Wuchereria
(helminth: nematode)

Overview

Nematodes are triploblastic pseudocoelomate unsegmented worms that undergo protostomial embryonic cleavage and grow by cuticular moulting (ecdysis). Two groups identified by the presence/absence of sensory phasmids have partly been ratified by molecular studies recognising three subclasses: Enoplia and Dorylaimia (both without phasmids) and Chromadoria (most with phasmids). Many phasmidian parasites of vertebrates are grouped in the chromadorian order Rhabditida; including tylenchinids, rhabditinids and spirurinids. The latter contains the infraorder Spiruromorpha: an enigmatic clade linked by molecular characters, but all having indirect life-cycles involving one or more intermediate hosts, the first invariably being an arthropod. Most possess two trilobed lips (sometimes greatly reduced), a bipartite oesophagus (anterior muscular, posterior glandular) and non-bursate males with coiled tails and two dissimilar spicules. Several superfamilies are recognised: including filarioids (without lips) living in subcutaneous, intermuscular, vascular or lymphatic systems of mammals. Two main families include the oviparous filariids (lay eggs) and the ovoviviparous onchocercids (eggs hatch internally releasing pre-larvae called microfilariae). Infections by the onchocercid genus *Wuchereria* are transmitted by mosquitoes and cause lymphatic (Bancroftian) filariasis in humans, involving severe lymphoedema (elephantiasis) of the appendages.

Classification:

Domain:	Eukaryota (membrane-bound nucleus)
Supergroup:	Amorphea (unikonts with single flagellum, or nonflagellated amoebae)
Kingdom:	Metazoa (multicellular eukaryotes, heterotrophs, notably animals)
Group:	Protostomia (triploblastic, spiral cleavage)
Subgroup:	Ecdysozoa (cuticle moulted = ecdysis)
Phylum:	Nematoda (unsegmented, pseudocoelomate roundworms, tubular digestive tract, dioecious)
Class:	Chromadorea (spiral amphids, three oesophageal glands, usually annulated bodies, free-living and parasitic)
Order:	Rhabditida (Secernentea, Phasmidea) (secretors, with phasmids, bipartite oesophagus, single testis)
Suborder:	Spirurina (mostly parasitic in vertebrate hosts)
Infraorder:	Spiruromorpha (enigmatic clade linked by molecular characters, indirect cycles with IHs)
Superfamily:	Filarioidea (tissue-dwelling filarial parasites, lack lips)
Family:	Onchocercidae (adults loose in tissues or in nodules, viviparous (live birth of microfilariae))
Genus:	*Wuchereria* (parasitic in lymphatics of humans, mosquito IH)
Species:	*W. bancrofti* (causes lymphatic filariasis (elephantiasis) in humans)

Parasite biodiversity and host range: Most Metazoa are multicellular triploblastic animals with differentiated tissues, many being bilaterally symmetrical with a body cavity. Most invertebrate animals are protostomes as their embryonic development involves spiral determinate cleavage. Those that moult their external cuticles during their life-cycles (process known as ecdysis) are grouped together in the unique clade Ecdysozoa, including the nematodes (roundworms), onychophorans (velvet worms), tardigrades (water bears) and arthropods (myriapods, chelicerates, crustaceans and hexapods, all with jointed limbs). Nematodes (roundworms) are unsegmented tubular worms with a fluid-filled body cavity (pseudocoelom) that acts as a hydrostatic skeleton. They have longitudinal muscles and typically exhibit a sideways thrashing motion. They have well developed digestive tracts with various partitions: the foregut comprising the mouth (often with lips and papillae), buccal capsule (sometimes with ridges, rods, plates, spears, stylets or teeth) and oesophagus (glandular, muscular or both); the midgut (nonmuscular absorptive section); and hindgut (rectum) emptying through a subterminal anus (cloaca in males). Most nematodes are dioecious and form separate sexes. Male worms have a single testis (sometimes 2), an elongate vas deferens often equipped with a seminal vesicle and ejaculatory duct (glandular and/or muscular), 1-2 copulatory spicules (sometimes with an accessory gubernaculum), and bursate species with elaborate posterior claspers. Female worms are usually didelphic (some monodelphic or polydelphic) with 2 ovaries, 2 oviducts usually with spermatheca, 2 uteri opening into a common vagina and a vulva often equipped with a muscular ovejector. Female worms are oviparous or viviparous and produce numerous eggs or larvae, respectively. Larval stages undergo several moults (L1-L4) before maturing into adult worms. Some nematodes have direct life-cycles where eggs or larvae infect definitive hosts (per os or per cutaneous), but many have indirect cycles where larvae first develop in invertebrate intermediate hosts before infecting definitive hosts (by ingestion, injection or deposition). Many nematode species are free-living in terrestrial and aquatic habitats, while some species from diverse groups have become plant or animal parasites. Two nematode groups identified by the presence/absence of sensory phasmids have partly been ratified by molecular studies recognising three subclasses: Enoplia and Dorylaimia (both without phasmids) and Chromadoria (most with phasmids). Most Enoplia are free-living marine organisms but some are found in freshwater, and on land as plant parasites. The Dorylaimia comprise numerous freshwater and terrestrial species, including major groups of plant and animal parasites. The Chromadoria is represented by many marine groups as well as a terrestrial group of plant and animal parasites. The taxonomic ranks of many nematode assemblages vary considerably depending

on which classification system has been followed. Molecular phylogenetic studies, however, have supported the separate classification of most groups, particularly at the level of superfamily. Collectively, species from at least 16 superfamilies are considered to pose serious threats to human and animal health as infectious diseases.

