

Phylum Platyhelminthes

Most parasitic platyhelminths belong to one of three classes: Monogenea, Cestoidea or Digenea. In older texts, Digenea and Monogenea are often united under the Trematoda. However, Monogenea are more closely related to Cestoidea because both have a caudal hook-bearing structure, the cercomer, at some stage of their development. In Monogenea this becomes a prominent opisthaptor whereas in Cestoidea, it is lost during development and is absent in the adult.

cercomer

Much of the classification of these groups is based on reproductive anatomy and it is therefore important to understand these structures in some detail. Platyhelminthes with few exceptions are hermaphroditic; individuals bear both male and female reproductive systems. Usually both systems develop simultaneously or the male system develops first (protandrous) but in Gyrodactylid monogenea, the female system develops first (protogynous). Although it varies in detail the basic reproductive anatomy is similar in all parasitic Platyhelminthes. The male system consists of one to many testes which lead to a common sperm duct that empties at the male genital pore. Often an intromittent organ is associated with this pore; this is referred to as a penis if it is protrusible, and as a cirrus if it is protrusible and eversible.

hermaphrodite

protogynous

protandrous

cirrus

penis

The female system consists of one or more ovaries that lead through an oviduct to a uterus that empties to the outside at a uterine pore. Glandular follicles, the vitellaria, produce cells that help to form the egg shell. These empty into a vitelline duct that empties into the oviduct near the level of the ootype, the region where the egg is fertilized. Usually a vagina is present and serves to bring sperm to the seminal receptacle that opens into the oviduct near the ootype. In some Digenea, the uterus takes on this role and there is no vagina. In each of the worms you study, make sure you understand where sperm are formed, how they leave the male system and enter the female system and how eggs are formed and leave the female system.

uterine pore

vitellaria

ootype

vaginal pore

Laboratory 4. Class Digenea

Slides to study: *Schistosoma mansoni*, slide 45 (male), 46 (female), 47 (eggs)
Echinostoma revolutum, slide 41.
Fasciola hepatica. Slide 37.
Quinqueserialis quinqueserialis, Slide 42.
Dicrocoelium lanceolatum, Slide 40.
Paragonimus westermani, Slide 44.
Clonorchis sinensis, Slide 34 (wholemound), 35 (bile system)

Living material: Local snails.

Digenea are endoparasitic in all classes of vertebrates and a few are parasitic in invertebrates. Adults occur in a great variety of tissues/sites in their vertebrate hosts. Digenea use molluscs as their first intermediate host where they usually occur in the digestive gland and/or ovotestis.

Digenea are distinguished by the presence of 2 prominent muscular suckers, the oral sucker which surrounds the mouth and the ventral sucker, or acetabulum, on the ventral surface. An acetabulum is absent in some groups (e.g. Notocotylidae). Digenean life cycles are highly varied but follow a common pattern. A ciliated miracidium develops in the egg and usually hatches in the external environment. The miracidium is a non-feeding stage that must locate and penetrate into an appropriate mollusc intermediate. It develops into a sporocyst in the digestive gland. Sporocysts are little more than a sac of larvae and may give rise to daughter sporocysts, rediae or cercariae depending on the species. Rediae are distinguished from sporocysts by the presence of a mouth and digestive system. They are usually motile and can give rise to daughter rediae or cercariae depending on the species. Cercariae are the end product of asexual reproduction.

acetabulum

miracidium

sporocyst

redia

cercaria

They leave the mollusc and reach the final host in one of three ways. Some (e.g. Schistosomes) penetrate the final host directly. Others (e.g. Fasciolidae, Paramphistomatidae) encyst as metacercariae on aquatic vegetation; these are typically parasitic in herbivorous hosts. The majority of Digenea encyst as metacercariae on second-intermediate hosts, and reach the final host through predation.

metacercaria

Live material. We have provided you with snails to dissect and examine for Digenean larval stages. Carefully break the shell apart in a small petri dish of water to expose the body of the snail. Rediae and sporocysts are large sac-like stages whereas cercariae are usually tailed and move around vigorously. You may have to dissect several snails to find one that is infected. Make a vaseline-ringed coverslip preparation of the material you find and examine it with the help of a compound microscope.

