

**LABORATORY THREE
INVERTEBRATE ZOOLOGY: BIOLOGY 3114
PHYLA PLATYHELMINTHES, NEMERTEA &
NEMATODA**

Please read over the appropriate chapters and/or pages on the Phyla Platyhelminthes, Rhynchocoela, and Nematoda in your lecture text, laboratory manuals and any handout material in advance of participating in this laboratory exercise. This will make your completion of this exercise much more timely and successful.

LABORATORY EXERCISES

*** Indicates a Required Drawing that Must be Placed in Your Laboratory Notebook**

PHYLUM PLATYHELMINTHES

Class Turbellaria: Order Tricladida

- *1. Examine, draw and label a whole-mounted freshwater triclad flatworm, *Planaria* sp.
- *2. Examine, draw and label a cross-section of a specimen of *Planaria* sp. through the region of the proboscis. There are three cross-sections on this slide, one through the anterior end above the proboscis, one through the proboscis in the middle of the flatworm and one below the proboscis in the posterior end. The **middle section of the three sections** is the **one passing through the proboscis**. It is the section on which to concentrate your observations. It is best viewed under the **medium power objective lens** of your compound microscope.
- *3. Note the behavior of living *Planaria*, *Dugesia*, or *Tigrina* sp. freshwater flat worms, particularly locomotion, the response to strong light or dark, and movements of the head. To observe the flatworm's behavior, place the flatworm in a Syracuse dish with a small amount of aged aquarium water available on the living specimen desk (Do not use tap water. The chlorine it contains will irritate the flatworm and affect its behavior). Place a small piece of meat or boiled egg yolk in a Syracuse dish filled with aquarium water containing the living flatworm. Record its behavior as it senses the food, finds it and feeds on it.
4. Note features of a preserved specimen of the Texan terrestrial triclad flatworm, *Bipalium tricolor*, on demonstration.

Class Cestoda

*5. Observe, draw and label the following portions of sections of the dog tape worm, *Taenia pisiformis* which have been whole-mounted on a slide. **These slides are very expensive and cannot be replaced; so please handle them with extreme care.** There are three sets of sections taken from various portions of the tape. The smallest contains the head or scolex with immature proglottides (reproductive segments) being budded behind it. The sections of tape of medium width contain mature proglottides with well developed male and female reproductive organs while the tape sections of greatest width contain mature proglottides in which most of the reproductive organs have been lost and all that remains is a uterus full of fertilized eggs carrying well developed **onchosphere larvae**.

Draw and label one example of each of the following structures:

A. **Scolex** B. **Mature Proglottid** C. **Gravid Proglottid**

*6. Observe, draw and label a slide mounted whole specimen of *Taenia cysticercus* larva with the scolex inverted into the bladder (or everted from the bladder) under the low power objective lens of your compound microscope. The scolex is everted in the gut when the primary host eats the tissue of the secondary host infested with cysticerci. The scolex attaches to the primary host gut wall and begins budding proglottides to form the mature tape worm.

7. Examine a whole mounted specimen of *Dipylidium caninum*, another species of dog tape worm. Compare its anatomical features to those of *Taenia pisiformis* studied in exercise # 19.

8. Examine the whole specimen of *Taenia pisiformis* on demonstration.

9. Examine the various specimens of adult tapeworms from different vertebrate primary hosts on demonstration.

Class Trematoda

*10. Observe draw and label a whole mount of the Chinese human liver fluke, *Opisthorchis sinensis* as viewed under the low power objective lens of your compound microscope.

*11. Observe, draw and label the stages in the life cycle of a typical fluke.

A. Free-swimming **miracidium larvae** hatching from eggs released by the adult. This stage generally seeks a freshwater snail host (*Biomphalaria glabrata* which may be viewed live on demonstration). It embeds in its tissues and develops into a **sporocyst**

B. **Sporocysts** live in the snail host's tissues and internally bud **redia** which accumulate within the sporocyst

C. **Redia** and **Cercaria**: Redia bud **cercariae** internally. The cercariae eventually leave the redia and bore out of the snail's tissues. Cercaria look like miniature flukes with a muscular tail. The cercaria use their muscular tail to swim to a fish host to encyst under skin as a **Metacercaria**.