CLASSIFICATION* OF SUPERFAMILIES OF PARASITIC NEMATODES
Class: Enoplea (Aphasmidea, Adenophorea) (gland-bearers, cylindrical oesophagus, no phasmids, setae, two testes)
Subclass: Dorylaimia (five or more oesophageal glands, buccal stylet (odontostyle), free-living or parasitic)[clade I(2)]
Order: Trichinellida (Trichocephalida, Trichurida) (single spicule, stichosome oesophagus, L1 with buccal stylet)
Superfamily: Trichinelloidea (oesophagus with short anterior muscular and long posterior glandular portions)
Class: Chromadorea (spiral amphids, 3 oesophageal glands, usually annulated bodies, free-living and parasitic)
Order: Rhabditida (Secernentea, Phasmidea) (secretors, phasmids present, amphids anterior, bulbous oesophagus)
Suborder: Rhabditina (free-living or parasitic in invertebrates/lower vertebrates)[clade V(9)]
Infraorder: Rhabditomorpha ('rod-shaped' buccal cavity)
Superfamily: Rhabditoidea (open tube stoma, excretory system with lateral canals)
Superfamily: Strongyloidea (bursate males, prominent buccal capsules, parasites of mammals, birds, reptiles)
Suborder: Spirurina (animal parasites, many use invertebrate intermediate hosts (IH))[clade III(8)]
Incertae sedis Superfamily: Dracunculoidea (elongate parasites of vertebrate tissues, freshwater crustacean IH)
Infraorder: Ascaridomorpha (large roundworms, three large lips, numerous caudal papillae)
Superfamily: Ascaridoidea (ascarids, eggs thick-shelled, larvae may undertake hepato-pulmonary migration)
Superfamily: Heterakoidea (preanal sucker anterior to cloaca in males, direct cycle, infection by egg ingestion)
Infraorder: Gnathostomatomorpha ('jaw-mouthed' due to unique bulbous armed heads)
Superfamily: Gnathostomatoidea (first IH copepod, often use paratenic hosts)
Infraorder: Oxyuridomorpha (pinworms, pointed tails, oesophagus with terminal bulb, males with single spicule)
Superfamily: Oxyuroidea (common in mammals, birds, reptiles, amphibians)
Infraorder: Spiruromorpha (enigmatic clade linked by molecular characters, indirect cycles with IHs)
Superfamily: Acuarioidea (small parasites mostly of birds, with cephalic cordons, ptilina or serrated shields)
Superfamily: Camallanoidea (conspicuous phasmids, L1 with dorsal tooth, ovoviviparous, L1-L3 in copepod)
Superfamily: Filarioidea (tissue-dwelling filarial parasites, lack lips, infect tissues/vessels, arthropod IH)
Superfamily: Habronematoidea (unique head structures with small pseudolabia and median lips)
Superfamily: Physalopteroidea (stomach worms in mammals, insect IH)
Superfamily: Spiruroidea (pseudolabia, bipartite oesophagus, infect birds (crop/gizzard), arthropod IHs
Superfamily: Thelazioidea (eye-worms of birds and mammals, transmitted by insects)
Suborder: Tylenchina (fungal, plant and animal parasites)[clade IV(10,11,12)]
Infraorder: Panagrolaimomorpha (free-living or parasitic (insects, reptiles, amphibians, mammals))
Superfamily: Strongyloidoidea (dauer stages, lip region without processes, striated cuticle)

*Contemporary genotypic classification schemes recognize strong monophyletic clades at the level of superfamily and infraorder, while previous phenotypic classification schemes had ranked many as separate orders.

Molecular phylogenetic studies have grouped a variety of superfamilies into the infraorder Spiruromorpha whose members are parasites of vertebrates with indirect life-cycles involving larval development within invertebrate intermediate hosts. Most members were previously classified within the order Spirurida: either within the suborder Camallanina (worms with conspicuous phasmids, uninucleate oesophageal glands, larvae without cephalic hooks, usually with copepodid intermediate hosts); or the suborder Spirurina (worms with inconspicuous phasmids, multinucleate oesophageal glands, larvae with cephalic hooks or spines, usually with non-copepodid intermediate hosts). Ten spirurinid superfamilies are recognised: Gnathostomatoidea and Physalopteroidea (buccal cavity weakly cuticularized, 2 large lateral pseudolabia); Habronematoidea and Acuarioidea (buccal cavity well cuticularized, 2 large lateral pseudolabia); Filarioidea, Rictularioidea, Aproctoidea and Diplotriaenoidea (buccal cavity well cuticularized, without pseudolabia); Thelazioidea (long cylindrical buccal cavity well cuticularized, body without caudal alae); and Spiruroidea (short buccal cavity well cuticularized, body with caudal alae).

The superfamily Filarioidea contains long thread-like nematodes which are predominantly tissue-dwelling parasites infecting the body cavities, subcutis, intermuscular tissues, blood vessels or lymphatic systems of terrestrial hosts. These worms are known colloquially as 'filariae', 'filarids' or 'filarioids' [Note: take care with terminology as the cognate family Filariidae (esp. genus *Filaria*) are known colloquially as 'filariids', and the unrelated metastrongyle (lungworm) family Filaroididae (genus *Filaroides*) are known colloquially as 'filaroids']. Adult filariae have a cylindroid pharynx with an anterior muscular portion and a posterior glandular portion. Males often have spirally-coiled tails, well-developed alae and dissimilar spicules. Females of most species are ovoviviparous (eggs hatch within body of parent) releasing pre-larval stages known as microfilariae (sometimes sheathed). Filariae have indirect life-cycles whereby microfilariae are taken up by blood-sucking or tissue-feeding invertebrates (arthropods, esp. mosquitoes) which act as intermediate hosts for the development of infective L3 larvae. Ten families are recognised: Filariidae and Onchocercidae infecting mammals, birds, reptiles and amphibians; Setariidae infecting mammals;

Aproctidae infecting birds; and Creagrocercidae, Drilonematidae, Homungellidae, Mesidionematidae, Scolecophilidae and Ungellidae infecting terrestrial annelids. Examples of filarioid genera covered in this resource are compared in the following table.

