# CEPHALOPOD DISSECTION BIOL 337

**(adapted from Fox 2001, http://webs.lander.edu/rsfox/invertebrates/lolliguncula.html)**

Dissections are one of your best opportunities in the course to be a scientist: posing hypotheses about what structures are and using various kinds of information to test your hypotheses—*where is it located? what color is it, and how does that color differ from other structures? does it connect to other structures, and can you trace connections? is it blind-ended? does it have an opening? a lumen? what does the lumen contain?* Specimens may not exactly match diagrams because the animal is a different sex or species or was collected in a different season. I would prefer that you go through the exercise of figuring out the anatomy through deduction (and possibly getting names wrong) than my simply confirming guesses. Don't be surprised when I don't tell you what something is, though I would be happy to listen to and check your reasoning.

You will dissect a frozen specimen of a squid (species depends on place of collection), which is sold locally as food or fishing bait. Place your specimen into a dissecting pan with tapwater and use magnification to look carefully at structures and their connections. You can use the diagrams in your textbook in addition to those here. Remember that structures may differ from diagrams.

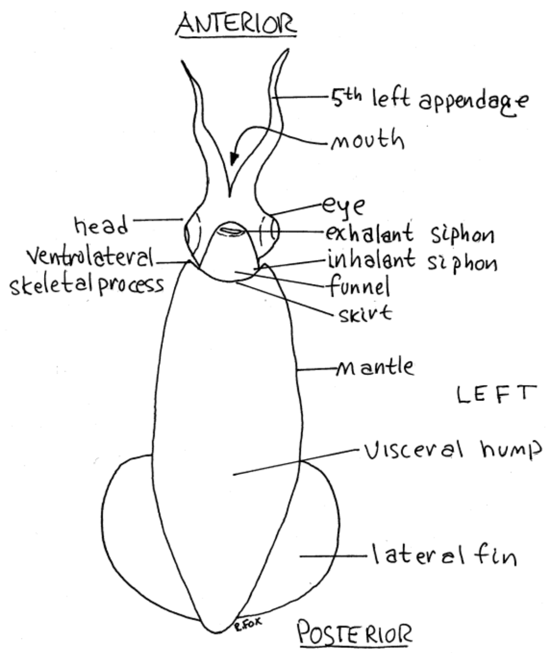



**Fig. 1. Ventral view of a female *Lolliguncula brevis*.**

## Orientation and locomotion

The squid body is **fusiform** (torpedo-shaped and streamlined), an adaptation for fast swimming. Recall how this body shape results from a transformation of the body axes. Orient yourself to the *functional* body axes (anterior to posterior, right to left, dorsal to ventral). Which *anatomical* body axes are compressed, and which elongated, relative to the homologous axes in other molluscs, to create the fusiform body shape?

Modified foot. The cephalopod foot has been modified and fused with the head to form a **tentacled** structure used in feeding, communication, and reproduction. Find the eight **arms** plus the two longer tentacles (Fig. 1). Under a microscope, examine the stalked **suckers** with their hardened chitinous **teeth,** as well as the pigmented **chromatophores** on the skin. Pop out the **chitinous ring** and examine the teeth under a dissecting microscope.

Swimming. Before opening the mantle cavity, recall how squid locomote. Slow swimming is achieved by undulations of the fins, but rapid movement involves jet propulsion using the **funnel** and mantle cavity. How are muscles used to achieve rapid locomotion? Diagram the following sequence with cartoons: **radial muscles** *dilate* the mantle cavity, bringing water in beneath the **mantle collar** (or skirt)—locate the collar opening. **Circumferential muscles** around the mantle cavity then suddenly contract, closing off the path for water flow out of the mantle collar--locate the **cartilagenous ridges and grooves** on the neck and funnel that ensure a tight fit. This muscle contraction also forces water at high speed out of the mantle cavity through the **funnel**, which can be directed at different angles to change the direction of movement.

✂ Orient your animal in the dissecting tray with the funnel up and head pointed away from you. Make a longitudinal incision through the funnel and through the mantle wall all the way to the posterior tip of the animal. This incision opens up the **mantle cavity**. To keep the mantle cavity opened and the structures separable, pin the mantle flaps down. Keep all structures under water.