You will find representatives of 4 orders of Digenea in the slide boxes. Examine the material carefully and develop a dichotomous key that distinguishes the species.

Order Strigeata

Members of this order are characterized by a furcocercous, or fork-tailed, cercaria.

furcocercous cercaria

Schistosomatidae Members of this family are the only dioecious Digenea. The male is larger than the female and carries his mate in a cleft on his ventral surface, the gynecophoral canal. Close relatives of the Schistosomatidae occur in blood vascular systems of fishes (Sanguinicolidae) and turtles (Spirorchidae); like most Digenea, members of these two families are hermaphroditic.

gynecophoral canal

Schistosoma mansoni (**Slide 45, 46, 47**) lives in mesenteric veins draining the large bowel of man. Females deposit their eggs in the smaller venules and the eggs migrate through the tissues to the intestinal lumen where they are passed in the faeces. Snails of the genus *Biomphalaria* serve as intermediate hosts and cercariae penetrate the final host directly.

Slide 45. Note the gynecophoral canal that extends posteriorly from the ventral sucker. There are 8 testes and the common sperm duct opens just behind the ventral sucker.

Slide 46. The female is more slender than the male. The ovary is near the level of the ventral sucker and empties posteriorly into the oviduct which flexes anteriorly to meet the ootype which also receives the vitelline duct. A single egg is usually present in the uterus. Vitellaria extend throughout the body posterior to the ventral sucker.

Slide 47. The egg bears a prominent lateral spine. Different groups of schistosomes can be distinguished by the form of this spine. Thus in addition to the lateral-spined forms, there are terminal spined (*S. haematobium* in man, *S. bovis* in cattle) and vestigial spined schistosomes (*S. japonicum* in man).

Order Echinostomata

Cercariae in this order have simple tails and large bodies.

Echinostomatidae. These are recognized by the collar of spines around the mouth, a characteristic possessed even by the cercarial stage. *Echinostoma revolutum* occurs in the intestine of a variety of semi-aquatic vertebrates (especially ducks) and larvae mature in a similarly broad array of mollusc hosts. However, work by McCarthy (1990)¹ suggests that some strains in different snails may represent different species. Metacercariae encyst in planaria, snails, molluscs, fish and tadpoles. The species is occasionally reported in man who acquires the parasite by eating undercooked snails or mussels.

Slide 41. Vitellaria extend through the posterior four fifths of the worm. The ovary is spherical and located in the middle of the body and empties posteriorly into an oviduct which leads to the ootype. Laurer's canal, a short tube (usually blind ending) that leads from the ootype to the dorsal side of the worm and is thought to be a vestigial vagina, is present but difficult to discern in this material. The uterus empties to the exterior at the genital pore just anterior to the ventral sucker. The testes are oval and lie in tandem behind the ovary. A short cirrus is present.

Laurer's canal

Fasciolidae. These are large parasites of the liver and bile duct of mammals.

Fasciola hepatica. **Slide 37.** The sheep liver fluke is one of the largest Digenea. Humans occasionally become infected by eating contaminated vegetation such as watercress, but more stringent regulations on growing conditions have minimized this occurrence

¹McCarthy, A. M. 1990. Speciation of echinostomes: evidence for the existence of two sympatric sibling species in the complex *Echinoparyphium recurvatum* (Von Linstow, 1973) (Digenea: Echinostomatidae). *Parasitology* **101**, 35-42.

through much of the world. The ovary is branched and opens through an oviduct to an ootype which also communicates with the vitelline duct and Laurer's canal. The uterus is highly coiled and runs anteriorly to the genital pore anterior to the ventral sucker. The testes are highly branched and situated in tandem posterior to the ovary. Cirrus and seminal vesicle are prominent. The egg (**Slide 38**) is large and operculate. Note also the miracidium (**Slide 39**).

Other species. Fasciolids are common in wild ungulates throughout the world. A North American species, *Fascioloides magna* of elk, is one of the largest. It lacks the "shoulders" of *F. hepatica*. One species, *Fasciolopsis buski* occurs in the intestine of man; its metacercariae are encysted on aquatic vegetation such as water chestnut.

