Exercise 3

Aim: To study pollen tube growth on stigma

Principle: Pollen grains germinate and form pollen tubes after they get deposited by the process of pollination on compatible stigma. Pollen tube, made up of cellulose, is an extension of the inner wall of pollen grain (intine). It emerges through one of the germ pore and passes through tissues of stigma and style to reach the ovule. The growing pollen tube is observed by staining with cotton blue.

Requirement: 5–6 excised styles with stigma of *Petunia*/grass/maize/sunflower/*Abelmoschus* (Lady's finger), beaker, water, slides, cover slips, cotton blue stain, microscope, brush, needle.

Procedure

(i) Place the stigmas in boiling water in a beaker for softening the tissues for 5–10 minutes.

(ii) Stain with cotton blue for 3–5 minutes and wash with water to remove excess stain.

(iii) Mount one stigma in a drop of glycerine on a slide. Place a cover slip on the stigma and gently press the cover slip on the material. Observe the slide under a microscope.

(iv) If you fail to observe pollen tubes mount another stigma.

Observation

Look for long blue-coloured tubular structures traversing through the tissues of stigma and style (Fig. 3.1).

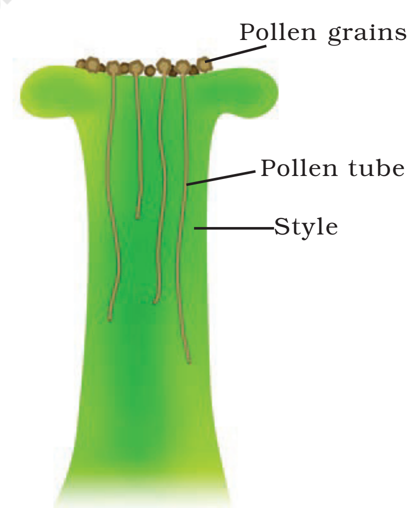

Fig.3.1 Growth of pollen tube in the style of a carpel

Discussion

Pollen tubes are seen amidst the stylar tissue. Many pollen tubes may be seen. Trace the origin of pollen tubes to the pollen grains present around the surface of the stigma.

Questions

1. Can pollen grains of one plant species germinate on stigma of other species? Give reasons.
2. Do all pollen tubes reach the ovules?
3. Are all the pollen tubes of equal length? If not, why?

Exercise 4

Aim: To study the discrete stages of gametogenesis in mammalian testis and ovary

Principle: In all male and female organisms gamete formation takes place in their gonads, i.e., testis and ovary respectively. The process of gamete formation, called gametogenesis involves meiotic cell division. The gametogenic development in testis is called spermatogenesis and in ovary it is oogenesis. They exhibit marked differences and can be examined in transverse section (T.S.) of these organs.

Requirement: Permanent slides of T.S. of testis and ovary, compound microscope, lens-cleaning paper and cleaning fluid

Procedure

(i) Clean the slide and microscope's eye and objective lenses with the help of lens cleaning paper using any cleaning fluid.

(ii) Place the slide on the stage of the microscope and observe first under lower magnification and then in higher magnification. Observe various stages of gamete development.

(iii) Record your observations in the notebook and draw labelled diagrams.

Observation

T.S. of testis

(i) You will observe a large number of seminiferous tubules under lower magnification. Observe a complete tubule in higher magnification and view various stages of gamete development from periphery towards lumen (Fig. 4.1) and identify the following types of cells namely, Germinal epithelium, Spermatogonial cells, Primary spermatocytes, Secondary spermatocytes, Spermatids and Spermatozoa.

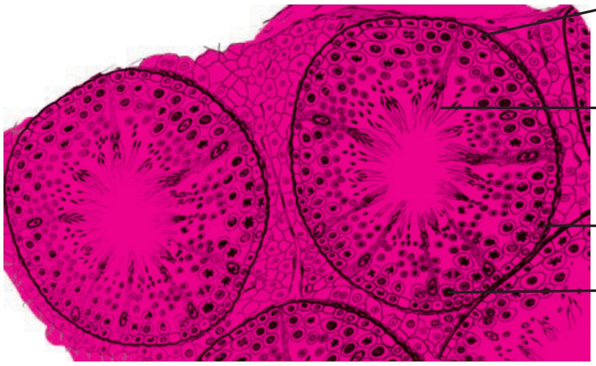

Fig. 4.1 T.S. of mammalian testis

(ii) In T.S. of testis the space between tubules are filled with blood vessels and a specific cell type called Leydig's cell or Interstitial cells.

