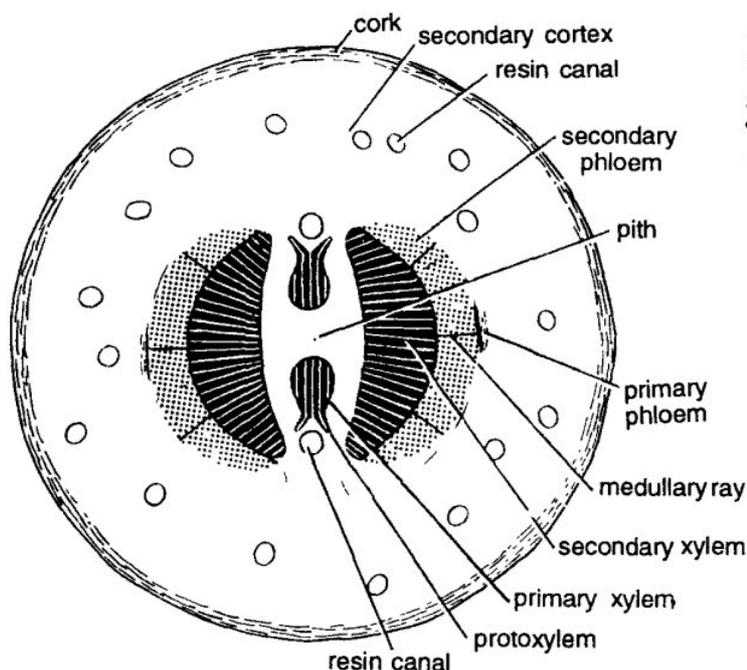


Fig. 3. *Pinus*. T.s. of old root (diagrammatic).

4. **Secondary cortex** follows cork. It is parenchymatous and a few layered deep.
5. **Many resin canals** are found in the secondary cortex.
6. **Primary phloem** occurs in two patches. The tissues are mostly crushed and obliterated.
7. **Secondary phloem** that follows is a few layered deep ring. It consists of sieve tubes, sieve plates, phloem parenchyma and albuminous cells.
8. Secondary phloem and secondary xylem are separated by a cambium.
9. **Secondary xylem** is composed of tracheids arranged in regular rows. It is traversed by uniseriate medullary rays.
10. **Pith** is small and parenchymatous. Two groups of primary xylem are situated on opposite radii.
11. **Each primary xylem** group is Y shaped. The divided arm faces the outer side (away from the pith).
12. **Resin canal.** The characteristic of the pine root is the presence of large resin canal between the divided arm of Y, close to each primary protoxylem group.

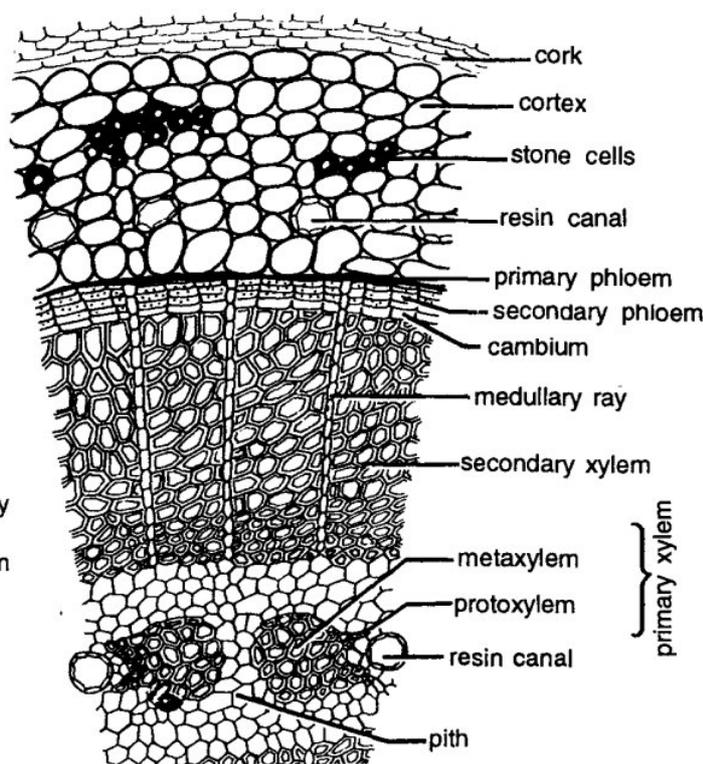


Fig. 4. *Pinus*. T.s. of old root (a part cellular).

#### Exercise 4

**Object :** Study of anatomy of the young long shoot.

#### Work procedure

Cut a T.s. of younger part of the long shoot towards the apex, stain in safranin-fast green combination, mount in glycerine and study.

#### Comments

1. **Outline** is wavy due to the presence of scaly leaves.
2. The stem is differentiated into epidermis, cortex and stele.
3. **Epidermis** is the outermost single layer. It is thickly cuticularized.
4. **Cortex** is multilayered and lies below the epidermis. The outer few layers forming hypodermis are sclerenchymatous. Inner layers are thin walled and parenchymatous in which

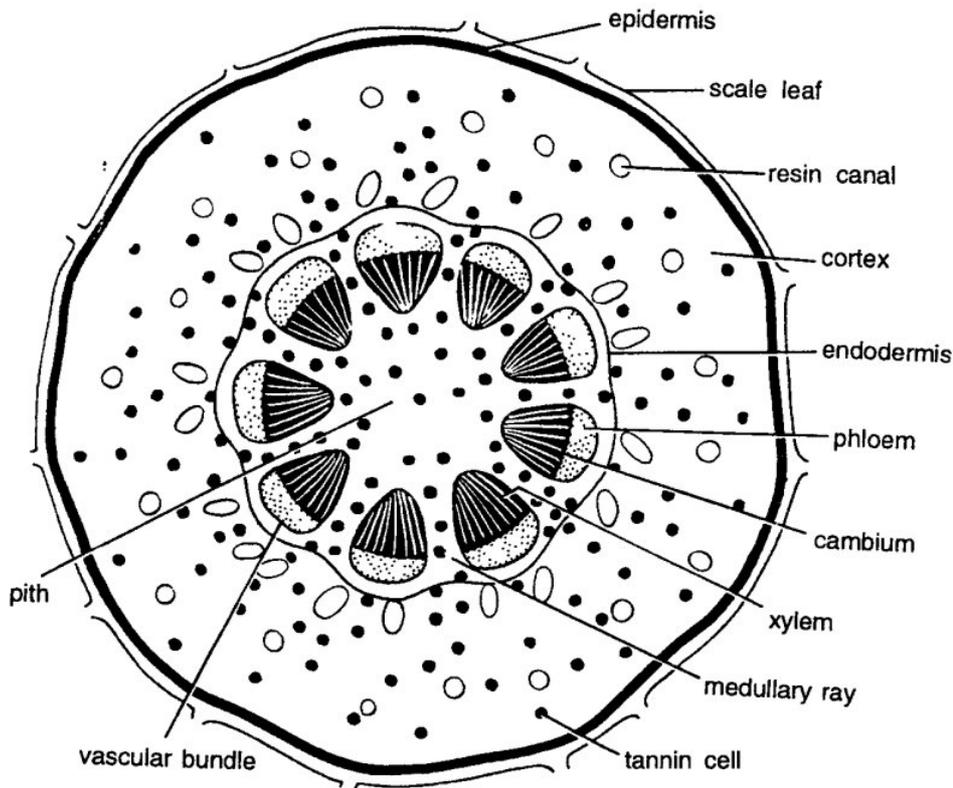


Fig. 5. *Pinus*. T.s. of young long shoot (diagrammatic).

- large number of resin canals and leaf traces are distributed irregularly.
5. **Resin canal.** The cavity of resin canal is bounded by a glandular, resin secreting epithelial layer. Outer to this layer are one or two layers of sclerotic cells.
  6. In *Pinus*, resin canals are present in the cortex and secondary wood of both stem and root and on margins of the primary xylem in the root.
  7. **The stele** is ectophloic siphonostele.
  8. **Endodermis** is present but is undistinguishable and so also a few layered pericycle located inner to it.
  9. **Vascular cylinder** is composed of 5-8 vascular bundles, separated by medullary rays. Vascular bundles are arranged in a ring.
  10. **Each vascular bundle** is conjoint, collateral endarch and open.
  11. **Xylem** is composed of tracheids and xylem parenchyma only, vessels are absent.
  12. **The phloem** is made up of sieve tubes, sieve plates and phloem parenchyma. Albuminous cells are also present.

13. **Pith** lies in the centre and is parenchymatous. It is connected with the cortex but narrow medullary rays separate the vascular bundles.

### Exercise 5

**Object :** Study the anatomy of the old long shoot.

### Work procedure

Cut a T.s. of the old part of the stem, stain with safranin-fast green combination, mount in glycerine and study.

### Comments

1. **The section** shows cork, cortex, primary and secondary vascular tissues and pith.
2. **Cork.** The outmost region is formed by the successive layers of cork. It consists of thick and suberized cells.
3. **Cork cambium** follows cork. It is made of a few layers of regularly arranged cells.
4. **Secondary cortex** present below is parenchymatous.

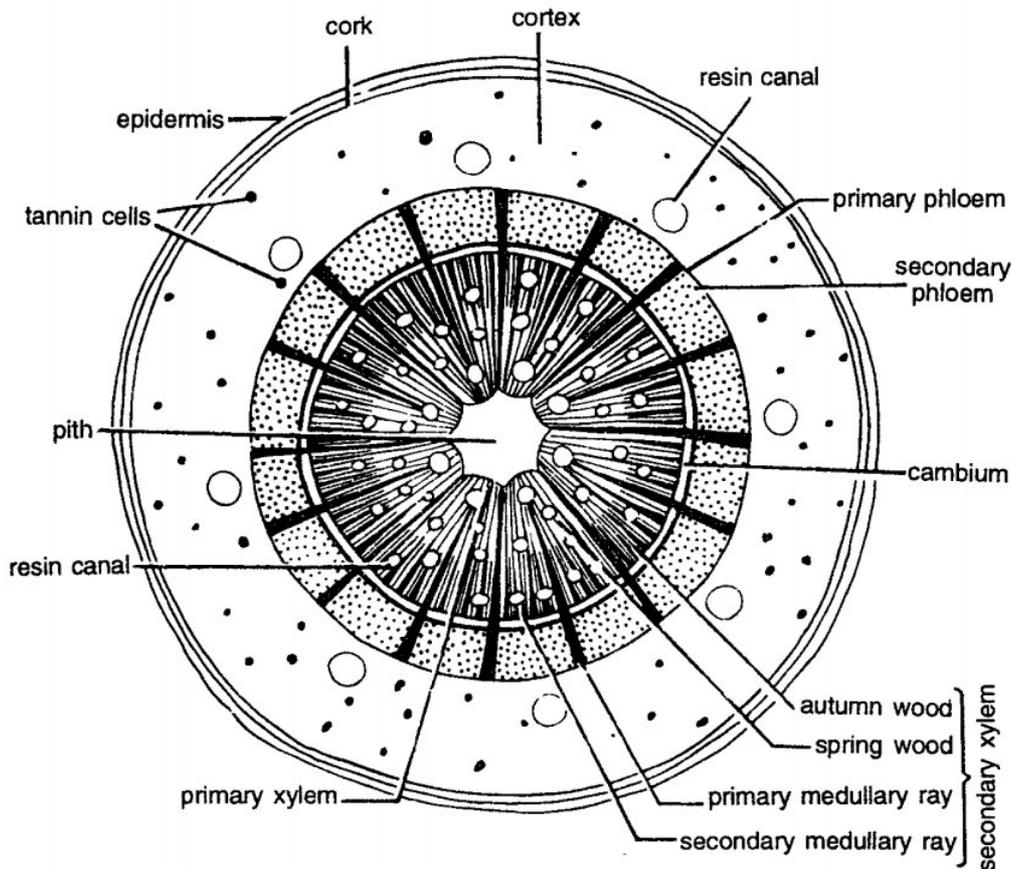


Fig. 6. *Pinus*. T.s. of old long shoot (diagrammatic).

5. **Primary cortex** is parenchymatous and many layered. The resin canals occur irregularly distributed in this region.
6. **Primary phloem** that lies inner to primary cortex occurs as small patches of crushed tissues.
7. **Secondary phloem** occurs as a well distinguished ring.
8. **Phloem** is composed of sieve tubes and phloem parenchyma.
9. **Cambium** separates the secondary phloem on its outer side and secondary xylem on its inner side.
10. **Secondary xylem** shows distinct and sharp annual rings. Thin walled and large xylem elements form a ring of spring wood. Thick walled and small xylem elements form a ring of autumn wood. The wood is pycnoxylic (compact).
11. **Rings of secondary xylem** — autumn and spring wood alternate one another and together form annual ring.
12. **Secondary xylem** (wood) is composed of tracheids and xylem parenchyma. Vessels are completely absent. Hence it is called non-porous wood.
13. **Medullary rays** traverse xylem and phloem. Primary medullary rays run from primary xylem to secondary phloem.
14. **Primary xylem** groups are endarch and lie just near the pith.
15. **Resin canals** are scattered in the primary and secondary xylem as in the cortex.
16. **Pith** is small, parenchymatous and many cells are filled with tannin.

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### Exercise 6

**Object : Study of R.L.s. of the wood.**

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#### Work procedure

Cut a thin section of wood along any one of the radii, stain in safranin-fast green combination, mount in glycerine and study.

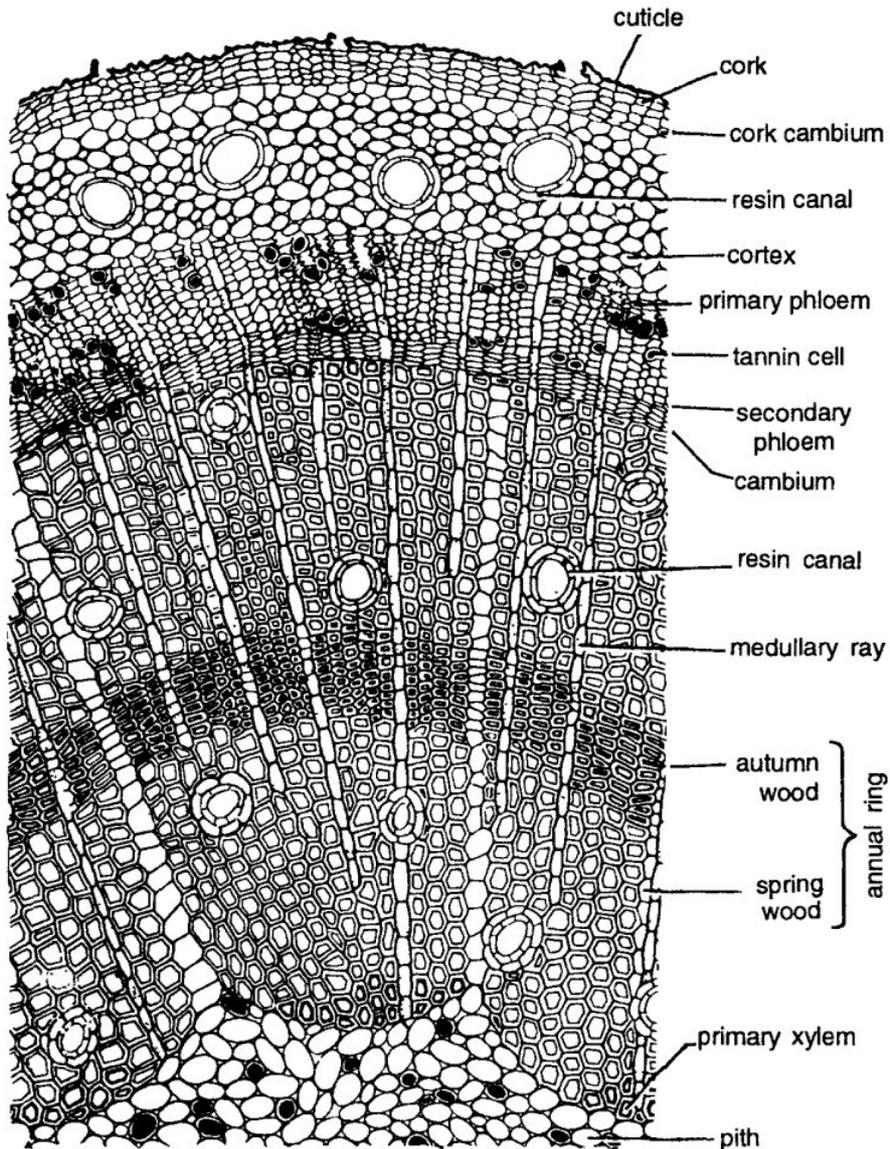


Fig. 7. *Pinus*. T.s. of old long shoot a part cellular.

### Comments

1. It shows presence of secondary xylem, ray tracheids and medullary rays.
2. **Xylem** is composed of tracheids with bordered pits on their radial walls. The bordered pits in this section are seen in surface view.
3. **Bordered pits** are circular areas surrounded by special cellulose thickenings called crassulae or Bars of Sanio. If pits are close to one another, the bars fuse to form Rims of Sanio.
4. **Medullary rays** run horizontally. In radial longitudinal plane they are cut length-wise and their length and height can be noticed. They are uniseriate.

5. **Each medullary ray** is made up of ray cells, ray tracheids and parenchyma.
6. **Ray tracheids** are present on both the sides of the medullary ray cells, only in the region of

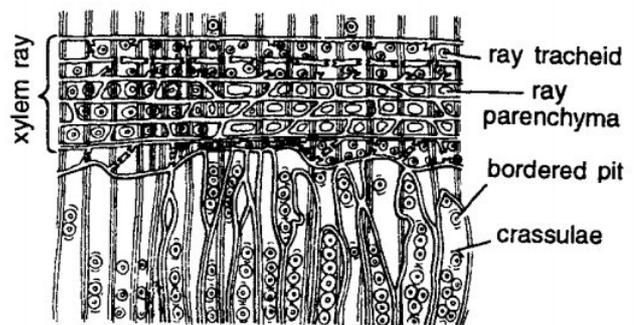


Fig. 8. *Pinus*. R.L.s. of wood (a part cellular).

xylem. These cells are thick, narrow and long. They show bordered pits.

7. **Ray parenchyma** occurs between the tracheids. These cells are thin, broad, small and living.
8. **Medullary ray**, in the region of phloem replaces ray tracheids with albuminous cells. They are small and contents are dense. (Ray parenchyma associated with these cells is filled with large amount of starch).

### Exercise 7

**Object : Study of T.L.s. of wood.**

#### Work procedure

Cut a thin section of wood along the tangent in the outer region, stain in safranin-fast green combination, mount in glycerine and study.

