Phylum Nematoda

The Nematoda, or roundworms, are a major eukaryotic group and display a startling variety of life histories. Many are free living and abound in soils and sediments in terrestrial, freshwater and marine habitats. As parasites they occur in every multicellular group. and occupy diverse tissue sites. Nematodes may be found in every major organ of the vertebrate body.

Nematodes are distinguished by their smooth cuticular body covering which may or may not be broken by annulations. They have a very simple body plan. They are pseudocoelomates, and possess a fluid filled body cavity which functions as a hydrostatic skeleton. The organ systems are simple consisting of one or two cell types. The digestive system consists of a pharynx of varied structure that leads through and intestine to a culticularly lined rectum at the posterior end.

The vast majority of nematodes are gonochorists. The reproductive system is a hollow tube of endothelium: at the blind end germ cells (oogonia and spermatogonia) divide to produce oocytes and spermatocytes that will undergo meiosis. In females, the system is typically doubled: ovaries are separated from uteri by an oviduct in which fertilization typically occurs. Uteri flow into a common vagina that serves as a canal for expulsion of eggs and for movement of sperm upwards to the oviducts. In males the reproductive system is typically single: the testis opens into a seminal vesicle where sperm are stored. Males also have accessory copulatory structures, the spicules, which are used to transfer sperm to the vagina of the female.

Phylogenetic relationships in the group are poorly understood (see Blaxter *et al.*[1] for a recent hypothesis) but most worker divide the group into 2 major classes, which your text refers to as the Rhabditea and the Enoplea, distinguished by differences in structure of the sensory system and excretory system.

All nematodes pass through 5 stages separated by 4 moults. Preadult stages are referred to as larval or juvenile stages and differs from the adult stage in size and in the extent of development of the

[1] Blaxter, M. L., Paul De Ley, James R. Garey, Leo X. Liu, Patsy Scheldeman, Andy Vierstraete, Jacques R. Vanfleteren, Laura Y. Mackey, Mark Dorris, Lind M. Frisse, J. T. Vita and Kelley Thomas. A molecular framework for the phylum Nematoda. *Nature*, **392**, 71–75.

reproductive system. Parasitic life histories are remarkably varied (for a brief review of animal parasitic groups, see Adamson[2]); they may be direct or indirect using a variety of intermediate hosts and some life cycles depend on vectors.

The major groups of parasitic nematodes are divided into orders which tend to have characteristic host preference, tissue sites and life cycles.

Laboratory 7. Nematoda

Slides to study: *Strongyloides stercoralis*: Slide 73 (parasitic female), 74 (Free-living males and females), and 75 (worms in intestine).
Ancylostoma duodenale: Slide 76 (male), 77 (female).
A. caninum: SLide 78 (male and female).
Necator americana: Slide 79 (male and female), 80 (eggs), 81 (larvae).
Enterobius vermicularis: SLides 72 (male and female), 95 (eggs).
Ascaris lumbricoides: Slides 69 (cross section of adult), 70 (eggs), 71(larvae in lungs).

Living Material: Pinworms of *Periplaneta americana*.

Rhabditea

The vast majority of parasitic nematodes belong to this class. Their free-living relatives are small soil dwelling species of which the laboratory model, *Caenorhabditis elegans* is an example. Free living rhabditeans live between soil particles or in fresh water sediments and feed on bacteria. Presumably, the earliest parasites in this group fed on bacteria in the hosts gut. Later, lineages arose that exploited extra-intestinal sites such as the lungs, etc.

[2]Adamson, M. L. 1986. Modes of transmission and evolution of life histories in zooparasitic nematodes. *Can. J. Zool.* **64**, 1375–1384.

Rhabditida

Strongyloides stercoralis is a tiny worm that inhabits the mucosa of the small intestine (Slide 75). Typically infections are without symptoms but worms may reach very high abundances within infected hosts and produce a relapsing colitis. In some immunocompromised patients, an autoinfecyive cycle can occur that can produce infections heavy enough to kill the patient.