Genus	Definitive hosts	Adults (location)	Microfilariae (location)	Periodicity	Vectors	*Wolbachia* symbiotes
Family Onchocercidae						
Wuchereria (2 spp.)	primates	2.5-10 cm (lymphatics)	210-320 µm sheathed (blood)	nocturnal, subperiodic	mosquitoes	present
Onchocerca (35 spp.)	primates, carnivores, ungulates, rodents	1.5-80 cm (subcutis, ligaments)	105-440 µm unsheathed (skin)	-	flies, midges	present
Mansonella (29 spp.)	primates, carnivores, ungulates, rodents	3-8 cm (subcutis, serosa)	170-300 µm unsheathed (blood/skin)	-	midges, flies, mosquitoes	present
Dirofilaria (34 spp.)	primates, carnivores, ungulates, rodents, lagomorphs, marsupials	4-31 cm (blood vessels)	180-385 µm unsheathed (blood)	-	mosquitoes, flies	present
Dipetalonema, Acanthocheilonema (57 spp.)	primates, carnivores, ungulates, rodents, cingulates, marsupials	1-7 cm (subcutis, serosa)	85-300 µm unsheathed (blood)	-	flies, fleas, lice, ticks	absent
Brugia (10 spp.)	primates, carnivores, rodents	1-9 cm (lymphatics)	170-380 µm sheathed (blood)	nocturnal, subperiodic	mosquitoes	present
Loa (3 spp.)	primates, ungulates, rodents	2-7 cm (subcutis, eye)	250-300 µm sheathed (blood)	diurnal	flies	absent
Family Filariidae						
Parafilaria (4 spp.)	ungulates	2-7 cm (subcutis)	40-58 x 23-33 µm larvated eggs (skin)	diurnal	flies	absent
Stephanofilaria (7 spp.)	ungulates	0.2-1.4 cm (subcutis)	45-195 µm sheathed (skin)	-	flies	absent
Family Setariidae						
Setaria (42 spp.)	primates, ungulates, rodents, lagomorphs	4-19 cm (body cavities)	140-310 µm sheathed (blood)	-	mosquitoes	absent

Members of the family Onchocercidae form adult worms that live loose in body cavities or in tissue nodules. Female worms release microfilariae which disperse into the blood or dermal connective tissues (unlike filariids which live in the skin close to where they deposit eggs or larvae). Some 88 onchocercid genera are divided into 7 subfamilies: Onchocercinae and Dirofilariinae (syn. Loainae) mostly in mammals but some in birds and reptiles, Waltonellinae and Icosiellinae in amphibians, Oswaldofilariinae in reptiles, Splendidofilariinae and Lemdaninae in birds, reptiles and mammals (former subfamily Setariinae in large mammals recently elevated to family status as Setariidae). Members of the subfamily Onchocercinae are characterised as forming males with markedly dissimilar spicules and long tails lacking caudal alae (while members of the subfamily Dirofilariinae form males with highly developed caudal alae). Some 43 genera occur in the subfamily Onchocercinae: namely, *Acanthocheilonema, Ackertia*, *Agamofilaria*, *Andersonfilaria*, *Bisbalia*, *Breinlia* (incl. *Johnstonema*), *Brugia*, *Cercopithifilaria*, *Chabfilaria*, *Cherylia*, *Courduriella*, *Cruorifilaria*, *Cystofilaria*, *Deraiophoronema*, *Dessetfilaria*, *Dipetalonema*, *Elaeophora* (syn. *Cordophilus*, *Alcefilaria*), *Filarissima*, *Fuscicorpa*, *Josefilaria*, *Litomosa*, *Litomosoides* (syn. *Vestibulosetariam Finlaynema*), *Mansonella*, *Microfilaria*, *Migonella*, *Molossinema*, *Monanema*, *Onchocerca* (syn. *Wehrdikmansia*, *Acanthospiculum*), *Paramadochotera*,

Paraochoterenella, *Paraprocta*, *Paulianfilaria*, *Pseudolitomosa*, *Rumenfilaria*, *Sandnema, Serofilaria*, *Skrjabinofilaria* (syn. *Cortiamosoides*), *Sprattia, Strianema*, *Wuchereria* and *Yatesia* in mammals, *Struthiofilaria* in birds, and *Macdonaldius* (syn. *Saurofilaria*) in reptiles. Three groups of human filariasis are distinguished on the basis of their tissue tropism: cutaneous dermal filariasis (onchocerciasis in Africa, Asia, Central and South America, loiasis in Africa, *streptocerca* mansonelliasis in Africa); lymphatic filariasis (wuchereriasis in Africa and Asia, brugiasis in South Asia); and serous filariasis (*perstans* mansonelliasis in Africa, Central and South America, *ozzardi* mansonelliasis in Central and South America). It is estimated that 1 billion people in tropical and subtropical countries are exposed to filarial infections and at least 200 million are infected (predominantly *Wuchereria bancrofti, Brugia malayi* and *Onchocerca volvulus*). In humans, species of *Wuchereria* and *Brugia* are responsible for causing lymphatic filariasis (known regionally as Bancroftian and Brugian (Malayan or Timorian) filariasis), involving inflammation of the lymphatics (lymphangitis) and blockage of lymph flow, frequently leading to massive swellings (elephantiasis) of the groin, genitalia (common in Bancroftian filariasis) and lower limbs (common in Brugian filariasis). *W. bancrofti* causes Bancroftian filariasis, which represents the majority (> 90%) of lymphatic filariasis cases.

