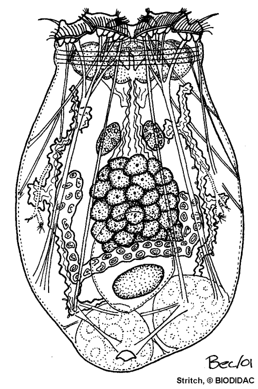
Basal Lophotrochozoa

The Rotifera and Platyhelminthes are well supported as part of the Lophotrochozoa. Most molecular studies have supported the basal position of the branches containing these two groups relative to the branch containing Mollusca and Annelida. However, there is some controversy surrounding the question of the monophyly of the branches containing these two groups. Some studies have supported a clade Platyzoa, comprising Platyhelminthes, Rotifers, Acanthocephala, Gnathostomulida, and Gastrotrichs. However, other studies have found the Platyzoa to be polyphyletic, placing the Platyhelminthes on a branch just basal to the branch containing Mollusca and Annelida, and placing the Rotifera in the most basal clade of lophotrochozoa along with the Acanthocephala and Gnathostomulida. As a result, many current trees show these branches as dotted lines, or polytomies. Hopefully advancements in phylogenetic techniques and additional molecular evidence will help to clarify the relationships of these groups.



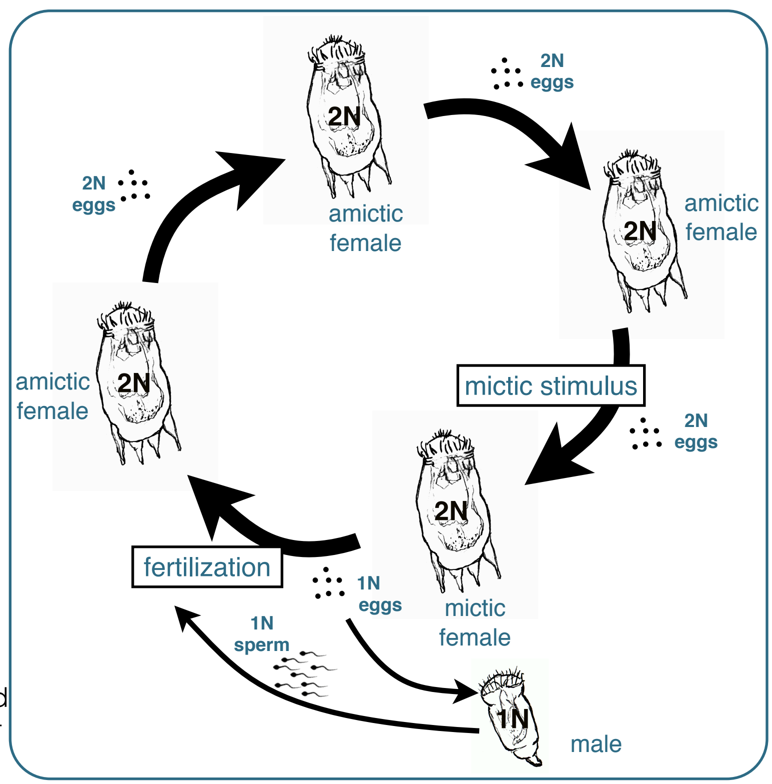
planktonic rotifer

phylum rotifera

Rotifers

Rotifers represent the smallest multicellular animals. They are common in freshwater environments although some species are marine or even "terrestrial". (Some rotifer species are common on terrestrial mosses, living in the water film which surrounds them.)

One of the most prominent features of rotifers is the **wheel organ** or **corona**, a circular arrangement of cilia around the mouth that generates a current which directs food particles into the mouth. The corona is also used for locomotion in motile rotifers.

