PROTOSTOMES: LOPHOTROCHOZOA

Objective: After completing this exercise, you should be able to do the following:
- Understand and use orientation terms for bilateral organisms.
- Describe features of the Lophotrochozoa phyla: Platyhelminthes, Annelida, and Mollusca.
- Compare the anatomy of the representative animals describing similarities and differences in organs and body form that allow the animal to carry out body functions.
- Discuss the relationship between body form and the lifestyle or niche of the organism.

Introduction
This lab is your first introduction to animals that are triploblastic (contain 3 embryological tissue layers) and bilaterally symmetrical (lineage Bilateria) (Figure 1). This lineage is divided into two monophyletic groups based on differences in early development and the origin of the mouth and the anus. An embryonic structure, the blastopore, develops into a mouth in the protostomes and into an anus in the deuterostomes.

Molecular studies have led taxonomists to create two large clades (single branch of the phylogenetic tree forming a monophyletic group) within the protostomes (figure 2), the Lophotrochozoa and the Ecdysozoa (studied in the next lab). The Lophotrochozoa get their name from 2 structures: the lophophore (figure 3) and the trochophore larva (figure 4). A lophophore is a tuft like structure that surrounds the mouth and functions in suspension feeding. A trochophore larva is a characteristic planktonic larval shape (figure x) with cilia ringing the equator of the organism. Though both lophophores and trochophore larvae are implicated in the name of this lineage, many members of this group have lost these characteristics over the course of their evolutionary history.
Figure 2. Phylogenetic tree of the Protostomes

Rotifers (phylum Rotifera), flatworms (Phylum Platyhelminthes), segmented worms (phylum Annelida), and molluscs (phylum Mollusca) have been classified in the Lophotrochozoa. Though Figure 2 indicates that a coelom (body cavity) is the synapomorphy for this group, the nature of the body cavity is not a characteristic that indicates major phylogenetic branching. A true coelom or pseudocoelom may have been independently gained or lost many times in evolutionary history.
A. ORIENTATION TERMINOLOGY

The terms defined below are used for bilaterally symmetrical animals both invertebrates and vertebrates (figure 5 and 6). Use these terms as you describe animals studied.

**Right/Left:** always refers to the animals right or left, not yours
**Anterior:** toward the head.
**Posterior:** toward the tail.
**Dorsal:** backside
**Ventral:** belly side

Terms related to position in the body

**Proximal:** near the trunk, attached portion, or point of reference
**Distal:** farther from the trunk, attached portion, or point of reference
**Superficial:** lying on top or near the body surface
**Deep:** lying under or below

Planes and Sections

A **section** is a cut through a structure. A **plane** is an imaginary line through which a section can be cut. Anatomists generally refer to three planes or sections (figure 3):

- **Sagittal plane:** divides the body into left and right portions or halves. This is a longitudinal section from anterior to posterior.
- **Frontal plane**: a longitudinal cut from anterior to posterior, this divides the body into dorsal and ventral portions or halves
- **Transverse plane**: Also called a cross section, this divides the body into anterior and posterior portions or cuts a structure across its smallest diameter.

Figure 5: Orientation and plane in a bilaterally symmetrical invertebrate

Figure 6: Orientation in a bilaterally symmetrical quadruped
B. PHYLUM PLATYHELMINTHES – FLAT WORMS

The phylum Platyhelminthes includes the parasitic and the free-living (not parasitic) flatworms. These animals are acelomate (do not have a coelom), dorso-ventrally flattened, and have a 2-way gut (there is no anus).

Today you will study the free-living planarians as a representative flatworm. Planaria are found under rocks, leaves and debris in freshwater ponds and creeks. They move over these surfaces using a combination of longitudinal and circular muscles in their body wall and cilia on their ventral sides.

Procedure:

1. Refer to page 91 in your photo atlas as you work through this exercise.

2. Add a dropper of culture water to a watch glass. Use a dropper or pipette to obtain a living planarian from the class culture – your instructor may show you how to do this.

