

OBJECTIVES

Observe representative specimens of the Phylum Platyhelminthes. Identify the structures referred to below, and make sketches as necessary to aid recall. Learn the life cycles of important parasitic species. If you are not familiar with a term, use your text or other resources and find a definition.

MATERIALS:

Phylum Platyhelminthes (flatworms) ~25,000 species. Classes Turbellaria, Trematoda, Monogenea, Cestoda. Characteristics: Bilateral symmetry, dorsoventrally flattened, cephalized. Three tissue layers- two are epithelial layers (ectoderm & endoderm) and the third is a non-epithelial parenchyme (mesoderm). Acoelomate, lack circulatory system. Incomplete digestive tract (gastrovascular cavity). Protonephridial osmoregulatory system. Most flatworms are parasitic. Sexual reproduction is usually monoecious and oviparous or ovoviviparous. Asexual reproduction by "transverse fission" (a type of budding) and great capacity for regeneration after transection because of abundant totipotent cells called "neoblasts".

Class Turbellaria

Mostly free-living species in marine, freshwater, and terrestrial habitats. Ciliated epidermis used in locomotion. The presence and form of the gastrovascular cavity has been used to define orders within the Turbellaria (e.g. Acoela, Tricladida, Polycladida). These traditional classifications are not phylogenetic and are being revised. The Acoela has been proposed as a separate phylum based on molecular comparisons.

Order Tricladida- tripartite gastrovascular cavity.

- *Dugesia* (=Planaria) live and whole mount. These free-living freshwater turbellarians are often used in research and teaching. Note the protrusible pharynx at midbody & the three major branches of the digestive tract. Note the pigment cups of the simple eyes which permit orientation toward or away from light.
- *Dugesia* X-sections slide. The slide has several sections from different positions. Cuts anterior to the pharynx should show just one branch of the GVC, while those posterior should show two major branches. There is no body cavity other than the digestive tract (gastrovascular cavity). Find a section that goes through the pharynx, and understand the difference between the pharyngeal cavity and a coelom.

The dorsal epidermis contains rod-shaped secretions, the rhabdites (rhabd = rod). When expelled at the surface, rhabdites become a sticky mucus which may help trap small invertebrate prey or discourage predators. Note also the clusters of adhesive gland cells situated at the lateral edge of the ventral epidermis. These are part of a cilia-free adhesive zone that encircles the worm. These cells secrete an adhesive that helps the animal grip the substratum. The ventral epidermis has gland cells that secrete mucus.

A thick layer of body wall muscles, consisting of outer circular and inner longitudinal fibers, lies just inside the epidermis. Inside the muscle layer, the interior of the worm is filled with a mesodermal tissue (parenchyma) having a loose, open appearance. Many large epidermal gland cells are submerged into it but open to the surface through the epidermis. Dorsoventral muscles can be seen passing vertically through the parenchyma connecting the muscle layers of the dorsal and ventral body walls. These muscles maintain the flat shape of the triclad body.

Scattered about in the interior are sections through the intestinal branches. The clear space in the interior of each is the gut lumen and is surrounded by an epithelium (gastrodermis) of large, vacuolated cells, which are secretory, absorptive, or phagocytic.

- Live *Dugesia* from Lake Taneycomo. Observe locomotion under a dissecting scope and on the compound scope. Smaller worms are better. Look for the epidermal cilia on higher magnification. It is difficult to make out details unless you really flatten a very small specimen under a cover slip. Watch for symbiotic ciliates on the body surface. Try to distinguish the GVC contents from the parenchyma. You might be able to identify flame cells of the protonephridial system- the flame cell cilia flicker like a flame, hence the name.
- *Bdelloura* whole mount. This species is a marine triclad that lives on the gills of "horseshoe crabs". The posterior margin has adhesive glands and is used for attachment. The worms are not parasites but rather ectocommensals feeding on protozoa and fragments of the host's food. Compare with *Dugesia*. If you close the iris you can see the central nervous system- paired longitudinal nerve cords- as relatively transparent streaks.

Order Rhabdocoela- simple unbranched gastrovascular cavity. Dr. Barnhart will show video of the rhabdocoel *Macrostomum* feeding.

The other three classes of flatworms besides Turbellaria (i.e. Monogenea, Trematoda, Cestoda) are exclusively parasitic. They are linked as a clade called Neodermata (why?)

Class Trematoda-Digenetic trematodes.

