Brugia
(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 *Brugia* are transmitted by mosquitoes and cause lymphatic (Malayan/Timorian) 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:	*Brugia* (parasitic in lymphatics of humans/cats, mosquito IH)
Species:	various species cause lymphatic Malayan/Timorian filariasis 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						
Brugia (10 spp.)	primates, carnivores, rodents	1-9 cm (lymphatics)	170-380 µm sheathed (blood)	nocturnal, subperiodic	mosquitoes	present
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
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).

The genus *Brugia* is characterised by the formation of elongate thread-like adult worms with transversely striated cuticles and round mouths bearing 2 circles of papillae. Female *Brugia* worms 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 (nocturnally periodic or subperiodic) attuned to differences in oxygen tension between venous and arterial blood during the sleeping/waking habits of the host, and thus their availability to day- or night-biting mosquito species. Some 10 *Brugia* spp. have been described in the lymphatic systems of a small range of mammals (primates, carnivores and lagomorphs) mainly in South-East Asia, but also in the Americas. In particular, the species *B. timori* causes Timorian filariasis in humans in Indonesia, and *B. malayi* causes Malayan filariasis in humans throughout Asia (South-East Asia, Philippines, Sri Lanka, India, East Indies, China, Japan and Korea). Recent molecular studies have identified 2 major *Brugia* clades: *malayi-timori-buckleyi* (parasites of primates and lagomorphs in Asia) and *pahangi-beaveri* (parasites of carnivores in Asia and America).

***Brugia* species**	**Definitive Hosts (DH)**	**Location**	**Vectors/Intermediate Hosts (IH)**	**Distribution**
Subgenus *Brugia*				
B. beaveri (syn. *Onchocerca*)	Carnivora: felid (cat, bobcat), mustelid (American mink), procyonid (raccoon); Rodentia: murid (Mongolian gerbil); Primates: hominid (human)	lymph nodes, subcutis	Diptera: culicid (*Aedes aegypti*)	North America
B. ceylonensis	Carnivora: canid (dog), felid (cat); Primates: hominid (human)	lymph nodes	Diptera: culicid (*Aedes aegypti, Anopheles stephensi, Mansonoides annulifera, uniformis*)	Sri Lanka
B. guyanensis	Carnivora: procyonid (South American coati); Primates: hominid (human)			South America
B. lepori	Lagomorpha: leporid (eastern cottontail, swamp rabbit)	abdominal lymphatics, subcutis		North America
B. malayi (incl 2 strains differing in periodicity) (Malayan filariasis, elephantiasis)	Carnivora: canid (dog), felid (cat, flat-headed cat, leopard cat), mustelid (European polecat), viverrid (small-toothed palm civet, Asian palm civet, Malayan civet); Rodentia: murid (jird, southern multimammate mouse, Natal multimammate mouse, Mongolian gerbil); Pholidota: manid (Sunda pangolin); Primates: callitrichid (common marmoset), lorisid (Sunda slow loris), cercopithecid (crab-eating macaque, rhesus macaque, silvered leaf monkey, gray langur), hominid (human)	lymph nodes and vessels, mf in blood (sheathed, nocturnally periodic or subperiodic)	Diptera: culicid (*Aedes aegypti, kiangensis, kwaiangensis, togoi, Anopheles anthropophagus, barbirirostris, barbirostris, campestris, donaldi, kweiyangensis, lesteri, nigerrimus, quadrimaculatus, sinensis, Armigeres subalbatus, Coquillettidia crassipes, Culex erythrothorax, quinquefasciatus, tarsalis, tritaeniorhynchus, Mansonia annulata, annulifera, bonneae, dives, indiana, longipalpis, uniformis, Ochlerotatus oceanicus, togoi*), Hemiptera: cimicid (*Cimex hemipterus, C. lectularius*)	South and East Asia (esp. Malaya/Timor)
B. pahangi	Carnivora: canid (dog), felid (cat, leopard cat, flat-headed cat, wild cat, lion, tiger), viverrid (Asian palm civet, small-toothed palm civet, large Indian	lymph nodes and vessels (sometimes heart, lungs,	Diptera: culicid (*Aedes aegypti, Anopheles barbirirostris, crucians, dirus, gambiae, quadrimaculatus, stephensi,*	South-East Asia