- D. **Metacercaria encysted in fish skin.** If eaten by the primary host, the metacercaria excysts in the intestine, drills through the primary host's gut wall to enter its circulatory system in which it migrates to the appropriate host target tissue or organ (liver in *Opisthorchis*) to take up life as an adult fluke.
12. Examine a whole mount of a specimen of the sheep liver fluke, *Fasciola hepatica*, under the low power objective lens of the compound microscope or under your dissecting microscope. Compare its structures with those of *Opisthorchis sinensis*.
 13. Examine and draw a male and female human blood fluke, *Schistosoma mansoni*. This species is an unusual platyhelminthid in that it is gonochoristic with separate male and female individuals rather than being hermaphroditic as are almost all platyhelminthid species. The female permanently lies in a ventral groove in the male's body. When they enter the circulatory and lymph systems of its human primary host, schistosomes cause severe inflammation which eventually debilitates then kills the human host as the number of schistosomes in the body increases. The cercaria of schistosomes have forked tails and drill directly into the primary host (there is no metacercaria). It is the chief killer of human beings in the tropics.

Terminology Required for the Phylum Platyhelminthes

The following terms should be understood and applied where appropriate in your examination of the Platyhelminthes: **anterior, posterior, dorsal, ventral, eye spots (ocelli), mouth, pharynx, proboscis, proboscis cavity, triclad gut, genital atrium, testes, vas deferentia, penis, ovaries, vitelline glands, oviduct, male genital opening, female genital opening, anterior sucker, ventral sucker, ootype, uterus, shelled mature eggs, scolex, proglottid, excretory canals, vagina, seminal receptacle, and shell gland.**

In *Planaria* cross-sections you should find: **dorsal, ventral, circular muscles, longitudinal muscles, diagonal or transverse muscles, proboscis, proboscis cavity, circular and longitudinal muscles of the proboscis, epidermis, rhabdites, gut branches, cilia for crawling on the ventral epithelium, mesodermal parenchyma, ventral nerve chords, and gland or mucus cells in epithelium.**

PHYLUM NEMERTEA

Class Anopla

- *14. Examine, draw and label the external features of the common **Atlantic "ribbon worm"**, *Cerebratulus* sp., noting the mouth, anus, proboscis opening, ocelli on the anterior end, and rhynchocoel cavity.
- *15. If available, examine, draw and label a cross-section of the anterior end of a rhynchocoel showing the **foregut lacunar plexus, mouth, foregut, ventral brain ganglion, proboscis pore, cephalic blood lacuna, epidermis, rhynchodaeum, cilia, longitudinal muscle layer, rhynchocoel and proboscis.**

PHYLUM NEMATODA

- *16. Dissect ***Ascaris lumbricoides*** male or female specimens (some students should volunteer to dissect a male specimen). Make a ventral-lateral longitudinal incision to one side of the mid-ventral line along the ventral length of the worm from the anterior to posterior end. The ventral side of the female contains the genital opening about 1/3 of the length of the worm from the anterior end. The hooked tail in the posterior end of the male hooks toward the ventral side. After making your incision use dissecting pins to pin back the body wall along the length of the worm on both sides to reveal its internal organs. Provide a labeled diagram of the internal structures of both a dissected male and female specimen of *Ascaris*. Well dissected specimens of both sexes will be placed on demonstration for students to study the anatomy of a specimen of the opposite sex from that which they dissected.
- *17. Observe and make a labeled drawings of cross sections of male and female *Ascaris lumbricoides* (on slides) showing all internal anatomical features.
- *18. Observe and make a labeled drawing of a cross section of *Ascaris lumbricoides* at the level of the esophagus (anterior region).
- *19. Examine and make notes regarding the behavior of free-living nematodes as available (***Turbatrix aceti***, ***Anguillula*** sp., ***Rhabdites*** sp., or ***Cephalobus*** sp.) paying particular attention to the use of the longitudinal muscles in opposition to the thick flexible, highly elastic cuticle in locomotion.
20. Examine and make labeled drawings of whole mounts (slides) of human hookworms, ***Necator americanus***. This species has chitinous teeth in the mouth which it uses to abrade the villi of the human gut wall. It then feeds on the blood released from the wound. Large infestations can cause gut hemorrhaging. Humans get hookworms by walking with bare feet in areas where larvae are present on the ground. Larvae attach to the soles of the feet, drill through them to enter the blood stream and are carried in the blood circulation to the gut where they bore out through the gut wall, taking up life as an adult worm. Eggs are released with the feces of the host and hatch into long-lasting larvae which remain on the ground until they encounter a suitable host.
- *21. Examine and make a labeled drawing of a whole mount (slide) of adult female and male specimens of ***Trichinella spiralis***. Adult specimens of this species live in the gut of humans, pigs, rats and mice. They do little harm to the host as adults. However, they release fertilized eggs which hatch into small worms which enter the blood stream of the host and are carried by the blood circulation to encyst in calcareous capsules in the host's voluntary muscles. Build-up of encysted larvae in human muscle can cause paralysis. Thus, the same individual acts as both primary host and secondary host. If a primary host eats flesh with encysted *Trichinella*, the young worms excyst in the gut and take up the life of an adult. Each female can produce 80,000 eggs in her life-time. *Trichinella* can encyst in pork and that is why pork must be thoroughly cooked before it is eaten to prevent *Trichinella* infection.
- *22. Examine and make a labeled drawing of a section of pig muscle with encysted larval ***Trichinella spiralis*** noting the larval worm and calcified capsule within which it is

