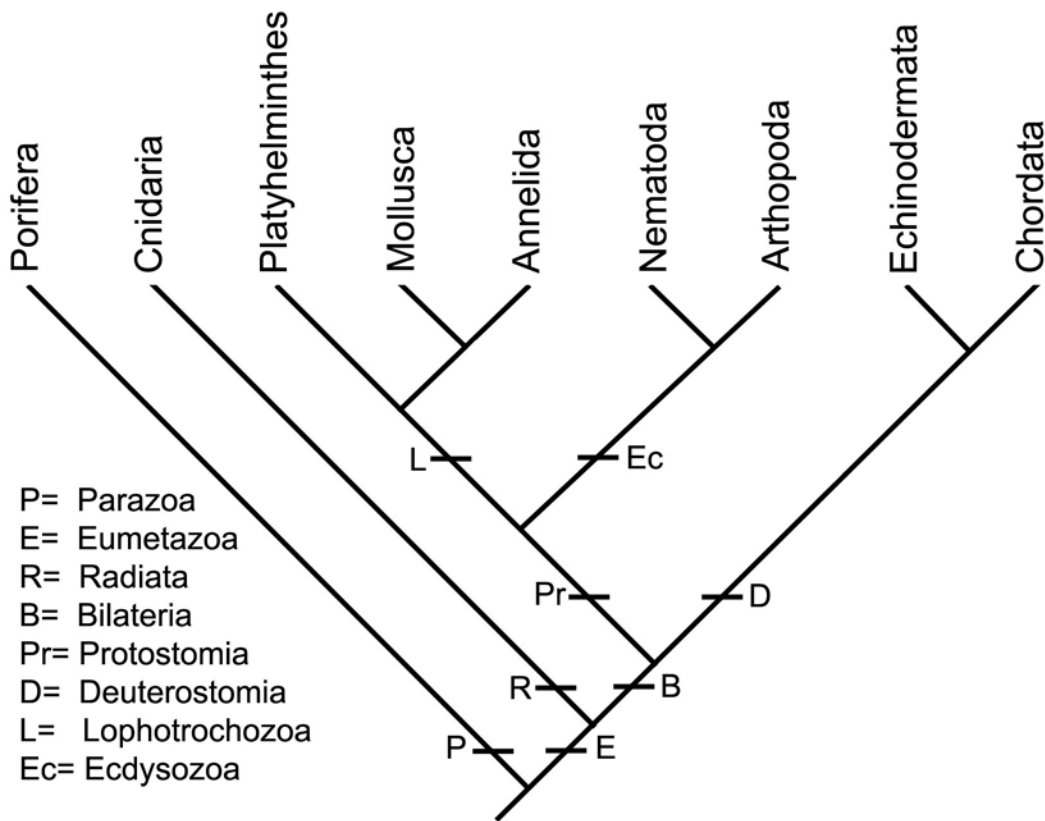


**Biology 3B Laboratory**  
**Invertebrates I: Porifera, Cnidaria, Platyhelminthes, Mollusca**

**Objectives**

- To understand the basic differences among the invertebrate animal phyla
- To investigate and learn the obvious external and internal characteristics of sponges, anemones and jellies, flatworms and molluscs
- To investigate at the microscopic level the organization and function of selected tissues and cells within these groups

**General Introduction to the Laboratory Observation of the Animals**



**Figure One.** Cladogram of the Major Animal Phyla based upon SSU-rRNA

Animals originated in the oceans of the Precambrian era about 1.5 billion years ago. The first animals were multicellular, eukaryotic and heterotrophic. They were the first “predators.” By the beginning of the Cambrian period (543 mya), sponges and cnidarians were already present. During the end of the Precambrian and the beginning of the Cambrian, a huge diversification of the animals took place. This is called the Cambrian Explosion, although it spanned the Cambrian-Precambrian boundary (565 to 525 mya). Most extant phyla are directly traced to this period. During this period, animals experimented with tissue formation, body symmetry, gut tube formation, major feed structures, molting strategies and skeletal arrangements. These evolutionary experiments,

through natural selection, resulted in the major animal lineages represented in Figure One. Although biologists identify approximately 35 extant phyla within the animal kingdom, we will look closely at only those nine shown in Figure One. (Warning: We will occasionally look at a few animals in other phyla! Yes, you will be responsible for those.) In this lab we will take a look at four phyla. The first two, Porifera and Cnidaria are complete natural groups; however, for logistical reasons we will split the lophotrochozoan phyla, covering only the Platyhelminthes (flat worms) and the Mollusca. The Annelida (segmented worms) will be covered in the next lab, Invertebrates II.

## **PHYLUM PORIFERA**

### **Animals without tissues**

The “monophyletic origin of animals hypothesis” asserts that all animal groups evolved from the one protistan clade. They diversified into distinct branches, one of which produced the sponges (Phylum Porifera). Since no other animals appear to have evolved from the sponges, they are considered to be an evolutionary dead end. Members of this phylum are among the simplest animals. They consist of loose aggregations of cells with little or no tissue organization. There is some “division of labor” among the cells, but there are no organs.

The basic body form of all sponges is a sac-like structure consisting of three layers, an outer layer of epidermal cells; an inner layer of cells, many of which are flagellated cells called **choanocytes**; and a middle layer of amoeboid cells that form skeletal structures of various sorts. These layers are perforated by a large number of small **pores**. The cavity of this sac is called the **spongocoel** and has at least one opening to the outside, called an **osculum**.