	civet, binturong), mustelid (European polecat); Rodentia: murid (brown rat, moon rat, wood rat, mouse, jird, Natal multimammate mouse, Mongolian gerbil), sciurid (black giant squirrel), cricetid (golden hamster); Eulipotyphla: erinaceid (moonrat); Pholidota: manid (Sunda pangolin); Primates: callitrichid (common marmoset, white-lipped tamarin), lorisid (Sunda slow loris), cercopithecid (dusky leaf monkey), hominid (human)	testes), microfilariae in blood	*Armigeres obturbans, subalbatus, Culex pipiens, quinquefasciatus, Mansonia annulatus, longipalpis, uniformis, Psorophora confinnis*)	
B. patei (syn. *Wuchereria*)	Carnivora: felid (cat), viverrid (genet), canid (dog)	lymphatics, microfilariae in blood (sheathed)	Diptera: culicid (*Aedes pembaensis, togoi, Anopheles gambiae, Mansonia africanus, uniformis*)	Africa
B. timori (Timorian filariasis, elephantiasis)	Primates: hominid (human), cercopithecid (silvery lutung); Rodentia: murid (Mongolian gerbil); Carnivora: felid (cat)	lymph nodes and vessels, microfilariae in blood (nocturnally periodic)	Diptera: culicid (*Aedes togoi, Anopheles barbirostris*)	Indonesia
B. tupaiae	Scandentia: tupaiid (common tree shrew, large tree shrew)	lymphatics	Diptera: culicid (*Aedes aegypti, albopictus, togoi, Armigeres subalbatus*)	South-East Asia
Subgenus *Brugiella*				
B. buckleyi	Lagomorpha: leporid (Indian hare); Carnivora: mustelid (Asian small-clawed otter)	heart and blood vessels		Sri Lanka

Parasite morphology: The filarial nematode *Brugia* forms 3 different stages in its developmental cycle: adult worms, pre-larvae (microfilariae) and several larval stages (L1-4). Microfilariae are elongate and variable in size depending on species, ranging from 170-380 µm in length by 3-7 µm in width (*B. timori* 265-380 µm long, *B. tupaiae* 283-322 µm, *B. beaveri* 200-325 µm, *B. ceylonensis* 220-275 µm, *B. pahangi* 186-260 µm, *B. malayi* 175-230 µm). They are often coiled or dorsally flexed and are enclosed by a delicate sheath (eggshell membrane) that is usually smooth and translucent, but sometimes granular (e.g. *B. pahangi*). These pre-larval stages have striated cuticles, rounded heads and tapering pointed tails, with a small rounded mouth, pharyngeal thread, cephalic space (sometimes with spines and tooth e.g. *B. malayi*), band-like nerve ring, sac-like innenkorper, excretory cell and pore, and nuclear column (genital primordia)). The nuclear column is tightly packed with nuclei extending to the tip of the tail (not in a continuous row) and 2 conspicuous terminal nuclei isolated at the tip (unlike *Wuchereria* microfilariae which lack terminal nuclei). Different species exhibit variations in the sizes and locations of microfilarial internal structures, especially in the cephalic space (larger in *B. timori*) and innenkorper (longer in *B. pahangi*). Microfilariae exsheath and develop into short sausage-shaped first-stage larvae (L1) measuring 140-150 x 15-20 µm in which the nerve rings and innenkorper become obscure. They moult into elongate second-stage larvae (L2) measuring 400-450 x 25-30 µm with bluntly rounded heads and posterior ventrally-curved spikes. These in turn moult into third stage larvae (L3) measuring 1.0-1.9 mm x 22-30 µm with 2 anterior circles of 4 cephalic papillae, transversely striated cuticles, a glandular oesophagus, and tails with 3 rounded papillae (less prominent in *Brugia* than *Wuchereria*). Adult worms are white, delicate and thread-like in shape ranging from 10-90 mm in length (but usually shorter than *Wuchereria*). They are covered by a smooth, transversely striated cuticle, have a bulbous head with well-defined papillae, long cephalic spaces, a buccal ring, an oesophagus clearly divided into 2 parts (thin anterior muscular, thicker posterior glandular) and the posterior end is ventrally curved. They have longitudinal muscles and move with a conspicuous threshing (S-shaped) motion. Mature worms are sexually dimorphic and live coiled up in pairs, with females being larger than males (40-90 mm x 130-300 µm cf. 10-30 mm x 70-80 µm). The exception is *B. buckleyi* which grows to almost twice that length. Males have a gubernaculum and 2 dissimilar unequal copulatory spicules (left spicule thinner with shorter distal section) and they have ~ 11 caudal papillae (fewer than *Wuchereria*). Female worms have long reproductive organs with the ovary located posteriorly and connected through oviducts and uteri to the vulva located anteriorly (but posterior to the nerve ring). Gravid females are ovoviviparous, producing embryonated eggs which hatch internally releasing microfilariae.

