

B-SG-1ST SEMESTER

PRACTICAL FILE

ZOOLOGY

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GENERAL METHOD OF MICROSCOPIC PREPARATION

PERMANENT SLIDE (STAGE) -

1. Killing
↓
2. Fixing
3. Washing (Tap Water)
4. Staining (Barax Carmine)
5. Destaining
6. Dehydration
7. Mounting on the slide
8. Labelling

CHART FOR SINGLE STAINING

Fix the Material



Wash in tap water



Dehydrate in 30 % Alcohol



Dehydrate in 50 % Alcohol (3-4 minut)



Dehydrate with 70 % Alcohol



Stain in basic Carmine / eosin / picric indigo Carmine



Destain in Acid Alcohol (100 %)



Wash with 70 % to 90 %



Dehydrate with 90 %



DeAlcoholize / xylol



Mount in Canada balsam



Time 5 - 15 minut - For each reagent.

CHART FOR DOUBLE STAINING

In Double staining both nuclear and cytoplasmic stain are used

↓
Fix the Material

↓
Wash in tap water

↓
Stain in Hematoxylin

↓
De Stain in Acid water

↓
Wash in tap water (Bright Blue colour)

↓
Dehydrate with 30 % Alcohol

↓
70 % Alcohol

↓
wash with 90 % Alcohol

↓
100 % Alcohol

↓
Clear or alcoholize with xylol

↓
Mount in Canada balsam

↓
Time 5 - 15 minutes for each reagent.

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EXPERIMENT - 1

To Prepared a slide of root tip of Onion by Squash Method and to identified study and draw the various Mitotic stage.

Material Required —

Onion root tips fixed in Carnoy fluid (6- part Oxidate Alcohol + 3 part glacial Acetic Acid + 1 part chloroform) Aceto Carmine stain, cover slip, blotting paper, spirit lamp, watch glass, 1 N HCl, 45 % Acetic Acid and Filter paper.

Procedure —

Take large size Mature Onions about the week before the experiment cut all the dried from the stem at the base of the bulb. Now place these onions on the Mouth of Coupling Jar's field with water in such away that the stem portion must be dipped continuously in water in about a weeks time new roots wood develop and wood start growing down ward in the water. Take the Onion and cut the Milky white portion of root tips up to 5 mm length. cut each piece into smaller

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Pieces and fix them in Carnoy Fixative for half hour. Transfer the Material to 90% Alcohol and then to 70% Alcohol. Keeping the Material for 10 minutes in each. Now preserve the Material in 70% Alcohol at the time of proceeding for experiment in the laboratory take few pieces from 70% Alcohol in a watch glass in few drops of 1N HCl and leave for 5 min. by this procedure the Material will become soft. Now drain off the HCl and wash the pieces with a little distilled water at least twice or thrice. Now put the root tip on a clean slide on right hand side and pour a few drops of 2% aceto-carmine over the slide gently on spirit lamp for few minutes at least 3 to 4 times but never let it boil. Cool and leave for 10 minutes. Then stain with the help of filter paper and put few drops of 45% Acetic Acid. Place a cover slip on the Material on the put above the cover slip a piece of blotting paper or filter paper folded two to three times. Three times press the cover slip gently with your thumb to break the Cell Membrane. The blotting paper

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As filter paper would save the cover slip blacking and would also absorb the excess stain which will ooze out from the cover slip take care that you while pressing seal the cover slip with nail polish. If you want to keep the slide for some time fix the slide under low power of Microscope and focus now change to high power and observe the various stage which would appear as shown in the attached photograph draw various stage and write down comment on each of them.

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DIFFERENT STAGE OF MITOSIS IN ONION

ROOT TIP

1. PROPHASE —

Prophase can either be early or late prophase.

(A) Early Prophase —

1. The Cell is of normal shape and size.
2. The nucleus becomes enlarged.
3. Nuclear Membrane and nucleus are distinct.
4. Thin thread like chromosome are visible within the nucleus.

(B) Late Prophase —

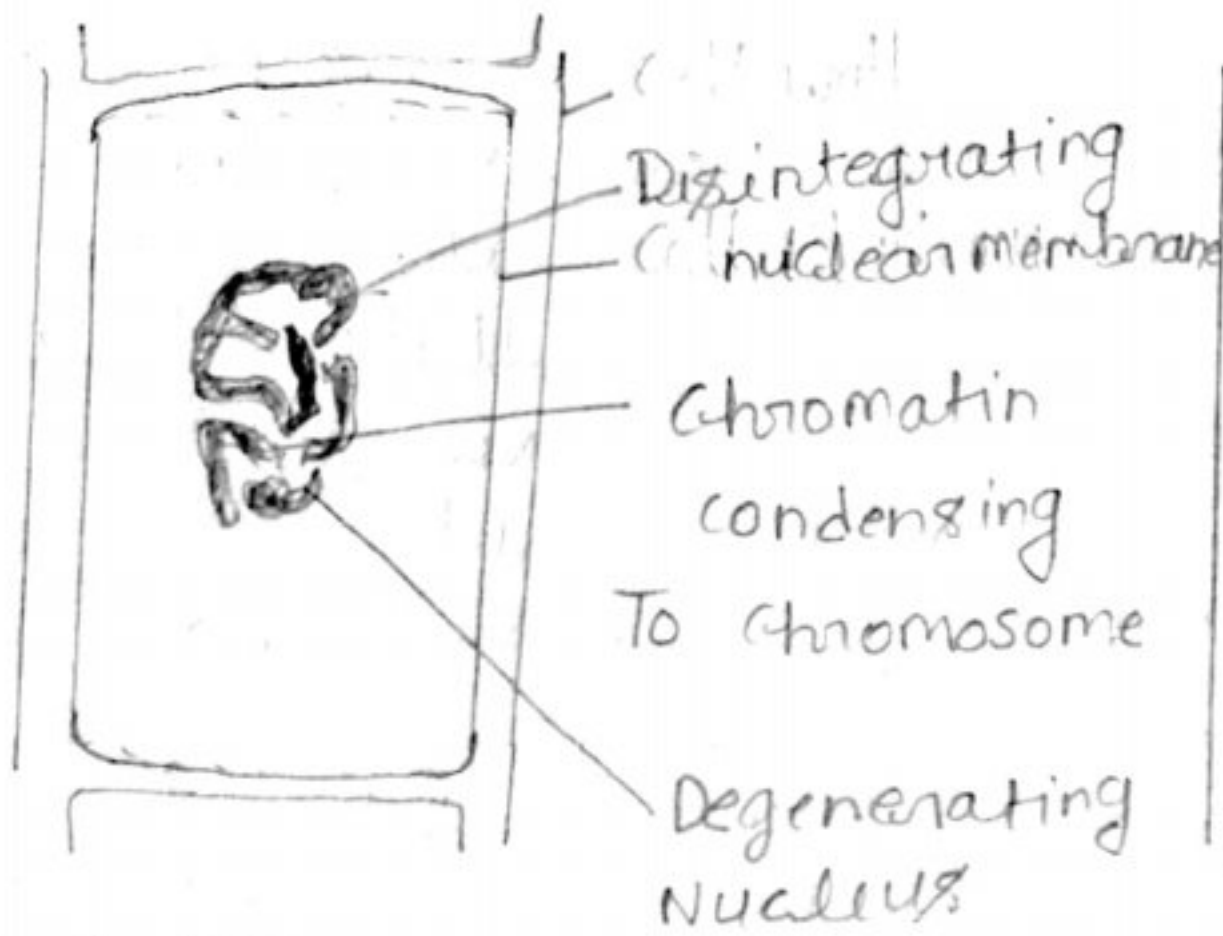
1. The Nucleus disappears and nuclear Membrane disintegrates.
2. Chromosome Condense into thick thread like structure.
3. Spindle Formation begins.

2. METAPHASE —

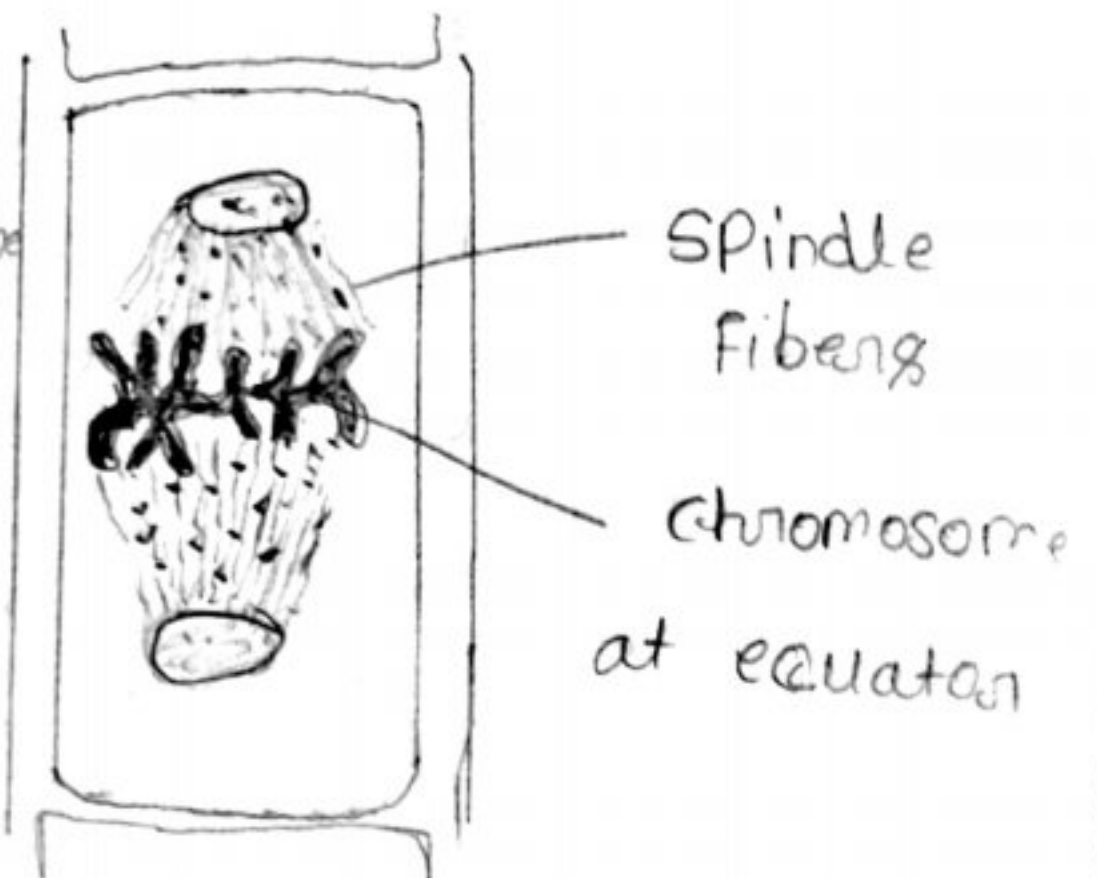
Prophase is followed by Metaphase

1. Spindle Formation is completed.
2. Chromosomes Condense further and now appear as rod like structure. Each chromosome is

