Diversity—Mollusks, Arthropods, and Echinoderms

LABORATORY

26

OVERVIEW

In Laboratory 25, several phyla of multicellular animals illustrated important evolutionary advances in organization—from the loosely organized tissuelike structures found in sponges to the two-layered (diploblastic) structure characteristic of cnidarians and the three-layered (triploblastic) structure of flatworms and all other organisms with organs and organ systems. The appearance of a functional body cavity in the nematodes (roundworms) and other wormlike groups was another particularly significant evolutionary milestone. From this point on the "trunk" of the family tree of animals, invertebrates can be divided into two discrete phylogenetic lines, the **protostomes** and the **deuterostomes** (review Figure 25-1 in Laboratory 25).

Protostomes were introduced in the last laboratory with the study of annelid worms. In this evolutionary line, the coelom develops as a schizocoel. In addition, mitotic divisions during the cleavage of the egg produce daughter cells whose fate is more or less committed to the production of a specified part of the body at division (the cell division is **determinate**). Cleavage is also said to be **spiral** due to the characteristic alignment of the mitotic spindles during division. In the eight-celled embryo, the top layer of cells spirals as though it were twisted to the right or left relative to the lower layer of cells. In addition, the blastopore (the opening into the developing gut cavity of the gastrula) becomes the mouth, hence the name protostome (first mouth) for this phylogenetic line.

In deuterostomes, the coelom develops as an evagination (outpocketing) of the primitive gut, or *enteron,* and is called an enterocoel. The mitotic divisions of the fertilized egg produce cells whose fate is not determined at the time of division, hence early cell division is said to be **indeterminate.** Cleavage is **radial:** daughter cells remain aligned above one another. The blastopore becomes the posterior opening of the gut and the mouth end forms later in development—hence the name deuterostome (second mouth) for this evolutionary line that leads eventually to humans (Figure 26-1).

In the present laboratory, you will complete your study of protostomes (phylum Mollusca and phylum Arthropoda) and begin a more detailed examination of the deuterostomes with the phylum Echinodermata.

STUDENT PREPARATION

To prepare for this laboratory, read the text pages indicated by your instructor. Familiarizing yourself in advance with the information and procedures covered in this laboratory will give you a better understanding of the material and improve your efficiency. Review Figure 25-1 carefully.

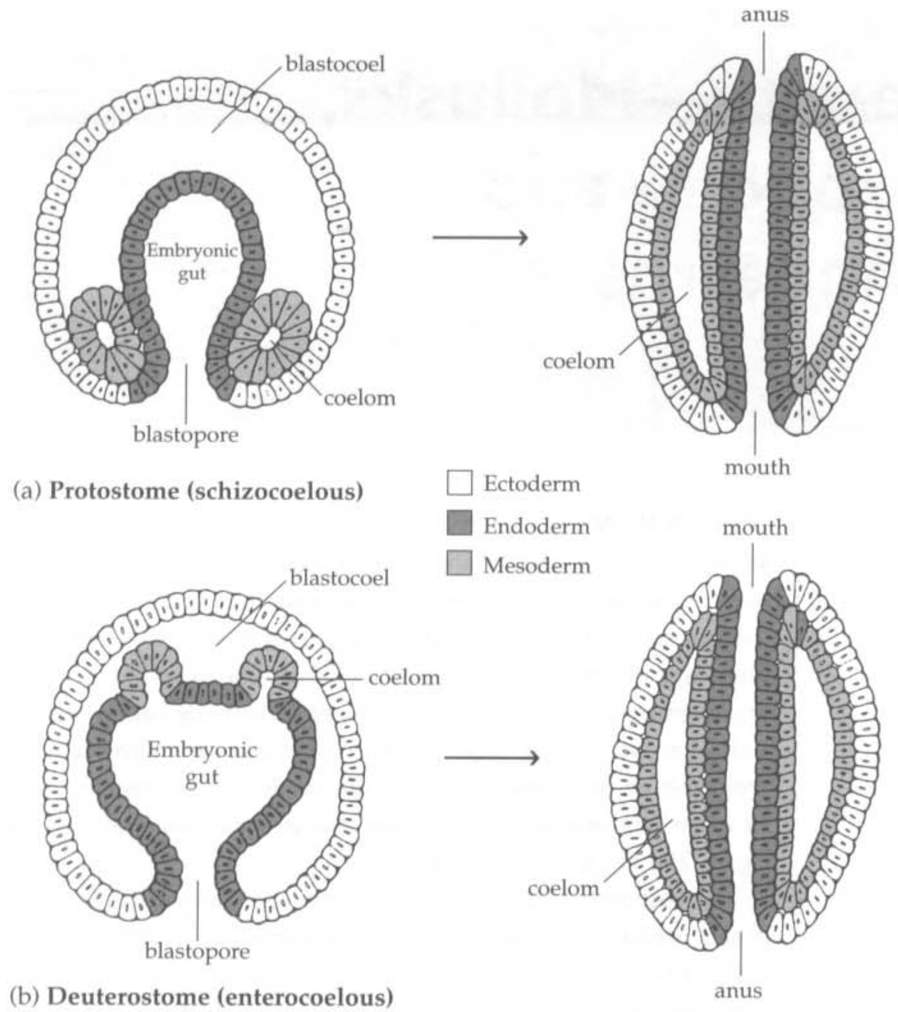


Figure 26-1 *(a) In protostomes, the mesoderm takes shape between the endoderm and the ectoderm, in the region around the blastopore. The coelom arises from a splitting of the solid mesoderm. As the cells continue to multiply, the blastocoel—the original embryonic cavity of the blastula—is obliterated. The blastopore of protostomes becomes the mouth.*

(b) In deuterostomes, the mesoderm originates from outpocketings of the embryonic gut. These outpocketings create cavities within the mesoderm that become the coelom. The blastopore becomes the anus, and the mouth develops elsewhere.

✓ EXERCISE A Phylum Mollusca

Mollusks represent the second largest phylum, consisting of more than 50,000 living species of marine, freshwater, and terrestrial animals. They are bilaterally symmetrical, coelomate, and apparently unsegmented.

The general body plan of a mollusk includes three regions: the **head-foot** (used in locomotion and food capture), the **visceral hump** or visceral mass (containing the major organ systems), and the **mantle** (soft tissue that secretes the calcium-containing shell present in many mollusks).

Mollusks have an open circulatory system with a chambered heart (one ventricle and two atria) and their blood contains an oxygen-carrying respiratory pigment, hemocyanin. Excretory organs, the **metanephridia,** drain the relatively small coelom surrounding the heart and a portion of the intestine.

Gills are present in the mantle cavity of most mollusks. Mollusks may be filter-feeders, sediment-feeders, herbivores, or carnivores.

Mollusks with shells include species that have shells formed from several plates (**chitons**), hinged shells (**bivalves,** including clams, oysters, and scallops), conical, twisted shells (**gastropods,** including snails and limpets), and reduced or internalized shells (**cephalopods,** including squids, octopuses, and cuttlefishes). (See Figures 26A-1, 26A-2, and 26A-3.)

Objectives

- ☐ Distinguish between the shell types characteristic of chitons, bivalves, gastropods, and cephalopods.
- ☐ Explain what the markings on the inner surface of a clam shell represent.
- ☐ Explain how water is moved through a clam for respiratory and feeding purposes.
- ☐ Distinguish between an open and a closed circulatory system.
- ☐ Distinguish between torsion and coiling of the gastropod shell.
- ☐ Describe how a cephalopod makes use of the visceral mass of its foot and the mantle cavity.