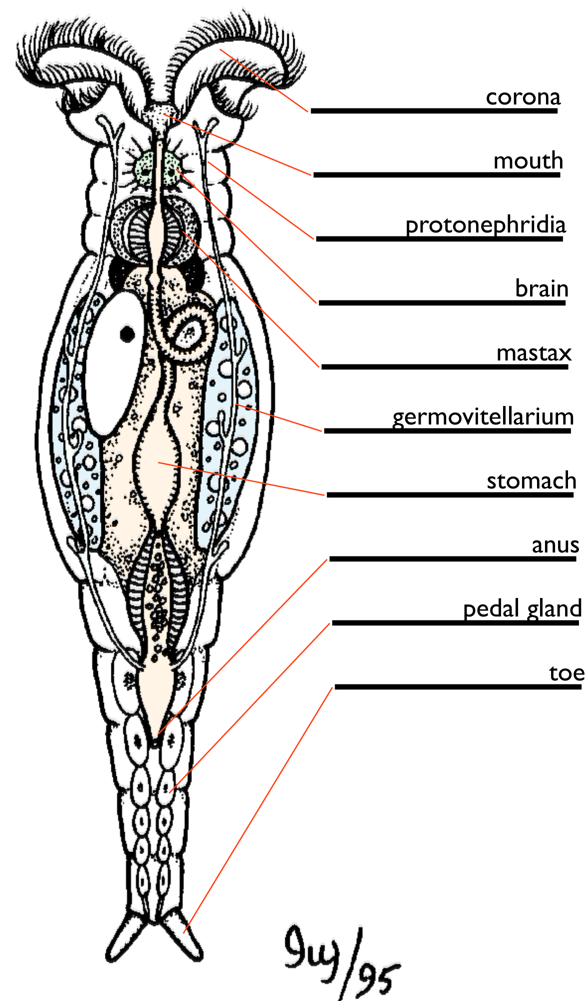


Rotifers comprise a great deal of diverse forms, and are generally classified as either sessile, floaters, or creepers. Sessile rotifers attach to a substrate by a holdfast. They are usually more wide-mouthed with a tapered **foot**. Planktonic floaters are usually more globular and less elongated. Creepers or swimmers are have more elongated bodies that can be extended or contracted. The **pedal glands** of creepers allow them to attach to a substrate while feeding. Creepers will often swim using their wheel organ, but may attach to a substrate or a piece of debris for an extended amount of time.

Mixis

Many rotifers are parthenogenetic, and exist only as females. However, even in the rotifer groups that possess males, males only exist during times of stress. These species undergo a special life cycle, alternating between asexual and sexual modes of reproduction. During relatively stable periods, there are only females in the population. These are referred to as **amictic** females, and produce diploid eggs that develop into amictic females. However, under stressful conditions, these females produce eggs that develop into **mictic** females. Mictic females produce haploid eggs. If the eggs are not fertilized, they develop anyway, but become males (sex in rotifers is determined by haplodiploidism). These males produce sperm to fertilize haploid eggs. If an egg is fertilized, it remains dormant until conditions improve, at which time it develops into an amictic female. This process allows rotifers to benefit from the genetic variation provided by sexual reproduction in stressful environments.

* Examine the prepared rotifer slide using the light microscope.



Livingstone, © BIODIDAC

Classification

The rotifers have traditionally been divided into three classes as listed below. However, there is some debate concerning the monophyly of the Rotifera. There is some molecular support for the idea that the Phylum Acanthocephala belongs inside the Rotifera. This issue remains unresolved, but would affect division of classes as shown below.

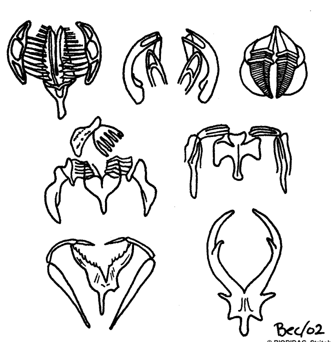
Class Seisonidea - Marine epizoic (i.e. living on another animal) rotifers comprising a single genus (*Seison*).

Class Bdelloidea - This group of freshwater and terrestrial rotifers are common in freshwater sediments. *Philodina sp.* which we will observe are members of this class. This group is parthenogenetic, with only female individuals. The bdelloids possess two germovittelaria, unlike the class Monogononta, whose members have only one. May be creepers, floaters or sessile.

Class Monogononta - Mictic rotifers are in this class. Monogononta possess only one germovitellarium, as their name implies. May be creepers, floaters, or sessile.

Organization

The bdelloid rotifers possess a well developed wheel organ with two distinct **trochal discs**. The wheel organ gets its name from its apparent spinning motion. However, it should be noted that the wheel organ does not actually spin. The motion of the cilia on its margin simply makes it appear as if it is spinning. These cilia generate a current of water that helps to channel food particles into the mouth.



various mastax forms

Inside the mouth, the **mastax** masticates food particles for easier digestion. Food passes through the esophagus into the stomach where most of the digestion and absorption of nutrients occurs. The stomach is the largest visible organ in the body. Just posterior to the stomach, food passes into the short intestine.

A set of four tiny toes on the end of the **foot** allow the rotifer to attach to a substrate for long or short periods of time. The **pedal glands** produce adhesive substances for attachment to a surface.

When attached to a substrate, you may notice the body of the rotifer extend or contract. Rotifers such as *Philodina* possess a bilobed brain with a pair of eyespots which help to sense the presence of light. They will often respond to stimuli by shortening the body. The apparent segmentation noticeable on the outside of the body is actually just segmentation of the **lorica**, a hard covering which surrounds the body and is secreted by the syncytial epidermis.

* Prepare a wet-mount slide using a sample of live rotifers (*Philodina sp.*). Draw your specimen and label the following structures: antenna, corona, brain, mastax, stomach, foot, lorica.