#### Comments

1. Tracheids and medullary rays are cut transversely in this plane.
2. **The bordered pits** are cut to show overarching borders, forming a dome-like structure. It encloses in the centre a small disc, called torus.
3. **Medullary rays** are uniseriate. Since they are

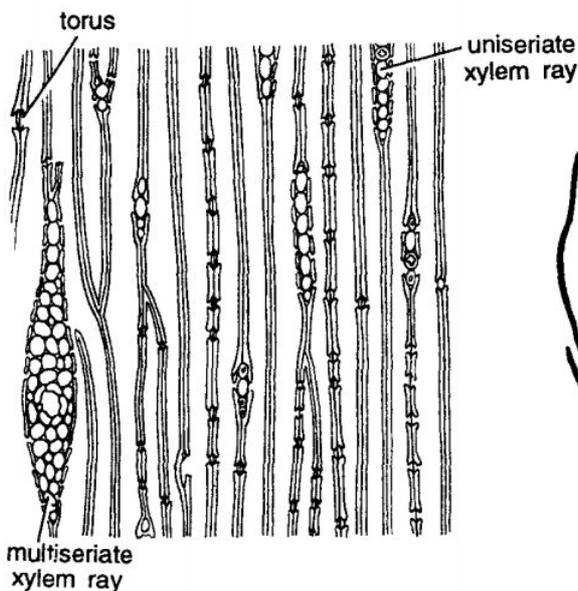


Fig. 9. *Pinus*. T.L.s. of wood (a part cellular).

cut transversely, their height and breadth can thus be determined.

4. **Each medullary ray** appears to be a short row of more or less rounded cells, three or four cells high.
5. Composition of medullary ray reveals centrally placed, thin-walled and living cells-the albuminous cells (in the phloem region) and the ray cells (in the xylem region).
6. These are surrounded on the lower and upper sides by thick walled and dead cells known as ray tracheids.

### Exercise 8

**Object : Study of T.s. of dwarf shoot at the base (before secondary growth).**

#### Work procedure

Take out a spur, invert it with needles downwards, the base is now the uppermost, cut T.s., at the base mount in glycerine and study.

#### Comments

1. **The section** almost resembles with that of the main stem.

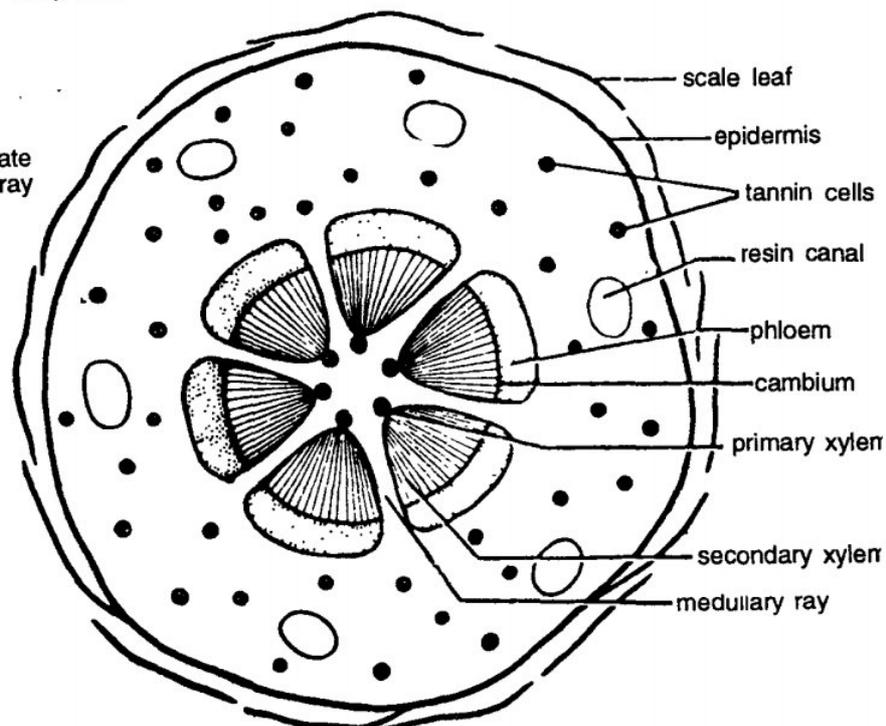


Fig. 10. *Pinus*. T.s. dwarf shoot showing secondary growth (diagrammatic).

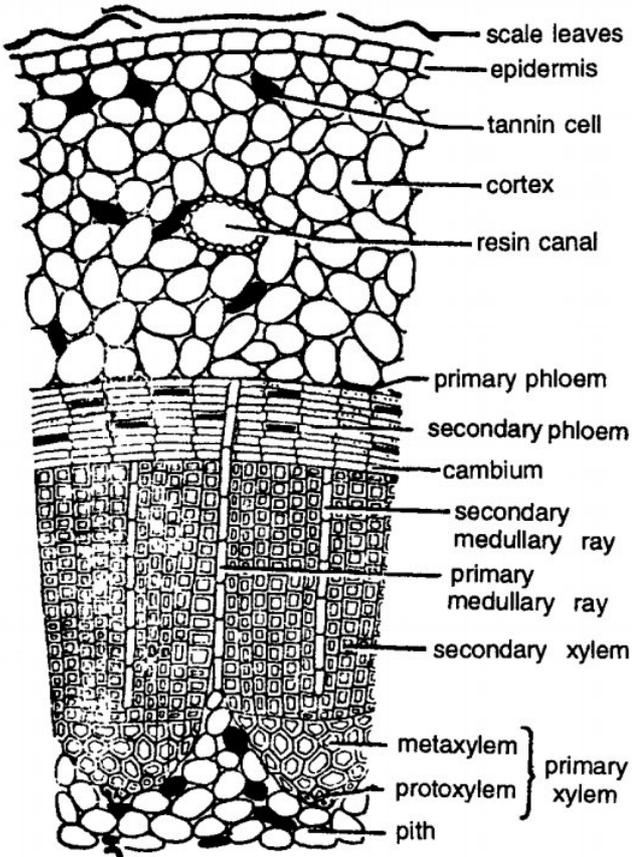


Fig. 11. *Pinus*. T.s. of dwarf shoot showing secondary growth (a part cellular).

2. **The outline** is wavy, due to ensheathing scaly leaves.
3. **The tissues** are differentiated into epidermis, cortex and stele.
4. **Epidermis** is made of single layer of thick walled cells.
5. **Cortex** follows epidermis. Outer few layers, close to epidermis are thick walled, while the inner layers are thin walled and parenchymatous.
6. **Resin canals** are present in the cortex. These are about six in number. Tannin cells are also irregularly scattered in this region.
7. **Stele** is an ectophloic siphonostele.
8. **Endodermis** is single layered and is followed by pericycle. Both the layers are indistinguishable.
9. **Vascular bundles** vary in number. They are generally six. Each vascular bundle is conjoint, collateral, endarch and open.
10. **Pith** is small. The cells are thick walled.

11. **Medullary rays** connect the pith and cortex and separate vascular bundles from one another.

### Exercise 9

**Object : Study of T.s. of dwarf shoot at base.**

### Work procedure

Cut a T.s. of dwarf shoot above the base, stain in safranin-fast green combination, mount in glycerine and study.

### Comments

1. Dwarf shoot also shows a little amount of secondary growth.
2. **The section** shows scale leaves, single-layered thick walled epidermis, few layers of cork cells and tannin-filled cells.
3. **Primary phloem** is crushed and form patches. Secondary phloem underlies it and forms a complete ring.
4. **Secondary xylem** is small and is separated by a thin ring of cambium from the phloem region. Medullary rays traverse the secondary xylem.
5. **Endarch protoxylem** group lies just near the pith. It is small and consists of thick-walled cells. Few cells are tannin-filled.

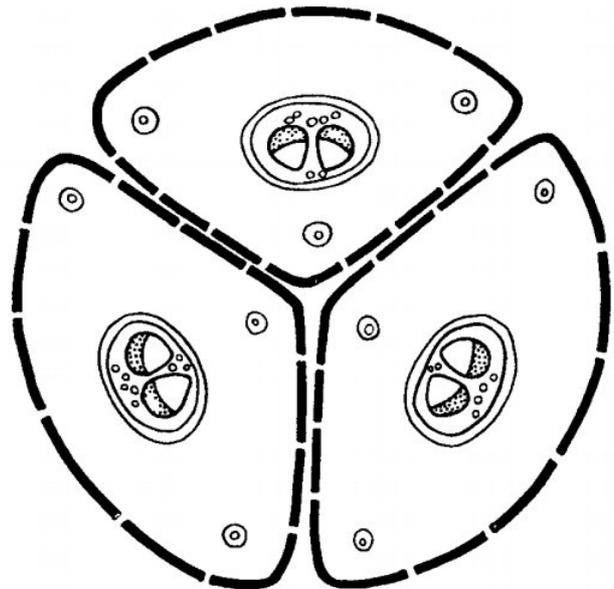


Fig. 12. *Pinus*. T.s. of dwarf shoot upper region (diagrammatic).

### Exercise 10

**Object :** Study of T.s. of the dwarf shoot at upper end.

#### Work procedure

Hold the dwarf shoot in upright position, cut a T.s., stain in safranin and fast green combination, mount in glycerine and study.

#### Comments

1. The structure is essentially similar to one found at its base.
2. The tissues of the dwarf shoot, towards the upper part, gradually become separated into equal parts, corresponding to the number of leaves in a spur (e.g. *Pinus gerardiana*, with trifoliar spur shows division of the dwarf shoot into three equal parts while in *P. quadrifolia*, with quadrifoliar spur, gets separated into four equal parts).
3. Each part shows distinct epidermis with stomata present all over.

4. Parenchymatous cortex fills most part of the section. Resin canals are located in the corners.
5. In the centre two conjoint, collateral and endarch vascular bundles are present. These are surrounded by distinct endodermis and pericycle.

### Exercise 11

**Object :** Study of T.s. needle (leaf).

#### Work procedure

Cut a thin T.s. of a needle, stain with safranin-fast green combination, mount in glycerine and study.

#### Comments

1. **The outline** of the section varies according to the species. (Triangular if spur is trifoliar, semi-circular if spur is bifoliar)
2. The needle is differentiated into epidermis, mesophyll and stele.
3. **Epidermis** is single with tangentially elongated and thickly cuticularized cells.

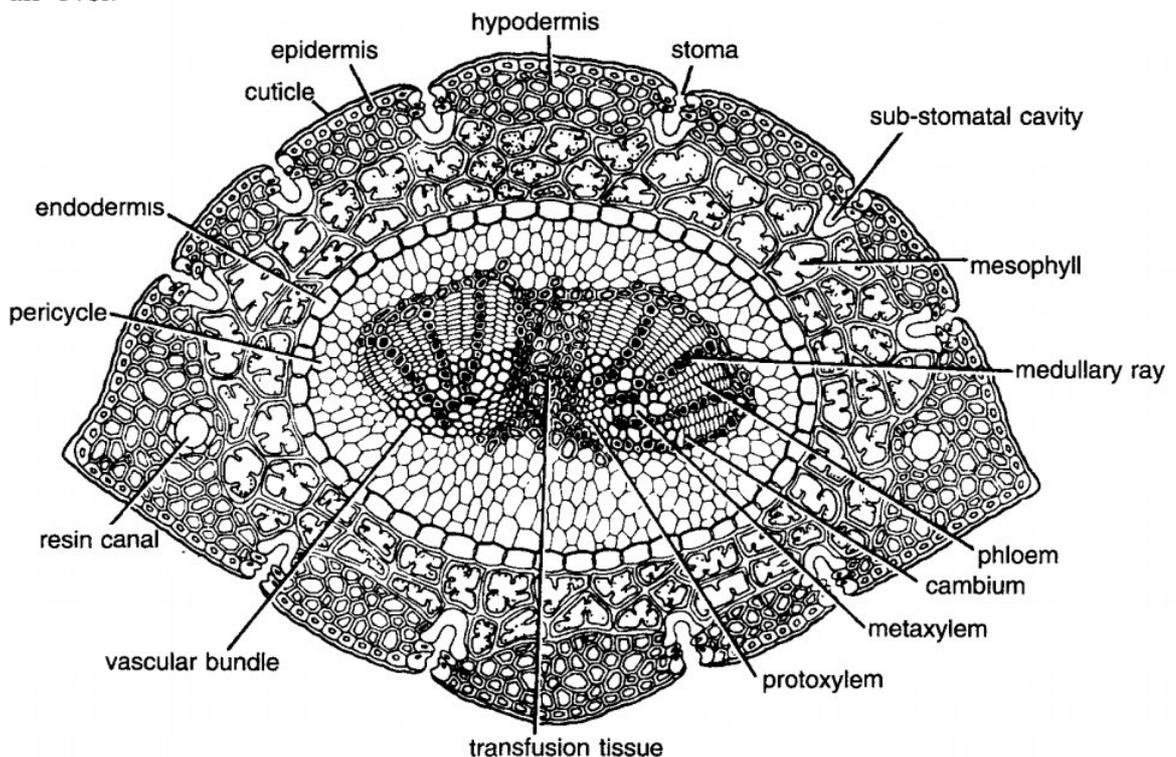


Fig. 13. *Pinus*. T.s. of needle (foliage leaf)-cellular details.

4. **Stomata** are sunken. These are present on all the faces of epidermis. The needle is thus said to be amphistomatic.
5. **Epidermis** is followed by hypodermis. It is few layered thick at the corners and 1-2 layered in other parts. Sub-stomatal chambers occur in this region. Cells are sclerenchymatous and fibrous.
6. **Mesophyll** lies below the hypodermis. It is made up of polygonal parenchymatous cells, densely filled with the chloroplasts. Numerous plate-like or peg-like infoldings project into the cell lumen (cavity) from the wall of the mesophyll cells.
7. **Resin canals** generally occur in the sclerotic hypodermis but also occur in the mesophyll tissue.
8. **Endodermis** is conspicuous. Cells are barrel-shaped and tangentially thickened. It is followed by a many layered, parenchymatous pericycle.
9. Generally two vascular bundles remain surrounded by this tissue. (In *P. strobus* there is only one vascular bundle).
10. **The vascular bundles** are separated from one another by a T-shaped thick walled transfusion tissue.
11. **Each vascular bundle** is conjoint, collateral and open. Protoxylem faces adaxial side. Phloem is located on the abaxial side.
12. **Xylem and phloem** groups are separated from one another by cambium at the base of the needle and by parenchymatous cells in the upper region.
13. **Secondary growth** is very little during which the medullary rays run between xylem and phloem.

**Features of special interest.** It shows the following xerophytic characters —

1. Narrow acicular form of the leaf.
2. Presence of thick cuticle.
3. Amphistomatic nature.
4. Sunken stomata.
5. Thick and sclerenchymatous hypodermis.
6. Infolded peg-like structures in mesophyll.
7. Presence of transfusion tissue.
8. Simple vascular system.

---

### Exercise 12

**Object : Study of male cone, microsporophylls and microsporangia.**

---

#### Work procedure

Dissect out the male cone, separate the microsporophylls, study the shape, size and microspores.

#### Comments

1. Male cones replace the dwarf shoots. Each male cone arises in the axile of a scale leaf. The main shoot, on which these are produced, continues to grow further.
2. Male cones are grouped in clusters on the shoots of the same year only.
3. Each male cone has single, centrally located cone axis around which many scaly microsporophylls are spirally arranged.
4. Each microsporophyll has an expanded triangular central part and stalk-like base. Terminal part projects into a tip.
5. Few lowermost sporophylls are sterile, and do not bear any male reproductive structures.
6. On the abaxial side, each microsporophyll bears two ovoid microsporangia or pollen sacs on its lateral sides.
7. Each microsporangium has its own wall which encloses many microspores
8. The young microspore is globular or spherical in shape and is uninucleate.
9. A mature microspore or pollen grain shows two wall layers- exine and intine, 2 prothallial cells and antheridial cell.
10. Pollen grain has a thick expanded exine in the form of wings on the sides, followed by a smooth intine.

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### Exercise 13

**Object : Study of L.s. of male cone.**

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#### Work procedure

Study a slide showing L.s. of male cone.

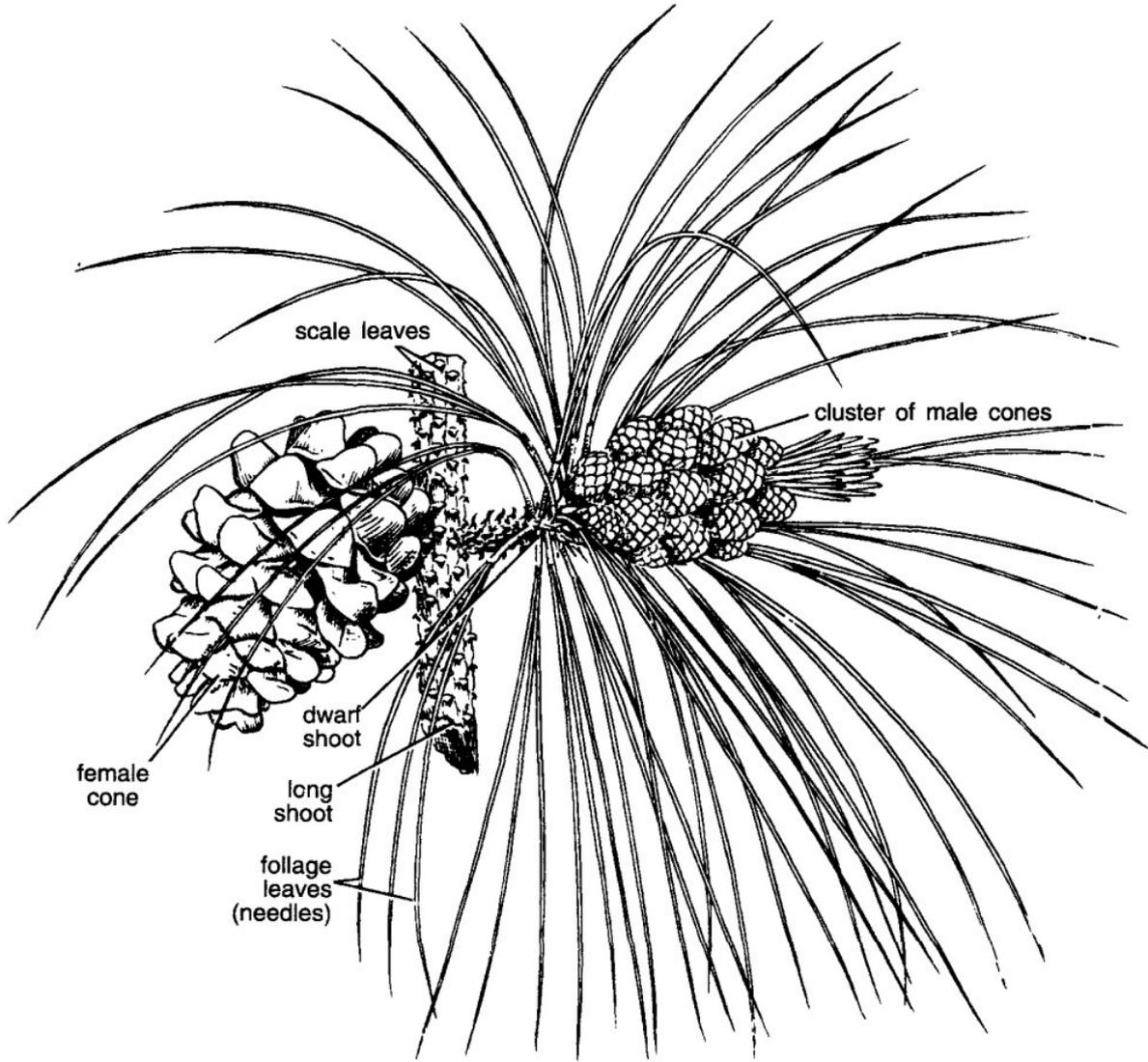


Fig. 14. *Pinus*. A twig with male and female cones.

