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Nemata

Filarioidea (Superfamily)

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Phylum Nemata

Superfamily Filarioidea

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Chapter 55

Filarioidea (Superfamily)

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Introduction

Filarioid nematodes are parasites of all classes of vertebrates except fish. Most of the species are parasites of wild animals, however some of them parasitize humans and domestic animals, triggering diseases. The majority of these filarioids are included in the family Onchocercidae. They are tissue-dwelling nematodes, with an indirect life cycle including hematophagous arthropods. Adults have been found in almost all tissues of their hosts; however, they prefer a particular location depending on the species. Usually, they are found parasitizing the body cavity, lymphatic vessels, nodules under the skin, or the right ventricle of the heart. They are viviparous, therefore first-stage juveniles (J_1), also known as microfilariae, are ingested by arthropods from the blood or skin of the definitive host. Later, the J_1s develop into J_2s in different organs of the arthropod, such as the Malpighian tubules in mosquitoes or the muscle cells in ticks, until they molt into J_3s, which migrate near the mouthparts of the vector to be transmitted to a new vertebrate host.

The filarioids have developed unique and highly evolved biological features compared with their parasitic spirurid ancestors. Adults are confined to the internal body of their hosts and have adapted their life cycle to transmission with a motile embryo, the microfilaria, which is accessible to hematophagous arthropods. There are 2 groups of filarioids, one of which is included in the family Filariidae, which includes nematodes that produce skin lesions. In this family, females inhabit the subcutaneous tissue and make a hole in the skin to deposit the eggs and/or juveniles which attract the arthropod vector, such as individuals in the family Muscidae. In contrast, nematodes in the family Onchocercidae have evolved to inhabit a more internal position of adult worms in the body of the hosts. Females deposit their embryos in the connective tissue drained by the initial lymphatic vessels, and the vermiform shape of the embryo or microfilaria allows individuals to reach the peripheral cutaneous lymphatic or blood vessels, thus becoming readily accessible to the vector animal. Microfilaria-like juveniles are an evolved character within the Onchocercidae. In this group, vectors may belong to a variety of arthropods, like biting midges, blackflies, fleas, mosquitoes, lice, mites, and ticks, creating lesions or perforating the skin to suck the infected lymph or blood.

Morphology

Compared to other nematodes, the morphology of filarioids is simple. They are long and slender worms, with **sensing structures** at the anterior extremity which are poorly developed. Their length can be variable from 5 mm to more than 50 cm, with males being smaller than females. Males display a posterior region that is coiled or J-shaped. The anterior extremity of filarioids possesses 2 rings of **papillae**; the internal ring with 4 **labial papillae**, and the external ring with 4 **cephalic papillae** usually located around the **oral opening**. Between them there are 2 lateral **amphids** (Figure 1). However, some species have a smaller number of head papillae. The number and arrangement of the papillae are important characters when identifying members of the species. Filarioids display a small **buccal capsule**. The capsule is constituted of 4 segments: An anterior segment that is transparent and corresponds to the **invaginated cuticle**, and 3 **cuticularized segments** that are more or less developed, depending on the genus. The buccal capsule is sometimes absent in some filarioids, such as specimens in the genus *Mansonella* (Figure 2). The stoma rests on the **esophagus**, which is long and occasionally differentiated in an anterior muscular portion and a posterior glandular

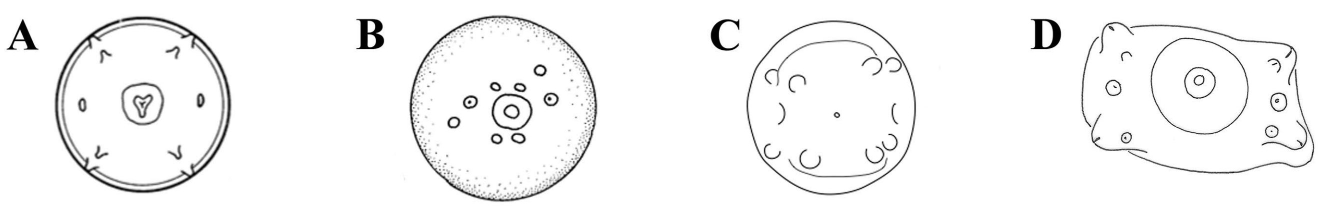

Figure 1. Apical views of some filarioids showing the arrangement of sensitive structures. A) *Brugia beaveri*. B) *Litomosoides odiale*. C) *Mansonela (Tupainema) dunni*. D) *Dipetalonema yatesi*. Sources: A) Adapted from Ash and Little, 1964; B) adapted from Notarnicola and Navone, 2002; C) adapted from Bain et al., 2015; D) adapted from Notarnicola et al., 2007. License: CC BY-NC-SA 4.0.

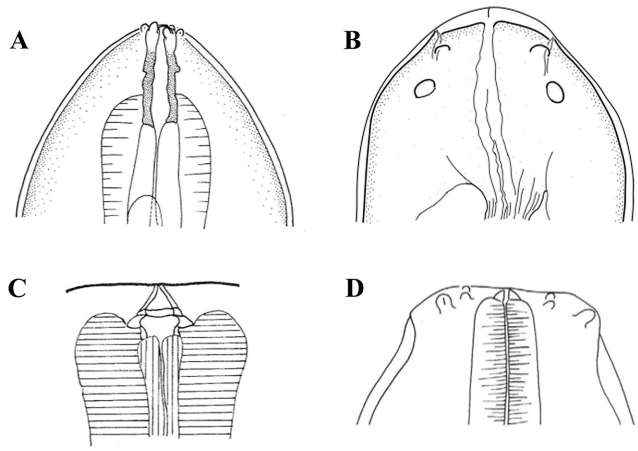

Figure 2. Anterior extremities of some filarioids showing the buccal capsule and the papillae. A) *Litomosoides odilae*, buccal capsule tubular; B) *Mansonella (Mansonella) interstutium*, buccal capsule absent; C) *Litomosa filaria*, buccal capsule constituted by 4 cuticularized segments;D) *Dipetalonema yatesi*, buccal capsule minute. Sources: A) Adapted from Notarnicola and Navone, 2002; B) adapted from Bain et al., 2015; C) adapted from Bain et al., 1966; D) adapted from Notarnicola et al., 2007. License: CC BY-NC-SA 4.0.

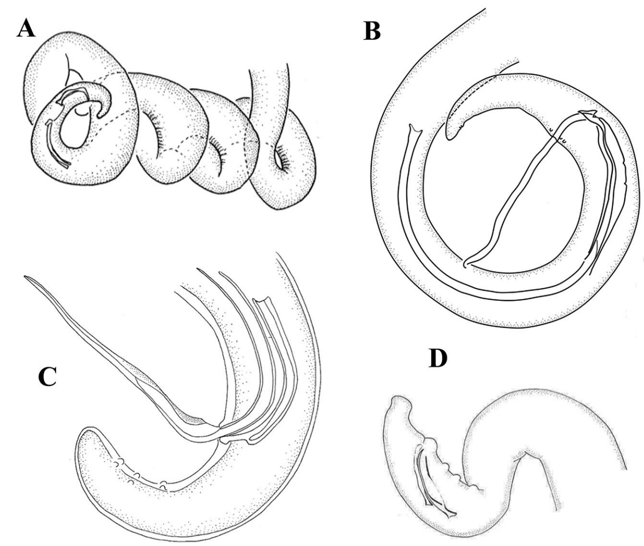

Figure 3. Male posterior ends showing the spicules. A) Posterior extremity of *Litomosoides odilae* showing the coiled region with area rugosa; B) Posterior extremity of *Mansonella (Pseudolitomosa) musasabi* showing the spicules in lateral view. Left spicule longer and different from right; C) Posterior extremity of *Litomosoides salazari* in lateral view; D) *Piratuboides huambensis* posterior region lateral view, with spicules few dissimilar but unequal. Sources: A) Adapted from Notarnicola and Navone, 2002; B) adapted from Bain et al., 2015; C) adapted from Notarnicola et al., 2010; D) adapted from Petit et al., 1983. License: CC BY-NC-SA 4.0.

portion. Neither the esophagus nor the **intestine** present diverticula. The intestine ends in a **cloaca** in males and in an **anus** in females. Filarioids display a **nerve ring** located anteriorly at the level of the esophagus.

The reproductive system in filarioids is amphidelphic. The male has 2 **testes**, 1 which is anterior and usually visible at the level of the esophagus-intestine junction, and the other that is posterior and visible near the **tail**. This continues with a duct which passes posteriorly without convolutions and opens into the cloaca. The cloaca possesses a **spicular pouch** where the **spicules** and the **gubernaculum** lie. The spicules in filarioids are unequal (meaning different in shape), and dissimilar (meaning different in size). The right spicule usually is shorter than the left spicule (Figure 3). The gubernaculum is sometimes present in some Onchocercinae species. Males also have cloacal papillae placed anterior, around, and/or posterior to the cloacal opening. In some species, there is a cuticle structure called the **area rugosa** all along the median ventral line generally extended into the posterior coiled region of the male. The area rugosa is constituted by **transversal ridges** in *Litomosoides* (Figure 4) or *Dipetalonema*, or by tiny **cuticular bosses**, as in *Litomosa* (Figure 5). Both structures, the papillae and the area rugosa, serve to attach the female during copulation (Figure 6). The length and shape of the spicules, as well as the number of cloacal papillae and the presence or absence of the area rugosa, are characters that help in the identification of the different genera and species.

The reproductive system in the females is convoluted. The anterior **ovary** is located near the level of the esophagus-intestine junction and continues backward in an **oviduct** and the **uterus**. In gravid females, eggs can be observed all along the uterus in different stages of development, such as, in the proximal portion, oval **eggs** containing **blastomera**; in the median portion, oval eggs containing the J_1; and in the distal portion, the extended microfilariae. The uterus continues to be situated within a long muscular **ovijector** and the **vagina**, which in some filarioids is a simple muscular tube (such as in specimens of *Ochoterenella* spp.) while in others it is more complex, differentiated into a **vagina vera** and a **vagina uterine**, as in *Dipetalonema* (Figure 7). The vagina is opened to a vulva at the anterior region, generally at the level of the esophagus or just posterior to it.

Microfilariae can be sheathed or unsheathed or not in the egg membrane, respectively. Females release thousands of microfilariae that migrate to the bloodstream, such as in *Dipetalonema*, or to the skin, such as in *Onchocerca*. Microfilariae are slender and fusiform; the anterior end is rounded usually with a hook, and the posterior end is pointed or blunt. Its length varies from 50 µm to more than 400 µm, depending on the species. The **sheath** is tightly applied to the body.