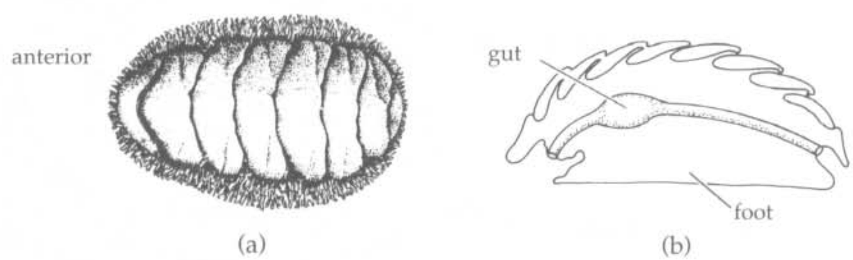
PART 1 Chitons

Procedure

Study the preserved chitons (class Polyplacophora) on demonstration. In most chitons, several plates or valves cover the upper body, suggesting a type of "segmentation," although this pattern is not reflected by internal structures (Figure 26A-1). These plates may be protective adaptations, allowing the organism to better fit the ocean bottom or to wrap itself into a ball. Identify the mouth and general head region. The specimen on demonstration has a well-developed foot. Locate the foot on the ventral surface. The outer covering of the body beneath the shell is the **mantle.**

a. How many plates are present in the shell? ______

Figure 26A-1 *(a) External body plan of the chiton. (b) The chiton in cross section.*



PART 2 Bivalves

Procedure

1. Examine a clam (class Bivalvia) shell.
   a. Is a clam a bivalve mollusk or a gastropod? ______
   b. The concentric rings on the shells of mollusks are referred to as growth rings. Which part of the shell do you suppose forms first? ______

Why does the outside of the shell have lines? The clam shell is composed of three layers: an outer horny layer, middle prismatic layer (mostly calcium carbonate), and inner pearly layer (this is where the pearl buttons on your shirts come from). The first two layers are laid down by the cells in the edge of the mantle, so as the clam grows the mantle makes layers in the outward direction. The entire mantle lays down the pearly substance. If a parasite or foreign object gets stuck in the mantle of a clam or an oyster, concentric rings of pearly material are secreted around it and a "pearl" is produced.

2. Orient the shell so that the anterior part, called the **umbo,** or beak, corresponds to the orientation of the shell in Figure 26A-2a. As you examine features in the shell, match the letters in the text to the letters in Figure 26A-2b; write the name of each anatomical structure on the diagram.

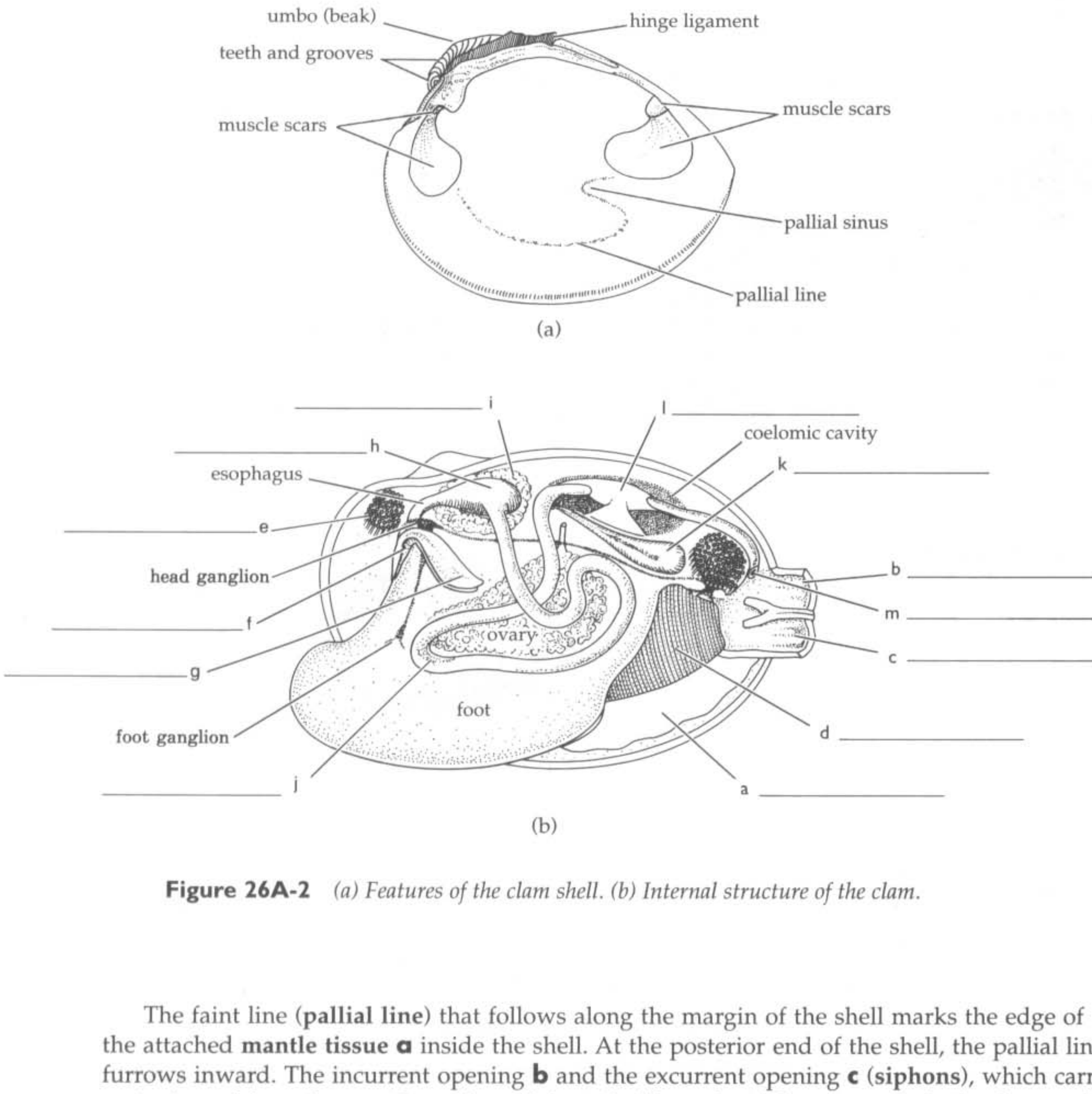


Figure 26A-2 *(a) Features of the clam shell. (b) Internal structure of the clam.*

The faint line (**pallial line**) that follows along the margin of the shell marks the edge of the attached **mantle tissue a** inside the shell. At the posterior end of the shell, the pallial line furrows inward. The incurrent opening **b** and the excurrent opening **c** (**siphons**), which carry water to and from the mantle cavity, originate in this region. Water currents bathe the mantle and **gills d** to facilitate gas exchange, and water passed through the gills is filtered through **mucous nets** to remove food particles that are subsequently eaten. Trace the path of food movement in Figure 26A-2b.

3. Locate the prominent **muscle "scars" e** on the inside surface of the shell. The contraction and relaxation of the muscles (whose points of attachment you see inside the valve) are responsible for closing and opening the shell. The two halves of a bivalve shell are held together by the **hinge ligament** whose scar may be visible along the inner dorsal edge of the shell. Also along this inner edge or lip are "teeth" and grooves that fit together with the corresponding "teeth" and grooves of the opposing valve.

4. Observe a preserved clam. Identify the following structures: **mouth f, palps g, stomach h, digestive gland i, intestine j, excretory organ k, heart l,** and **anus m.**

   The saclike stomach is surrounded by the digestive gland. The intestine runs from the stomach through the foot and passes through the cavity of the heart or *pericardial cavity* (the heart is actually wrapped around the intestine). Intestinal wastes are eliminated at the excurrent pore. Excretory organs (paired) lie beneath the heart, each resembling a tube coiled back on itself. The anterior portion of each organ collects waste from the blood and pericardial cavity, and the posterior portion eliminates waste into the dorsal gill passage.

   The circulatory system is open. The heart has three chambers (one ventricle and two atria). Blood is pumped anteriorly and posteriorly in open sinuses. When the large blood sinus in the foot is engorged with blood, it swells and makes locomotion possible.

   The nervous system consists of only three ganglia (foot, head, and visceral ganglia) connected to each other by long neurons. Sensory cells containing small amounts of limestone are found in the food and are used for balancing. A small patch of cells near the visceral ganglion is sensitive to chemicals.

5. Observe the compressed, bladelike form of the large muscular **foot** used in burrowing. Movement of this organ depends on blood pressure changes and on the pedal protractors and retractors, muscles that extend from each side of the foot to the shell on the opposite side and attach near the anterior adductor muscles.

   c. How is the foot used in burrowing? ______________________________

   ____

6. Examine other bivalve mollusk shells on demonstration. You are probably familiar with many of these.

   d. List these representatives. ______________________________

   ____

EXTENDING YOUR INVESTIGATION: FILTER-FEEDING

Clams are filter-feeders and feed mostly on microscopic particles. If live clams are available, you can follow the ciliary action using fine carbon particles. Form a hypothesis that predicts the pathway that carbon particles will take if the clam treats them as food.

HYPOTHESIS:

NULL HYPOTHESIS:

What do you **predict** will happen when carbon particles are placed on the surface of the gills?

Identify the **independent variable** in this investigation.

Identify the **dependent variable** in this investigation.

Use the following procedure to test your hypothesis:

PROCEDURE:

1. Use a candle or several wooden matches to apply a small amount of carbon film on the outside of a beaker or test tube.
2. Insert a one-piece scalpel or cartilage knife between the shells of a live clam. Angle the blade against the inside of one side of the shell and carefully cut the adductor muscles that hold the shells closed. **Do not cut toward your hand!**
3. Open the clam and place it in a dish with just enough water (seawater for a marine clam) to fill the shell and moisten the exposed gills.
4. Use a dissecting needle or small brush to remove a few carbon particles from the smoked beaker prepared in the first step. Gently place these particles on the posterior surface of the gills.