The genus *Wuchereria* is characterised by the formation of elongate thread-like worms with a defined head and mouth bearing 2 circles of papillae. The genus has been considered to be monotypic containing only one species, *W. bancrofti*, which infects the lymphatic vessels of humans (and rarely langur monkeys), although some also recognize the species *W. kalimantani* in leaf monkeys. Other species reported in early studies have mostly been transferred to the genus *Brugia*, or are considered *species inquirenda* as only microfilariae were found. Female *W. bancrofti* are ovoviviparous and release sheathed microfilariae into the blood which are taken up by mosquito vectors in which infective larvae develop. Different strains demonstrate variable periodicity in microfilaraemia (nocturnal or diurnal) attuned to differences in oxygen tension between venous and arterial blood during the sleeping/waking habits of the host, thus favouring night- or day-biting mosquito species in different geographic regions. *W. bancrofti* is common in tropical regions (found in some 76 countries bounded roughly by latitudes 30-41°N and 28-30°S), notably where climatic requirements are suitable for mosquito intermediate hosts, in and around temporary pools or standing water.

***Wuchereria* species**	**Definitive Hosts (DH)**	**Location**	**Vectors/Intermediate Hosts (IH)**	**Distribution**
W. bancrofti (syn. *Filaria, W. pacifica, W. philippinensis, W. vauceli, W. kalimantani*?) (Bancroftian filariasis, elephantiasis)	Primates: hominid (human), cercopithecid (silvered leaf monkey, gray langur)	lymphatics, mf in blood (sheathed, variable periodicity)	Diptera: culicid (*Aedes aegypti, aquasalis, bellator, cooki, darlingi, futunae, kochi, niveus, poecilius, polynesiensis, pseudoscutellaris, rotumae, samoanus, scapularis, scutellaris, tabu, togoi, tongae, tutuilae, upolensis, vigilax, Anopheles aconitus, anthropophagus, arabiensis, balabacensis, bancroftii, barbirirostris, bwambae, candidiensis, dirus, donaldi, farauti, flavirostris, fluviatilis, funestus, gambiae, jamesi, koliensis, kweiyangensis, letifer, leucosphyrus, maculatus, melas, merus, minimus, nigerrimus, nili, pauliani, philippinensis, punctulatus, sinensis, stephensi, subpictus, vagus, wellcomei, whartoni, Culex annulirostris, antennatus, bitaeniorhynchus, fatigans, modestus, pallens, pipiens, quinquefasciatus, sitiens, tritaeniorhynchus, vishnui, Coquiollettidia juxtamansonia, Mansonia pseudotitillans, titillans, uniformis, Ochlerotatus fijiensis, harinasutai, niveus, oceanicus, poicilius, samoanus, scapularis, togoi, vigilax, Psorophora jamaicensis*); Hemiptera: cimicid (*Cimex lectularis*)	South-East Asia, spread to Pacific Islands, China, Japan, India, Africa and then Central and South America, Caribbean, (no longer found in Australia or North America)
W. kalimantani	Primates: cercopithecid (silvered leaf monkey)	lymphatics, testes, mf in blood (sheathed, nocturnally periodic)	Diptera: culicid (*Aedes balabacensis, togoi*)	Borneo
W. lewisi sp. inq.	Primates: hominid (human)	adult not detected, mf in blood	mosquito?	Brazil

Parasite morphology: *Wuchereria* spp. form 3 morphologically distinct developmental stages: microfilariae, larvae and adult worms. Gravid female worms do not lay eggs but are ovoviviparous and release advanced embryos or pre-larvae (known as microfilariae). These stages are elongate, measuring 210-320 x 6-10 μm, and are ensheathed (enclosed within a transparent egg membrane). They have a striated cuticle, rounded anterior end, short cephalic region, neck-like constriction and pointed tail (tapers to a delicate rounded tip). Microfilariae have nuclear columns loosely dispersed along the body but not inside the tip of tail (no terminal nuclei like *Brugia* and *Loa*). These pre-larvae undergo a series of moults to form sausage-shaped first-stage larvae (L1) measuring up to 300 μm long, elongate second-stage larvae (L2) measuring up to 600 μm long, and then elongate third-stage larvae (L3) measuring 1,000-2,000 x 18-23 μm. The infective L3 have truncated trapezoidal heads, circular oral openings lined with papillae on the outer edge, a glandular oesophagus and blunt tails with three bulbous caudal papillae (two latero-ventral and one dorso-terminal). They moult to form fourth-stage larvae (L4) and then adults (L5), both stages marked by the transformation of the truncated conic head to a rounded appearance. Adults worms are long and thread-like in appearance, measuring 25-100 x 0.1-0.4 mm. They are covered by a translucent white smooth cuticle with very fine transverse striations and have slightly swollen heads with a rounded oral opening bearing 2 circles of distinct papillae. Mature worms are sexually dimorphic, with females being larger than males (80-100 mm cf. 25-40 mm) and having rounded tails rather than curved tails. Both sexes live coiled together in host lymphatic tissues. Males have conspicuous unequal copulatory spicules and a cloaca ringed with sensillae. Females are ovoviviparous and produce embryonated eggs which hatch internally freeing microfilariae which are released from the anterior vulva (located just posterior to the nerve ring).