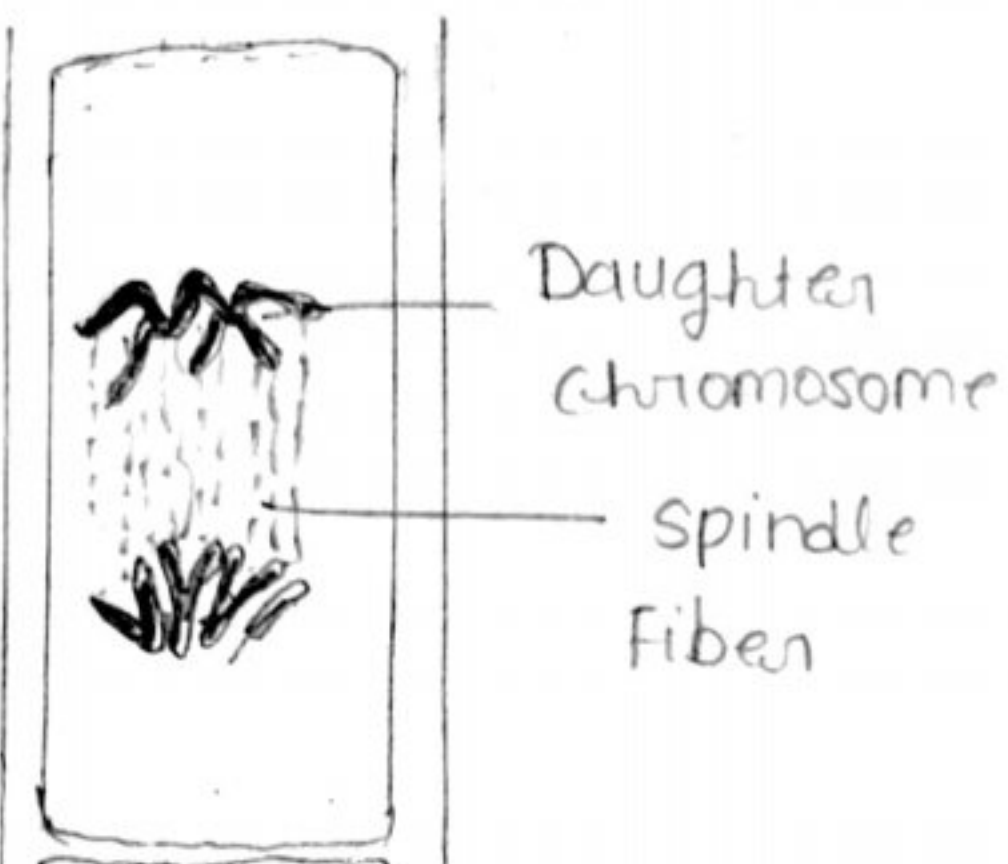
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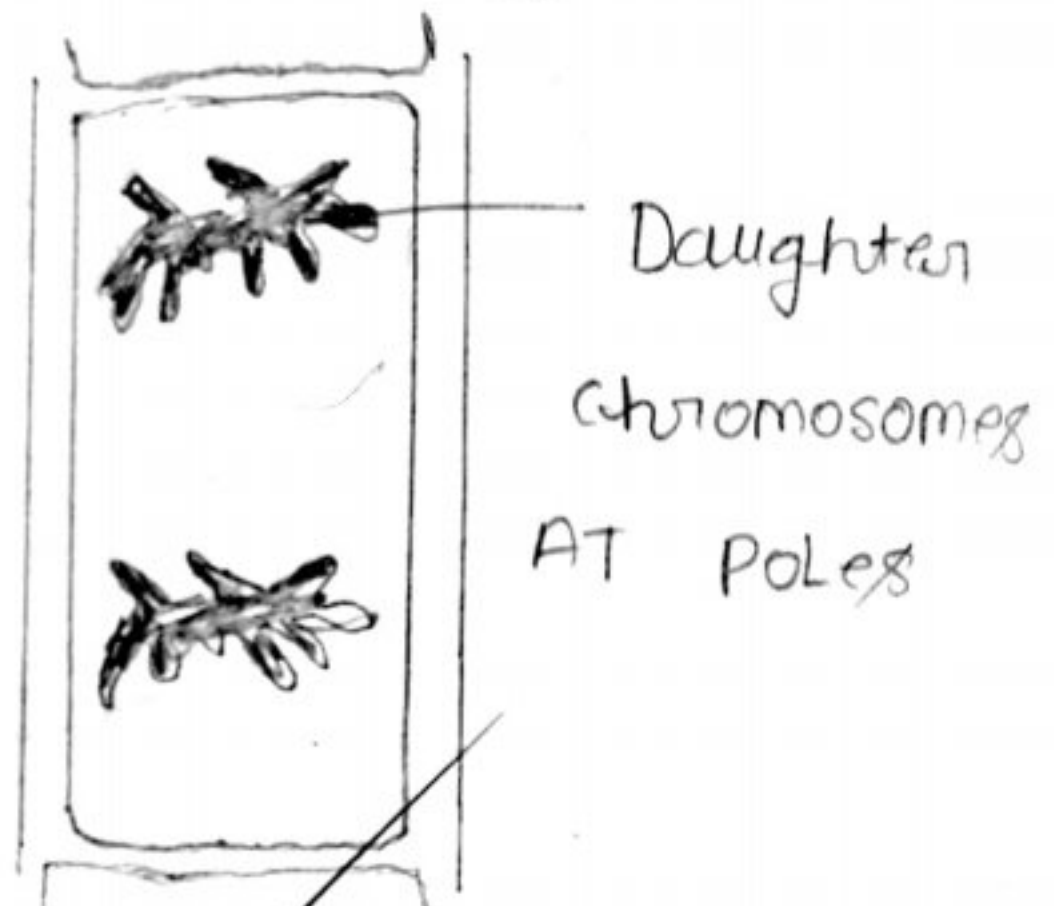
PROPHASE



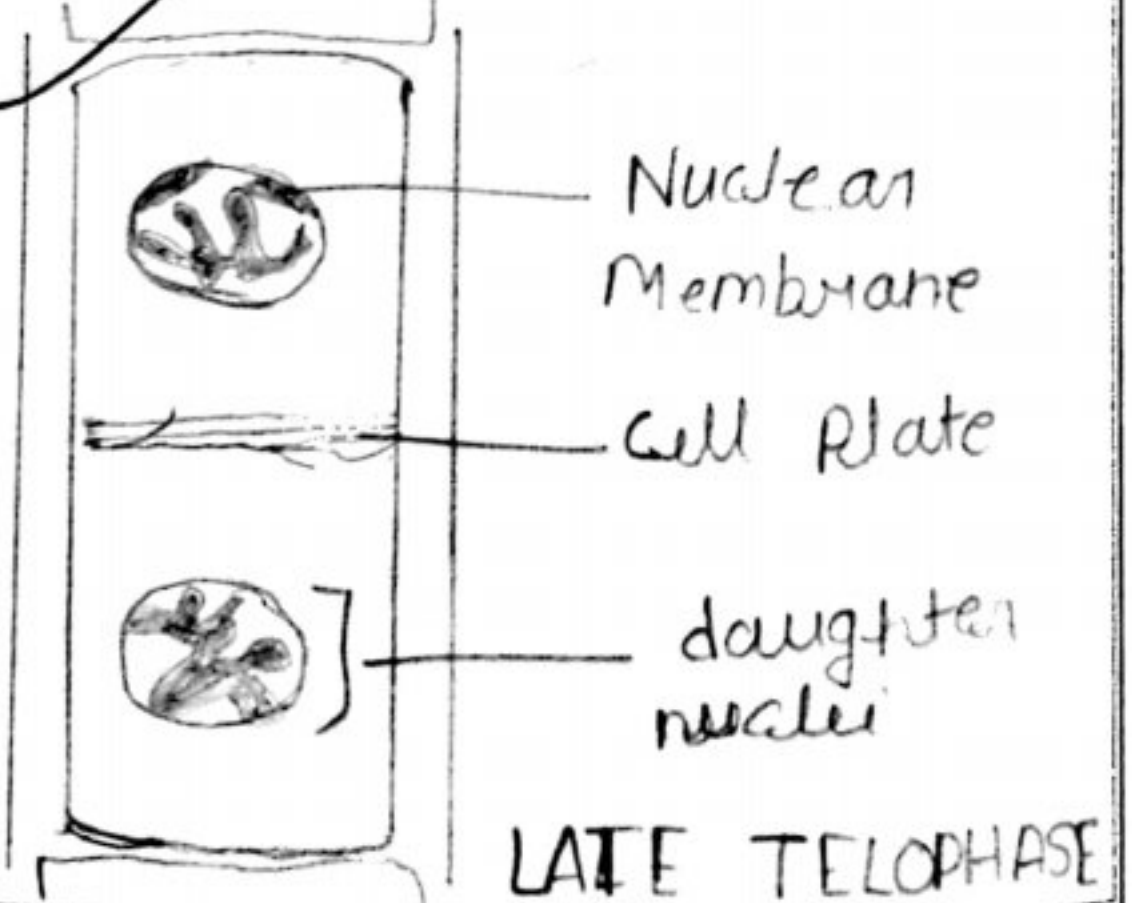
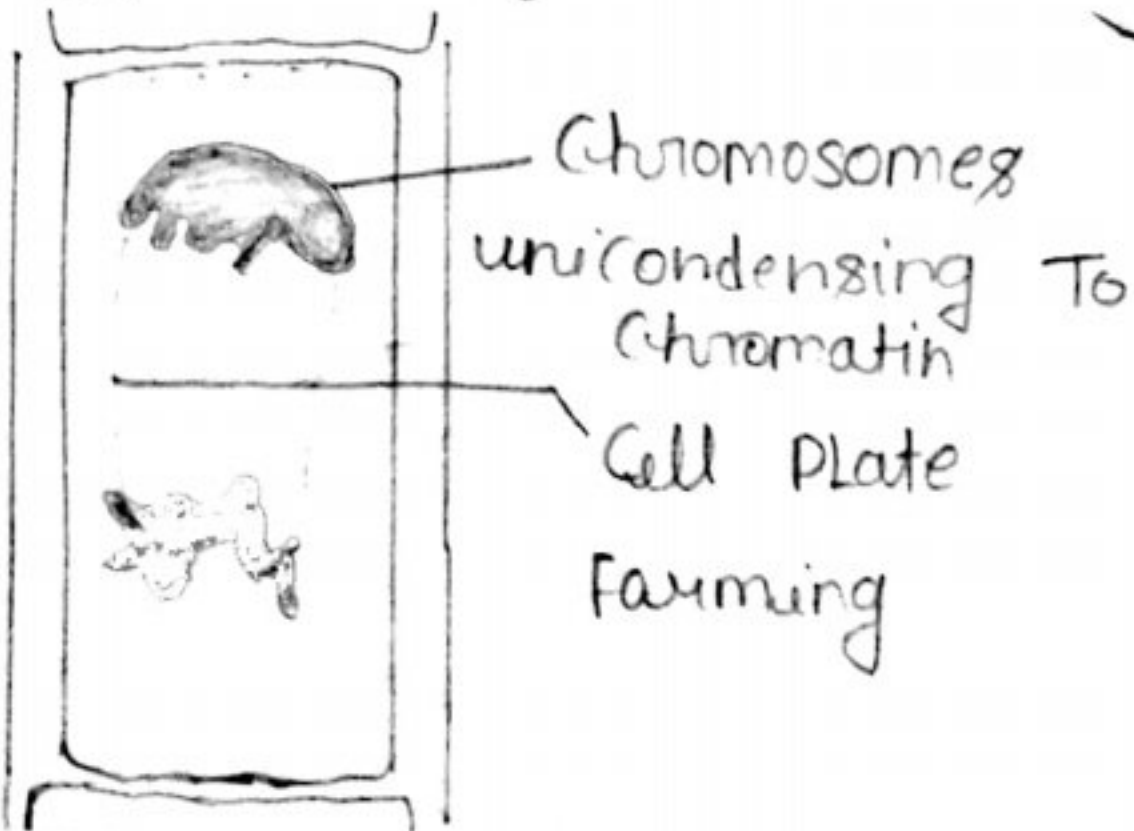
METAPHASE



EARLY ANAPHASE



LATE ANAPHASE



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Now called bivalent.