RESULTS: Determine how the carbon particles move across the gills.

What did you observe happening to the carbon particles?

Do your results support your hypothesis?

Your null hypothesis?

Was your prediction correct?

What do you **conclude** about the way in which filter feeders like the clam obtain food?

✔ PART 3 Gastropods

Procedure

1. Observe the gastropod mollusk shells (class Gastropoda) on display.

   a. Does the shell coil to the right or to the left? ____________

   The direction of shell coiling is determined as early as the eight-cell stage in development by maternal messenger RNA contained in the egg. The coiled nature of the shell disappears in some gastropods, with the adult shell representing a single large expanded whorl as in the abalone or slipper shells.

   In some gastropods, such as terrestrial slugs and marine nudibranchs, the shell may be completely lost. Note the many ornate ridges and bumps on the shells of gastropods on display. Look for the channels and grooves in the shells that house siphons through which water is flushed to aerate the gills.

2. Examine living pulmonate land snails on display. These snails do not have gills. Instead, the mantle cavity functions like a lung.

   b. When a snail is crawling about, what parts of its body are outside the confines of the shell?

   ____

   c. Would you say that cephalization (development of the head) is more apparent in gastropods than in bivalves? ______ *Why?* ______________________________

3. Move a blunt probe toward the snail you are observing. Which part of the body retracts into the shell first? Notice the disk-shaped plate (**operculum**) covering the opening of the shell.

   d. Does the operculum completely seal off the snail's body from its surroundings when the animal is disturbed? ______ *Is the operculum continuous with the shell itself?* ______

   e. Where does the operculum go when the snail is crawling along? ______________

4. Snails and slugs secrete mucus that is laid down by the foot. The slime is protective—a slug can pass unharmed over the edge of a sharp razor—and also contains hormones (pheromones) that guide other snails along the same trail. Can you find the slime trail that is formed as the animal crawls across the substrate?

✓ PART 4 Cephalopods

Procedure

1. Examine the squid on display. Cephalopod mollusks (class Cephalopoda) are among the most advanced invertebrates. The name means "head-footed," for in these animals the foot, which is divided into a number of "arms," is wrapped around the head (Figure 26A-3). The cephalopod head has complex sensors and the nervous system is well developed. Cephalopods are even capable of learning complex tasks.

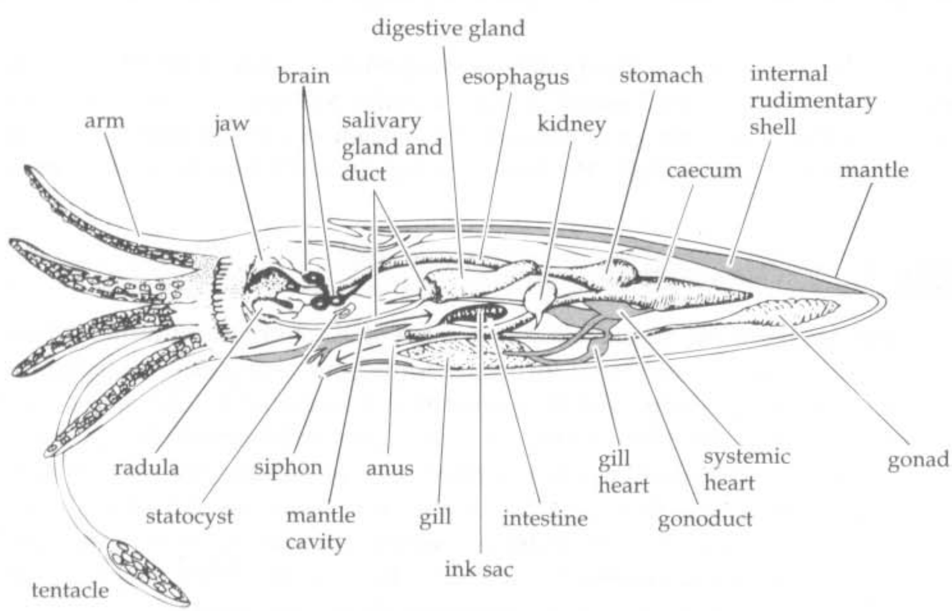


Figure 26A-3 *Internal structure of a squid. In the cephalopods, the head is modified into a circle of arms, and part of the head-foot forms a tubelike siphon through which water can be forcibly expelled. The arrows indicate the direction of water movement.*

2. The shell, characteristic of most mollusks, is only a thin vestige or "pen," buried in the body tissues of the squid. However, in the cuttlefish, a close relative of the squid, this internal shell is calcified. If you have cared for a pet bird, you may have provided this skeleton ("cuttlebone") for your pet to use in sharpening its beak and as a source of calcium. Examine a squid's "pen" and a cuttlefish's "cuttlebone" on demonstration.

3. The mantle, which secretes the "shell," is thick and muscular and serves a protective function, compensating for reduced shell size. When the mantle is relaxed, water enters the mantle cavity and, as it contracts, the edge of the mantle becomes tightly sealed to the body, forcing water out the funnel. Gently probe this area with your fingers to see these structures. When the animal is excited, the mantle contracts strongly and water is forced out of the funnel, pushing the animal in the direction opposite to that of the water jet. When attacked, the squid may emit dark ink from an ink sac opening into the funnel, thereby escaping as the predator is confused by this "smoke screen."

4. Examine the head. The squid has eyes constructed much like human eyes, although they developed in quite a different way.

   The squid has internal cartilaginous supports and cartilage protects the brain.

5. If a dissected squid specimen is available, locate the gills, ink sac, intestine, gill hearts, stomach, and systemic heart (see Figure 26A-3).

6. The octopus and chambered nautilus are also cephalopod mollusks. An external shell has been retained in the nautilus, but is lost in the octopus.

   a. *What structure represents the foot in the octopus?* ______________________

   The mantle of the octopus is fused to the body wall, and the entrance to the mantle cavity is more restricted than in other cephalopods. The octopus can swim by creating a jet of water, but typically it crawls over rocks and retreats into its den when attacked.

✓ PART 5 The Scaphopoda

This class of mollusks, the "tooth shells," have shells shaped like an elephant's task. Study the tooth shells on display.

The shell is open at both ends. The poorly developed head bears a number of extensible filaments used to capture prey. A radula for feeding and a muscular foot for burrowing are also present. The gills have been lost and the mantle serves as a respiratory organ as water currents are constantly maintained in and out of the upper end of the shell. The lower end of the shell remains buried in the sand.

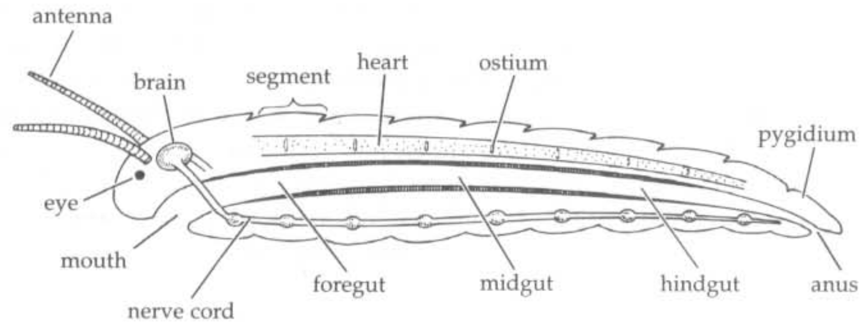
EXERCISE B Phylum Arthropoda

Arthropods are by far the most numerous and diverse of all animals, with more than 750,000 known species. Marine, freshwater, or terrestrial forms are found in every conceivable habitat due to their high degree of evolutionary adaptability and their great mobility, including, for some, the ability to fly.

The segmented arthropod body, covered by a chitinous **exoskeleton,** is typically divided into three parts: the **head, thorax,** and **abdomen.** Each of these may be subdivided into several segments to which are attached jointed appendages that carry out a variety of functions. As arthropods grow, they shed their chitinous exoskeleton by the process of **molting.** During this growth, many arthropods may also undergo a marked change in form (**metamorphosis**). If this is the case, the larva, a feeding stage, often bears no resemblance to the adult produced when metamorphosis is completed.