Site of infection: Adult *Wuchereria* worms occur in humans in tightly coiled nodular masses in major lymphatic ducts, mostly in afferent lymph vessels, usually in the lower limbs or groin region in men or the upper limbs and breasts in women. Mature worms also occur sometimes in lymphatic vessels in the peritoneal cavity or subcutaneous tissues. Gravid female worms release microfilariae which pass through the lymphatic ducts and reach the peripheral blood stream where they circulate. Some microfilariae exhibit nocturnal or diurnal periodicity and sequester in deep capillaries (esp. in the lungs) during the day or night, respectively. Larval development occurs in the thoracic (flight) muscles of mosquito vectors and infective L3 stages migrate through the body cavity (haemocoel) to reach the proboscis.

Pathogenesis: *W. bancrofti* has been associated with a wide variety of clinical manifestations, ranging from asymptomatic subclinical infections to acute lymphatic inflammation to chronic granulomatous reactions, lymphoedema and the extensive disfiguring enlargements of extremities (elephantiasis) typically associated with Bancroftian filariasis. Pathological changes occur due to a combination of direct effects (live parasites in lymph vessels), indirect effects (metabolites released from dying parasites and their endosymbiotic *Wolbachia* bacteria), host inflammatory and immune responses, and secondary infections (bacterial and fungal). Live parasites often elicit few host reactions, but significant inflammation occurs when worms die (posing a serious complication to drug therapy). Symptomatic infection has been associated with recurrent incidences of fever, malaise, lymphadenitis (enlargement of lymph nodes) and lymphangitis (inflammation of lymphatic system) typically affecting the lower extremities (sometimes with genital or breast involvement). Lymphatic filariasis is debilitating, but not typically fatal, and the progression of disease may be divided into 3 phases: incubation; acute; and chronic.

The incubation phase may last for up to a year as worms mature and start producing microfilariae. Around two-thirds of infections remain asymptomatic while others have mild vague symptoms, often involving fever. Some patients recover spontaneously and are unaware of having been infected. The incubation phase has been associated with immuno-tolerance or hypo-responsiveness due to suppressed Th1 responses. *In utero* exposure to worm antigens has even been associated with tolerance to post-partum infection. Nonetheless, even asymptomatic infections may cause subclinical pathology with damage to the lymphatics, notably lymphangiectasia (dilatation of lymph vessel walls) with alternating areas of fibrosis and hyperplasia, and the kidneys, with haematuria, proteinuria and glomerulonephritis (possibly associated with increased immune complexes in the circulation). In endemic areas, asymptomatic individuals remain amicrofilaraemic for extended periods, being known as ‘endemic normals’ with no symptoms and no microfilariae although they test positive to worm antigens. Over time, however, this tolerance might break down and patients may become symptomatic

The acute (or inflammatory) phase commences after female worms have matured and produced microfilariae. Worms and their metabolites, as well as antigens from endosymbiotic *Wolbachia* bacteria, provoke intense inflammatory responses in the lymphatic system, especially in the lower body. Patients experience periodic attacks of lymphangitis and lymphadenitis as well as exhibiting chills, fever, toxaemia, skin infections, painful lymph nodes, localized swellings in arms and legs, tender skin covering lymphoedematous extremities, and sometimes orchitis (inflammation of testes) and epididymitis (inflammation of spermatic cord). These symptoms may persist for 5-7 days and then subside, but often recur at frequent intervals. The parasites cause significant lymphatic damage with concomitant immune dysfunction, which predisposes the host to secondary infections; particularly 2 distinct syndromes: ADLA and AFL. Acute dermatolymphangioadenitis (ADLA) is characterised by diffuse plaque-like inflammatory lesions (with classic signs of fever, redness, swelling and warmth) when skin on the limbs becomes secondarily infected by bacteria (*Staphylococcus* or *Streptococcus*) or fungi, with symptoms lasting up to two weeks. ADLA is often accompanied by pain, fever, chills, inflammation of the lymph vessels and nodes, and potentially oedema. Recurrent episodes can lead to permanent damage, and has been linked to chronic lymphoedema and elephantiasis. Acute filarial adenolymphangitis (AFL) is rarer and characterised by less severe lymphatic inflammation (often an isolated nodule or cord), regional pain, hydrocele and oedema, generally resolving

within a week. These symptoms are due to a granulomatous responses (involving macrophages, eosinophils, neutrophils and lymphocytes) resulting in granuloma formation, fibrosis and temporary lymphatic obstruction. Only dead or dying worms elicit these responses, so this syndrome can paradoxically be triggered as a side effect to the treatment of infections.