T.S. of Ovary

(i) In the section of ovary, there is a mass of tissue lined with germinal epithelium. Inside that you will observe an ovum, which is a cell surrounded by one to several layers of follicular cells. As the ovum matures, the number of surrounding follicular cell layer increases (Fig. 4.2).

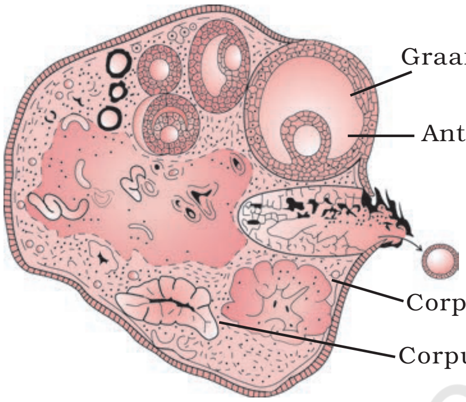

Fig. 4.2 Section of mammalian ovary

(ii) In the later stage of follicular development a cavity called **antrum** appears.

(iii) The cavity gets further enlarged and the follicle grows bigger. This is the stage of **Graafian follicle** ready to release the ovum (ovulation).

(iv) In the next stage, you may notice a Corpus luteum, and/or Corpus albicans, which differ from each other and also from Graafian follicle in their features.

Discussion

Spermatogenesis is a continuous process after attainment of puberty, and that is why gamete development and spermatozoa are observed in a single seminiferous tubule. In case of ovary, the follicular development stages are observed.

Questions

1. What would happen if meiosis fails to occur in gametocyte?
2. At which stage of follicular development, is ovum released?
3. Spermatogenesis is a continuous process. Justify the statement.
4. Draw a labelled diagram of T.S. of testis.
5. Draw a labelled diagram of T.S. of ovary.
6. What would happen if sperms are devoid of their tail?
7. What are the consequences of failure of ovulation?

Exercise 5

Aim: To study and identify various stages of female gametophyte development in the ovary of a flower

Principle: In flowering plants, female gametophyte (embryo sac) is a microscopic structure situated deep inside the ovule. An ovule generally has one female gametophyte. Development of female gametophyte begins with megaspore mother cell. Most common type of female gametophyte is the monosporic, 8-nucleate, 7-celled type.

Requirements: Permanent slides of V.S. of ovary, photographs/chart or models showing stages of female gametophyte development and microscope

Procedure

(i) In a V.S. of ovary we generally find several ovules. Carefully observe each ovule and locate as many stages of female gametophyte development as possible.

(ii) Draw the diagrams as observed under microscope.

Observation

(i) Record the features of ovule like number of integuments, nucellus and micropylar and chalazal poles. Fig 5.1 shows the female gametophyte (embryo sac) as seen in a V.S. of an ovule. Different stages of development of female gametophyte are shown in Fig. 5.2.

(ii) Observe the placement of embryo sac close to the micropylar pole.