The arthropod circulatory system is open: a distinct muscular heart (Figure 26B-1) pumps blood through open spaces in the tissues—the **hemocoel.** The coelom, correspondingly, has been reduced and is represented in most arthropods only by the cavity of the gonads. The digestive tract of arthropods is well developed and modified into several distinct parts. The nervous system and associated sense organs are particularly well developed and control a variety of complex behaviors, including flight in winged insects.

Figure 26B-1 *Generalized body plan of an arthropod.*



The group of organisms we collectively call arthropods appears to have diverged into at least four separate subphyla, often regarded as phyla. The subphylum Cheliceriformes (or phylum Chelicerata) includes the classes Merostoma (horseshoe crabs), Arachnida (spiders, mites, ticks, scorpions, and their relatives), and Pycnogonada (sea spiders). Chelicerates lack antennae and mandibles (jaws). Instead, the first pair of appendages, the **chelicerae,** are in the form of pincers or fangs. The subphylum (or phylum) Crustacea includes crabs, lobsters, shrimps (class Malacostraca) and barnacles (class Cirripedia), and a number of other small aquatic invertebrates. The crustacea are **mandibulates** and are characterized by **biramous** (two-branched) appendages, two pairs of antennae and mandibles (jaws), and a pair of compound eyes. Members of the subphylum Uniramia* include the insects (class Insecta), centipedes (class Chilopoda), and millipedes (class Diplopoda). Insects are **uniramous,** having only one pair of antennae and unbranched (uniramous) appendages.

A fourth group of arthropods (subphylum Trilobitomorpha or phylum Trilobita) is an extinct taxon of primitive arthropods that were abundant in the Cambrian and Ordovician Periods and are known from their rich fossil remains.

The origin of arthropods remains a mystery. Systematists originally believed that members of the phylum Onycophora, a group of wormlike animals with claw-bearing legs and a thin chitinous cuticle, represented a type of common ancestor, a "missing link" between annelids and arthropods. Ribosomal RNA evidence, however, indicates that onycophorans are true arthropods. Members of a second phylum, Tardigrada, the "water bears," are similar to the onycophorans. Most likely these two groups are similar in structure to early arthropods but do not represent common ancestral lines.

In examining representatives of three major groups of arthropods (Crustacea, Arachnida, Insecta), we will concentrate on characteristics that are particularly useful in distinguishing arthropod species: the numbers and types of appendages, the degree of external segmentation, and the types of sensory organs.

Objectives

- ☐ Distinguish among the external body plans of the crustaceans, arachnids, and insects.
- ☐ Discuss the importance of molting in arthropod life cycles.
- ☐ Describe the respiratory system of insects.
- ☐ Give reasons for the success of insects.

✓ PART 1 Crustacea: External Morphology of the Crayfish

Crustaceans exhibit a great range of sizes, from lobsters and crabs to tiny water fleas and brine shrimp, hundreds of which can be cultured in a small laboratory beaker.

**If Uniramia is considered a phylum, two subphyla are identified: Myriapoda (centipedes and millipedes) and Insecta.*

Crustaceans also exhibit a wide array of modifications to their jointed appendages. These are associated not only with feeding and swimming, but also with reproduction, respiration, and burrowing. Among the characteristics that distinguish crustaceans from other arthropods are two pairs of antennae and biramous appendages. Mouthparts called **mandibles** are used to crush and grind food.

Crayfishes, used in this study, are freshwater relatives of lobsters. They patrol the bottoms of shallow creeks and ponds, where they feed mainly on aquatic plants and and on animal carcasses. When high water temperature and turbulence become a problem, crayfishes burrow into the mud. Their burrows also serve as shelters from their predators, including fishes, turtles, water snakes, and humans.

Procedure

1. Carefully remove a crayfish from the jar of preserved specimens available on the demonstration table. Place it dorsal side up in a dissecting tray (or Petri dish) and use the dissecting microscope to make your observations.
2. Note the general body plan of the crayfish. It is divided into a **cephalothorax** (anterior half) and an **abdomen** (posterior half). The dorsal portion of the cephalothorax of crustaceans is heavily mineralized (with calcium and phosphate salts) and is called the **carapace.** Running laterally across the carapace is a suture (the **cervical groove**) which marks the nearly complete fusion of the head and thorax.

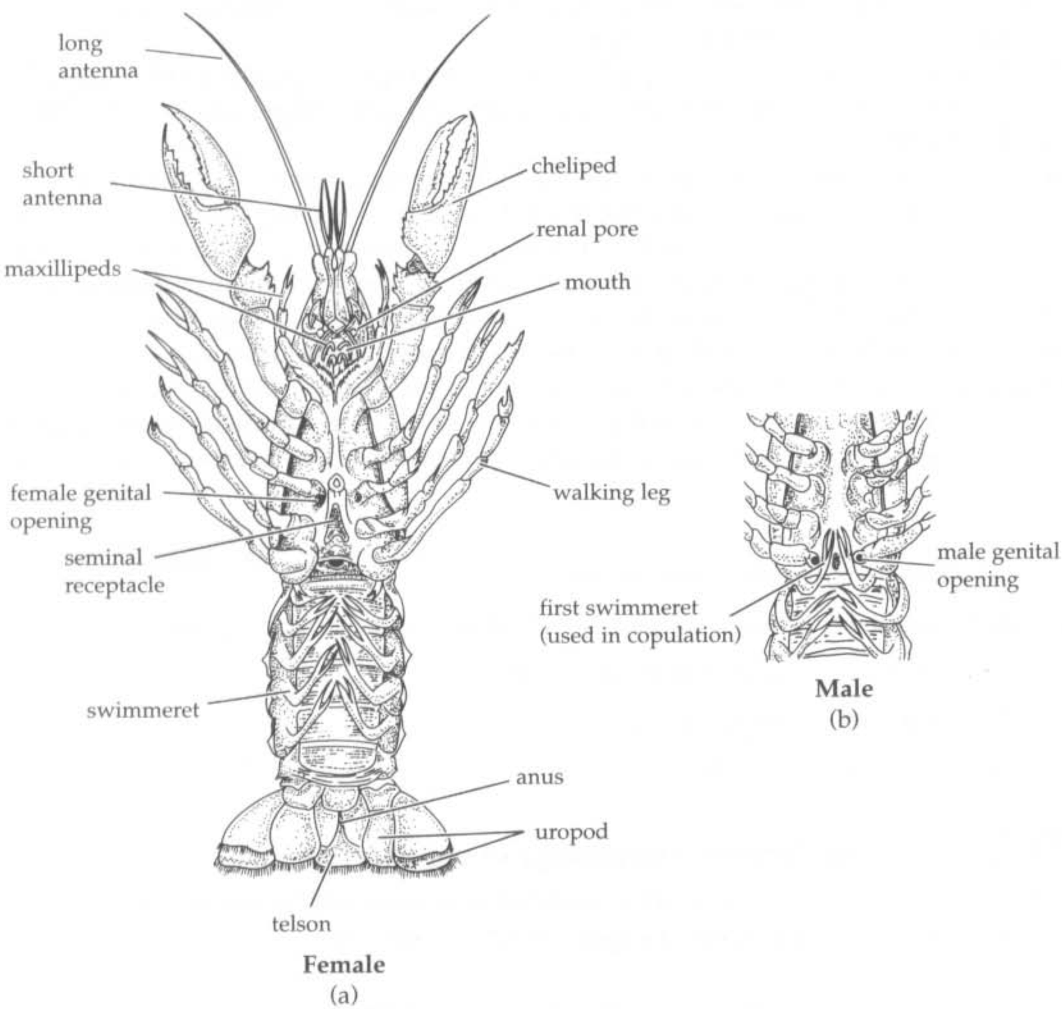


Figure 26B-2 *External features of (a) a female and (b) a male crayfish.*

3. Examine the head of your crayfish under a dissecting microscope. The carapace ends anteriorly in a protective hood, the **rostrum.** On either side of the rostrum is a compound eye, situated on a stalk that allows the eye to move. Gently use your forefinger and thumb to peel off the outer layer of the eye (the cornea). Note the many small rectangular facets that make up the structure of the compound eye.
4. Crustaceans have two pairs of antennae, used primarily as sensory appendages. Briefly compare the structure of the two types of antennae. At the base of one of the long antennae, locate the small pore that functions in the excretion of nitrogenous waste and excess water.
5. In addition to antennae, crayfishes have 17 other pairs of appendages; three pairs make up the inner jaw (mandibles and maxillae), three pairs make up the outer jaw (maxillipeds), five pairs are associated mainly with crawling and digging, and the most posterior (abdominal) six pairs are used mainly in swimming. It is best to examine these with the crayfish positioned so that the ventral side is facing you (Figure 26B-2). As you study each type of appendage, relate its structure to its function.
6. Summarize the characteristics of crustaceans in Table 26B-1.