encysted.

23. Examine adult filarial worms, *Dirofilaria immitis* on demonstration. These are the nematode 'heart worms' which infect the blood vessels of dog's hearts in Texas. In the tropics, similar filarial worms can infect a human being's circulatory system causing **elephantiasis, river blindness, loa loa worm** or **fire worm** disease and, in North America, **pin worms (*Enterobius*)**, the common, relatively harmless nematode gut parasite of children and school teachers. Female *Enterobius* release eggs into the feces which causes the anus to itch. Uninhibited children use their hands to scratch their anus, transferring eggs to their hands. When they put their hands into their mouth, they auto-reinfect themselves. If you touch their hands and put your hands in your mouth you infect yourself, thus teachers working around children are likely to be occasionally infected by pin worms (almost an occupational disease).
24. Examine specimens (on slide) of the larval form of the human filarial worm *Wuchereria bancrofti* in human blood. These larvae are picked up by blood sucking flies and carried to another host where they are injected into their blood stream as the fly begins to bite. Mosquitos disperse heart worm to dogs in the same manner in Texas and elephantiasis worms to human beings in the tropics.

Terminology for the Phylum Nematoda

The use of the following terms should be understood and applied where appropriate in your examinations and drawings of nematodes.

In **WHOLE SPECIMENS**: **anterior-posterior, dorsal-ventral, mouth, pharynx, intestine, anus, excretory canal, lateral line, pseudocoel, longitudinal muscles, vagina, two horns of the uterus, thread-like oviducts and ovaries, penial spicules** or spines, **seminal vesicle, vas deferens, testes, lateral lines, dorsal and ventral longitudinal nerve chords** and genital **openings**.

In **CROSS SECTIONS**: **dorsal-ventral, eggs, ovary, oviduct, uterus, testes, vas deferens, sperm, lateral line, excretory duct, dorsal and ventral longitudinal nerve chords, rachis, cuticle, hypodermis, longitudinal somatic muscles**.

**CLASSIFICATION AND DIAGNOSTIC CHARACTERISTICS OF THE
PHYLA PLATYHELMINTHES AND NEMERTEA STUDIED IN THIS
LABORATORY EXERCISE**

PHYLUM PLATYHELMINTHES: Triploblastic, acoelomate, bilaterally symmetrical, spiral cleavage, unsegmented, gut with one opening, no circulatory system, hermaphroditic, dorsal-ventrally flattened

CLASS TURBELLARIA: Free living, nonparasitic flat worms found in air, in water and in terrestrial habitats, proboscis opens from a mid-ventral chamber, ciliated outer epithelium.

ORDER TRICLADIDA: Mostly freshwater and terrestrial species, three branched gut, proboscis projects from middle of body on the ventral side, includes terrestrial species. *Planaria*, *Trigrina* and *Dugesia* sp., *Bipalium tricolor*.

ORDER POLYCLADIDA: Large, greatly flattened marine flat worms, oval in shape, with the gut subdivided into many branches, numerous eye spots. *Bdelloura* sp.