Site of infection: Adult *Brugia* worms live coiled together in pairs in the lymphatic vessels, and sometimes the lymph nodes, of their mammalian (definitive) hosts, typically in the extremities of the legs and arms. The exception is *B. buckleyi* which infects in the heart and coronary circulation. Microfilariae are released into the lymph and make their way to the blood stream via the thoracic duct. They occur in the peripheral circulation at peak concentrations mostly at night (exhibiting nocturnal periodicity or

subperiodicity). Larval stages develop in the thoracic muscles of their mosquito (intermediate) hosts and infective L3 migrate through the haemocoel to the proboscis.

Pathogenesis: *Brugia* infections in domestic and wild animals are usually inapparent and worms are mostly detected serendipitously at post-mortem. Two-thirds of infections in humans are asymptomatic or subclinical, nonetheless, pathological changes still occur due to the combined effects of live worms and host responses to dead and dying worms, microfilariae and their bacterial endosymbionts. In particular, persistent inflammation causes progressive damage to lymph vessels which may eventually result in acute or chronic disease, characterised by lymphoedema (tissue swelling) and elephantiasis. *Brugia* spp. cause lymphatic filariasis in humans, often called Brugian filariasis, with regional variants known as Malayan and Timorian filariasis. These conditions are similar to Bancroftian filariasis caused by *Wuchereria bancrofti*, but genital involvement, hydrocoele and chyluria are rare. The incubation period (time from infection to disease presentation) varies considerably as worms take months to mature (3-6 months for *B. malayi* and 6-12 months for *B. timori*) and adult worms are able to evade the host immune system for several years causing little immunopathology. Adult worms cause lymph vessel dilatation which compromises lymph transportation and immune surveillance, thus rendering the host more susceptible to secondary bacterial and fungal infections. When worms die, they release potent immuno-stimulatory ligands not only from the worms themselves but also from their endosymbiotic *Wolbachia* bacteria. This results in significant inflammation of lymph nodes (lymphadenitis) and the lymphatic system (lymphangitis) with immune cell infiltration, fibrosis, endothelial cell hyperplasia, vessel obstruction, lymph retention and lymphoedema (swelling). The earliest sign is often lymph varicose, and lymph (chyle) is released into surrounding tissues if vessels rupture. Chyluria (cloudy urine due to the presence of chyle) may also develop. Acute presentation involves episodic attacks of fever and chills, inflamed lymph nodes and localised swellings. Infections usually progress from asymptomatic microfilaraemia to acute adenolymphangitis (ADL) characterised by fever and lymphoedema associated with lymphadenitis as a result of secondary bacterial infection. Lymphadenitis may involve supraclavicular, axillary, epitrochlear, antecubital, abdominal, pelvic, inguinal and cervical lymph nodes, which become swollen, tender and painful. Lymphangitis may initially appear as a focal oedema but which continues to spread distally, commonly affecting the scrotum and/or limbs. Chronic infections and/or repeated episodes of ADL can lead to marked lymphoedema with elephantiasis evident as gross swelling and thickening of the tissues and overlying skin, particularly of the limbs, scrotum or breasts. Chronic oedema eventually results in irreversible changes to the skin, such as fibrosis, sclerosis, scarring, verrucous dermatitis and cellular infiltration. Elephantiasis caused by *B. malayi* and *B. timori* usually involves the legs below the knees and involvement of the genitals is uncommon. Elephantiasis of the arms below the elbows may sometimes occur in *B. malayi* infections. The lymphatic system normally performs immune surveillance for foreign material, but this function becomes compromised as lymph pools and is no longer or improperly screened. Patients become susceptible to secondary opportunistic infections by bacteria and fungi, which further exacerbates skin lesions. It has been estimated that some 40 million people worldwide develop elephantiasis (~10% due to Brugian filariasis) which typically causes high morbidity but low mortality. While infections may be acquired in childhood, symptoms generally do not appear until adolescence or early adulthood. Patients become increasingly are unable to provide for themselves or their families and may be ostracized by local communities.