Table 26B-1 Comparison of the External Morphology of Arthropods

Characteristics	Crustaceans	Arachnids	Insects	Myriapods
Main divisions of the body				
Main body divisions that show external segmentation				
Locomotor structures				
Other appendages				
Number of pairs of antennae				
Mouthparts				
Number and type of eyes				

✓ PART 2 Arachnida: External Morphology of a Spider

Arachnids are a group of eight-legged terrestrial arthropods that are, for the most part, fierce predators of other arthropods. The most distinctive characteristic of these carnivores is a pair of appendages, the **chelicerae,** used in procuring and eating prey. Arachnids include not only spiders, but also scorpions, harvestmen (daddy longlegs and their relatives), ticks, and mites.

Many arachnids use venom to paralyze their prey. **Silk,** another common arachnid product, may be used for prey capture, reproductive activities, and dispersal.

Procedure

1. Use a pair of forceps to carefully remove a preserved spider from its vial. Place it in a Petri dish containing alcohol and a cotton pad. With the ventral side resting against the cotton, orient it so that the front edge of the cephalothorax is toward you. Use the dissecting microscope to examine the spider.

2. In arachnids, as in crustaceans, the body is divided into two parts, the cephalothorax and abdomen.

   a. *How can you distinguish the cephalothorax of your specimen from its abdomen?*

   ____

   b. *To which part of the body are the eight legs attached?* ____________________

3. Position the spider against the cotton pad so that the anterior edge of the cephalothorax is turned upward and the spider is "looking up" into the microscope. Note that the eyes of spiders are simple rather than compound. They occur in pairs of different sizes.

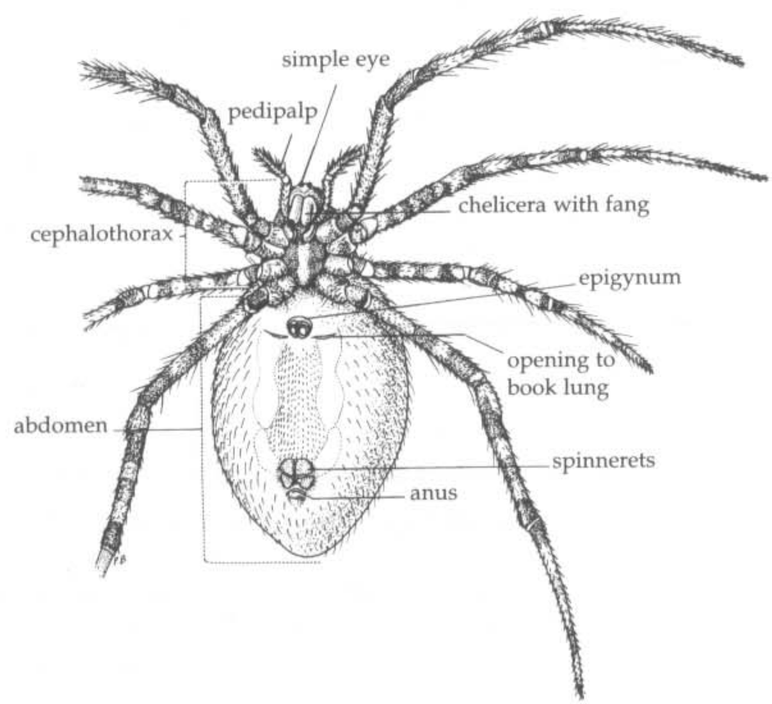
   c. *How many eyes can you find?* ______ *How are these eyes arranged?* ____________________

   d. *Which pair of eyes is the largest?* ____________________ *(In some spiders, the posterior pair of eyes is well back on the sides of the cephalothorax.)*

4. The first pair of appendages, the **chelicerae,** are below the first pair of eyes. Turn your specimen over onto its dorsal surface (so that you view its ventral surface as in Figure 26B-3). Gently push aside the other appendages with your dissecting needle to find where the chelicerae are attached to the body. In spiders, these stout but powerful appendages are sharply pointed to form fangs used to inject venom into prey. In other arachnids, the chelicerae are not fangs but claws.

   The mouth of a spider lies directly posterior to the chelicerae. It is a small hole that might be difficult to see because of the many hairs that surround it. These hairs are used in filtering out solid materials—spiders consume only the liquefied portion of their prey.

Figure 26B-3 *External features of a female spider.*



5. On either side of the chelicerae are the **pedipalps,** short leglike appendages used for a number of purposes. In male spiders, the tips of the pedipalps contain an organ (the **tarsal organ**) used to store sperm. This organ and the sensory hairs around it give the tip of the pedipalp the appearance of a tiny boxing glove.

   If your specimen does not appear to be a male, examine its abdomen for a furrowed area bordered by patches of color (Figure 26B-3). This is the **epigynum,** whose two tiny openings lead into the female reproductive tract. The male inserts its tarsal organs into the opening of the epigynum to deliver sperm.

6. On either side of the epigynum, look for the openings to the **book lungs.** (They are in the same location in males as in females.) A book lung consists of a highly folded membrane with a rich supply of blood capillaries. It is used for respiratory gas exchange.

7. At the tip of the abdomen is a pair of **spinnerets,** appendages that secrete silk from glands within the abdomen. Each pair is specialized to secrete a different type of silk suited to a specific purpose.

   e. How many spinnerets are present in your specimen? ______

   Posterior to the last pair of spinnerets is the **anal tubercle** with an anal pore at its tip. This pore is usually closed and not visible.

8. Summarize arachnid characteristics in Table 26B-1. Compare these with the structural characteristics of crustaceans.

PART 3 Chelicerata: Biochemical Taxonomy of the Horseshoe Crab (*An Optional Exercise*)*

Although *Limulus* (the horseshoe crab) appears to have many anatomical and physiological similarities with crustaceans, biochemical evidence suggests that it is more closely related to the arachnids. With which group does *Limulus* belong?

Organisms demonstrating biochemical similarities among their proteins are thought to be more closely related to each other than to other organisms. The number and sequence of amino acids and the manner in which a protein folds are all a reflection of the "recipe" in an organism's DNA. Similar proteins have similar amino acid sequences dictated by similar DNA sequences, and scientists believe that similar DNA sequences indicate a common evolutionary origin.

The more biochemically similar two organisms are, the more similar their enzymes will be. In this exercise you will test for the activity of lactic acid dehydrogenase (LDH), a multi-subunit enzyme present in the muscle tissue of all animals. During strenuous exercise, LDH catalyzes the anaerobic conversion of pyyruvic acid to the lactic acid. In this reaction, lactic acid is formed when electrons carried by NADH are accepted by the pyruvic acid:

$$\begin{array}{lcl} \mathrm{CH_3CCOOH + NADH + H^+} & \rightleftharpoons & \mathrm{CH_3CHOOH + NAD^+} \\ \quad\;\; \| & & \quad\;\; | \\ \quad\;\; \mathrm{O} & & \quad\;\; \mathrm{OH} \\ \text{pyruvic acid} & & \text{lactic acid} \end{array}$$

This reaction is reversible, and in this exercise you will study the conversion of lactic acid to pyruvic acid. During this reaction, electrons are transferred from lactic acid to NAD^+ and then to phenazine metasulfate (PMS) and, finally, to dichloro-phenol-indophenol (DPIP). As DPIP accepts electrons and is reduced, its color changes from blue to clear.

The tertiary structures of LDH subunits vary slightly among different organisms. These tertiary structures are determined by the interactions of amino acids, which, in turn, depend upon primary

This experiment was developed by Mary Ellen Hart and Scott E. Pattison. Funding was provided by the National Science Foundation and California State University system under the auspices of the Institute for Cellular and Molecular Biology, California State Polytechnic University, Pomona.

structures and thus upon DNA sequences. The structural variations in LDH among types of organisms result in differences in the interaction with NAD^+ and, consequently, in the degree of color change in DPIP. To investigate the degree of evolutionary similarity among organisms, you will compare LDH activity in spiders (Arachnida), crabs (Crustacea), and *Limulus* (Merostomata).

Objectives

- ☐ Use biochemical evidence to elucidate evolutionary relationships.
- ☐ Demonstrate that anatomical similarities do not always indicate close taxonomic or evolutionary relationships.