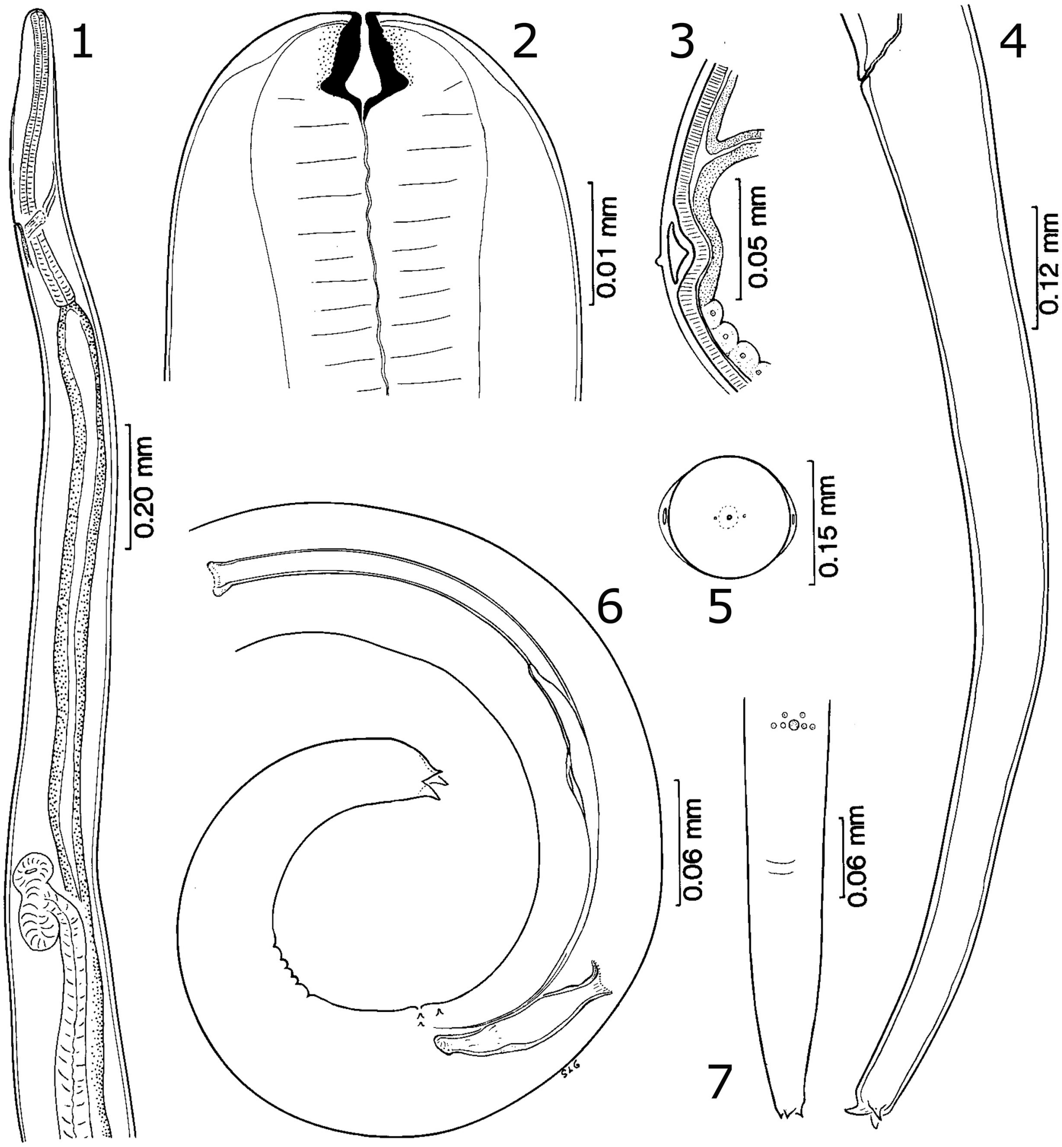

Figure 4. General morphological characters of a filarioid nematode of the genus *Litomosoides* that were obtained from pocket gophers collected in Weld County, Colorado, United States. Plate of *Litomosoides westi*: 1) Anterior end of female showing nerve ring, excretory pore, and vulva; 2) anterior end of female showing degree of development of stoma; 3) cross section of female showing lateral internal cuticular ridge; 4) posterior end of female showing species specific tail with three terminal points; 5) en face view of female; 6) posterior end of male showing coiled aspect, morphologically dissimilar spicules, and small cloacal papillae; 7) posterior end of male showing ventral view. Source: S. L. Gardner, HWML. License: CC BY.

When present, they could be visible at the anterior or posterior ends and appear as a delicate membrane. The internal anatomy of the microfilaria is unique, distinguished by several **internal nuclei** and **primordial organs** (Figure 8). From the anterior to the posterior end, it is possible differentiate the **nerve ring**, the **excretory vesicle** and the **excretory cell**, the inner body composed of few cells, a large stained **G1 cell**, and a row of 3 large, **stained cells** (R2–R4) similar to G1, connected to a clear area called the **anal vesicle** (Figure 8). The function of the G1 cell is unknown, but R2–R4 cells develop in the **rectum** and part of the reproductive system. It has been suggested that the inner body serves as a food reserve (Bain, 1972; McLaren, 1972; Anderson, 2000). The disposition and number of nuclei at the tip of the tail, plus the

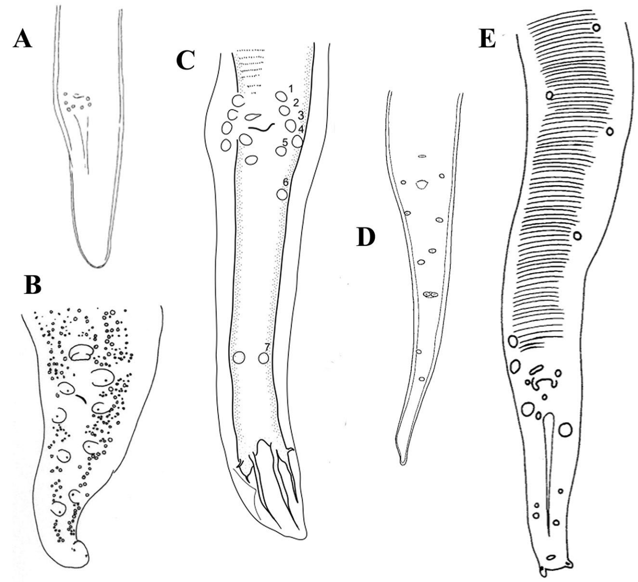

Figure 5. Different arrangements of male cloacal papillae, ventral views. A) *Litomosa goodmani* with a group of pericloacal papillae; B) *Ochoterenella esslingeri* with one precloacal papilla, 4 pairs of large symmetric postcloacal papillae, and ventral ornamentation made of irregular smaller bosses; C) *Mansonella (Mansonella) llewellyni*, precloacal *area rugosa* constituted by a row of small longitudinal ridges, caudal alae present, caudal papillae grouped near the cloaca, phasmids well developed at tip tail; D) *Litomosoides oxymycteri* with an attenuated tail, 1 precloacal papilla, one pair of ad-cloacal, and 4 pairs of asymmetric postcloacal papillae; E) *Orihelia anticlava* presenting precloacal *area rugosa* constituted by a row of small longitudinal ridges, 6 unpaired precloacal papillae, and several asymmetric postcloacal papillae, some larger than others, caudal lappets at tip tail. Sources: A) Adapted from Martin et al., 2006; B) adapted from Souza Lima et al., 2012; C) adapted from Bain et al., 2015; D) adapted from Notarnicola et al., 2000; E) adapted from Notarnicola and Navone, 2003. License: CC BY-NC-SA 4.0.

presence or absence of a sheath, are systematic characters of importance, mostly in species that parasitize humans.

One major characteristic of the Onchocercidae is the periodicity of the microfilariae, which refers to them flooding into the peripheral circulation at certain times of the day or night and disappearing from them at other times. The movement of the microfilariae appears to be associated with physiological changes of the host, as well as with the activity of the vector animals. The other notable characteristic is the longevity of the microfilariae, since they can live circulating in blood for several months after the adults have died. Both features are adaptations of microfilariae that allow them to be transmitted efficiently to the vectors.

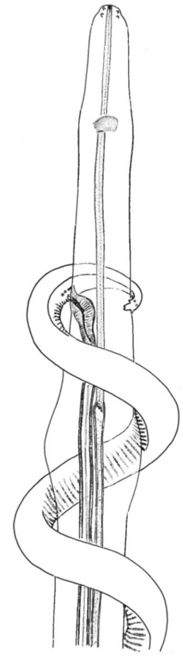

Figure 6. Schema of coupled male and female filarioids. Male cloaca positioned just opposite the female vulva during copulation. The area rugosa and cloacal papillae help for the attachment to female. Source: Adapted from Bain and Chabaud, 1988. License: CC BY-NC-SA 4.0.

Taxonomy of Suborder Filariata

See Anderson (2000) for a good reference to many of the topics following as well as Hodda (2022) for classification also for many life cycles and other important data on treatment and pathology see the web pages of the United States Centers for Disease Control and Prevention (2021).

Family Filariidae

This family includes filarioids parasitizing the subcutaneous tissues of certain mammals. Adults are small to medium-sized, and females possess a vulva located anterior to the nerve ring or near the oral opening, which facilitates the release eggs or juveniles in the skin (Figure 9). Adults and juveniles are located near one another. The family is composed of 2 subfamilies with only 5 genera (Table 1). Filarioids in this family are known to produce diseases clinically characterized by the occurrence of bleeding spots on the surface of the skin, or dermatitis.

Individuals of *Filaria taxideae* (in the subfamily Filariinae), for example, produce inguinal lesions in the skin of American badgers. Females are found in nodules containing embryonated eggs and few first juvenile stages. It is known that females live in the muscle fascia embedded in the dermis of their host and migrate to the epidermis evoking an

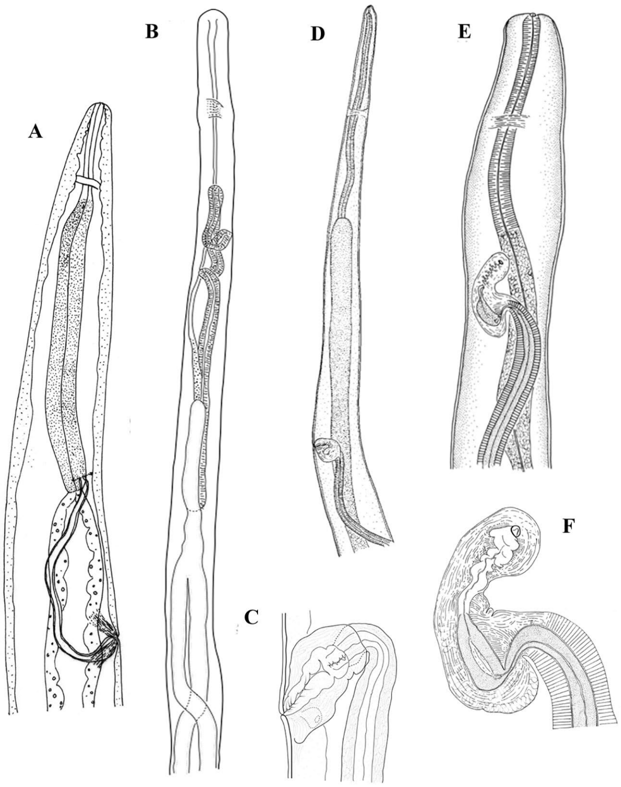

Figure 7. Anterior extremities showing the esophagus, position of the vulva, and shape of the vagina. A) *Ochoterenella esslingeri* possesses an esophagus divided in a short anterior muscular portion and a long posterior portion, the vagina is a simple muscular tube that opens posterior to the esophageal-intestinal junction; B, C) *Mansonella (Mansonella) ozzardi*. B) Anterior end showing a long fibrous esophagus with the vulva at mid-length of the esophagus; C) Vagina uterina simple; D) *Litomosoides oxymycteri* has an esophagus divided and vulva posterior to the esophageal-intestinal junction, vagina globular; E, F) *Dipetalonema robini*. E) Anterior end showing a divided esophagus, vulva at the level of the esophagus near the muscular-glandular division; F) Detail of the vagina, vagina vera conforming a chamber and vagina uterina muscular with a sinuous tube. Sources: A) Adapted from Souza Lima et al., 2012; B, C) adapted from Bain et al., 2015; D) adapted from Notarnicola et al., 2000; E, F) adapted from Vanderhoeven et al., 2017. License: CC BY-NC-SA 4.0.

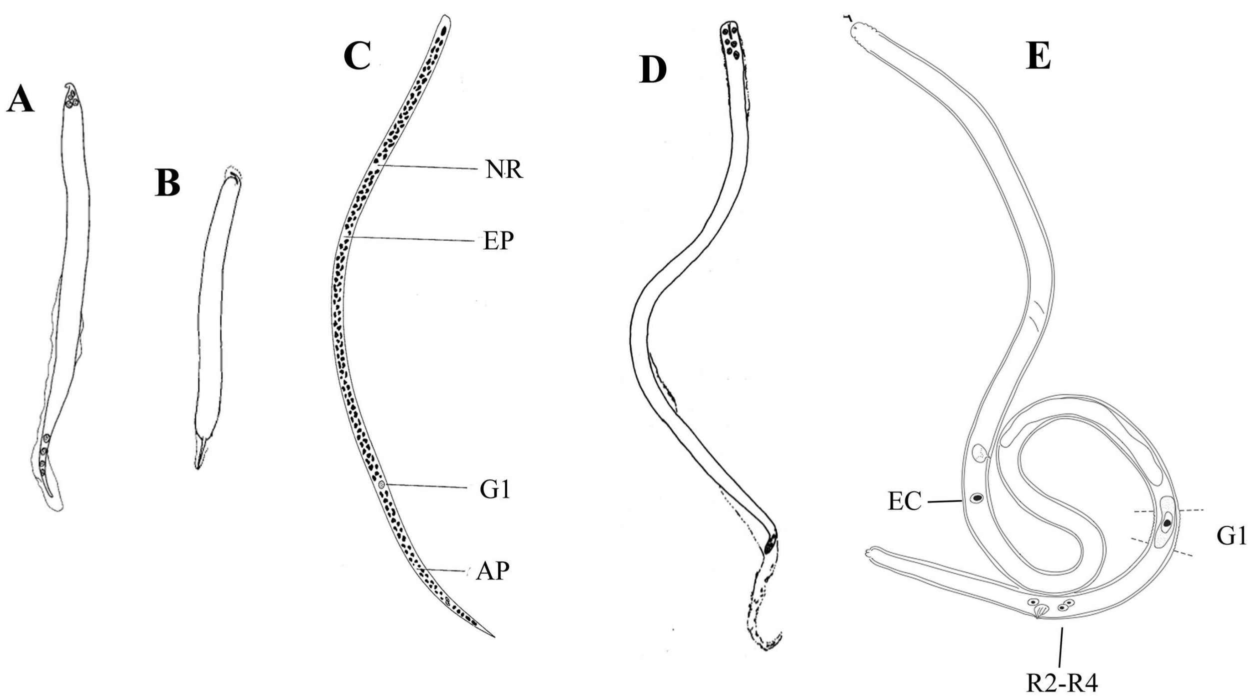

Figure 8. Different shapes of microfilariae. A) Uterine microfilaria of *Litomosoides oxymycteri*, sheath visible at tail; B) Uterine microfilaria of *Litomosoides solari* possessing a tail abruptly attenuated to a sharp point, sheath visible only at tip tail; C) Skin microfilaria of *Onchocerca lienalis*, unsheathed, tip tail with five nuclei aligned in a line; D) Uterine microfilaria of *Ochoterenella esslingeri*, sheathed; E) Blood microfilaria of *Mansonella (Tetrapetalonema) colombiensis*, unsheathed. NR: nerve ring; EP: excretory pore; G1: G1 cell; AP: anal pore; EC: excretory cell; R2-R4: R cells. Sources: A) Adapted from Notarnicola et al., 2000; B) adapted from Guerrero et al., 2002; C) adapted from Eberhard, 1979; D) adapted from Souza Lima et al., 2012; E) adapted from Bain et al., 2015. License: CC BY-NC-SA 4.0.