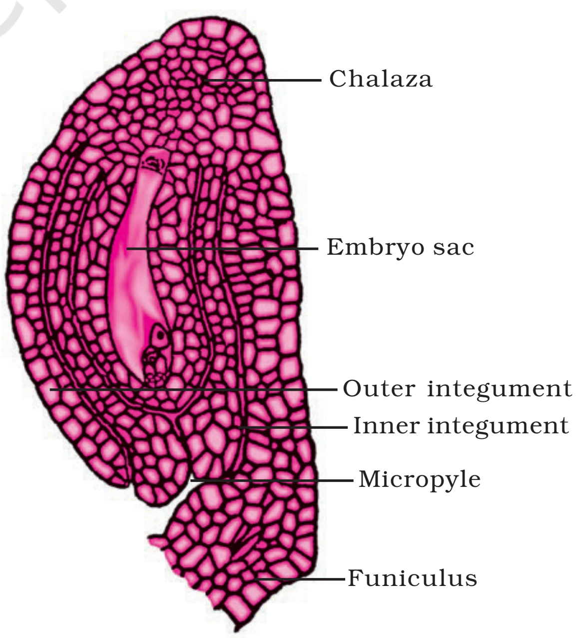

Fig. 5.1 V.S. of an ovule

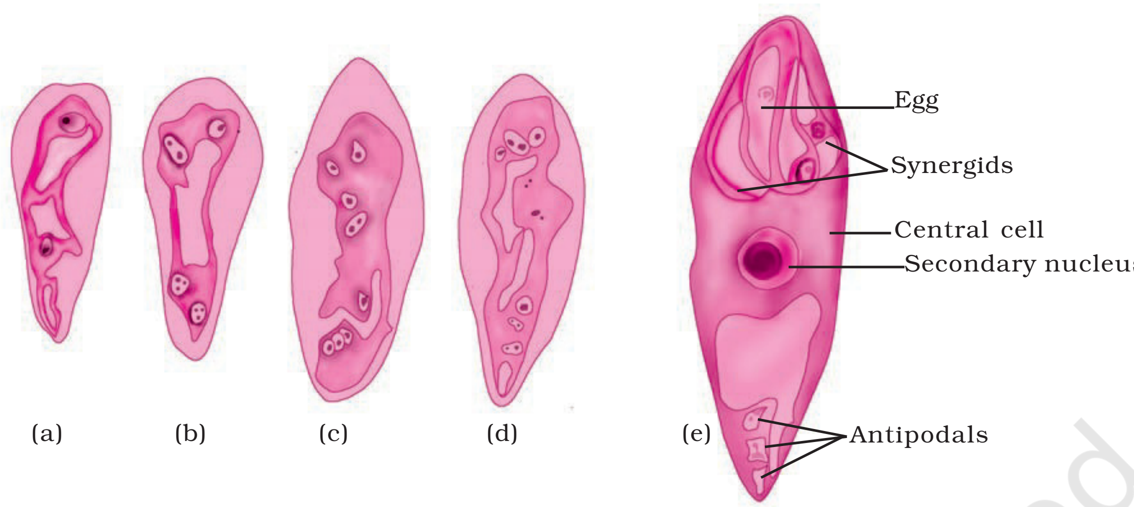

Fig. 5.2 Stages of gametophyte development: (a) megaspore with 2 nucleus (b) 4-nucleate stage (c) 8- nucleate stage (d) 8- nucleate stage showing 3+2+3 distribution of nuclei (e) mature embryo sac.

(iii) Note the contents of embryo sac, namely, an egg apparatus (2 synergids and egg) at micropylar end, secondary nucleus in the center and three antipodal cells at the chalazal end (Fig. 5.2).

Questions

1. Explain the difference between gamete and a gametophyte.
2. Name two differences between synergids and egg.
3. What is the function of polar nuclei?

Exercise 7

Aim: Study of stages of meiosis using permanent slides

Principle: Meiosis is a type of cell division in which the number of chromosomes is halved (from diploid to haploid) in the daughter cells, i.e., the gametes. The division is completed in two phases, meiosis I and meiosis II. Meiosis I is a reductional division in which the chromosomes of homologous pairs separate from each other. Meiosis II is equational division resulting in the formation of four daughter cells. Stages of meiosis can be observed in a cytological preparation of the cells of testis tubules or in the pollen mother cells of the anthers of flower buds.

Requirement: Permanent slides of meiosis and compound microscope

Procedure

Place the slide on the stage of the microscope and search for the dividing cells using lower magnification. When dividing cells are located observe them under higher magnification.

Observation

Observe various stages of meiosis and identify them on the basis of the specific features given in the table 7.1. A significant number of cells will be in the Interphase. These cells have a centrally positioned densely stained nucleus. In case of slide of animal tissue a few mitotically dividing spermatogonial cells may also be seen.

Table 7.1 Different stages of meiosis and their features

Meiosis I	
1. Prophase I	Unlike the prophase of mitosis, it is a comparatively complex phase characterised by a number of events. Five sub-phases can be identified in it. **(a) Leptotene** (*leptos* = slender *tene* = band or thread) (i) The nuclear membrane and nucleolus are not distinctly observable (Fig. 7.1 a). (ii) Fine network of thin threads are seen uniformly distributed in the nucleus.These are chromatin threads, which may be observed as more prominent structures in the later stages.