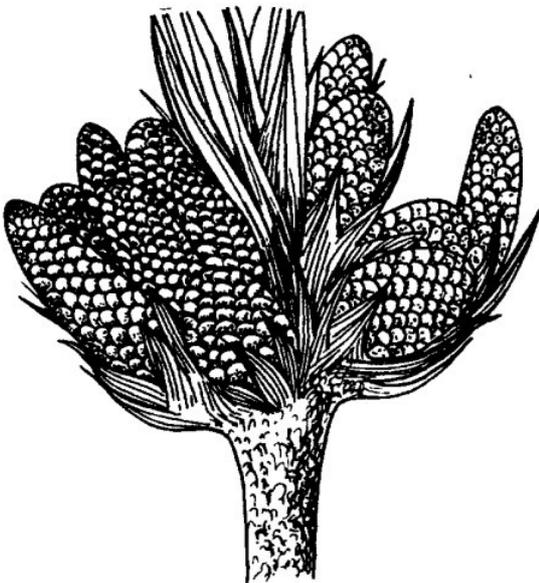


Fig. 15. *Pinus*. Male cones in cluster.

### Comments

1. It shows a cone axis bearing microsporophylls.
2. The cone axis is centrally located.
3. Microsporophylls are spirally arranged. These are scaly, triangular and expanded.
4. It is attached to the cone axis by a stalk-like base.
5. The outer expanded part is sterile and is known as apophysis.
6. Microsporangia are present on the lower or abaxial surface.
7. Each microsporangium has a wall that encloses a cavity.
8. The wall consists of epidermis, wall layers and tapetum.
9. The cavity shows numerous microspores in various stages of development.

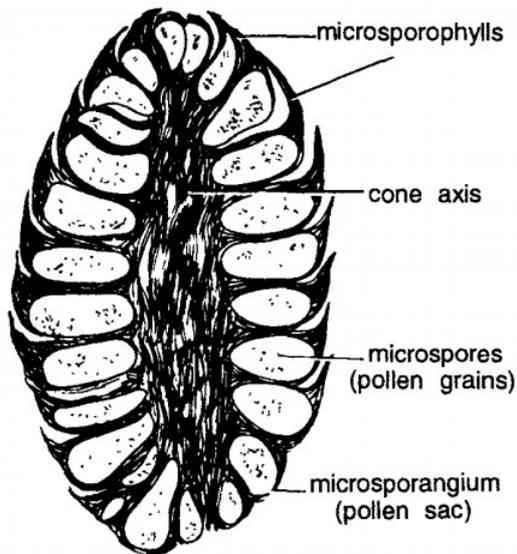


Fig. 16. *Pinus*. L.S. of male cone.

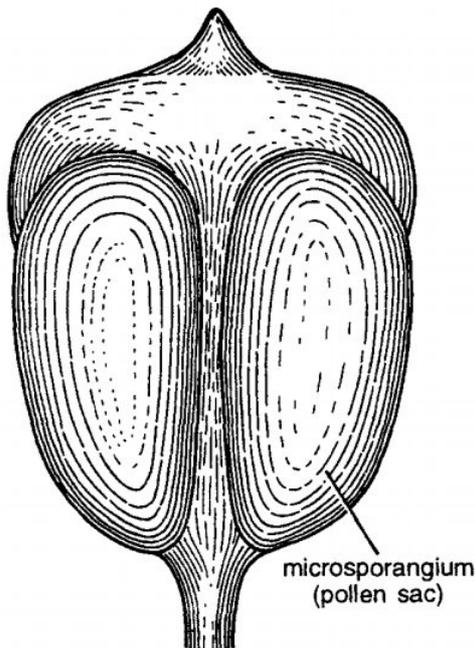


Fig. 17. *Pinus*. A microsporophyll with two microsporangia as seen from lower side.

#### Exercise 14

**Object :** Study of morphology of the female cone.

#### Work procedure

Study the external features of 1st, 2nd and 3rd year female cones. Note the position, arrangement and structure of sporophylls.

#### Comments

1. Female cones are larger than the male cones. They are borne at the apices of the young elongated shoots, replacing the shoot of unlimited growth (long shoots).
2. Single shoot may bear one to four female cones which are reddish-green in colour and mature in three years.
3. In the first year, cones are compact and sporophylls are closely arranged.
4. The second year cones are large in size and woody in nature but sporophylls are still compactly arranged.
5. In the third year, cone becomes loose. Sporophylls separate from one another due to elongation of the cone axis.
6. Each female cone consists of many sporophylls, arranged spirally around the cone axis.

#### Exercise 15

**Object :** Study L.S. of female cone.

#### Work procedure

Study a prepared slide of L.S. of female cone.

#### Comments

1. Female cone is made of centrally located cone axis and spirally arranged sporophylls.
2. Each sporophyll consists of two kinds of paired scales : (i) bract scale or cone scale and (ii) ovuliferous scale or seminiferous scale.
3. Many small and thin bract scales are arranged spirally around the cone axis. They are directly borne on the cone axis. Each of these is present on the abaxial (lower) side of the ovuliferous scale.
4. On the adaxial (upper) side of the bract scale, a thick, large, woody and triangular ovuliferous scale is present.
5. The ovuliferous scales in the middle part of the cone are the largest and get gradually smaller towards its base and apex.
6. Ovuliferous scale and bract scale are fused for a little distance near the cone axis while free at a distance away from it.

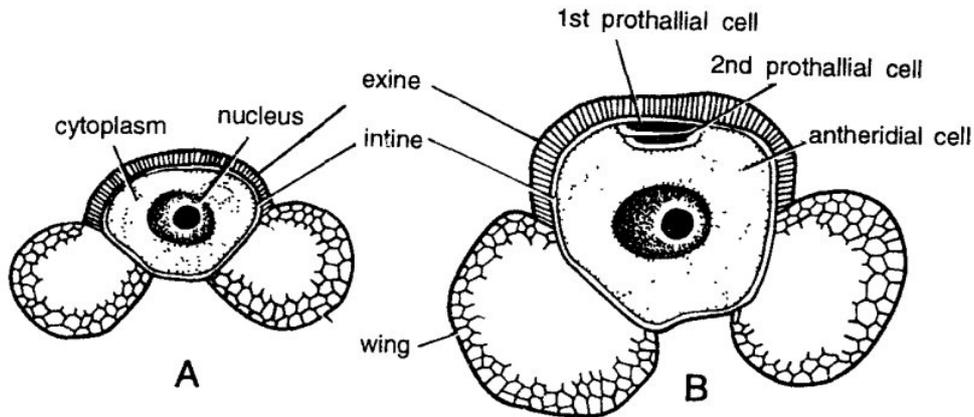


Fig. 18. *Pinus*. Microspores (pollen grains), A. Young. B. Old.

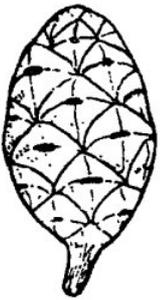


Fig. 19. *Pinus*. 1st year female cone.



Fig. 20. *Pinus*. 2nd year female cone.

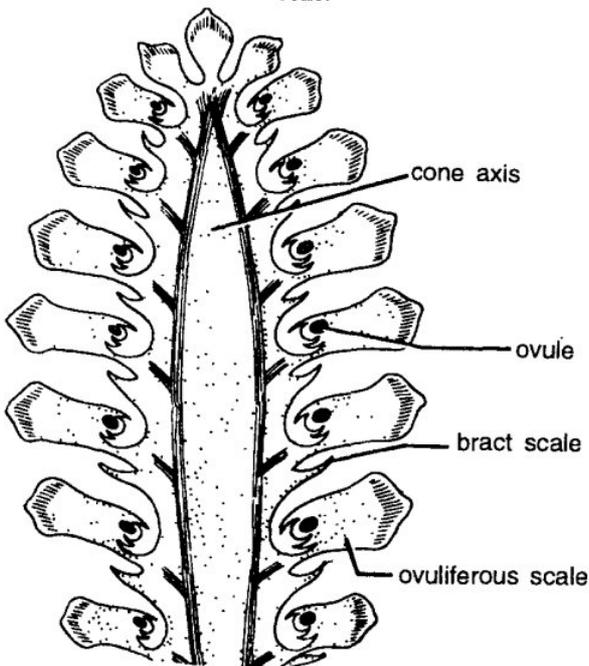


Fig. 22. *Pinus*. L.S. of female cone.

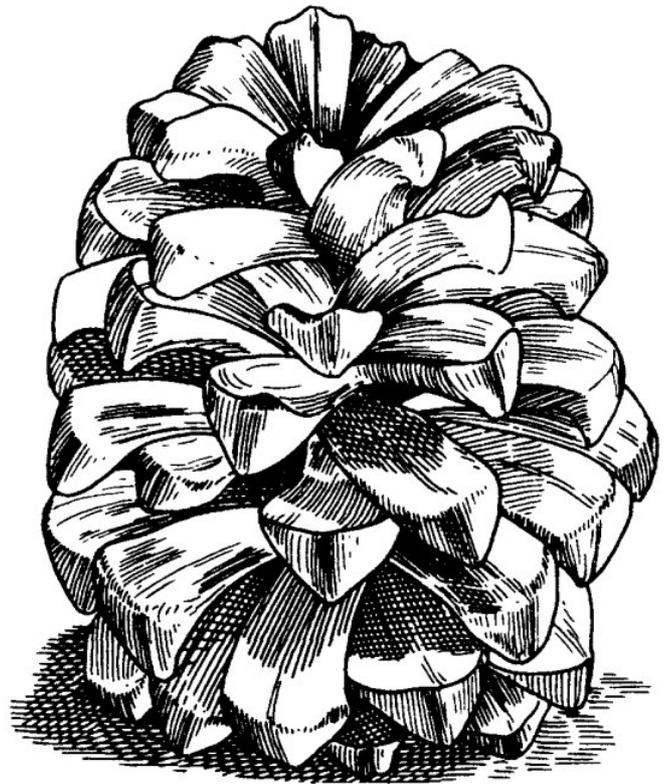


Fig. 21. *Pinus*. 3rd year female cone.

7. Ovuliferous scale is shortly stalked and rest of the part is expanded.
8. At the base of this expanded, triangular part, two naked and sessile ovules are present. These are situated on the adaxial, (upper) surface of the ovuliferous scale, at its base, with their micropyles directed towards cone axis.
9. The terminal part of the ovuliferous scale is broad and sterile and is known as apophysis.

**Exercise 16****Object : Study of L.s. of ovule.****Work procedure**

Study a prepared slide of L.s. of ovule. Note integuments, nucellus, female gametophyte and archegonia.

**Comments**

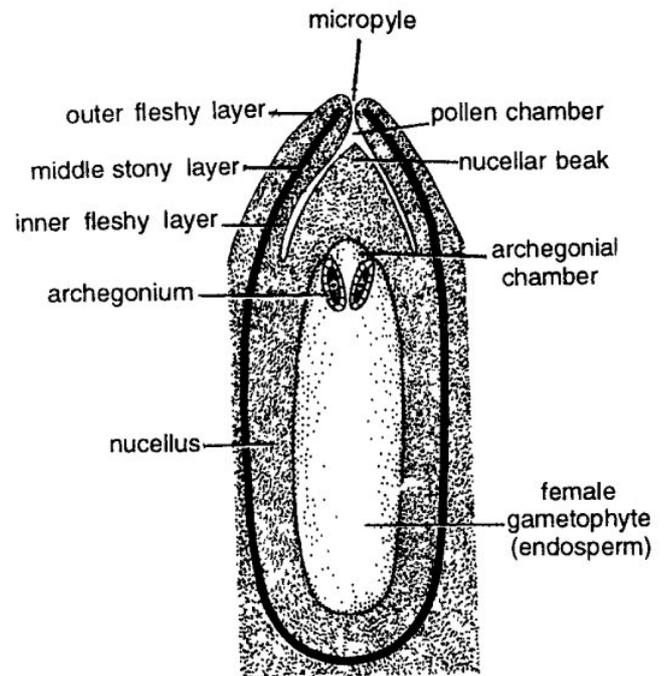
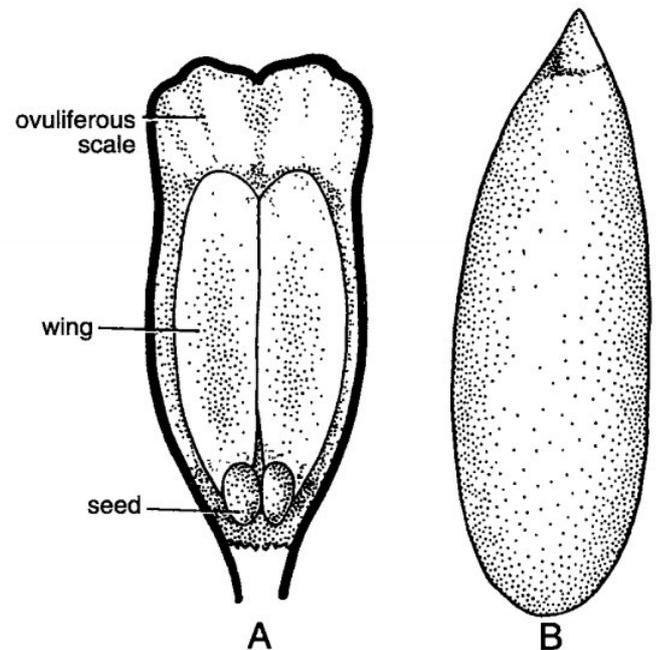
1. Ovule is elongated in shape.
2. It is unitegmic and the integument is three layered. The outermost layer is thin. The middle layer is stony and prominent. The innermost layer is fleshy and well developed.
3. Nucellus is fused with inner layer of the integument, except at its tip where it forms an elongated and slender micropyle, directed towards the cone axis.
4. In the nucellar region lies a small cavity just opposite the micropyle. It is known as pollen chamber.
5. Female gametophyte (endosperm) is differentiated from nucellus. About 2-5 archegonia are situated in this region at the micropylar end near the base of the archegonial chamber

**Exercise 17****Object : Study of seed.****Work procedure**

Study the position and arrangement of seeds on ovuliferous scale, also study a prepared slide of L.s. of seed

**Comments**

1. Fertilized ovules get transformed into seeds which are situated on the adaxial side of the ovuliferous scale at its base near the cone axis.
2. Seeds are small, elongated and winged. The wing is a thin layer of tissue which splits off from the adaxial face of the ovuliferous scale. (Seed can be best studied by cutting longitudinal section of the seed of *P. gerardiana*; vern. chilgoza).

**Fig. 23.** *Pinus*. L.s. of ovule.**Fig. 24.** *Pinus*. A. Ovuliferous scale bearing two winged seeds. B. seed.

3. The seed is covered with red and brown testa.
4. Inner fleshy layer of the integument still persists. It is membranous, thin and papery, termed as tegmen.
5. The nucellus is present as a thin layer and forms a nucellar cap at the micropylar end.
6. The larger part of the seed consists of oily endosperm.

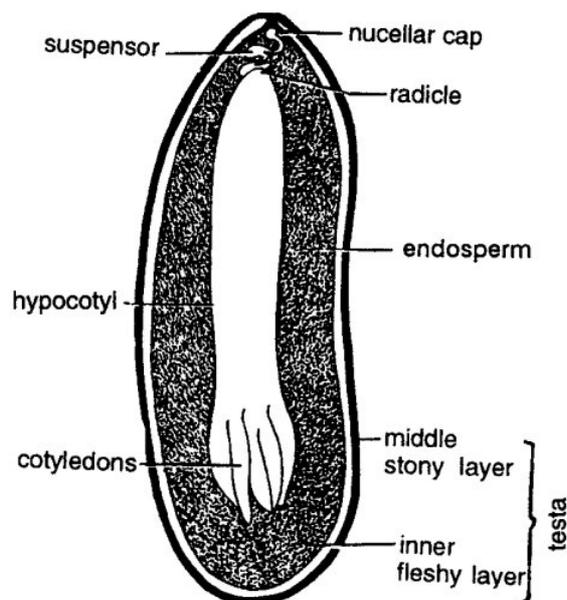


Fig. 25. *Pinus*. L.s. of seed.

7. The suspensor is long and becomes coiled. Embryo is differentiated into radicle, plumule and cotyledons (3-8 in number).
8. In between the radicle and plumule, is present a well developed hypocotyl.

### Exercise 18

**Object : Study of seedling.**

#### Work procedure

Study a newly germinated seed. Observe various organs.

#### Comments

1. Seedling shows three parts — (i) roots (ii) hypocotyl and (iii) leaves.
2. The **roots** are well branched and arise from the radicle.
3. **Hypocotyl** gives rise to unbranched, slender and thin primary shoot.
4. **Leaves** are green and needle-like which are borne in whorls on the primary shoot. These are cotyledonary leaves.
5. **The primary leaves** or first spur shoots arise in the axils of some of these juvenile (cotyledonary) leaves and are borne in spiral series on the primary shoot.

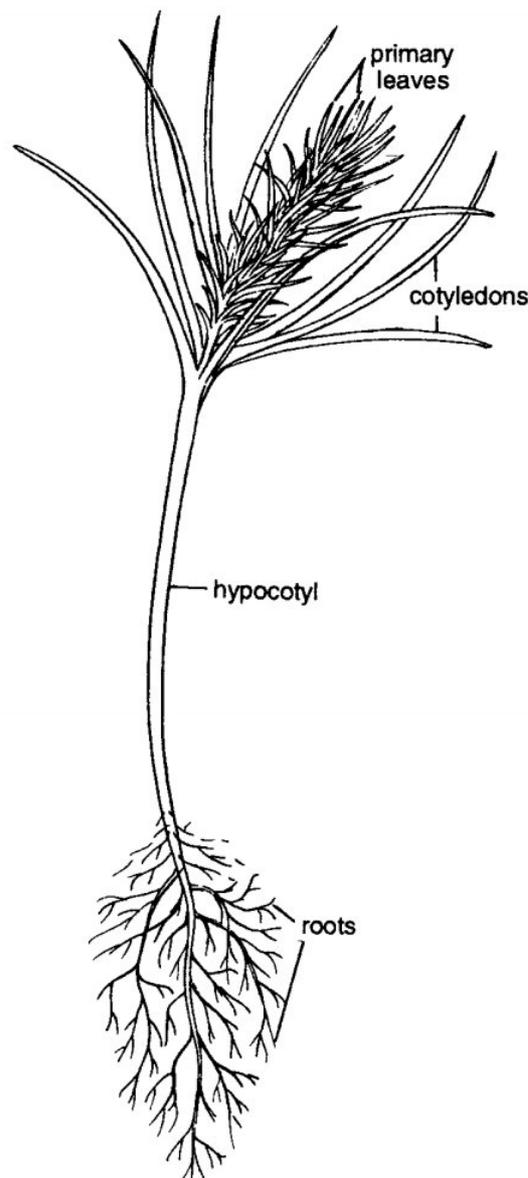


Fig. 26. *Pinus*. Older seedling to show cotyledons and spirally arranged primary leaves.