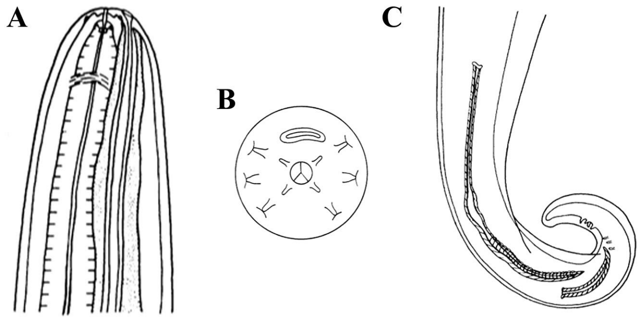

Figure 9. *Filaria taxideae*. A) Female anterior extremity showing the anterior position of the vulva; B) Female apical view, vulva located dorsal to the oral opening, 4 inner labial papillae and 4 external cephalic papillae, 2 lateral amphids; C) Male tail with dissimilar and unequal spicules. Source: Adapted from Keppner, 1969.

inflammatory response due to the presence of both adults and eggs (Keppner, 1971). Similarly, species of *Parafilaria* (also in the subfamily Filariinae) have been described from the subcutaneous tissue on the upper parts of the body of horses, cattle, and water buffalo from Eurasia, Africa, and South America. In contrast to *Filaria taxideae*, females of *Parafilaria* migrate from the dermis and settle with their anterior ends immediately below the epidermis where they release embryonated eggs. The females pierce the skin of the nodule, causing bleeding which attracts the dipteran intermediate hosts (Figure 10). The first stage juvenile (J_1) is unsheathed and is carried in blood flowing from the skin. Second stage juveniles (J_2) are found in the body cavity and the fat body of *Musca* and *Haematobia* fly species, and the third stage juveniles (J_3) are found near the mouthparts (Anderson, 2000). Species of *Stephanofilaria* cause dermatitis, such as *S. stilesi* which causes dermatitis along the ventral midline, between the brisket and navel of cattle. In this species, adults are located in the dermis, just beneath the epidermis. The microfilariae are 50 µm-long and enclosed in a spherical sheath.

The development of the parasites coincides with the activity of the vector, that is, during the spring and summer. During these seasons, flies bite parasitized hosts, which become infected in 10 to 15 days, although the prepatent period for these filarioids is variable. Adults grow in 5 to 7 months post infection, depending on the species, and in 8 to 9 months the scratches characteristic of infection appear on the skin of cattle. The bloody spots are distributed in the hump, at the level of the neck, shoulders, withers, back, and rump, depending on the species. In *Parafilaria bovicola*, a parasite from cattle in Europe and Africa, the lesions tend to bleed when exposed to sunlight (Nevill, 1979).

Table 1. Genera included in the family Filariidae**.**

Subfamily	Genus	Hosts
Stephanofilariinae	*Stephanofilaria*	Parasites of Bovidae
Filariinae	*Filaria*	Parasites of carnivores and rodents
	Suifilaria	Parasites of Suidae
	Parafilaria	Parasites of ruminants and equids
	Pseudofilaria	Parasites of antelope

These worms are not overtly pathogenic; afflicted hosts do not become sick and consequently show no particular clinical symptoms. However, lacerated skin can become infected secondarily with bacteria, fly juveniles, and other pathogens. Moreover, it is known that symbiotic cleaning birds, like oxpecker or cattle tyrant, are also attracted by the spots due to the presence of insects and ticks, making the spots larger.

Several countries in Europe and Asia are endemic for these filarioids. Although the disease is not lethal, the nodules are painful and irritating, and slaughtered carcasses containing the worm are downgraded during inspection. From the point of view of conservation, these parasitoses are important. For example, in the 1960s, and more recently in 2012, there was a filariosis outbreak associated with *Stephanofilaria dinniki* (in the subfamily Stephanofilarinae) in threatened species of white and black rhinoceroses in Meru National Park in Kenya (Round, 1964; Mutinda et al., 2012) (Figure 10).

The therapy recommended for these parasitoses against the adult worms is ivermectin in different doses according to the host (such as cattle or horses), as well as high doses of levamisole and fenbendazole. It is also recommended to control flies and ticks to reduce the entry points of infective juveniles.

Family Onchocercidae

The Onchocercidae includes a diverse group of nematodes with more than 80 genera split among 8 subfamilies. The adult worms are small- to medium-sized. Females possess a vulva situated in the anterior region at the level of the esophagus, although occasionally may be found in the equatorial region. Males have a posterior extremity coiled with or without caudal alae. Microfilariae inhabit the skin, lymph, or blood. Unlike the Filaridae, adults live far away from the juvenile stages, inhabiting the body cavity, heart, skin, muscles, eyes, lymphatic system, and other regions of the host's body.

One of the 8 subfamilies, the Oswaldofilariinae, is confined to reptiles, another 2, the Waltoneliinae and Icosieliinae, to amphibians, while the Splendidofilariinae and the Lemdaniinae are parasites of reptiles, birds, and mammals. The Setariinae are confined to large mammals. Due to their great

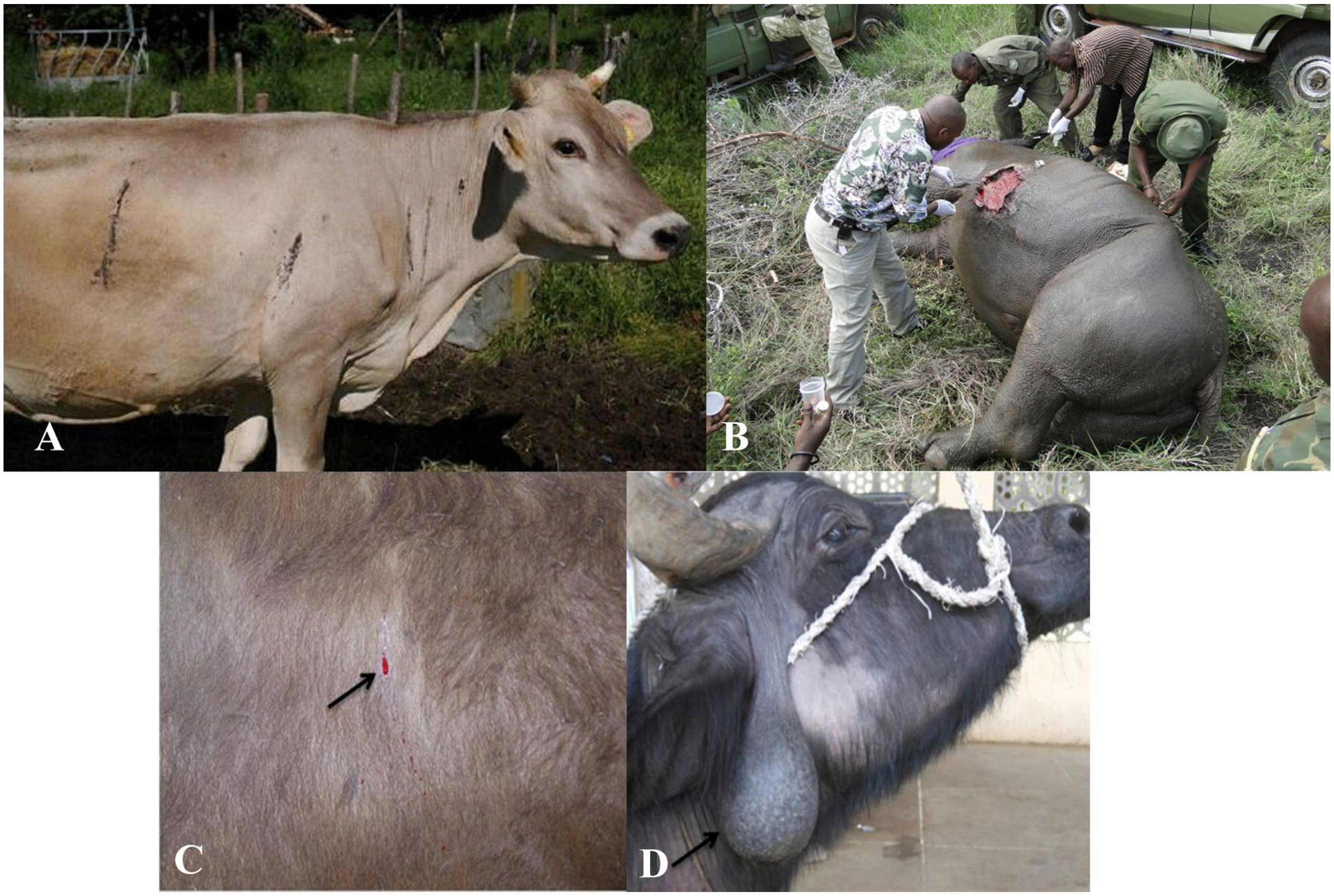

Figure 10. A) Adult female Buffalo from India with a growth below right ear with multifocal bleeding points over the skin; B) Bleeding spot in adult female Buffalo from India caused by *Parafilaria bovicola*; C) Bleeding spot from rhinoceros in Kenya caused by *Stephanofilaria dinniki*. Sources: A, B) Chandratre et al., 2017; B) Mutinda et al 2012. Licenses: A, B) CC BY-NC-SA; C) CC BY 4.0.

diversity and the numerous diseases they cause, the most important subfamilies are the Onchocercinae and Dirofilariinae. Both subfamilies are mainly parasites of mammals, although a few genera occur in birds and reptiles.

Subfamily Oswaldofilariinae

This subfamily includes filarioids that parasitize lacertilians and crocodiles. The location of adults is variable, being found in the connective tissue, heart, aorta, mesentery, intestinal wall, or body cavity. They are transmitted by mosquitoes. Members of the Oswaldofilariinae are distinguished by a vulva located in the middle or posterior region of the body, an esophagus that is well developed and divided, no caudal alae, sometimes large caudal papillae forming a subterminal group, and spicules that are often stout, unequal, and dissimilar from one another (Figure 11).

The subfamily is composed of 7 genera all parasitizing lizards, with the exception of *Oswaldofilaria*, which has 3 species in crocodiles. *Oswaldofilaria* is the most diverse genus with 13 species distributed in Australia, Africa, and South America (notably, a Gondwanian distribution). *Befilaria* comprises 3 species, 1 in the Neotropical region and 2 in the Ethiopian region; *Piratuboides* is present in South America and Australia only, with 1 and 2 species, respectively. The remaining genera have a restricted distribution. *Piratuba* includes 7 species in the Neotropics, *Conispiculum* includes 2 species, *Gonofilaria* has only 1 species occurring in India, and a single species in *Solafilaria* is found in lizards from Madagascar.

As mentioned above, it has been demonstrated that these filarioids are non-pathogenic to their hosts. A survey carried out in 110 *Tropidurus torquatus* from Brazil revealed that adult filarioids of *Oswaldofilaria chabaudi* were found 35% in the body cavity and 65% in the muscular aponeuroses of which 58% were found in the thighs and 7% at the base of the tail (Pereira et al., 2010). Microfilariae circulate in the blood. Experimental development in different species of *Oswaldofilaria* involved mosquitoes of the genera *Aedes*, *Culex*, and *Anopheles*. Juvenile stages were found in the adipose tissue or muscles of the dipterans.