Worms feed on the submucosal tissues. The parasitic stage (Slide 74) is a parthenogenetic female, which releases eggs that hatch to produce first-stage larvae that typically leave the host with the faeces. These larvae may unbergo 2 moults to reach the third (infective stage) or they may undergo 4 moults to reach adulthood and reproduce as free-living adults (males and females) (Slide 73); after one or more rounds of such development, larvae develop to the infective third stage. Infection occurs by percutaneous penetration. Worms then travel by blood and lungs or through the tissues to reach the gut where they mature to parthenogenetic adults. In immunocompromised patients some of the larvae produced by females become directly infective (autoinfection).

Strongylida

This Order includes the bursate nematodes characterized by the presence in males of a caudal bursa, a modification of the posterior end that aids males in handling the females during copulation. In addition to the hookworms (Ancylostomatoidea), there are several Superfamilies (Trichostrongyloidea, Oesophagostomatoidea, Strongyloidea, and Metastrongyloidea) that are important as parasites of domestic livestock.

The Hookworms are easily distinguished by a bulbous buccal capsule that is used to attach to the gut of the host. These worms are blood-suckers and possess cutting teeth that breach the epithelium; antocoagulants produced by the worms ensure them a steady stream of food, and hookworms infections produce anaemia in their hosts.

Worms enter the host by cutanbeous penetration and travel via tissue migration and the blood stream to the lungs where they break into the airspaces and are carried by the bronchial escalator into the back of the throat; they are then swallow and carried to the small intestine where they attach and develop. In some hookworms (e.g.,

A. duodenale) larvae may encyst in the tissues during their migration to the lungs especially in hosts that are already infected. Such larvae can become mobilized by the hormonal environment of pregnant or lactating hosts and can produce infections in the developing faetus by transplacental migration, or in newborns by transmammary transmission.

Humans are plagued by 2 species that can be distinguished by the shape of their cutting teeth.

Necator americana (slides 79, 80 and 81), in spite of its name is probably of Old World origin and in endemic to many parts of Africa, India and SouthEast Asia. It has been introduced into the Americas with the slave trade and represents most of the cases that are diagnosed in the Americas. Note the broad cutting plates in the buccal capsule).

Ancylostoma duodenale (Slides 76 and 77) is also of Old World origin. Although it has been introduced into the Americas with the slave trade, it is much less prevalent there than is *N. americana*. You should be able to make out 2 pairs of pointed teeth in the buccal capsule. A related species, *A. caninum* (Slide 78) occurs in dogs. This parasite is uncommon in Canada but occurs commonly throughout the United States and Europe. Humans can become infected with this species; infective larvae penetrate but remain trapped in the skin where they wander leaving a red meandering trail behind them (creeping eruption). This trail is often secondarily invaded by bacteria and becomes intensely itchy.

Oxyurida

The so-called pinworms were recognized by Hippocrates and Aristotle some 400 years B.C. The human species[3] is *Enterobius vermicularis* (Slide 72), a fine democratic parasite that happily infects people regardless of their economic background. In fact, day care settings provide excellent transmission opportunities for this worm. Worms occur in the large intestine. Females mate and accumulate eggs in their uterus. When they are ready to lay their eggs they migrate out of the anus and deposit them in a mss aroiund the anus. The perineal region becomes itchy and infected hosts reinfect them-

[3]See Adamson, 1989. Evolutionary patterns within the Oxyurida. Biofacies of a haplodiploid taxon. *Advances in Parasitology* **28** for an account of the diversity of life histories in the group.

selves when they scratch and later put their fingers to their mouths. Needless to say pinworms tend to be a bigger problem with young children who are unable to curb their habits in this regard. Pinworm infections have been put forward as the root of sleeplessness and a variety of nervous ailments. Diagnosis is accomplished by finding eggs associated with bedclothes or underwear, or by examining the peri-anal region of the suspected host at night while they sleep. Effective non-toxic treatments exist but reinfections are common in those behaviourally disposed to transmission.