The sponges are taxonomically classified based on the type of skeletal materials produced. These include **calcareous spicules**, **siliceous spicules**, or proteinaceous **spongin fibers**. This leads to the basic sponge taxonomy, which includes three classes.

Sponges in the Class Calcarea have calcium carbonate spicules, which have three or four rays. All of these sponges are marine. The Class Hexactinellida have siliceous spicules, which are 6 rayed. These sponges are all marine and most often cylindrical in form and found in deep water. The Class Demospongiae are typically called “bath sponges” because they were used by humans for bathing. These sponges have spongin fibers, or siliceous spicules, or both. They represent over 90% of the sponges in the world, and one family is found in fresh water.

Within each class, the sponges can be further differentiated by body form. In **asconoid** sponges the body wall is not folded; in **syconoid** sponges the body wall is folded into canals; and in **leuconoids** sponges the canals formed by the folded body wall are extensively branched. **Ostia** are the openings into the pores of asconoid sponges; they are the openings into the canals of syconoid and leuconoid sponges.

In all sponge types, the body is designed to facilitate feeding. Water is pulled into the pores and canals by the beating of the flagella of choanocytes. The water moves into the spongocoel and is eventually forced out through the osculum. As the water passes across the choanocytes, food particles (microscopic algae, bacteria, and organic debris) adhere to the cells and are eventually taken into food vacuoles for intracellular digestion.

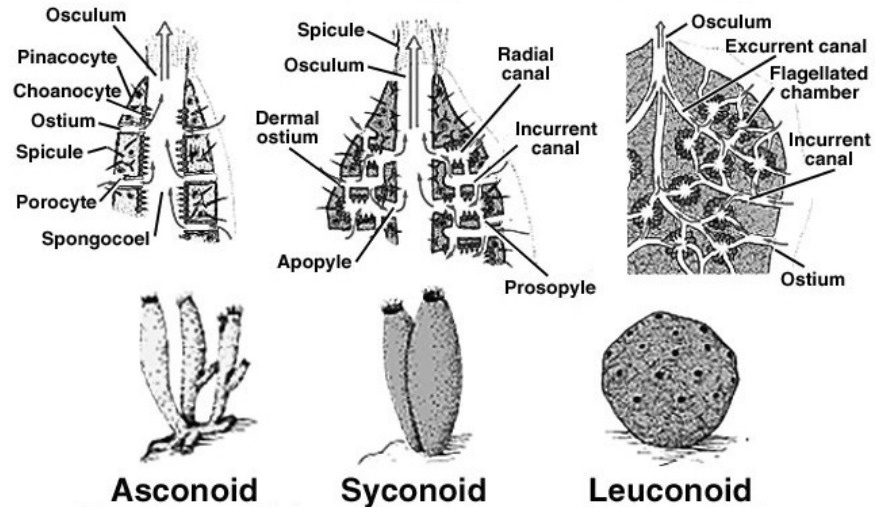


Figure Two. Sponge body plans

## Observations of the Porifera The Asconoid Sponges

Asconoid sponges have the simplest organization. Water enters the sponge through the ostia, drawn into the spongocoel by the beating of choanocyte flagella. Water is expelled through the single apical osculum. Look at Figure 2 and be sure you understand the function of this simple sponge.

- Examine *Leucosolenia*, a simple asconoid sponge. Look at the whole mount and longitudinal section. Find the **spongocoel**, **osculum**, **ostia**, and (at higher power) **choanocytes**.

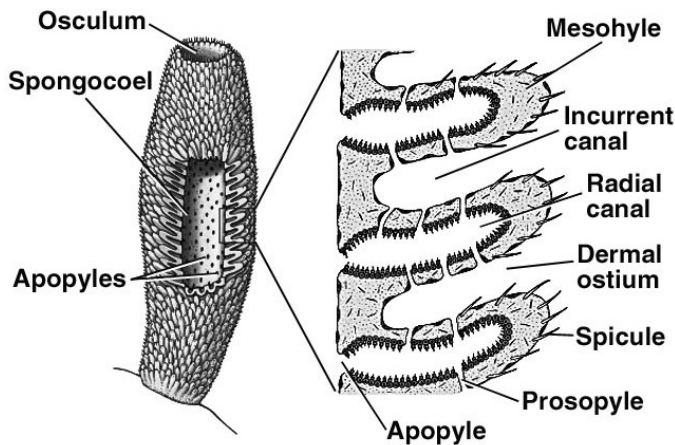


Figure Three. Structure of a syconoid sponge

## The Syconoid Sponges example: *Scypha* (Class Calcarea)

Syconoid sponges have a tubular design similar to the ascon sponge, but the body wall is folded. The "folds" form **radial canals**. **Choanocytes** line the radial canals rather

than the **spongocoel**.

- Examine a preserved specimen of *Scypha* (aka *Grantia*). Note the exterior surface of this sponge; the rough texture is due to **spicules** that protrude through the body wall (see Figure 3). Look closely for the small dermal **ostia**. You should be able to identify the basal disc, which is the point of attachment to the substrate. On the opposite end of the sponge, you will see the **osculum**.
- Examine a prepared slide of the longitudinal section of *Scypha* using a compound microscope at low power. Draw the section, labeling the **spongocoel**; the **radial canals** that radiate from the spongocoel and the **apopyles** (the openings into the radial canals); the **ostia** and the **incurrent canals** they open into; and the **prosypyles** (the small openings connecting the radial canals to the incurrent canals). Using high power look for the choanocytes that line the radial canals.
- Examine the slide labeled *Scypha* spicules. Draw one of the spicules. How many spines are present? What material makes up these spicules?