Endoparasites of vertebrates, many causing important diseases of man and domestic animals. Rather than a posterior holdfast the digeneans have two suckers- one around the mouth (oral sucker) and another midventrally (acetabulum). Complex life cycles- at least two, and often three hosts are involved in the life cycle.

Generalized life cycle of a digene: Adults produce eggs which hatch a ciliated miracidium larva. Miracidium penetrates the first intermediate host, usually a snail, develops into sac-like sporocyst. Internal budding gives rise asexually to many daughter (secondary) sporocysts or to daughter redia, which are similar to a sporocyst but have a mouth & small digestive tract. Redia (or secondary sporocysts) give rise internally to many cercariae. Cercariae are basically tiny

flukes but with a tail for swimming. They emerge and may penetrate the definitive host, as in schistosomes. In other species, the cercaria may penetrate a second intermediate host and encyst as a metacercaria. If the metacercaria is ingested by a suitable final host the fluke matures. Life cycles vary among the taxa- particularly the number of asexual generations.

Clonorchis sinensis Chinese liver fluke, a significant human parasite in Asia. Learn the life cycle and anatomy.

- Whole mount of adult worm *Clonorchis sinensis*- Both male and female organs are present- copulation is mutual. You should be able to find the labeled structures. Male organs include paired testis, seminal vesicle, prostate gland & cirrus (=penis). Female organs are the ovary (germarium), seminal receptacle, yolk glands, and uterus (usually full of eggs). Notice the characteristic shape of the eggs, which are about 30 microns long. Helminth eggs are commonly used to diagnose infections.

Prosthogonimus macrorchis Lives in the oviducts of many species of birds, including chickens, ducks, and pheasants. The presence of this parasite can result in a marked reduction in (or complete cessation of) egg laying, so the parasite could have an economic impact in some instances. Cercaria develop in snails, encyst in dragonflies, which are eaten by the bird.

- Whole mount slide of adult *Prosthogonimus*. Included here because it is large and the internal anatomy is very clear- compare the reproductive structures with *Clonorchis*.

Schistosoma mansoni. The schistosomes live in blood vessels of vertebrates and are unusual among flatworms in being dioecious (having separate sexes). Several species infest man, causing diseases known as schistosomiasis. An estimated 200 million people are affected, causing a world health problem second only to malaria. The female worm is smaller and lies in a groove (gynecophoric canal) in the male's body- perpetual conjugal bliss. Learn the life cycle. These worms can be cultured in mice for research, so we have prepared slides of the life cycle stages:

- *Schistosoma mansoni* whole mounts of male and female adults (from blood vessels of the vertebrate host). Note the slender body form, an adaptation to the station in the host body. Find the oral sucker and acetabulum characteristic of Digenea. Observe the gynecophoric canal of the male. Notice the characteristic spine on the eggs (if present) in the females. The eggs of *S. mansoni* are about 150 microns long.



Sections of host liver with *Schistosoma* eggs in granulomas within the tissue. Notice the highly porous structure of mammalian liver- the spaces in the tissue are blood spaces called sinuoids, that bring the blood in close contact with the liver cells. Notice also the granulomas, round balls of fibrous scar tissue surrounding the schistosome eggs. Remember this is a thin slice of a 3-D object. Some or all of the egg itself may be present in the plane of section –try to find one that shows the distinctive spine. The liver is a “dead end” for the eggs. Where did they want to go?

- Slides of miracidium, sporocysts in snail tissue, and cercariae. What does "swimmer's itch" have to do with schistosomes?

Trematode metacercariae: Many trematodes have a resting larval stage within an intermediate host (after the snail but before the definitive vertebrate host).

- Preserved fish showing "black spot". The condition called black spot in fish is caused by digenetic trematodes in the genus *Uvulifer*. The definitive host is most often a bird, and the parasite's eggs are passed in the bird's feces. The first intermediate host is a snail, and the second intermediate host is a fish. The cercariae penetrate the fish, lose their tails and transform into a "neascus" type metacercaria. The bird is infected when it eats the fish. The cysts of *Uvulifer* often turn black and are visible on the fish's skin.
- Prepared slide of *Clinostomum* This large metacercaria is called "yellow grub" and commonly seen encysted in muscle or on viscera of freshwater fish such as bass. A related species called *Bolborhynchus confusum* which infects catfish recently became an issue in aquaculture in southern states (leading to introduction of an exotic fish, the black carp, to control the snail host).
- Unidentified metacercariae in the fat body of a garter snake.