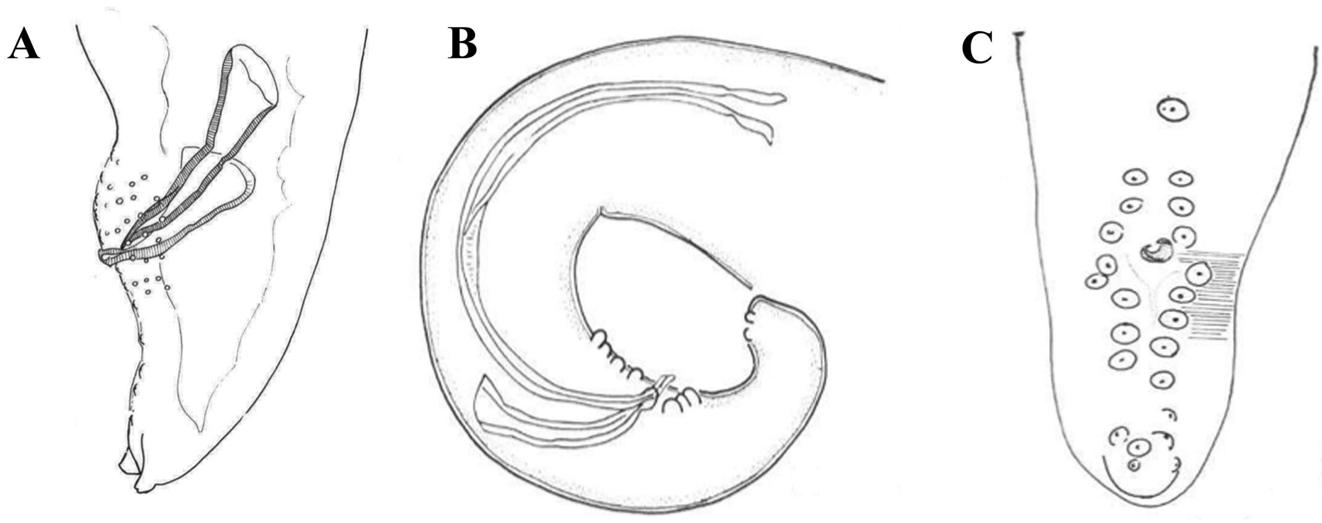

Figure 11. Posterior extremity of males from Oswaldofilariinae. A) *Conispiculum ramachandrani*, lateral view; left spicule slightly longer than right, unequal; numerous minute papillae around the cloaca and lappets at tip tail; B) *Oswaldofilaria versterae*, lateral view, showing pre and postcloacal papillae; left spicule long dissimilar and unequal than right; C) *Befilaria puertoricensis*, ventral view showing 2 lines of cloacal papillae. Sources: A, B) Adapted from Bain et al., 1982; C) adapted from Bain and Chaniotis, 1975. License: CC BY-NC-SA 4.0.

Subfamily Icosielliinae

This subfamily includes a single genus, *Icosiella*, including 9 species parasitizing the subcutaneous aponeurosis of amphibians from Palearctic, Occidental, and Australian realms. Adult worms are short with the posterior end of the body conical and blunt in females and protuberant in males. The buccal capsule is absent, 2 median cephalic spines are present, the esophagus is divided into a short anterior muscular and a long posterior glandular portion, and the anus is subterminal (Figure 12). Nine species were described from subcutaneous tissues from frogs of the family Ranidae, mainly in the genus *Rana*. Vectors in the life cycle of *I. neglecta* were shown to include *Forcipomyia* (biting midges in the family Ceratopogonidae) and *Sycorax* (in the family Psychodidae); these were observed to feed on the head of frogs. Second-stage juveniles (J_2) were detected in the muscles of the flies (Desportes, 1941; 1942).

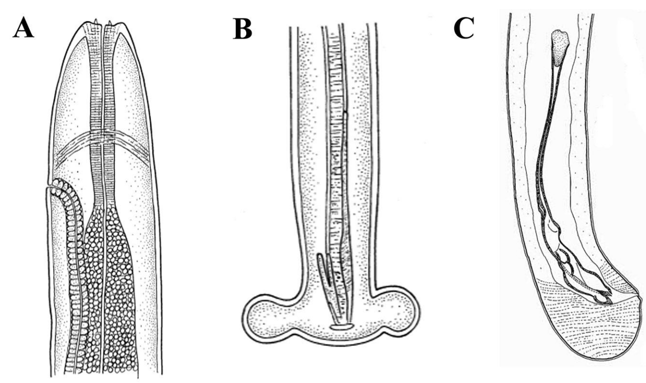

Figure 12. Examples of Icosielliinae species. A, B) *Icosiella turgeocauda*; A) Anterior end of female showing the muscular and glandular esophagus, and the muscular vagina; B) Posterior end of male, ventral view showing two lateral swollen, and dissimilar and unequal spicules; C) Male posterior end of *I. intani,* lateral view showing the spicules. Sources: A, B) Adapted from Bursey et al., 2003; C) adapted from Purnomo and Bangs, 1996. License: CC BY-NC-SA 4.0.

Subfamily Waltonelliinae

Members of this subfamily are parasites from the body cavity and mesentery of frogs and toads included in the families Bufonidae, Leptodactilidae, Racophonidae, and Ranidae. Adults are characterized by the presence of large cephalic papillae, lateral and caudal alae, and thin and dissimilar spicules (Figure 13). They are distinguished from members of the Icosielliinae by their long tail and the absence of cephalic spines.

There are 5 genera: *Waltonella*, *Ochoterenella*, *Madochotera*, *Foleyellides*, and *Paramadochotera*. Currently, *Ochoterenella* is the only genus reported in Central America and South America. The life cycle is only known for some species of *Waltonella*. They are transmitted by mosquitoes of the family Culicidae allowing microfilariae to develop in the body cavity (in *W. brachyoptera*), in the muscles (in *W. ranae*), or in the fat body (in *W. flexicauda*). Second- and third-stage juveniles (J_2 and J_3) may be found in mosquitoes 15 days post-infection and during the prepatent period in frogs in approximately 7 to 8 months.

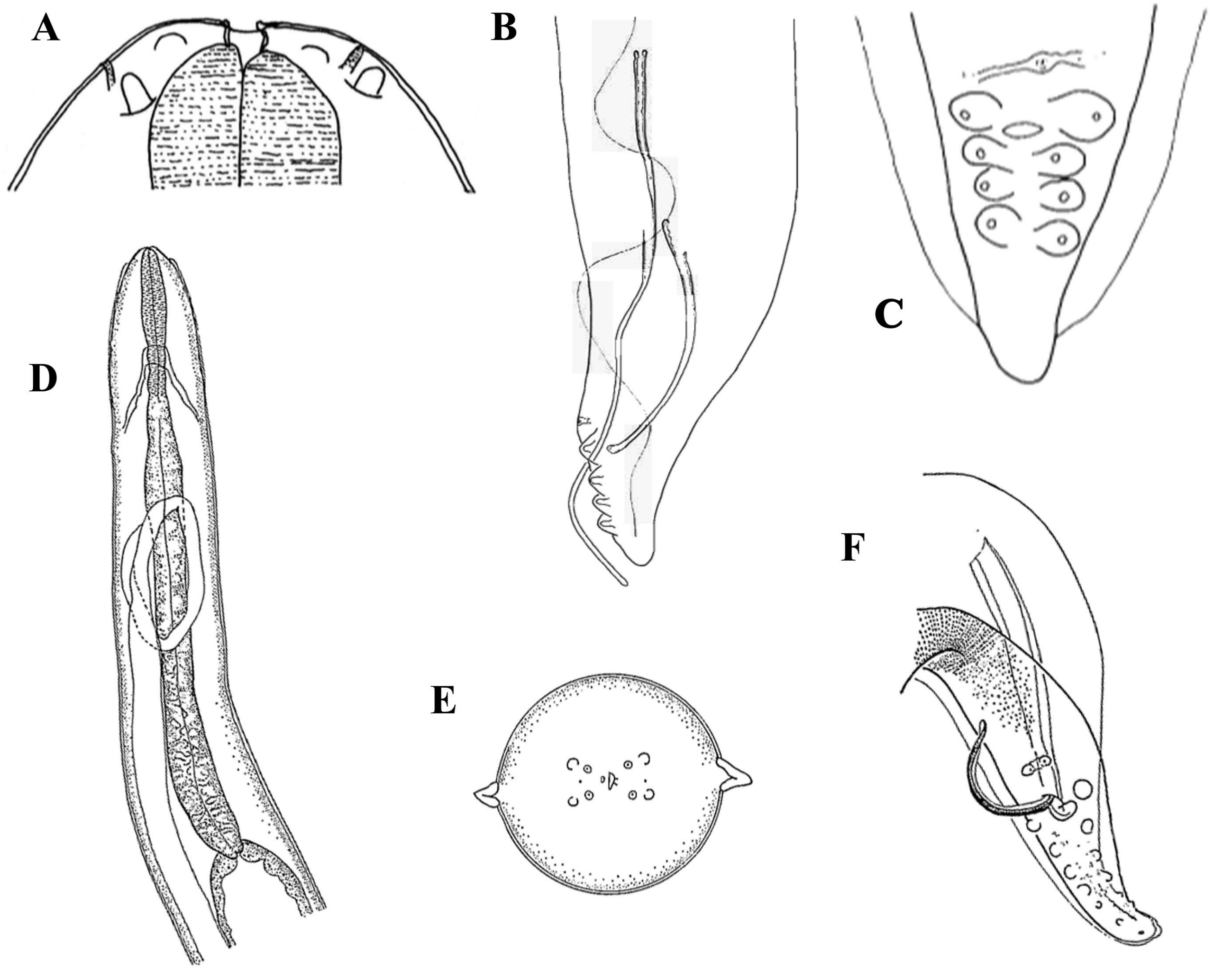

Figure 13. Waltoneliinae species. A) *Ochoterenella esslingeri* anterior extremity showing the buccal capsule and head papillae; B, C) Male posterior end of *Madochotera pichoni*. B. Lateral view showing the spicules and lateral alae; C) Ventral view with large symmetric cloacal papillae; D-F) *Foleyellides striatus*, male; D) Anterior extremity showing the divided esophagus; E) Apical view of the head showing the head papillae and the lateral alae; F) Tail with spicules and caudal papillae. Sources: A) Adapted from Souza Lima et al., 2012; B, C) adapted from Bain and Prod'hon, 1974; D) J. Notarnicola; E) adapted from Esslinger, 1986. License: CC BY-NC-SA 4.0.

Subfamily Setariinae

This subfamily comprises 2 genera: *Setaria* with more than 40 species parasitizing artiodactyls, mainly bovines, hyracoids, and equines; and *Papillosetaria* with only 3 species parasitic in artiodactyls. They are normally found in the abdominal cavity, but also rarely can be found in the eyes, lungs, and skin. Adults are medium- to large-sized, characterized by a complex cephalic structure composed of median or lateral cuticular elevations (spines) and well-developed cephalic papillae. The vulva is near the muscular esophagus, the male tail is rounded without caudal alae, and the spicules are markedly dissimilar from one another (Figure 14). Sheathed microfilariae are 200–231 μ-long; they circulate in the blood until mosquitoes feed on them (*Aedes* spp., *Culex* spp., and *Anopheles* spp.). Microfilariae invade the hemocoel and later the fat body where development takes place. After 12 days, the J_2 is developed and moves again to the hemocoel where it stays for 5 to 12 days more until it reaches the J_3 stage. The infective juveniles are 1.65–2.32 mm-long with numerous tubercules on the tip of the tail. These juveniles then invade a new host and migrate to the final location, the abdominal cavity. The prepatent period varies between 7 and 8 months, and the longevity of adult worms is 1.5 years (Osipov, 1966).

Two species are distributed worldwide: *Setaria equina*, a parasite of the abdominal cavity of horses, and *S. labiatopapillosa* from cattle. *Setaria digitata* parasitizes cattle in Asia. Adults are non-pathogenic, thus, filariasis goes unnoticed unless detected by the presence of microfilariae in blood smears.