Procedure

1. Label four test tubes: control, *Limulus*, spider, and crab.
2. Add 3 ml of lactic acid solution to each of the four test tubes.
3. If you are using the spectrophotometer to quantitate the DPIP reaction, use a tube containing the lactic acid solution to "zero" the spectrophotometer (see Laboratory 4) at 600 nm. Record the data for all four tubes in the first row of Table 26B-2.
4. Add six drops of DPIP indicator solution to each tube. Cover each tube with a small piece of Parafilm and invert the tube to mix the solutions.
5. Record the color of each of the sample tubes in Table 26B-2. If you are using a spectrophotometer, also record the absorbance.
6. Add 10 drops of spider supernatant to the tube marked "spider" (the supernatant contains homogenized muscle tissue). Cover the tube with Parafilm and invert to mix. Immediately note the initial color and record the absorbance in Table 26B-2.
7. Add 10 drops of crab supernatant to the tube marked "crab," 10 drops of *Limulus* supernatant to the tube marked "*Limulus*," and 10 drops of distilled water to the tube marked "control." Cover each tube with Parafilm and invert to mix. Note the initial color and record absorbance in Table 26B-2.
8. At 5, 10, and 15 minutes, note the color and record the absorbance for each tube.

Table 26B-2

	Spider		*Limulus*		Crustacean		Control	
	Absorbance /	Color	Absorbance /	Color	Absorbance /	Color	Absorbance /	Color
Lactic acid solution								
Lactic acid solution + DPIP								
Lactic acid solution + DPIP + supernatant: initial reading								
5 minutes								
10 minutes								
15 minutes								

a. Based on observed color differences or on absorbance data, is Limulus *more closely related to the spiders (Arachnida) or to the crabs (Crustacea)?* ______________________

b. Anatomically, which group of organisms does Limulus *more closely resemble?* ______________

c. How would you explain the fact that anatomically, Limulus *resembles one group of organisms, but biochemically, it is more closely related to another group of organisms?*

____

____

✓ PART 4 Insecta: External Morphology of the Grasshopper

Of all invertebrates, insects are the most diverse and the most complex. Almost 90 percent of all known animal species belong to this group. The enormous success of insects as a group can be attributed, in part, to their diverse and specialized types of locomotor and feeding appendages and their well-developed sensory organs. The most important reason for their success, however, is their capacity for flight.

Procedure

1. Use your dissecting microscope to examine a preserved grasshopper. Orient the specimen in a Petri dish so that its head is to the left as you view it. From Figure 26B-4 and the discussion that follows, locate each of the structures indicated in the text in boldface type. On Figure 26B-4, write in the names of the structures accompanied by letters in the text.
2. The insect body is covered by a strong, protective **cuticle** secreted by cells of the epidermis. The major chemical component of this cuticle is chitin. A layer of pigment covers the chitin and gives the insect its characteristic color. On the outer surface, a waxy layer protects the insect from desiccation.

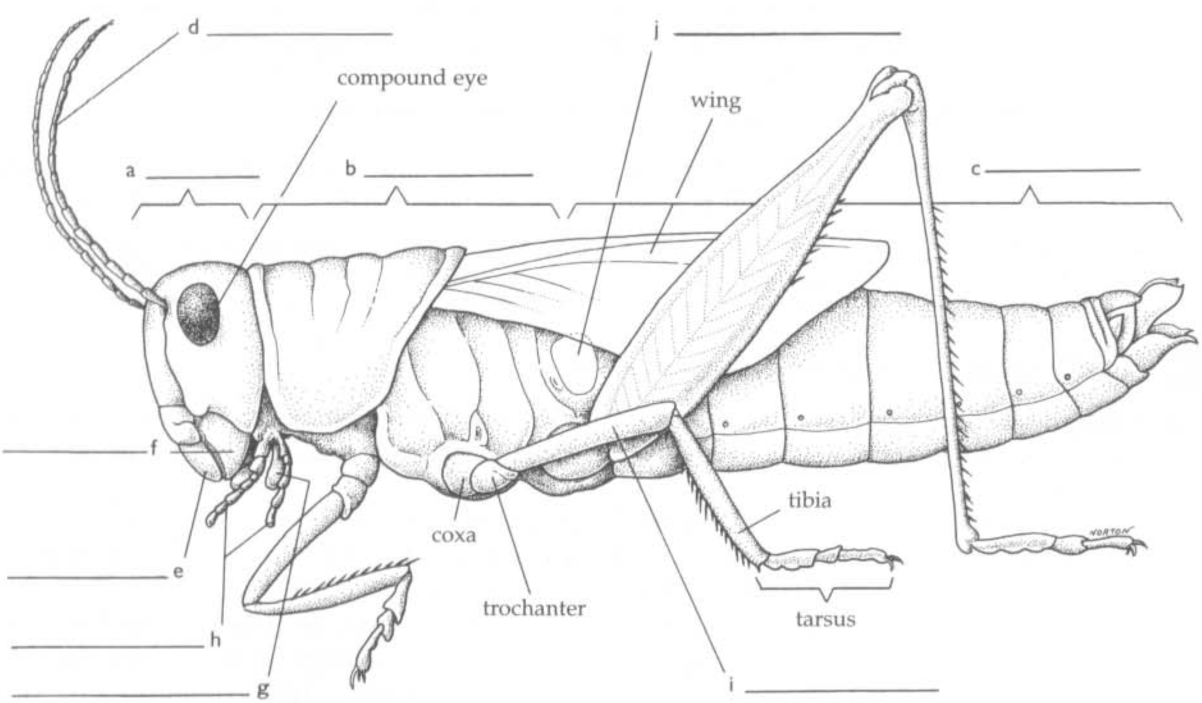


Figure 26B-4 *External anatomy of the grasshopper.*

3. The body of an insect is divided into three parts: the **head a, thorax b,** and **abdomen c.**

   a. How does this body plan differ from that of crustaceans and arachnids?

   ____

4. The insect head bears both sensory organs and mouthparts. Locate the **antennae d** (first segment) and the single pair of compound eyes (second segment.) Insects also have simple eyes (**ocelli**) that are much smaller than compound eyes and lack the multiple sense elements found in compound eyes. Ocelli may be very sensitive to low light levels and may also enhance the sensitivity of the compound eyes.

   b. How many ocelli do you see above the antennae? ______

5. Now, using a dissecting needle, carefully manipulate and identify the mouthparts. The third segment bears the upper lip, **labrum e,** and the fourth a pair of toothed, horny jaws, **mandibles f,** which are accompanied by accessory jaws, **maxillae,** on the fifth segment. Both the maxillae and the lower lip, **labium g,** bear sensory **palps h,** delicate, segmented appendages used in manipulating and "smelling" food.

6. Look at the dorsal part of the thorax of your grasshopper. This portion of the thorax is a hardened shield called the **prothorax.** In flying insects, the thorax bears one or two pairs of **wings.** A grasshopper has hardened forewings overlying translucent membranous hindwings. Veins in the hindwing are formed by a network of chitinous tubules that provide strength without adding much weight.

   The thorax bears three pairs of **legs,** one pair on each thoracic segment. All insects have six legs. In the grasshopper, the first two pairs are typical walking legs and the third is specialized for jumping. Identify the leg parts in Figure 26B-4. Do you see the many spinelike and toothlike structures used for grasping? The upper segment of the grasshopper hindleg, the **femur i,** is enlarged; it contains the muscles used in jumping.

7. Now examine the abdomen of your grasshopper. It is divided into several segments externally, but not internally. The first segment of the abdomen of a grasshopper bears a relatively large sense organ, the **tympanum j,** used in detecting airborne vibrations (sound). Along the side of the abdomen are a number of tiny holes, or **spiracles,** the openings of the insect's respiratory system, a network of chitin-lined tubules that spread throughout the body.

   c. How many pairs of spiracles are present on each segment of the grasshopper? ______

   Appendages on the posterior tip of the abdomen are associated with reproduction and sensory detection. You will not need to study these in detail.

8. Add the characteristics of insects to Table 26B-1 and compare them with the features found in crustaceans and arachnids.

✓ PART 5 Diplopoda and Chilopoda

The **diplopods** (millipedes) and **chilopods** (centipedes) are flightless terrestrial arthropods sometimes collectively called the **myriapods.** Myriapods may be either herbivorous (millipedes) or carnivorous (centipedes).

The head is connected to an elongated trunk composed of a short thorax and long abdomen with many leg-bearing segments. In centipedes, each trunk segment bears one pair of legs; in millipedes each segment has two pairs of legs. The head bears a pair of antennae and sometimes ocelli, but compound eyes are almost never present. The mouth is surrounded by an upper lip (labrum) and lower maxillae and mandible. In chilopods, appendages of the first trunk segment (maxillipeds) are modified into poison claws for food gathering. Some centipedes are dangerous to humans—their venom is quite potent.

Examine a millipede and a centipede. Compare their external body plans with those of the crustaceans, arachnids, and insects. Summarize their characteristics in Table 26B-1.