Class Monogenea- Monogenetic trematodes.

Mostly ectoparasites on fishes & other aquatic vertebrates, feeding on blood. Simple life cycle and single host ("monogenetic"). Ciliated larva swims to find host. Adults have highly developed organs of attachment including anterior adhesive glands and the posterior opisthaptor.

- *Polystomoides* ("many mouthed") lives in the mouth of freshwater turtles and has an elaborate posterior holdfast (opisthaptor) with multiple hooks and suckers for anchorage in this turbulent environment. Compare with the diagram and identify mouth, oral sucker, pharynx, GVC, and the male and female reproductive organs.
- Other monogenes may be set up as demos to show the variety of body forms and opisthaptors. Most species live on gills & skin of freshwater fishes. Infestations are common and may be especially heavy on aquarium & hatchery fishes, which may "cough" or strike themselves against the bottom in attempting to dislodge the worms. Note the prominent hooks on the holdfasts.

Class Cestoda- tapeworms.

The most specialized group of Platyhelminthes. Lack digestive tract, feed saprobically (direct absorption of nutrients). Metameric (segmented). Body consists of anterior scolex (holdfast) and the strobila which is composed of repeated identical proglottids (segments). Monoecious, with complete set of reproductive organs in each proglottid. Eggs pass in host feces and are

ingested by an intermediate host, which may be an arthropod, a vertebrate, or other animal, depending on the species. Egg hatches an oncosphere larva, which penetrates the host gut and becomes an encysted larva. As this point the intermediate host & the cyst may be eaten by either the definitive host or in some cases another intermediate host in which the larvae may reencyst. When the larval cyst is eaten by a suitable definitive host, the adult (sexually mature) worm develops in its intestine.

- *Taenia pisiformis*. Slides: 1) w.m. of scolex and 2) of mature proglottids. Compare mature proglottid with diagram of reproductive organs.
- Preserved specimen: squirrel liver with cysticercus larvae. Definitive hosts usually canids including dogs. Intermediate host is usually rabbits. A closely related species that parasitizes man is *Taenia solium*, which looks similar and has a similar life cycle. So, look at *T. pisiformis*, but learn the *T. solium* life cycle.
- *Taenia solium* (pork tapeworm) is fairly common in rural U.S. & Mexico. Know the life cycle. Adults live in the gut of man- eggs pass in the feces and may be ingested by pigs, the usual intermediate host. The oncosphere develops into a cysticercus larva (bladderworm) in the pig's muscle and enters the human digestive tract via poorly-cooked pork. Adult worms are up to 7 meters long but are not normally very pathogenic. Disease (cysticercosis) may develop if eggs reflux into the stomach and hatch; the resulting oncospheres frequently encyst in the brain (see the preserved squirrel liver). The related beef tapeworm (*Taenia saginata*) is less dangerous.
- *Diphyllobothrium latum*. w.m. of scolex. Broad fish tapeworm of man. Life cycle involves a copepod crustacean, a fish, and a mammal. Man obtains the worm by eating the second intermediate host (fish), containing plerocercoid larvae encysted in the tissues. The worms are large and can cause severe anemia by competing for vitamin B12 (needed for red blood cell formation). The plerocercoid larvae of some species of *Diphyllobothrium* can cause a human disease called sparganosis, which is contracted by drinking water containing copepods with proceroid larvae, which then burrow into the tissues and encyst. In this case the human serves as a second intermediate host.
- *Echinococcus granulosus* (hydatid worm) w.m. slide of adult worm. (see handout for anatomy and life cycle- learn the life cycle). Normal adult hosts are canids, including domestic dogs. Adult worms are tiny, with only three proglottids. Larval forms are parasites of various mammals including man, causing a serious disease called hydatid cyst disease. If eggs are ingested the oncosphere penetrates tissues and develops into a cyst that may grow to a diameter of 15 cm or larger. The cyst reproduces asexually- budding inside the cyst gives rise to millions of cysticerciae, each capable of forming another cyst or an adult worm. What happens if this apparent "tumor" is biopsied (punctured)?
- Plerocercoid larva of other tapeworm species. These larvae are frequently encountered in tissues of fishes but are not dangerous. Definitive hosts are birds and mammals other than man.