### Identification

**Division—Gymnosperms.** (1) Absence of vessels, (2) Ovules naked, (3) Seeds attached to woody scales, (4) Scales generally form a cone.

**Class—Coniferopsida.** (1) Leaves needle shaped, (2) Wood pycnoxylic (compact), (3) Presence of resin canals, (4) Compact male and female cones, (5) Non-flagellate male gametes, (6) Seeds bilaterally symmetrical.

**Order—Coniferales.**

**Family—Pinaceae.** (1) Resinous wood, (2) Plants monoecious, (3) Sporophylls spirally arranged, (4) Microsporophylls with two microsporangia, (5) Pollen grains winged, (6) Female cone woody, (7) Polyembryony present, (8) Seed dry and winged.

**Genus—*Pinus*.** (1) Plants sporophytic and monoecious. Male and female reproductive organs in cones, (2) Branches dimorphic, (3) Long shoots with secondary xylem, annual rings are formed, wood pycnoxylic and resinous, (4) Dwarf shoots with a little secondary growth, (5) Leaves are of two types, (6) Scale leaves brown and membranous, (7) Foliage leaves are acicular, xerophytic, mesophyll cells with peg-like ingrowths, 2 resin canals and T-shaped transfusion tissue, (8) Male cones borne laterally, in clusters, microsporophyll bears two microsporangia on abaxial side, (9) Pollen grains winged, (10) Female cones borne single and terminal, (11) Bract scales and ovuliferous scales spirally arranged, (12) Two naked ovules on the adaxial side of the ovuliferous scale, (13) Seeds dry and winged.

### Hints for Collection

In India *Pinus* is represented by five species which grow wild in north-east and north-west Himalayas. The species are *P. gerardiana* (Chilgoza in Hindi), *P. roxburghii* (= *P. longifolia*), *P. wallichiana* (*P. excelsa*), *P. insularis* (= *P. khasya*) and *P. armandi*. In plains it is cultivated for its ornamental value.

### *Ephedra* (Jointed Fir)

#### Classification

Division	–	Gymnosperms
Class	–	Gnetopsida
Order	–	Gnetales
Family	–	Ephedraceae
Genus	–	<i>Ephedra</i>

#### Exercise 1

**Object :** Study of external morphology.

#### Work procedure

Study the external features of the plant, note the jointed nature of stem, scale leaves and underground tap root, observe the positions of male and female strobili.

#### Comments

1. Plants are small, bushy, trailing or climbing shrubs attaining a height of not more than 2

meters. However, *E. antisiphilitica* is a small tree, reaching a height of 3-5 meters.

2. The plant body is branched and possesses only minute leaves at the nodes. It therefore, resembles superficially with the species of *Psilotum* and *Equisetum*.
3. It is differentiated into stem, leaves and underground roots.
4. The stem remains anchored by a deep tap root and many adventitious roots.
5. The stem is delicate, slender and green when young. It is ribbed irregularly and is differentiated into short nodes and long internodes.
6. Two or three branches arranged in whorls arise from the nodes in the axils of leaves. The branches are shed off during dry season.
7. Older part of the stem may bear many branches. It becomes hard and woody due to secondary growth.
8. Leaves are borne in a whorl of 2-4 at each node.

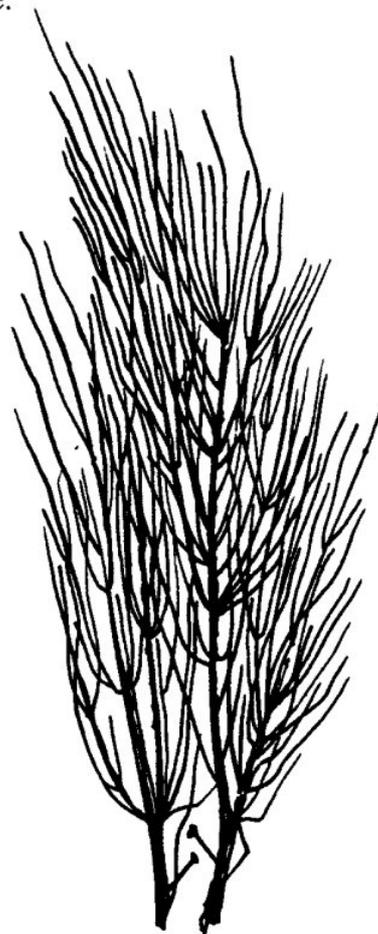


Fig. 1. *Ephedra*. Plant showing shrubby habit.

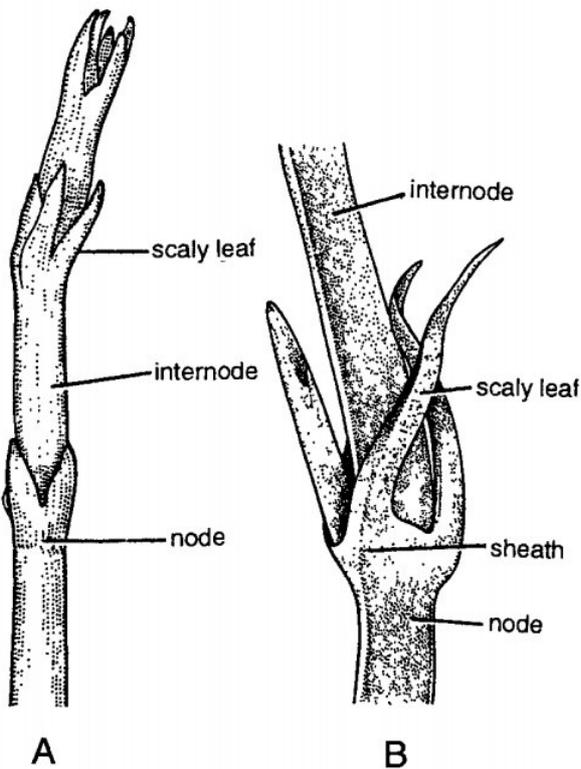


Fig. 2. *Ephedra*. A part of stem showing A. Nodes and internodes. B. A node with scaly leaves.

9. Leaves that are small and scale-like are connate at the base and thus form a sheath around the node.
10. Each scale leaf is traversed by two parallel and unbranched veins.
11. Foliage leaves are completely absent.
12. The male and female reproductive organs are borne in small strobili.
13. The plants are mostly dioecious and bear only one type of reproductive organs. They may also be monoecious when they bear both kinds of strobili.

### Exercise 2

**Object :** Study of T.s. of young stem.

#### Work procedure

Cut a T.s. of the younger part of the stem, stain in safranin-fast green combination, mount in glycerine and study.

#### Comments

1. **The outline** of the section shows ribbed nature of the stem.

(B-14)

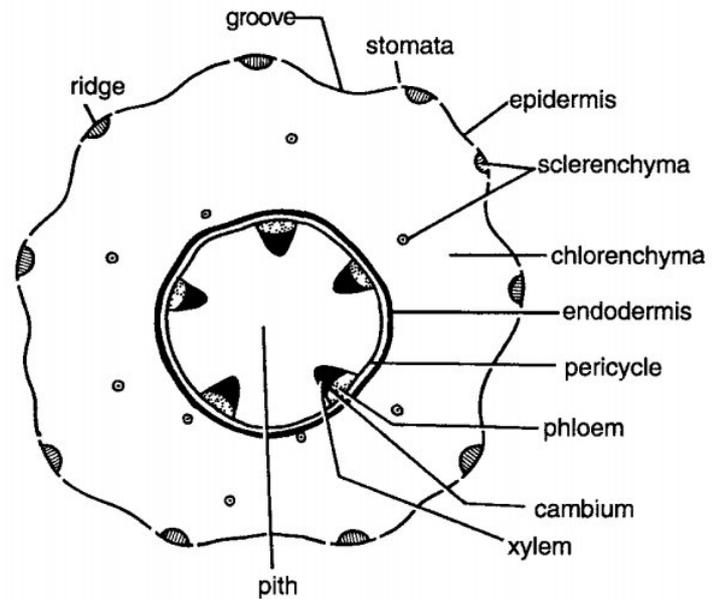


Fig. 3. *Ephedra*. T.s. of young stem (diagrammatic).

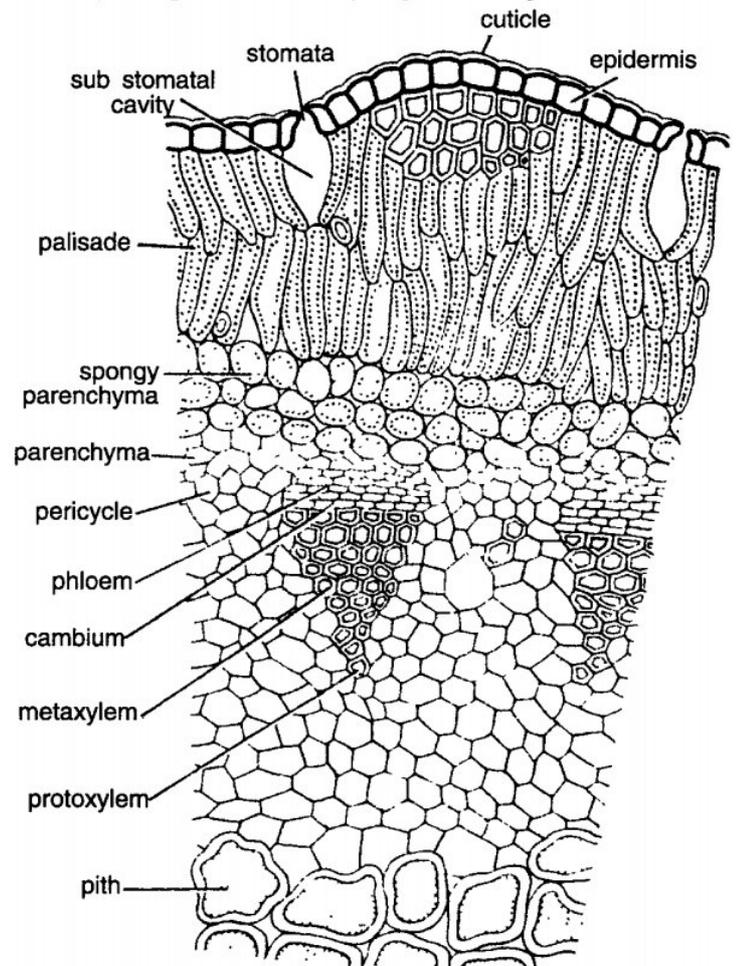


Fig. 4. *Ephedra*. T.s. of young stem (part cellular).

2. The tissues are differentiated into epidermis, cortex and stem.

3. **Epidermis** is the outermost layer. It is very thick and heavily cuticularized.
4. **The stomata** are sunken. These interrupt epidermis frequently and occupy a position just on the slopes of the ridges.
5. **Hypodermis** is sclerenchymatous and occur in small groups below the ridges.
6. **Cortex.** Rest of the cortical tissue is chlorenchymatous. The cells are often radially elongated and contain abundant chloroplasts. Large intercellular spaces are present between these cells.
7. A few patches of sclerenchyma occur dispersed in the cortex (specially in young axis rendering hardness and resistance).
8. **Stele** is ectophloic siphonostele. It is composed of many vascular bundles, their number being variable.
9. **Endodermis** is single layered and is followed by a pericycle.
10. **A few vascular bundles** are arranged in a ring. Each is conjoint, collateral, endarch and open.
11. **External phloem** group is separated from internal xylem group by a narrow layer of cambium.
12. **Pith** is parenchymatous and occurs in the central region.
13. **Nodal diaphragm.** The characteristic anatomical feature is the presence of diaphragm-like plate of cells at the base of each internode or at the node (nodal

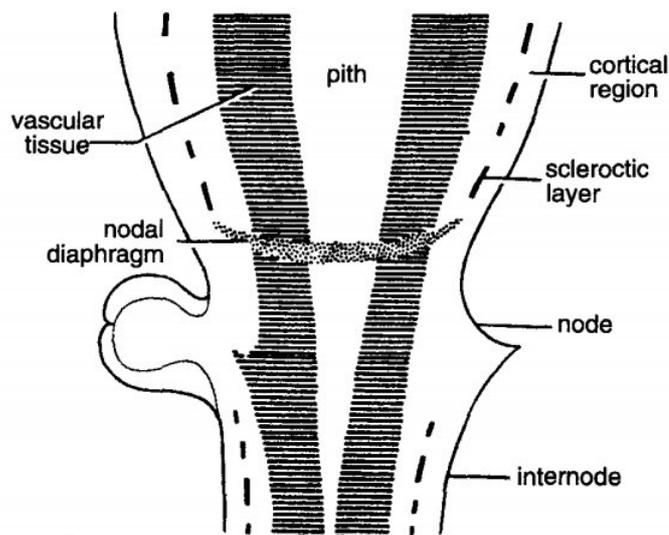


Fig. 5. *Ephedra*. L.s. nodal region (diagrammatic).

diaphragm). This helps the plant to shed off the branches at the nodes.

### Exercise 3

**Object : Study of T.s. of stem showing secondary growth.**

### Work procedure

Cut a T.s. of the older part of the stem, stain in safranin-fast green combination, mount in glycerine and study.

### Comments

1. **The section** shows epidermis, cortex and primary and secondary vascular tissues.
2. **The epidermis** and cortex remain unchanged. However, after (3-4 Years) of growth, cork develops just outside the phloem and outer tissues (epidermis, cortex, etc.) are, therefore, cast off.
3. **Sclerotic cells** (stone cells) develop just above the zone of secondary tissue.
4. **Primary phloem** occurs as obliterated patches.
5. **Secondary phloem** forms a zone below. Phloem is composed of sieve tubes and phloem parenchyma.
6. **Annual rings** are distinct comprising autumn and spring wood each. These are formed in the secondary xylem (wood).
7. **The secondary xylem** shows a thin walled spring wood and thick walled autumn wood, successively formed in alternating zones.
8. **Autumn wood** is made of smaller cells, while those of spring wood are bigger in size.
9. **The tracheidal cells** of the secondary wood are associated with broad vessels. Though absence of vessels is characteristics of the Gymnosperm, *Ephedra*, (i.e. order Gnetlaes) itself is an exception.
10. **Vessels** are most abundant in the spring wood and a few or none at all in the autumn wood. Spring wood is often ring porous.
11. **Tracheids and vessels** have uniseriatey or irregularly distributed bordered pits. Protoxylem elements of primary xylem show spiral, annular or reticulate tracheids.

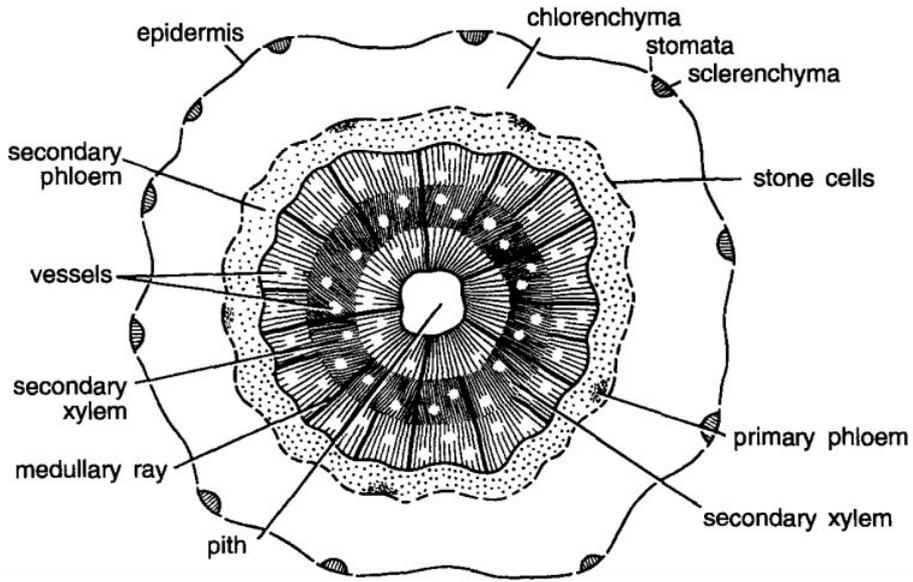


Fig. 6. *Ephedra*. T.s. of old stem (diagrammatic).

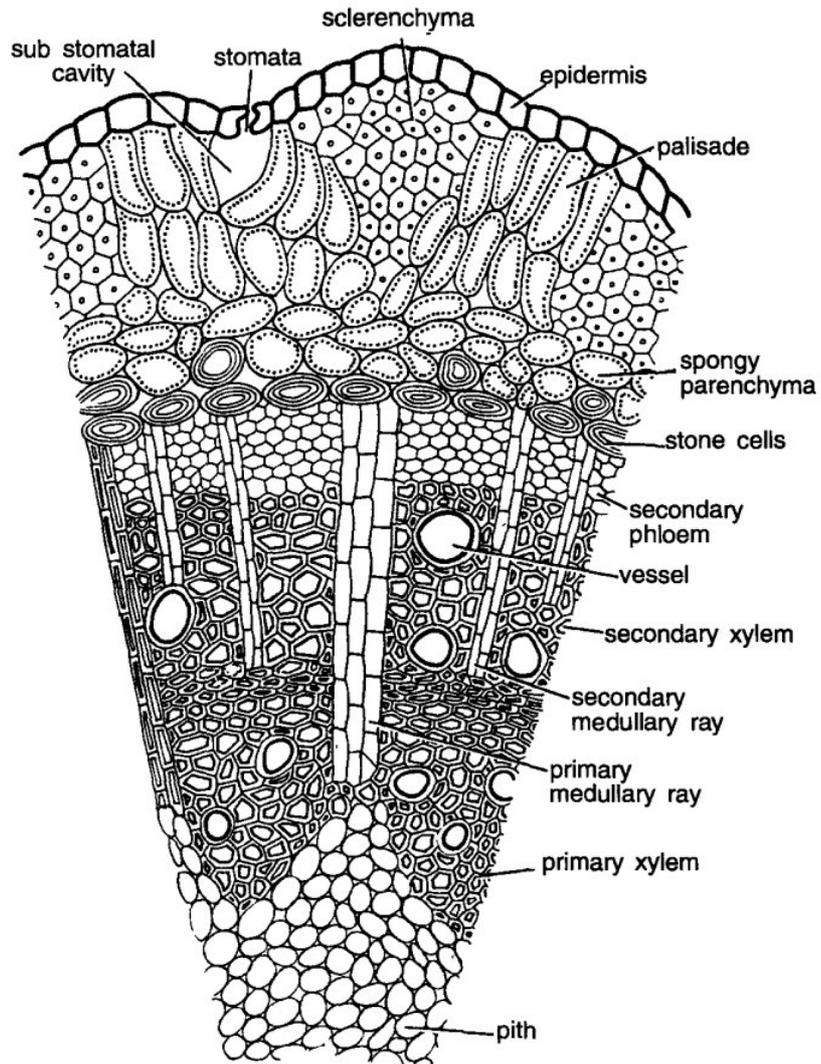


Fig. 7. *Ephedra*. T.s. of old stem (a part cellular).

12. **Medullary rays** traverse the wood. Primary medullary rays run from primary xylem to primary phloem while secondary medullary rays run from secondary xylem to secondary phloem.
13. **Medullary rays** are uniseriate in the young stem but are very broad and long (multiseriate) in the old stem.
14. **Primary xylem** groups are present at the end of the secondary wood near the pith. These are endarch.
15. **Pith** is large and parenchymatous. It occupies the centre.