The chronic (or obstructive) phase occurs well after patency (formation of adults and microfilariae), although afflicted patients are usually amicrofilaraemic. The host lymphatic system becomes progressively damaged over the course of several years, the damage often exacerbated by secondary bacterial and fungal infections. Chronic disease has been associated with heavy infections, recurrent ADLA episodes, and hyper-immunoresponsiveness. If not treated, chronic infections can result in permanent tissue damage with significant cellular and fibrous infiltration producing lymph varices, lymphoedema (fluid retention and tissue swelling), hydrocoele (grossly swollen scrota), epididymitis (inflammation of the spermatic cord), orchitis (inflammation of the testes), elephantiasis (especially of the limbs and scrotum in men, and the limbs, vulva and breasts in women), and sometimes chyluria (lymph passed in urine). Worms and host inflammatory responses cause significant changes (remodeling) to lymph vessels with damage to walls and valves affecting lymph pressure, transport and drainage leading to backflow, blockages and consequently lymphoedema. The movement of interstitial fluid may also be reduced across lymphatic epithelia, exacerbating lymphoedema, and weakened lymphatic vessels may rupture releasing chyle (lymph) into surrounding tissues. In swollen tissues, the accumulation of protein-rich lymph recruits keratinocytes, fibroblasts and adipocytes, with hyperkeratosis and hyperplasia leading to further thickening. Lymphocytes accumulate underneath the epidermis contributing to the extensive inflammation. The overlying skin becomes fibrotic, granulomatous, thickened, hardened, verrucous, cracked, ulcerated and highly susceptible to secondary infections. Elephantiasis is grossly disfiguring and a major cause of morbidity and disability in endemic regions, with negative social stigma and spiraling health costs. Although only some 10% of infected people develop elephantiasis after many years of continual filarial reinfection, the condition is nonetheless seen in an estimated 40 million people (90% due to *W. bancrofti*). It is most prevalent in those older than 40 years of age, and is rarely seen in people < 25 years old. On rare occasions, a condition known as tropical pulmonary eosinophilia may develop when microfilariae migrate to the lungs and invoke hypersensitivity responses with paroxysmal cough, wheeze, interstitial fibrosis, cellular infiltrates, reduced pulmonary function and weight loss.

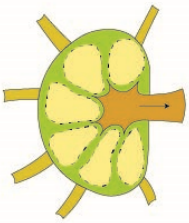
The recent discovery of unique endosymbiotic rickettsial α-proteobacteria (*Wolbachia*) in many filarial nematodes, including *Wuchereria*, has interesting implications for both disease progression and treatment. In addition to worm antigens, metabolites (endotoxins and lipopolysaccharides) released from live and dying bacteria have been shown to contribute to host pathology by provoking inflammatory responses, fibrosis, extravasation, lymphatic endothelial cell hyperplasia and lymphoedema. This presents a problem for chemotherapy as parasites killed by anthelmintic drugs break down releasing their bacterial symbionts. Several studies have shown that concomitant treatment with the antibiotic doxycycline leads to reductions in plasma lymphangiogenesis factors with reduced lymph vessel dilation. Further studies are required to assess the interdependent relationships between filarial worms and their symbiotic (mutualistic) bacteria.

Developmental cycle and mode of transmission: *Wuchereria* has a heteroxenous indirect life-cycle involving cyclic transmission between mammals (definitive hosts) harboring adult parasites and haematophagous mosquito vectors (intermediate hosts) harboring larval stages. Adult worms in humans produce thousands of ensheathed microfilariae which are released into the lymph but enter the blood stream via the thoracic duct. Once in the circulation, the microfilariae display circardian periodicity with peaks in concentration in peripheral blood at certain times of the day whereas they sequester within deep capillaries (especially in the lungs) at other times. Three major subtypes of *W. bancrofti* have been detected: nocturnally periodic, nocturnally subperiodic and diurnally subperiodic strains. The nocturnally periodic strain (microfilariae at highest concentrations in peripheral blood between hours of 10 pm and 2 am) is transmitted by night-biting mosquitoes, principally *Culex* spp. which flourish in the poor drainage systems of overcrowded tropical towns in Asia, East Africa and the Americas, *Anopheles* spp. in rural areas, particularly sub-Saharan Africa, and *Mansonia* spp. in West Africa. The nocturnally subperiodic strain (microfilariae present in peripheral blood at all times but with peak concentration around midnight) was once common in Thailand and the Andaman and Nicobar Islands of India where the mosquito *Ochlerotatus* (*Aedes*) *niveus* and related species served as vectors. The diurnally subperiodic strain (microfilariae present in peripheral blood at all times but with peak concentrations between noon and 8 pm) are transmitted by day-biting mosquitoes, principally *Aedes polynesiensis* which breeds in rural brush areas of the Pacific islands (Fiji, Samoa, Philippines, Tahiti). Microfilariae ingested by mosquitoes exsheath in the intestines in 2-6 hours and then invade the thoracic muscles in 4-17 hours where they develop to sausage-shaped L1 in 5-6 days, moult to elongate L2 in 8-10 days and then to L3 in 11-14 days. Infective L3 migrate through the haemocoel to the proboscis where they are deposited onto the skin of the mammalian host when the mosquito feeds (contaminative rather than inoculative transmission). This form of transmission is considered relatively inefficient so a large number of infective mosquito bites are thought necessary to establish infection. The larvae invade the skin often through the feeding puncture site and migrate to the lymphatic system (varices of lymphatic glands of the groin and epididymis of males and labial and mammary glands of females). They moult twice and mature to thread-like adult worms over at least 6-8 months. Mature adults live intertwined and nest together in major lymphatic ducts (preferentially in the intrascrotal lymphatics and inguinal, axillary and epitrochlear lymph nodes). After mating, gravid female worms may produce up to 10,000 microfilariae each day for up to 10 years. Microfilariae are released into the lymph and make their way to the bloodstream via the thoracic duct (lymphatic drainage duct). The prepatent period (time from infection to first release of microfilariae) ranges from 8-12 months.

