Phylum Platyhelminthes

Class Turbellaria

1. Order Acoela – no representatives
2. Order Rhabdocoela – no representatives
3. Order Alloecoela – no representatives
4. Order Tricladida
   a. living planaria (*Dugesia*)
   b. planaria whole mount slides showing digestive tracts
   c. planaria cross-section, prepared slides
   d. *Bdelloura candida*, prepared whole mount slides (commensal on the ventral side of the horseshoe crab *Limulus*)

5. Order Polycladida – no representatives

Class Trematoda

Order Monogenea

Order Digenea

1. *Clonorchis sinensis* (Chinese liver fluke)
   a. prepared whole mount slide
   b. metacercaria larva – prepared slides
   c. rediae – prepared slides

2. *Schistosoma mansoni* (blood fluke) – whole mount slide

3. *Fasciola hepatica* (sheep liver fluke)
   a. prepared whole mount slide
   b. rediae, ova, miracidia, cercaria

Class Cestoda

Subclass Eucestoda

1. *Taenia* – prepared whole mount slides
2. cysticerci larvae of tapeworm
3. *Dipylidium caninum* (cucumber tapeworm)
From the phylum platyhelminthes we will look at three of the five classes: the free-living (i.e. nonparasitic) TURBELLARIA, and two classes of highly specialized parasites, the TREMATODA (flukes) and the CESTODA (tapeworms). Generally, the term “flatworm” is restricted to the class turbellaria, in spite of its being the common name of the phylum. Flukes and tapeworms, along with certain other groups of parasitic worms, are commonly called helminthes, a convenient nontaxonomic term for worm parasites.

The platyhelminthes demonstrate remarkable adaptations to both a free-living intertidal or fresh water existence, and a parasitic mode of life. The phylum also displays: 1) the organ level of complexity, 2) bilateral symmetry, and 3) the simplest type of body organization built largely of mesoderm. These three characteristics are critical steps in the development of complex organisms.

Certain characteristics of free-living flatworms have suggested to some workers that platyhelminthes are ancestral to the more complex animal phyla, and hence to all animals of greater complexity than a jellyfish or sea anemone. The increased specialization of mesoderm with the development of efficient organs is the most marked distinction between flatworms and jellyfish or sponges. Turbellaria are the simplest animals with a brain and central nervous system (CNS), a well-organized excretory-osmoregulatory system, and a complex reproductive system. Most body functions of flatworms are performed by specialized tissues or organs; a significant difference from the most complex cnidarians in which organs are more or less absent and highly specialized individuals (zooids) accomplish feeding, reproduction, defense, and other functions.

Accompanying bilateral symmetry in flatworms is cephalization associated with improved sensory perception, increased muscular coordination, more rapid movement, and greater efficiency in food processing, excreting wastes, and controlling metabolic processes.

Associated with greater structural complexity is a pattern of life employing a CNS-directed mobility in the active seeking of prey. This is a distinctly different pattern of behavior from that of coelenterates which sting prey accidentally encountered in their primarily nondirected swimming, floating, or sessile activities.

To understand the factors permitting increased complexity in invertebrate phyla, the structural development of mesoderm tissue and body cavities is important. In platyhelminthes, mesoderm fills the space between body wall and gut (the potential body cavity space) with a mass of cells lacking clearly defined cell membranes. The platyhelminth organization, considered the most primitive type among higher animals is called acoelomate (without a true coelom). Further integration of organ system is found in the phylum ASCHELMINTHES where a body cavity is partially lined with mesoderm. This body plan is termed pseudocoelomate. In animals possessing a true coelom (coelomates) structures lying between the body covering (epidermis) and intestinal layer (endodermis) are enclosed by mesodermal sheets called mesenteries, and in these animals specialized and discrete organs are individually enclosed in a mesodermal lining and suspended within the body cavity.

In today's laboratory we will examine structure and function in the three platyhelminthes classes.

