

Lab

Ascaris - a Parasitic Roundworm**Pre-lab Discussion**

The 20,000 known nematode species inhabit terrestrial, marine, and freshwater environments and are found in almost all moist habitats. The taxon includes numerous plant and animal parasites, many of which are of medical or agricultural importance, but most are free-living (non-parasitic). Most nematodes, or roundworms, are long, slender, almost featureless externally, tapered at both ends, and round in cross section. The body cavity, if present, is a pseudocoelom. Because the body cavity acts as a circulatory medium, it is also called a hemocoel. The pseudocoelom forms between the mesodermal and endodermal germ layers.

The body is covered with a thick extracellular **cuticle** secreted by a syncytial epidermis that is molted during juvenile development. The epidermal nuclei are sunken below the epithelial layer into the four longitudinal epidermal cords that extend the length of the animal. The body wall has well-developed longitudinal muscles but lacks the circular muscles **found in segmented worms**.

The gut is complete with a terminal anterior mouth and a subterminal posterior anus. The digestive tract is formed by an ectodermal foregut and hindgut and an endodermal midgut.

The simple brain is formed by cerebral ganglia that form a ring around the pharynx. There are several longitudinal nerve cords, the most important of which are the two ventral nerve cords that contain many ganglia throughout the entire length of the nematodes body. The nerve cords are located in the longitudinal epidermal cords, along with the epidermal nuclei. Cytoplasmic innervation processes from the longitudinal muscles extend to the longitudinal nerve cords and serve the function of motor neurons, which are absent. Sensory structures may include unique chemosensory cells and sensory bristles around the mouth.

Most nematodes lack cilia or flagella, even in the sperm. Roundworms produce ammonia that will be excreted by simple diffusion across the body wall. Water regulation is accomplished by an excretory canal system. There are no nephridia or flame cells present in nematodes.

Nematodes are typically separate sexes and fertilization is internal. Sexual dimorphism is common. Nematode sperm have no flagella and probably employ amoeboid locomotion. Development is direct and includes four juvenile and one adult instar separated from each other by molts. Most nematodes are small (<3 mm) and free-living but some of the parasitic species, such as the *Ascaris*, may reach lengths of 20 inches.

Purpose

Become familiar with the external and internal structures of an *Ascaris*.

Materials

Preserved specimen of *Ascaris*
Dissection tray

Dissection kit
Straight pins

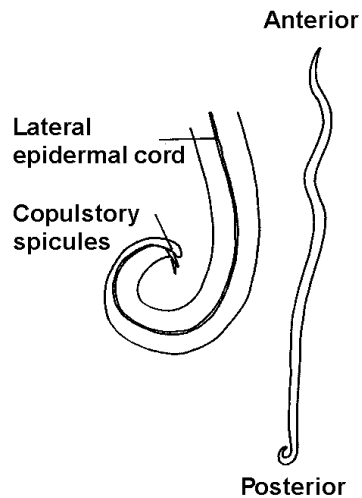
Hand lens
Dissecting scope

Procedures

Part A. External Anatomy

1. Place a preserved adult *Ascaris* in a long narrow dissecting pan of tap water. While not essential, the dissection is best conducted with a dissecting microscope. Preserved worms are delicate and should be handled carefully.
2. Look at the surface of the worm with the dissecting microscope and note that it is firm and resists deformation. It is covered with a thick proteinaceous cuticle which plays an important role, in the absence of circular muscles, in containing the high hydrostatic pressure of the hemocoel. Look for the characteristic ornamentation of the cuticle, which in this species consists of fine circumferential ridges. The cuticle of preserved specimens is fragile and rough handling will cause it to break or peel away.
3. Determine the sex of your specimen. Females reach larger sizes than males. The posterior end of a male is curved ventrally and looks like a shepherd's crook (see Figure 1). Two tiny copulatory spicules may be visible protruding from the anus on the inside curve of the crook. The posterior end of females is not noticeably curved.