Notocotylidae, *Quinqueserialis quinqueserialis*, Slide 42. These Digenea occur in the caecum of muskrat, *Ondatra zibethica*, which acquire the infection by ingesting aquatic vegetation upon which metacercariae have encysted. There is no ventral sucker. The branched ovary occurs in the posterior part of the body and empties anteriorly through an oviduct into the ootype which also receives a vitelline duct. Laurer's canal is present but difficult to discern. The uterus extends anteriorly and becomes highly muscular before emptying at the uterine pore. Testes are branched and positioned on either side of the ovary. There is a prominent cirrus.

Order Plagiorchiata

Cercariae often possess an oral stylet (xiphidiocercariae).

Dicrocoelidae. *Dicrocoelium lanceolatum* (Slide 40) is a parasite in bile ducts of ungulates such as sheep. The life cycle involves mollusc and ant intermediate hosts. These are long slender flukes. The ovary is post-testicular but in the anterior half of the body, and opens posteriorly into an oviduct that leads to the ootype. A seminal receptacle is present. The uterus extends posteriorly before flexing anteriorly and opening at the genital pore. Testes are obliquely arranged.

Troglotrematidae, *Paragonimus westermani*, Slide 44. This species is normally parasitic in lungs of carnivorous mammals such as

Felidae, but occurs frequently in humans in Asia. Cercariae leave the snail and penetrate a crustacean second intermediate host. The final host becomes infected when eating infected crustaceans. Diagnosis of human disease is usually made by finding the characteristic eggs in faeces.

Order Opisthorchiata

Opisthorchiidae. Members of this family occur in gall bladder and bile ducts (rarely the intestine) of all classes of vertebrates.

Clonorchis sinensis, **Slide 34.** An important parasite in the bile duct of man. A variety of fish-eating mammals can serve as reservoirs. These are long slender flukes. The ovary is pre-testicular in the posterior half of the body. Laurer's canal and a seminal receptacle are prominent. The uterus coils anteriorly to empty at the genital pore anterior to the ventral sucker. Testes are highly branched.

Slide 35. Sectioned material demonstrates the parasites in the bile system. The egg (**Slide 36**) is small with an operculum at its narrow end.

Hemiuridae, Slide 43. These are parasites in the intestine of fishes and use copepods as second intermediate hosts. Depending on the slide box you will have a specimen of *Hemiurus*, *Brachyphallus* or *Derogenes*. In all, the vitellaria form 2 compact masses posterior to the testes and ovary. The ovary is pretesticular and the uterus has descending and ascending coils.

Laboratory 5 & 6. Monogenes & Cestodes

Slides to study: **Monogenea**

Entobdella hippoglossi. Slide 33.

Gyrocotylidae

Gyrocotyle parvispinosum, Slide 50.

Cestoidea

Phyllobothrium, Slide 51.

Diphyllobothrium latum, 53 (wholemound), 68 (plerocercoid).

Glaridacris, slide 52.

Proteocephalus sp., Slide 54.

Dipylidium caninum, Slide 65.

Monezia expansa, Slide 66.

Hymenolepis nana, Slide 62.

Taenia pisiformis, Slide 55.

Echinococcus granulosus. Slide 59, 60 (hydatid).

E. multilocularis, slide 61 (hydatid).

Living material: *Gyrodactylus alexanderi* from the three spined stickle-back, *Gasterosteus aculeatus*.

Bothriocephalus sp. from the tubesnout,
Aulorhynchus .

Monogenea are typically ectoparasitic on ectothermic vertebrates, although one species occurs in the eyes of Hippopotomus, and a few species are true endoparasites (e.g. *Dictyocotyle* in the body cavity of rays; *Polystoma* in the urinary bladder of frogs). Most species occur on the gills or body surface of fishes. Life cycles are direct and most involve a free swimming stage, the oncomiracidium, that leaves the egg to locate the host. In Gyrodactylids there are no oncomiracidia and reproduction occurs directly on the body (or gills) of the host. *oncomiracidium*

Monogenea are recognized by a body that is divided into an anterior region containing reproductive and digestive organs, and a posterior region, the opisthaptor, that serves as a holdfast. The opisthaptor varies in structure from one species to the next, and is an important taxonomic feature. *opisthaptor*

Gyrodactylus

This species is extremely small and best appreciated through living material. You have been provided with specimens of *Gasterosteus*

aculeatus, three-spined stickleback, that are frequently infected with *Gyrodactylus*. Anaesthetize a fish using MS 222, and place it in a small petri dish. Look for the worms on the fins or at the base of the fins and tail; you may have to examine several fish before you find a parasite. Make a vaseline-ringed coverslip preparation of a single worm and examine with the help of a compound microscope. Note the posterior opisthaptor with its two large hooks and circle of smaller hooks.