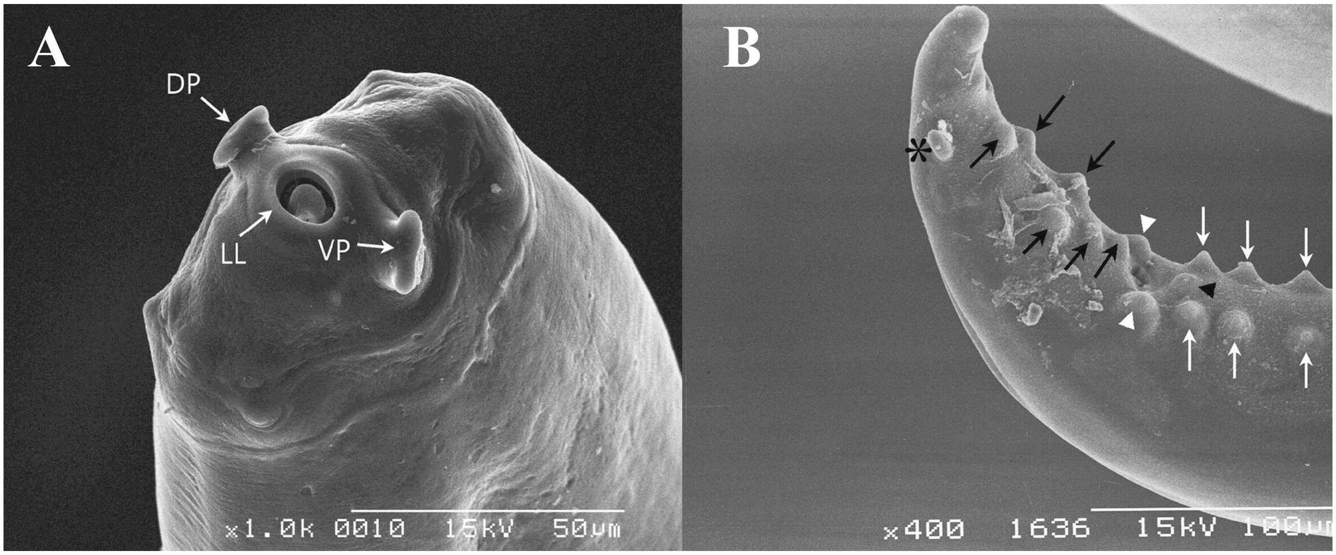

Figure 14. Scanning electron micrograph of *Setaria digitata* found in horse from Korea. A) Anterior end of a male adult showing the anterior structure; DP: Dorsal projection, LL: Lateral lips, VP: Ventral projection; B) Male tail showing the papillae and a pair of lateral appendages near the tip tail (asterisk). Three pairs of precloacal papillae (white arrows), a pair of ad-cloacal papillae (white arrowheads) and 3 pairs of postcloacal papillae (black arrows), plus a central papilla just in front of the cloaca (black arrowhead). Source: Shin et al., 2017. License: CC BY 4.0.

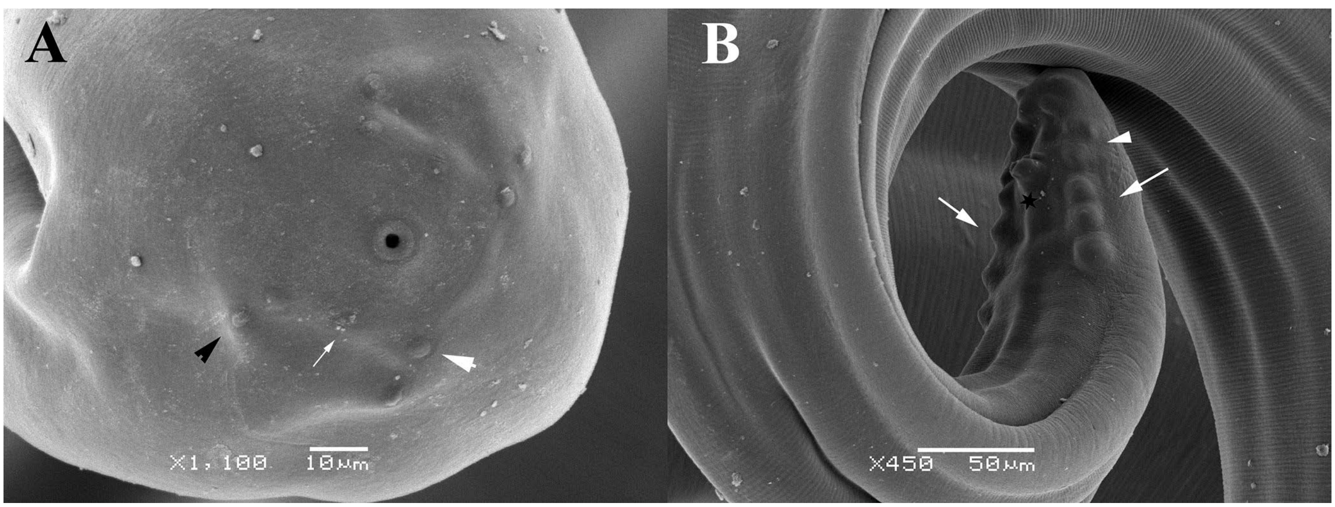

Figure 15. Scanning electron micrograph of *Pelecitus* sp. found in *Arremon flavirostris* (Emberizidae) from Argentina. A) Apical view showing 4 labial papillae (white arrowhead), 4 cephalic papillae (black arrowhead), and amphids (white arrow); B) Posterior extremity of male with protruded cloaca (black asterisk), large precloacal papillae (white arrows), and a group of 2 pairs of postcloacal papillae (white arrowhead). Source: J. Notarnicola. License: CC BY-NC-SA 4.0.

The major pathogenic effect occurs when immature stages migrate erratically in the pleural cavity, central nervous system, urinary bladder, and other organs. *Setaria cervi*, a common parasite of the body cavities from *Alces alces*, *Capreolus* spp., and *Cervus* spp. in Europe and Asia, is frequently found invading the central nervous system with concurrent infections with *Elaphostrongylus cervi* (Metastrongylidae) causing neurological disease (Blažek et al., 1968).

Subfamily Dirofilariinae

Nematodes of this subfamily include males with a short tail and a well-developed caudal alae, which distinguish them from other members of the Onchocercidae. They also have large and pedunculate caudal papillae and spicules that are markedly dissimilar from one another (Figures 15 and 16). Representatives of this subfamily include 1 genus parasitizing reptiles, 1 genus in birds, and 8 in mammals (Table 2)

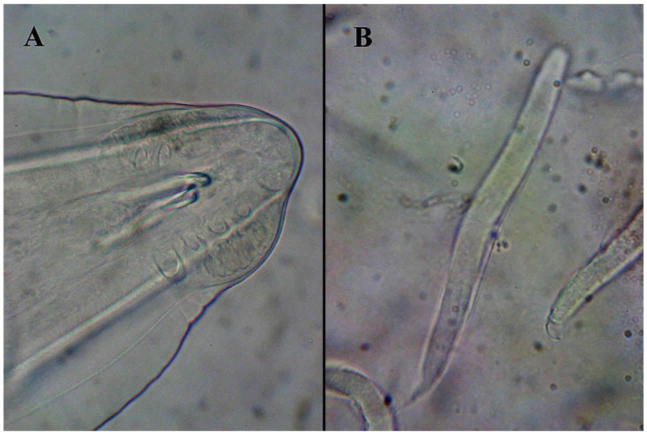

Figure 16. A) Photograph of *Pelecitus* sp. found in *Arremon flavirostris* (Emberizidae) from Argentina, posterior extremity of a male showing symmetrical caudal alae, spicules dissimilar and caudal papillae; B) Photograph of uterine microfilaria of *Pelecitus fulicaeatrae* found in *Podiceps occipitalis* (Podicipediformes) from Argentina. Source: J. Notarnicola. License: CC BY-NC-SA 4.0.

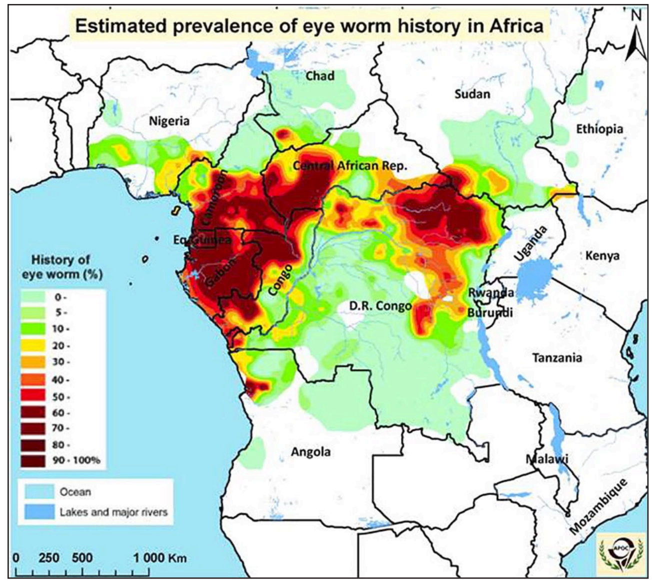

Figure 17. Distribution of *Loa loa* based on prevalence data collected in more than 4,700 villages in 11 African countries. Source: Adapted with data from WHO, 2010, https://www.who.int/apoc/raploa/Africa_EN_map.jpg?ua=1. License: CC BY-NC-SA 4.0.

(Anderson, 2000). Most of the genera are parasites of the subcutaneous tissues or muscles, with the exception of *Edesonfilaria* spp., which is located in the body cavity of arboreal dermopterans, chiropterans, and primates from the Indo-Malaysian region, and the cosmopolitan *Dirofilaria immitis* which parasitizes the right ventricle of the heart and pulmonary artery of carnivorous mammals.

This subfamily includes 2 species that are of epidemiological importance for humans: *Loa loa* and the zoonotic *Dirofilaria immitis*. Adult worms of *L. loa* live in subcutaneous tissues of humans producing edematous swellings on the body known as Calibar swellings or loiasis. Occasionally they migrate through the eyes in the conjunctiva and cornea. The species is endemic of the rainforest of West Africa and equatorial Sudan. Usually, it is diagnosed by the presence of microfilariae in blood smears or adults in the subconjunctiva. It is estimated that between 3 and 13 million people are infected at any one time with filariasis (Klion and Nutman, 2011). Infection is hidden in a large proportion of patients, which are asymptomatic. According to the World Health Organization (WHO, 2022), loiasis (also called African eye worm) is potentially endemic in 11 African countries, recording more than 40% of prevalence in Gabon, Equatorial

Table 2. List of Dirofilariinae genera with their localization and their hosts.

Genus	Localization	Hosts
Bostrichodera	Muscles	Parasites of edentates
Dirofilariaeformia	Pulmonary artery	Parasites of rodents Sciuridae
Edesonfilaria	Body cavity	Parasites of arboreal dermopterans, chiropterans, and primates
Macacanema	Muscles	Parasites of primates
Skjabinodera	Inguinal fascia and renal fat	Parasites of ungulates
Loa	Subcutaneous tissues	Parasites of primates
Foleyela	Subcutaneous and intermuscular connective tissues; body cavity	Parasites of reptiles chameleonids
Pelecitus	Tendons and muscles near leg joints and feet	Parasites of birds and mammals
*Dirofilaria**	Subcutaneous tissue and heart	Parasites of mammals
Loaina	Subcutaneous tissue and muscles	Parasites of lagomorphs

* *Tawila tawila* Khalil 1932 was transferred to *Dirofilaria tawila* by Webber, 1955.

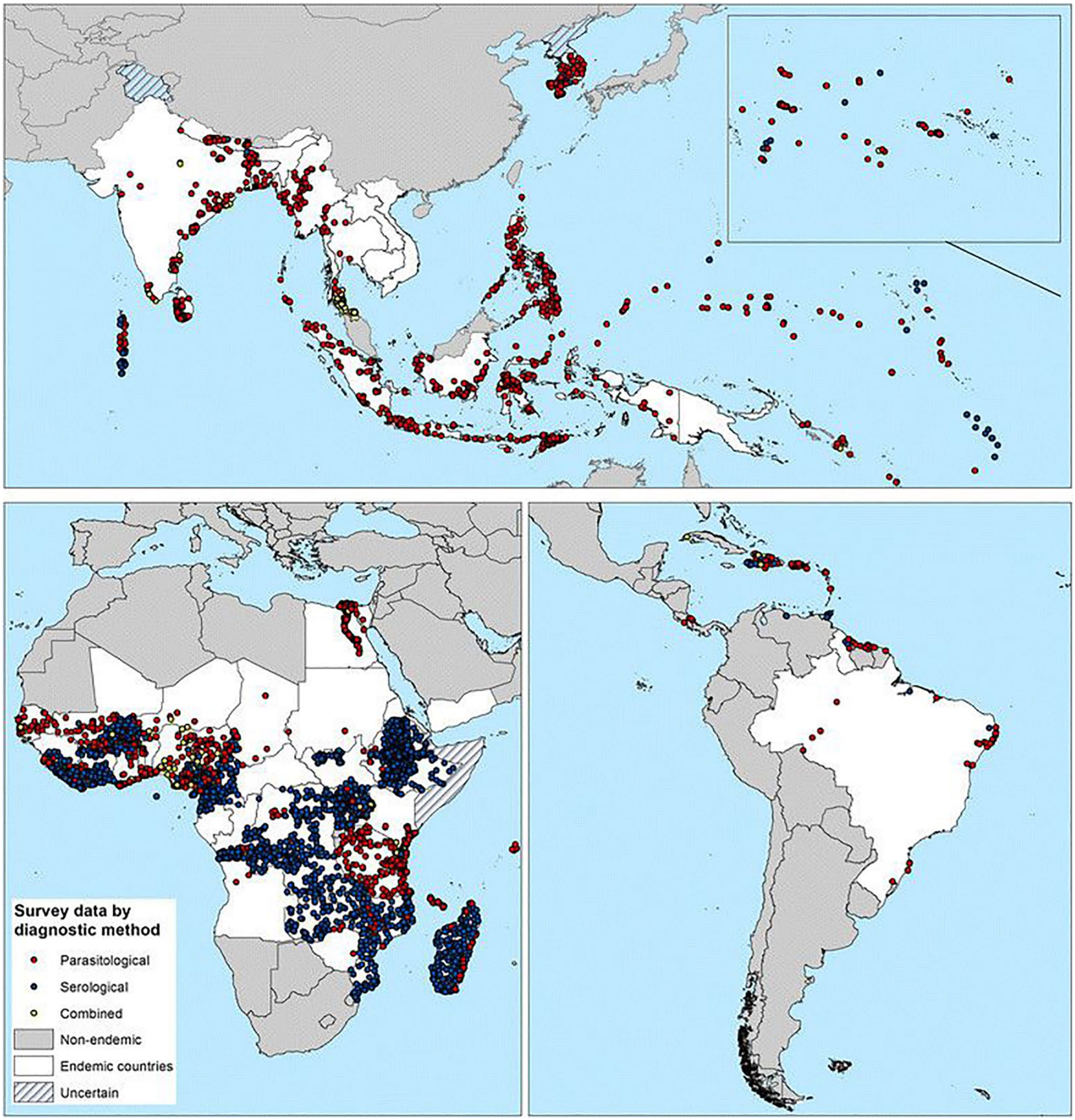

Figure 18. Global atlas of lymphatic filariasis (LF). Global distribution of LF and data points by diagnostic method. Data were identified for 66 of the 72 countries currently endemic and for a further 17 countries where LF is no longer endemic. Red = parasitological methods; blue = serological methods; and yellow = combination of methods. Source: Cano et al., 2014. License: Graphics and text, CC BY 4.0; data, CC0.