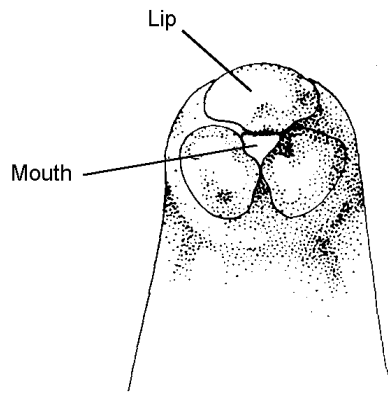
Figure 1. View of the left side of a male *Ascaris*



4. Distinguish between the anterior and posterior ends. The curled posterior end of male makes this easy for that sex but females are straight with similar anterior and posterior ends. In both sexes, the mouth is terminal at the anterior end but the posterior end has no terminal opening. Viewed head-on with the help of a hand lens, the mouth can be seen to be surrounded by three small lips. It is easier to view the mouth with a hand lens than with the dissecting microscope because of the problem of orienting the long worm vertically on the stage.

The three lips of *Ascaris* are formed by fusion of the six lips of the ancestral nematodes. The arrangement of the mouth and its lips is radially symmetrical. The subterminal anus of both sexes is located slightly anterior to the posterior tip of the worm (see Figure 2). It is a transverse ventral slit and is the best landmark for recognizing the ventral surface. Its position just behind the tip on the ventral surface is referred to as subterminal. The mouth, which is at the extreme tip of its end, is terminal.

Figure 2. *Ascaris* mouth structures



5. The female genital pore known as the vulva, is located on the midventral line about 1/3 of the animal's length posterior to the mouth. It is a small pore best found with magnification. The female reproductive system opens to the exterior independently of the gut and there is no **cloaca** in this sex. The male reproductive system does not have its own external genital pore and sperm must exit the animal via the anus. Two copulatory spicules may extend from the anus in some specimens.
6. Knowing the ventral, anterior, and posterior locations will allow you to find dorsal, right, and left. Find the plane of symmetry and the dorsal midline. The four epidermal cords in the body wall are visible from the exterior as thin, pale stripes. These are the dorsal, ventral, and two lateral longitudinal epidermal cords. They are faint, but discernable with good lighting.

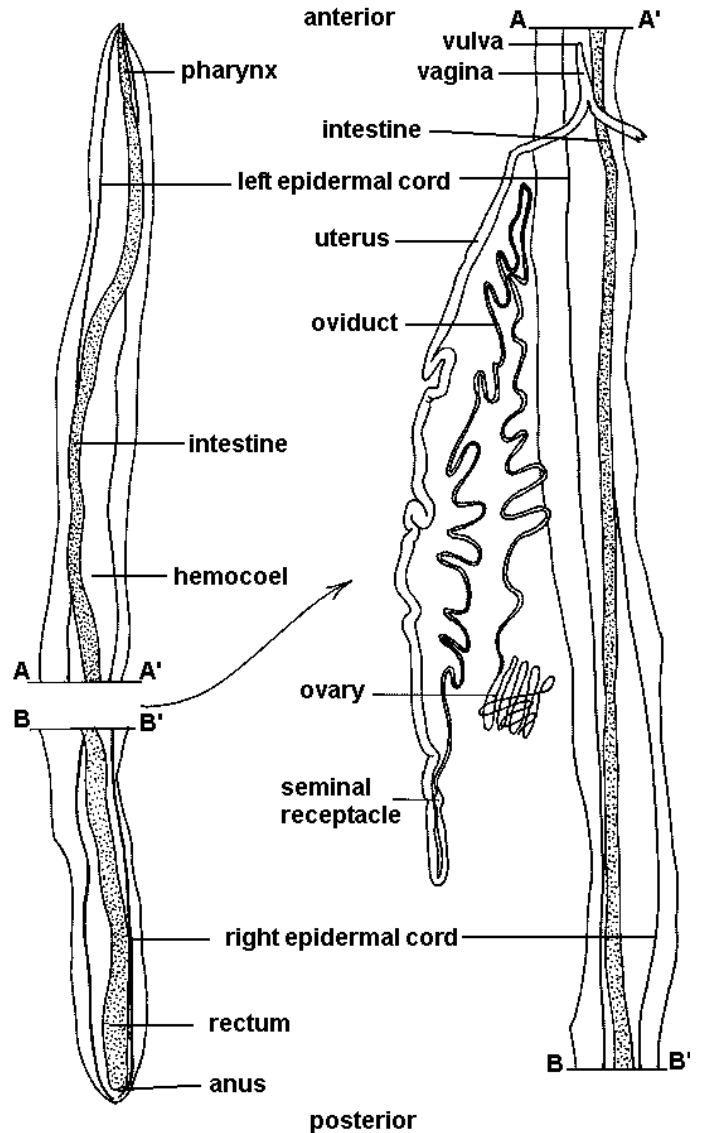
The two lateral cords are easiest to see. A tiny pore belonging to the excretory canal system is located immediately posterior to the mouth on the ventral midline but is usually not visible.