3. Using your stereoscopic microscope observe the planarian.
   a. Describe its locomotion. Is it directional?
   b. What is the position of its head?
   c. Observe the eyespots and auricles. What might function might these serve?
   d. Does its body appear to contract?

4. What new features with regard to symmetry that you did not see in the two phyla previously studied do you see with planarian?

5. Add a small piece of liver or hard boiled egg to the water near the planarian. It may begin to feed by extending a long tubular pharynx out of the mouth, a circular opening on the ventral side of the body. It will curve its body over the liver and extend the pharynx, which may be visible in the stereoscopic microscope.

6. Draw and label the bold terms above your observations in your notebook.

7. Return the planarian to the class culture after observing its feeding behavior.

8. Obtain a prepared slide of a whole planarian. Using a compound microscope set to the scanning objective to observe this slide. Locate the pharynx inside the pharyngeal pouch. At distal end of the pharynx you will see the mouth. The proximal end of the pharynx opens into a dark-colored, branched intestine. Notice that there is no anus. What does this say about the structure of the digestive tract?

9. Notice how much interior of the organism the intestines take up. It is here that digestion takes place.
10. The anterior blunt end of the animal is the head end. At each side of the head is a projected **auricle**. It contains a variety of sensory cells, chiefly for touch and chemical sense. Between the auricles on the dorsal surface are two pigmented **eyespots**. These are pigments cups into which retinal cells extend from the brain, with the photosensitive end of the cells inside the cup. Eyespots are sensitive to light intensities and the direction of the light source but can form no image. Beneath the eyespots are two cerebral ganglia that serve as the brain. Two ventral nerve cords extend posteriorly from the brain. These are connected by transverse nerves to form a ladder-like nervous system.

**11. Draw and label the bold terms above your observations in your notebook.**

12. Obtain a prepared slide of a planarian cross-section and study it using the compound microscope.

![Figure 7. Cross-section of a planarian.](image)

13. Platyhelminthes have three well-defined embryonic tissue layers, enabling them to have a variety of tissues and organs. Locate the **epidermis** (derived from ectoderm), the **gastrodermis** (derived from endoderm) which lines the intestine, and **muscles** (derived from the mesoderm). Note that you cannot see any coelom. The “spaces” you see are either the pharyngeal pouch, an in-folding of the epidermis, or the inside of the intestine (Figure 7).

**14. Draw and label the bold terms above your observations in your notebook.**
15. Excretory organs consist of two lateral excretory canals and “flame cells” that move fluid through the canals to a pore (Figure 8).

16. Planarians can reproduce both asexually and sexually.

17. Respiratory, circulatory and skeletal systems are lacking.
   a. How do these organisms perform the function of these “missing” systems?

18. **Complete the summary table that you started last week in your notebook, filling in all information for characteristics of the Platyhelminthes.**

C. PHYLUM ANNELIDA – SEGMENTED WORMS

The phylum Annelida includes earthworms and their relatives, leeches, and a large number of mostly marine worms known as polychaetes. Various species of polychaete are known as lugworms, clam worms, bristle worms, fire worms and sea mice. Most species are marine animals, living free in the open ocean or borrowing in ocean bottoms. Others live in fresh water or on soils. Leeches are parasitic and live on the blood or tissue of their hosts.

Annelids are coelomates with the coelom divided into separate compartments by partitions called **septa**, which give the "segmented worms" their segmented appearance. You should observe that each segment contains its own excretory, nervous, circulatory and muscular structures. Some regions of the animal display tagmatization; specialization of segments for specific functions.

Most earthworms and leeches are hermaphroditic with both male and female gonads. Polychaetes usually have separate sexes; many polychaetes hatch into a trophophore larva which later metamorphoses into a juvenile annelid. Some polychaetes, however, can reproduce asexually, by budding.
Today you will study *Lumbricus terrestris*, commonly called an earthworm. These animals burrow through soils rich in organic matter. As you observe this animal, note features that are adaptations to a burrowing, terrestrial lifestyle.