Living Material. Pinworms infect a variety of hosts including the American cockroach, *Periplaneta americana*. We have cockroaches for you to examine for worms. Prepare a wet mount of the nematodes from the hindgut and use this preparation to introduce yourself to the basic anatomy of nematodes. Note the muscular pumping and grinding gizzard like bulb at the base of the oesophagus. Can you locate the ovaries, oviducts and uteri? Can you distinguish males from females?

Ascaridida

This is a large, diverse order of parasitic nematodes that occur in all classes of vertebrates. The only important species from the point of view of human disease is *Ascaris lumbricoides*, the large intestinal roundworm of man. This worm can reach length of half a meter (Slide 69 is a cross section through the body) and occurs in the intestine where it feeds on intestinal contents. Heavy infections can obstruct the bowel and even lead to perforation. The latter condition produces a peritonitis which is rapidly fatal if not treated.

Ascaris lumbricoides infections in humans occur through accidental ingestion of eggs contaminating the surroundings. Larvae hatch from the egg in the duodenum, penetrate the mucosa and are carried by the lymph and blood systems to the lungs where they penetrate into airspaces, are carried up the bronchial escalator and are swallowed. They mature in the small intestine. Eggs (Slide 70) have a characteristic convoluted outer shell; they are long-lived and persist in the soil for more than a year. Large numbers of larvae moving through the lungs (Slide 71) can give rise to a verminous pneumonia.

Other Ascaridoidea. Although *Ascaris Lumbricoides* is the only important Ascaridoid in terms of human disease, several other species can be important under certain conditions. Anisakidae are parasites of fish eating fish, birds and mammals; anisakid life cycles typically involve passage through a fish intermediate host. Humans can ingest such larvae (i.e., when we eat sushi or sashimi, or well prepared pickled Herring) which then burrow into the stomach wall. A single worm can cause intense epigastric pain and although these infections resolve with the eventual death and or encapsulation of the worm, the pain involved is often unbearable and surgical removal is a common option.

Domestic cats and dogs become infected with *Toxacara cati* and *T. canis*. Dogs and cats become infected by a variety of different routes (including trans-placental, carnivory, direct faecal oral contamination). Humansd are occasionally infected by ingesting eggs contaminating their environment; larvae hatch from the eggs and burrow through the intestinal wall but instead of proceeding with their normal migration to the lungs, they become waylaid and wander aimlessly throughout the tissues. This condition which can occur in hookworms also is known as visceral larva migrans.

Laboratory 8. Nematoda continued

Slides to study: *Cystidicola*, male and female, slide 82.
Onchocerca volvulus, Slide 83 (tumour), 84 (microfilariae)
Wuchereria bancrofti, slide 85 (microfilariae)
Trichuris trichura, Slides 86 (male), 87 (female), 88 (histological section of worm in situ), 89 (eggs).
Trichinella spiralis, Slides 90 (male), 91 (female), 92 (larvae in muscles).

Rhabditea, continued

Spirurida

This is the most diverse of parasitic nematode orders and includes several important pathogens of humans. Life cycles are always indirect and use a variety of arthropods as intermediate hosts. Typically, eggs passed in faeces are ingested by the intermediate host; larvae hatch from the egg and penetrate into the body cavity where they develop to the third stage. When a suitable vertebrate host ingests an intermediate host carrying these infective third stage worms the cycle is complete: the worms migrate to their final site in the hosts and develop to adulthood. Members of the Filarioidea use haematophagous arthropods as vectors.

Cystidicola

This worm occurs in the swim bladder of salmonid fishes throughout the holarctic. Eggs are carried via the pneumatic duct to the intestine and leave the host in the faeces. The eggs are filamented: you can see this if you focus carefully on eggs in the midbody region of the female in slide 82. These filaments may add buoyancy or may allow them to be picked up more efficiently by the intermediate hosts which are amphipods. $L1$ to $L3$ occurs in the amphipods and transmission to the final host occurs when infected amphipods are ingested.