Fig. 7.1 Sub-phase of Prophase I (a-d) – actual microscopic view on left side and its diagrammatic representation on the right hand side

	(b) Zygotene (*Zygon* = paired) This stage is characterised by the pairing of the homologous chromosomes, which can be seen as paired chromatin threads (**bivalents**) (Fig. 7.1b). **(c) Pachytene** (*pachy* = thick) The chromatin threads get condensed and appear shortened and thick. Pairs of homologous chromosomes can be seen. Each chromosome has two chromatids and thus each bivalent consists of four chromatids. This configuration is called tetrad (Fig. 7.1c). **(d) Diplotene** (*diplos* = double) The homologous chromosomes (each made up of two chromatids) show distinct separation from each other except at few regions where attachments are seen (Fig. 7.1d). These are **chiasmata** (sing. chiasma) representing the site of exchange of the parts between two homologous chromosomes (i.e. crossing over). **(e) Diakinesis** *(Dia = opposite; kinesis= separation or movement)* (i) The homologous pair of chromosomes appear more shortened, thick and prominent (Fig. 7.1d). (ii) Chiasmata can be still observed. (iii) All the homologous pairs appear scattered in the cell.
2. Metaphase I	Homologous chromosomes are still in pairs, and are arranged along the equatorial plane of the cell (Fig. 7.2a). At this stage, the number of bivalents can be counted. Chiasmata may still be seen in a few bivalents.
3. Anaphase I	The chromosome pairs appear to have moved towards the two opposite poles of the cell. At the later stage, the anaphase - I may show the assembly of chromosomes at two poles (Fig. 7.2b). This results into the reduction of number of chromosomes to half. This stage can be identified by the presence of two chromatids in each chromosome.
4. Telophase I	The chromosomes present at the two poles appear decondensed and form two distinct nuclei (Fig. 7.2c). **Note**: *After the telophase I stage there may or may not be cytokinesis. Thereafter the cell enters into the second meiotic division.*
Meiosis II	
1. Prophase II	(i) Distinct thread- like chromatin fibres or rod- shaped chromosome are seen.

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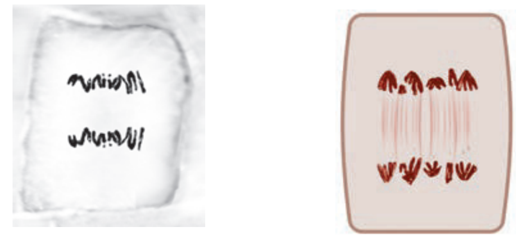
(a) Metaphase I

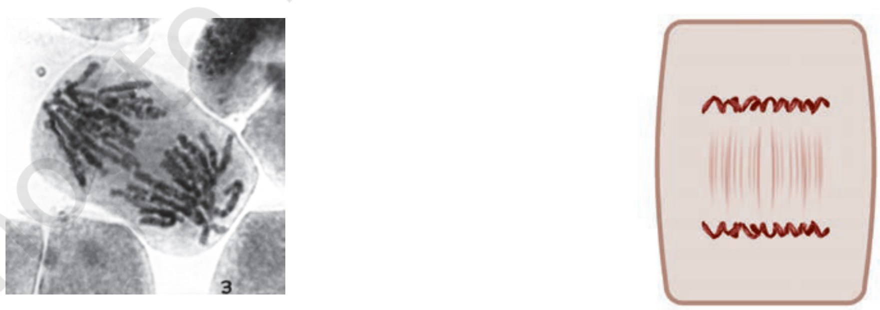


(b) Anaphase I

(c) Telophase I

Fig. 7.2 Phases of Meosis I (a-c) – actual microscopic view on left side and its diagrammatic representation on the right hand side.

(a) Metaphase II



(b) Anaphase II

Fig.7.3 Phases of Meosis II (a,b) – actual microscopic view on left side and its diagrammatic representation on the right hand side.

2. Metaphase II	This phase is similar to that of mitotic division (i) The chromosomes having two chromatids attached at the centromere are observed arranged at the equatorial plane of the cell. **Note**: *Metaphase II of meiosis can be differentiated from metaphase-I on the basis of the following features:* (ii) Each chromosome of metaphase II has two chromatid (Fig. 7.3a) whereas in metaphase I these are paired homologous chromosomes each having two chromatids thus forming tetrad. (iii) In the metaphase I of meiosis, a few chiasmata are observed, where as no chiasmata are observed during metaphase II.
3. Anaphase II	The two chromatids of each chromosome after separation appear to lie at the two poles of the cell (Fig. 7.3b). **Note**: *Anaphase II can also be distinguished from the anaphase I of meiotic division on the basis of chromatids: In anaphase I, each chromosome has two distinct chromatids, but in anaphase II, each chromosome is represented by one chromatid only.*
4. Telophase II	The separated chromosomes appear decondensed and form nuclei (Fig. 7.3c).