Part B. Internal Anatomy

1. Position a worm in a dissecting pan of water with its ventral side down. The water's depth need only to cover the top of the worm. Males must be rotated a little to accommodate the curl of the tail.
2. Hold the worm gently with thumb and forefinger of one hand and use a sharp pointed probe to scrape a longitudinal middorsal incision through the cuticle and longitudinal muscles of the body wall in the anterior third of the body. Do your best to keep the incision on the dorsal midline even though there are not many landmarks to guide you. The two lateral epidermal cords, which will be revealed by your incision are large and conspicuous ridges on the inside of the body wall that can be used for orientation. You should make the dorsal incision so a lateral epidermal cord is equidistant from either side of it. You will not be able to see the cords at first and must wait until the incision is long enough to spread the walls apart. In the anterior end of the body there are few organs to damage but you should be careful nevertheless. You can develop your skill with the sharp pointed probe in this region where the possibility of damage is lessened. The cuticle tends to resist the cutting motion of the pin so that it separates unevenly but several scrapes in the same place will penetrate it. The longitudinal muscles inside the cuticle, on the other hand, help guide the pin in the correct direction and there is no connective tissue or circular muscles to impede the passage of the pin.
3. Extend the incision anteriorly to the mouth. Deflect the cut edges of the body wall and pin them to the wax using straight pins inserted at 45 ° angle. Be careful as you pin the body wall aside. The internal organs, especially the gut, are very delicate and break easily. Further, the gut often adheres to the body wall and is easily pulled past its breaking point by movement of the body wall.
4. When you have opened and pinned the anterior third of the worm, extend the incision posteriorly to the end of the body, deflecting and pinning the walls. Opening the middle region of the worm is a bit more difficult because it is packed with the reproductive system (see Figure 3).

Figure 3. Dorsal dissection of a female *Ascaris*.

The right side of the reproductive system is omitted and the left side has been moved to the left to expose the gut. The reproductive system has been simplified and untangled for clarity.



Digestive System

1. The gut is a long, straight tube running from mouth to the anus. It is composed of an anterior foregut, a midgut, and a hindgut. Find the mouth at the anterior end of the *Ascaris*. The foregut comprises the buccal cavity and the pharynx which are lined with the cuticle.

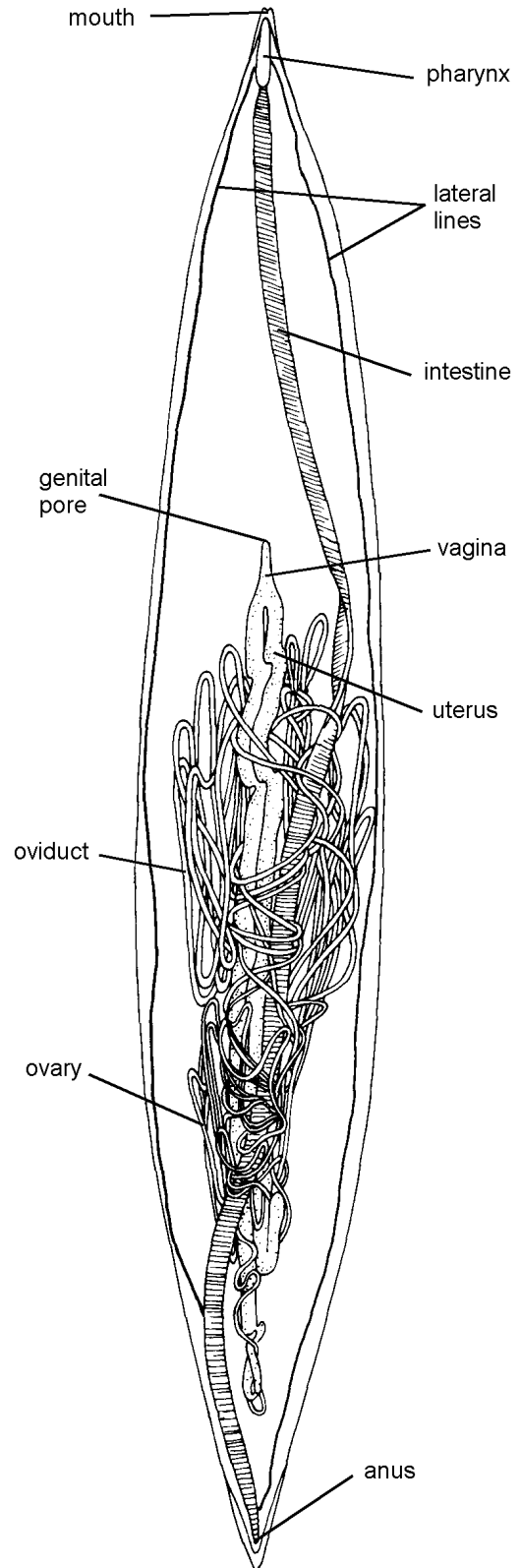
The mouth opens into the small, inconspicuous, thin-walled buccal cavity. Immediately posterior to the buccal cavity is the longer, thicker-walled pharynx whose heavily muscularized walls are used to suck food into the gut in opposition to the high hydrostatic pressure of the hemocoel. The posterior end of the pharynx is swollen slightly and the pharyngeal lumen is triangular in cross section.