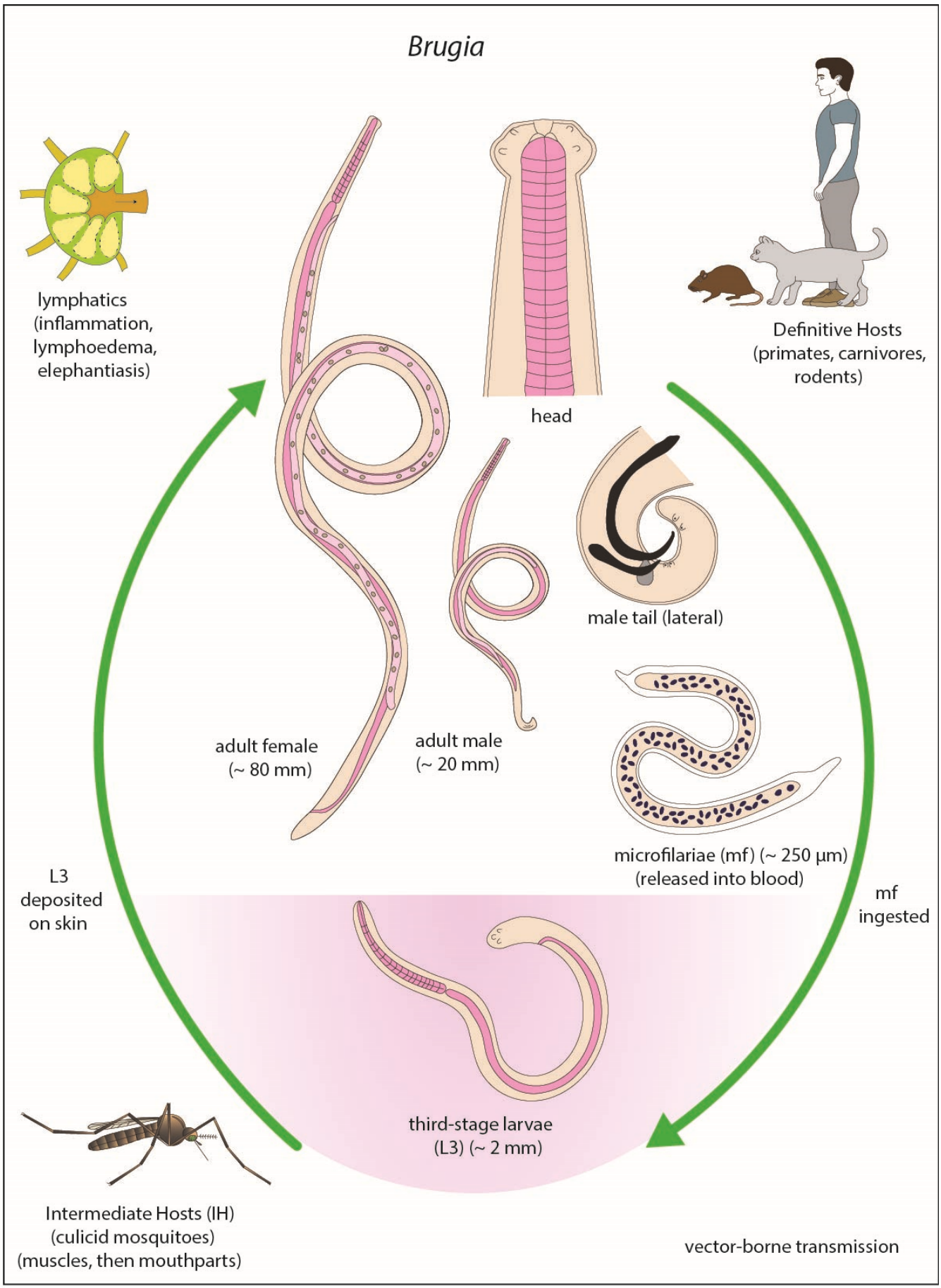
The discovery of unique endosymbiotic rickettsial α-proteobacteria (*Wolbachia*) in many filarial nematodes, including *Brugia* spp., presents a conundrum for disease progression and management. The bacteria are present in all worm developmental stages and they are passed from mother to offspring. They are considered to be mutualistic symbiotes essential for worm development and longevity. However, when worms die, the bacterial symbionts break down releasing surface proteins that have been shown to trigger innate immune responses and Th1 pro-inflammatory responses. The bacteria therefore contribute to disease development. This presents a problem for treatment as chemotherapy should include both anthelmintics to kill worms and antibiotics to kill bacteria, but avoiding heroic doses so the host is not overwhelmed by inflammatory molecules.