**Procedure:**
1. Refer to page 99 in your photo atlas and Figure 9 as you work through this exercise.
2. Obtain a stereoscopic microscope and a preserved earthworm and identify its anterior end by locating the **mouth**, which is overhung by a fleshy dorsal protuberance called the

![Figure 9. Earthworm anatomy](image_url)
prostomium. The anus at the posterior end has no such protuberance. Also, a swollen glandular band, the clitellum (structure that secretes a cocoon that holds eggs), is located closer to the mouth than the anus.

3. Using scissors, make a mid-dorsal incision along the anterior third of the animal. You can identify the dorsal surface in a couple of ways: the prostomium is dorsal and the ventral surface of the worm is usually flattened.

4. Cut the prostomium. Pin the body open in the dissection pan (place pin in an oblique angle to the pan). You may need to cut through the septa that divide the body cavity into segments.

5. Using a stereoscopic microscope look for the small brain just behind the prostomium on the surface of the digestive tract. Note the two nerves that pass from the brain around the pharynx and meet ventrally. These nerve tracts continue posteriorly as a ventral nerve cord underlying the floor of the coelom.

6. Look for the large blood vessel on the dorsal wall of the digestive tract. You may be able to see the enlarged lateral blood vessel (heart) around the anterior portion of the digestive tract.

7. Identify the pharynx (the muscular region that pumps food to the esophagus). The crop is a storage area for food while the gizzard mashes the food into a paste. The intestine is where digestion and absorption of nutrients takes place.

8. Organs called nephridia carry out excretion in the earthworm. A pair of these minute, white, coiled tubes is located in each segment of the worm body. To view these organs cut out an approximately 2cm piece of the worm posterior to the clitellum and cut it open along its dorsal surface. Cut through the septa and pin the piece to the dissection pan to facilitate observation with the stereoscopic microscope. The coiled tubules of the nephridia are located in the coelomic cavity. Nephridia collect excreted waste and discharge it to the outside through small pores.

9. **Draw and label the bold terms above your observations in your notebook.**

10. Obtain a prepared slide of an earthworm cross-section and study it using the compound microscope.

11. Locate the thin cuticle lying outside of and secreted by the epidermis. Recall the habitat of this organism and speculate about the function of the cuticle?

12. Find the coelom by locating the gap between muscle layers both inside the epidermis and also outside the surface of the intestine.

13. Identify circular muscles and longitudinal muscles. What structure do these muscles work against in order for the animal to move?

14. Locate the ventral nerve cord, lying in the floor of the coelom, just inside the muscle layer.
15. **Draw and label the bold terms above your observations in your notebook.**

16. **Complete the summary table that you started last week in your notebook, filling in all information for characteristics of the Annelida.**

**D. PHYLUM MOLLUSCA – MOLLUSCS**

The phylum Mollusca is one of the most diverse groups of animals on the planet, with at least 85,000 known living species. It includes familiar organisms as snails, octopuses, squid, clams, scallops, oysters, and chitons along with lesser-known groups like the monoplacophorans, a group once thought to be extinct for millions of years until one was found in 1952 in the deep ocean off the coast of Costa Rica. Most species are marine. Others live in fresh water or on land. Many mollusks are of economic importance being favorite human foods.

Molluscs share four characteristic features: (1) a hard **shell** for protection; (2) a thin structure called a **mantle**, which secretes the shell; (3) a **visceral mass** in which most organs are located; and (4) a muscular **foot** used for locomotion. Molluscs are **coelomate**, although the coelom is reduced.

In this exercise you will dissect a clam, a species in the bivalve lineage; a group with a two-shells called called **valves**. Most clams are marine, although many genera live in freshwater lakes and ponds.