2. Look at a commercially prepared slide of a cross section made through the pharynx. Note the thick muscular pharyngeal walls and the triradiate lumen. When filled with food, the lumen expands and becomes circular.
3. The midgut, or intestine, begins immediately posterior to the pharynx. It is a long dorsoventrally flattened tube that extends posteriorly almost to the anus. Unlike the foregut, its walls consist solely of a simple columnar or cuboidal epithelium and its basal lamina. There is no associated muscle or connective tissue.
4. The intestine is the region of hydrolysis and absorption. In the middle of the body your view of the intestine is probably obscured by the reproductive system but you can find it again posterior to this region (see Figure 4). *Ascaris* subsists chiefly on monomers (sugars and amino acids) from the intestinal contents of its host. These are absorbed by the microvilli of the midgut endothelial cells.
5. The intestine extends posteriorly to join the short hindgut, or rectum. In females, the rectum is difficult to differentiate from the intestine but in males the rectum is a **cloaca** which receives the male gametes and the undigested waste products of the intestine before opening to the exterior via the anus. The rectum is also lined with the cuticle.

Respiratory System

Energy metabolism is anaerobic and there are no special organs involved with gas exchange.

Figure 4. The female digestive tract



Circulatory System

The unpartitioned pseudocoelom of nematodes does not need a circulatory system. Transport is thought to be accomplished by diffusion in small species and by movement of the hemocoelic fluid in larger species.

Excretory System

The excretory system consists of an enormous H-shaped canal system. The uprights of the “H” are longitudinal canals located in the lateral epidermal cords and extend over the entire length of the worm. The two longitudinal canals connect with each other via a transverse canal near the anterior end of the worm. A short excretory duct leads from the transverse canal to the excretory pore on the anterior ventral midline. The system is thought to be used for water balance within the body of nematodes. The excretory canal system is difficult to observe in gross dissection of preserved specimens. The excretory pore is located immediately posterior to the mouth on the ventral midline but it is difficult to find.

Nervous System

Study of the nervous system of *Ascaris* requires specially prepared material and will not be attempted. The central nervous system consists of a three part nerve ring that is wrapped around the pharynx. Try to find the brain. Dorsal, ventral and lateral longitudinal nerves arise from the brain and extend posteriorly in the epidermal cords. Of these the ventral nerve cord is most important and is a double ganglionated cord. The dorsal cord is single and unganglionated. A small lateral nerve cord is present in each lateral epidermal cords.

Locomotion

The locomotory system comprises the pressurized hemocoel, which is a **hydrostatic skeleton**, the antagonistic dorsal and ventral longitudinal muscles of the body wall, and the elastic cuticle, which contains the hydrostatic pressure and opposes the longitudinal muscles. When one muscle contracts, the opposite side of the body lengthens to relieve the hydrostatic pressure and the cuticle on that side stretches. Alternate contractions of dorsal and ventral muscles results in waves in the dorso-ventral plane passing along the length of the body. The arrangement of the protein fibers in the cuticle allow changes in the length of the worm but not its diameter. This results in the characteristic dorso-ventral thrashing motion that can be translated to efficient forward movement in a viscous medium or an environment, such as wet sand or the walls of a host's intestine, with solid or resistant surfaces to push against. Nematodes, except for the very small, are relatively helpless in pure water and are not capable of directed motion but have efficient locomotion when there is a substratum to push against.