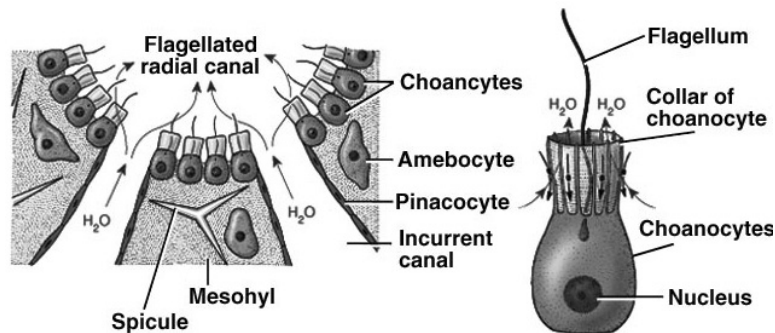


Figure Four. Choanocytes in sponges

### The Leuconoid Sponges

example: the "bath sponge" (Class Demospongiae)

Leuconoid sponges represent the most complex body form. The canal system is extensively branched. Small incurrent canals lead to **flagellated chambers** lined by choanocytes. Flagellated chambers discharge water into excurrent canals that eventually lead to an osculum. Usually there are many oscula in each sponge. The "bath sponge" is an example of a leuconoid sponge. The skeleton of this sponge is made of a soft protein, called spongin, rather than calcium carbonate or silica.

- Examine **all** demonstration materials showing the leuconoid body form.

### Class Hexactinellida

The Venus Flower Basket, *Euplectella sp.*, is an example of this siliceous sponge. It is found in deep water grows to about 15 cm in length. It has an intricate cylindrical mesh-like skeleton of glassy silica; a pair of mated shrimp are often

trapped inside the spongocoel. At the base of the sponge's skeleton is a tuft of fibers that extends outward like an inverted crown. Typically, these fibers are between two and seven inches long and about the thickness of a human hair. Recently, scientists at Bell Labs (Aug 2003) discovered that these fibers rival all known manmade optical fibers.

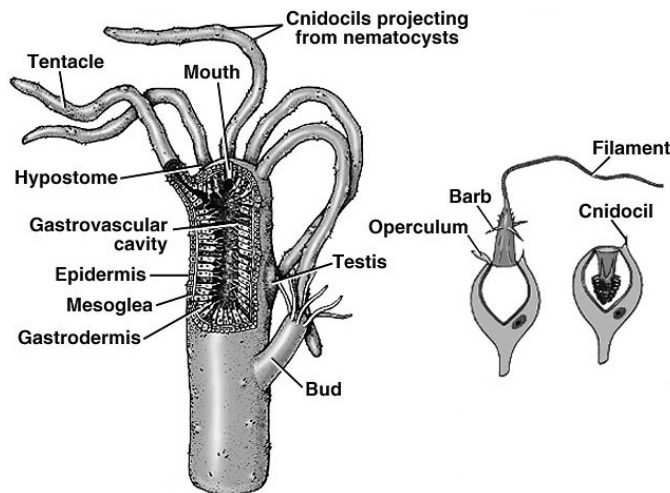
- Observe the skeleton of *Euplectella* on display. Look carefully at the basal fibers and the interwoven fibers of the sponge body. Can you find the remains of the pair of shrimp that once inhabited the spongocoel? Sketch a small part of the interwoven pattern of the skeleton of this unique animal.

## PHYLUM CNIDARIA

### Radially symmetrical animals

Members of the Phylum Cnidaria are considered to be more "advanced" than the poriferans for two major reasons. First, they are the first animal to show tissue level organization, although they have no organs. Second, the adult forms are derived from two distinct embryonic germ layers, the ectoderm and the endoderm hence, they are diploblastic. Higher phyla are triploblastic (derived from three distinct embryonic germ layers).

The organisms in the phylum Cnidaria are characterized by radial symmetry. Terms for direction use the mouth as a point of reference. The end of the organism which contains the mouth is **oral**; the opposite end of the animal is **aboral**. Radial symmetry refers to the fact that any plane passing through the oral-aboral axis divides the animal into two equal halves, or that the body tends to radiate out from the oral-aboral axis like spokes of a wheel.



**Figure 5.** Anatomy of *Hydra*

either cellular, or more often, acellular. Unique organelles, called **nematocysts** are found in cells called **cnidocytes**. Cnidocytes are especially abundant on tentacles, but may be generally distributed throughout the epidermis and gastrodermis.

The basic body plan of the cnidarians is a sac-like structure, with a **gastrovascular cavity**. The gastrovascular cavity has a single opening which serves as both mouth and anus; it is often surrounded by **tentacles**. The body wall has an external cell layer, the **epidermis**; an internal cell layer, the **gastrodermis** that lines the gastrovascular cavity; and a layer between these two, called the **mesoglea** which may be