✓ PART 6 Insect Mobility

The capacity for flight has provided insects with the ability to disperse themselves widely and to exploit food resources more efficiently. Flight demands a great deal of energy and oxygen. To fill this demand, insects have developed a highly efficient system for exchanging gases between cells and the environment. This is the **tracheal system,** a finely branching network of tiny tubules through which oxygen passes directly to the cells of muscle and other tissues throughout the body. The tracheal system is independent of the pumping of the heart and operates solely on the principle of diffusion (with muscular activity aiding in the ventilation of larger tracheoles). Although this system is efficient, diffusion into and out of small tracheoles limits the overall body size that an insect can attain.

a. What is the largest insect you have seen or read about? ______________________________

In addition, the tracheal system makes insects particularly vulnerable to various diseases, particularly those caused by fungi that invade body tissues through these tubes.

Procedure

1. Look at the prepared slide of an insect spiracle.

   b. What is the external location and orientation of the spiracle on the insect's body?

   ____

   c. How is the chitin arranged within the spiracle? ______________________________

   d. What might be a purpose for the tiny hairs lining the air space in the spiracle?

   ____

2. Look at the prepared slide of an insect tracheal system.

   e. How is the chitin arranged within individual tracheae and tracheoles? ______________

   f. What might be the purpose of this type of arrangement? ______________________

   g. Describe the type of branching within the tracheal system.

   ____

3. Examine the preserved insect specimens on display in the laboratory. List those that have wings and indicate how many pairs of wings are present. Record your results in Table 26B-3.

Table 26B-3

No Wings	One Pair of Wings	Two Pairs of Wings

h. From what division of the body do the wings extend? ______________________

i. What other parts of the body do the wings cover? ______________________

j. What secondary function does this suggest for the wings? ______________________

✓ EXERCISE C Protostomes: Minor Groups

There are several groups of protostome invertebrates that do not fit into any of the large phyla we have discussed. These include several phyla of wormlike organisms: the Pogonophora (the largest of which are found along hydrothermal rifts in the ocean floor), the Sipuncula or "peanut worms," and the Echiura and Pentastomida (parasites that inhabit the respiratory systems of reptiles). Tardigrades (Tardigrada), tiny soil organisms called "water bears," are often found in the water films of mosses. (These are a favorite of most biology students). Recall that the tardigrades and onycophorans (Onycophora), which resemble slugs with legs, appear to be similar to primitive arthropods, although their role as ancestral "missing links" is questionable.

Four additional phyla, the Bryozoa, Entoprocta, Phoronida, and Brachiopoda, all possess a tentacular filter-feeding apparatus called a **lophophore.** Often these phyla are grouped together as lophophorate coelomates. However, they are not closely related to one another; bryozoans and entoprocts are more closely related to deuterostomes. Bryozoans or "moss animals" include colonial and *sessile* (attached to a substrate) organisms with a mouth surrounded by tentacles. Entoprocts are similar to the bryozoans but, unlike the bryozoans, both the mouth and anus are located within the lophophore. The phoronids live within chitinous tubes in marine waters, while the brachiopods or "lamp shells" are encased by the dorsal and ventral valves of their shell (rather than left and right valves as in bivalve mollusks). Brachiopods are widely distributed as fossils of the Paleozoic and Mesozoic eras.

Objectives

- ☐ Define "protostome."
- ☐ Identify minor phyla by describing an organism for each.

Procedure

1. Observe the pictures or preserved specimens of this diverse group of organisms on display in the laboratory.
2. Make a wet mount of moss leaves (or use a prepared culture) to observe tardigrades, the "water bears."

✓ EXERCISE D Phylum Echinodermata

If you examine the phylogenetic tree presented at the beginning of Laboratory 25, you will see that our study of echinoderms takes us to a new branch of the tree. Recall that three major differences in early embryonic development and form typify these organisms. First, unlike the protostome organisms we have studied thus far, echinoderms are **deuterostomes.** During development, the blastopore contributes to the formation of the anus rather than of the mouth as in protostomes. Second, the cleavage pattern typical of deuterostome zygotes differs from that of protostomes: cleavage is **radial** rather than spiral. Third, the true coelom of deuterostomes is derived from a mesodermal outpocketing of the primitive gut, or **enteron;** the deuterostomes are called enterocoelous coelomates. Recall that the coelom of annelids, arthropods, and mollusks (all protostomes) in schizocoelous, formed from a splitting of a solid mass of mesoderm within the body.

The phylum Echinodermata includes four major groups of marine bottom dwellers or burrowers: the starfishes and brittle stars (class Stelleroidea); sea urchins and their relatives, the sand dollars (class Echinoidea); sea lilies (class Crinoidea); and sea cucumbers (class Holothuroidea). Echinoderms are noted for their spiny protective skins, their five-part structure, and the presence of numerous small appendages, the tube feet, which function as part of a water vascular system derived from the coelom (Figure 26D-1). The tube feet are used for locomotion, feeding, and respiration. The coelom carries out circulatory, respiratory, and excretory functions.

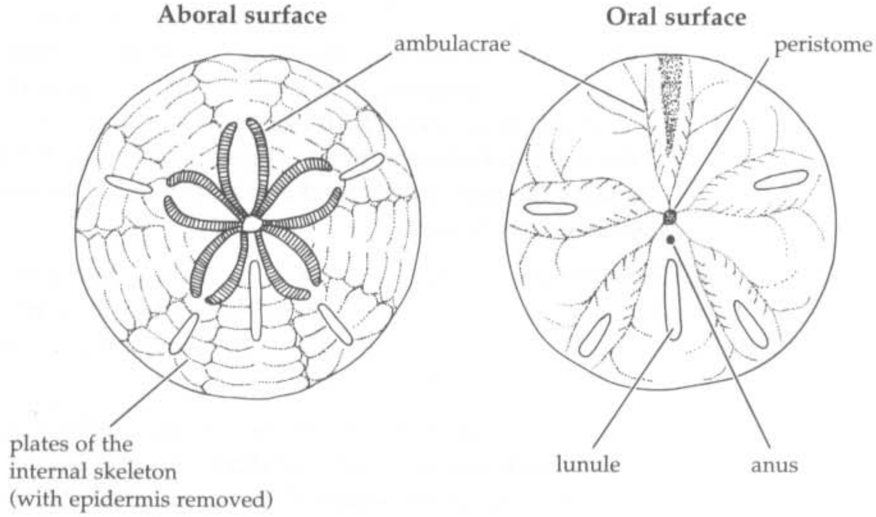


Figure 26D-1 *The aboral and oral surfaces of the sand dollar.*

Objectives

- ☐ Define "deuterostome."
- ☐ Explain why echinoderms form a new branch of the phylogenetic tree.
- ☐ Give three major characteristics of organisms grouped as echinoderms.
- ☐ Describe radial symmetry among the echninoderms.

Procedure

1. Examine the echinoderms on display in the laboratory.
   a. *Which forms are radially symmetrical?* ______
   b. *Which are bilaterally symmetrical?* ______
   c. *Which have arms?* ______
   d. *Which are spherical?* ______
   e. *Which are disklike?* ______

2. Unlike arthropods, which have exoskeletons, echinoderms have internal skeletons. The skeleton is composed of flattened calcareous plates called **ossicles.** Spines are outward extensions of these plates and are characteristic of the echinoderms, often called the "spiny-skinned" animals. In addition to spines, some echinoderms (the Stelleroidea and Echinoidea) also have **pedicellaria** extending from their surfaces. These are small "pincers" that aid in capturing food and keeping the body surface clean. Look at the rounded surface of a sand dollar (Figure 26D-1). Do you see the faint lines indicating an array of interconnected plates? Or a separate sheet of paper, make a sketch of this part of the skeleton. In the living sand dollar, as in other echinoderms, these plates are covered by an epidermis and would not be visible. Insert your drawing into the manual.

3. The terms dorsal and ventral are not usually used to describe radially symmetrical organisms. Instead, the terms **oral** (on the same side as the mouth) and **aboral** (on the side opposite the mouth) are preferred. The mouth of radially symmetrical echinoderms is on the lower surface. Identify the oral and aboral surfaces of the sand dollar.

Radiating outward from the center of the sand dollar on both sides is a pattern of "arms" (**ambulacrae**) resembling the arms of a starfish. Along each edge of the ambulacrae is a row of tiny perforations through which the tube feet project.

Gonopores, from which eggs and sperm are shed, may be seen where the ambulacrae join at the center of the organism. The narrow elongated holes in the skeleton, called **lunules,** are channels through which food can be moved from the upper (aboral) surface to the mouth on the lower (oral) surface.