Reproductive System

The reproductive system is a continuous tube with the gonads so gametes are not released into the hemocoel. Both male and female systems are long tapered tubes lying coiled in the body cavity. The upstream, solid, free ends of the tubes are small in diameter but expand and become hollow as they extend downstream toward the genital pore. The solid upper ends are the ovaries or testes. The hollow, larger regions are specialized for various purposes, including transport and storage of gametes.

Mitotic divisions of primordial germ cells in the gonads produce diploid cells which move down the reproductive ducts undergoing **gametogenesis** on the way. Study the reproductive system of your specimen and then look at a dissection of the opposite sex. You should be familiar with both sexes.

Female

1. Female *Ascaris* have a Y-shaped reproductive system consisting of two tubes, each with an ovary, oviduct, and a uterus forming an arm of the Y. The two arms join to form a common unpaired vagina which is the stem of the "Y". The vagina empties to the exterior via the single genital pore. It is convenient to trace the system backwards beginning at the genital pore but keep in mind that female gametes travel in the opposite direction. The upper, small diameter ends are referred to as "upstream".
2. Relocate the approximate position of the **vulva** on the midventral line and look on the inside surface of the body wall to find a short tube attached to the body wall. This tube is the **vagina** and the vulva opens into it. The vagina is formed by the union of two large, convoluted tubular uteri. Each **uterus** extends posteriorly, decreasing in diameter, almost to the posterior end of the pseudocoelom.
3. Upstream of each uterus is the smaller diameter **oviduct**. A small swelling, the **seminal receptacle**, is situated at the junction of the oviduct and uterus. The oviducts extend anteriorly, without much change in diameter, to about the level of the vagina where they turn and run posteriorly again. The two oviducts are coiled around the uteri, the gut, and themselves.
4. Near the middle of the body each oviduct decreases in diameter to become an **ovary**. The ovaries are solid and form a mass of small-diameter threads in the middle of the worm. Oogonia are produced by mitotic divisions of primordial germ cells in the upper ovary. The oogonia move downstream to the upper oviduct where mitotic divisions produce many primary oocytes. The primary oocytes undergo meiosis eventually forming haploid gametes. Fertilization occurs in the seminal receptacle before when the egg is called a secondary oocyte. Once fertilized, it enters the uterus where it develops a chitinous eggshell and undergoes its final meiotic cell division to become an egg. Embryonic development begins in the uterus. The uterus will contain shelled "eggs" in all stages of embryonic development.

Male

1. The reproductive system of male *Ascaris* resembles that of females but is only one tube rather than two. The solid free upstream end of the tube is the **testis**. It is a small, white solid thread coiled in the posterior third of the body cavity. As it twists back and forth, it gradually increases in diameter. Eventually it turns anteriorly and becomes the **vas deferens**, or sperm duct. This region of the male duct is hollow and contains male sex cells undergoing spermatogenesis. It makes several loops in the middle of the body cavity, some of which extend posteriorly to the region of the testis.
2. The last loop of the vas deferens turns posteriorly again and expands abruptly to become the **seminal vesicle**, a large diameter tube that runs straight posteriorly in the posterior third of the animal. Its diameter increases as it extends posteriorly and spermatozoa are stored in it. Before reaching the posterior end of the body, the male duct decreases in diameter once again and becomes the ejaculatory duct. This muscular duct extends posteriorly from the end of the seminal vesicle to join the **cloaca** immediately anterior to the anus. A region with secretory epithelium, the **prostate gland**, lies between the seminal vesicle and the ejaculatory duct. Two retractile copulatory **spicules** are located beside the cloaca. The spicules are housed in deep pouches opening from the cloaca. During copulation these are extended out of the anus and into the vulva of the female to hold the vulva open.