Filarioidea

The so-called filarial worms include several of the most important nematode pathogens of humans. Adults occur in various tissue sites in a variety of vertebrate hosts. However, only terrestrial vertebrates

are infected. Transmission is effected by haematophagous arthropod vectors (mosquitoes, Blackflies, Deer- and Horseflies, biting midges, etc).

Onchocerca volvulus parasitizes humans in West Africa, parts of East Africa and Yemen and isolated areas of Mexico, Venezuela and Colombia in the New World. The worm is clearly of Old World origin and was presumably introduced into the New World by the slave trade. Worms are transmitted by Black flies where development from $L1$ to $L3$ occurs; these flies have aquatic larvae that need fast flowing streams of well-oxygenated water to develop and this requirement explains the more local distribution of the worms; i.e. they tend to occur in areas with fast flowing water. An East African strain has developed and is vectored by a Black fly that develops in the gill cavity of certain fresh water crabs in an area otherwise unsuitable for *Obchocerca* transmission.

Onchocerca volvulus adults occur beneath the skin where they become surrounded by a tumour-like growth of connective tissue (note the proliferation of collagen and fibrocytes in slide 83). Females release unsheathed microfilaria (see slide 82) that accumulate in the skin where they are picked up by Blackflies. [4] Pathology is associated with chronic infection and arises largely from microfilariae that block local blood flow and elicit chronic infammation. As a result of this, the skin around a tumour can become itchy, and undergoes secondary changes in texture and thickness. The eye acts a s a trap for microfilariae and chronic host reaction to the presence of microfilariae eventually leads to blindness. *Onchocerca* is a leading cause of blindness and some 270,000 of the estimated 18 million people worldwide infected with these worms are blind.

Lymphatic Filariasis. Lymphatic filariasis is caused by several filarial species: most notable are *Wuchereria bancrofti* and *Brugia malayi*. The disease occurs throughout Asia (India, Sri Lanka, China, Southeast Asia and Oceania. Adult worms occur in the lymphatics, particularly of the groin and lower extremities. Females deposit sheathed microfilariae (slide 85) into the lymphatics; microfilariae are carried into the bloodstream and transmission is effected

[4] Unlike mosquitoes, which use a long proboscis to penetrate a blood vessel directly, Blackflies are "pool" feeders, which create a small lesion and lap up serum and blood that exudes into it.

by mosquito vectors (different genera and species are involved depending on the zoogeographic area and species of worm).

Pathology varies greatly: of 120,000,000 people infected with filarial worms in this group, about 75,000,000 show relatively little pathology whereas some 45,000,000 million show signs of inflammation ranging from mild to severe (elephantiasis characterizes about 12,000,000 infected people). The disease is caused by a chronic blockage of lymphatics, not by adult worms, but by microfilariae that get trapped in lymphatics and die. These elicit a strong chronic response from macrophages leading to connective tissue buildup, improper lymph drainage of affected tissues and pressure atrophy of swollen tissues.

Enoplea

Trichurida

This group is recognized by the presence of one or more columns of glandular cells that parallel the oesophagus. This structure is referred to as the stichosome and the individual cells as stichocytes. STichocytes secrete material that aid in digestion and that modulate the host reaction to the parasite.

Trichuris trichura

Some 1 billion people are infected with this species, and most infections occur in developing countries. Mortality rate is 1 in a 1000 but the disease produces considerable morbidity.

The worms are relatively large (30–50 mm), with extremely narrow anterior and broader posterior ends, hence the common name "whipworm". The generic epithet, "Trichuris" means thread tail and stems from early workers having mistaken the anterior for the posterior end of the worm. These parasites occur in the large bowel where they embed their anterior ends beneath the mucosal surface and feed on cells in the lamina propria. The protruding posterior ends of the worms find each other for mating and females deposit eggs into the intestinal lumen where they pass out with the faeces.