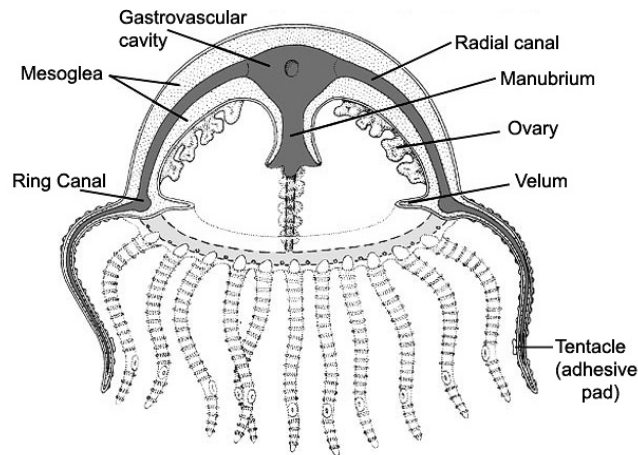
The life cycle of a typical cnidarian alternates between an often sessile **polyp** stage and a free-swimming **medusa** stage. The existence of two distinct forms such as this is known as polymorphism. Both stages exhibit the body plan described above; however, the polyp stage is cylindrical and attached at the aboral end to a substrate, while the medusa stage is flattened in appearance with the mouth oriented downward. The polyp is an asexual stage, while the medusa is a sexual stage. In some cnidarian classes, either the polyp or the medusa stage may be reduced or completely absent. You will examine the three classes of the Phylum Cnidaria: Class Hydrozoa, Class Scyphozoa, and Class Anthozoa.

## Observations of the Cnidaria

### CLASS HYDROZOA

Most members of this class exhibit both the polyp and medusa stages; however, *Hydra* exists only in the polyp form.

- Look at the preserved examples of *Hydra*. Observe a cross section slide of *Hydra*. Identify the inner layer of cells, the **gastrodermis** that surrounds the **gastrovascular cavity**. Find the **epidermis**, which is the outer layer of cells. Between these two layers find the **mesoglea** the acellular (middle-glue). In the epidermis it may be possible to see the **cnidocytes**, within these cells are the **nematocysts**.



**Figure 6.** Anatomy on *Gonionemus*

- Place a *Gonionemus* medusa in a watch glass and examine its structure under a dissecting microscope. See if you can tell "which end is up", that is, locate the upper or convex surface, the **exumbrella**, and the concave **subumbrella**. Sketch the specimen and label the **velum**, a circular shelf-like rim attached to the margin of the umbrella and directed inward; the **manubrium**, a dark-colored projection hanging down from the center of the subumbrella cavity. The free end is the mouth; the **ring canal**, which runs around the circumference of the umbrella; the four **radial canals**, which extend to the margin of the umbrella and connect with the ring canal; the **tentacles**, which arise from the umbrella margin; the **statocysts** (organs of balance), located between the bases of the tentacles; the

**gonads**, folded, ribbon-like structures suspended beneath the radial canals.

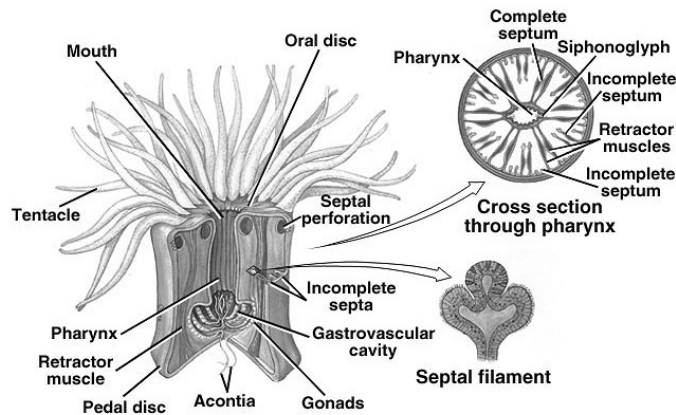
## CLASS SCYPHOZOA

Animals in this class are entirely marine. The polyp stage is reduced or absent.

- Obtain and observe a preserved specimen of *Aurelia*. Compare *Aurelia* with *Gonionemus*. Note the absence of a **velum**.

## CLASS ANTHOZOA

The term anthozoa literally means "flowering animals" in reference to the brightly colored forms exhibited by some members of this class. They exist in the sessile polyp stage only; no medusa stage is present. The class Anthozoa is a large class whose representatives include the sea anemones and corals.



**Figure Seven.** Anatomy of *Metridium*

- Examine a specimen of the sea anemone *Metridium*. Draw the specimen and label the **oral disc** and the **tentacles** attached to it; the **mouth** (the opening in the oral disc) that leads into a passageway leading into the gastrovascular cavity; and the **pedal disc (basal disc)**, which is the point of attachment to the substrate.
- Examine the other anthozoans present in the lab. In particular please see the colonial Sea Pansy, *Renilla* and any examples of corals.

## PHYLUM PLATYHELMINTHES

### Triploblastic acoelomate animals

The Platyhelminthes include free-living flatworms, like the planarians, and the parasitic tapeworms and flukes. The term **flatworm** refers to the fact that the body is dorsoventrally flattened. Phylogenetically, the flatworms are the first organisms to have tissues organized into organs and the first to demonstrate bilateral symmetry. **Bilateral symmetry** means that one plane passing through the longitudinal axis of an organism divides it into right and left halves that are mirror images. It is characteristic of active, crawling, or swimming organisms and usually results in the formation of a distinct head (**cephalization**) where

accumulation of nervous tissue and sensory structures occurs. This reflects the importance to the organism of seeing where you are going, rather than where you have been. The Platyhelminthes and all phyla above them on the evolutionary tree are bilaterally symmetrical or have evolved from bilaterally symmetrical ancestors.