**Features of special interest.** It shows the following xerophytic characters.

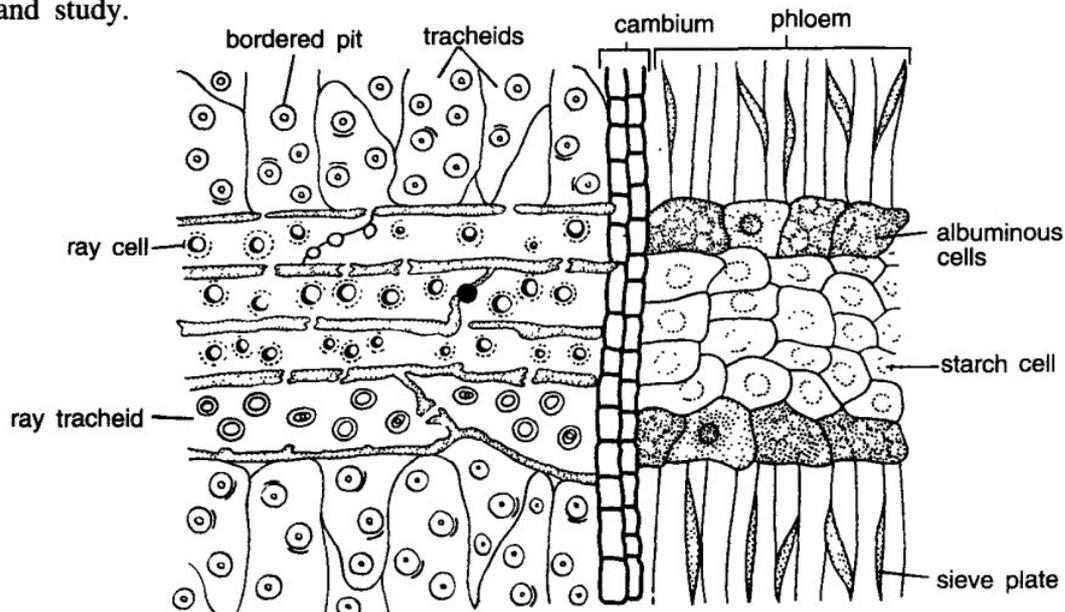
1. Thickly cuticularized epidermis.
2. Sunken stomata.
3. Palisade and spongy parenchyma in the cortex.
4. Patches of sclerenchyma.
5. Shedding of branches.
6. Presence of nodal diaphragm.
7. Vessel in the secondary wood.

#### Exercise 4

**Object : Study of R. L.s. of wood.**

#### Work procedure

Cut a R.L.s. of wood along any one of the radii. Stain in safranin-fast green combination, mount in glycerine and study.



**Fig. 8.** *Ephedra*. R.L.s. of wood.

#### Comments

1. It shows the presence of secondary xylem and medullary rays.
2. Secondary xylem consists of tracheids with bordered pits on their radial walls.
3. The bordered pits are circular or slightly elliptical. These may form reticulations (mostly due to the dissolution of walls of cavities of the pits) and such perforations, being characteristic of *Ephedra*, are known as Ephedroid perforations. Bordered pits are either scattered or arranged in 2 or 3 tight rows. (They are never polygonal due to mutual compression).
4. Special cellulose thickening - Bars of Sanio are also present below the pits.
5. A few vessels present show bordered pits which are scattered or may remain arranged in 2 or 3 rows. The apertures of the bordered pits are commonly horizontally oriented. End walls are also perforated.
6. Medullary rays are uniseriate or multiseriate. These run horizontally. In this plane medullary rays are cut lengthwise and their length and height can be observed.
7. Medullary rays may range up to 40-50 cells in height.
8. Each medullary ray in the region of secondary xylem is composed of ray cells and ray tracheids dispersed regularly.

9. Ray cells are thick walled as well as thin walled. These occur in the same medullary ray. Their tangential walls possess bordered pits or slit-like openings.
10. Ray tracheids are thick walled. Their radial and tangential walls are pitted, pits being bordered.
11. In the region of phloem, medullary ray is made of starch cells surrounded by albuminous cells on both sides.

### Exercise 5

**Object : Study of T.L.s. wood.**

#### Work procedure

Cut a T.L.s. of wood by passing the razor along any one of the tangents, stain in safranin-fast green combination mount in glycerine and study.

#### Comments

1. In this plane tracheids, vessels and medullary rays are cut transversely.
2. Bordered pits are seen in surface view.
3. The bordered pits show usual over-arching dome-shaped structure and a small disc-torus.
4. Medullary rays are transversely cut and as such their height and breadth can be determined.

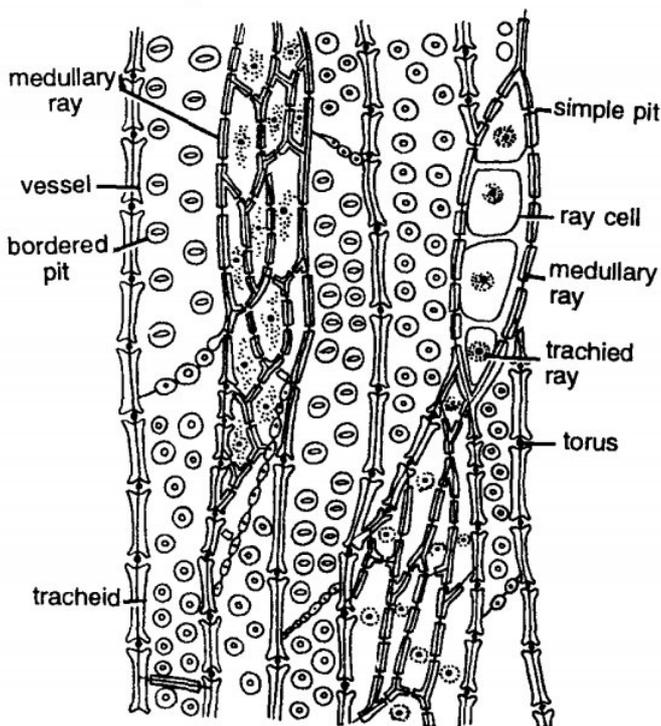


Fig. 9. *Ephedra*. T.L.s of wood.

5. Rays are elongate and many tangential walls show simple slit-like pits.

### Exercise 6

**Object : Study of male strobilus.**

#### Work procedure

Study the position, structure and organization of male strobilus. This can be done by studying external morphology of the strobilus and a slide of its longitudinal section.

Reproductive parts are borne in strobili. One of the following conditions may be found :

(i) Usually male and female strobili are different; in such a case, the strobilus may be termed as monosporangiate. These strobili may be borne on two different plants (dioecious sps.).

(ii) Sometimes one plant may bear both the strobili (monoecious sps.).

(iii) A few plants, sometimes, bear both the reproductive parts in one strobilus only (bisporangiate strobilus), e.g. *E. foliata* and *E. intermedia*. In such cases, male flowers are situated below the female flowers which occur at the higher level in the same strobilus.

#### Comments

1. The strobilus resembles inflorescence, spike.
2. Each strobilus consists of an axis which bears decussately arranged sterile scales and stamens.
3. Male spike (male strobilus or staminate strobilus) arises in the axil of scale leaf.
4. Each spike is generally round in shape but may be ovoid or spherical.
5. A spike has a short axis with many scaly bracts. The bracts are arranged in decussate pairs. The number of pairs varies from 2-12.
6. In the axil of each bract arises a single male flower.

### Exercise 7

**Object : Study of male flower.**

#### Work procedure

Place a male strobilus under the dissecting microscope, separate the sterile scale to isolate a

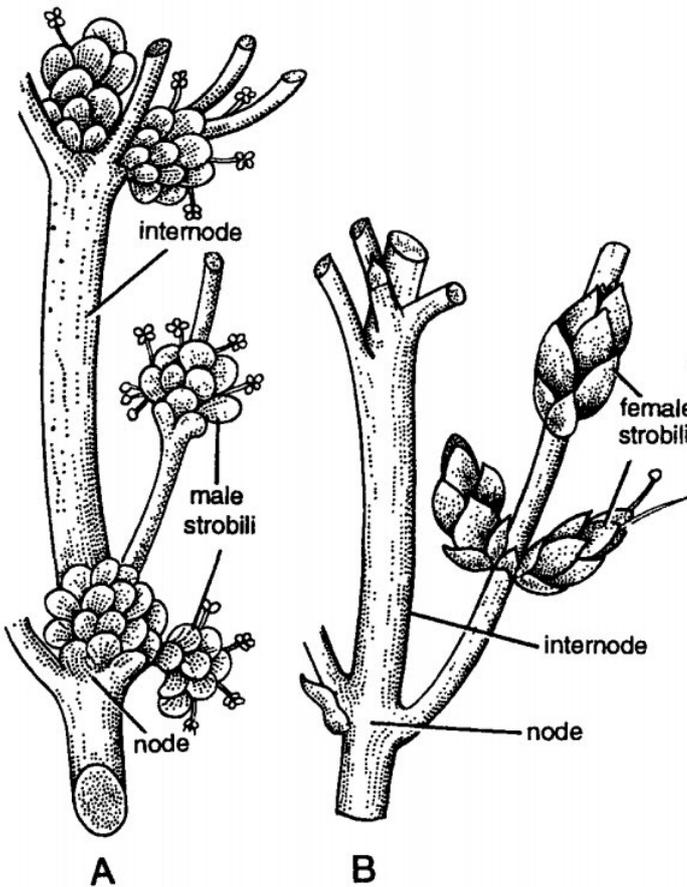


Fig. 10. *Ephedra*. Reproductive structures. A. A branch with male strobili. B. A branch with female strobili.

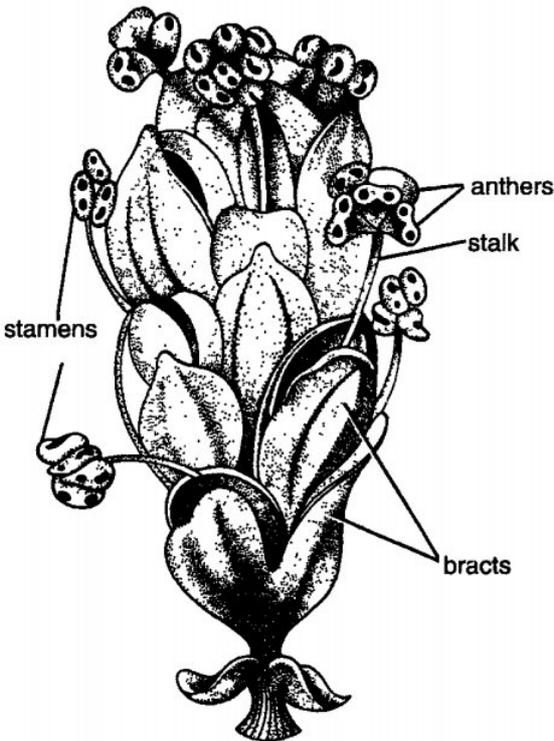


Fig. 11. *Ephedra*. A male strobilus.

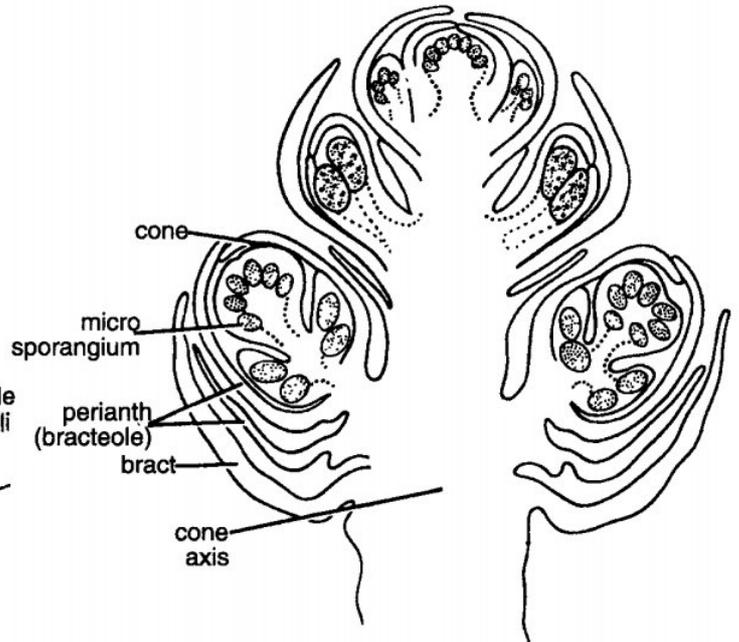


Fig. 12. *Ephedra*. L.s. of male strobilus.

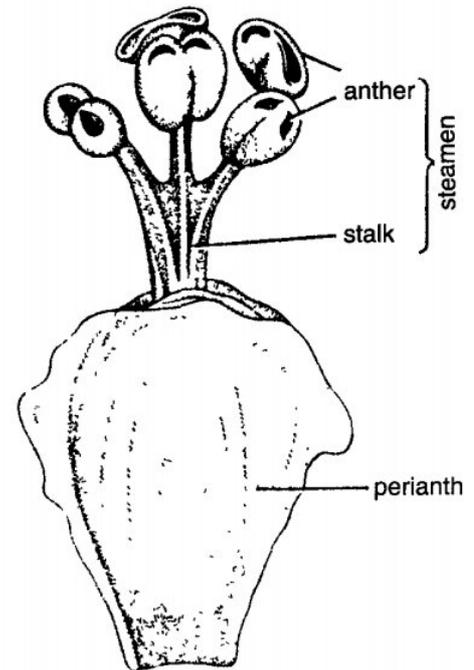


Fig. 13. *Ephedra*. A male flower

single male flower, stain in safranin, mount in glycerine and study. Also study slide of L.s. of a stamen.

**Comments**

1. A male flower has a perianth of bract scale which encloses a stamen.

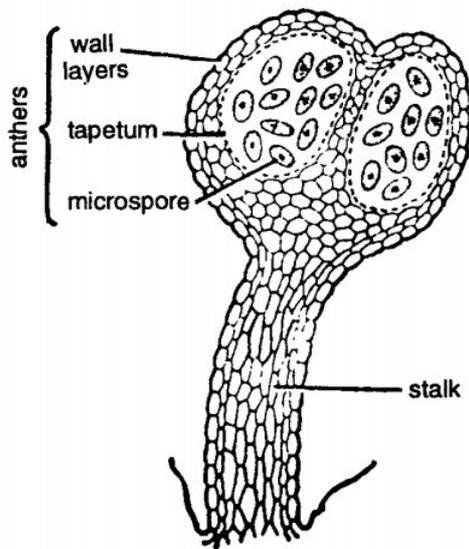


Fig. 14. *Ephedra*. L.S. of stamen.

2. **Stamen** consists of a stalk (variously termed as column or antherophore). It bears 2-5 microsporangia or anthers at its tip.
3. **Each microsporangium** is a bilocular structure. It has two wall layers and a prominent tapetal layer which encloses pollen grains or microspores.
4. **Each microspore** is elliptical and has an outer thick and ribbed exine and a thin intine.
5. A microsporangium opens by apical part (apical dehiscence).

#### Exercise 8

**Object : Study of female strobilus.**

#### Work procedure

Study the position, structure and organization of the female strobilus. This is done by studying the external features and a slide of its longitudinal section.

#### Comments

1. The strobilus resembles spike inflorescence.
2. Each strobilus consists of an axis bearing decussately arranged sterile bracts (scales) and ovules.
3. The female or ovulate strobili arise in the axil of scale leaves. The female strobilus is also sessile and not so richly branched as the male.

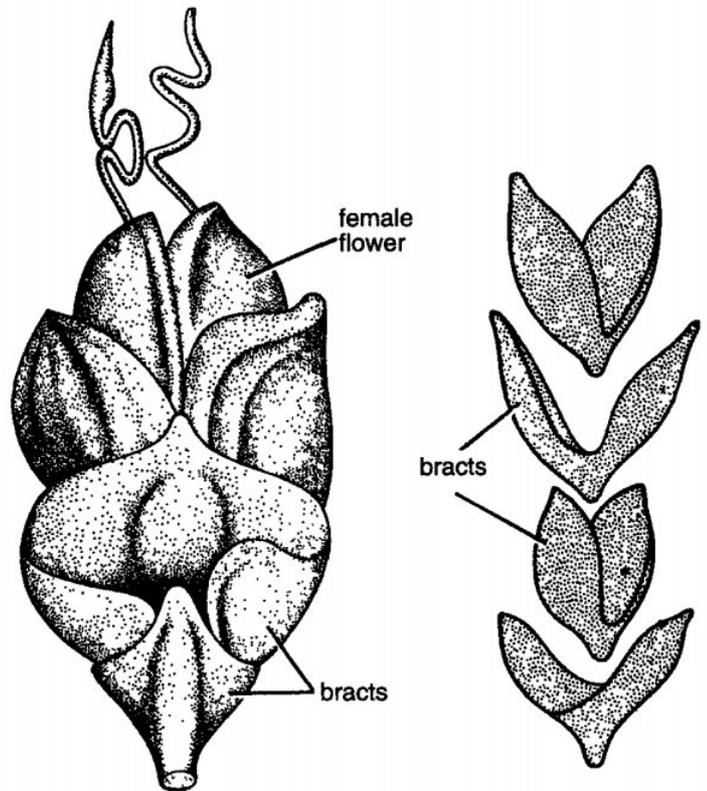


Fig. 15. *Ephedra*. Female strobilus. A. Female strobilus as it appears on plant. B. Bracts separated to show arrangement.

4. The apex of ovulate strobilus is mostly acute.
5. The spike has a short axis on which about 4-7 pairs of bracts are arranged decussately.
6. Lower most 1 or 2 pairs are sterile while terminal pairs bear short stalked ovules. The bracts are generally dry, winged and may be variously coloured.
7. Each bract mostly encloses two ovules out of which one may be abortive.

#### Exercise 9

**Object : Study of L.S. of ovule.**

#### Work procedure

Study a prepared slide of L.S. of ovule.

#### Comments

1. **Ovule** is covered by two integuments.
2. **Outer integument** (involucre or perianth) is a cup-like structure, attached at the base of the ovule and free above.
3. **Inner integument** is delicate, composed of two segments. It prolongs into a tubular process and

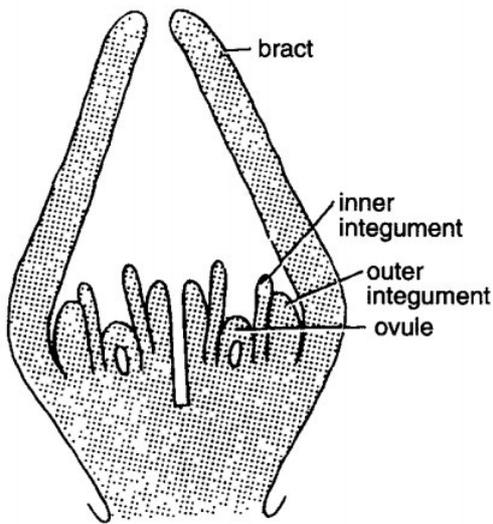


Fig. 16. *Ephedra*. L.s. of female flower.