Perhaps the most interesting feature of these worms is their mode of reproduction. Worms are born as grandmothers. That is, they are born with a juvenile worm already in utero that is itself pregnant. Read Harris (1985)² for an account of reproduction in this worm. Ovary and vitellaria are fused in a common mass, the ovovitellarium that feeds through an oviduct into the uterus. Typically, a single uncleaved egg is present at the base of the uterus which also contains a well developed, pregnant adult. The testis is single, located behind the ovovitellarium and a sperm duct leads anteriorly to a protrusible penis. The digestive system is blind and consists of a subterminal oral opening, a muscular pharynx and two caeca.

ovovitellarium

Entobdella hippoglossi

Slide 33. This large monogenean lives on the body surface of Halibut. The opisthaptor is circular with a wrinkled (rugose) surface and bears two pairs of large hooks. An oviduct and short uterus lead from the single ovary to a common genital pore on the left side. Testes are paired and empty through paired sperm ducts to a common sperm duct that leads to the genital pore. A protrusible penis is present. The mouth is a transverse slit and intestinal caeca are branched.

²Harris, P. D. 1985. Observations on the development of the male reproductive system in *Gyrodactylus gasterostei* Glaser, 1974 (Monogenea; Gyrodactylidae. *Parasitology* **91**, 519–529.

Class Cestoidea

Tapeworms lack a mouth and digestive system, and absorb nutrients through the tegument. An important unifying character of the Class is the presence of larval hooks. In tapeworms, except cyclophyllideans, hooks are arranged on a caudal structure, the cercomer; 10 hooks are present in Cestodaria, whereas 6 are present in most Eucestoda. In cyclophyllidean Eucestoda, there is no cercomer but the larval oncosphere bears 6 hooks. Tapeworms may be monozoic or polyzoic. In monozoic worms there is only a single set of reproductive organs, whereas polyzoic worms are divided into a variable number of proglottids each with one or more sets of reproductive organs. Cestodaria are monozoic and most Eucestoda are polyzoic. Life cycles, with few exceptions, are indirect and often involve an invertebrate first intermediate and vertebrate second intermediate host.

monozoic
polyzoic

In ovo development gives rise to an oncosphere larva which is typically ingested by the intermediate host while still in the shell. In Pseudophyllidea, the oncosphere is surrounded by a ciliated epithelium and hatches to a free-swimming coracidium. Larvae of Cestodaria are also ciliated; they bear 10 hooks and are referred to as lycophores. In all but cyclophyllidean Eucestoda, oncosphere larvae penetrate into the body cavity of the first intermediate host (typically a crustacean) and develop into procercooids, elongate larval forms with short round caudal appendages (the cercomer). When this stage is ingested by the second intermediate host, typically a vertebrate and often a fish, procercooids move into the body cavity, lose their tails and develop their oral structures (proboscis or scolex) becoming plerocercoids. After ingestion by the final host plerocercoids develop into adults in the intestine. Cyclophyllidea have a unique series of larval stages and their development will be discussed below.

oncosphere
coracidium
lycophore

procercooid
plerocercoid

After you have examined the material below, prepare a dichotomous key to the class Cestoidea using characters taken from the material at hand.

Subclass Cestodaria

There are two orders, Amphilinidea and Gyrocotylidea, the former parasitic in the body cavity of turtles and primitive fishes such as catfish and sturgeon, the latter parasitic in the spiral valve and bile ducts of chaemerid (Holocephali) fishes. You will be examining *Gy-*

rocotyle parvispinosum from local ratfish, *Hydrolagus collicii* (Slide 50.). The only known Cestodaria life cycle is that of *Amphilina foliaceae* of sturgeon. The lycophore within the egg shell is ingested by an amphipod and develops in the haemocoel as a proceroid, Within the amphipod, the tail is shed, the proboscis develops and the animalis now referred to as a plerocercoid. After the amphipod is ingested by the sturgeon the plerocercoid burrows through the fish's gut and reaches maturity in the coelom. Adult worms must burrow through the body wall of the host to deposit their eggs.