Guinea, southern Cameroon, eastern Central African Republic, the Republic the Congo, northwestern Democratic Republic of the Congo, and southwestern Sudan (Figure 17).

The heartworm *Dirofilaria immitis* is a common parasite of dogs and other mammals of several orders, including humans (Artiodactyla, Carnivora, Edentata, Lagomorpha, Perissodactyla, Primates, and Rodentia). Most animals exhibit no signs of disease when infected, however, some of them experience respiratory distress, cough, and other symptoms. Human dirofilariosis has been reported worldwide. Cases presenting with subcutaneous infestations in the Old World are attributed to *D. repens*, a subcutaneous worm from dogs,

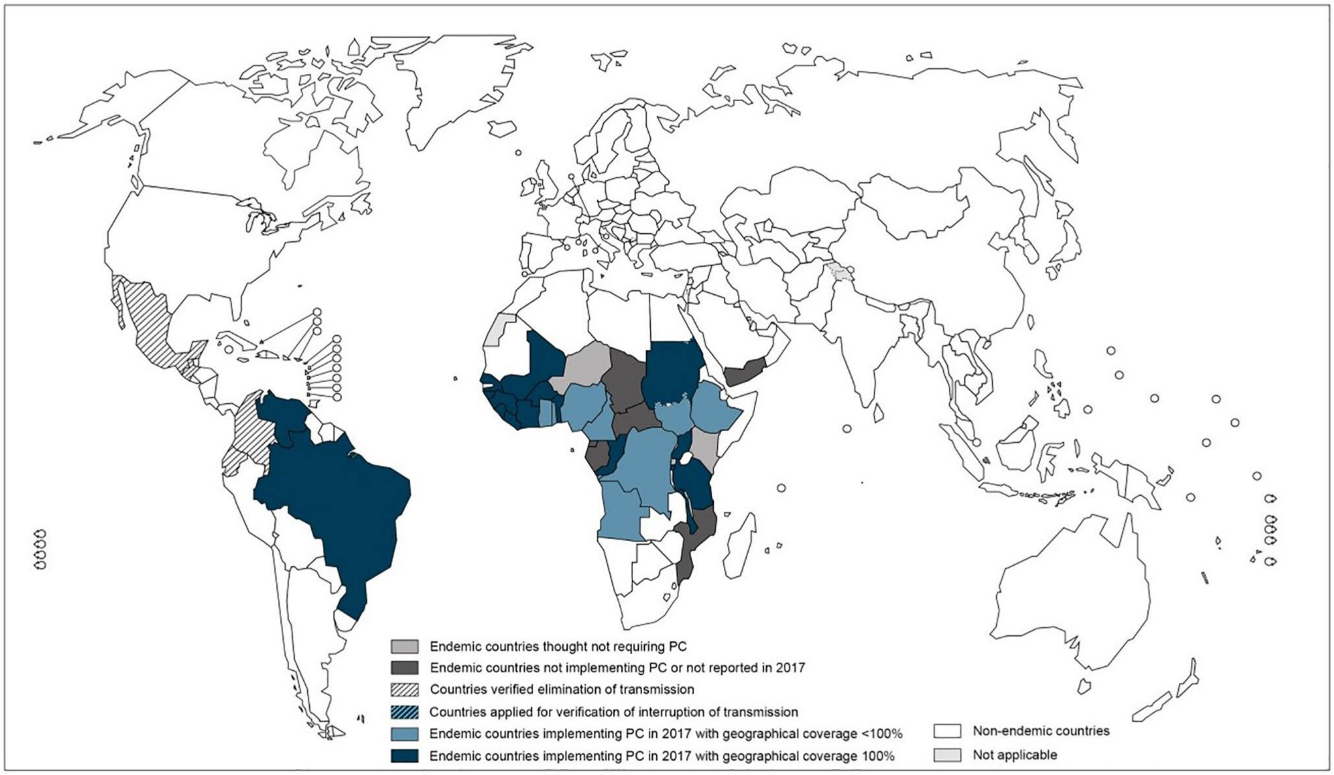

Figure 19. Map of the distribution and status of preventive chemotherapy for onchocerciasis worldwide. Source: Adapted from WHO, https://www.who.int/news-room/fact-sheets/detail/onchocerciasis. Permissions: Cf WHO terms of acceptable uses (non-commercial, educational).

whereas pulmonary dirofilariosis in the New World is associated with *D. immitis* (Dantas-Torres and Otranto, 2013).

The life cycle of filarioids within Dirofilariinae generally includes mosquitoes as intermediate hosts. In *Dirofilaria* spp. juvenile stages develop in Malpighian tubules and, depending on the country and habitat, different genera act as vectors, specifically, *Culex*, *Aedes*, *Anopheles*, *Ochlerotatus*, *Stegomyia*, *Jarnellius*, and *Aedimorphus*. In *Foleyella*, J_2s and J_3s develop in the fat body of mosquitoes, while *Loaina* prefers the hemocoel. In the case of *Loa loa* species, it develops in the horsefly, while *Pelecitus* develops in Mallophaga lice.

As an example of a life cycle, adults of *Pelecitus fulicaeatrae* are found in the tendons near the ankle of coots and grebes. Both male and female *P. fulicaeatrae* worms are short and coiled, and males possess an asymmetrical caudal ala and large pedunculate cloacal papillae. In other species of *Pelecitus*, the caudal alae are symmetrical (Figure 16). Microfilariae are about 92–122 µm-long and occur in the skin and the feathered portions of the lower leg, usually located near the feather follicles (Figure 17). The louse *Pseudomenopon pilosum* (order Amblyocera, suborder Mallophaga) inhabits the base of feathers ingesting tissues of the bird with microfilariae. Second-and third-stage juveniles (J_2 and J_3) of *P. fulicaeatrae* were recovered from the fat body in naturally infected lice. Parasitized lice can be transferred from adult coots to coot chicks, infecting the young birds. Immature adult worms can be found after 20 days post-infection in the ankles, while microfilariae appear in the skin after 7 to 8 months (Bartlett and Anderson, 1989).

Subfamily Onchocercinae: Cause of Several Neglected Diseases in Humans

This subfamily includes more than 30 genera parasitizing mammals. Some of them induce what are termed **neglected diseases** of humans, which is defined by the United States Centers for Disease Control and Prevention (CDC, 2021) as diseases caused by parasites, viruses, or bacteria that cause substantial illness for approximately 15% of the world's inhabitants that results in trapping them in a cycle of poverty.

The worms in this subfamily have a long non-alate tail and dissimilar and unequal spicules (see Figures 3 and 4 above). Adult worms are usually located in the body cavity of the host; however, some species inhabit subcutaneous tissues or the lymphatic vessels and nodes.

Seven species are responsible for infection in humans: *Wuchereria bancrofti, Brugia malayi*, *B. timori*, *Onchocerca volvulus*, *Mansonella (Mansonella) ozzardi*, *M. (Esslingeria) perstans*, and *M. (E.) streptocerca*. Each is described briefly below.

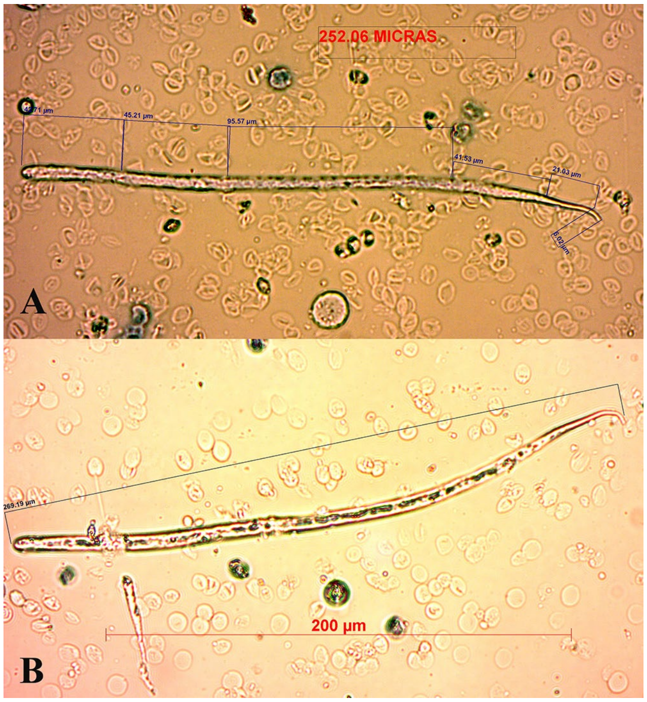

Figure 20. Microfilariae photographs. A) From *Dirofilaria immitis*; B) From *Acanthocheilonema reconditum*. Microfilariae from *D. immitis* is characterized by a cephalic end rounded, straight tip tail, and longer body length; *A. reconditum* possess a cephalic end obtuse, tail J-shaped and shorter body length. Source: S. Costa. License: CC BY-NC-SA 4.0.

Wuchereria bancrofti, Brugia malayi, and *B. timori* cause lymphatic filariasis, or elephantiasis, due to the parasites living in the lymphatic system (vessels and nodes), disrupting the system's normal function. Infection is usually acquired in childhood causing hidden damage to the lymphatic system. Later in life, people develop lymphedema and elephantiasis as well as hydrocele and scrotal elephantiasis. Patients usually suffer physical disability that contributes to poverty (Yonder and Pandey, 2023).

Ninety percent of lymphatic filariasis is caused by *Wuchereria bancrofti*; the remaining 10% by *Brugia malayi* and *B. timori*. Bancroftian filariasis is transmitted by several different species of mosquitoes: *Culex* spp., widespread in urban and semi-urban areas; *Anopheles* spp., mainly found in rural areas; and *Aedes* spp., found on endemic islands in the Pacific. *Brugia malayi* is transmitted by mosquito species of the genus *Mansonia*, whereas *B. timori* is transmitted by *Anopheles* mosquitoes (Anderson, 2000).

Lymphatic filariasis is a major problem in tropical and subtropical countries, extending throughout central Africa, the Nile delta, Turkey, India, Southeast Asia, the East Indies, the Philippine and oceanic islands, Australia, New Guinea, Brazil, Guyana, Venezuela, and some countries in Central America (Figure 18). It is estimated that 120 million people are infected worldwide; of these, almost 25 million men have genital disease and almost 15 million, mostly women, have lymphedema or elephantiasis of the leg (Anderson, 2000).

Onchocerca volvulus specimens are usually found in subcutaneous tissues producing onchocerciasis, or river blindness, which is a filariosis characterized by pruritus, dermatitis, lymphadenopathy, and ocular lesions. It is not a fatal disease; however, it can cause disfigurement of the skin and visual impairment, including permanent blindness. It is transmitted to humans through exposure to repeated bites of infected blackflies of the genus *Simulium*. The WHO (2022) reports that 20.9 million people were infected with *O. volvulus* worldwide in 2020, 14.6 million infected people had skin disease, and 1.15 million had vision loss. Onchocerciasis is distributed in 31 countries of Africa, Yemen, and some countries of Latin America (Figure 19). Implementation of different programs for control, eradication, and treatment of the disease were carried out by the WHO and governments, contributing to Colombia, Ecuador, Mexico, and Guatemala being free of onchocerciasis (WHO, 2022).