Anhydrobiosis

Rotifers possess the ability to desiccate and withstand long periods without water. Especially in terrestrial species, **anhydrobiosis** is an important adaptation that allows rotifers to exist in an environment with only intermittent water availability. When observing rotifers on a wet-mount slide, after a few minutes when the water begins to evaporate, the rotifers may start to become less active. As the slide dries, the rotifers will encyst themselves to withstand the drying process.

phylum platyhelminthes

The flatworms are regarded as one of the most simply organized members of the Bilateria. They are bilaterally symmetrical triploblastic organisms that are found in a variety of environments. The term "flatworm" refers to the flattening of the body that is characteristic in this group (Platyhelminthes = Gr. [*platy*, flat] + [*helmins*, worm]). The flat body of flatworms is an adaptation that allows relatively larger body size, while maintaining a relatively high surface-area-to-volume ratio. The relatively large surface are of flatworms is important, considering that they possess no circulatory system, no coelom, and exchange materials principally by diffusion across the body surface.

Classification

Flatworms are currently divided into four classes. Most evidence supports the Monogenea and Cestoda as sister taxa, with the Trematoda as a basal group, and the Turbellaria basal to the other three groups. However, the Turbellaria may represent several lineages, one of which is more closely related to the branch containing the parasitic flatworms. The classes are as listed below:

Class Turbellaria - This class contains the free-living flatworms. Turbellarians may be marine, freshwater, or terrestrial. The term "planaria" is used to refer to freshwater and terrestrial turbellarians with a spade-shaped head. The Turbellaria are probably polyphyletic. There are two orders defined in part by the structure of the gut: Polycladida (multiple branching gut) and Tricladida (triple branching gut).

Class Monogenea - Monogenic flukes. The Monogenea are ectoparasites often found on the gills of aquatic hosts. Unlike the "true flukes," monogenic flukes have only one host, as their name implies. They attach to a host using a special posterior attachment organ called an opisthaptor. Monogenic flukes feed mainly on mucus secretions and debris present on the gills.

Class Trematoda - Flukes. The digenic flukes usually have two hosts: a intermediate host and a definitive host. The intermediate host is often a mollusk, and the definitive host is usually a vertebrate. The adult fluke attaches to the primary host using its oral sucker. Flukes may have complex parasite-host life cycles.

Class Cestoda - Tapeworms. Cestoid flatworms are usually intestinal parasites of vertebrates. There are two regions of the body, the scolex and the strobilum. Tapeworms attach to their host using hooks and suckers located on the scolex. Since they generally inhabit the small intestines of vertebrates, tapeworms do not possess a digestive system. Pre-digested nutrients are simply absorbed from the intestinal lumen of the host.

Organization

Flatworms possess a relatively simple digestive system, consisting of a **gastrovascular cavity** with a single opening, the **mouth**. The gastrovascular cavity possesses three (triclad) or more trunks (polyclad) and is extensively branched to increase its surface area, allowing for more efficient digestion and absorption of nutrients. Absorption of nutrients occurs across the columnar epithelial cells that comprise the gastrodermis. Digestion is both extracellular and intracellular. Nutrients diffuse into adjacent tissues without the help of a circulatory system, which is lacking in the flatworms.

Turbellarians possess the most extensive digestive system in flatworms. They possess a mid-ventral extensible **pharynx**, that is used to ingest food items. It is enclosed in the pharyngeal sheath and can be extended through the mouth.

The parasitic lifestyle of tapeworms and flukes often makes digestion unnecessary, since nutrients can be absorbed directly from their host. The flukes usually have a wishbone-shaped gastrovascular cavity with less branching. The tapeworms lack a digestive cavity altogether.

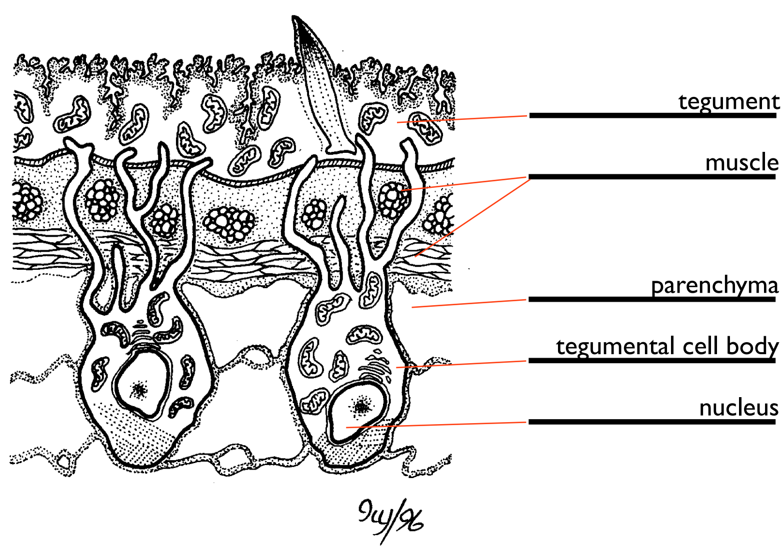
✱ Observe the planaria WM slide with the gastrovascular cavity stained.