Platyhelminthes, Turbellaria, Tricladida

Figure 1. Whole mount of *Dugesia*

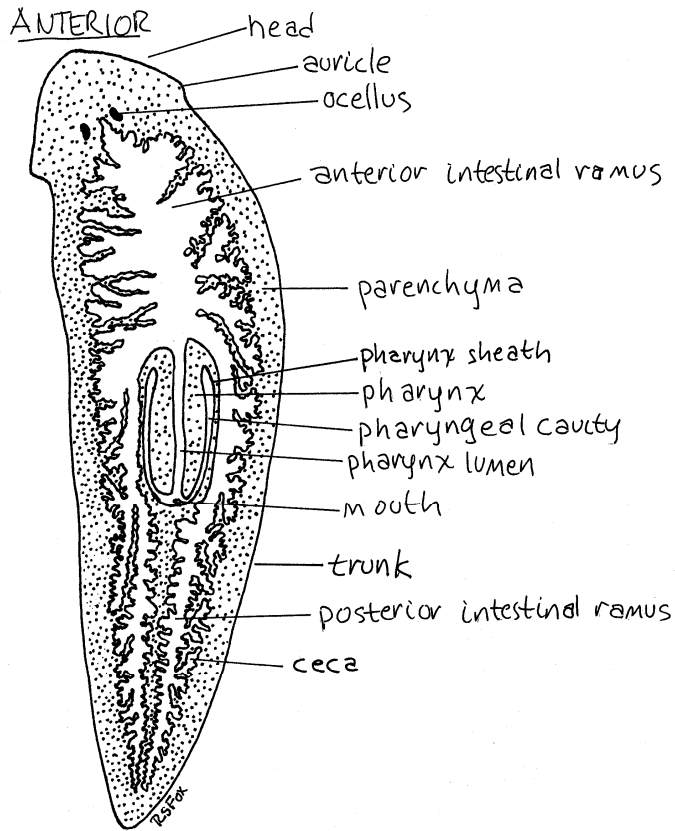


Figure 2. Whole Mount of *Bdelloura*

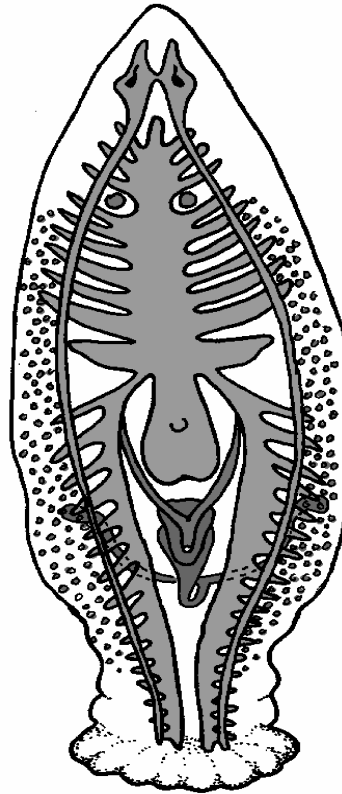


Figure 2. Cross section through the posterior body of *Dugesia*.

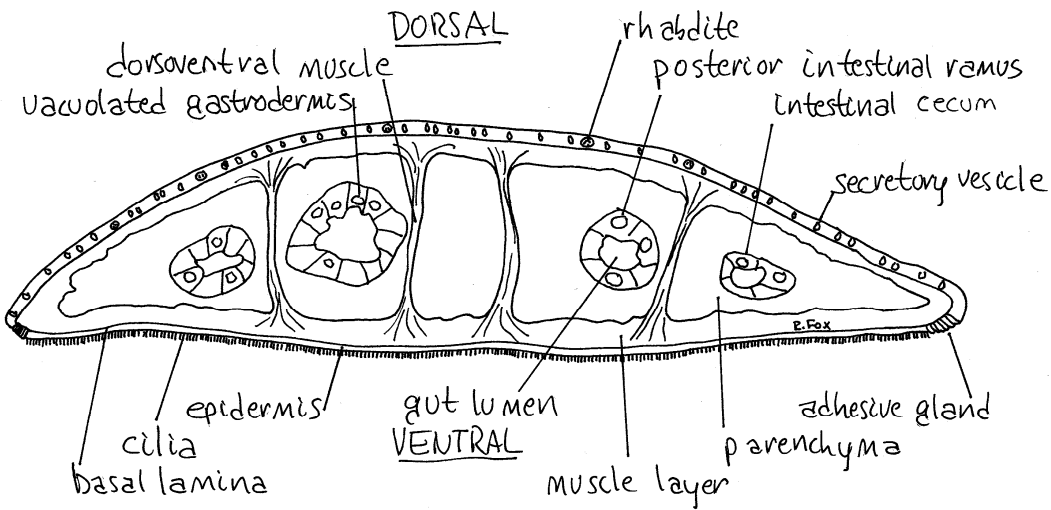


Figure 3. Cross section through the pharyngeal region of *Dugesia*.