3. ANAPHASE —

Metaphase is followed by Anaphase.

1. Chromosome split at the Centriosome.
2. The Chromosome move towards opposite poles by the contraction of Spindle Fibres.
3. During this movement the Centrioles face the poles.

4. TELOPHASE —

Anaphase is followed by Telophase.

A. Early Telophase —

The Cell become elongated.
Chromosome collect at the opposite poles of the Cell.

B. Late Telophase —

Each group of Chromosome gets surrounded by a nuclear membrane.
The nucleolus reappears.

5. CYTOKINESIS —

The reappearance of the nuclear membrane produce 2 daughter nuclei.
This is followed by division of cytoplasm by formation of Cleavage Furrow.

Result —

Clearly observe and note down the stage of Mitosis.

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EXPERIMENT - 2

To Prepare a Slide of Meiotic stage through squash Method from testis of Chironomous larva of grasshopper.

Material —

Testis of grasshopper or chironomous larva, Carnoy fluid, Azito Carmine, Niddles, slide, watch glass, Acetic Acid, Coverslip, Filter paper or blotting paper, spirit lamp, Normal saline, 1% Citrate and 2.2 Trisodium citrate.

Procedure — For Grasshopper Testis —

Dissect out testis of grasshopper and keep in watch glass in saline transfer them into an other watch glass containing 1.0 ml of fresh sodium citrate solution so that their nuclei may swell up and chromatids may spread over larger area transfer the testis into carnoy fluid for 20 minutes to 1 hour. dehydrate the testis through 90% and 70% alcohol for minutes each now from 70% Alcohol transfer the testis to 2% azito Carmine solution for 20 min. Destain of the excess stain and pour 45%.

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Acetic acid and leave the testis in it for 10 minutes now place 1 testicular follicle on a clean slide in 45% acetic acid and put a cover slip over it, put a piece of filter or blotting paper over the cover slip and put your thumb on cover slip and gently press the cover slip. The excess fluid shall be blotted off by filter paper now seal the cover slip with nail polish and observe under Microscope.

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DIFFERENT STAGES OF MEIOSIS IN GRASSHOPPER TESTIS —

MEIOSIS I —

It is also called Reductional division. It has the following stages: Prophase I, Metaphase I, Anaphase I and Telophase I.

A. Prophase I —

It is a complex stage of a long duration that includes 5 substage, which are as follows.

(i) Leptotene (Leptonema) —

1. Chromosome appears as thin thread like structure.
2. Chromosome have a beaded appearance due to the presence of chromosome along their entire length.