The excretory organs of flatworms are **protonephridia**. The main purpose of protonephridia is to maintain water balance, especially in freshwater planaria, in which large amounts of water are secreted. The protonephridia consist of two long trunks of **tubules** spanning the length of the body on the left and right. Fluid enters this tubule system through flame cells, located at the blind ends of certain tubules. The **flame bulb** consists of a **flame cell** (or cap cell) that forms the end of the tubule, with a set of joined flagella, directed toward the interior of the tubule. These flagella beat back and forth to create flow of interstitial fluid into the tubule. Along the length of the tubule, the composition of the filtrate is changed by selective absorption and secretion of materials. The filtrate exits the body via a **nephridiopore** in the outer body wall. Multiple nephridiopores are located down the length of the body on either side.

✱ Examine the live planaria using a dissecting scope.

Planaria adhere to a substrate using the mucous covered ventral aspect of their body. They glide along this mucous membrane through ciliary action. The ventral surface of the planarian produces tiny rod-shaped adhesive structures called **rhabdites**. These rhabdites allow the worm to attach to a substrate. Flatworms can also swim via undulatory movements of the body.

Sensory structures in planaria are mostly concentrated on the anterior end of the body. The **auricles** contain chemosensory cells and cells for tactile sensation. Also noticeable on the anterior end are two large **eyespots** or **ocelli**. These clusters of photosensitive cells are used to sense light and dark. Most planaria exhibit **negative phototaxis**. While observing a live specimen, it can be difficult to keep the worm in the field of view due to its tendency to move away from the light source.



Reproduction in flatworms can be asexual or sexual. Planaria reproduce asexually through **binary fission**. Regenerative abilities allow planaria to regrow missing parts of the body, so that when an individual splits in multiple parts either because of injury or for reproductive purposes, each part regrows what it is missing. If the body is split into anterior and posterior halves, the posterior end grows a new head, and the anterior end grows a new posterior end. Most flatworms are **monoecious** (i.e. there are no sexes). **Hermaphroditic** individuals cross-fertilize to produce embryos.

Flukes

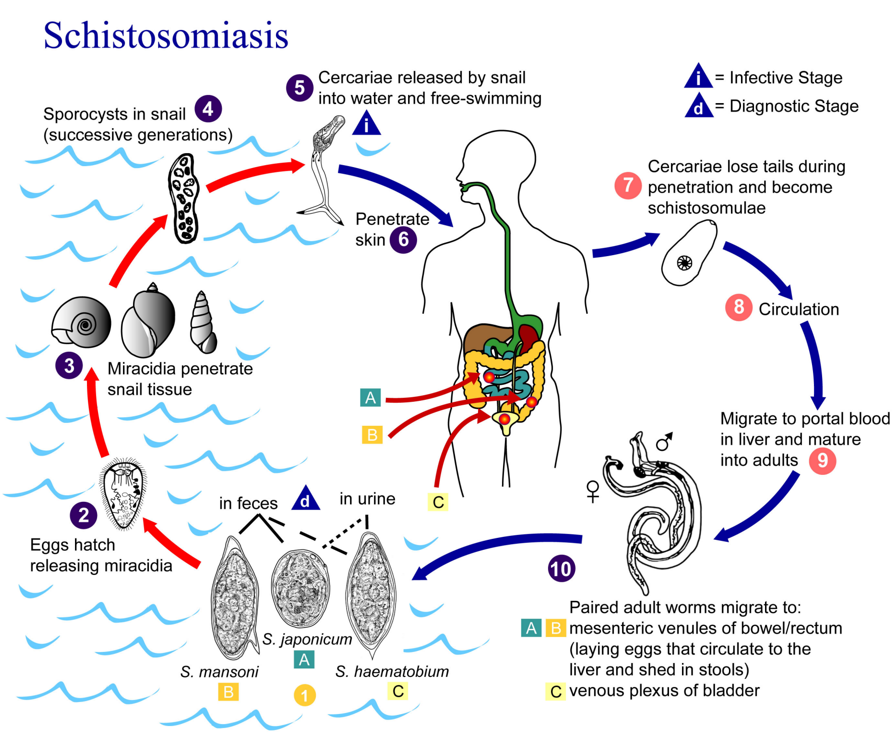
The digenean flukes have flat, roughly elliptical bodies. An oral sucker allows them to attach to a host. The outermost layer of the body is a syncytial **tegument** with spines (but no cilia). A layer of muscle tissue lines the interior of the tegument. The tegument possesses multiple nuclei, which are contained in "**cell bodies**" located just inside the layer of muscle tissue. Parenchyma cells pack the space between the gut (and other organs) and this layer of muscle. The parenchyma also fill in the spaces between the cell bodies of the tegument.