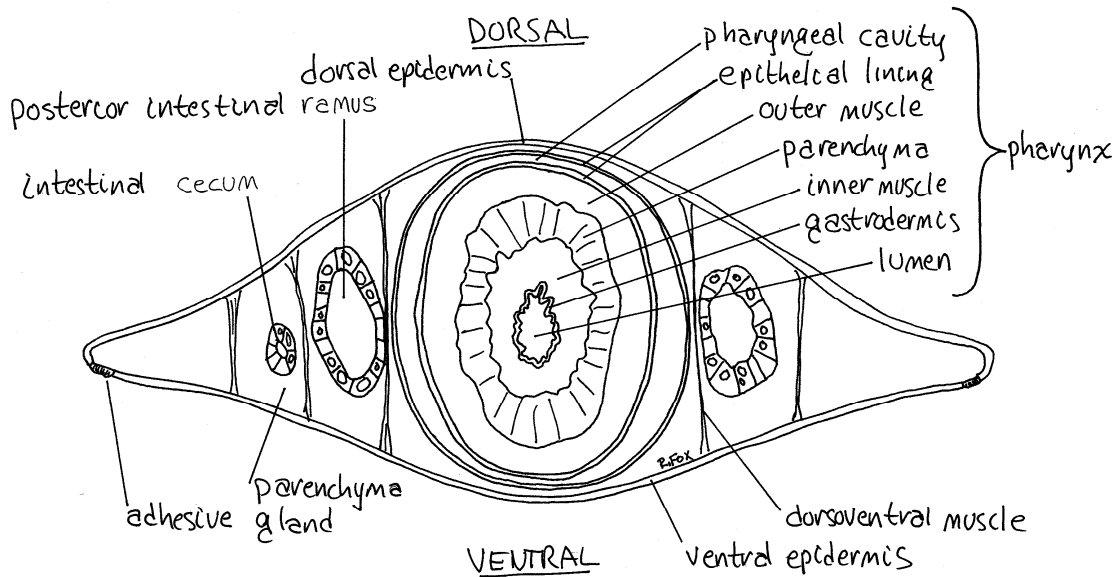


Figure 4. Life Cycle of Chinese liver fluke- *Clonorchis sinensis*.