CLASS CESTODA: Endoparasitic flatworms of vertebrate guts, no gut tube, all nutrients absorbed directly through the body wall, nonciliated outer epithelium, scolex with hooks and suckers for attachment to the vertebrate host's gut wall, body divided into a series of reproductive segments called proglottides budded posterior to scolex, life cycle with oncosphere and cysticercus larval stages in an intermediate host. *Taenia pisiformis*, *Dipylidium caninum*

CLASS TREMATODA: Endoparasite of vertebrate circulatory systems, tissues and organs, two ventrally directed suckers for attachment, anterior mouth opening, gut divided into two branches, epithelium not ciliated, life cycles with several characteristic larval stages (miracidium, sporocyst, redia, cercaria and metacercaria), larval stages generally parasitize two intermediate hosts the first of which is usually a mollusk, while the second may be a vertebrate or arthropod. *Opisthorchis sinensis*, *Fasciola hepatica*, *Schistosoma mansoni*.

PHYLUM NEMERTEA: Triploblastic, acoelomate, bilaterally symmetrical, spiral cleavage, unsegmented, linear, once-through gut with an anterior mouth and posterior anus, presence of an protrusile proboscis for prey capture held in the dorsal rhynchocoel cavity not associated with the mouth or gut, marine,

CLASS ANOPLA: Proboscis without piercing stylets at tip. *Cerebratulus* sp.

PHYLUM NEMATODA: Pseudocoelomate; Triploblastic; Vermiform; Round in cross section; Lack cilia; Only longitudinal muscles in body wall; gonochoristic. *Ascaris lumbricoides*, *Anguillula* sp., *Rhabdites* sp., *Cephalobus* sp., *Necator americanus*, *Trichinella spiralis*, *Dirofilaria immitis*, *Wuchereria bancrofti*

Student Name and Signature:	[X] Required Drawing [] Optional Drawing
PHYLUM PLATYHELMINTHES CLASS TURBELLARIA ORDER TRICLADIDA <i>Planaria sp.</i> Whole mount of a typical freshwater flatworm	Placement Sheet 3-1 Draw the flatworm noting and labeling internal organs and external structures

Student Name and Signature:	<input checked="" type="checkbox"/> Required Drawing <input type="checkbox"/> Optional Drawing
PHYLUM PLATYHELMINTHES CLASS TURBELLARIA ORDER TRICLADIDA <i>Planaria</i> sp. Cross-sections through body Anterior to the Proboscis Through the Proboscis Posterior to the Proboscis Observe the cross-section through the region of the proboscis and proboscis cavity	Placement Sheet 3-2 Use this cross-section to examine and learn the internal features of the flatworm.

Student Name and Signature:	[] Required Drawing [X] Optional Drawing
PHYLUM PLATYHELMINTHES CLASS TURBELLARIA <i>Bipalium tricolor</i> Terrestrial flatworm common in the southeastern United States and Texas Preserved whole specimen	Placement Sheet 3-4 Demonstration This species is relatively common in Texas and is found during warm months in moist highly organic soils. Thus, it is found in well watered gardens with much peat moss. It is very common in green houses. It is completely safe for human being to handle

Student Name and Signature:	[X] Required Drawing [] Optional Drawing
PHYLUM PLATYHELMINTHES CLASS CESTODA <i>Taenia pisiformis</i> Whole mounts of various sections of an adult specimen of the Dog Tape Worm	Placement Sheet 3-5 Find and draw: A. Head with Scolex B. Mature Proglottid with well developed reproductive organs C. Gravid Proglottid showing uterus full of fertilized eggs

Student Name and Signature:	[X] Required Drawings [] Optional Drawings
PHYLUM PLATYHELMINTHES CLASS CESTODA Cysticercus Larva of <i>Taenia pisiformis</i> With scolex inverted or everted scolex	Placement Sheet 3-6 An inverted Scolex is the normal position when embedded in the tissues of the secondary host. The scolex becomes everted when the tissues of the secondary host are eaten by the primary host and the cysticercus enters the gut.

Student Name and Signature:	[] Required Drawing [X] Optional Drawing
PHYLUM PLATYHELMINTHES CLASS CESTODA <i>Dipylidium caninum</i> Whole mount of adult dog tape worm	Placement Sheet 3-7 Compare the anatomical features of <i>Dipylidium caninum</i> with those of <i>Taenia pisiformis</i> .