fluke cercaria

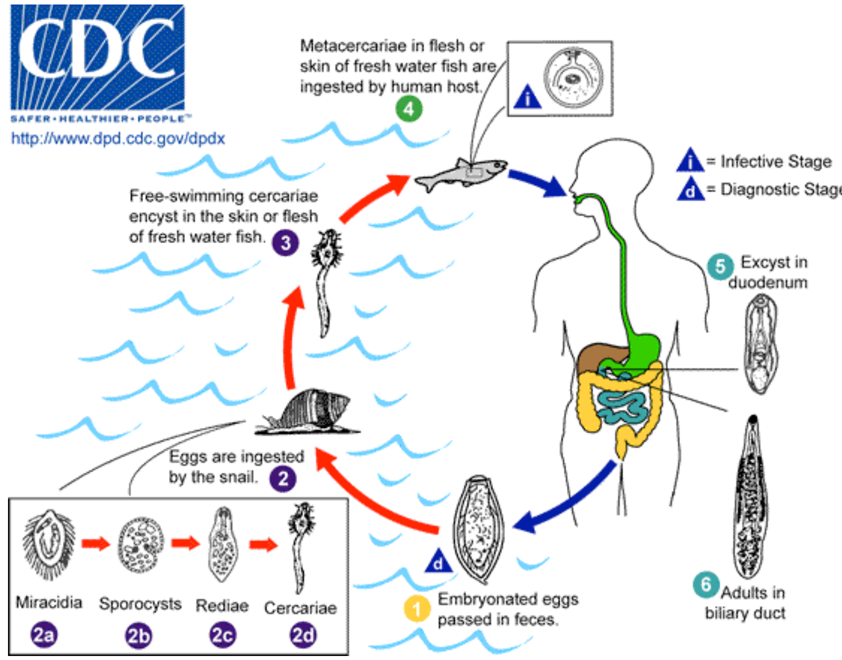
The Trematoda are all parasitic, and most have complex parasitic life cycles. In most cases, a fluke egg hatches into a free-living ciliated **miracidium** larvae. The miracidium larva usually enters into an intermediate host, often an aquatic gastropod. Inside the intermediate host, the miracidium develops into a **sporocyst**. The sporocyst uses asexual reproduction to produce **rediae** and more sporocysts. Each redia produces **cercariae** through asexual reproduction. Cercariae leave the body of the gastropod and enter the surrounding water. Cercariae (secondary larvae) may burrow into the primary host directly, or burrow into another intermediate host. Once inside a host, the cercariae encyst in the host's tissues as a **metacercaria**. If ingested by a suitable host, the metacercariae develop into the adult fluke.

Schistosoma sp.

Schistosoma sp. are blood flukes that usually infest the liver and blood vessels of their host. The clinical name for this condition is **schistosomiasis**, a common ailment in the tropics. The eggs produced by *Schistosoma sp.* can clog capillaries in the liver, resulting in scarring (cirrhosis). The eggs can also cause become lodged in the wall of the intestine or other parts of the body resulting in infection. Schistosomiasis can result in damage to the spleen, liver, genitals, bladder, brain, skin, and lungs. The intermediate hosts of schistosomes are snails. Schistosome cercariae usually burrow directly into the primary host. In the primary host, the cercariae develop into adult flukes.



Schistosomes are dioecious and sexually dimorphic. The female is very small relative to the male. Male *Schistosoma sp.* have a special canal running along the ventral surface of the body, the **gynecophoric canal**. The female resides in the gynecophoric canal, producing eggs that are fertilized by the male. The eggs are then released in the feces or urine of the host.

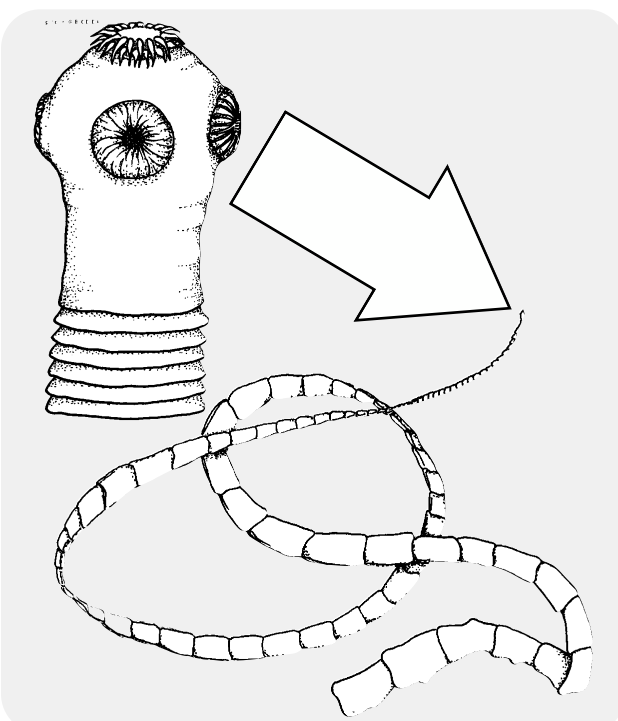


✱ Observe the prepared microscope slides demonstrating the phases of the life cycle of *Schistosoma*.

✱ Observe the prepared *Schistosoma* wm. *in copula* slide.

Clonorchis sinensis

Another group of flukes that are common parasites of humans are liver flukes (e.g. *Clonorchis sinensis*). *Clonorchis* has two intermediate hosts. Eggs are eaten by snails and hatch into miracidium larvae. The miracidia develop into sporocysts which produce multiple redia. Redia produce cercariae which migrate out of the snail and penetrate the skin of fish. Inside the fish, the cercariae encyst in muscle tissue as metacercariae. The flukes are transmitted to humans through the ingestion of undercooked fish (usually carp). The adult resides in the bile duct of the primary host, feeding on bile produced by the liver.