Three *Mansonella* species cause mansonellosis. *Mansonella (Mansonella) ozzardi* and *M. (Esslingeria) perstans* reside in body cavities and the surrounding tissues, while *M. (E.) streptocerca* lives in the dermis and subcutaneous tissue. Infections by *M. (E.) perstans* are often asymptomatic, however, are at times associated with angioedema, pruritus, fever, headaches, arthralgias, and neurologic manifestations. Those produced by *M. (E.) streptocerca* can cause skin manifestations, including pruritus, papular eruptions, and skin pigmentation changes. *Mansonella (M.) ozzardi* can cause arthralgias, headaches, fever, pulmonary symptoms, adenopathy, hepatomegaly, and pruritus. Adult filarioids live for several years and reside in various tissues. Biting midges of the family Ceratopogonidae transmit all 3 *Mansonella* species and blackflies of the family Simuliidae play an important role in the transmission of *M. (M.) ozzardi* in Latin America. *Mansonella (E.) perstans* is endemic in Sub-Saharan Africa as well as a northern part of the Amazon rainforest stretching from equatorial Brazil to the Caribbean coast of South America. *Mansonella (E.) streptocerca* is limited to continental Africa, occurring in the tropical rainforest areas of central and west Africa as well as in Uganda, while *M. (M.) ozzardi* has a patchy geographic distribution across Latin America. It has been recorded from southern Mexico to northwestern Argentina, but has not been reported in Chile, Uruguay, or Paraguay. The parasite also occurs on several Caribbean islands and elsewhere in Latin America (Anderson, 2000).

There is a lack of data about the prevalence of the filarioid disease mansonellosis and the morbidity and mortality

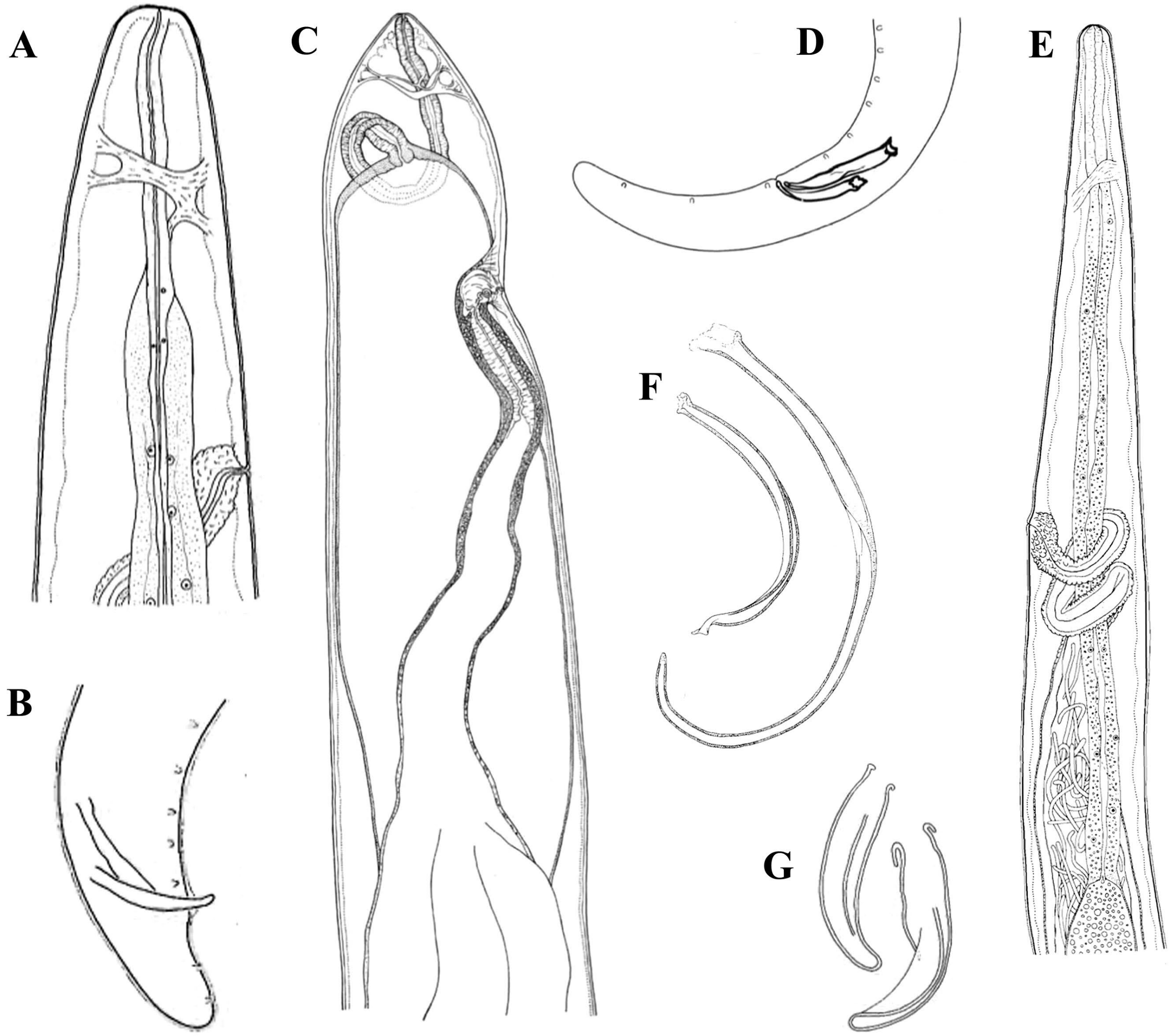

Figure 21. Examples of Splendidofilariinae species. A, B) *Dessetfilaria guianensis*; A) Female anterior extremity showing the muscular and glandular esophagus and the vulva; B) Male tail, lateral view showing the similar and equal spicules; C, D) *Meningonema peruzzii*; C) Female anterior extremity; D) Male tail, lateral view showing the spicules; E, F) *Chandlerella bushi*. E) Female anterior extremity; F) Spicules, dissimilar; G) *Splendidofilaria chandenieri* spicules, similar and equal. Sources: A, B) Adapted from Bartlett and Bain, 1987; C, D) adapted from Orihel and Esslinger; E, F) adapted from Bartlett and Anderson, 1987; G) adapted from Bartlett and Bain, 1987. License: CC BY-NC-SA 4.0.

associated with it. The lack of knowledge of these effects is due in part to a high prevalence of asymptomatic cases, the lack of a clinical profile that makes diagnosis difficult, plus the similarity of the microfilariae to *Mansonella (Esslingeria) streptocerca* (in Africa) and *M. (Mansonella) ozzardi* (in Latin America) with that of *Onchocerca volvulus*. Additionally, mansonellosis parasites have been shown to interfere with some onchocerciasis immunodiagnostic assays, may interfere with the diagnostics used in other neglected tropical disease controls, and negatively affect the efficacy of vaccine programs (specifically, HIV and tuberculosis vaccines) (Ta-Tang et al., 2018).

The method for diagnosing lymphatic filariasis and *Mansonella (Esslingeria) perstans* and *M. (Mansonella) ozzardi* diseases is usually the finding of microfilariae in peripheral blood smears thick- or thin-stained with Giemsa or hematoxylin-and-eosin. Concentration techniques, such as Knott's technique or filtration through a Nucleopore® membrane, may also be efficacious in cases with a low burden of microfilariae. As lymphatic filariasis exhibits a nocturnal periodicity, an accurate diagnosis is best achieved on smears collected at night (10:00 pm–2:00 am). For *Mansonella* this is not necessary because it is a non-periodic filariasis. These

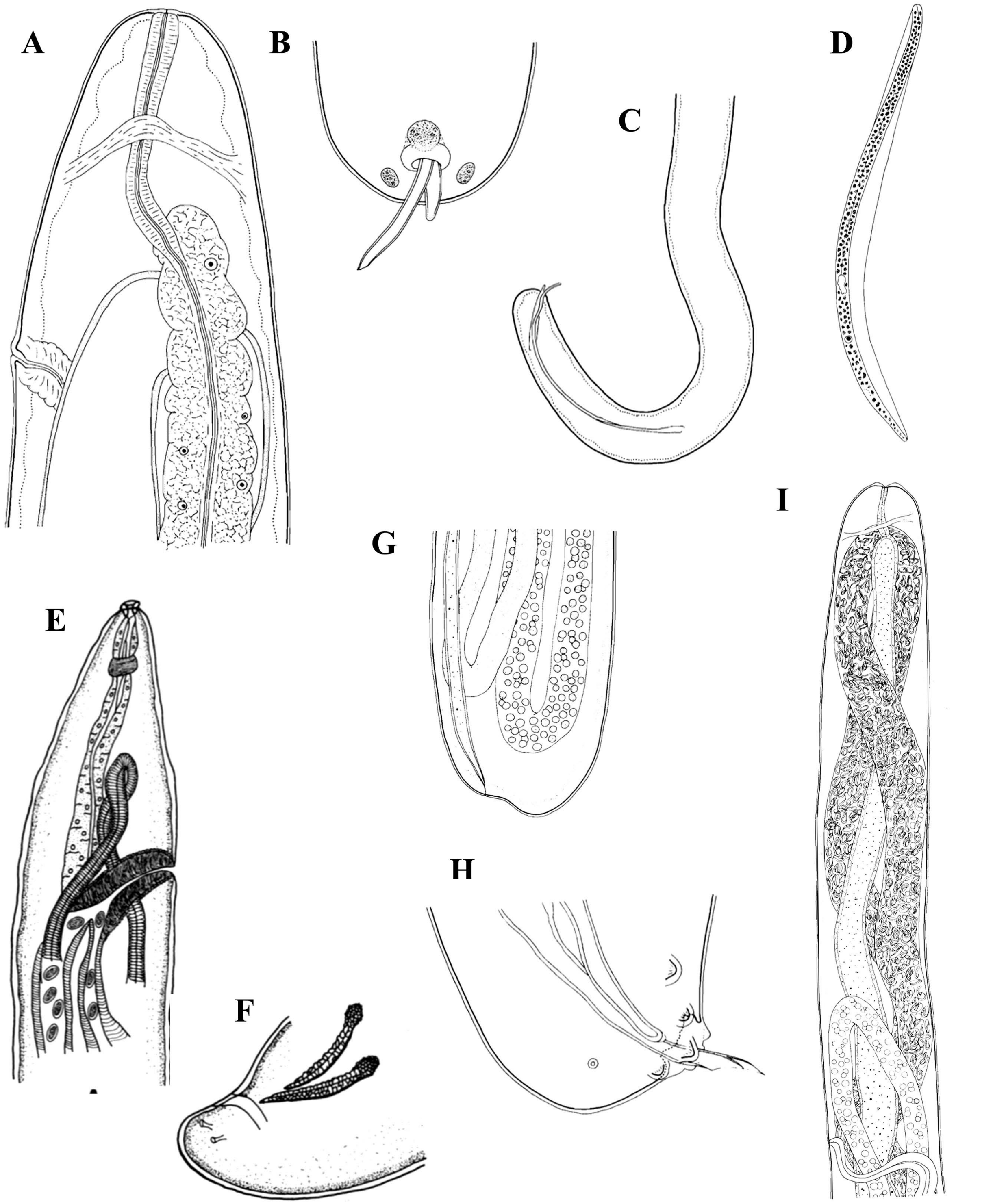

Figure 22. Examples of Lemdaniinae species. A–D) *Lemdana wernaarti*; A) Female anterior extremity showing the differentiation of the muscular and glandular esophagus and position of the vulva; B) Male tail with cloacal papillae; C) Male posterior extremity with dissimilar spicules; E, F) *Aprocta intraorbitalis*. E) Female anterior extremity; F) Male tail with similar spicules and cloacal papillae. G–I) *Eulimdana lari*. G) Female tail showing the anus at tip tail; H) Male tail with similar spicules and cloaca at tip tail; I) Female anterior extremity showing the differentiation of the muscular and glandular esophagus and uterus full of micrifilariae. Sources: A–C) Adapted from Bartlett and Anderson, 1987; E, F) adapted from Hernandez-Rodriguez et al 1986; G–I) adapted from Bartlett et al., 1985. License: CC BY-NC-SA 4.0.