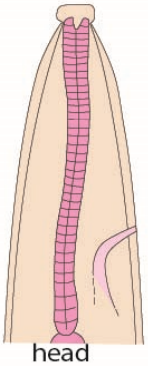
Differential diagnosis: Despite the characteristic gross deformities (elephantiasis) associated with long-standing chronic *W. bancrofti* infections, early infections are difficult to diagnose because of the long incubation period (up to 12 months), the absence of clinical symptoms during this period, the absence of microfilariae in many patients (esp. 'endemic normals') and, when present, their variable occurrence due to their nocturnal or diurnal periodicity. Blood samples collected from patients around midnight in the tropics/subtropics (nocturnal periodicity) and around 4 pm in the Pacific (diurnal subperiodicity) may be examined microscopically for the presence of characteristic sheathed microfilariae (with no terminal nuclei). Blood samples may be examined as thick or thin smears or following concentration by filtration through polycarbonate membranes (5 uμ pore size) or by centrifugation of lysates (saponin, formalin or hypotonic saline lysis). Smears may be stained with Giemsa, Delafield's haematoxylin or Field's rapid stain, while concentrates may be coloured with methylene blue or cresyl blue. Medical imaging techniques have been used to examine infected tissues for worms. In particular, high-frequency ultrasound revealed nests with live adult worms performing a signature 'filarial dance' (rapid threshing). Dead and calcified worms may be detected upon X-ray examination. A range of immunological tests have been developed to detect host antibodies against infection, including intradermal tests, immunochromatography and enzyme immunoassays, but their diagnostic sensitivities and specificities were poor. More recently, similar technologies have been adapted to detect parasite antigens in host blood samples, especially enzyme immunoassays and rapid immunochromatographic card tests using monoclonal or polyclonal antibodies. Molecular biological techniques have also been used to detect adult and larval DNA in samples following amplification by single, nested, multiplex, restriction fragment, reverse transcriptase, real-time polymerase chain reaction (PCR) or loop-mediated isothermal amplification (LAMP) of specific gene sequences (18S and 5.8S ribosomal RNA and internal transcribed spacer regions, repetitive sequences (SspI, pWb12, pWb-35, long DNA repeat (LDR), abundant larval transcript-2) or genes encoding surface proteins (e.g. Gp29).

Treatment and control: Infections by adult *Wuchereria* worms and microfilariae have been successfully treated using several anthelmintic drugs: notably the diethylenediamine diethylcarbamazine (DEC), the macrocyclic lactone ivermectin, and the benzimidazole-methylcarbamate albendazole or combinations thereof. These drugs quickly kill microfilariae but generally slowly kill macrofilariae (adult worms), so repeated treatments are required, particularly in endemic areas where re-infections are common. Patient compliance to treatment may waver as various side effects arise; including pruritus, nausea, fever, dizziness, headache, muscle and joint pain. Mass treatments have been used to great effect in several countries where drugs are given on a monthly, semi-annual or annual basis, sometimes in combination with DEC-fortified salt (although this is contraindicated in areas where onchocerciasis or loiasis are present due to worsening of eye or brain lesions). A novel strategy recently adopted has been to also treat patients with antibiotics to destroy the endosymbiotic *Wolbachia* bacteria within the worms. These bacteria are present in all life stages, are transferred from mother to offspring and appear to be essential to healthy growth and development of the parasite. Treatment with tetracyclines (tetracycline, doxycycline) and ansamycin (rifamycin) have been shown to inhibit the motility, viability and release of microfilariae and impede their development. The reduced survival and/or fecundity of the worms may substantially reduce transmission rates. Nonetheless, treatment with anthelmintics and/or antibiotics may have unintended consequences as much of the disease pathology is caused by host reactions to dead and dying worms and bacteria. Patients should be carefully monitored for worsening of their conditions. Despite the apparent efficacy of drug treatments, patients with established hydrocoele and elephantiasis require major interventions. These conditions cause severe disfigurement and in societies with many stigmas attached, so patients often hide their afflictions. Many are also poverty-stricken and simply cannot afford to be treated. It has been shown that recurrent secondary bacterial infections are associated with the development and worsening of elephantiasis, so patient management must include improved hygiene, regular washing and disinfection, careful inspections to detect and treat lesions, and appropriate exercise and physiotherapy to help slow, and sometimes reverse, the onset of elephantiasis. Elastic pressure bandages may be used to combat lymphoedematous swellings of affected limbs. However, incapacitating deformities usually require radical surgery to remove granulomatous, fibrous and calcified tissues, but such options are not always applicable in advanced cases. Most preventive strategies are designed to interrupt transmission patterns by reducing mosquito vector populations or avoiding mosquito bites. Various chemical insecticides have been used to kill adult mosquitoes (aerial spraying or fogging machines) or larval/pupal stages (aquatic larvicides, oils, solvents or even polystyrene beads), despite emergent problems with insecticide resistance. Mosquito breeding sites may be eliminated through improved hygiene and sanitation to reduce standing or polluted water bodies. Protection against mosquito bite has been found to be most effective using repellents (sprays, lotions), physical barriers (window screens, impregnated bed nets or long-sleeved clothing) and by curbing outdoor activities during peak mosquito feeding times. Public education campaigns, coupled with mass chemotherapy, has helped reduce the incidence of lymphatic filariasis in many countries.