Questions

1. What is the significance of meiosis?
2. What is synapsis and crossing over?
3. How can anaphase I and anaphase II be distinguished from each other?
4. Indicate distinguishing feature of metaphase I of meiosis and metaphase of mitosis.
5. How many daughter cells are produced at the end of meiosis?
6. The daughter cells produced at the end of meiosis are genetically different. Explain.
7. What is the significance of synapsis?

Exercise 8

Aim: To study the blastula stage of embryonic development in mammals, with the help of permanent slide, chart, model or photograph

Principle: The zygote undergoes a few cycles of mitotic divisions to form a solid ball of cells called morula. The cells continue to divide and at a later stage a cavity is formed within it. This stage is blastula. The internal structural details of blastula can be observed in its transverse section.

Requirement: Permanent slide, chart/model of T.S. of blastula, compound microscope, lens cleaning fluid and paper

Procedure

Observe the slide under lower magnification of the microscope. In case of chart/models/photographs, note the feature of blastula in your practical record and draw labelled diagram.

Observation

In transverse section, the blastula appears as a sphere with a cavity, called blastocoel within it (Fig. 8.1). Notice an outer layer of blastomeres called trophoblasts. A cellular mass, adhered to the trophoblast is present on one end of the blastula. It is called inner cell mass.

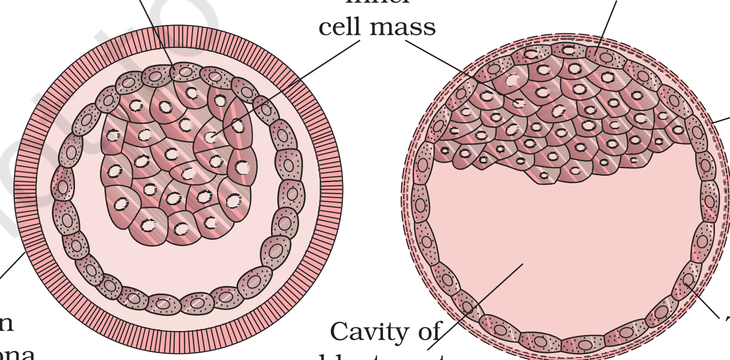


Fig.8.1 Blastula stage of a mammal

Questions

1. What are the differences between blastula and morula?
2. What are the main structures you observe in T.S. blastula?
3. Match the stages in column I with features in column II

Column I		Column II	
(a)	Trophoblast	(i)	Dividing cells of the morula
(b)	Morula	(ii)	Outer layer of blastula
(c)	Blastocoel	(iii)	Solid ball of cells
		(iv)	Cavity

Exercise 14

Aim: To identify common disease-causing organisms and the symptoms of the diseases

Principle: There are quite a large number of organisms that are parasitic/pathogenic to humans. These organisms substantialy damage the human body and cause diseases, which may even be fatal sometimes. These organisms exhibit characteristic features in their external morphology. Symptoms of the diseases caused by them are also specific.

Requirement: Preserved specimens/permanent slides/photographs of *Ascaris*, *Entamoeba*, *Plasmodium*, Ring-worm fungus and compound microscope

Procedure

Observe the preserved specimens/slides/photographs and note down the features in the practical record book. Take care to observe all the minute details and draw labelled diagrams of the pathogens.

Observation

A. *Entamoeba*

Observe the following features of the parasite in the slide or photograph:

(i) It is unicellular.

(ii) Shape of the cell is irregular due to pseudopodia.

(iii) A single nucleus is present eccentrically in the cell.

(iv) *In the nucleus a peripheral ring of granule of nucleoprotein and central karyosome are observed. Rest of the space in the nucleus looks empty (Fig. 14.1).

(v) A few food vacuoles may be seen in the cytoplasm. Contractile vacuoles are absent.

(vi) *Mature quadrinucleated cysts may be present.

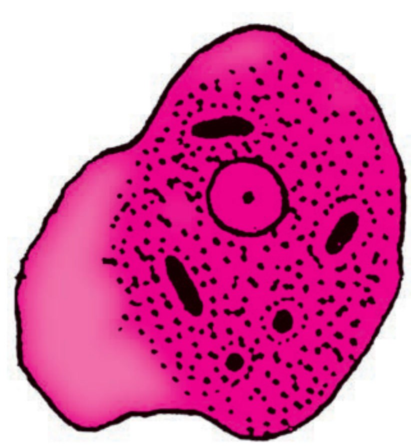


Fig.14.1 An *Entamoeba*

Note: *Entamoeba* is an intestinal parasite in humans and causes amoebic dysentery. The symptoms of the disease are frequent loose, mucus filled watery stools, abdominal pain and spasms.