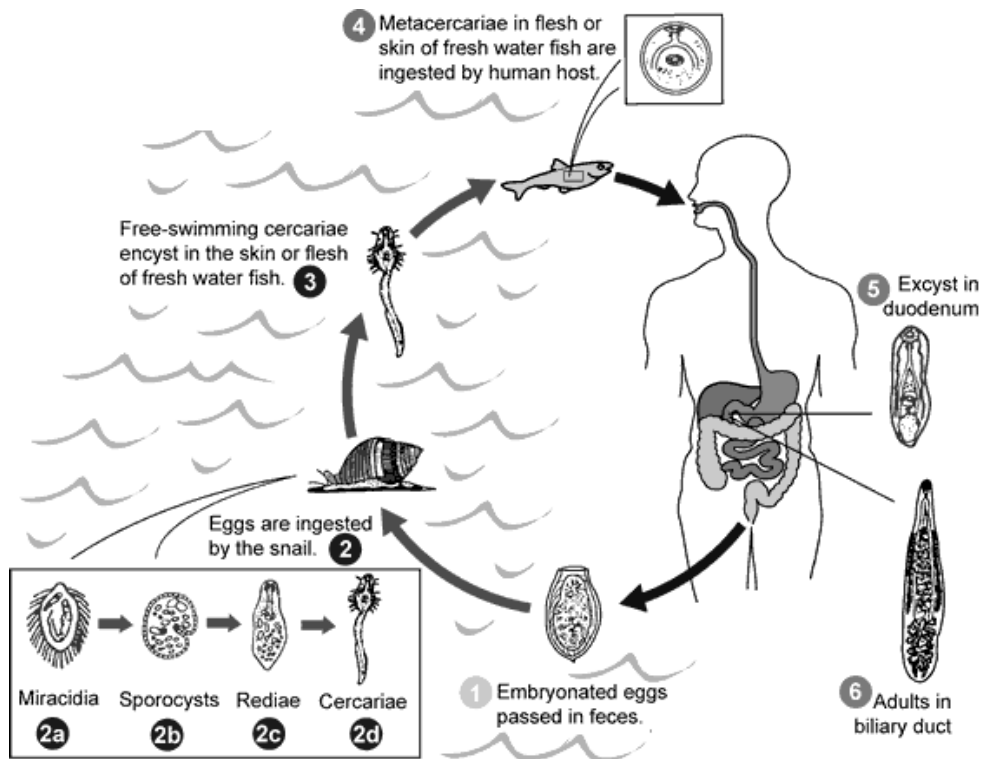
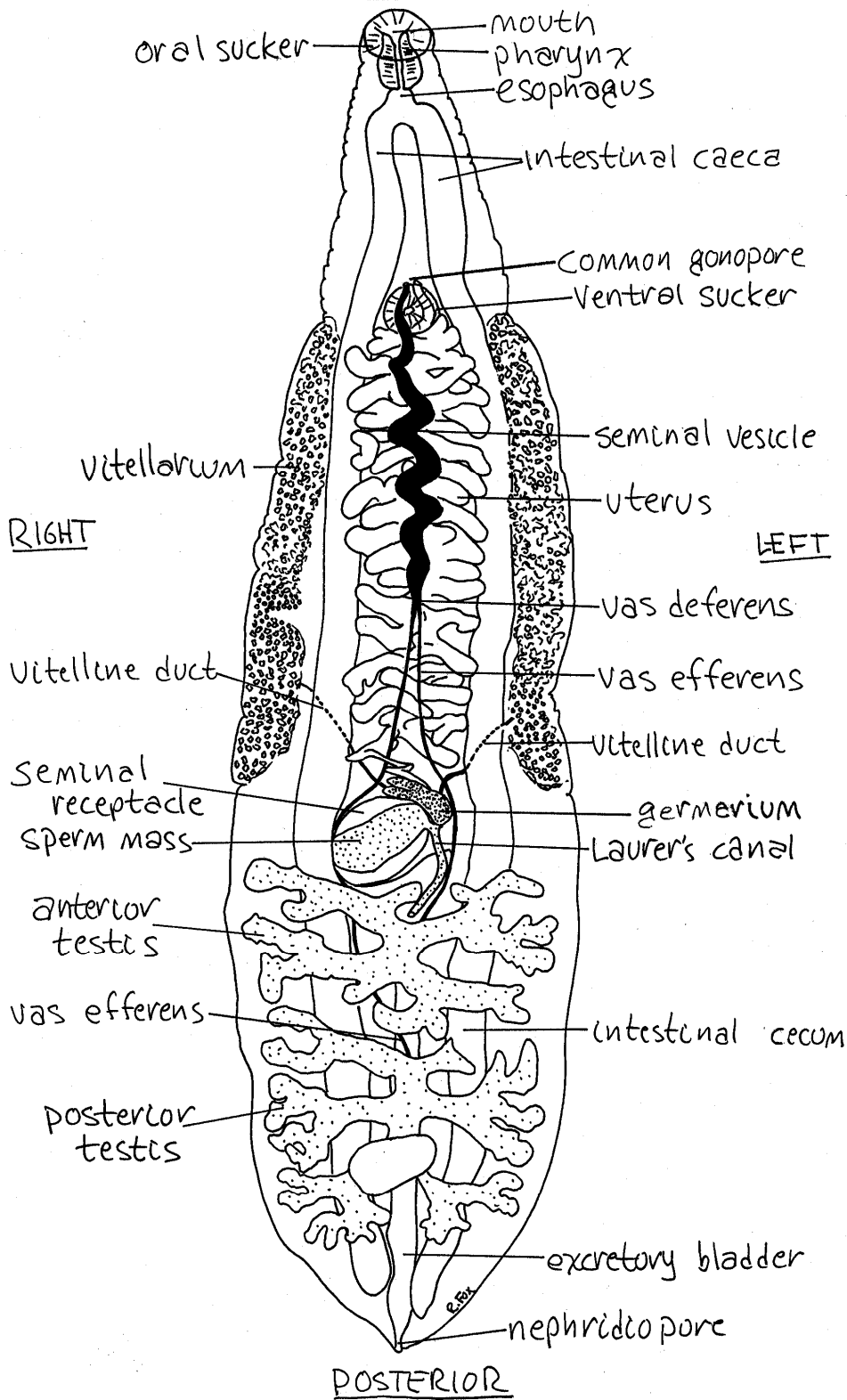