4. Examine the skeleton (**test**) of a sea urchin. Compare its form to that of the sand dollar. Like the sand dollar, the ossicles bear spines, but they are more pronounced. Depending on the species, these can be narrow and pointed, long or short, and may sometimes be very thick and heavy as in the pencil urchins.

5. Examine the oral surface of the sea urchin. At its center is an open ring, the **peristome.** In the live organism, the peristome contains the gills and is covered by a membrane. Inside the ring is a circle of large, mineralized teeth shielding the opening of the mouth. The teeth of both the sand dollar and sea urchin are part of a complex feeding apparatus called Aristotle's lantern. Look again at the oral surface of the sand dollar. Compare its central structure with that of the sea urchin.

   f. How many teeth are present in the sand dollar? ______

   Surrounding the peristome of living echinoids are rings of tube feet. Tube feet are also scattered over much of the oral surface in sea urchins. Can you see the holes in the test through which the tube feet project? Tube feet near the mouth are used like tiny hands to move food toward the opening. The others are used like tiny suction cups in locomotion. Tube feet are exposed directly to the water and have permeable membranes through which oxygen can diffuse. The anus of the sea urchin is near the center of the aboral surface; it may not be visible.

6. Compare the structure of the sand dollar and sea urchin with that of the sea star (starfish, class Stelleroidea) on display. On the oral surface, identify the ambulacral grooves and spines. In the living starfish, tube feet project from these grooves. Examine the aboral surface and find the **madreporite,** a small round plate placed somewhat off-center on the central disk. The madreporite is a perforated plate that guards the opening through which water enters the water vascular system (Figure 26D-2).

7. Related to the sea stars are the brittle stars. They lack the pedicellaria and skin gills (**papulae;** thin areas of the body wall projecting outward between ossicles) present in sea stars. Also, their tube feet do not have suckers and are sensory in function rather than locomotory. Examine the brittle star on display. Notice that the arms are sharply distinct from the central disk. Both sea stars and brittle stars are able to regenerate lost parts.

8. Examine a preserved specimen of the sea cucumber (class Holothuroidea). Sea cucumbers lack arms, spines, and pedicellaria. They lie on the ocean floor (looking much like cucumbers) and filter-feed by means of branched tentacles, which are really modified tube feet. Some are burrowers. The ossicles are small and are buried in the leathery body wall.

   Sea cucumbers have an interesting way of discouraging predators—they eviscerate (cast out part of their internal organs). The predator is left holding the viscera while the sea cucumber escapes. Lost parts are then quickly regenerated.

9. The sea lilies (class Crinoidea) are the most primitive group of echinoderms. Some are stalked and sessile, while others (feather stars) are motile. Crinoids trap detritus and planktonic organisms in the center of their feathery arms and then move it toward the mouth through a ciliated ambulacral groove with the assistance of many suckerless tube feet. In all other groups of echinoderms, the tube feet are simply locomotory or sensory, not ingestive, in function. Examine the preserved feather star specimen on demonstration.

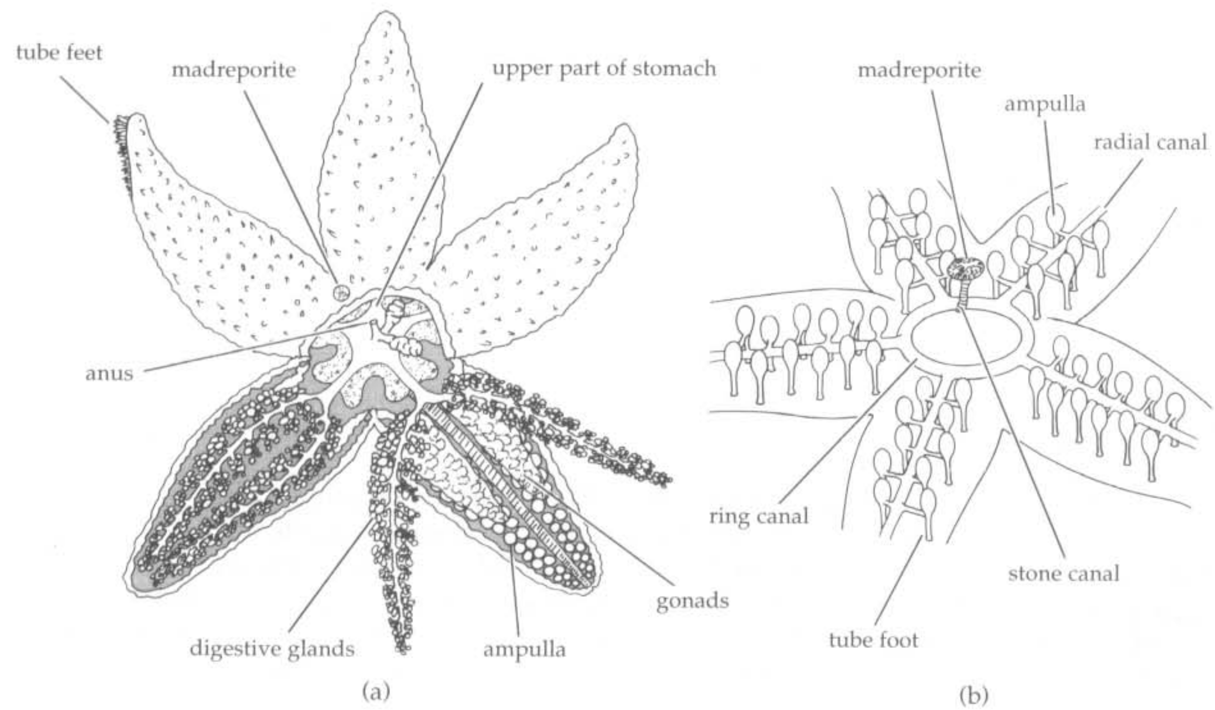


Figure 26D-2 *The sea star, (a) external and (b) internal anatomy. The water vascular system is filled with fluid similar to seawater, and some seawater can enter the madreporite to maintain water pressure. The internal canals—stone canal, ring canal, and radial canals—are interconnected. Radial canals terminate in a series of tube feet and bulbs* (ampullae) *separated from the canal by a valve. During locomotion, the ampullae contract and the valves close, forcing water into the tube feet, which extend and adhere to the substrate by means of mucous adhesion and suction.*

Laboratory Review Questions and Problems

1. Protostomes, including the annelids, arthropods, and mollusks, can be distinguished from deutoerostomes, including echinoderms and chordates (to be studied during the next laboratory period). Fill in the following table to summarize the differences.

	Protostomes	Deuterostomes
Type of coelom		
Type of cleavage in egg		
Type of development		
Fate of the blastopore		

2. The shells of chitons are segmented into eight plates, which may be buried in the mantle. What is the significance of this observation with respect to their placement in the annelids, arthropods, and mollusks?

3. Cephalopod mollusks are adapted for swimming by propulsion and for carnivorous eating habits. Explain how the foot and mantle cavity are modified for these purposes.

4. What unifying characteristics do you find among the arthropods?

5. If you were to assign someone the task of efficiently dividing a large, diverse group of objects or organisms into smaller groups according to specific characteristics, you might devise a **dichotomous key.** This method uses pairs (*dichot-*, two) of mutually exclusive descriptions to subdivide larger groups into successively smaller subgroups. These descriptions are based on the possession of, or lack of, particular characteristics. With each subdivision, the characteristics become more limited, until each "path" of characteristics can lead to only one specific member or the smallest identifiable group.

For example, suppose you are given a rubber bulb, a rubber stopper, a glass rod, a glass slide, and piece of glass tubing. Your dichotomous key might look like this:

1a object rubber	go to 2
1b object glass	go to 3
2a object squeezable	rubber bulb
2b object solid	rubber stopper
3a object flat	glass slide
3b object cylindrical	go to 4
4a object hollow	glass tubing
4b object solid	glass rod

Notice that each alternative choice excludes the other. Each message on the right indicates the "path" to be followed through the key until the object is definitively identified.

Devise a dichotomous key to separate the five classes of arthropods that you have studied:

1a ______________________________

1b ______________________________

2a ______________________________

2b ______________________________

3a ______________________________

3b ______________________________

4a ______________________________

4b ______________________________

5a ______________________________

5b ______________________________

6. What two characteristics distinguish crustaceans from other arthropods?

7. Distinguish between the general body plan of crustaceans and arachnids and that of insects.

8. Echinoderms are deuterostomes and represent a different branch of the phylogenetic tree from the other phyla you have studied. How do they differ from these other phyla?

9. What common characteristics are shared by the four classes of echinoderms you studied?