✱Observe the prepared slides demonstrating the phases of the life cycle of *Clonorchis*.

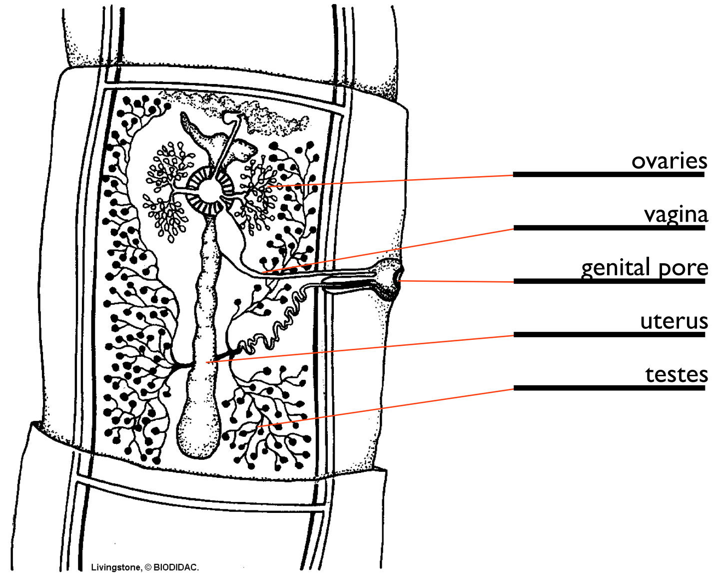
Tapeworms

Members of the class Cestoda are mostly vertebrate intestinal parasites. Tapeworms represent a group that is extremely well adapted to its parasitic lifestyle. The adult tapeworm attaches to the wall of the small intestine, and absorbs pre-digested nutrients from the lumen of its host. As a result, tapeworms possess no mouth, stomach, or digestive tract. Nutrients are simply absorbed across the tegument. The surface area of the tegument is greatly increased by the presence of tiny spines called **microtriches**, enhancing efficiency of absorption.

The body of a tapeworm consists of two parts, the **scolex** and the **strobila**. The scolex is used for attachment to the host, via suckers and/or hooks. Although the scolex might appear to represent the anterior end of the tapeworm body, this is actually incorrect. The scolex is probably homologous to the posterior end of the body of monogenic flukes. The structure of scoleces varies greatly among species, with different combinations of attachment organs, including hooks, suckers, and tentacles. Relative to the length of the strobila (which can be several meters long), the scolex is very small.

✱ Observe the *Taenia sp.* WM prepared slide. Note the scolex.

The strobila consists of a chain of **proglottids**, reproductive segments that are generated in the germinative zone at the base of the scolex. Mature proglottids contain both **ovarian** and **testicular** tissue. Tapeworms are hermaphroditic and monoecious. Although capable of self-fertilization, it is more common for tapeworms to cross-fertilize if conspecifics are present. However, when isolated they will self-fertilize.



Mature proglottids are shed at the distal end of the strobila. Once fertilized, a proglottid develops into a sort of egg case. Fertilized eggs are released when the exterior wall of the proglottid is ruptured.

* Observe the prepared slide of *Taenia sp.* WM with mature proglottids. Draw a mature proglottid and label the ovaries, testes, genital pore, uterus.

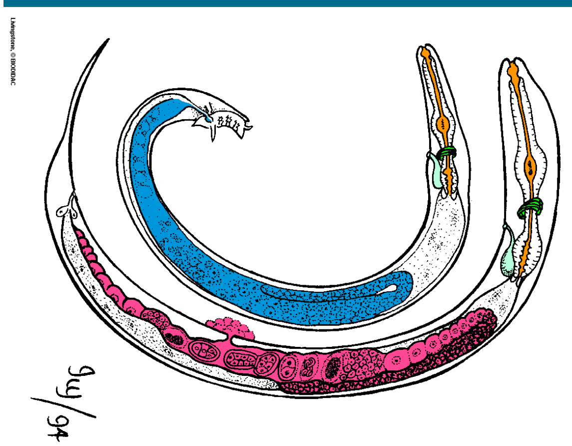
A mature tapeworm proglottid will have visible ovaries and testes. The ovaries are roughly bean-shaped, and stain darker than the surrounding tissue. The grainy tissue surrounding the ovaries are the testes. On one side of the proglottid, about halfway down its length is an opening called the genital pore. The genital pore is a common opening for the vagina and the sperm duct.

* Observe any preserved tapeworm specimens available.