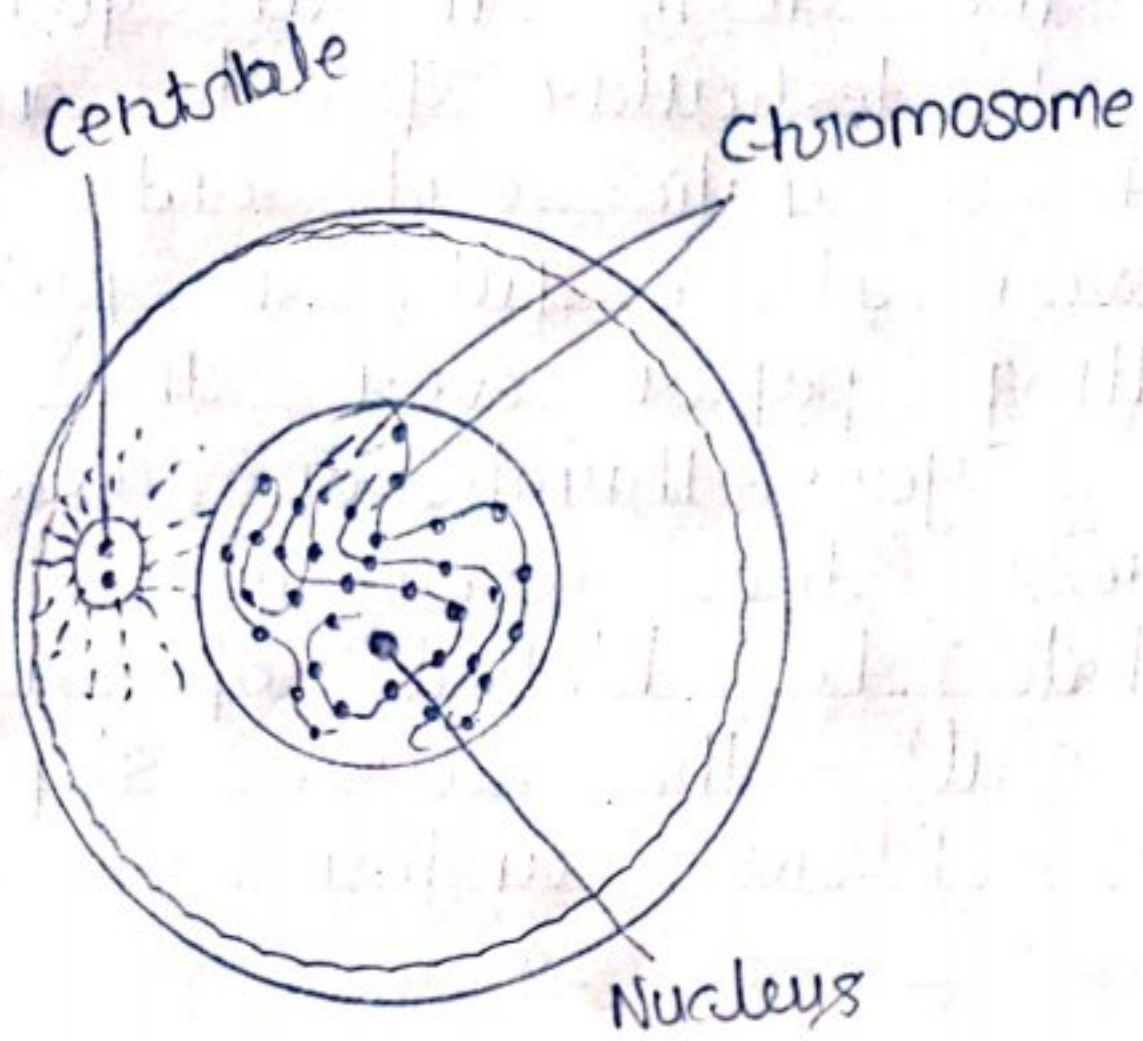
(ii) Zygotene (Zygonema) —

1. Chromosomes condense further.
2. Pairing of homologous chromosome called synapsis occurs.

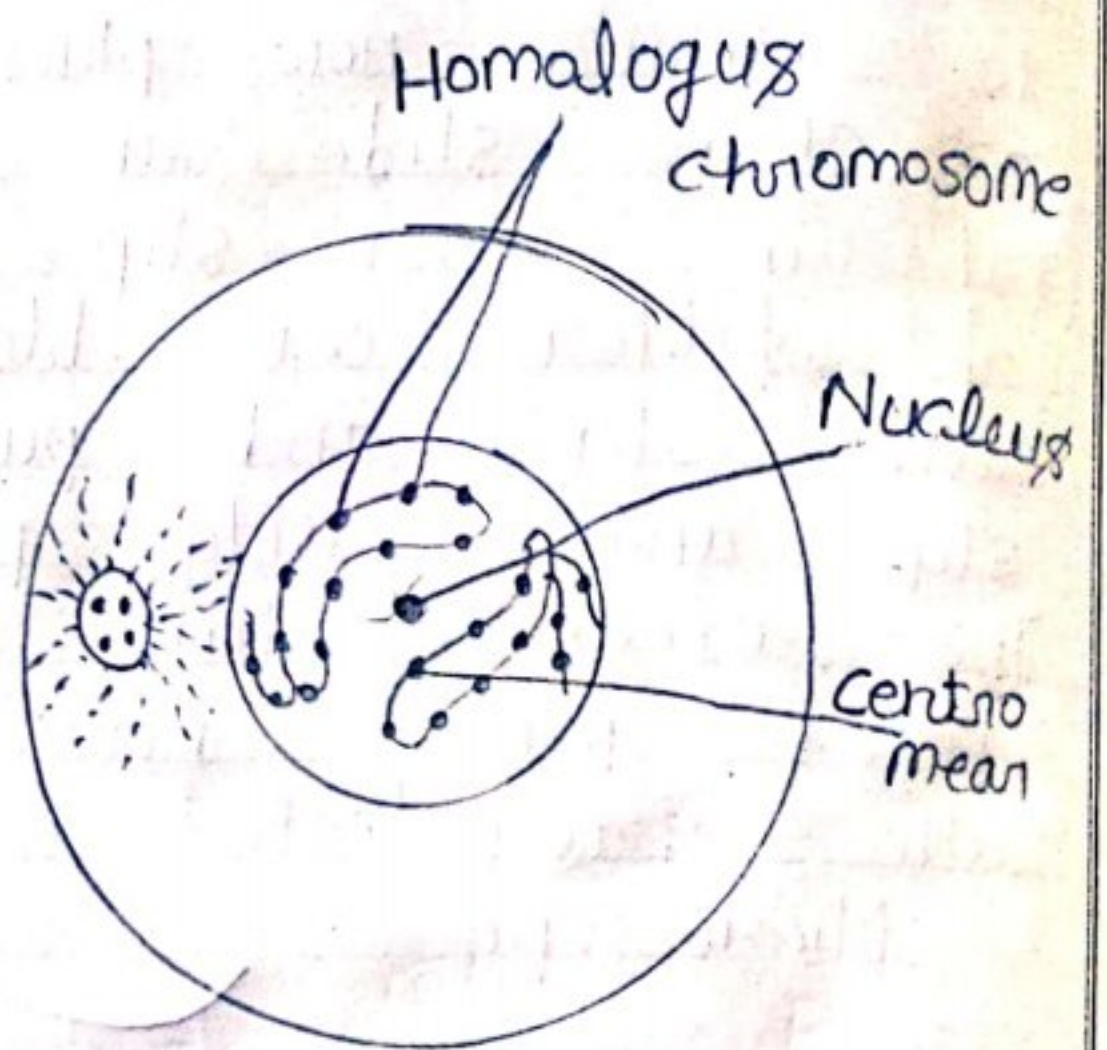
(iii) Pachytene (Pachynema) —

1. Chromosome condense further.
2. Each chromosome is seen to be made up of two chromosomes joined by a centromere.

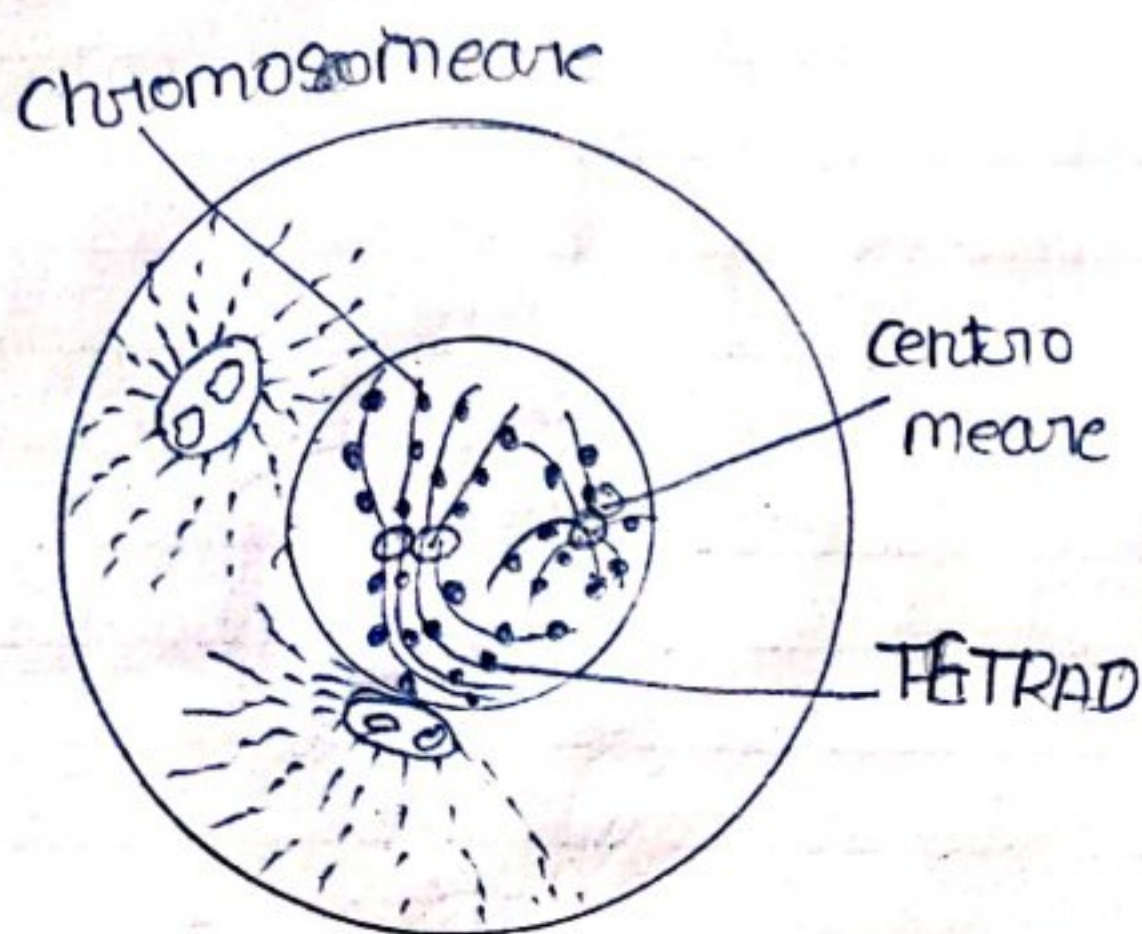
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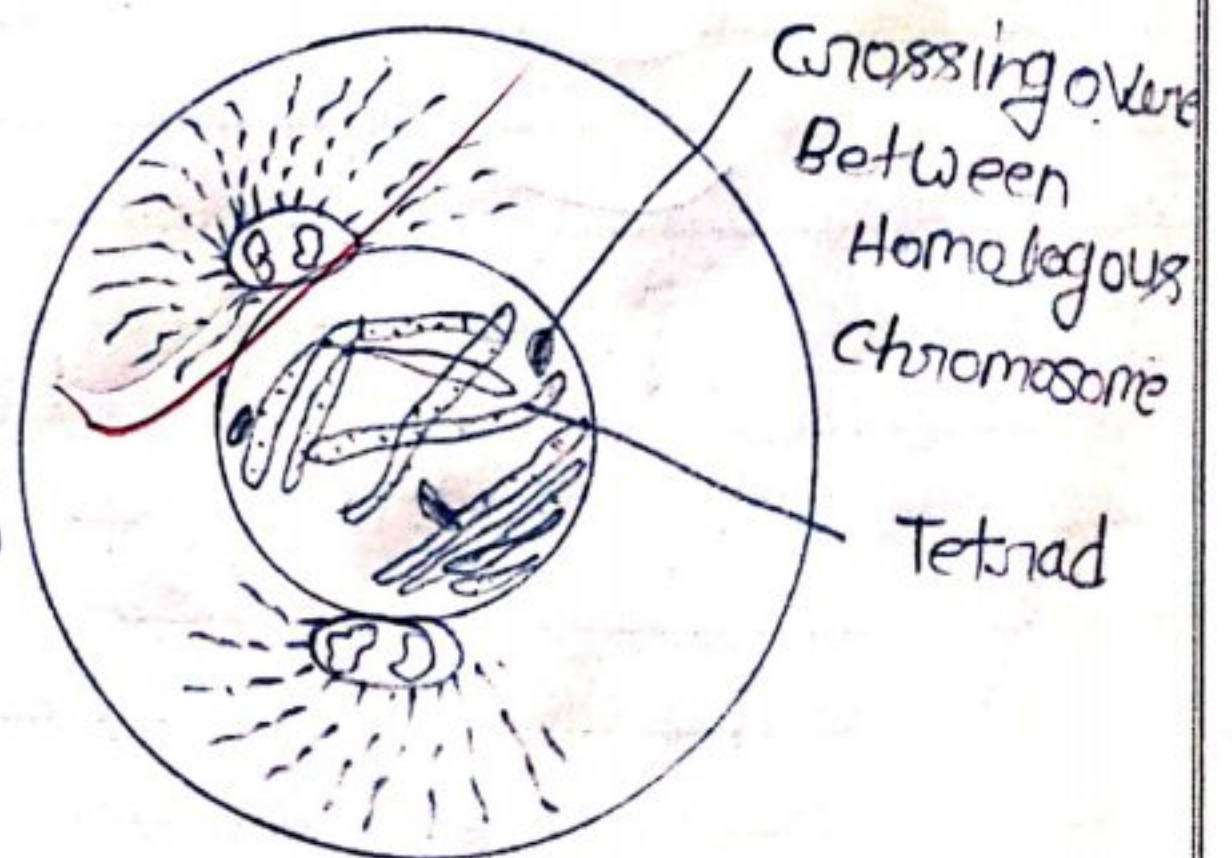
Leptotene