In the Platyhelminthes, different tissues cooperate in a given function. This results in the organ level of organization. Three major sets of organs characterize the phylum. The **excretory system** consists of flame cells and their associated ducts. The **nervous system** consists of a pair of anterior ganglia, usually with two nerve cords running the length of the organism. Nerve cords are interconnected by transverse nerves to form a ladder-like structure. The **digestive tract** is incomplete (a single opening serves for ingestion of food and elimination of wastes).

The Platyhelminthes are **triloblastic** and **acoelomate**. There are three primary germ layers: **ectoderm**, **endoderm**, and **mesoderm**. As with the Cnidaria, the ectoderm gives rise to the outer epithelium, and the endoderm gives rise to the lining of the gut tract. The third germ layer, the mesoderm, gives rise to the tissue between the ectoderm and the endoderm, including muscle, excretory structures, and undifferentiated cells referred to as parenchyma. The term **acoelomate** refers to the fact that there is no body cavity (fluid-filled space) between any of the primary germ layers.

## Observations of the Platyhelminthes

### CLASS TURBELLARIA

The class Turbellaria is a plesiomorphic group, in that it retains the defining features of the phylum, such as anteriorly located sense organs and a well-developed muscular system. The remaining classes in this phylum are composed of specialized parasites that have lost many features seen in free-living animals. A simple example of this group is *Dugesia*, a planarian flatworm.

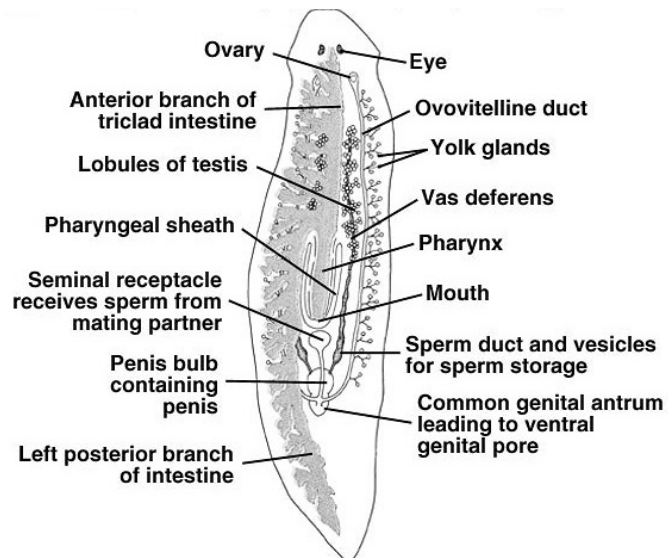
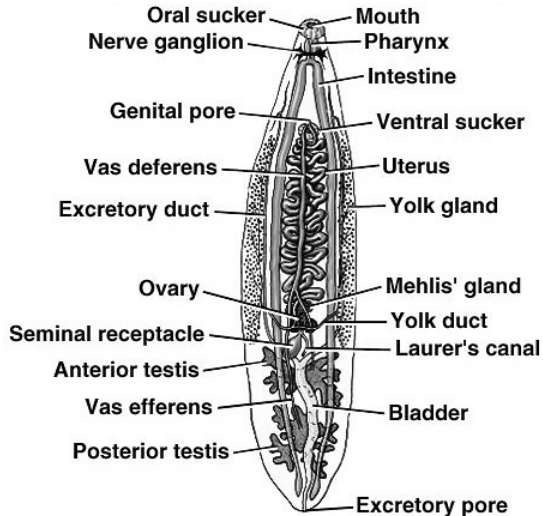


Figure Eight. Anatomy of Planarian, *Dugesia*

- Observe the model of a planarian (*Dugesia*). Identify the **head**, **auricles**, and **eye spots**. Also find the **digestive tract** and the **pharynx**, **pharyngeal chamber**, and highly branched **gut**.
-

## CLASS TREMATODA The Digenetic Flukes

Flukes are all parasitic, primarily attacking vertebrates, including man. The flukes in the class Trematoda are digenetic, meaning they have a life cycle requiring two or more hosts. In such a cycle the final host is termed the primary or definitive host, while the other (or others) are intermediate hosts. Flukes of this



group are typically endoparasites (living inside the host). They have highly specialized reproductive systems, very high reproductive capacity, and complex life cycles in which most of the intermediate stages are capable of asexual reproduction. Some of the structures well-developed in free-living flatworm types are found to be considerably reduced or even absent in these parasitic forms.

Figure Nine. A Liver Fluke

- Examine a prepared slide of *Fasciola hepatica*, a sheep liver fluke. This specimen demonstrates typical parasite features, including the absence of sensory organs, reduction of locomotor and digestive systems, expanded reproductive system, and presence of holdfast organs. Sketch the specimen and label the **gut**, the prominent reproductive structures (**ovary** and **testes**), and the **oral sucker** and **ventral sucker**.

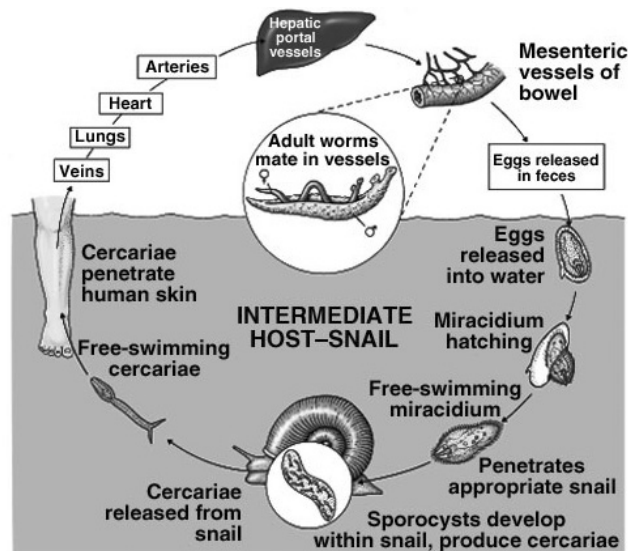


Figure Ten. The Life Cycle of *Schistosoma mansoni*

- Examine the whole mount slides of **Schistosoma mansoni**, the human blood fluke. Look carefully the life cycle of this organism (Figure 10). On

the male animal identify the **gynecophoric canal**, in which the female normally resides.