Student Name and Signature:	[] Required Drawing [X] Optional Drawing
PHYLUM PLATYHELMINTHES CLASS CESTODA <i>Taenia pisiformis</i> Demonstration of whole specimen of Dog Tape Worm	Placement Sheet 3-8 Demonstration Note the series of proglottid segments along the length of the body. Where do think the immature, mature and gravid segments are?

Student Name and Signature:	[] Required Drawing [X] Optional Drawing
PHYLUM PLATYHELMINTHES CLASS CESTODA Mixed whole specimens of adult tape worms	Placement Sheet 3-9 Demonstration Note the similarities and differences in the morphology of different tape worm parasites

Student Name and Signature:	<input checked="" type="checkbox"/> Required Drawing <input type="checkbox"/> Optional Drawing
PHYLUM PLATYHELMINTHES CLASS TREMATODA <i>Clonorchis sinensis</i> Whole mount of adult human Liver Fluke	Placement Sheet 3-10 Draw whole specimens noting all described internal and external structures.

Student Name and Signature:	<input checked="" type="checkbox"/> Required Drawings <input type="checkbox"/> Optional Drawings
PHYLUM PLATYHELMINTHES CLASS TREMATODA Life Cycle Stages of Parasitic flukes Eggs Miracidium Sporocysts with Redia Redia and Cercaria Encysted Metacercaria	Placement Sheets 3-11A-D Draw the different life-cycles stages of parasitic flukes on this page in the order that they occur in the life cycle. Use arrows to indicate successive life-cycle stages including: Egg released from primary host → Miracidium penetrates snail host → Sporocyst develops → Sporocyst buds redia → Redia leave sporocyst and bud cercaria → Cercaria leave redia and eventually leave the snail host → Cercaria find and encyst in a fish or crustacean host → Primary host (mammal or bird) eats fish or crustacean → metacercaria excysts penetrates gut wall and takes up life as and adult fluke in the primary host.

Student Name and Signature:	[] Required Drawing [X] Optional Drawing
PHYLUM PLATYHELMINTHES CLASS TREMATODA <i>Fasciola hepatica</i> Whole mount of the adult sheep liver fluke	Placement Sheet 3-12 Note internal structures under a dissecting microscope. Compare to those of <i>Opisthorchis sinensis</i>

Student Name and Signature:	[] Required Drawing [X] Optional Drawing
PHYLUM PLATYHELMINTHES CLASS TREMATODA <i>Schistosoma mansoni</i> (Human blood fluke) Male and female specimens whole mounted on a slide	Placement Sheet 3-13 The female of this species is held in a groove on the ventral side of the male. This gonochoristic condition in blood flukes is unusual as the vast majority of Trematode species are hermaphroditic. Schistosomes are unusual in that there is no metacercarial stage in the life cycle. The cercaria bores directly through the skin of the primary host (mammals or birds). This species is the number one killer of human beings in tropical countries. Massive infections result in immune responses that debilitate then kill the host.

Student Name and Signature:	[X] Required Drawing [] Optional Drawing
PHYLUM RHYCHOCOELA CLASS ANOPLA <i>Cerebratulus sp.</i> Marine Ribbon Worm Whole preserved specimens	Placement Sheet 3-14 Examine and draw external features from a dorsal-lateral perspective

Student Name and Signature:	[X] Required Drawing [] Optional Drawing
PHYLUM RHYCHOCOELA CLASS ANOPLA <i>Cerebratulus sp.</i> Marine Ribbon Worm Cross section of anterior end through the rychocoel	Placement Sheet 3-15 Draw the cross section of the rychocoel noting the separation of the rychocoel and gut.

Student Name and Signature:	[X] Required Drawing [] Optional Drawing
<p>PHYLUM NEMATODA</p> <p><i>Ascaris lumbricoides</i> Nematode gut parasite of pigs and man Preserved specimens for dissection</p>	<p>Placement Sheet 3-16</p> <p>Females are larger and straight while males are smaller and have a hooked posterior end. Several students in the laboratory should volunteer to dissect male specimens. Well dissected male and female nematodes will be placed on display and the anatomy of both should be drawn on this page.</p>

FEMALE**MALE**

Student Name and Signature:	[X] Required Drawing [] Optional Drawing
PHYLUM NEMATODA <i>Ascaris lumbricoides</i> Cross-sections of female and male bodies	Placement Sheet 3-17 Place labeled drawings of both male and female cross sections of the body of <i>Ascaris lumbricoides</i> on this page.