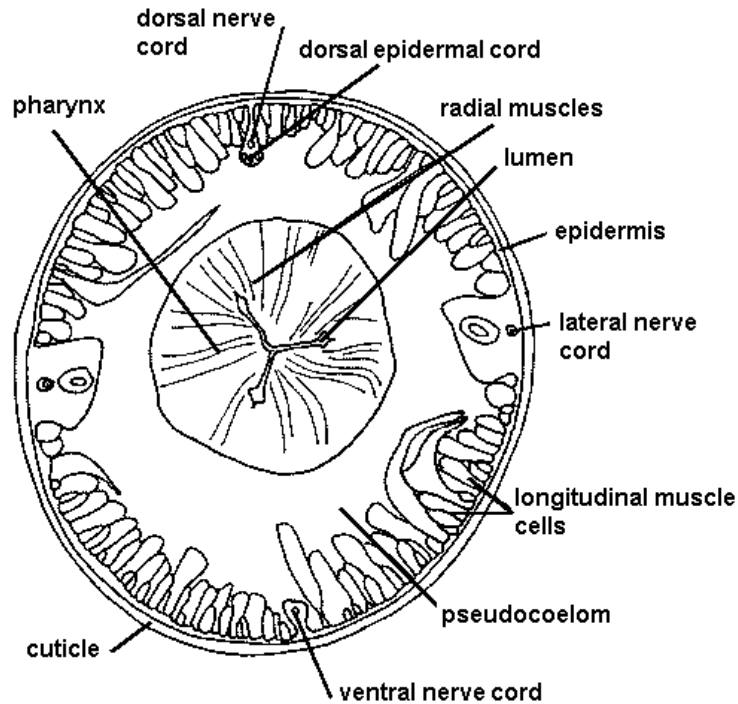
Optional Activity - Microscopic Study of the Internal Composition of a Nematode

Procedure

1. Obtain a prepared slide showing the cross sections of the male and female reproductive systems. Orienting these sections is sometimes difficult and you cannot rely on position on the slide for clues. The lateral epidermal cords are much larger than the dorsal and ventral cords and can be used to distinguish lateral from dorsal/ventral. Distinguishing dorsal from ventral is difficult but the best landmark is the gut, which is usually, but not always, in the dorsal half of the hemocoel. Fortunately it doesn't make much difference if you can't make the distinction between dorsal and ventral but it is important that you distinguish lateral from dorsal and ventral.

- The body wall can be studied on any of your cross sections but, if possible, it is most convenient to begin with the pharyngeal cross section (see Figure 5). The outermost layer of the body wall is the thick **cuticle**. It is a nonliving extracellular secretion that stains pink in most preparations. Immediately inside the cuticle is the thinner epidermis which secretes the cuticle. On most slides the epidermis appears as a thin, pale, pink layer but it may be separated from the cuticle by a white space. If present, this space is an artifact resulting from the slide-making process and in life there is no space between cuticle and the epidermis. Together the epidermis and cuticle make up the **integument**.
- The epidermis is a **syncytium** whose nuclei are located in the four epidermal cords sunken into the body cavity. The right and left lateral epidermal cords are large and easily located. The inconspicuous excretory ducts are in these cords. The dorsal and ventral epidermal cords are much smaller but can be found by careful inspection. The dorsal and ventral longitudinal nerve cords are usually visible in the dorsal and ventral epidermal cords respectively.

Figure 5 Cross Section of the Pharyngeal region of the *Ascaris*



- The thickest part of the body wall is the **longitudinal muscle** layer. This is a single layer of large cells which bulge far into the body cavity and occupy much of it.
- The white space in the interior of the worm is the **hemocoel** or body cavity, and in it are the digestive and reproductive systems. The pharynx is round in cross section and has thick muscular walls. At rest its lumen is collapsed and is triangular shaped. The lumen is dilated by contraction of the radial muscles in the pharyngeal walls.
- The intestine is usually flattened but may be dilated and very large. At high power you can see that the intestinal walls are composed of a columnar epithelium of very tall cells. The apical ends of the epithelial cells have microvilli and form an absorptive brush border which is visible as a dark line around the midgut lumen. The **pseudocoelom** is bounded on the outside by **mesodermal** cells and on the inside by the **endodermal** cells.
- Commercially prepared slides usually have one section each from male and female specimens. These sections are made through posterior regions of the body and include several sections through regions of the reproductive tubes.