### CLASS CESTODA The Tapeworms

These highly adapted endoparasites absorb their nutrients directly through their body walls from the host gut. They have lost their own digestive system and increased their reproductive capacity. A hard cuticle protects them from the host's digestive enzymes. The anterior region of a tapeworm's body is modified as a simple holdfast, known as the **scolex**. The rest of the tapeworm body is composed of a series of segments called **proglottids**. Proglottids are produced continuously behind the scolex, therefore the proglottids closest to the scolex are the least mature. As the proglottids mature, the male reproductive system develops followed by the female reproductive system. The most mature proglottids are little more than a uterus filled with eggs, and are called **gravid proglottids**.

- Examine a whole mount of *Taenia pisiformis*. Find the **scolex** and **proglottids** in different stages of development - immature, mature, and gravid proglottids. On the scolex identify the hooks and the sucker discs. Find a **mature proglottid** and identify the **testes**, **ovary**, **uterus**, and **genital pore**. Also observe an **immature proglottid** and a **gravid proglottid**.

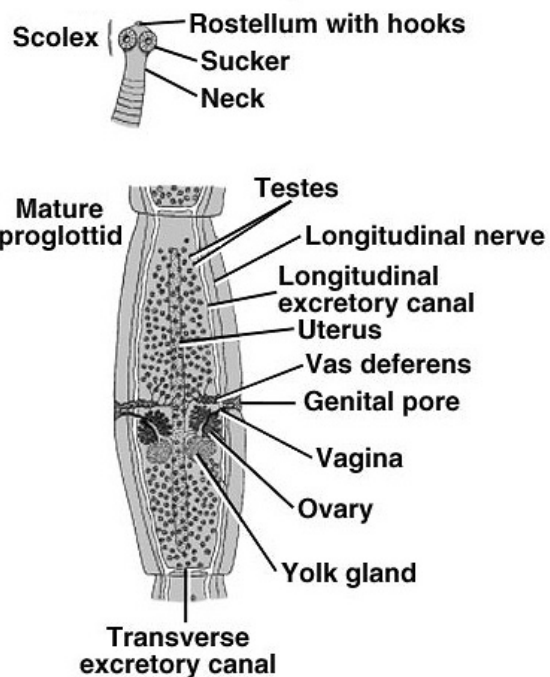


Figure Eleven. Mature proglottid of *Taenia*

### PHYLUM MOLLUSCA

#### Triploblastic eucoelomate animals

The molluscs are classified as triploblastic eucoelomate animals, as are all phyla remaining to be examined. Most molluscs are bilaterally symmetrical and have well-defined circulatory, respiratory, excretory, and digestive systems. With nearly 50,000 species, the molluscs are a large group, second only to arthropods. The name "mollusc" is derived from the Latin *molluscus* ("soft"), indicating that the molluscs are soft bodied animals. The group includes the snails, bivalves, chitons, squid, octopuses, and others. In some forms, the soft bodies are protected by a calcareous shell.

The organisms in the phylum Mollusca are characterized by having three main body areas: a head-foot (sensory and locomotion structures), a visceral mass

(excretory, digestive, and circulatory structures), and a mantle (which secretes the shell). The gills, which function in respiration, are located between the visceral mass and the mantle. In this lab we will look at four classes: Class Polyplacophora, the chitons; Class Gastropoda, the snails and slugs; Class Bivalvia, the clams, oysters, and allies; Class Cephalopoda, the squid, octopus, and chambered nautilus.

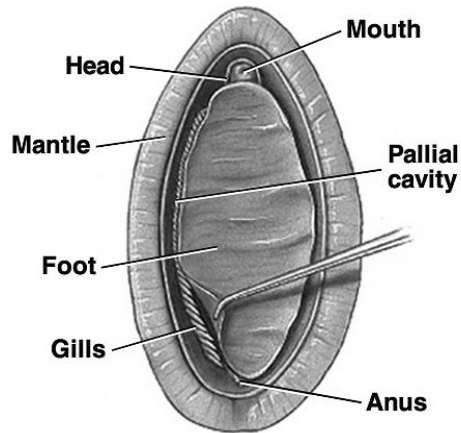


Figure Twelve. Chiton foot anatomy

### Class Polyplacophora

This class is considered the least advanced of the phylum. These animals are entirely marine, and have oval bodies with a shell consisting of eight dorsal plates. A broad, flat foot used in locomotion is located ventrally. The mantle cavity is reduced to a groove running on either side of the body between the foot and the margin of the animal.

### Class Gastropoda

This class includes snails and slugs. It is by far the largest class of molluscs. Gastropods are primarily marine, but some species also inhabit freshwater and terrestrial habitats. In all forms, the visceral mass is located enclosed in a coiled shell during early developmental stages. In most gastropod species the shell is retained in the adult, but in some, such as the common garden slug, it has been completely lost. Because of this, slugs are restricted to moist areas to prevent desiccation.