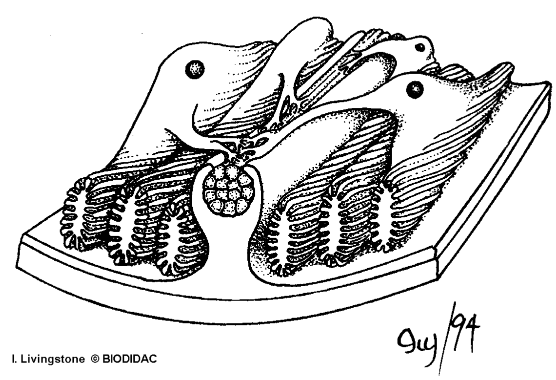
Ecdysozoa

The Nemata are ecdysozoans, a clade which contains Arthropoda, Onychophora, Tardigrada, Nemata, and Nematamorpha, among others. Although the clade Ecdysozoa was initially controversial, especially among morphological systematists, it is now well accepted and has a great deal of molecular support.

phylum nemata



The roundworms were formerly placed in a large phylum including all the pseudocoelomate protostomes, the Aschelminthes. This group include the classes Nematoda, Rotifera, Nematomorpha, Gastrotricha, Priapulida, and Kinorhyncha.



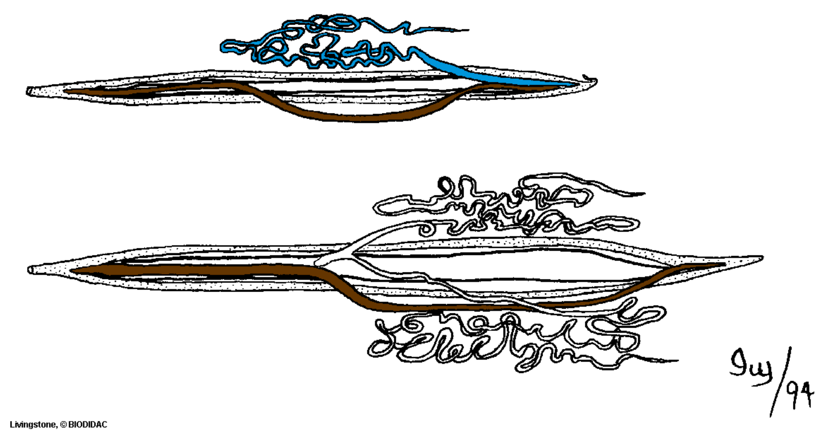
However, when this group was found to be polyphyletic, the classes were raised to the status of phyla. Since a phylum containing only the roundworms had already been described by Cobb (1919) as Nemata, this name should have been given priority. Therefore, the official recognized name of this phylum is Nemata. However, it is still very common to see the name

Nematoda used in many texts.

One of the morphological characteristics shared by ecdysozoans is the presence of a three-layered **cuticle**. In order to grow, ecdysozoans must shed this cuticle through a process called **ecdysis**. The cuticle of nematodes is composed of collagen, and is used as an external skeleton. Longitudinal muscles work in opposition to this stiff cuticle.

✱ Prepare a wet-mount slide of *Turbatrix* (vinegar eel) live specimens and observe using a compound microscope. Note: do not add water.

Organization



The cuticle of nematodes is secreted by the outermost layer of the body, a syncytial **hypodermis**. Longitudinal dorsal and ventral **hypodermal cords** enclose dorsal and ventral nerve cords. These hypodermal cords also contain the nuclei of the syncytial hypodermis. Nematodes also possess two lateral longitudinal hypodermal cords which contain the excretory ducts. **Longitudinal muscles** connect to the dorsal and ventral nerve cords via **protoplasmic arms**. Muscles work in opposition to a hydrostatic pseudocoel and the collagenous cuticle. When longitudinal muscles contract on one side of the body, the body flexes. When the muscles relax, the cuticle resumes its original shape. This allows for sinusoidal movements of the body, or simply flexing the body one way or the other.

Nematodes have a complete digestive tract, with a mouth and an anus. However, ecdyzosoans possess no cilia, and pseudocoelomates possess no musculature around the gut. As a result, food moves through the digestive tract as a result of packing and pressure provided by the fluid in the pseudocoel.

✱ Obtain a preserved *Ascaris sp.* specimen. Observe the specimen using a dissecting scope.

Ascaris sp. is an intestinal roundworm commonly found in humans. In some parts of the world, it is the most common human nematode parasite.

The nematodes possess few obvious external characteristics. The anterior end of the body possesses sensory papillae, which may be visible under a dissecting scope. Males and females can often be distinguished by the hooked tail which is characteristic of males. Also, females possess a genital pore at the midpoint of the ventral surface of the body (this is often difficult to visualize).

Ascaris Dissection

Determine the sex of your specimen by examining the posterior end of the body for the presence of a hooked tail. If a hooked tail is not present, the specimen is most likely a female. However, since the hooked tail is not always evident in preserved specimens, it should be used only as an initial guess. If the specimen has a hooked tail, it should be inspected under a dissecting scope for the presence of a pair of **copulatory spicules** (hooks) near the anus. Once the specimen is opened, the sex can be confirmed on the basis of gonad morphology.

At the anterior end of the body, the mouth is surrounded by three **lips**, which are visible under the dissecting scope.