CLASS TURBELLARIA
The free-living flatworms are lined by a ciliated epidermis with rhabdites and mucus glands and a ventral mouth. They are usually pigmented and have eye spots. The orders of turbellaria are based on the structure of the digestive system and are: 1) Acoela (no digestive tract); 2) Rhabdocoela (single digestive tube); 3) Alloecoela (straight digestive tube with short branches); 4) Tricladia (three-branched digestive tube) and 5) Polycladia (manybranch, mostly marine forms). We will examine the common freshwater Dugesia as a representative turbellarian.

Examine a living specimen of the common freshwater planarian Dugesia in a watchglass containing pond water. Can you identify the anterior end? What structures are associated with the anterior end? Can you see the pharynx? Is the pharynx stationary or can it move? Where is the mouth located?
To examine the ventral surface of *Dugesia*, lightly elevate the cover slip using playdough, place a drop of water or two and a planaria on the coverslip, and then lightly press a second coverslip over the first. If done carefully, you can use this mount to examine either side of *Dugesia*. Observe the ventral and dorsal surfaces carefully. How do they differ? Identify the structures on the ventral surface.

To examine the internal anatomy of *Dugesia* examine the prepared slide of a cross section and a longitudinal section of *Dugesia*. Notice the single-layered, cellular epidermis in some species the epidermis may be syncytial (multinucleated). Characteristic of the epidermis of turbellarians are rhabdites, which are curved rods embedded in the epidermis. Little is known about the function of rhabdites. Since they discharge to the exterior when the animals are stressed, a protective function is suggested though mucous production has also been attributed to the rhabdites. Try to locate small regions of dorsal surface devoid of rhabdites and cilia on the margins of the body. These are the locations of adhesive glands, whose secretions aid the animal in adhering to the substrate. Locate the following structures on your slide: gastrovascular cavity, gland cells, mesenchyme, nerve cord, circular muscles, longitudinal muscles, and pharynx. Why might you not be able to find all these structures?

Try to locate the cerebral ganglia of *Dugesia* on the specimen you have sandwiched between coverslips of the longitudinal prepared slide. If it is dried or otherwise damaged, prepare a new setup. Locate as many CNS structures as possible. The coordinating function of the nervous system of flatworms is most easily studied by observing behavior. With a paintbrush, transfer a living specimen of *Dugesia* to a watchglass and observe its movements. Using a probe examine the reaction of *Dugesia* to physical stimulation. Is *Dugesia* equally sensitive to mechanical stimulation throughout its body? Carefully examine the locomotion of *Dugesia* and describe how you think it is accomplished. How and what muscles are used? How are cilia used? The relative roles played by cilia and muscles in locomotion may be determined by treating individuals with a solution of 1-2% magnesium chloride, which paralyzes the muscles. Explain your results. What do you think the role of mucus glands is in locomotion? To observe mucus tracks, add some talcum powder to a dish of water, mix well and allow the suspension to settle to the bottom of the dish. Pour off the water. Transfer a fresh planarian to the talc suspension dish and note the tracks produced by the organism.
Now attempt to examine the conditioning of behavior with *Dugesia*. Expose a *Dugesia* in a watchglass to a strong directed light (use the dissecting scope light). What is the reaction? Are *Dugesia* photonegative or photopositive?

The digestive tract of turbellarians (except acoels) consists of a midventral mouth, a muscular pharynx, and a blind-ending intestine. As mentioned previously, the branching arrangement of the gastrovascular cavity is the main basis for separating the free-living flatworms into orders. Examine the branching patterns of the turbellarian slides available to you in the laboratory. To examine the feeding process in *Dugesia*, place starved individuals (starved for at least 48 hrs) in a watchglass of water. Place them in the middle of the container, and then introduce small bits of fresh boiled liver or egg on one edge of the container. Do the *Dugesia* move directly to the food? Patiently examine the prey searching and feeding behavior of *Dugesia*. Describe what you see.

Turbellarians have simple life cycles, particularly by platyhelminth standards. They do, however, have considerable powers of asexual reproduction. Some species of *Dugesia* rarely reproduce sexually, but commonly reproduce asexually by transverse fission. *Dugesia* are unusual in that the sexual reproductive system appears only during the spring and summer months when it sexually reproduces. Like almost all flatworms *Dugesia* are hermaphroditic.