### Class Bivalvia

Members of this class are characterized by a shell consisting of two valves or halves. Bivalves use a muscular foot for locomotion. Siphons are used to draw in a stream of water which is passed over the gills for feeding and respiratory purposes.

### Class Cephalopoda

The cephalopods are considered to be the most advanced class of molluscs. These organisms have a highly evolved visual system, and tentacles with suction cups. They are all marine. They are fast swimmers and use jet propulsion as a means of locomotion.

## Observations of the Mollusca

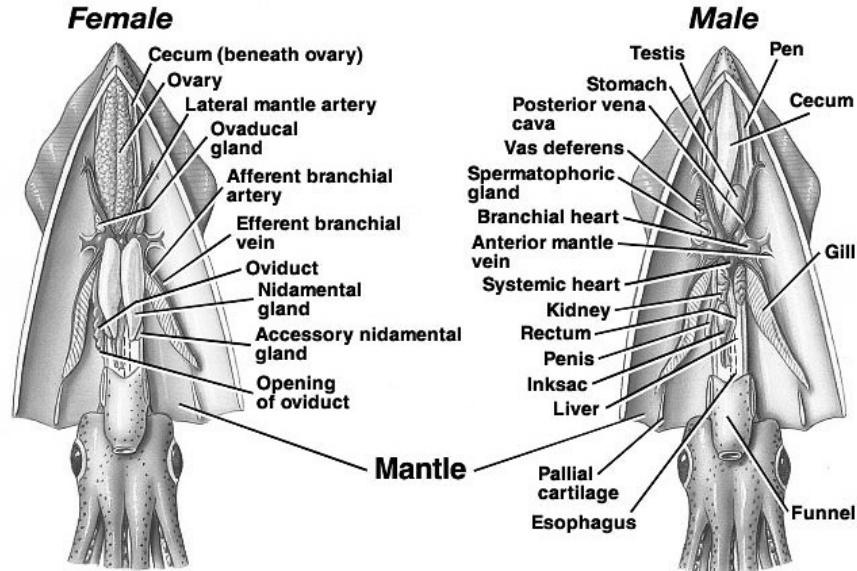
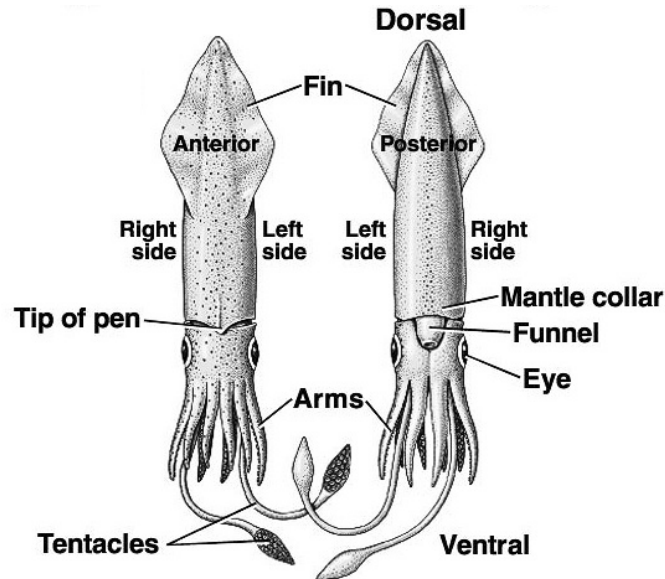
### Class Polyplacophora

- Examine the chitons on display. In some cases you will see only the eight **dorsal plates** diagnostic of the class. In the preserved jars you will also observe the **ventral foot** and perhaps the **mouth** on the anterior end (Figure 12.)

## Class Gastropoda

- Observe several of the snail shells in present in the lab. Notice the type of coiling and external decoration of these shells.

## Class Cephalopoda



**Figure Thirteen.** External and Internal anatomy of squid, *Loligo*

- Examine the preserved squid (*Loligo*). Find the following structures
  - eight **arms**
  - two **tentacles**
  - **mantle** (enclosing the visceral mass)
  - **lateral fins**

- **eyes**, located just anterior to the mantle
- **siphon**, protruding from below the mantle

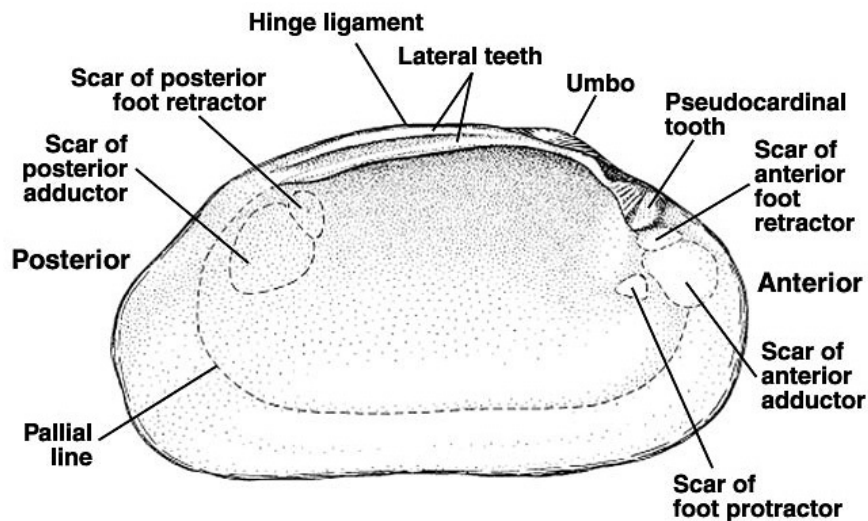
Water drawn into the mantle cavity can be forcefully expelled through the siphon when muscles of the mantle contract, resulting in jet propulsion. The siphon can direct the jet of water in different directions.