Gyrocotyle parvispinosum: The anterior extremity is provided with an eversible muscular proboscis and the posterior end bears a membranous outgrowth that serves as an attachment organ. The testes are spherical bodies throughout the parenchyma in the anterior half of the body, and empty into a common sperm duct that leads anteriorly and becomes highly coiled before emptying through a pore associated with a tiny papilla. Ovarian follicles form a mass in the posterior part of the worm and feed into an oviduct into which a seminal receptacle and a vitelline duct open. The uterus extends anteriorly and empties to the outside through a large uterine pore. Insemination occurs through a vaginal pore on the left lateral side.

seminal receptacle

Subclass Eucestoda

These are the true tapeworms. The vast majority are polyzoic, comprised of a scolex which serves as a holdfast and a strobila that is divided into many proglottids. Each proglottid potentially contains a complete set of male and female reproductive organs. Proglottids are added on in the "neck" region between the scolex and strobila, and mature as they move down the body. The male system develops first and anterior proglottids often contain only male organs. Older proglottids will contain both reproductive systems and anatomy of mature proglottids is often obscured by developing eggs. Eggs may be released through a uterine pore (e.g. as in *Diphyllobothrium*), or the entire gravid proglottid may break off (apolysis) releasing eggs as it breaks up. You have slides representing 5 orders of tapeworms: Tetraphyllidea, Pseudophyllidea, Caryophyllidea, Proteocerphalata and Cyclophyllidea. Examine these slides and develop a dichotomous key to the tapeworms based upon them.

scolex
strobila
proglottid

apolysis

Order Tetraphyllidea. This order and the order Trypanorhyncha are restricted as adults to sharks and rays. No complete life cycle is known but larval stages (plerocercoids) have been found in molluscs and a variety of fish, and the proceroid stage is thought to occur in crustacea.

Phyllobothrium, **Slide 51.** These plerocercoids were collected from the body cavity of salmon. The scolex is adorned with 4 leaf like bothridia and a spherical apical organ is visible at the anterior end. *bothridium, —a*

Order Pseudophyllidea. *Diphyllobothrium latum*, formerly known as *Dibothriocephalus latus*, is the broad fish tapeworm, a parasite of fish-eating mammals including man with larval stages in copepods (proceroid) and fish (plerocercoid).

Stickle-backs in the lower mainland are commonly infected with larval stages (plerocercoids) of *Schistocephalus* sp., a pseudophyllid genus that completes its development in piscivorous bird such as herons and loons. The plerocercoid is often startlingly large in comparison to its fish host and it has been suggested that infected fish are more susceptible to predation than are uninfected fish.

Slide 68. The plerocercoid is like a small bladder with a well developed scolex on the anterior end. The scolex is spatulate with two lateral bothria (singular: bothrium, a shallow cleft-like depression). *bothrium, —a*

Slide 53. The ovary is bilobed and empties through an oviduct into the ootype. The uterus is multicoiled and occupies the center of the proglottid, emptying at the uterine pore. Vitellaria form small follicles just beneath the tegument. Testes are more deeply located, more diffuse staining; the common sperm duct opens just anterior to the uterine pore. Insemination occurs through a vaginal pore that connects to the seminal receptacle in the region of the ovary. Eggs of this species (**Slide 67**) are ovoid and have an operculum; they give rise to a free swimming coracidium. Compare them to those of the cyclophyllideans *Hymenolepis* and *Taenia*.

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Order Caryophyllidea. These are monozoic tapeworms that resemble pseudophyllidea. The scolex is simple and smooth or with bothria.

Slide 52. The scolex of *Glaridacris* is broad and smooth and the reproductive organs similar in arrangement to those of *D. latum*. This species is parasitic in the gut of suckers and has larval stages (proceroid) in oligochaetes.

Order Proteocephalata. These are parasitic in freshwater fishes and more rarely in amphibia and snakes. Larval stages occur in copepods (proceroid) and fish or amphibians (plerocercoids).