Wuchereria

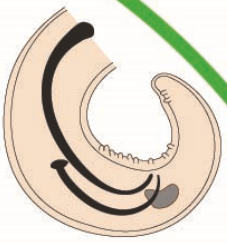


lymphatics
(inflammation,
granulomas,
lymphoedema,
elephantiasis)

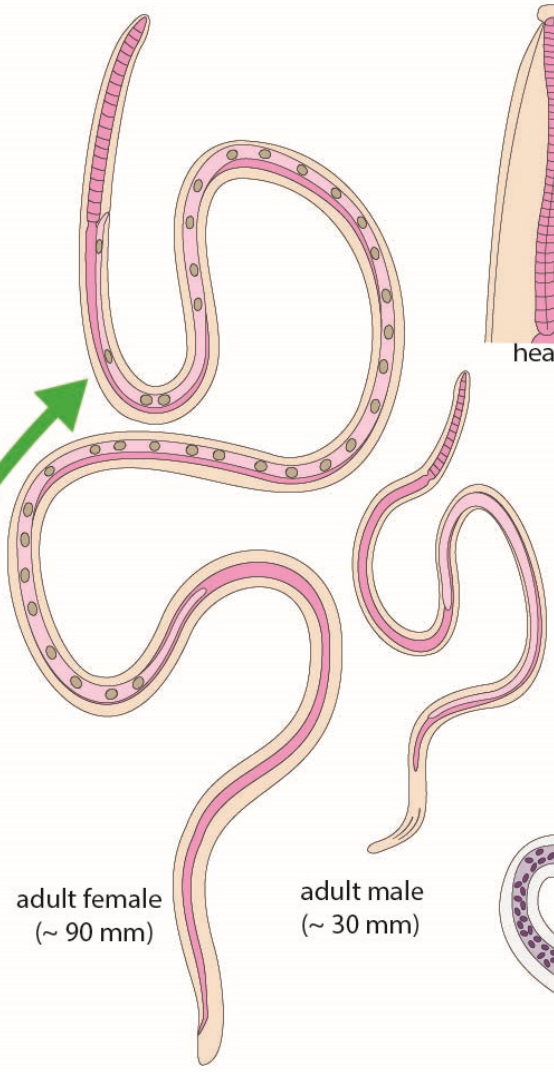


head

Definitive Hosts
(primates)

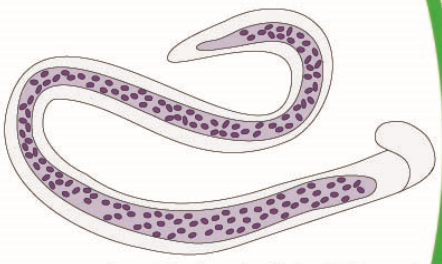


male tail (lateral)



adult female
(~ 90 mm)

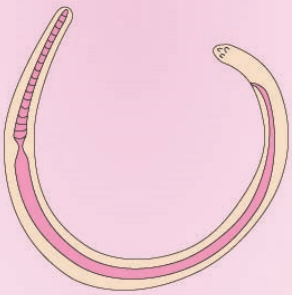
adult male
(~ 30 mm)



microfilariae (mf) (~ 250 µm)
(released into blood)

L3
deposited
on skin

mf
ingested

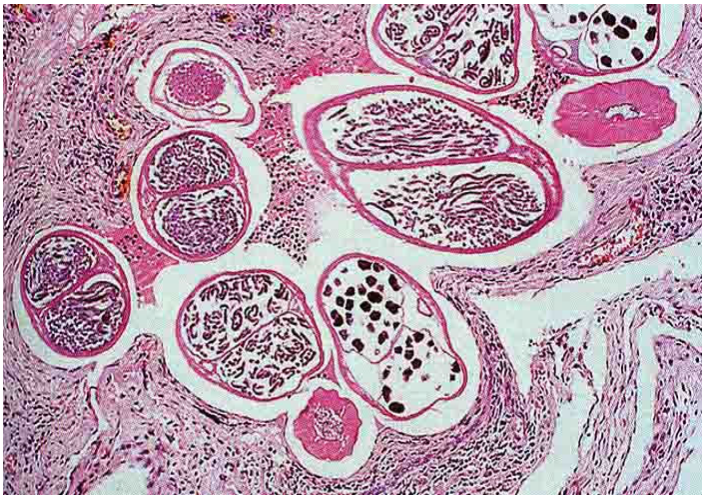


third-stage larvae
(L3) (~ 1.5 mm)

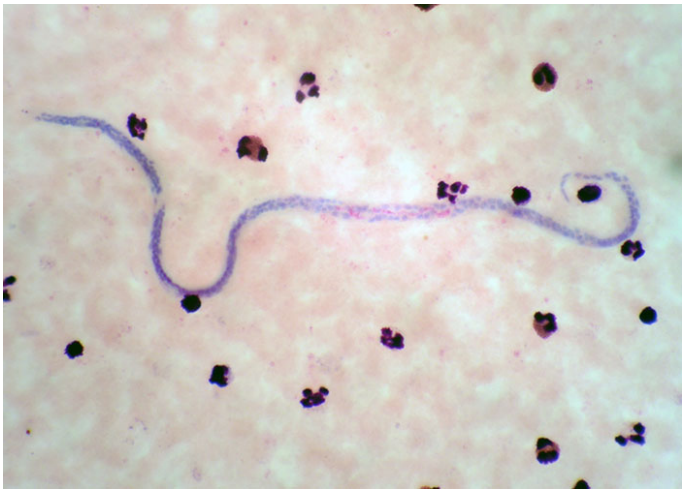
Intermediate Hosts (IH)
(culicid mosquitoes)
(muscles, then mouthparts)

vector-borne transmission

Wuchereria adult worms



Wuchereria sections of worms in lymph node



Wuchereria microfilaria