***Note:*** *Keep in mind that, by making this incision, you are* <u>*not*</u> *opening up the inside of the animal--the mantle cavity is an external, sea-water filled space. All of the structures you see are covered at least by epidermal tissue. However, because they are protected inside the cavity, these structures are not behind a thick dermal wall that covers the rest of the body.*

Sex. If your specimen is female and reproductive (Figs. 2,3) you may see a pair of large **nidamental glands** on top of the central viscera. These glands, plus two smaller, pinkish accessory glands at their anterior ends, secrete the egg capsules in which embryos are laid. If these structures are absent you likely have a male (Fig. 4); confirm this by finding the prominent **penis** on the specimen's right side.

Let nearby groups know which sex you have before proceeding, and be sure to look at an individual of the other sex. If you have a female, identify and then carefully remove the nidamental and accessory glands and set them aside so that organs beneath them can be seen.

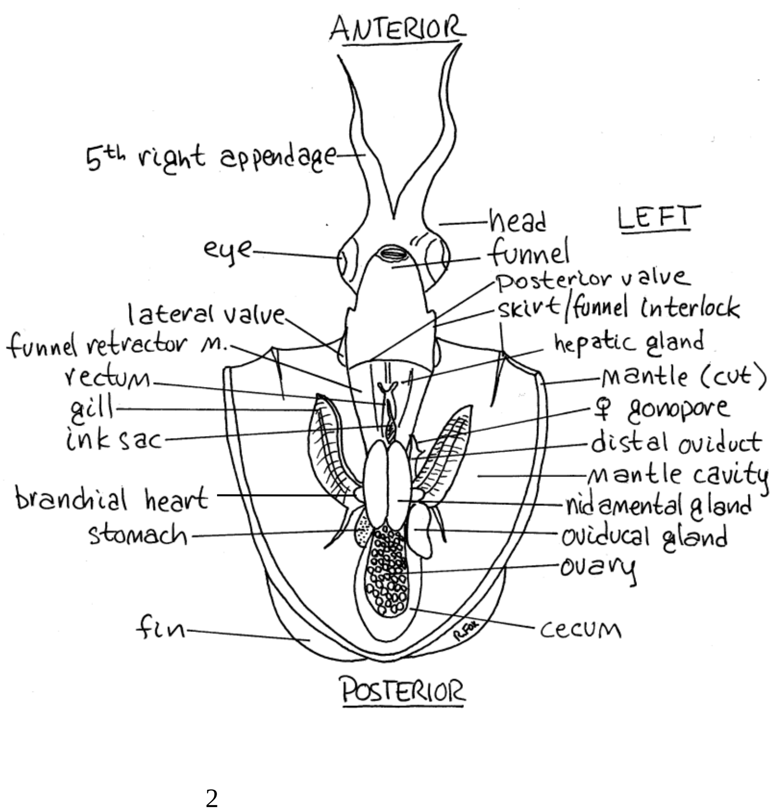



**Fig. 2. A female *Lolliguncula* with the mantle cavity open and viewed ventrally.**

## Circulatory, respiratory, and excretory systems

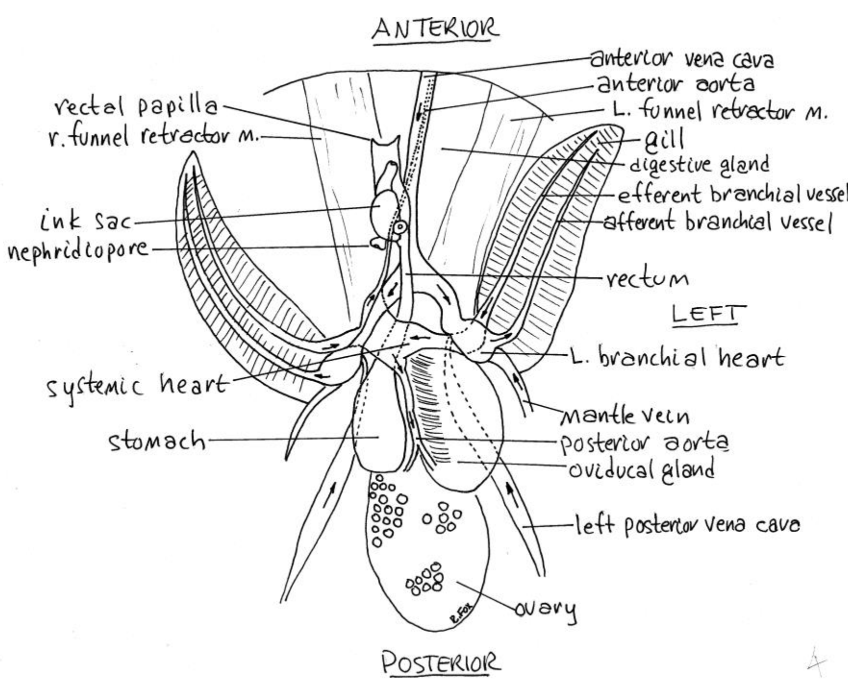
Most evident in the opened mantle cavity will be a pair of large **ctenidia** with numerous filaments. At the base of each gill is a swelling, the **branchial heart**, which pumps blood forcefully through the vessels and capillary bed inside the gill.