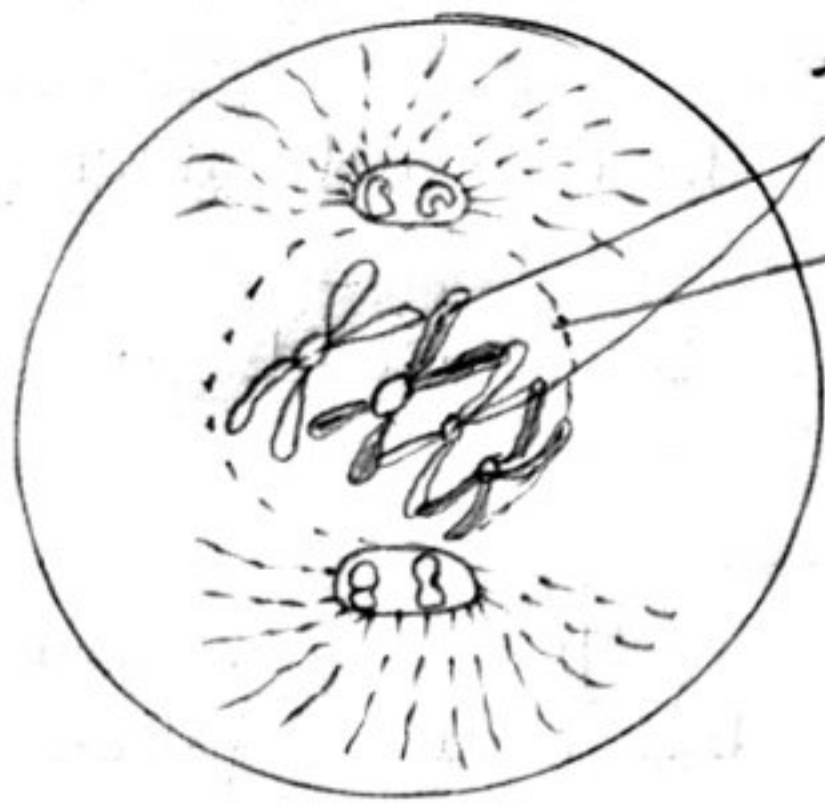
Zygotene



Pachytene

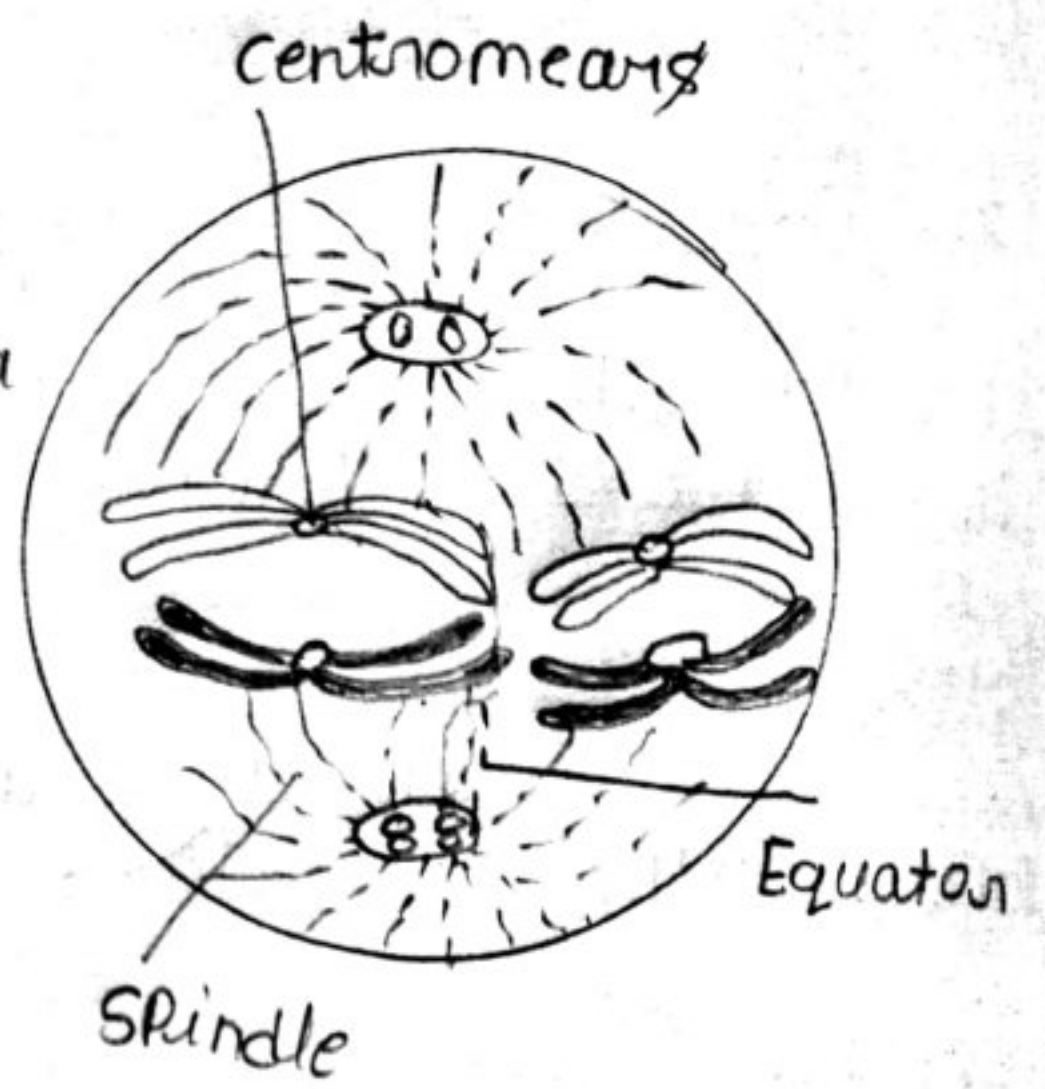


Diplotene

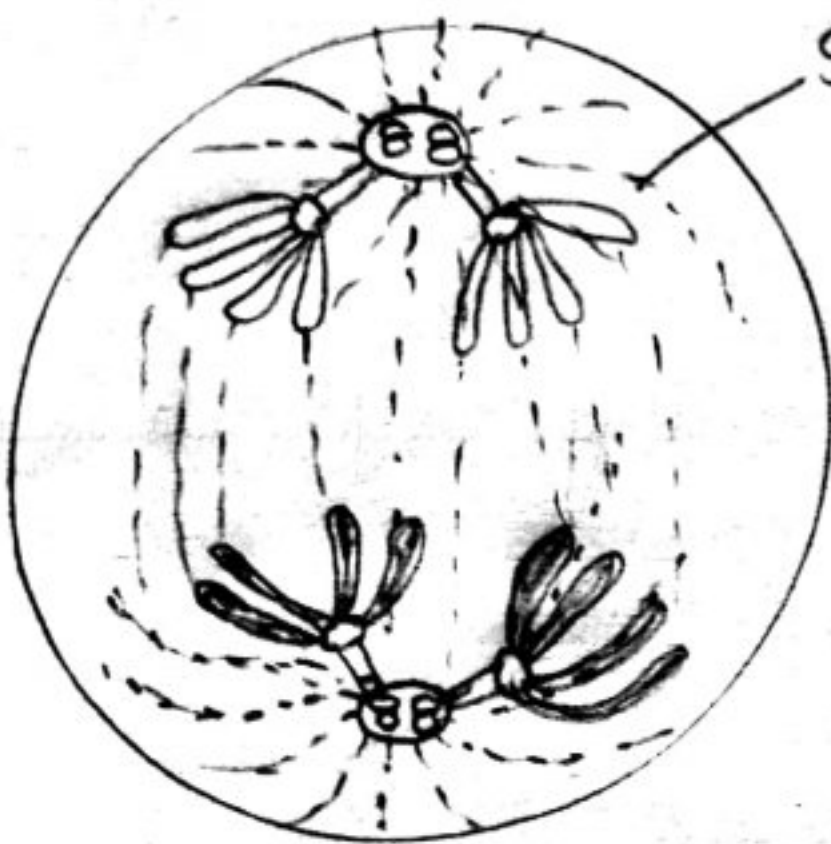


Tetrads
Disintegrating
nuclear
membrane

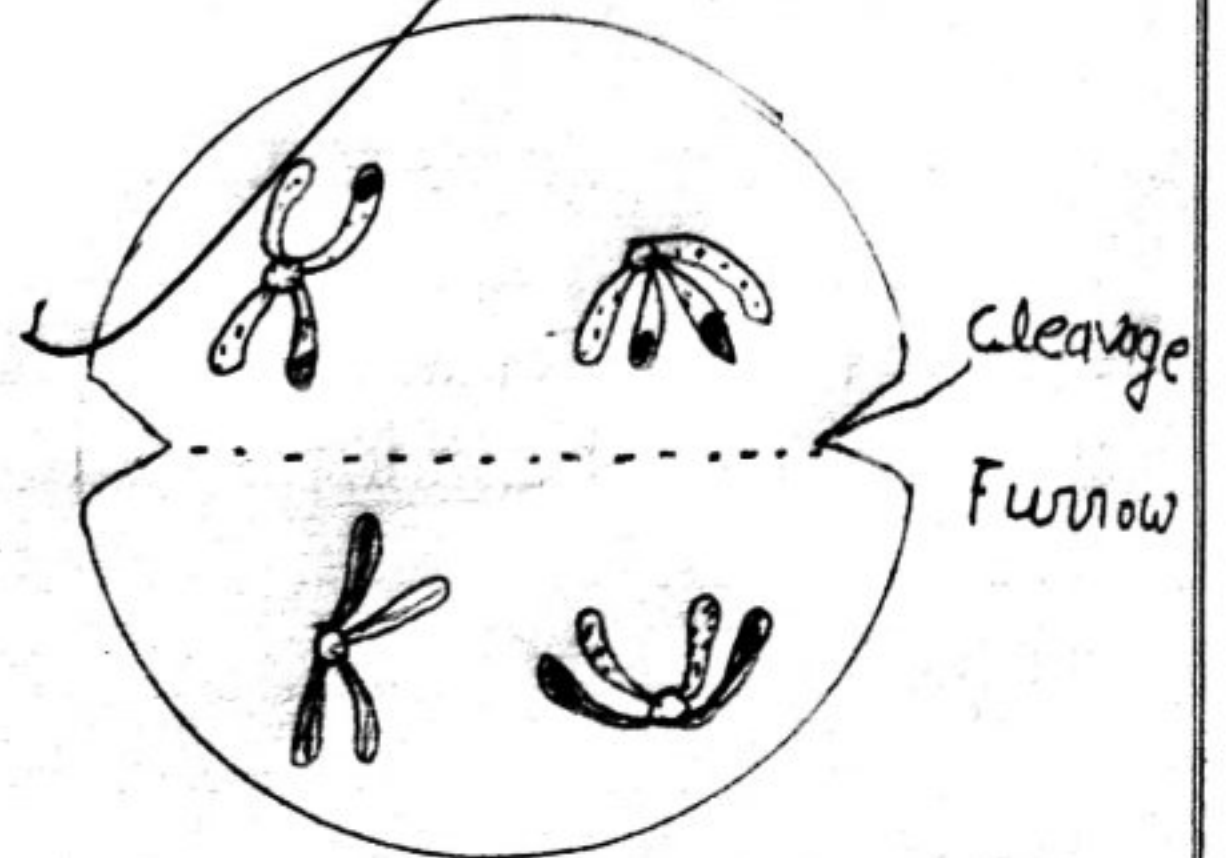
Diakinesis



Metaphase



Anaphase



Telophase

(iv) Diplotene -

Chromosome Condense Further.

Homologous Chromosome start separating after crossing over.

(v) Diakinesis -

Chromosome Condense Further.

The nuclear envelope disintegrate and end nucleolus disappears. This mark the end of Prophase.

B. Metaphase I -

Diakinesis is Followed by Metaphase.

Spindle Fiber apparatus is assembled.

C. Anaphase I -

The Spindle Fibers contract.

The Homologous Chromosome of each pair move to the opposite poles.

D. Telophase I -

1. Chromosome collect at the 2 poles and begin to uncondense.

2. Nucleolus and nuclear Membrane reappear.

3. The cell has 2 haploid nuclei.

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MEIOSIS II -

It is similar to Mitosis. It is also called "Equational Division".

A. Prophase II -

Chromosome condense into thick threads.
Nuclear Membrane and nucleolus disappear.

B. Metaphase II -

Spindle Formation is completed.
Chromosome condense further.

C. Anaphase II -

Chromatid separated by the splitting of centromere.
Spindle Fiber contract and the chromatid are pulled apart.

D. Telophase II -

Chromosome collect at the poles, uncondense and form a chromatin network.
Nuclear Membrane and nucleolus are formed again.

POLYTENE CHROMOSOME (SALIVARY GLAND CHROMOSOME) —

Polytene Chromosome were first seen in 1881 by E. G. Balbiani but he could not recognise them as chromosome in 1933 painter called the chromosome.

These are found in secretory cell of Malpighian Tubules, Rectum, Gut, Salivary gland. These are produced by endomitosis.

In this process the homologous chromosome pair and replicated again to again.

All replicated chromosome form a Giant Many filament.

The chromosome in *Drosophila* it contain 1024 chromosome identical chromatin.

The size of cells containing these chromosome also increases. These chromosome are easily seen in light

Microscope. When polytene chromosome are stained and seen in light

Microscope. Alternating dark band and light band (inter band). The band are also called as - Chromomeres represent heterochromatin.

The band are formed due to tight coiling of the 30nm fibers of all 104 chromatin.

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Right Arm of Chromosome
(3)

X Chromosome

Chromosome (4)

Chromocentromere

Left Arm

Right Arm

Band

Puff

Balbiani Ring

Band

Interband

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BALBIANI RING —

In addition to band and interband there are regions in the polytene chromosome which at times become swollen. These swollen areas are called puffs. Larger puffs are called as Balbiani Rings.

STUDY OF PARASITE

PLASMODIUM (PROTOZOAN PARASITE)

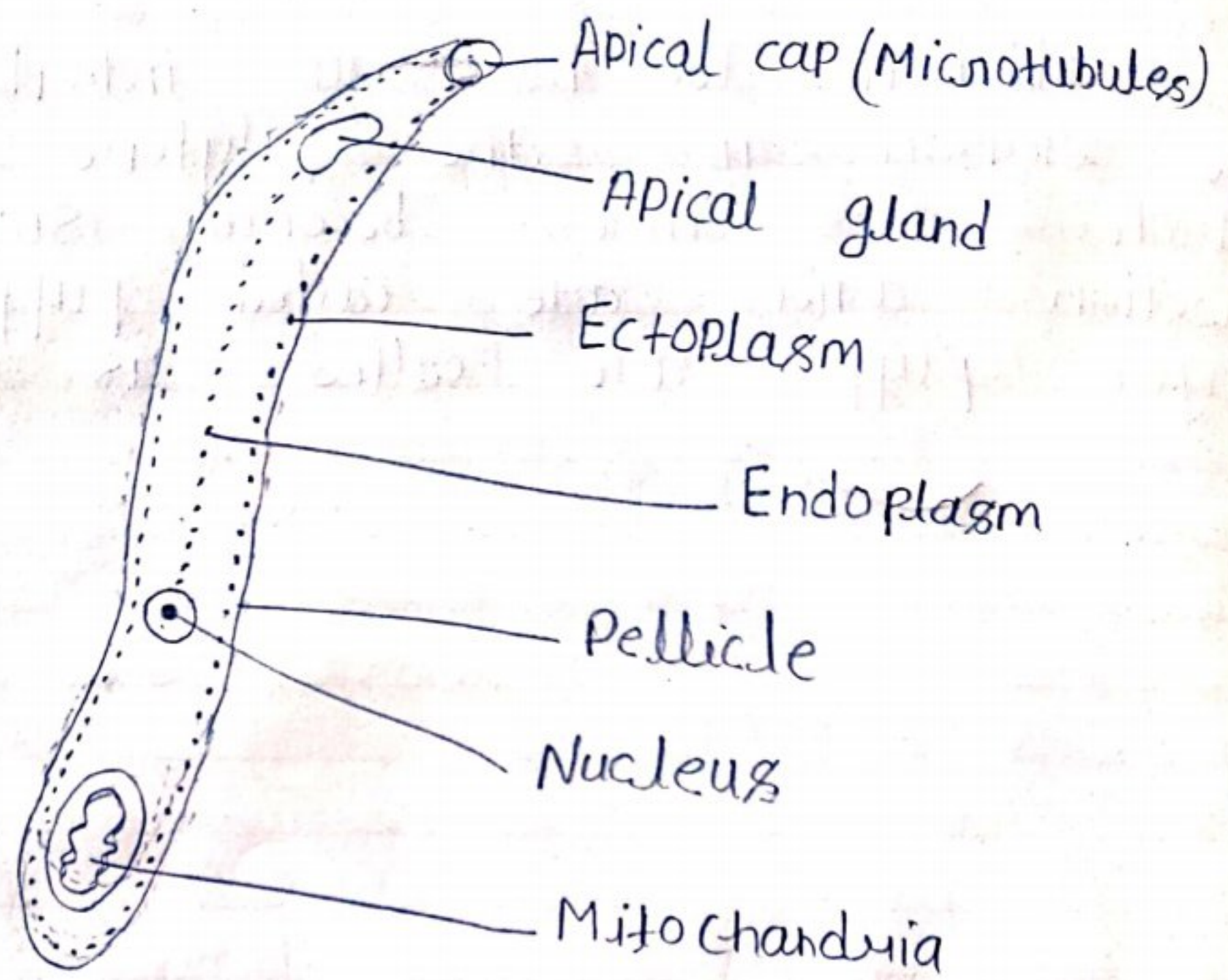
CLASSIFICATION —

Phylum	Protozoa
Class	Telosporea
Order	Eucoccididia
Type	Plasmodium vivax

Comments —

1. Unicellular, Microscopic, simple animalcule.
2. A blood parasite of man and other vertebrates, it causes Malarial Fever.
3. It is Digentic i.e. it completes its life cycle in two hosts - Man and Mosquito.
4. Its life cycle is divided into (A) Asexual cycle and (B) sexual cycle.