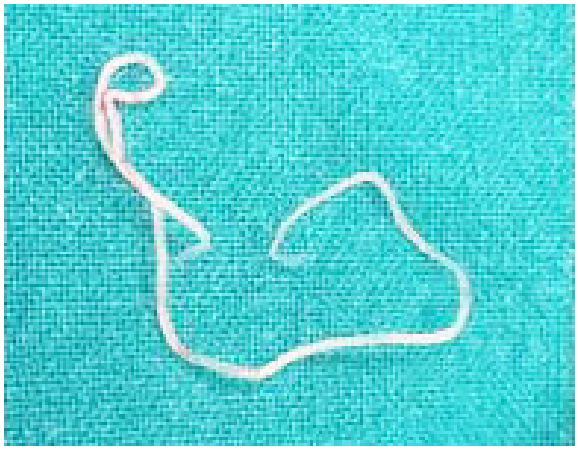
Developmental cycle and mode of transmission: Like other filarial worms, *Brugia* spp. have indirect heteroxenous life-cycles involving sequential development of adult stages in mammalian (definitive) hosts and larval stages in haematophagous mosquito vectors (intermediate hosts). Gravid female worms produce thousands of sheathed microfilariae which make their way from the lymph to the bloodstream via the thoracic duct. The concentration of microfilariae in the peripheral circulation may exhibit circadian (daily) variation with a periodicity attuned to the wake/sleep cycles of their mammalian hosts and the feeding behaviours of their mosquito vectors. *B. timori* is nocturnally periodic (microfilariae at highest concentration between 10 pm and 2 am) and is transmitted by night-biting mosquitoes (*Aedes, Anopheles*) breeding in moist areas of Indonesia. Two strains of *B. malayi* are recognized: a nocturnally periodic strain (microfilariae at highest concentration between 10 pm and 2 am) which is transmitted between humans and domesticated carnivores by mosquitoes (*Aedes*, *Anopheles* and *Mansonia* spp.) that breed in rural areas in open swamps or coastal rice-growing areas throughout Asia and that usually bite during night (anthropophilic vectors are mostly *Anopheles* mosquitoes); and a nocturnally subperiodic strain (microfilaraemic all the time but with higher numbers between noon and 8 pm) transmitted between humans and forest-dwelling animals by zoophilic vectors (*Mansonia* mosquitoes) breeding in swamp-forest areas in Malaysia, Indonesia and Philippines and biting mostly by night but sometimes in the shade by day. Other *Brugia* spp. are mosquito-borne but little is known about the periodicity of their microfilaraemias. Ingested microfilariae exsheath in the mosquito gut and migrate to the thoracic (flight) muscles, and sometimes the fat bodies, where they moult to second-stage larvae (L2) in 4-5 days and then third-stage larvae (L3) in 6-8 days. Infective L3 larvae move through the body cavity (haemocoel) to the proboscis by 9-21 days. When mosquitoes feed on mammalian blood, the infective larvae are released onto the skin and enter through the bite site or other adjacent wounds. This contaminative (rather than inoculative) form of transmission is considered to be