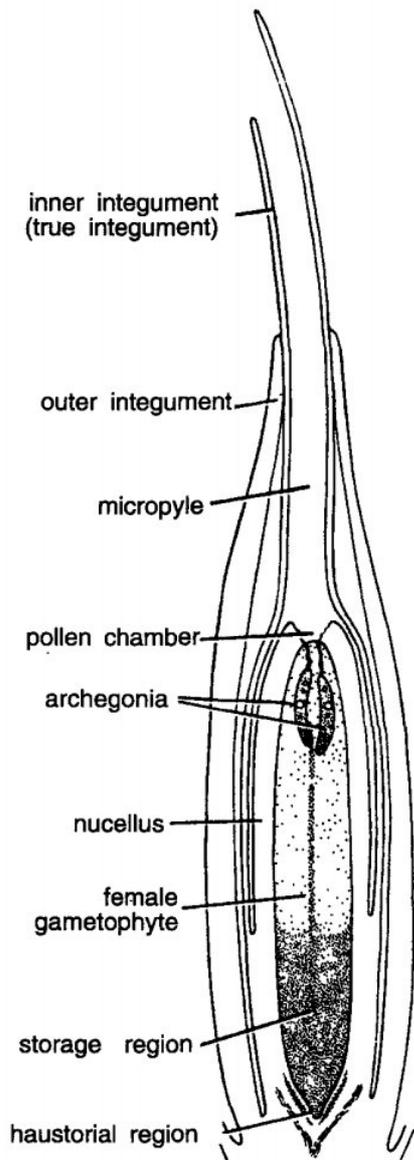


Fig. 17. *Ephedra*. L.s. of ovule

comes out beyond the bracts and involucre at the time of pollination.

4. **Micropyle** is an opening in between the integuments, in the upper region of the ovule.
5. **Nucellus** lies below the integuments. A small pollen chamber is present just below the micropyle in the tissue of nucellus.
6. **Female gametophyte** is a tissue situated below the pollen chamber. Two archegonia are present, just below the pollen chamber, in the female gametophyte.
7. **Haustorial region** lies opposite the micropylar end. It is occupied by tissue filled with stored food material. It also gives out haustorial processes for the absorption of food and is known as haustorial region.

### Exercise 10

**Object : Study of L.s. seed.**

#### Work procedure

Study a double stained prepared slide of L.s. of seed. (A seed is formed as a result of fertilization).

#### Comments

1. **Outer integument** encloses entire seed. It is thick walled.

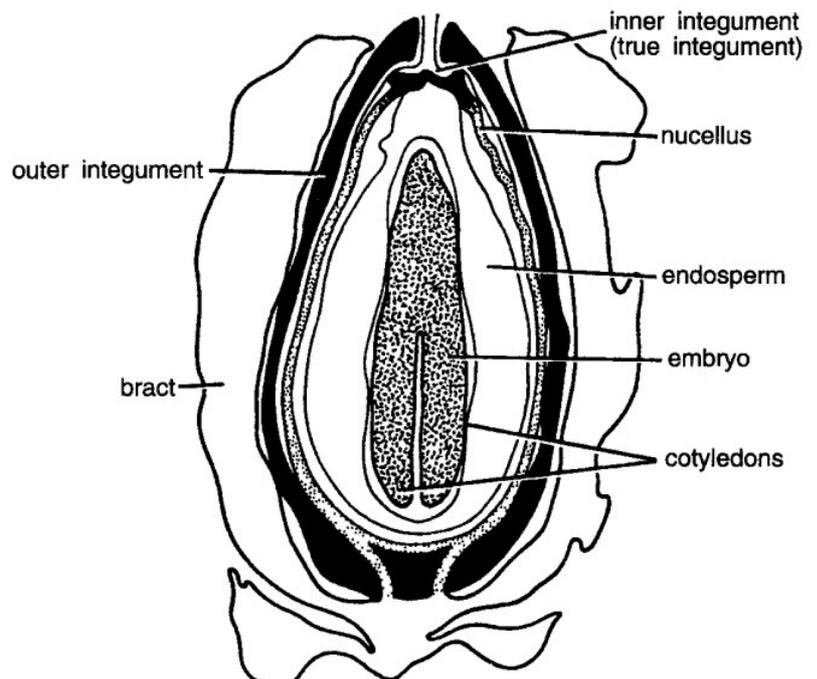


Fig. 18. *Ephedra*. L.s. of seed.

2. **The inner integument** ('true integument') persists at the micropylar end only.
3. **Nucellus** forms shrivelled layer in the form of a disorganized sheath of cells. It is located inside the inner integument.
4. **Female gametophyte** (endosperm) surrounds a big embryo which has two large cotyledons.
5. **Bracts** adjacent to strobilus are fleshy and thick in a completely mature seed. These form an additional envelope.

### Identification

**Division—Gymnosperms.** (1) Ovules naked, (2) Seed attached to a scales, (3) Scales form a strobilus.

**Class—Gnetopsida.** (1) Wood with vessels, (2) Flowers in compound strobili or 'inflorescence', unisexual, usually dioecious, (3) Ovules surrounded by several envelopes.

**Order—Gnetales.** (1) Plants woody trees, shrubs or lianes, (2) Leaves opposite or whorled, simple.

**Family—Ephedraceae.** (1) Plants either shrubs or woody climbers, (2) Leaves scaly, foliage leaves absent, (3) Nodal diaphragm present, (4) Stamens enclosed by bract, (5) Seeds covered with fleshy bracts.

**Genus—Ephedra.** Single genus.

### Hints for Collection

Six species of *Ephedra* viz. *E. pachyclada*, *E. intermedia* var. *tibetica*, *E. saxatilis* var. *sikkimensis*, *E. gerardiana* *E. nebrodensis* var. *procera* and *E. regeliana* grow wild in the north west Himalayan region and are all shrubby. *E. foliata* var. *ciliata* grows widely as a scandent shrub, climbing over small trees in the southern part of the Punjab and Rajasthan. It is usually cultivated in gardens and in other places.

### Preamble

Palaeobotany is the study of plant life of geological past (i.e. the study of plant fossils). The earth has undergone large climatic changes over billion years of its geologic past. During this period of drastic weather changes, most of the plants died and their remains were decomposed. However, a few plants which escaped decay and decomposition, remained preserved. These preserved plant specimen are called plant fossils. Generally, only certain plants made of harder tissues are preserved while those made of soft tissues get decomposed. The preserved plant parts are discovered independently at different times and places, hence each part is given a status of genus, representing form genus or artificial genus. Later, all these related parts are placed together and a full plant is visualized and reconstructed.

The age of the plant can be determined by using radioactive elements like uranium-238, uranium-235, thorium-232, potassium-40 and rubidium-87. Carbon-14 has also been used for time-measuring technique.

The major stratigraphic and time divisions used are given below.

The process of fossil formation is called fossilization. This had begun ever since deposition of sedimentary rocks began. According to the nature of fossilization, following fossil types have been recognized.

1. **Petrifaction fossil.** These fossils have well preserved external form and internal structure. This is due to replacement of plant material by some 20 minerals.

2. **Cast or incrustation.** This type of fossils are formed when a plant part gets covered by sand or mud. The cast or coat is hard and, therefore, used in the study of external morphology.

3. **Impression.** These are impressions of plant or plant parts when these fall on partially hard clay. Such types of fossils show clear venation patterns.

4. **Compression.** These are formed as a result of complete burial and the constant pressure of continuing sedimentation above it. The organs generally become flat.

5. **Coal, Amber, Graphite, Diatomaceous Earth,** etc. These are also types of fossils.

The fossils are of great importance in the study of evolution. These make it possible to know the time and the type of flora and fauna that flourished. It has, therefore, been possible to trace evolutionary series and reconstruct the changes which might have occurred during the past.

In this chapter, few fossils belonging to Pteridophytes and Gymnosperms are described.

## Classification

### 1 Division. PTERIDOPHYTA

Sub-division	Order	Family	Example
1. Psilophytosida	Psilophytales	Rhyniaceae	<i>Rhynia</i> , <i>Horneophyton</i>
2. Lycopsidea	Lepidodendrales	Lepidodendraceae	<i>Lepidodendron</i> , <i>Lepidocarpon</i>
3. Sphenopsida	Calamitales	Calamitaceae	<i>Calamites</i>

## II Division. GYMNOSPERMAE

Class	Order	Family	Example
1. Cycadopsida	Pteridospermales	Lyginopteridaceae	<i>Lyginopteris</i>
	Bennettitales	Williamsoniaceae	<i>Williamsonia</i>
		Cycadeoidaceae	<i>Cycadeoidea</i>

### Distinguishing Characters of Taxa

#### 1 DIVISION. PTERIDOPHYTA

- (1) True roots generally present
- (2) Plant body differentiated into stem, roots and leaves
- (3) True vascular strand present

#### Sub-division. Psilophytopsida

- (1) True roots absent

- (2) Sporangia borne at the tips of erect branches either singly or in pairs

#### Order. Psilophytales

- (1) Sporophyte dichotomously branched
- (2) Sporangia generally borne singly

#### Family. Rhyniaceae

- (1) Rhizoids unicellular, present on rhizomes
- (2) Aerial portion leafless

Examples. *Rhynia*, *Horneophyton*

Geological periods		Age (in million years)
Present Day		0
<b>CENOZOIC</b>	Quaternary	{ Post-Glacial { Glacial
	Tertiary	{ Pliocene { Miocene { Oligocene { Eocene Angiosperms dominant
<b>MESOZOIC</b>	Cretaceous	{ Upper { Lower
	Jurassic	{ Upper { Middle { Lower Gymnosperms and Pteridophytes dominant
	Triassic	{ Upper { Middle { Lower
<b>PALAEOZOIC</b>	Permian	{ Upper { Lower 182
	Carboniferous	{ Pennsylvanian { Mississippian Pteridophytes dominate Gymnosperms
	Devonian	{ Upper { Middle { Lower 255
	Silurian	Earliest records of land plants
	Ordovician	Algae
	Cambrian	Algae
Precambrian	{ Upper { Lower Traces of algae 510	

**Sub-division. Lycopsidea**

- (1) Leaves microphyllous
- (2) Sporangia borne singly on adaxial face of the sporophyll or in its axil
- (3) Sporophyll borne in strobili

**Order. Lepidodendrales**

- (1) Plants tree-like
- (2) Secondary tissues formed due to cambium
- (3) Leaves microphyllous and ligulate
- (4) Strobili heterosporous

**Family. Lepidodendraceae**

- (1) Aerial portion freely branched
- (2) Strobili at the tips of branches
- (3) Trunk and branches with spirally arranged leaf scars

Examples. *Lepidodendron*, *Lepidocarpon*

**Sub-division. Sphenopsida**

- (1) Stem branched, articulated, ridged and furrowed with distinct nodes and internodes
- (2) Leaves microphyllous, small, scaly and arranged in whorls at nodes

**Order. Calamitales**

- (1) Tree-like sporophytes with considerable secondary thickening of stem and branches

**Family. Calamitaceae**

- (1) Stem branched, branches in whorls at nodes
- (2) Stem shows endarch siphonostele

Example. *Calamites*

**II DIVISION. GYMNOSPERMAE**

- (1) Ovules naked
- (2) Seeds attached to a scale
- (3) Scales forming a strobilus

**Class. Cycadopsida**

- (1) Wood manoxylic
- (2) Large frond-like leaves
- (3) Seeds with radial symmetry

**Order 1. Pteridospermales**

- (1) Leaves large, frond-like, pinnately compound
- (2) Large leaf traces with one or more strand
- (3) Spores formed in sporangia, aggregated in synangia

**Family 1. Lyginopteridaceae**

- (1) Stem monostelic
- (2) Petioles with single vascular strand
- (3) Seeds small

Examples. *Heterangium*, *Lyginopteris*

**Family 2. Glossopteridaceae**

- (1) Leaves with a strong midrib
- (2) Stelar structure unusual, showing several plates of vascular tissues
- (3) Reproductive structure cupulate and bisexual

Example. *Glossopteris*

**Order 2. Bennettitales**

- (1) Tree trunk covered by a mantle of persistent leaf-bases
- (2) Microsporophylls in groups, at the tip, leaves frond-like
- (3) Megasporophylls in cone-like organization

**Family 1. Williamsoniaceae**

- (1) Stem delicate, branched
- (2) Inflorescence stalked or sessile, not sunk in the scales of persistent leaf bases

Example. *Williamsonia*

**Family 2. Cycadeoideaceae**

- (1) Trunk columnar
- (2) Trunk covered by mantle of leaf bases
- (3) 'Flowers' sunk in the distal part of the trunk

Example. *Cycadeoidea*

## PTERIDOPHYTES

### *Rhynia*

#### Classification

<i>Division</i>	—	<b>Pteridophyta</b>
<i>Sub-division</i>	—	<b>Psilophytopsida</b>
<i>Order</i>	—	<b>Psilophytales</b>
<i>Family</i>	—	<b>Rhyniaceae</b>
<i>Genus</i>	—	<b><i>Rhynia</i></b>

#### Exercise 1

**Object : Study of external features of the plant.**

#### Work procedure

Study the reconstruction of plant. Observe the differentiation of plant body into rhizoids, rhizome and leaf-less aerial branches.

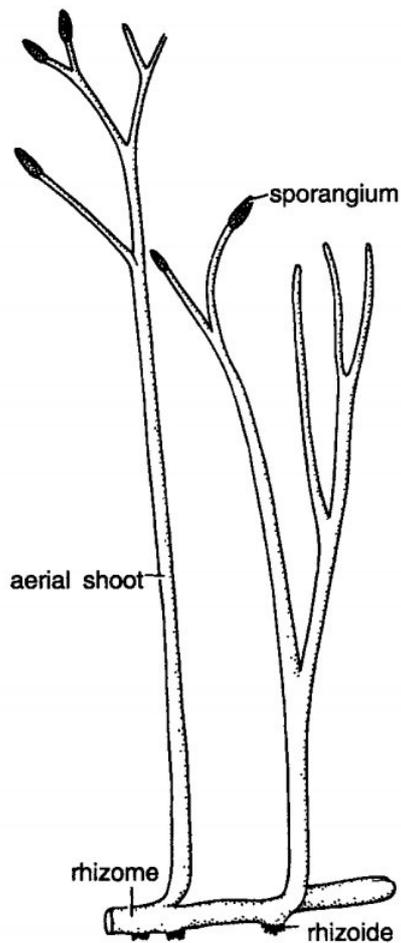


Fig. 1. *Rhynia*. External features of the plant.

### Comments

1. *Rhynia* is a fossil member (not found living in the present age), discovered from Rhynichert Beds (or upper Devonian era) in Aberdeenshire of Scotland, by Kidston and Lang in 1917.
2. The two species *R. major*, about 40-50 cms in height and *R. gwynne-vaughani*, about 20 cms in height, found at this station, were well preserved, hence their form and structure are well known.
3. The plant grew in swampy marshes. It was differentiated into horizontally creeping rhizome and an upright branched shoot without leaves.
4. There were no roots. Unicellular rhizoids were borne in patches, on underside of the rhizome.
5. The upright branches were dichotomously branched and gradually tapering. The sporangia terminated these branches.

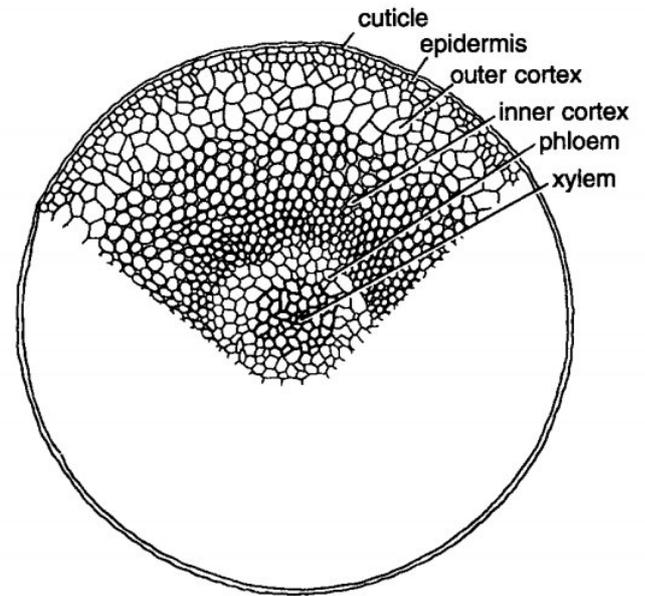


Fig. 2. *Rhynia*. T.s. of rhizome (a part is shown cellular).

### Exercise 2

**Object : Study of anatomy of rhizome and aerial shoot.**

### Work procedure

Study the prepared slides of transverse section of rhizome and aerial branch.

### Comments

1. Both aerial branches and rhizome were differentiated into epidermis, cortex and stele.
2. **The epidermis** was cuticularized with stomata scattered on the aerial shoot. They were absent from the rhizome.
3. **The cortex** was differentiated into outer and inner zone.
4. **The outer cortex** was only 1-4 layered and had compact parenchymatous cells.
5. **The inner cortex** had spherical cells with large intercellular spaces. The inner cortex is believed to be the chief photosynthetic region of the plant.
6. **The stele** was a typical protostele with xylem completely surrounded by phloem.

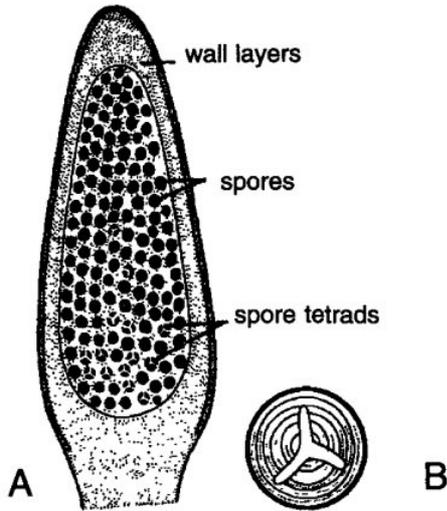


Fig. 3. *Rhynia*. Reproductive structures :  
A. A.L.s. sporangium, B. Single spore.

**Exercise 3**

**Object : Study of reproductive organ.**

**Work procedure**

It is sporangium borne at the tip of the branch.  
Study a slide of L.s. of sporangium.

**Comments**

1. It was nearly oblong in shape, broad at the base and pointed at the apex.
2. The wall of the sporangium was several layered, of which two could be easily distinguished.
3. The outer wall was cuticularized and the innermost acted as tapetum.
4. Inside the sporangium were spores, all of which were of the same size. Spores in tetrads were also seen in some specimens.
5. Columella was absent from the sprangium.

**Identification**

**Division—Pteridophyta.** (1) Generally true roots present. (except in Psilopsida), (2) True vascular strand present.

**Sub-division—Psilophytosida.** (1) True roots absent, (2) Shoot differentiated into subterranean rhizome and apical portion, (3) Sporangia borne terminally, (4) Homosporous.

**Order—Psilophytales.** (1) Sporophyte dichotomously branched, (2) Sporangia generally borne singly.

**Family—Rhyniaceae.** (1) Rhizoids unicellular, on rhizomes, (2) Aerial portion leafless.

**Genus—*Rhynia*.** (1) Subterranean portion not corm-like, (2) No columella in sporangia.

***Horneophyton lignieri***

**Classification**

Division	—	Pteridophyta
Sub-division	—	Psilophytosida
Order	—	Psilophytales
Family	—	Rhyniaceae
Genus	—	<i>Horneophyton</i>

**Exercise 1**

**Object : Study of the external features of the reconstructed plant.**

**Work procedure**

Study the features from a drawing of a reconstructed plant.