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PLASMODIUM

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ENTAMOEBIA HISTOLITICA

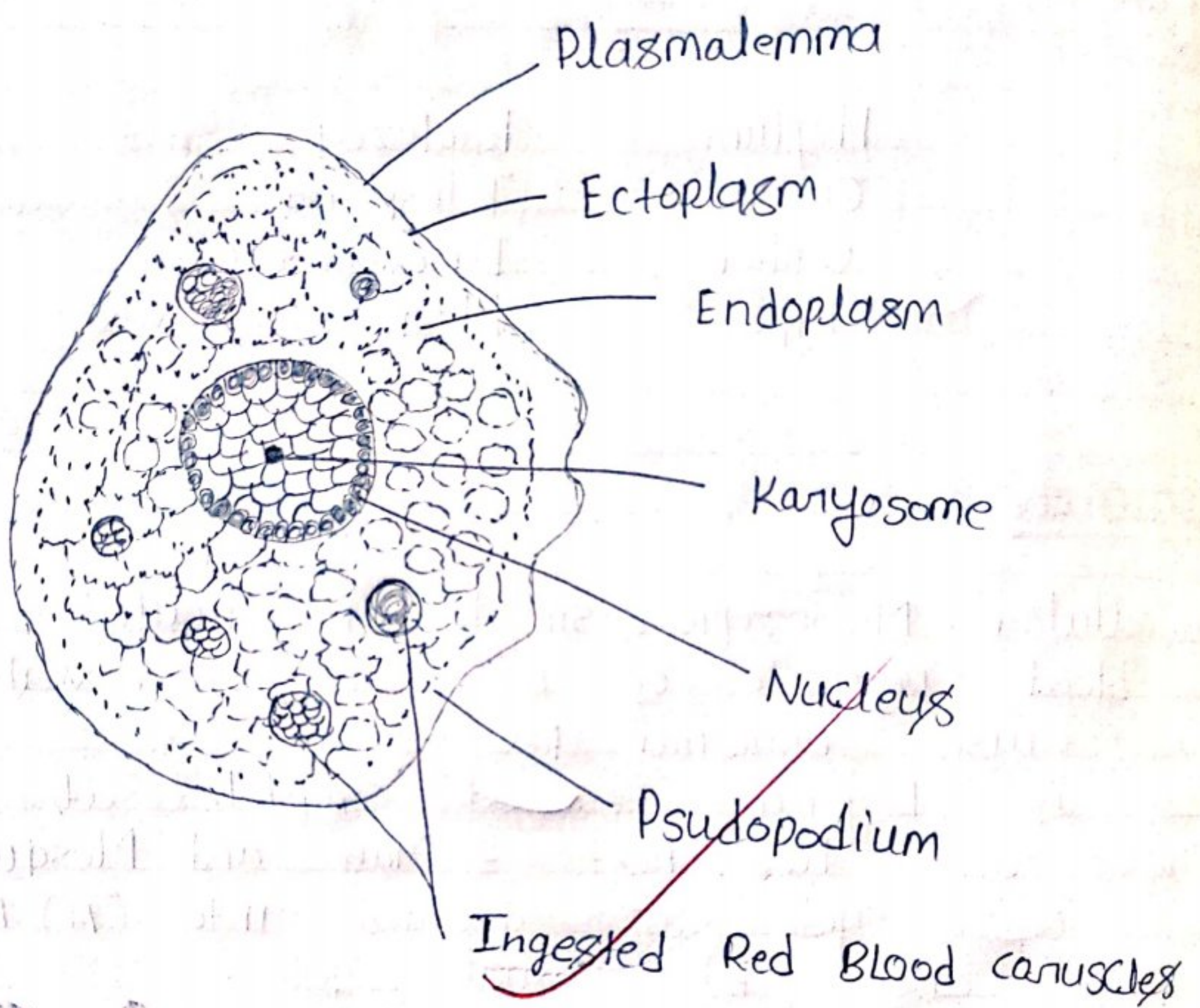
CLASSIFICATION —

Phylum	Protozoa
Class	Rhizopoda
Order	Lobosa
Type	Histolitica (Entameba histolitica)

Comments —

1. Unicellular, Microscopic, simple animalcule.
2. Entameba histolitica is a parasite in the human intestine and causes amoebic dysentery.
3. Cytoplasm is divided into clear hyaline ectoplasm and granular endoplasm.
4. Nutrition is holozoic and it feeds on the cell on the intestinal wall.
5. Nucleus is like the Trophozoite stage.
6. Tetranucleate cysts are the infective stage. They pass out with stools.

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ENTAMOEBA
HISTOLITICA

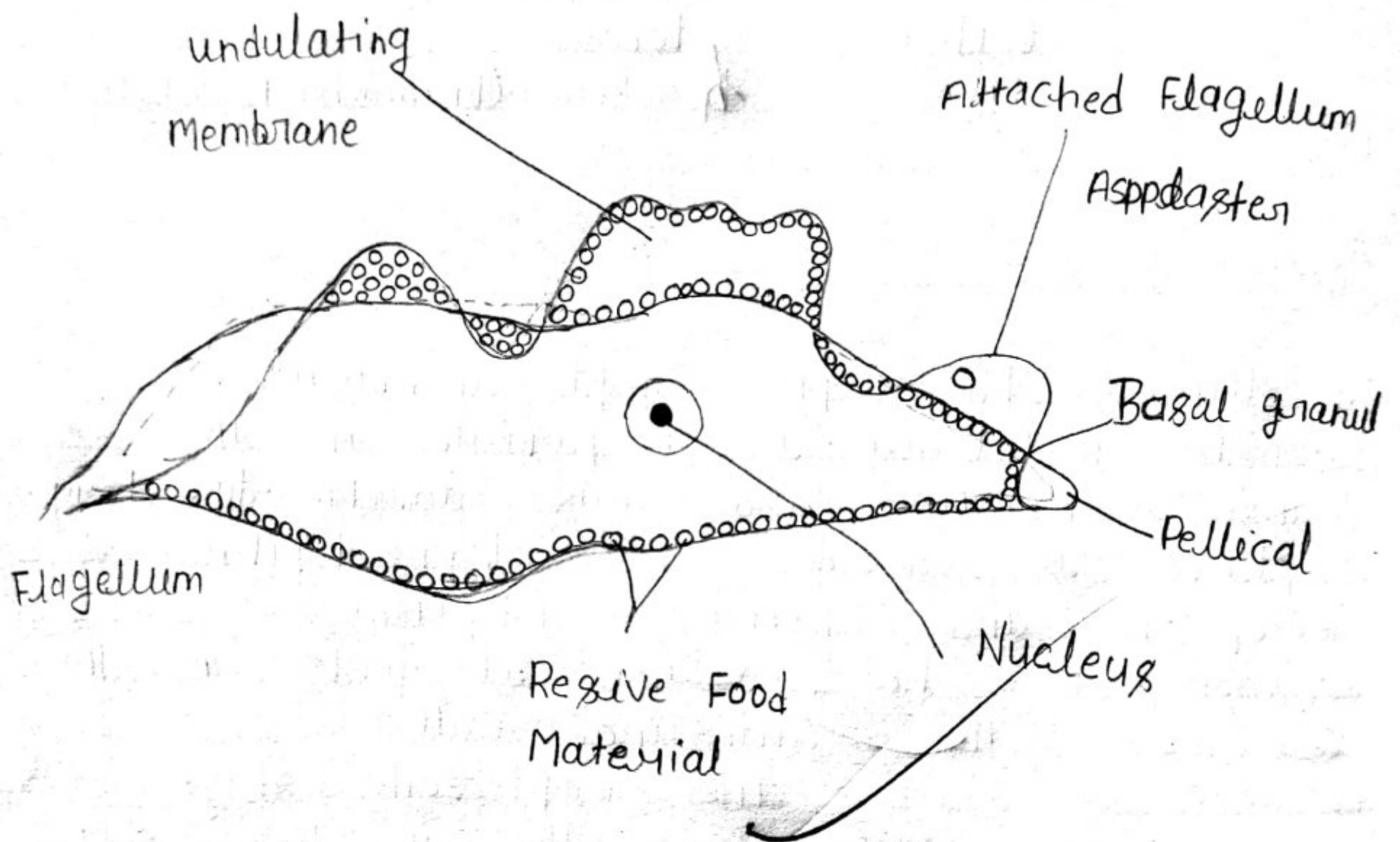
TRYPANOSOMA

CLASSIFICATION —

Phylum	Protozoa
Sub Phylum	Saccomastigophara
Class	Zoomastigophara
Order	Kinetoplastida
Type	Trypanosoma

Comments —

1. Unicellular, Microscopic, simple animalcule commonly found in Africa.
2. It is an endoparasite found in blood and other of Man and other vertebrate.
3. It is a ~~polymorphic~~ species existing in two distinct forms.
4. A thin elastic pellicle covers the whole body.
5. A single flagellum arises from a basal body placed near the posterior end.
6. Reproduction take place by longitudinal binary fission.