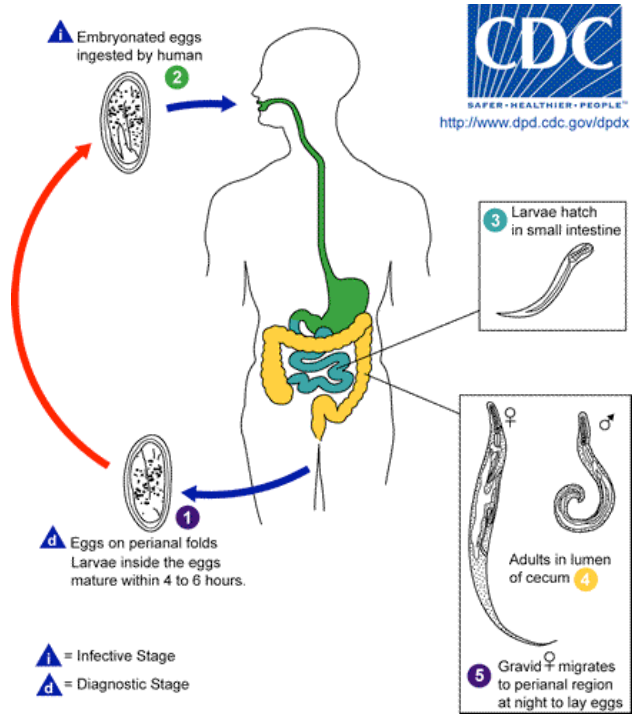
A sagittal incision can be made beginning at the tail and proceeding in an anterior direction using either an insect pin or a pair of sharp scissors, while holding the worm between two fingers. A scalpel is probably not a good choice for this delicate incision. Great care must be taken to prevent damage to the internal organs.

Once the body is opened, there are two sets of organs that are obviously visible: the digestive tract and the reproductive organs. The digestive tract is a single tube that runs down the length of the body cavity (pseudocoel). The reproductive organs are thinner and more contorted, with tapering ends. In males, there is a single limb to the reproductive organs. The distal, tapered end of this structure is the **testis**. The thicker proximal end of the organ is the **seminal vesicle**. These two portions are connected by the **vas deferens**. If the specimen is a female, the reproductive organs will consist of two trunks. The base of the structure is the **vagina**, which branches into two long uteri, tapering into **oviducts**, with **ovaries** at the ends of each.

✱ Draw your Ascaris specimen and label the parts of the reproductive organs and the digestive tract.

Parasitism

Many species of nematodes are parasitic, often infecting vertebrates, including humans. Some types of nematodes are very common in humans. The pinworm, *Enterobius sp.* is the most common nematode in Americans, infecting approximately 13% of Americans. Infection rates are significantly higher in children and institutionalized individuals. In these groups, infection rates can approach 50%. Pinworms are spread by inappropriate anal hygiene. Juvenile worms emerge on the anus of infected individuals and are passed from hand to mouth.



Pinworm (Enterobius) life cycle

Filarial roundworms produce larvae called **microfilariae**, which are released into the bloodstream and are spread by female flies that take blood meals to nourish their eggs (such as mosquitos). These microfilariae can also cause blockages in capillaries and lymphatic vessels resulting in severe edema. This condition is referred to as **elephantiasis**. Heartworms (*Dirofilaria sp.*) are another example of a filarial roundworm.

✱ Observe the preserved heartworm specimens.

Mermithid nematodes are very similar in lifestyle and appearance to Nematomorpha. The chief distinguishing characteristics for mermithids are the posterior **spicules** (in males) and lighter coloration than nematomorphs. Mermithids are parasitoid, and result in the death of their host. The adults are free-living and aquatic, producing larvae which enter into insects or spiders when ingested with water. Inside the arthropod, the larvae grow and mature eventually damaging the internal organs of the host. When ready to emerge, the adult causes the host to walk erratically, making it more likely that it will come in contact with water. In the water, the adult worm emerges from its host and swims away.

✱ Observe the specimen of a mermithid nematode emerging from the abdomen of a wolf spider.

questions

rotifers

1. What is haplodiploidism?

2. What happens to the fertilized eggs of a mictic female?

3. What is the mastax used for?

Platyhelminthes

1. Where is the mouth of a planarian located?

2. How do planaria respond when exposed to a light source?

3. Describe the basic life cycle of a liver fluke. (use drawings if necessary)

Nemata

1. What sex was your *Ascaris* specimen?

2. How are the muscles of nematodes different from most of the other groups we have observed in the lab?

3. How are heartworms spread?

4. How do mermithid nematodes differ from Nematomorpha?

Drawings

Philodina sp. live specimen

Kingdom: _____________
Phylum: ______________
Class: ________________
Order: ________________

Drawings

Taenia sp. mature proglottid WM.

Kingdom: _____________
Phylum: ______________
Class: ________________
Order: ________________

Drawings

Ascaris dissection

Kingdom: _____________
Phylum: ______________
Class: ________________
sex: ________________