**Comments**

1. *Horneophyton* with one species *H. lignieri* was found with *Rhynia* in the Rhynichert bed of Aberdeenshire, Scotland. It was previously named as *Horneo*.
2. The rhizome was a lobed parenchymatous body bearing non-septate rhizoids but with no continuous vascular strand of its own.

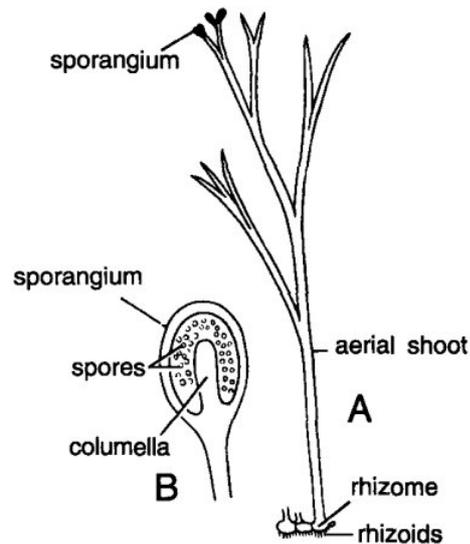


Fig. 1. *Horneophyton lignieri*. A. Restoration. B. L.s. sporangium.

3. The rhizome gave rise to upright shoots which ranged from 1 to 2 mm. in diameter and were dichotomously branched.
4. Sporangia were borne on the tips of these branches and were 1 to 2 mm in diameter and 2 or more mm in length.

### Exercise 2

**Object :** Study the fossil slide of L.s. of sporangium.

#### Work procedure

Slide of fossil of sporangium is available. Study various characters using microscope.

#### Comments

1. The sporangium was oval or slightly cylindrical spore sac, somewhat pointed distally.
2. The sporangial wall consisted of a cuticularised epidermis followed by several layers of thin walled cells. The innermost layer was a well defined tapetum.
3. The most striking feature was the presence of central columella projecting into the spore cavity from the base. The columella was continuous with the phloem of the main stem.
4. Surrounding the columella and within the sporangial wall were many spores.
5. The spores were about 50 m in diameter and occur as tetrads.

### Identification

**Division—Pteridophyta.** (1) Generally true roots present (except Psilopsida), (2) True vascular strand present.

**Sub-division—Psilophytopsida.** (1) True roots absent, (2) Shoot differentiated into subterranean rhizome and apical portion, (3) Sporangia borne terminally.

**Order—Psilophytales.** (1) Sporophyte dichotomously branched, (2) Sporangia generally borne singly.

**Family—Rhyniaceae.** (1) Rhizoids unicellular, (2) Aerial portion leafless.

**Genus—Horneophyton.** (1) Rhizome—a lobed parenchymatous body, (2) Rhizome without a continuous vascular strand of its own, (3) Presence of columella in the sporangium.

## Lepidodendron

### Classification

Division	—	Pteridophyta
Sub-division	—	Lycopsida
Order	—	Lepidodendrales
Family	—	Lepidodendraceae
Genus	—	Lepidodendron

### Exercise 1

**Object :** Study the external features of the plant.

#### Work procedure

Study the reconstruction of the plant. Observe the differentiation into stigmarian root system, Lepidophylloids leaves and *Lepidostrobus* strobili.

#### Comments

1. *Lepidodendron* with over 100 species, is the largest known genus. It is found in the shales and sandstones of carboniferous coal bearing formations.
2. The root system is included in the form genus *Stigmara*, leaves in form genus *Lepidophylloides* and the strobili in the form genus *Lepidostrobus*.
3. *Lepidodendron* was a tall tree reaching 40 meters. The trunk was straight that branched dichotomously only at the top. The branches were shorter.
4. The branches were covered with spirally arranged leaves, placed in the form genus *Lepidophylloides*. These were simple, ligulate, acicular to linear. A single vein traversed the length of a leaf.
5. Leaves were attached to the summit of pyramidal cushion-like leaf bases which were rhombic or diamond shaped. The leaf scars formed oblique or spiral rows on the stem. Actual leaf scar was above the middle line of rhombus. In the leaf scar, the bundle scar mark

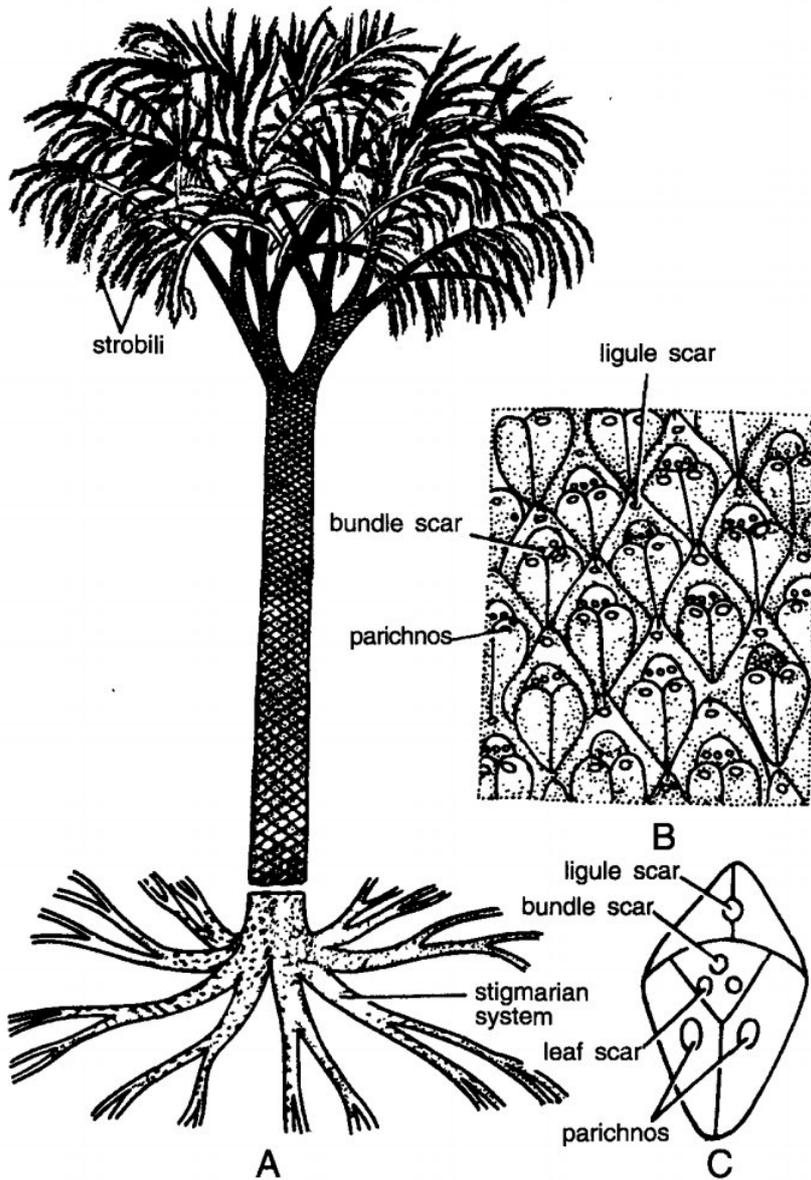


Fig. 1. *Lepidodendron*. A. Reconstruction of tree. B. Surface of the trunk showing scars formed by the leaf bases C. A leaf scar.

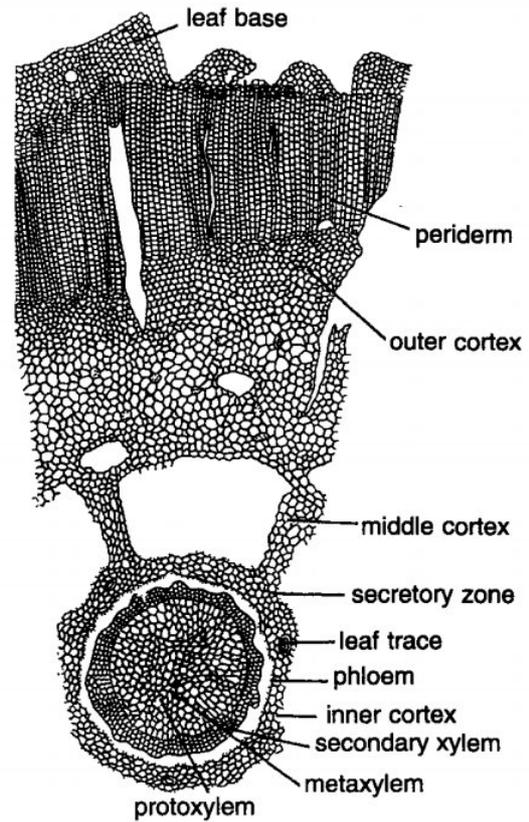


Fig. 2. *Lepidodendron*. *L. selaginoides* (= *L. vasculare*). T.s. of stem.

- was flanked by two scars and at the lower level by two more large scars which represented parichnos scars (strands of loosely arranged parenchyma).
6. Typical *Lepidodendron* trunk was attached to a typical stigmarian root system. The base of the trunk was divided into four massive rhizophores which later again divided dichotomously.
  7. The strobili borne terminally were found attached to a branch identical to *Lepidodendron* and are placed under the form genus *Lepidostrobus*.

**Exercise 2**

**Object : Study of the anatomy of stem.**

**Work procedure**

Study the prepared slide of transverse section of the main trunk or a branch.

**Comments**

1. It shows a protostele or siphonostele with exarch and polyarch protoxylem.
2. Metaxylem was present in the centre with many protoxylem points on periphery.
3. A ring of cambium situated outside produced a narrow zone of secondary xylem. Both primary

(B-14)

- and secondary xylem were made of scalariform and spiral tracheids.
4. A large cortex surrounded the vascular tissues. It showed four regions-
    - (i) Inner cortex : homogeneous and made of parenchyma except for leaf traces.
    - (ii) Secretory zone made of intermingled large and small cells, many of which were filled with a dark coloured substance.
    - (iii) Middle cortex that consisted of delicate cells which were often destroyed.
    - (iv) Outer cortex made of alternate radial masses of thick and thin walled elements.
  5. Outermost was the periderm formed by phellogen. It consisted of many elements on its inner side forming endophelloderm than on the outer side that formed exophelloderm.

### Exercise 3

**Object : Study the strobilus.**

#### Work procedure

Study the structure of a strobilus by observing and studying the slide of L.s. of strobilus.

#### Comments

1. The strobili borne by Paleozoic lepidodendrids are placed in organ genus *Lepidostrobus*. All known species are heterosporous.
2. Strobili occurred on terminal parts of certain smaller branches. The strobili were elliptical, 1 to 7.5 cm in diameter and 2.5 to 30 cm or more long.
3. Each consisted of a central axis around which sporophylls were found in spirals or whorls (in some cases verticillate).
4. The sporophyll was peltate with the upper terminal lobe overlapping the sporophyll above. The sporophylls were attached at right angles to the axis.
5. Each sporophyll bore a single sessile elongate sporangium on its adaxial face. Just beyond the sporangium, the sporophyll had a small ligule.
6. There was also a shorter downward prolongation of the lamina called heel,

(B-14)

- completely covered by the lamina of the sporophyll below.
7. The sporangium was a large sac-like structure wider than the sporophyll. The wall was of a single layer of prismatic cells. Some radiating flaps of tissue called trabeculae extended upward from the base of the sporangium.
8. The microsporangia and megasporangia are of the same size.
9. Microsporangium enclosed several hundred microspores. Megasporangium enclosed 4, 8, or 16 megasporos.
10. Megaspores showed gametophytic development while still within the megasporangium.

### Identification

**Division—Pteridophyta.** (1) True roots present, (2) Plant body differentiated into stem, roots and leaves, (3) True vascular strand present.

**Sub-division—Lycopsida.** (1) Leaves microphyllous, (2) Sporangia borne singly on adaxial face of the sporophyll or in its axil, (3) Sporophylls borne in strobili.

**Order—Lepidodendrales.** (1) Plants tree-like, (2) Secondary tissues formed by means of a cambium, (3) Leaves microphyllous, ligulate, (4) Strobili heterosporous.

**Family—Lepidodendraceae.** (1) Aerial portion freely branched, (2) Strobili at the tips of branches, (3) Trunk and branches with spirally arranged leaf scars.

**Genus—Lepidodendron.** (1) The vertical diagonal of the rhombus longer than the tranverse, (2) Cones borne on short lateral branches.

### Calamites

#### Classification

Division	—	Pteridophyta
Sub-division	—	Sphenopsida
Order	—	Calamitales
Family	—	Calamitaceae
Genus	—	Calamites

#### Exercise 1

**Object : Study the reconstructed plant.**

#### Work procedure

Study reconstruction from the standard books.

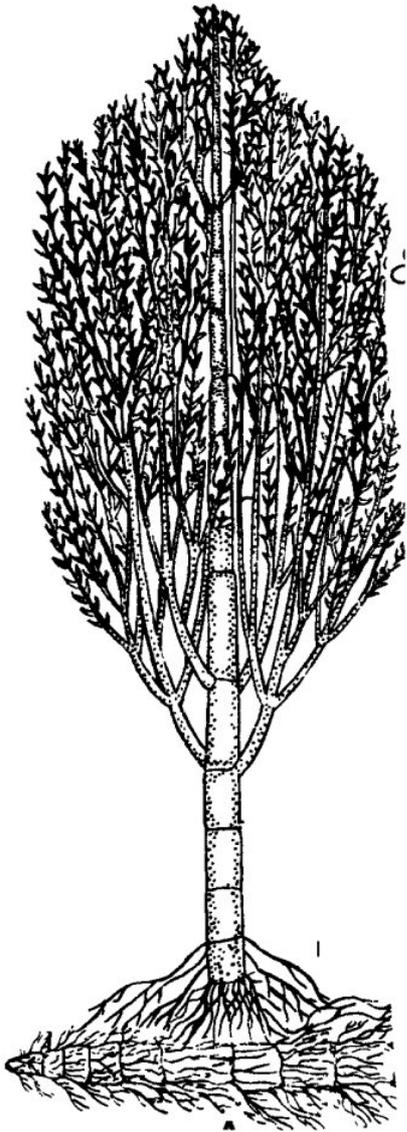


Fig. 1. *Calamites*. Reconstruction of the plant.

**Comments**

1. Genus *Calamites* appeared in Upper Devonian, and was most abundant during Carboniferous and became extinct early in the Triassic.
2. The plant was 20 to 30 meters tall tree.
3. The tree was arborescent with horizontal rhizome, aerial shoots and whorls of leaves.
4. Rhizomes were differentiated into nodes and internodes. Nodes bore whorl of adventitious roots.
5. Aerial branches were borne on the upper side of rhizome. These were generally constricted at the place where these joined the rhizome.
6. The lowermost nodes of the erect branches produced whorls of adventitious roots.

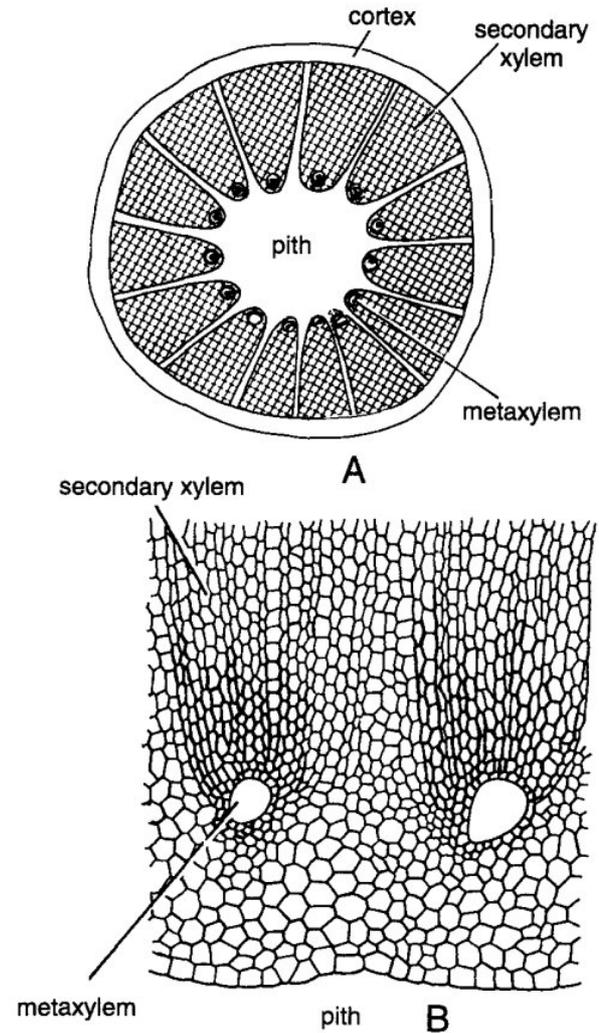


Fig. 2. *Calamites*. T. s. of stem. A. Outlines, B. A part cellular.

7. The aerial branches were highly articulated. The leaves were borne in whorls at the upper nodes. These also had branches borne in pairs.
8. Each node had a single whorl of leaves. The leaves were smaller and perhaps were photosynthetic organs. These were lanceolate, linear or spatulate with a single vein.
9. Genus *Calamites* is divided into subgenera on the basis of mode of branching and general growth pattern.
  - (i) *Eucalamites*—bore one to many branches at the node.
  - (ii) *Calamitina*—bore branches in whorls which were separated by a series of branchless nodes.
  - (iii) *Diplocalamites*—2 to 3 branches produced with branches alternating from node to node.

- (iv) *Crucicalamites*—large number of branches produced at each node.  
 (v) *Stylocalamites*—without branches or branches fewer and irregularly scattered.

### Exercise 2

**Object : Study of the stem anatomy.**

#### Work procedure

Study the prepared slide showing stem anatomy.

#### Comments

1. The petrified calamitean stem of three form genera viz. *Arthropitys*, *Calamodendron* and *Arthroxyulonn* have been recognised. They have been established to belong to *Calamites* and their special names are used only when necessary.
2. Petrified stems of *Calamites* are frequent in the coal balls and other carboniferous petrifications.
3. The stem was ridged and furrowed which alternate at each successive internodes.
4. The transverse section of internode shows endarch siphonostele with secondary xylem produced by vascular cambium.
5. There was a large hollow central pith cavity. It became solid at nodes forming a diaphragm.
6. Surrounding the pith was a ring of more than a dozen primary collateral vascular bundles.
7. The protoxylem was endarch and was represented by carinal canal formed due to dissolution of many elements.
8. The metaxylem was very less in amount. It was composed of scalariform and pitted tracheids.
9. Cambium occurred in between xylem and phloem.
10. Secondary xylem was formed on the inner face of cambium. It was made of tracheids and rays. Tracheids were arranged in radial rows. These showed scalariform thickenings or multiseriate bordered pits on the radial walls. Secondary xylem formed wedge-shaped zones separated by interfascicular rays.
11. The rays show considerable diversity in structure and serve as the basis for generic distinction.