TRYPANOSOMA

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GIARDIA

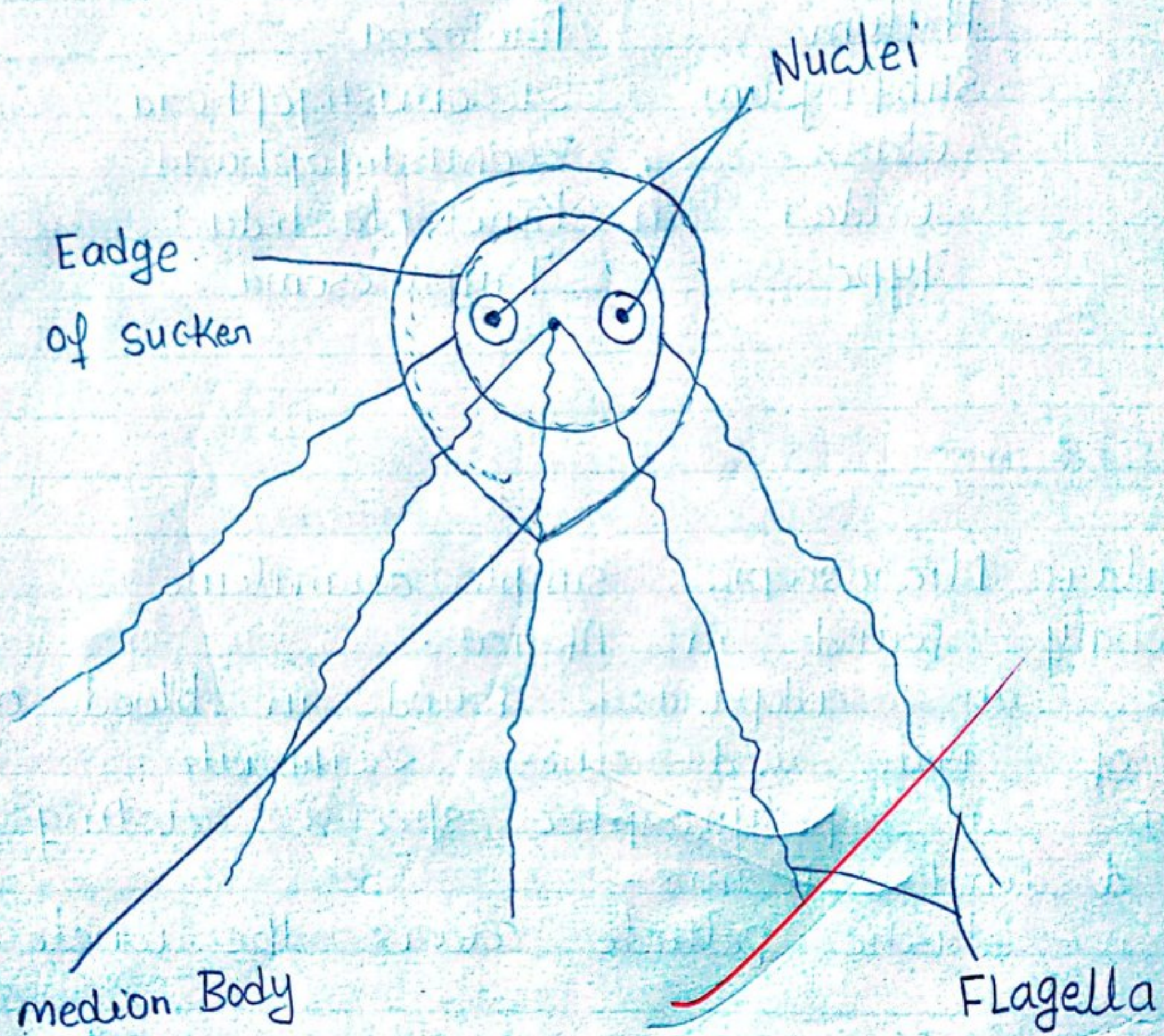
CLASSIFICATION —

Phylum	Protozoa
Class	Zoomastigophana
Order	Diplomonadida
Type	Giardia.

Comments —

1. unicellular, Microscopic, simple animalcule.
2. It lives in the intestine of man as an endoparasite and causes Giardiasis.
3. It has a pear shaped body measuring 9 - 21 microns in length and 5 - 15 microns in width.
4. It has two nuclei and bears 4 flagella on either side of the body.
5. An axostyle in the median region of the body forms its longitudinal axis.

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GIARDIA

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HELMINTHS PARASITE

FASCIOLA HEPATICA

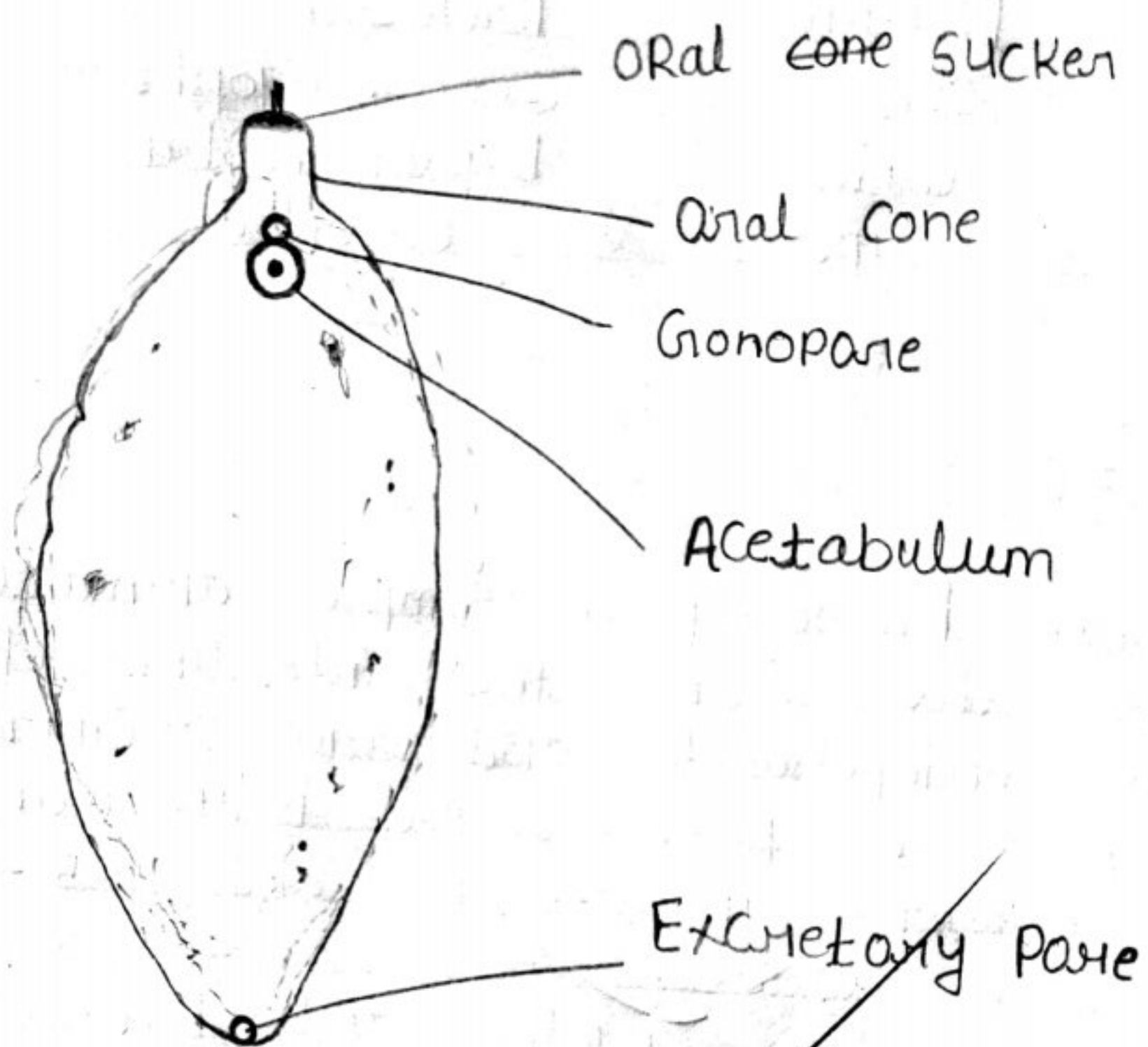
CLASSIFICATION —

Phylum	Platyhelminthes
Class	Trematoda
Order	Digenea
Type	Fasciola hepatica

Comments —

1. Fasciola is a common parasite flatworm.
2. Fasciola hepatica is oval, soft, flattened and leaf like.
3. It lives in liver and bile passage of vertebrate sheep, cattle, goat dog and man.
4. Fasciola hepatica is a digentic trematode.
5. Its life cycle complete in two host.
A primary host is vertebrate and
Secondary host is invertebrate.

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FASCIOLA HEPATICA

TAENIA SOLIUM

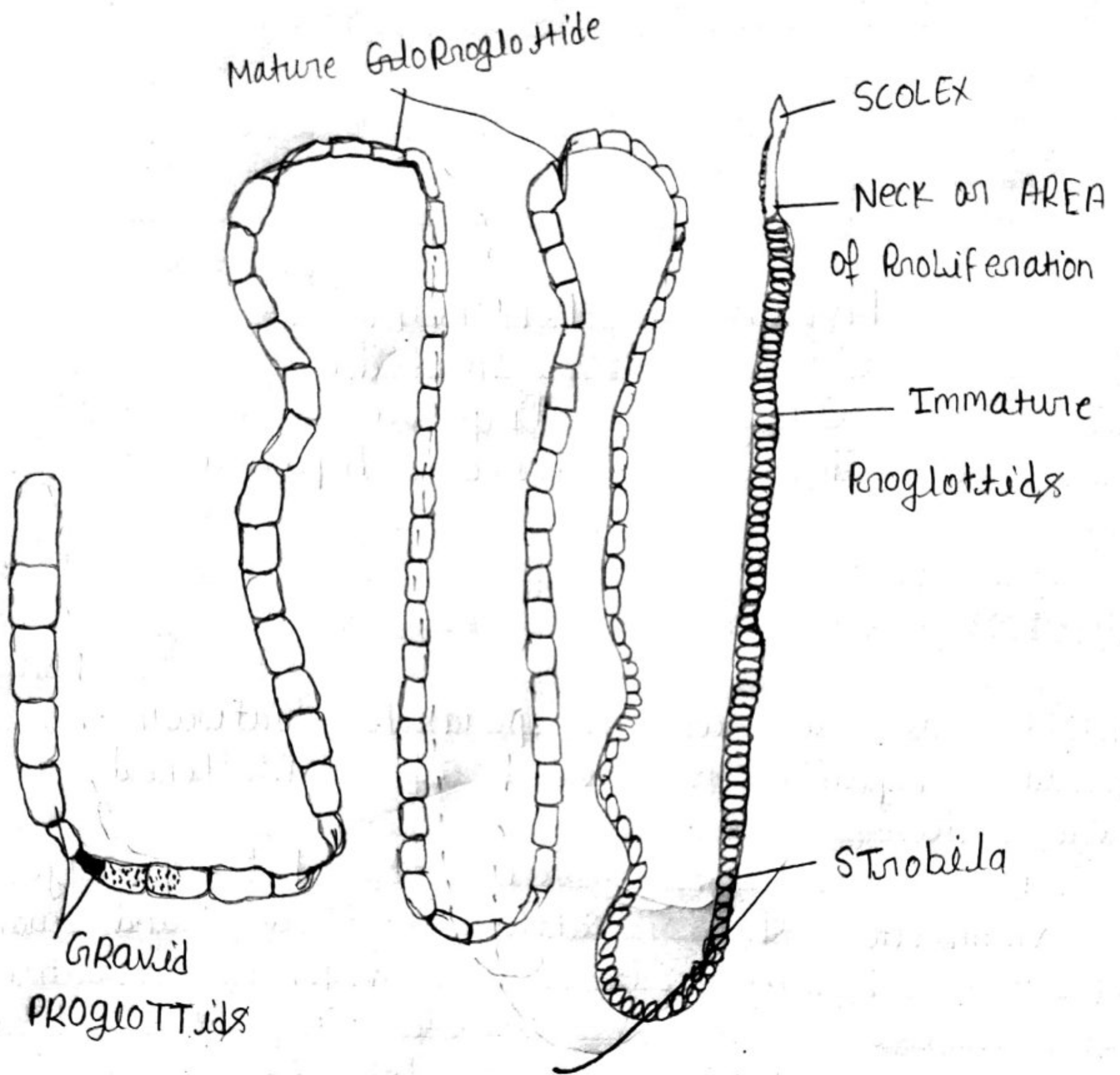
CLASSIFICATION —

Phylum	Platyhelminthes
Class	Cestoda
Order	Taeniidea
Type	Taenia solium

Comments —

1. Taenia Solium is a long, dorsoventrally flattened, ribbon-like.
2. It is found lives in the intestine of man leading an endoparasite life.
3. Taenia Solium is a digentic.
Its life cycle completes in two host - primary host is the Man and secondary host is the pig.
4. It is found in pork tapeworm of man.
5. Taenia solium is exclusively endoparasite.