Proteocephalus sp., **Slide 54.** The scolex bears 4 prominent suckers. Immature proglottids are featureless except for the 4 prominent excretory canals that run the length of the worm. The male system consists of tightly packed testes that feed into a common sperm duct that terminates in a muscular ejaculatory duct. The opening is lateral and may empty on the right or left side. The vagina opens anterior to the sperm duct and leads posteriorly to the region of the bilobed ovary. The vitellaria are restricted to lateral regions of the proglottid. Mature proglottids break off and pass in host faeces.

Order Cyclophyllidea. Members of this order are parasitic in intestines of amphibians, reptiles, birds and mammals. They are essentially parasites of terrestrial hosts and their eggs are surrounded by a thick shell; infective eggs contain an oncosphere larva that bears 6 hooks. They have a variety of intermediate host types, both vertebrate and invertebrate. Depending on the cestode species, the stage in the intermediate vertebrate host may be a cysticercus, a coenurus or a hydatid. The cysticercus is a bladder-like form with an invaginated scolex located at one end; a coenurus is similar but bears multiple scoleces. In a hydatid, brood pouches that develop within the bladder gives rise to multiple scoleces (20 or so) and each hydatid

cysticercus
coenurus
hydatid
cysticercoid

cyst may include thousands of scoleces. Larvae in invertebrate hosts (usually arthropods) are cysticercoids; these have no bladder but a simple invaginated scolex. The scolex has 4 prominent suckers and a terminal rostellum often armed with hooks may be present.

Dipylidium caninum. **Slide 65.** This is a common parasite of dogs and a related species occurs in cats. The larval stage is a cysticercoid and occurs in fleas. The final host is infected when it ingests an infected flea. The scolex has a prominent rostellum armed with hooks. Reproductive systems are paired, one opening on each side of the proglottid. Testes are scattered throughout each side of the proglottid and feed into a common sperm duct that empties anterior to the vaginal pore. Vitellaria are located in a compact mass posterior to each ovary. *rostellum*

Monezia expansa. **Slide 66.** Cysticercoids of this parasite of sheep and goats occur in oribatid mites and final hosts are infected when they ingest infected mites. The scolex is unarmed, with 4 large suckers. Proglottids are wider than long and contain paired reproductive organs similar to those of *D. caninum*.

Hymenolepis nana. **Slide 62.** Also known as *Vampyrolepis nana* this is a parasite of mice, with cysticercoids in flour beetles of the genus *Tribolium*. Humans often become infected from contaminated granaries. The scolex has a prominent armed rostellum and 4 large suckers. Three large spherical testes occur in the posterior part of the proglottid. The ovary is spherical and centrally located. **Slide 63** is of eggs of this species. Note the larval hooks on the oncosphere within the thick shell.

Taenia pisiformis **Slide 55.** This and the next species belong to the family Taeniidae, parasites of carnivores with larval stages in vertebrate prey species. *Taenia pisiformis* normally occurs in canids or felids and uses rabbits as an intermediate host. Cysticerci (**Slide 57.**) are large bladders in the body cavity, particularly the inguinal region. The scolex of the adult is armed and has 4 large suckers. Gravid proglottids are longer than wide; the reproductive system is single and the genital pores open on alternating sides of the proglottids. **Slide 56** is of eggs of *Taenia*.

Echinococcus granulosus. **Slide 59.** These tiny tapeworms consist of 3 proglottids and occur in canids and their ungulate prey which serve as intermediate hosts. The most posterior proglottid is gravid. The reproductive system is single but otherwise resembles that of *D. caninum*. The larval stage is a hydatid, a large tumour like bladder filled with developing scoleces. On exposure to eggs, man can become infected with hydatids and the result is a disease known as echinococcosis, common in some parts of Eurasia. The hydatid of *E. granulosus* (**Slide 60**) is unilocular and can be surgically removed. However, that of *E. multilocularis*, a parasite of foxes and lemmings, is multilocular, highly invasive and may even spread throughout the body by metastasis (**Slide 61**). Prognosis for those infected with multilocular hydatid is extremely grave. Compare the histological appearance of these two types of hydatid. Note especially the well-developed limiting capsule of the unilocular form.

*unilocular and
multilocular hydatid*