(B-14)

12. The tissues outside the secondary xylem showed inner cortex made of thin walled parenchyma with resin canals.
13. The cells of the outer cortex were also thin walled but were much smaller than the cells of the inner zone.
14. The cortex was as wide as xylem and formed thick bark (also termed periderm) that was smooth externally.

### Identification

**Division—Pteridophyta.** (1) True roots present, (2) Plant body differentiated into stem, roots and leaves, (3) True vascular strand present.

**Sub-division—Sphenopsida.** (1) Stem branched, articulated, ridged and furrowed with distinct nodes and internodes, (2) Leaves microphyllous, small, scaly and arranged in whorls at nodes.

**Order—Calamitales.** (1) Tree-like sporophytes with considerable secondary thickening of stem and branches, (2) Strobilus with central articulated axis which bore alternate whorls of sporangiophores and sterile bracts.

**Family—Calmitaceae.** (1) Stem branched, branches in whorls at nodes, (2) Stem shows endarch siphonostele.

**Genus—Calamites.** (1) Subterranean rhizome present, (2) Lower nodes producing adventitious roots, (3) Vallecular canal absent, (4) Periderm may or may not be formed.

## GYMNOSPERMS

### *Lyginopteris*

#### Classification

Division	—	Gymnospermae
Class	—	Cycadopsida
Order	—	Pteridospermales
Family	—	Lyginopteridaceae
Genus	—	Lyginopteris

### Exercise 1

**Object : Study of external morphology of the stem.**

#### Work procedure

Study the diagrammatic representation of the stem genus from standard books. Summarise the major points of significance.

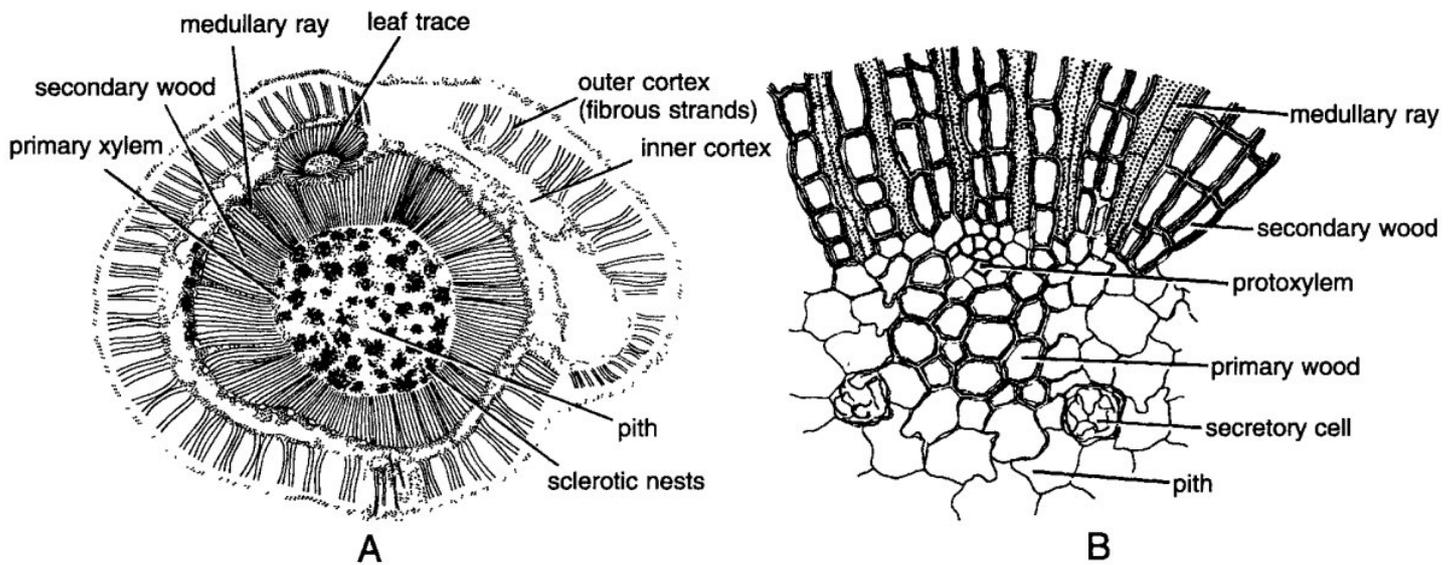


Fig. 1. *Lyginopteris*. A. T.s. of stem (outlines), B. T.s. stem— a part cellular.

### Comments

1. *Lyginopteris* is a palaeozoic pteridosperm. It is a stem genus of *Calymatotheca Hoeninghausi*. The descriptions of *Lyginopteris Oldhamia* are available.
2. The genus was described under *Dadoxylon oldhamium* by Binney in 1886. Later the genus was transferred to *Lyginodendron* on the basis of cortical impressions. Genus *Lyginopteris* was established by Potonie in 1899.
3. Petrifications and compressions of the stem were found in the lower and middle. Coal measures of Britain, continental Europe and north America.
4. The stem was long and generally slender. Diameter varied from 2 mm to 40 mm, sometimes up to 4 cms. Some specimen showed branching. The branches were axillary when present. These were arranged spirally.

### Exercise 2

**Object : Study the anatomy of stem.**

### Work procedure

Study the slide of T.s. of fossil stem or alternatively study the diagrams from the books and draw a list of important features of anatomy.

### Comments

1. The section shows three distinct regions—  
(i) central pith,  
(ii) centrally located vascular tissue and  
(iii) outer region of cortex.
2. A large parenchymatous pith was present in the centre. Many sclerotic nests were present scattered in this region.
3. Along the margins of the pith were present many strands of primary xylem. Protoxylem was mesarch.
4. Primary xylem was made of tracheids with bordered pits on their radial walls.
5. The strands of primary xylem were surrounded by a wide region of secondary xylem. It was made of tracheids with multiseriate bordered pits. Wide medullary rays which alternated with groups of primary xylem were also present.
6. Continuity of secondary xylem was interrupted by outgoing leaf traces giving an appearance of four large and unequal masses.
7. Outside the secondary xylem were present cambium and secondary phloem. Pericycle was situated outside the vascular tissues and was represented by groups of stone cells.
8. A band of internal periderm was present outside pericycle.
9. The cortex situated outside was divisible into inner cortex and outer cortex.

10. The inner cortex was poorly preserved and, therefore, the tissues were not distinct. However, it gave an impression as if made of large parenchymatous cells.
11. Outer cortex was made of a wide region of radially arranged fibrous strands. These were anastomosed and formed a distinct reticulum.
12. Numerous glands were present on the stem surface. The tip of the gland was spherical and was made of small cells. These were sessile as well as stalked.

### Identification

**Division—Gymnospermae.** (1) Ovules naked, (2) Seeds attached to scales, (3) Scales forming a strobilus.

**Class—Cycadopsida.** (1) Wood manoxylic, (2) Large frond-like leaves, (3) Seeds with radial symmetry.

**Order—Pteridospermales.** (1) Leaves large, frond-like, pinnately compound, (2) Large leaf traces with one or more strands, (3) Spores formed in sporangia, aggregated in synangia.

**Family—Lyginopteridaceae.** (1) Stem monostelic, (2) Petioles with single vascular strand, (3) Seeds small.

**Genus—Lyginopteris.** (1) Presence of stone cells in pith, (2) Wide fibrous bands in outer cortex.

## Cycadeoidea

### Classification

Division	—	Gymnospermae
Class	—	Cycadopsida
Order	—	Bennettitales
Family	—	Cycadeoidaceae
Genus	—	Cycadeoidea

#### Exercise 1

**Object :** To study the external features of stem.

#### Work procedure

Study the external features of the reconstructed plant from the figures and text available from standard text books. List various important features.

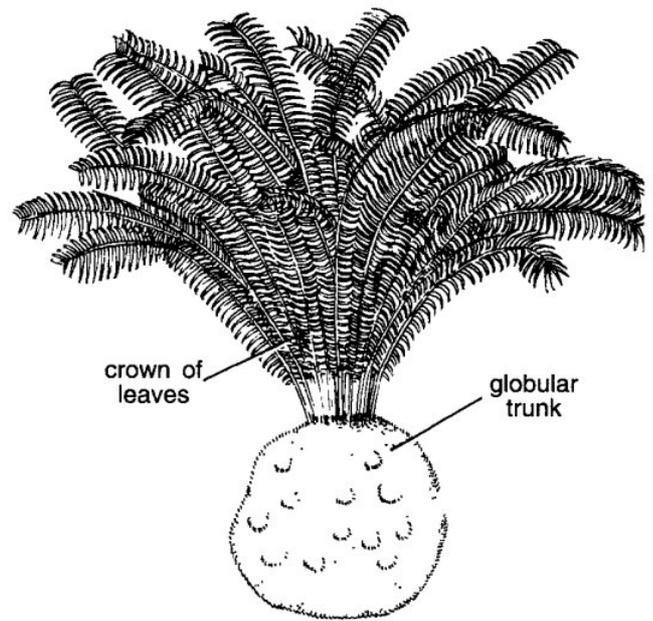


Fig. 1. *Cycadeoidea*. External features.

#### Comments

1. *Cycadeoidea* (= *Bennettitites*) is a fossil that includes many species. Many petrifications have been found of which some are complete plants. This fossil has been found in Jurassic and cretaceous strata of Dakota, America.
2. The stems were generally small ( less than three feet in height, sometimes 10 to 12 feet,) and thick ( about 2 feet). It remained covered with persistent leaf bases. A few specimen were branchless ( though in some 3 to 4 small branches arose from in a group from a basal stalk).
3. Persistent leaf bases were closely placed and formed a thick mantle. In between them were present flat scale-like hairs called ramentum.
4. Only young leaves have so far been found. These were present close to the apex. Leaves were pinnately compound and the venation was parallel.
5. The flowers were borne in the axils of leaves and completely embedded in the armour of persistent leaf bases.

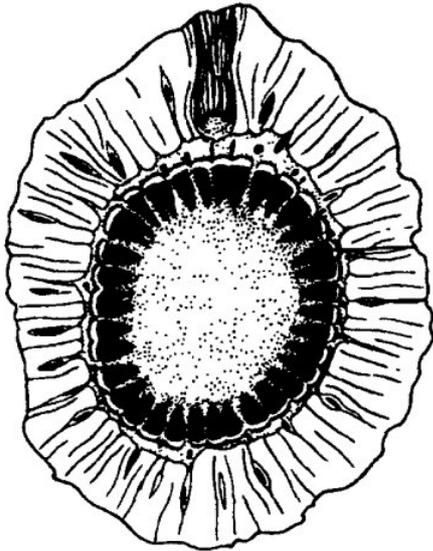


Fig. 2. *Cycadeoidea*. T.s. stem.

**Exercise 2**

**Object : Study of anatomy of transverse section of stem.**

**Work procedure**

Study the slide of T.s. of stem or study the diagrams available in the standard books.

**Comments**

1. The section is differentiated into three regions central pith, middle region consisting of wood and outer cortex.
2. Centrally located pith is smaller in diameter. It was surrounded by wood from all the sides.
3. Wood formed a small region that surrounded central pith. The region appeared broken by ray-like extensions projecting from central pith.
4. Endarch protoxylem was situated on the inner side of the wood, close to the pith. Vascular bundles were arranged in a ring and the xylem was arranged centrifugally.
5. Secondary xylem was made of scalariform tracheids and small rays. Rays were uniseriate or biseriate.
6. Medullary rays extended beyond cambium up to phloem. These alternate with parenchyma and vertical vascular tissues.
7. Cortex was made of parenchyma where gum canals and leaf traces were abundant.
8. Leaf trace occurred singly at the place of their origin but broke into many mesarch strands to arrange itself into horse-shoe shaped structure. The mouth of horse-shoe was directed outwards.

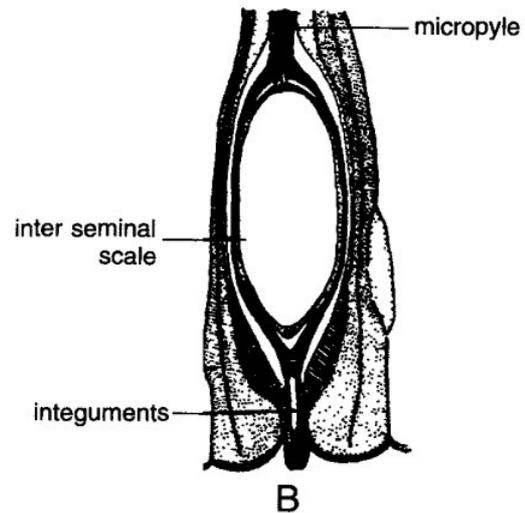
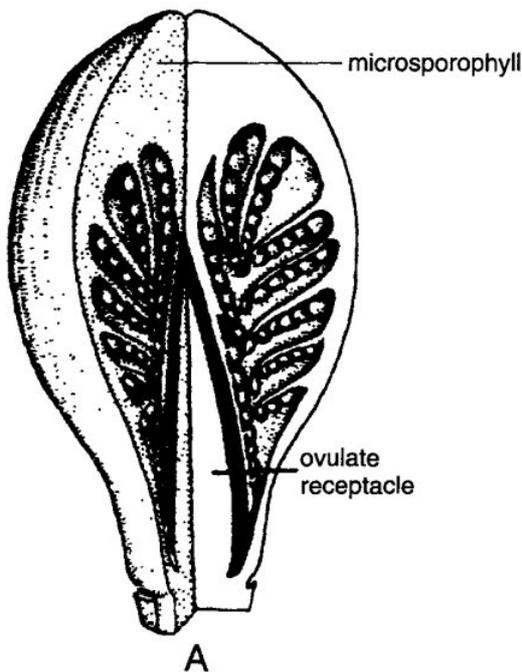


Fig. 3. *Cycadeoidea*. A. L.s. of cone, B. L.s. of Ovule.

### Exercise 3

**Object :** To study the reproductive parts.

#### Work Procedure

Study the diagrams from the book and prepare the list of main features.

#### Comments

1. Fruiting bodies were produced on the axillary shoots (In some species more than 100 fruiting bodies).
2. These appeared in a group between leaf bases. Fructifications were bisexual.
3. Many spirally arranged bracts were present on fertile shoots. These kept fructifications covered till they matured. On maturity the bracts of the flower opened and formed saucer-shaped perianth. The structures were modified androecium and gynoecium.
4. The pollen bearing structures bore a whorl of about 20 microsporophylls.. These were almost fused at the base. Each microsporophyll consisted of main rachis which bore two rows of trabeculae which in turn held two lateral rows of multicellular synangia. Each synangia was pear-shaped bearing 20 to 30 longitudinal pollen sacs.
5. The ovule bearing structure was made of conical or spherical receptacle with many stalked ovules and interseminal scales .
6. The ovule was orthotropous with a long micropylar beak. The integument of the ovule was fused with the nucellus except at the apex where it formed micropyle.
7. The seeds were small and invested with a basal cupule. It was somewhat elongated or oval in shape and possessed two cotyledons.

#### Identification

**Division—Gymnospermae.** (1) Ovules naked, (2) Seeds attached to scales, (3) Scales forming a strobilus.

**Class—Cycadopsida.** (1) Wood manoxylic, (2) Large frond-like leaves, (3) Seeds with radial symmetry.

**Order—Bennettitales.** (1) Tree trunk covered with mantle of persistent leaf bases, (2) Microsporophylls in groups, at the tip, leaves frond-like, (3) Megasporophylls in cone-like organization.

**Family—Cycadeiodaceae.** (1) Trunk columnar, (2) Trunk covered with mantle of leaf bases, (3) Flowers sunk in the distal part of the trunk.

**Genus—Cycadeoidea.** (1) Small, globular trunk, (2) Bisexual strobilus, (3) Orthotropous ovules.

## Williamsonia

#### Classification

Division	—	Gymnospermae
Class	—	Cycadopsida (Bennettitopsida)
Order	—	Cycadeoidales (Bennettitales)
Family	—	Williamsoniaceae
Genus	—	Williamsonia

### Exercise 1

**Object :** To study the external features of the plant.

#### Work procedure

Study the morphological features of the reconstructed plant, note the significant features and list them from standard books.

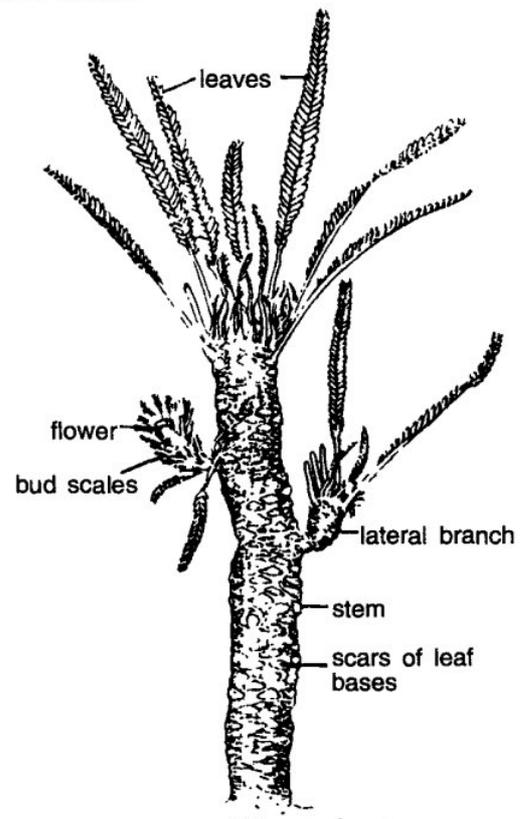


Fig. 1. *Williamsonia*. External features. Reconstruction.

**Comments**

1. Many species have so far been reported from upper Triassic and Jurassic beds of India, Europe and north America.
2. It was earlier placed under *Zamia gigas* by Williamson (1868). Later it was named in honour of its discoverer. *Williamsonia seawardiana* is the best known species described by late Professor Birbal Sahni from Rajmahal Hills, Bihar (India). Gupta (1953) later described another species from Rajmahal Hills and named it after Professor Sahni.
3. *W. seawardiana* had a sturdy columnar trunk similar to that of *Cycas*. It had a crown of pinnately compound leaves. The stem was covered with alternating areas of large (foliage) and small (scaly) leaf bases.
4. The leaf was placed in from genus *Ptilophyllum*.

It has linear and parallel veined leaflets similar to those found in *Zamia*.

5. The lateral branches were borne through leaf bases and possessed flowers. These branches were also covered with scales and bracts.
6. The flowers were unisexual.

**Identification**

*Division—Gymnospermae.* (1) Ovules naked, (2) Seeds attached to the scale, (3) Scales forming a strobilus.

*Class — Cycadopsida.* (1) Wood monoxyletic, (2) Large frond-like leaves, (3) Seeds with radial symmetry.

*Order — Bennettitales.* (1) Tree trunk covered by a mantle of persistent leaf bases, (2) Microsporophylls in groups, at the tip, leaves frond-like, (3) Megasporophylls in cone-like organization.

*Family —Williamsoniaceae.* (1) Stem delicate, branched, (2) Inflorescence stalked or sessile, not sunk in the scales of persistent leaf bases.

*Genus—Williamsonia.* (1) Stem sturdy and columnar, (2) Leaves pinnately compound, (3) Lateral branches with flowers.

(B-14/JMD/0708)