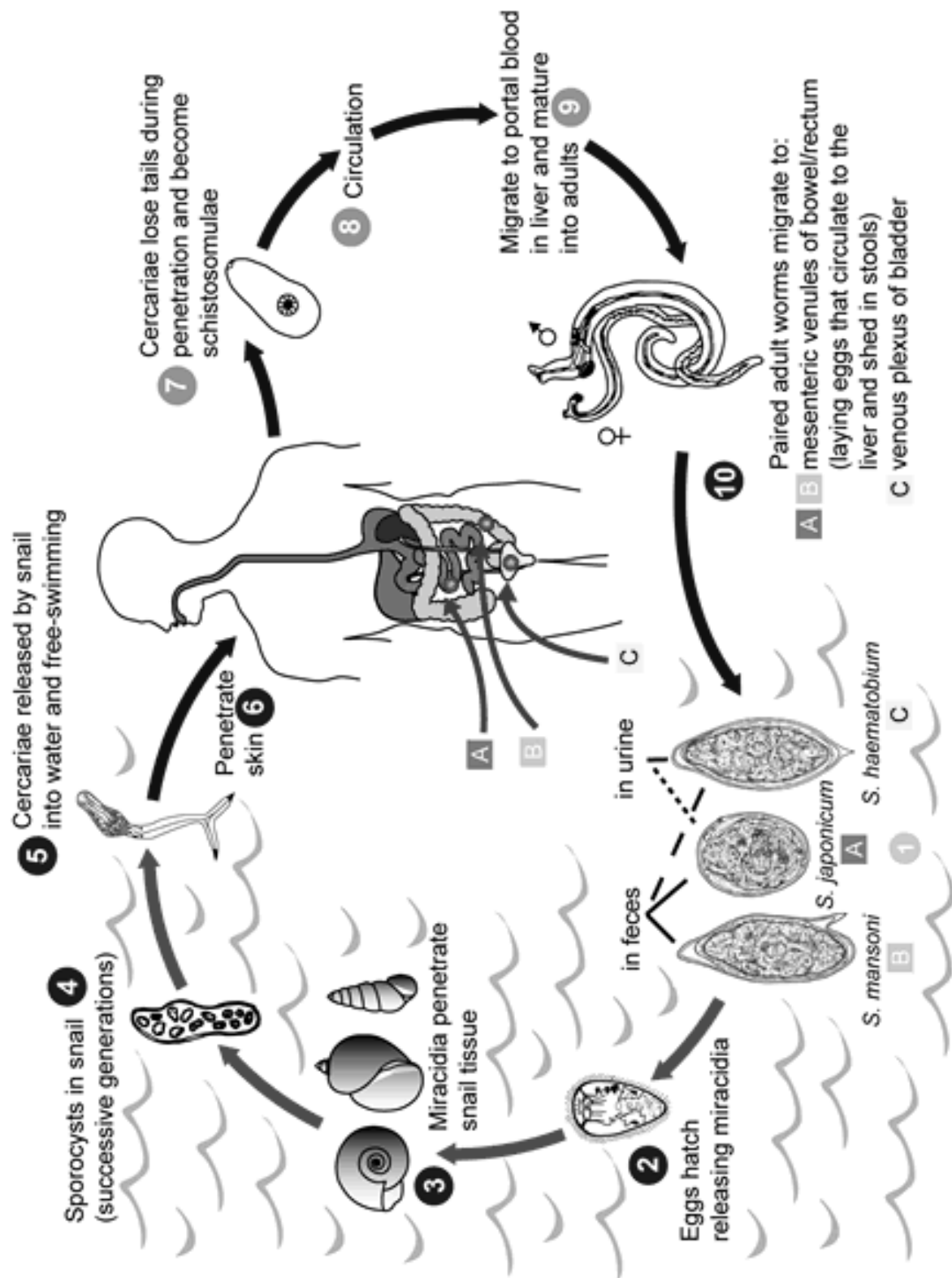
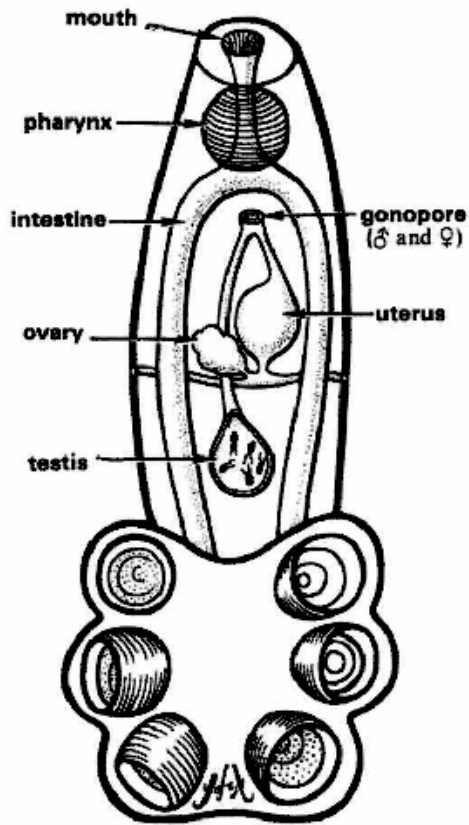