MALE CROSS SECTION**FEMALE CROSS SECTION**

Student Name and Signature:	[X] Required Drawing [] Optional Drawing
PHYLUM NEMATODA <i>Ascaris lumbricoides</i> Cross-section of pharyngeal region	Placement Sheet 3-18 Make a labeled drawing of the cross-section at the anterior region.

Student Name and Signature:	<input type="checkbox"/> Required Drawing <input checked="" type="checkbox"/> Optional Drawing
<p>PHYLUM NEMATODA</p> <p><i>Necator americanus</i> Whole mount of the male and female "hook worm" A human gut parasite</p>	<p>Placement Sheet 3-20</p> <p>Draw and label the anatomical features of both male and female specimens. Males have hooked tails from which protrude penal spines. <i>Necator americanus</i> feeds by using teeth lining the mouth (developed from the cuticle) to shred the villi of gut wall and then drinking the blood that is released. The cause intestinal hemorrhaging.</p> <p>Eggs are released with the feces and hatch out on the ground. The larval stage drills directly into the skin of the primary host when it comes in contact with it. Wear shoes when walking in unfamiliar places or where animal feces are present especially in areas without sanitary sewers in order to avoid infection.</p>

FEMALE**MALE**

Student Name and Signature:	<input checked="" type="checkbox"/> Required Drawing <input type="checkbox"/> Optional Drawing
<p>PHYLUM NEMATODA</p> <p><i>Trichinella spiralis</i> Nematode gut parasite of man and certain mammalian species Whole mount of male and female worms Gut parasites of pigs, mice and human beings</p>	<p>Placement Sheet 3-21</p> <p>Draw and label both male and female specimens. males tend to be smaller and have hooked posterior ends while females are straight. Adults are very small and generally do not produce deleterious symptoms when infecting the gut. It is the larvae of this species which form calcium carbonate-walled cysts in the muscles of secondary hosts that cause pathological symptoms (i.e., paralysis).</p>

FEMALE**MALE**

Student Name and Signature:	[X] Required Drawing [] Optional Drawing
<p>PHYLUM NEMATODA</p> <p><i>Trichinella spiralis</i></p> <p>Larval <u>Trichinella</u> worms encysted in pig muscle.</p>	<p>Placement Sheet 3-22</p> <p>Note the thick calcareous capsule around the larva. If enough of these accumulate in a muscle they can cause partial or complete paralysis. This species can encyst in man if he eats poorly cooked pork which contains encysted larvae (called "mealy pork" and is looked for by meat inspectors). Larvae hatch from cysts and mature and mate in gut. Impregnated females embed into the walls of the gut and release larvae into the blood. The larvae migrate to skeletal muscle and encyst. One female lays 80,000 eggs. Cook your pork thoroughly!</p>

Student Name and Signature:	[] Required Drawing [X] Optional Drawing
PHYLUM NEMATODA <i>Dirofilaria immitis</i> Adult filarial worms of dog heart	Placement Sheet 3-23 These "heart worms" live in the blood vessels of a dog's heart and as they accumulate they block blood flow to the heart, impairing delivery of oxygen to the heart and eventually resulting in heart attack. The larvae enter the blood stream where they are taken up by mosquitoes which pass them to another dog when they bite it. Dog heart worm medicine is usually arsenic or other form of poison given in doses that kill the worms but not the dog. Never overdose your dog with heart worm medicine as it can cause lethal poisoning.

Student Name and Signature:	[] Required Drawing [X] Optional Drawing
<p>PHYLUM NEMATODA</p> <p><i>Wuchereria bancrofti</i></p> <p>Larval stage of human blood filarial nematode parasite found free in the blood.</p>	<p>Placement Sheet 3-24</p> <p>This specimen is an example of the larval stages of a filarial worm. As is found in the blood you will find many blood corpuscles around it in this blood smear slide. They are hatched out in the primary host (in this case a human being) and live in the blood. Larvae are taken up when a biting fly such as a mosquito when it sucks the host's blood. Larvae are passed to another human host when the biting fly regurgitates them into the second host's blood stream in the process of sucking the second host's blood.</p>