Eggs are easily recognized by their prominent bipolar plugs and large size. Females produce from 3 to 20,000 eggs per day and can live for up to a year. The eggs can remain infective for months if they are deposited in moist soil in shade.

Some 1 billion people are infected world wide and most infections occur in developing countries. Typically, infections involve fewer than 100 worms and are relatively symptomless. However, more intense infections can produce a variety of ailments: bloody stool, chronic inflammation of the bowel as well as more systemic effects such as growth retardation, finger clubbing, learning impairment and even death (mortality due to trichuriasis is estimated at 1 per 100,000).

Examine slides (Slides 86, 87, 88 and 89) of adult worms. You should be able to make out the stichosome, although this species is very easily distinguished by its characteristic shape. Note the spicule which often protrudes from the cloaca at the posterior end of the worm. The most common method of diagnosis involves examination of faecal samples; eggs of this species are unmistakeable.

Trichinella spiralis

These are among the smallest roundworms parasitic in humans. They produce a disease known as trichinosis. Adults occur in the upper regions of the small intestine where they burrow beneath the mucosa feeding on the tissues. Mated females produce larvae (they are ovoviviparous) that are deposited in the tissues. Eventually these first stage larvae find their way to the blood stream and are disseminated throughout the body. Larvae penetrate an individual striated muscle cell and with the help of stichosomal secretions provoke formation of a nurse cell that nurtures and protects the parasite inside the cell. These infective stages can persists for many years.

Trichinella spp. have very reduced host specificity although different species tend to occur in diiferent host associations. The life cycle depends on scavenging food chains: first-stage larvae embedded in muscle ingested by a predator or scavenger develop to adulthood and produce infective larvae in the muscles of the scavenger. Often the predator/scavenger must die to make its infective larvae available for transmission. For example, *Trichinella* occurs relatively commonly in Black Bear in Canada. Presumably they get infected through feeding on voles and other small mammals. However, infective larvae in bear muscle are a dead end unless the bear dies whereupon its body is scavenged by a variety of small mammals who complete the life cycle. In other parts of the world, different scavengers are involved: the dingo in Australia, the Hyaena in Africa, Polar Bear in the north, etc.

Humans usually acquire infections through eating undercooked pork. Pigs maintain infections only because transmission is facilitated by farming practices. For example, offal may be fed directly to pigs in some parts of the United States; this is especially common in rural areas where informal associations get together to raise and slaughter pigs for the community. A common source of *Trichinella* infection are homemade sausages. In Canada all offal must be heat treated before it can be used in feed animals and cases of trichinosis are very rare.

Examine male and female (slides 90 and 91) and be sure you can distinguish the sexes in this species (note that the males lacks a spicule). You should also be able to recognize the stichosome. Note the larvae in the muscles. Is there any sign of inflammation around the infected cell (slide 92)?

Other Enoplea

Capillaria (Trichurida) is a large genus that occurs in all classes of vertebrates. LIfe cycles are varied: some are direct with transmission occurring by faecal-oral contamination; others involve intermediate hosts. One species, *C. philippinensis*, has been the cause of concern in human populations in the Philippines during a five year window in the mid-sixties. Humans acquire infections by ingesting undercooked fish containing larval worms. Females produce two types of eggs: one, a thick-shelled variety that passes out of the host, and another containing fully-developed larvae that hatches on deposition and gives rise to an endogenous cycle (autoinfection). Because of this autoinfective cycle, *C. Philippinensis* reaches extreme abundances in infected hosts with consequent pathological changes: degeneration of intestinal function, blood and ion loss through faeces.

Dioctophyme renale (Order Dioctophymatida) is a parasite of wolves and other carnivores that occurs in the kidney (typically the right) of the host. Worms may be close to a meter in length and replace the infected kidney tissue through pressure atrophy. Humans can become infected and worms behave much as they would in the normal final host. The life cycle requires an oligochaete intermediate host. However paratenic hosts (frogs, snakes, etc.) are probably crucial in chanelling the parasite to its proper final host.