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TAENIA SOLIUM

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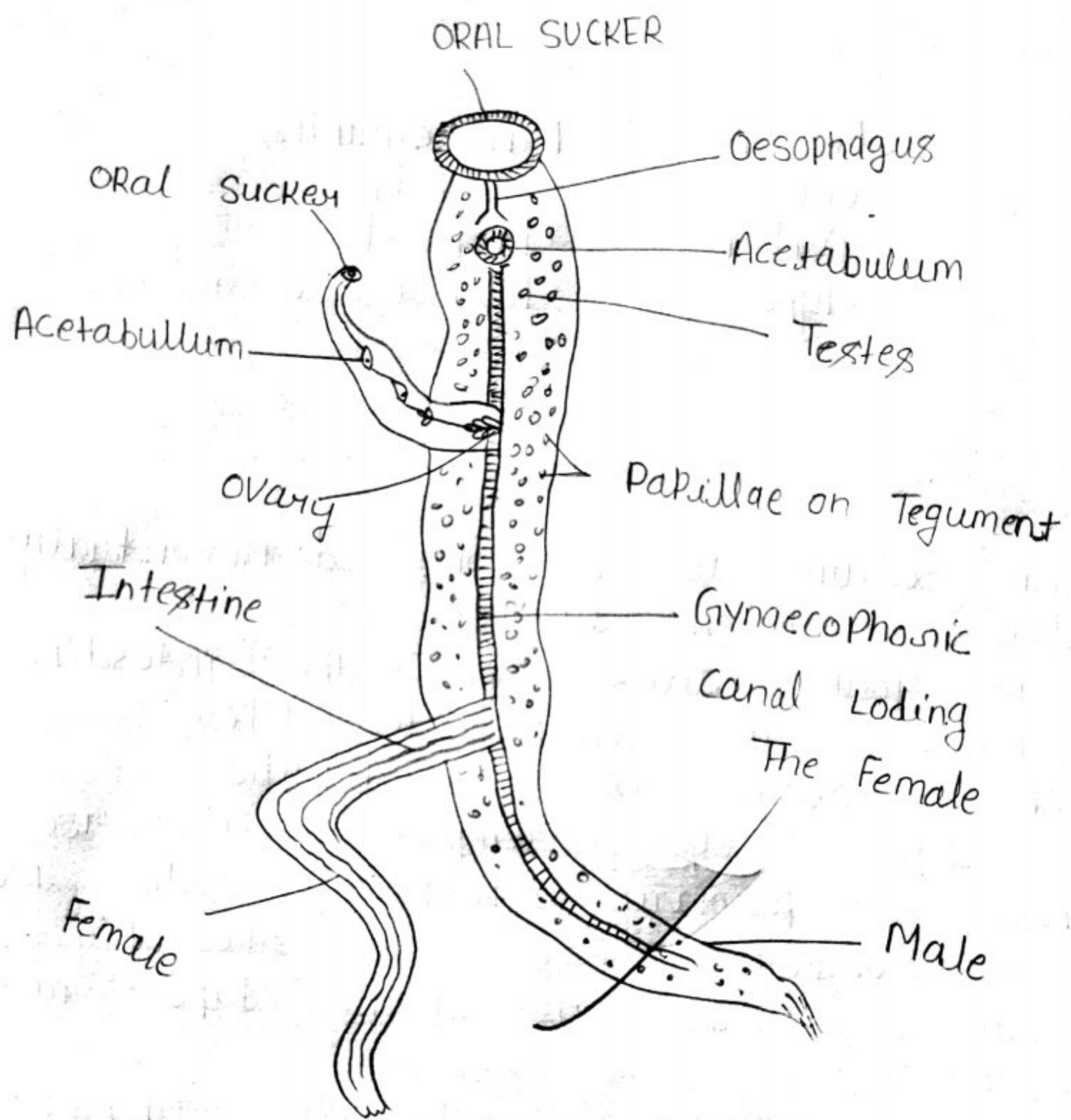
SCHISTOSOMA

CLASSIFICATION —

Phylum	Platyhelminthes
Class	Trematoda
Order	Digenea
Type	Schistosoma

Comments —

1. Bilaterally symmetrical, dorsoventrally flattened acoelomate worm.
2. It is commonly called the blood fluke of man.
3. It inhabits the blood vessels of the abdominal cavity and causes a disease called Schistosomiasis.
4. It shows well defined sexual dimorphism.
5. The tegument has many fine tubercles.



SCHISTOSOMA

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ECHINOCOCCUS GRANULOSUS

CLASSIFICATION —

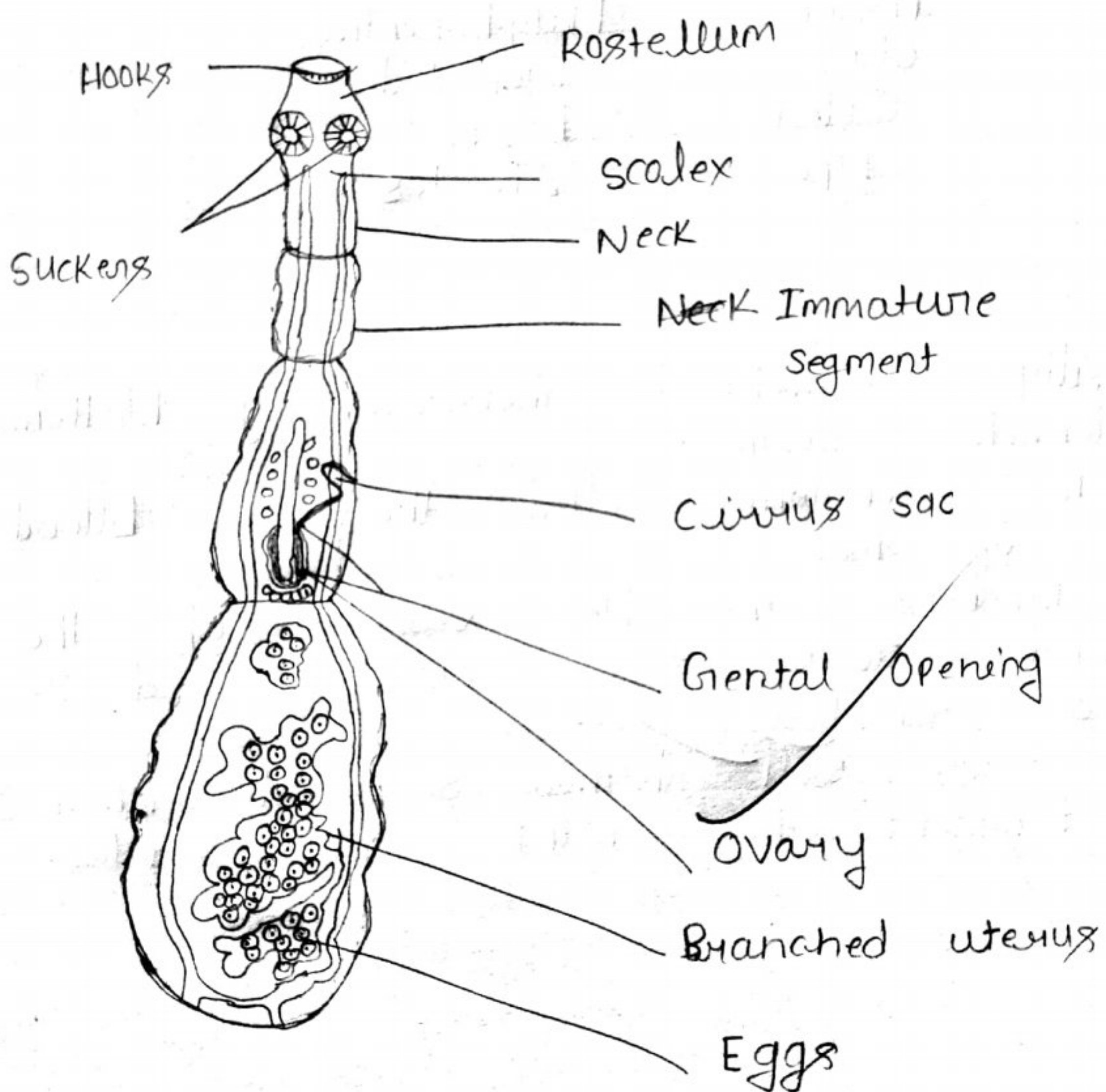
Phylum	Platyhelminthes
Class	Cestoda
Order	Cyclophyllidea
Type	Echinococcus granulosus

Comments —

1. The hydatid cyst is a single chambered, spherical ball full of cystic fluid.
2. A fully developed cyst has a three layered cyst wall containing pericyst, ectocyst and endocyst.
3. A Number of germinal vesicles called brood capsules are formed by budding from the endocyst.
4. Brood capsule can have numerous internal scolices with hooks and suckers.
5. These are infective stages that infect a new host.

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25/12/2021

Signature



TYPE OF CHROMOSOME ABERATION -

There are four Method by which structure of chromosome can be and are as follow -

1. Deletion
2. Duplication
3. Translocation
4. Inversion

1. DELETION -

Which shows loss of part chromosome deletion, It is also called as deficiency in this phenomenon chromosome breaking occurs due to radiation and chemical bond.

Deletion are of two type -

2. Terminal Deletion -

Loss of chromosomal part near the end called deletion.

A	B	C	D	E	F	○	G	H	I	J	K	L
---	---	---	---	---	---	---	---	---	---	---	---	---

B	C	D	E	F	○	G	H	I	J	K	L
---	---	---	---	---	---	---	---	---	---	---	---

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(b) Interstitial Deletion —

Loss of Chromosome from middle in a middle portion is called Interstitial deletion.

A	B	C	D	○	E	F	G	H	
---	---	---	---	---	---	---	---	---	--

A		C	D	○	E	F	G	F	
---	--	---	---	---	---	---	---	---	--

2. DUPLICATION —

In this phenomenon Chromosomal part duplicate in one Chromosome are of following type.

(a) Tandem Duplication —

In this case duplicated part lies just on the side of the original chromosome.

A	B	C	D	E	F	○	G	H	I	J	K	L
---	---	---	---	---	---	---	---	---	---	---	---	---

A	B	C	D	E	F	G	F	G	H	I	J	K	L	
---	---	---	---	---	---	---	---	---	---	---	---	---	---	--

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(b) Reverse Tandem Duplication —

In this case the duplicated part get reverse.

A	B	C	D	E	F	G	G	F	H	I	J	K	L
---	---	---	---	---	---	---	---	---	---	---	---	---	---

(c) Displace Duplication —

In this case duplicated part is not adjacent but it is displaced.

A	B	C	D	E	F	G	H	I	J	K	L
---	---	---	---	---	---	---	---	---	---	---	---

A	B	C	D	E	F	G		H	I	
---	---	---	---	---	---	---	--	---	---	--

3. TRANSLOCATION —

It is a segmental change of chromosome in which chromosome