relatively inefficient, and it has been estimated that hundreds of mosquito bites are required to establish infection. Infective larvae migrate to the lymphatic system where they moult to fourth-stage larvae (L4) in 6-9 days and then to immature adults (L5) in 18-40 days. Sexually mature adult worms develop over 2-3 months in animals but 3-12 months in humans. The prepatent period (time from infection to first release of microfilariae) ranges from 55-120 days, and gravid females may produce thousands of microfilariae per day and live for up to 5-15 years.

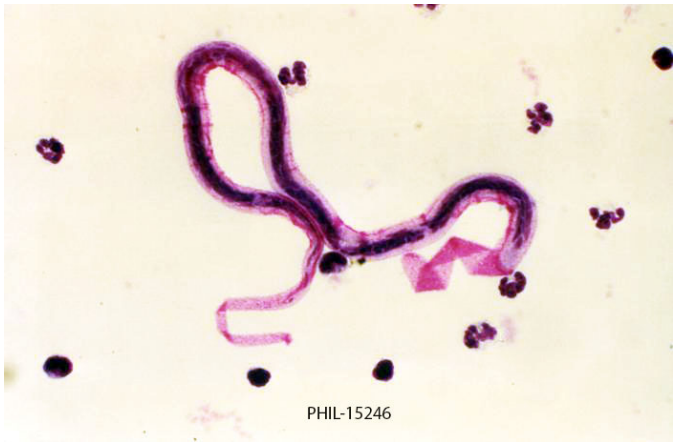
Differential diagnosis: Infections may be suspected on the basis of clinical symptomatology, such as swelling of limbs, but other conditions may present with similar symptoms, such as Milroy disease or Hodgkin lymphoma. It is usual for provisional diagnoses to be confirmed by microscopically examining blood samples for the presence of characteristic microfilariae. Peripheral blood is best collected around 9 pm to midnight to account daily variations due to nocturnal periodicity or subperiodicity. Microfilariae may be observed directly in thick or thin blood smears or following concentration by filtration through polycarbonate membranes (5 μm pore size) or by centrifugation of lysates (formalin, saponin or hypotonic saline lysis). Smears are best stained with Giemsa, Delafield's haematoxylin or Field's rapid stain, while concentrates may be counter-stained with cresyl blue or methylene blue. *Brugia* microfilariae are sheathed, often kinked, have an elongate cephalic space, crowded nuclear column and a pointed tail with 2 terminal nuclei. Despite the ease and versatility of blood examination techniques, they may exhibit low diagnostic sensitivity due to the absence of microfilariae during the long incubation period after infection and the persistence of asymptomatic amicrofilaraemic infections. Modern medical imaging techniques have been applied to the detection of adult worms and pathological changes in host tissues. Ultrasonography may reveal living adult worms performing a signature 'filarial dance' (*Brugia* spp. may be distinguished from *Wuchereria bancrofti* by differences in the amplitudes of worm movements, as the latter are larger in size when fully mature). X-ray examination may reveal dead and calcified worms in tissues. Lymphoscintigraphy (injection of radiolabeled albumin or dextran into tissues) has also been used to examine the extent of lymphatic dilatation, obstruction or other abnormalities in affected limbs. Several immunological tests have been developed to detect host antibodies or parasite antigens in blood samples, involving enzyme immunoassays and immunochromatographic tests, respectively. More recently, molecular biological techniques have been used to detect parasite DNA in samples from mammals and mosquitoes by polymerase chain reaction (PCR) amplification of nuclear genes encoding small subunit ribosomal RNA, internal transcribed spacer regions 1 and 2, trans-spliced leading Exon I, small heat-shock protein, and HhaI repetitive region.