Figure 5. Ventral view of an adult Chinese liver fluke, *Clonorchis sinensis*.

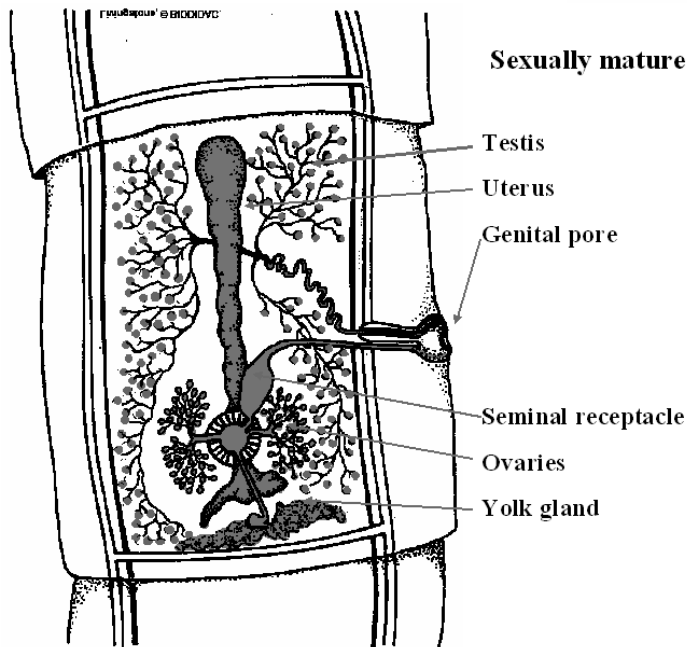
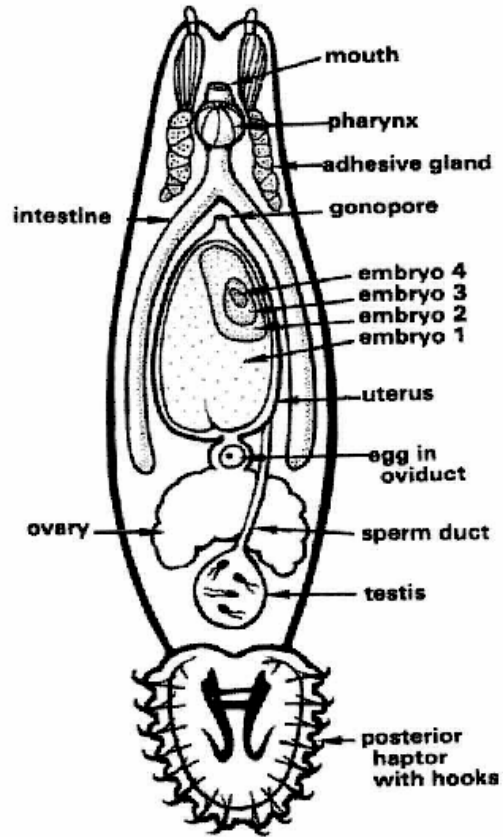




Polystoma from the mouth cavity of a turtle. (Modified after H.W. Stunkard)



Gyrodactylus



Sexually mature proglottid