Locate the thin tissue between the branchial hearts that encloses **coelomic spaces** that form the **nephridium** and **pericardial cavity** around the systemic heart. Recall how primary urine is produced by filtration through walls of the pressurized circulatory system, and then modified by the nephridium to produce a final urine. Using a dissecting scope, you may be lucky to find a pair of **nephridiopores** on the upper surface anterior to the branchial hearts.

After looking for these pores, carefully tear away this thin tissue layer from between the branchial hearts to expose the firm, yellowish **systemic heart**. Find the following structures, and draw a flowchart of the path of blood flow (see Fig. 3), using the following description. *To find blood vessels, try to trace their entry or exit from the hearts and gills.* (a) Venous blood returns from the body to the **branchial hearts** via anterior and posterior vena cavae. The **posterior vena cavae** might be filled with jelly-like coagulated blood (the anterior vena cavae pass through the nephridial tissue and are hard to find); (b) blood enters and then leaves the **ctenidia** via the **afferent** and **efferent branchial vessels**, respectively; (c) after entering the **atria** of the systemic heart, blood moves to the central **ventricle**, and is pumped by the ventricle to the body viscera via the **anterior and posterior aortae**.

➔ **TQ and Table**: Fill in the table and answer the TQ on your worksheet.

**Fig. 3. Ventral view of a female *Lolliguncula* with the mantle cavity opened. The nidamental glands have been removed.**