Examine a whole stained mount of *Bdelloura candida* on which you should be able to identify parts of the reproductive system and compare the specimen with *Dugesia*. How do these organisms differ structurally? How is that related to their lifestyle? What are the different lifestyles?

**CLASS TREMATODA**

The flukes are: all parasitic, have an external tegument, suckers and/or hooks for attachment, anterior mouth leading into a two-branched digestive tract, and single ovary and paired testes. There are two orders: 1) *Monogea* (usually single host with direct development, i.e., no distinct larval stages), 2) *Digenea* (more than one host, one of which is always a mollusc).

*Chlonorchis sinensis*

Examine a prepared slide of the human liver fluke *Chlonorchis sinensis*. This flatworm lives in the bile ducts of humans and feeds on the living tissues of the host. The anterior end of the worm tapers to a point and bears a sucker at its tip, whereas the posterior end is blunt. Is *Chlonorchis* bilaterally symmetrical? The oral sucker at the anterior end surrounds the mouth. Note the two branches of the gastrovascular cavity. As in all helminthes the reproductive structures take up most of the body space.
These parasites have essentially become reproductive machines; since the chance of a parasite individual to transfer its progeny from one host to another is remote at best, high reproductive capacity is an essential adaptation for survival. Can you observe any difference between the dorsal and ventral surfaces of *Clonorchis*? How does this compare with *Dugesia*? Suggest an explanation.

Now examine a cross section of *Clonorchis* and identify the internal structures. Pay particular attention to the body covering or tegument, which was lacking in *Dugesia*. What is the function of the tegument?

The reproductive system of trematodes differs from that of turbellarians chiefly in the enlargement of the main canal of the female system into a **coiled uterus**, capable of storing many fertilized eggs. Reexamine a whole mount of *Clonorchis* and identify the male and female components of the reproductive system. While self-fertilization appears to occur in trematodes, cross-fertilization is the rule. Copulation involves the insertion of the penis or cirrus into the terminal portion of the **Laurer's canal** or **uterus** of another fluke. Sperm are stored in the **seminal receptacle** and released when required.

Examine the lifecycle of *Chlonorchis sinensis*. Are these organisms monogeneans or digeneans? What hosts are required in order to complete the lifecycle and produce adults that will affect human health?

**Schistosoma mansoni**

In contrast to the above description of hermaphroditic trematodes, flukes in the family *Schistosomatidae* live in the hepatic portal system of birds and mammals and are noteworthy due to their sexual dimorphism. Schistosome parasites are extremely common and dangerous parasites of humans and infect hundreds of million of people annually. Examine the schistosome slides on demonstration in the laboratory.
As mentioned previously, the two classes of trematodes are characterized by different life cycles. The monogens are ectoparasites and have only a single aquatic, cold-blooded host and have direct development with no asexually reproducing larval stages. The digens are largely endoparasites and have at least two hosts and asexually reproducing larval stages.

Examine the typical diogenetic life cycle. Typically, the sexually reproduced eggs pass through the wall of the hosts intestine (creating secondary infections through encystment in the liver) or are released in the feces and hatches (using a small tooth-like structure, the lateral spine) to a ciliated, free-swimming miracidium. The miracidium is covered in epidermal plates containing multiple cilia and detects its snail host using chemoreception (attracted to the fatty acids secreted by the snails), attaches, using the terebratorium (which functions as a sucker) and penetrates the molluscan body using chemical secretions and mechanical boring. Inside the molluscan host the miracidium will grow into a sac-like sporocyst. There are two versions of the sporocyst, the mother and daughter sporocyst. The mother sporocyst will produce daughter sporocysts and is a way of increasing the overall yield of infective cells. The daughter sporocyst produces the cercariae, which are released from the snail host. The cercariae are released 25-30 days after the snail has been infected and are another non-feeding stage (as the miracidium). Their energy stores will become depleted over time, making it pertinent that they find their terminal host within 5-8 hrs. In some species, the cercariae encyst on an additional host or vegetation as metacercariae and enter the vertebrate host when ingested. Initial stages of human infection by cercariae is completed by penetrating the human epidermis, crossing the dermis and entering a blood vessel and as such migrating through the body until they have reached maturity.
There are human stages of a schistosome infection, including a lung stage which is not an adult schistosome and still needs to migrate through the blood vessels into the left side of the heart to the hepatic portal vein and into the liver, where they will reach maturity. Once present in the liver, the parasite undergoes true growth, with completion of the development of the gut, and maturation of the gonads. Once sexually mature, the adult male and female parasites pair up, and migrate against the flow of blood back along the hepatic portal vein, to its mesenteric branches around the intestine where egg laying commences. Unlike *C. sinensis* and *F. hepatica*, schistosome adults display separate sexes and also show sexual dimorphism.