**Procedure:**

1. Refer to page 97 in your photo atlas and Figures 10 and 11 as you work through this exercise.

2. Obtain a clam. Observe the external anatomy of the clam. Certain characteristics will become obvious immediately. Determine this organism’s symmetry, support system and the presence or absence of appendages. Does this species display segmentation (don’t confuse the growth rings with segments)?

3. Before you continue determine the **dorsal**, **ventral**, **anterior**, **posterior**, **right** and **left** regions of the animal. Identify the left and right **valves**. The valves are held together by a **hinge** near the **umbo**, the oldest part of each valve. The hinge and the umbo are located dorsally, and the valves open ventrally. The umbo is displaced anteriorly. Hold the clam ventrally with the umbo away from your body, and cup one of your hands over each valve. The valve in your right hand is the right valve; the valve in your left is the left valve. Two strong adductor muscles inside the shell hold the two valves together.
4. To study the internal anatomy of the clam, you must open it by prying open the valves. **Be cautious as you open the clam!** Hold the clam in the dissecting pan in such a way that the scalpel will be directed toward the bottom of the pan.

5. Insert the handle of your forceps or scalpel between the valves and twist to pry the valves farther open. Place your clam in the dissecting pan with clam’s dorsal side supported on the pan bottom. This will allow you to make your cuts with the scalpel blade directed toward the pan bottom and not your hand. Carefully insert the scalpel blade, directed toward the dorsal side of the animal, into the space between the left valve and a flap of tissue lining the valve. The blade edge should be just ventral to (that is, below) the anterior adductor muscle. The flap of tissue is the left **mantle**. Keeping the scalpel blade pressed flat against the left valve, carefully loosen the mantle from the valve and press the blade dorsally. You will feel the tough **anterior adductor muscle**. Cut through this muscle near the valve.

6. Repeat the procedure at the posterior end and cut the **posterior adductor muscle**. Lay the clam on its right valve and carefully lift the left valve. As you do this, use your scalpel to loosen the mantle from the valve. If you have been successful, you should have the body of the clam lying in the right valve. It should be covered by the mantle.

7. Look at the posterior end of the animal where the left and right mantle come together. Hold the two mantle flaps together and note the two gaps formed. These gaps are called **incurrent** (ventral) and **excurrent** (dorsal) **siphons**. What function do the siphons serve?
8. Lift the mantle and identify the **visceral mass** and the **muscular foot**.

9. Locate the **gills**, which have a pleated appearance. One functions of these structures is obvious, but they have a second function as well. As water comes into the body, it passes through the gills and food particles are trapped on the gill surface. The food is then moved anteriorly by coordinated ciliary movement.

10. Locate the **mouth** between the **labial palps**, two flaps of tissue just ventral to the anterior adductor muscle. The anus is located just above the posterior adductor muscle and will likely be hard to locate.

11. The **heart** of the clam is located in a sinus, or cavity, just inside the hinge, dorsal to the visceral mass. This cavity, called the pericardial cavity, is a reduced **coelom**. The single ventricle of the heart actually surrounds the intestine passing through this cavity. Thin auricles, usually torn away during dissection, empty into the heart via openings called ostia. Blood passes from sinuses in the body into the auricles. Is this an open or closed circulatory system?

12. Ventral to the heart and embedded in the mantle tissue are a pair of greenish brown tissue masses, the nephridia, or excretory organs. Nephridia remove waste from the pericardial cavity.

13. **Draw and label the bold terms above your observations in your notebook.**

14. Continue dissection by opening the visceral mass making an incision with the scalpel, dividing the mass into right and left halves. Begin the incision just above the foot and cut dorsally. You should be able to open the flap produced by this cut and see organs such as the **gonads**, **digestive gland**, **intestine**, and **stomach**.

15. **Draw and label the bold terms above your observations in your notebook.**

16. It is difficult to observe the nervous system in the clam. It consists of three ganglia, one near the mouth, one in the foot and one below the posterior adductor muscle. Nerves connect these ganglia.

17. **Complete the summary table that you started last week in your notebook, filling in all information for characteristics of the Mollusca.**