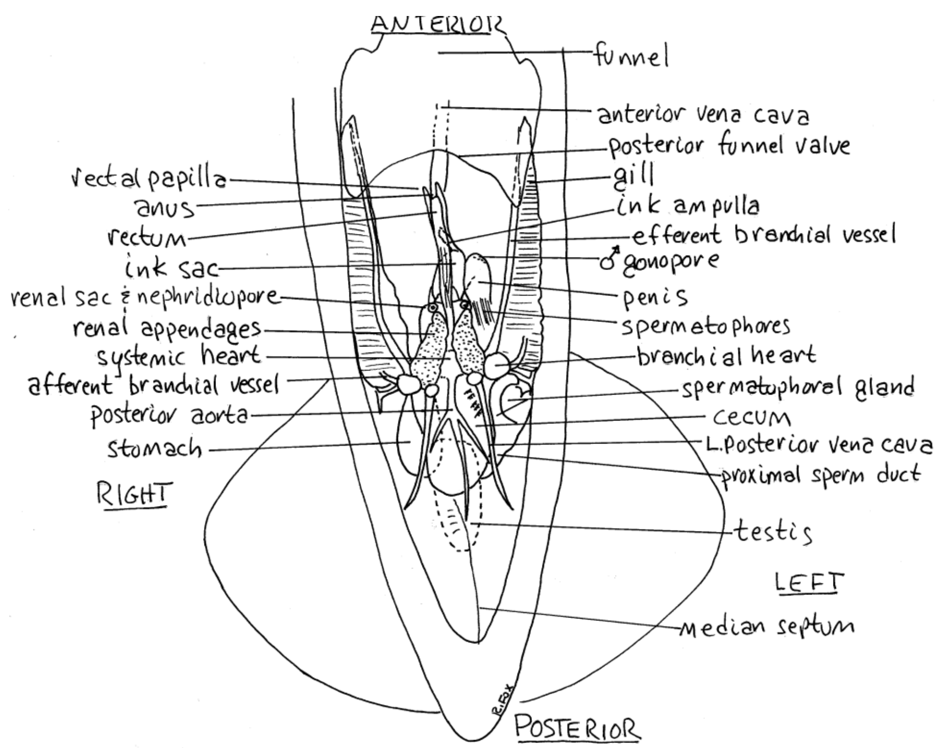



## Digestive system

Below the left branchial heart is a firm, whitish **stomach**. Cut the stomach open and examine its contents on a slide; you may find such meal remnants as fish scales or bones, crustacean exoskeletons, chromatophores, or the chitinous rims of squid suckers. Hanging from one side of the stomach is a large, delicate bag, the **cecum**, within which most digestion occurs. Leaving the cecum, the **intestine** heads anteriorly, with the **anus** at the base of the funnel (two flaps seal the anus opening when water pressure increases in the mantle cavity). Note the silvery **ink sac** alongside the intestine, storing dark ink to be released through the anus and funnel when danger threatens. **Do not disturb this ink sac, yet.** To locate the large white **liver**, the largest digestive gland, cut into the viscera beneath the intestine.

✂ Now cut the squid's head in half by continuing your initial incision, removing the spherical **buccal mass**. Note the large parrot-like **beaks**. Cut the buccal mass in half and a small, rough, tongue-like **radula** can be found inside. Try to dissect out both the beak and the radula. The beak is one of the best illustrations of sclerotization you will see in the course, as the business end is dark, hard, and sclerotized, while the flexible parts that attach to muscle are clear and unsclerotized. In the neck region, surrounding the esophagus, can be seen three pairs of large, fused **ganglia** that constitute the brain. Note that they are covered by **protective cartilage material**--*functionally analogous* to a skull. Does the size of the brain surprise you, given that cephalopods represent likely the most intelligent invertebrate taxon?

**Fig. 4. Ventral view of a male *Lolliguncula* with its mantle cavity opened.**




## Reproductive system

Be sure to see both sexes—if you have a female, show neighboring "male" groups where the nidamental glands and accessory glands were located before removal.

**In the female** identify the large egg-filled posterior **ovary**, the **oviducal gland** that secretes a membrane around each egg, and the more anterior **gonopore**. Eggs pass out the gonopore to be enclosed in a gelatinous mass secreted by the nidamental glands before the mass is deposited.

**In the male** find the large, cream colored **testis** below the cecum. Sperm leave each testis and enter the vas deferens which carries the sperm to the coiled **spermatophoral gland**, where the sperm are encased in complex spermatophores. These spermatophores are then stored in the **spermatophoric sac**, which connects to the **penis**. Find and squeeze the spermatophore sac - can you see any spermatophores at the penis opening?

Cut open the spermatophore sac, remove a spermatophore, and put it on a slide. Note the enclosed **sperm mass** as well as other structures that attach the spermatophore to the female and aid ejaculation of the sperm. During mating the male will eject spermatophores through the penis and funnel, grasp the spermatophores with a modified arm (the hectocotylus arm), and transfer them to the female. In some species the hectocotylus arm is the first arm to the right of the funnel when the squid is oriented as it is for dissection. Examine the tip of this arm in male specimens. Note that there are slight elongations and other modifications of the suckers for the transfer of spermatophores (Fig. 5).

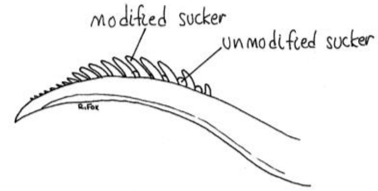



**Fig. 5. Tip of the left fifth (hectocotylus) arm.**

Like other cephalopods, squid lack larval stages. Most squid lay groups of gelatinous "egg fingers" on the bottom, within which development occurs. Be sure to examine the squid egg fingers when you look through other displayed material.