Systematic position

Phylum	–	Protozoa
Class	–	Rhizopoda
Type	–	*Entamoeba histolytica*

* Distinctive feature of the pathogen

B. *Plasmodium vivax*

(i) It is an intracellular endoparasite seen easily within the RBC of the infected person.

(ii) It is unicellular.

(iii) The most diagnostic stage of the parasite is "signet ring" stage in the erythrocytes, within which it appears as a rounded body (Fig. 14.2).

(iv) It has a big vacuole inside, and the cytoplasm is accumulated at one place containing the nucleus. Because of the above mentioned features, the parasite appears as a ring.

Search the stage in the blood film slide, find the signet-ring stage, and draw its labeled diagram.

Note: It is a protozoan parasite causing malaria in humans. When an infected female anopheles mosquito bites a healthy person, it injects the infective stage, sporozoite, into the peripheral blood vessels. The infective stage undergoes several rounds of multiplication in liver and erythrocytes.

Symptoms: Intermittent high fever with chills followed by profuse sweating at an interval of alternate days.

Systematic position

Phylum	–	Protozoa
Class	–	Sporozoa
Type	–	*Plasmodium vivax*

C. *Ascaris*

The external features of round worm are as follows:

(i) Body long (20 to 40 cm), cylindrical (5 to 6 mm diameter) with no segmentation (Fig. 14.3).

(ii) Sexes are separate; the females are longer than the males.

(iii) Both the ends are pointed; posterior end of male is ventrally curved.

(iv) Mouth is situated at the anterior end, and is surrounded by three lips, one present mid-dorsally and rest two lips are situated ventrolaterally (for viewing these lips a magnifying lens is needed).

(v) Single longitudinal lines are present on the dorsal, ventral and on the two lateral sides, all along the length of the body. Out of these the lateral lines are comparatively more distinct than the others lines.

(vi) Excretory pore is present on the ventral surface slightly behind the anterior end.

(vii) In addition to the ventrally curved posterior tip, the male worm has a pair of penial spicules very close to the cloacal opening.

(viii) In case of female specimen a female genital aperture is present mid-ventrally about one third distance from the anterior end.

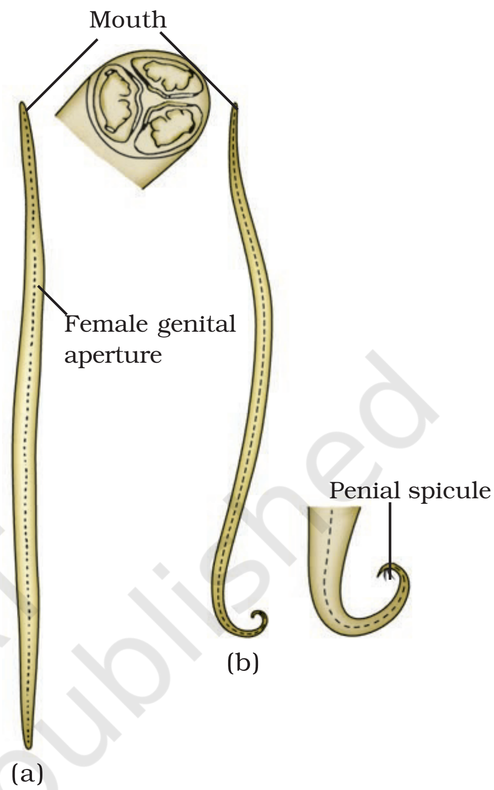


Fig.14.3 *Ascaris* (a) Female (b) Male

Systematic position

Phylum – Aschelminthes

Class – Nematoda

Type – *Ascaris lumbricoides*

Note: Round worm or Ascaris is one of the common parasite found in the intestine of human beings.

Symptoms: (a) Irregular bowel, (b) Occasional vomiting, (c) Anaemia

Trichophyton *(Ringworm fungus)*

It is a fungus that feeds on keratin of the skin of human beings. The features as observed under the microscope are:

1. Texture of hyphae is waxy, glabrous to cotton like.
2. Unstained hyphae are white, yellowish brown to reddish brown in colour.

Systematic position

Kingdom	–	Fungi
Class	–	Deuteromycetes
Type	–	*Trichophyton rubrum*

Symptoms

Ringworm is a contagious fungal infection of the skin. Infected area of skin is itchy, red, raised, scaly patches (with sharply defined edges). It is more red on the periphery than in the center creating a ring like appearance.