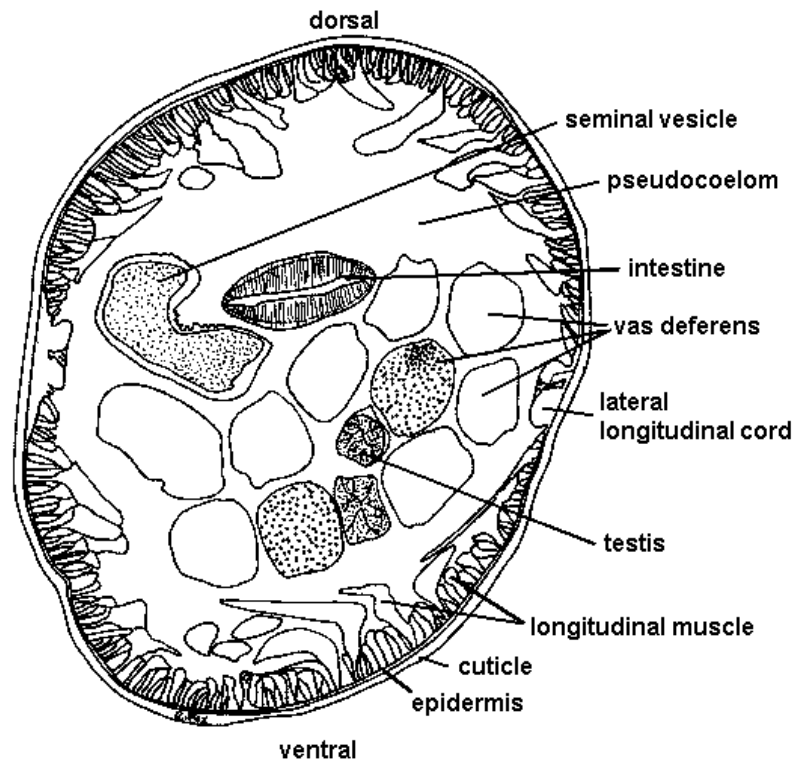
8. Study the cross section through a male *Ascaris* (see Figure 6). Male cross sections are usually smaller in diameter than female and lack the large egg-filled uteri (see Figure 7). The male reproductive system is a long coiled tube. The plane of the cross section will have passed through this tube many times and each specialized region of the tube will be represented many times. The number of times depends on the level of the section.

9. The upstream end of the tube is the **testis** which is solid, small in diameter, and enclosed by an epithelium. It is filled with small, spherical primordial germ cells and has no lumen. Mitotic divisions of the germ cells produce spermatogonia which move downstream to undergo spermatogenesis. Several sections through the testis may be present.

10. The next region of the male tube is the **vas deferens**. It is slightly larger in diameter than the testis and is also enclosed by a thin epithelium. Its interior is filled with spermatogonia and their daughters undergoing spermatogenesis. The organization of the contents of the vas deferens is looser than that of the testis and its sex cells are larger. A lumen is present although it may not be apparent since it is filled with developing germ cells. There should be several sections through the vas deferens in the hemocoel of your specimen.

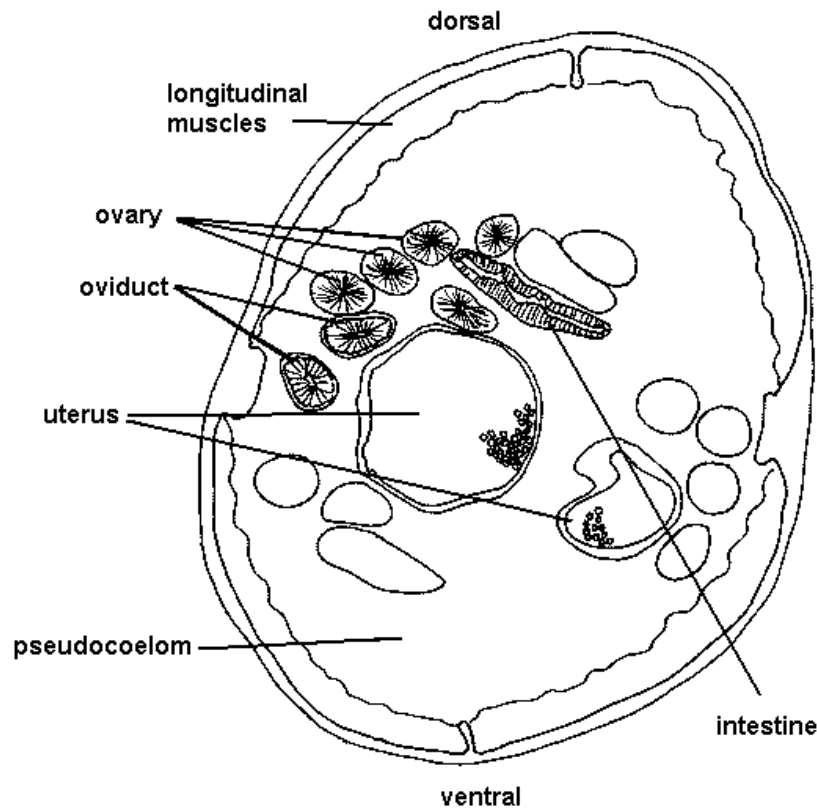
11. The next region of the male duct is the **seminal vesicle**. Amoeboid spermatozoa are stored here. Most slides have a single section through the seminal vesicle but one made too far anteriorly will have none. In life, the seminal vesicle is larger in diameter than the other regions of the male system but in preserved material it often contracts and may be smaller. The epithelial walls of this region are much thicker than those of upper regions of the reproductive tube and this is the best way to recognize it. The **ejaculatory duct**, which is the farthest downstream region of the male system, is too far posterior to be present in the same cross section as the testis and vas deferens.