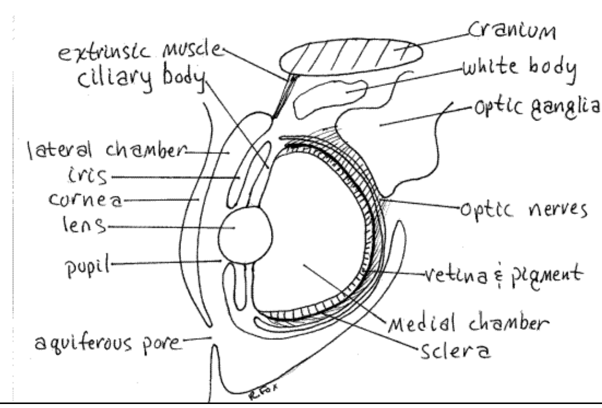
## Nervous system, ink sac, pen, and eye

Four additional structures remain to be examined briefly in your squid.

Giant axons. On the side of the animal, where the visceral mass joins the inside of the mantle wall, you will see on each side of the body a large ganglion with giant nerve fibers (axons) leading from it. These ganglia and giant nerve axons entering the mantle wall cause the contraction of the mantle muscles during jet propulsion. Due to the large size of these nerve axons, they are frequently used in neurophysiological research.

Eye. Remove an eye to examine its anatomy. The cephalopod **camera eye** (Fig. 6) is similar in construction to the vertebrate eye, yet it differs in certain key details that reflect its remarkable, independent evolution. These details include the position of the optic nerves (behind rather than in front of the retina), the use of muscles to change the position rather than the shape of the lens, and the ability to see polarization in light due to the arrangement of pigment and sensory cells.

**Fig. 6. Sketch of a cephalopod eye in section.**




The eyeball is surrounded by a transparent **cornea** on the lateral surface and by the tough, protective white **sclera** elsewhere. The **iris**, visible inside the cornea, includes a tough, silvery layer containing **iridiocytes**, which also line the back of the retina and prevent light entry from any direction except through the pupil. The **pupil** is an opening in the iris. Although behind the cornea, the lens is actually in contact with seawater because a small **aquiferous pore** located on the cornea allows seawater to enter.

✂ Cut through the iris, beginning at the outer edge of the pupil, to reveal the interior, **medial chamber**, of the eye (Fig. 6). The lens is supported by ciliary ligaments and muscles that form the **ciliary body**. The inner wall of the interior of the eye is the sensory pigmented **retina**. It is a layer of alternating sensory and pigment cells analogous to the vertebrate retina.

At the back of each eye is a very large number of individual optic nerves that connect to each retinal cell. This spread of nerves is consistent with the location of nerves behind the retina, unlike in the vertebrate eye where nerves sit in front of the retina and must be bundled together to pass through the retina (causing the "blind spot" in your vision).

<u>Ink sac</u>. Carefully remove the ink sac from its location near the intestine, place it in a dish *without* water, and pierce it. Note the mucus-rich ink that escapes. Squid ink is thought to serve an important role in defensive escape by confusing the predator when the squid leaves a viscous blob of ink behind when it escapes.

<u>Pen</u>. By pulling the body viscera away from the mantle, remove the squid's dorsal internal, non-calcified, chitinous **pen**, which is homologous to the calcified shell of other molluscs. This structure is used for a flexible reinforcement of the expanded and heavily muscularized mantle cavity. The pen is one example of the evolutionary process of shell reduction seen among cephalopods, with octopuses having lost the shell entirely on one end and the chambered nautilus retaining a coiled shell as a flotation device on the other. Compare the squid pen to the calcified **cuttlebone** on display, removed from the cuttlefish (*Sepia*), which is also homologous to the mollusc shell. Cuttlebones function as internal buoyancy devices that aid positioning in the water column.

- ➔ Using the **squid pen**, write your name with **squid ink** on your laboratory drawings page!