- Compare the external anatomy of an octopus to the squid, noting the difference in the shape of the mantle and the octopus's lack of fins.

## Class Bivalvia

You will now complete a dissection of the clam as directed below.

- Examine the clam externally. Find the two **valves**, the **hinge ligament** that holds them together, the swollen **umbo** at the anterior end of the hinge, and the **lines of growth**.

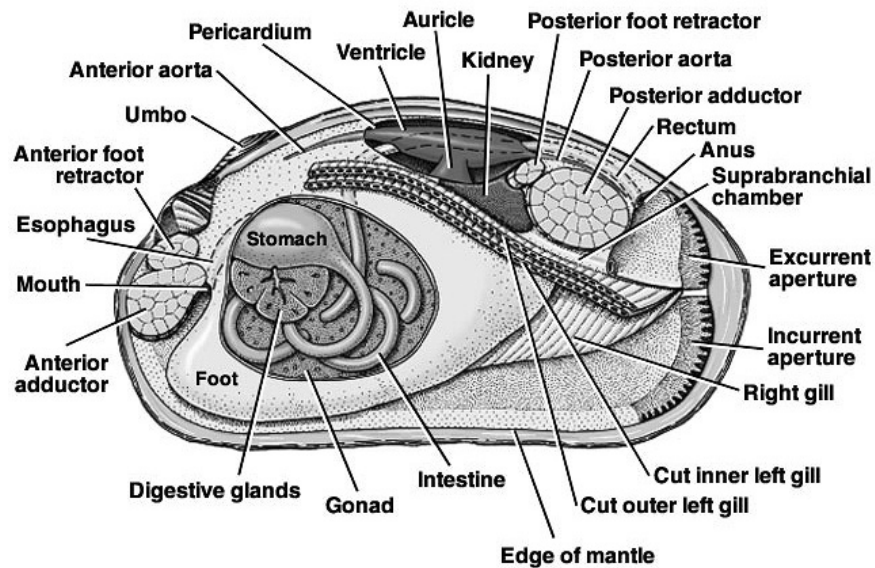


**Figure Fourteen.** Internal shell anatomy of clam

- Using the position of the hinge and umbo, determine which side is **dorsal**, which side is **ventral** which end is **anterior**, and which end is **posterior**.
- Internal Structures**
- Locate the position of the **anterior adductor muscle** and **posterior adductor muscle** through the narrow opening between the valves. Slip a scalpel between the mantle and the left valve. Use the scalpel to gently pry the adductor muscles away from the valve to which they are attached. Loosen the mantle over the entire area of the left valve and open the clam.
  - Examine the inner surface of the empty valve. Notice its smooth nacreous surface. Observe the position of the various muscle scars, including **anterior adductor muscle scar**, **posterior adductor muscle scar**, **anterior protractor muscle scar**, **anterior foot retractor muscle scar**,

and **posterior foot retractor muscle scar**. Also note the **pallial line**, which is the point where the mantle attaches to the shell.

- Look at the **mantle**, including the **pallial muscle**. The mantle of the left and right valves comes together posteriorly to form a ventral **incurrent aperture** and a dorsal **excurrent aperture** that allow water to enter and exit the mantle cavity. Water enters through the incurrent aperture and leaves through the excurrent aperture.



**Figure Fifteen.** Internal anatomy of the clam

- The space between the mantle and the body is the mantle cavity. Lift the mantle to expose the visceral mass, foot, gills, and associated structures. The muscular, wedge-shaped **foot** is at the ventral aspect of the body. The soft tissue making up the bulk of the body is the **visceral mass**. Between the mantle and the visceral mass lie two **gills**. At the anterior margin of the visceral mass, note the smaller, flap-like, **labial palps**. Labial palps surround, and direct food toward, the mouth. Water coming in from the incurrent aperture reaches the ventral aspect of the gills and passes dorsally through the gills into a **suprabranchial chamber**. Water is then directed posteriorly and out of the mantle cavity through the excurrent aperture. In the process, suspended food particles are filtered and gas exchange occurs. Food particles are transported by cilia to **food grooves** along the dorsal margin of the gills. Cilia in the food grooves transport food to the labial palps.
- Return the mantle to its original position and locate the **pericardium**, a thin membrane dorsal to the visceral mass. The circulatory system of bivalves is an open system in which blood leaving the heart flows freely between the organs. Carefully open the pericardium to see the heart. The **heart** wraps around the intestine where the intestine emerges from the visceral mass. The **intestine** is running posteriorly to empty at the excurrent aperture. The heart consists of two parts, a thick-walled

- ventricle** surrounding the intestine and two thin-walled **auricles** attached at either side of the ventricle. If you were careful in removing the pericardium, you should see both. Look for a dark mass of tissue ventral to the pericardial sac. This is an excretory organ known as a nephridium. Nephridia remove metabolic waste products from the blood and release the waste into the mantle cavity near the excurrent aperture.
- Finally, use your scalpel to make a sagittal section of the foot and visceral mass. Within the visceral mass note the cut sections of intestine. The yellowish-brown tissue surrounding the intestine is **gonad**.