Females are larger and grooved and males are generally located within the gynecophoric canal (groove). Due to their smaller size and their general location, parasitic structures (oral sucker and acetabularium) are also generally smaller in males. Be sure you examine and understand the life cycles outlined in this handout and examine the demonstration slides of different trematode lifecycle stages and determine their important features and the reason for these features.
*Fasciola hepatica*

Averaging 30mm in length and 13 mm in width, *Fasciola hepatica* is one of the largest flukes in the world. The adult worm has a very characteristic leaf shape with the anterior end being broader than the posterior end and an anterior cone-shaped projection. This fluke possesses a powerful **oral sucker** at the end the **anterior cone** and a **ventral sucker** or **acetabularium** at the base of the cone, which allow it to attach to the lining of the biliary ducts. Each worm possesses ovaries and testes that are highly branched and allow for individual flukes to produce eggs and sperm independently. The branches of the alimentary canal (intestines) are generally found only in the anterior cone and obscured within the rest of the body. As in *Chlonorchis sinensis* the female reproductive organ (**ovary**) is found in the anterior portion of the animal, the male **testes** in the posterior portion. Unlike in *C. sinensis* the **yolk glands** obscure the reproductive organs.
CLASS CESTODA

*Taenia solium*

The tapeworms are: parasites on vertebrates; usually intestinal; usually segmented, with each segment (proglottid) lined with tegument and bearing one or two complete hermaphroditic reproductive systems; anterior scolex with suckers or hooks for attachment; no digestive system or sense organs; and the life cycle is complex with no larval multiplication in most forms.

Examine a preserved specimen of the pork tapeworm *Taenia solium*, which shows the typical morphology of this class of flatworms. The body consists of a linear series of proglottids that become progressively smaller, so that the most anterior tip of the animal consists of a microscopic scolex. Study the scolex under the dissecting microscope and note the attachment structures (hooks and suckers, see structural detail below). Immediately posterior to the scolex is the neck, which buds off proglottids that contain the male and female reproductive machinery. Adult tapeworms do not have a digestive track.

Like flukes, tapeworms are hermaphrodites. Each tapeworm is composed of different segment-like structures referred to as proglottids. The sum of the proglottids is called a strobila, which is thin, and resembles a strip of tape. Proglottids range from immature to gravid from the head of the tapeworm to the posterior end. Each mature proglottid of a tapeworm contains at least one complete set of reproductive organs (male and female). Identify the structures of the male and female reproductive system.
on a prepared slide of a mature proglottid. Immature proglottids are produced immediately behind the scolex and initially do not show developed sexual structures.

You should be able to observe this on the prepared slides of a *Taenia* scolex. As the proglottids mature, their reproductive structures become evident. As in flukes, eggs from the ovary move down the oviduct and into the yolk duct. Sperm, having entered the vagina after copulation, migrate from their storage point
in the seminal receptacle, through the oviduct and into the yolk duct where fertilization occurs. Eggs are then quickly coated with yolk and a shell. Finished eggs are then pushed along into the uterus. In the uterus the eggs develop into larva still in the shell. The larva hatches from the shell after being stimulated to do so in the stomach of an intermediate host, which became infected by accidentally ingesting eggs or segments from the feces of the previous host. The released larva then penetrates the intermediate host's intestinal wall, invades the body tissues, and forms **cysticercus** (bladder worms) in muscle or other tissue.

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