Figure 6 Cross section of a Male *Ascaris* Nematode



12. Study the cross section of a female *Ascaris* and its reproductive system. It should be recognizable by its two large egg-filled uteri. The female reproductive system consists of two long coiled tubes (see Figure 8). The plane of the cross section passes through these tubes many times and each region of a tube will probably be represented many times. The number depends on the level of the section. Most sections should include cuts through the ovaries, oviducts, and uteri. The twisting, coiled nature of the reproductive system results in multiple sections through the ovaries and oviducts, but probably not the uteri. Unlike that of the male, the female reproductive system is double so there are two ovaries, two oviducts and two uteri. There is only one vagina and genital pore but these will not be represented on the slide.
13. The smallest sections are of the **ovary**. These are easily recognized because they are solid whereas the oviducts and uteri are hollow (albeit so full of eggs they may seem to be solid). The ovary has a central cellular core, known as the rachis, from which radiate a single layer of long narrow primordial germ cells. Their nuclei are usually easy to see. Mitotic divisions of these cells produce oogonia which move downstream as they undergo oogenesis. The ovary and oviduct are surrounded by thin epithelia which are often pulled away from the germinal cells leaving a white space between them. This space is an artifact. The rachis may appear to be a tiny lumen but it is not. If you look closely, you will see pale, lightly staining cells in it. If there are no cells here and the center of the organ is white, you are looking at an oviduct, not an ovary.
14. The **oviduct** has much the same appearance as the ovary but it is a little larger in diameter and is hollow. Its epithelium is similar to that of the ovary and is thick but it has a small lumen instead of a rachis. Around the lumen is a single thick layer of oogonia (upstream) or oocytes (farther downstream).
15. The **uteri**, of which there should be two in most cross sections, are much larger than either oviducts or ovaries. They are filled with large, shelled “eggs” in various stages of oogenesis and development and the uterine wall is thick and muscular.

Figure 8 Cross Section of a Female *Ascaris* Nematode



Life Cycle

The *Ascaris* life cycle involves only one host which becomes infected when it ingests *Ascaris* eggs in its food or water. These hatch in the intestine and juvenile worms migrate to the liver where they enter the host's circulatory system. They are carried in the blood to the lungs where they enter the lumen of the alveoli. From here they crawl to the pharynx, then follow the gut lumen to return to the small intestine where they mature into adult roundworms and feed on chyme.

Tremendous numbers of worms may be present in a single host. After maturation, copulation occurs and females produce and release shelled eggs which leave the host in the feces. When fully developed and infective, an *Ascaris* "egg" contains a tiny juvenile worm capable of infecting a new host. *Ascaris* eggs are difficult to kill and remain viable in soil for as long as 20 years and are widespread in the environment. Development is direct with no distinct larva. The juveniles undergo four molts to become adults. Ingestion of eggs and subsequent infection is most common in children due to their habit of playing in grass and sand and indiscriminately ingesting a variety of potentially contaminated materials.

Observation

1. Is your nematode a male or female?
2. What is the purpose of the spicules in the male *Ascaris*?
3. Would you describe the digestive tract of a nematode as complete or incomplete?
4. How can a nematode pass through the acid environment in the stomach and survive?
5. After you compared the male to a female *Ascaris*, what was the most noticeable difference between them?
6. Do some research and find out what the *Ascaris* eats.
7. Did the *Ascaris* have a distinct head end?

Analysis and Conclusion

1. List two characteristics that best describe the phylum Nematoda.

2. A mature female *Ascaris* has reproductive organs that total 50% of her body weight. Why do you think the reproductive system of the *Ascaris* is so extensive?

3. Describe the digestive system in the *Ascaris*. Why don't they have a stomach or liver, or pancreas?

4. Explain why most successful parasites don't kill their host.

5. Distinguish between the following terms and which of these terms describes the nematodes?
 - a. acoelomates

 - b. pseudocoelomates

 - c. coelomates