Treatment and control: The drug of choice for the treatment of Brugian filariasis is the anthelmintic diethylenediamine diethylcarbamazine (DEC), which is effective against both adult worms and microfilariae, thus ameliorating disease and curtailing transmission. Adverse side-effects are rare so chemotherapy has been widely employed in mass drug administration programs in endemic areas, sometimes using DEC-fortified table salt. However, caution is advised in some regions as DEC may worsen onchocercal eye disease and can cause serious side-effects in patients with loiasis. Alternatively, the macrocyclic lactone ivermectin and the benzimidazole-methylcarbamate albendazole have been shown to be effective, but less so against microfilariae so they are often used in combination with DEC. Another treatment strategy recently adopted has been to target the endosymbiotic bacteria (*Wolbachia*) inside the worms. The bacteria appear to have a mutualistic symbiotic relationship essential for the growth and development of most filarial worms. Treatment with the antibiotics tetracycline, doxycycline or rifamycin has been associated with reduced survival and fecundity of adult worms, with substantial decreases in the numbers of microfilariae produced. A complicating side-effect of such treatment is that material released from dead and dying bacteria has been shown to contribute to host inflammatory responses and disease development. In patients with significant lymphoedema and elephantiasis, the management of secondary bacterial and fungal infections has proven critical for preventing acute adenolymphangitis (ADL), reducing skin inflammation and generally improving their quality of life. This involves strict hygiene with regular (daily) washing with soap and water and treating all local injuries with antibiotics (topical or systemic). Swelling may be alleviated by keeping limbs elevated, wearing stocking or compression bandages and using proper footwear. Radical surgery may be required in advanced cases to remove excessive granulomatous, fibrous or calcified tissue but, regrettably, surgical options are not always suitable. At present, there are no vaccines available to prevent infections, but various strategies have been adopted in endemic countries to break transmission cycles either by vector population control or preventing mosquito bites. Insecticides sprayed on and around houses may substantially reduce adult mosquito numbers, although there are growing concerns about the emergence of insecticide resistance. Aquatic breeding sites may be treated with chemicals (larvicides, solvents, oils or even polystyrene beads) or eliminated by improved sanitation or non-flooding irrigation in agriculture. A variety of procedures may be adopted to protect humans against mosquito bites, particularly the use of long-life or residual insect repellents (sprays, lotions), physical barriers (impregnated bed nets, window screens), wearing clothing to cover exposed skin and curbing outdoor activities during peak mosquito feeding times. Few options are available, however, for preventing infections in domestic animals and wildlife which may act as reservoirs of infection in rural or wilderness areas. Nonetheless, the incidence of lymphatic filariasis has been substantially reduced in many countries with pervasive public education campaigns coupled with regular mass chemotherapy.

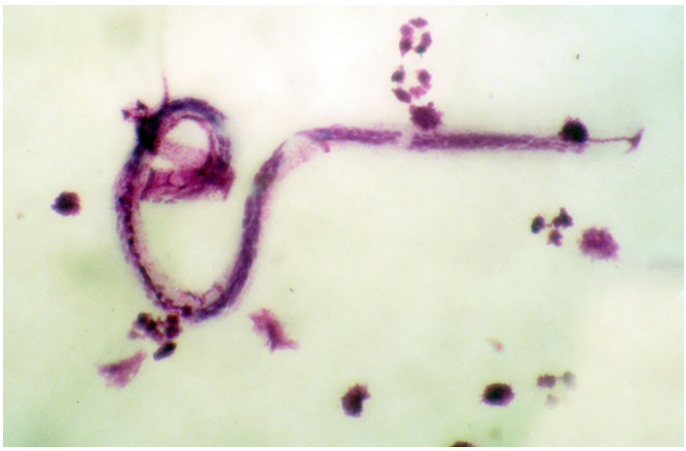

Brugia
lymphatics
(inflammation,
lymphoedema,
elephantiasis)
head
Definitive Hosts
(primates, carnivores,
rodents)
male tail (lateral)
adult female
(~ 80 mm)
adult male
(~ 20 mm)
microfilariae (mf) (~ 250 µm)
(released into blood)
L3
deposited
on skin
mf
ingested
third-stage larvae
(L3) (~ 2 mm)
Intermediate Hosts (IH)
(culicid mosquitoes)
(muscles, then mouthparts)
vector-borne transmission



Brugia adult worm



Brugia microfilaria



Brugia microfilaria