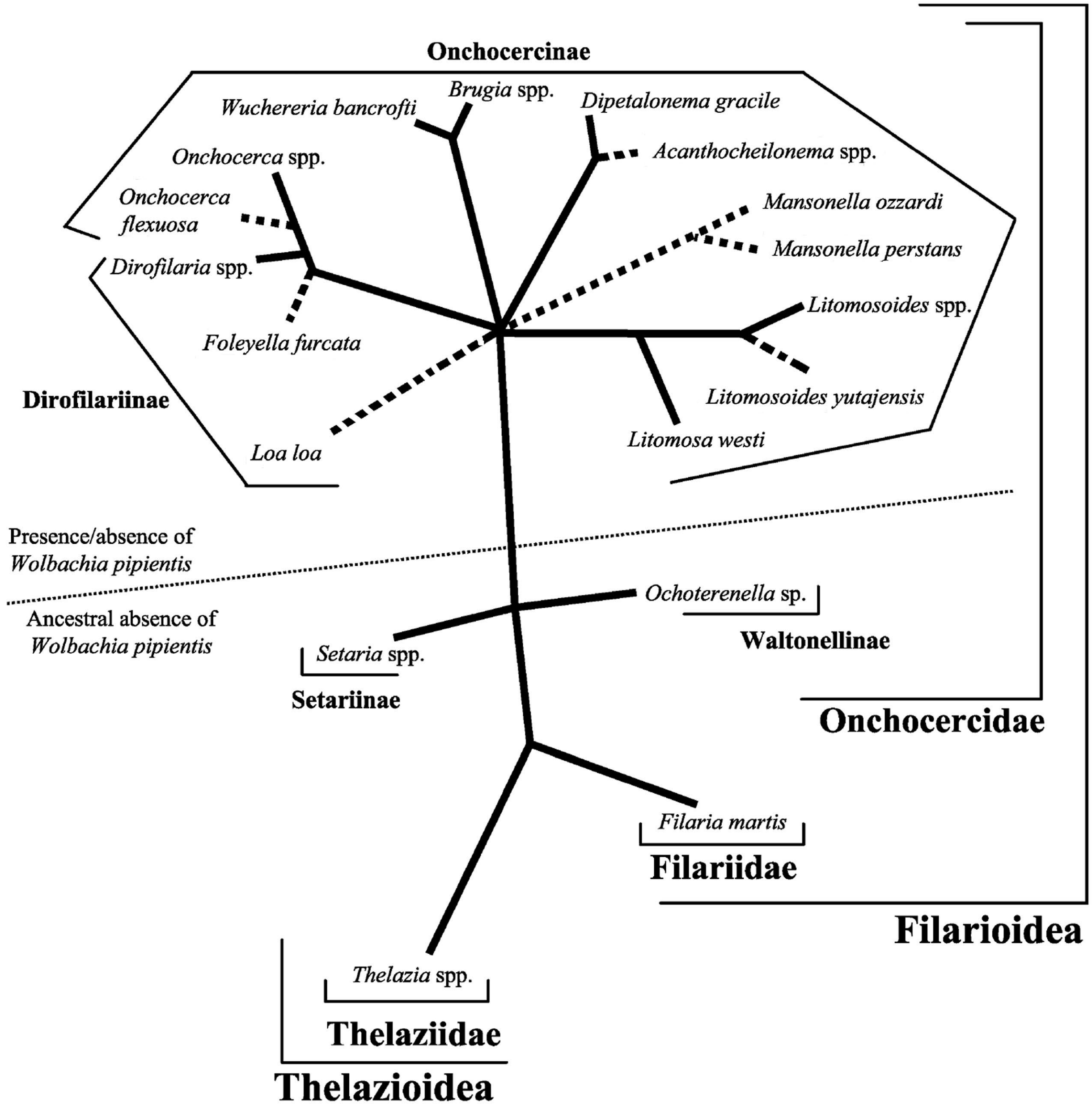

Figure 23. Hypothetical evolution of *Wolbachia pipientis* infection mapped on the phylogenetic tree of Filariae and related nematodes. *Wolbachia pipientis* could have been ancestrally absent from the lineages leading to *Thelazia* spp., *Filaria martis*, *Setaria* spp. and *Ochoterenella* spp. and it could have been acquired on the lineage leading to the Onchocercidae family, and then lost several lineages (*Litomosoides yutajensis*, *Mansonella* spp., *Acanthocheilonema* spp., *Onchocerca flexuosa*, *Foleyella furcata*, and *Loa loa*, dashed lines). The positions of *Mansonella* spp. and *O. flexuosa* are based only on their taxonomic affiliations. Source: Adapted from Casiraghi et al., 2004. License: CC BY-NC-SA 4.0.

methods are cheaper and faster than those using antigen detection, such as the immunoassay for circulating filarial antigens, which can be detected in blood samples collected at any time of day, unlike microfilariae with nocturnal periodicity. However, in many countries, antigen detection diagnosis tests are not licensed, making the diagnosis more difficult due to the similarity of the microfilariae species in areas with several filarioses. Adults may be identified in biopsied specimens of lymphatic tissue. For the pathogens causing onchocerciasis and *M. (E.) streptocerca*, diagnoses are performed by detection of microfilariae in skin snips or adults in biopsy specimens of skin nodules. Microfilariae of *Onchocerca* do not exhibit any periodicity, similarly to *Mansonella* (CDC, 2020).

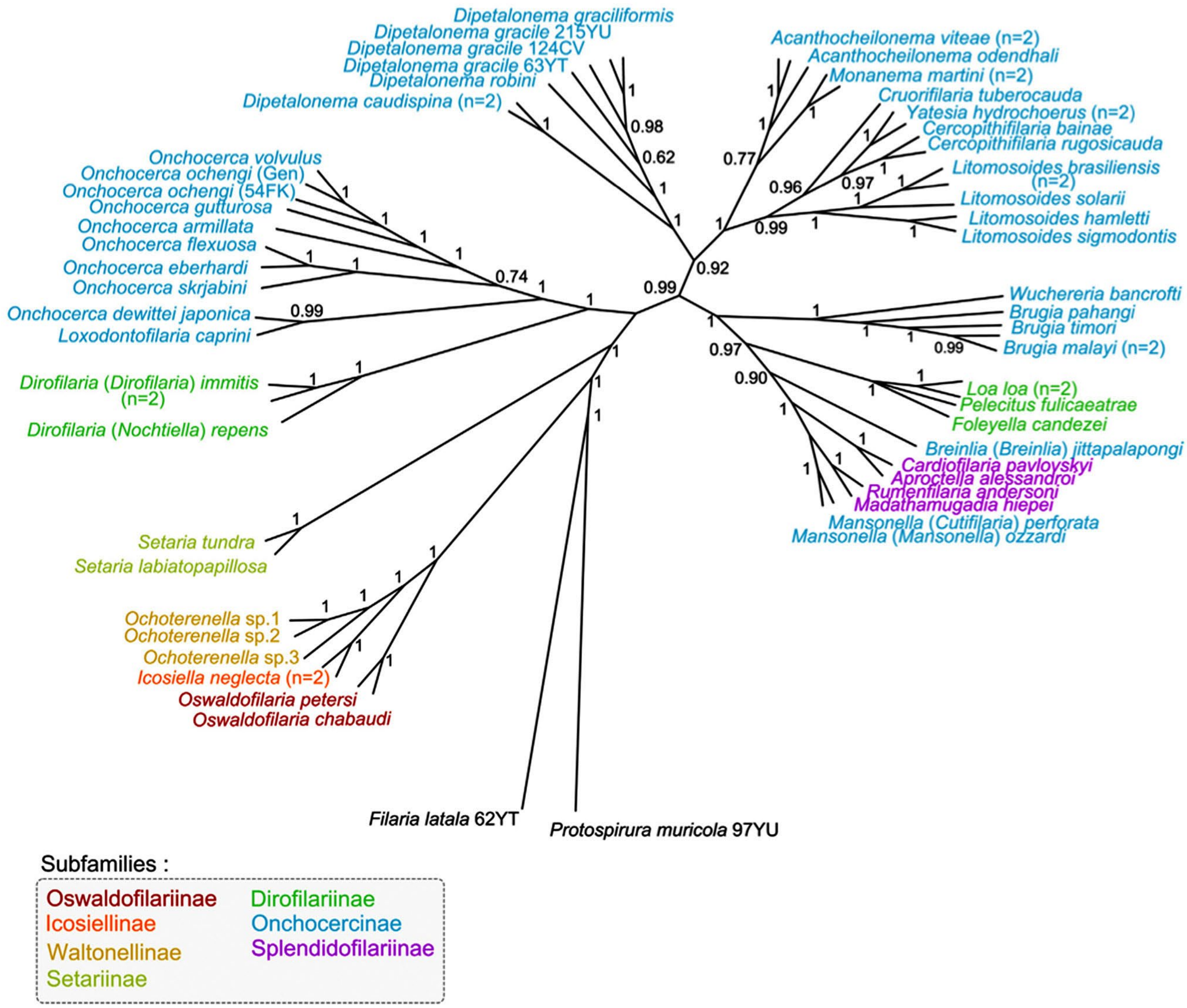

Figure 24. Phylogeny of family Onchocercidae based on partitioned concatenated datasets of 12S rDNA, coxI, rbp1, hsp70, myoHC, 18S rDNA, and 28S rDNA sequences using Bayesian inference. The total length of datasets is approximately 4,950 bp. Sixty onchocercid specimens (representing 48 species) were analyzed. *Filaria latala* and *Protospirura muricola* were used as outgroups. The topology was inferred using Bayesian inference. Nodes are associated with Bayesian posterior probabilities based on one run of 5 million generations. The onchocercid subfamilies are indicated by colors: Blue for Onchocercinae, dark green for Dirofilariinae, purple for Splendidofilariinae, pale green for Setariinae, yellow for Waltonelliinae, orange for Icosielliinae, and red for Oswaldofilariinae. Source: Adapted from Lefoulon et al., 2015. License: CC BY-NC-SA 4.0.

Among filarioids infecting dogs, *Acanthocheilonema reconditum* is a species whose microfilariae have been frequently confused with *Dirofilaria immitis* (Figure 20). This species has also a global distribution. It is important to have a correct identification of the microfilariae since *A. reconditum* is a non-pathogenic species. Adults parasitize the subcutaneous tissues and fascia of canids while microfilariae circulate in blood. The fleas *Ctenocephalides felis* and *C. canis* are the vectors for *A. reconditum*, whereas the role of ixodid ticks (that is, *Rhipicephalus*) as vectors for this filarioid species has been definitively rejected. The full development of microfilariae to the J_3 infective forms occurs in experimental infected fleas in about 15 days. Moreover, the localization and size of developing juveniles inside the infected flea suggest that this arthropod might act as an intermediate host through the ingestion of infected fleas rather than inoculation during a blood meal on dogs. This route of *A. reconditum* transmission is unique, differing from that of other filarioids, which are actively transmitted through the bites of mosquito vectors (Anderson, 2000).

The remaining filarial genera within the Onchocercinae are parasites of mammals, with the exception of *Macdonaldius* which is a parasite of reptiles. Most of the genera display a distribution restricted to a continent. For example, *Litomosoides*, a parasite of the body cavity of bats, rodents, and marsupials represented by 42 species, is distributed on the American continents from the southern United States to central Argentina, while *Dipetalonema* sensu stricto, with 6 species, and *Mansonella (Tetrapetalonema)*, with 13 species, parasitize Platyrrhinii monkeys from Central America to parts of South America. Other genera are monospecific, such as *Orihelia anticlava* which is widespread in South America and is a parasite of the body cavity from armadillos. In contrast, *Filarissima lainsoni* and *Migonella fracchiai* are only known by single unique records from a Brazilian coendou and a Paraguayan bat, respectively (Anderson, 2000).

Subfamily Splendidofilariinae

Members of this subfamily parasitize reptiles, birds, and mammals, including humans. It includes 21 genera with more than 90 species. Females contain a vulva in the anterior region of the body, the tail in both sexes is relatively long, caudal alae are absent, and the spicules are little different in size and morphology (Figure 21). Worms may be found in the body cavity of birds, sometimes in capsules in hidden locations (such as in *Madathamugadia*), while in reptiles they have been found in the mesenteric vessel of the intestine, the heart of turtles (such as in *Cardiofilaria*), or subcutaneous tissues in gecko lizards (such as in *Thamugadia*). In mammals they may be found in the body cavity (as in *Micipsella*) or the central nervous system (as in *Meningonema*) (Anderson, 2000).

The life cycle of these filarioids involves different vectors, such as mosquitoes in *Aproctella* and *Cardiofilaria*, or ornithophilic ceratophogonids in *Chandlerella* and *Splendidofilaria*. *Splendidofilaria fallisensis*, a parasite of the subcutaneous tissues of wild and domestic ducks of North America, utilize ornithophilic simuliids of the genus *Simulium* as vectors. Blackflies crawl under the feathers and engorge on infected blood. Microfilariae penetrate the stomach of the simuliid and develop in the haemocoel. After 7 to 14 days, depending on the temperature, J_3 appears near the mouthpart of the blackfly. Microfilariae appear in the blood of the ducks 30–36 days after they are inoculated with J_3, whereas in *S. californiensis*, a parasite of the heart from California quail, the prepatent period is about 6 months.

The genus *Meningonema* is commonly found parasitizing the central nervous system of African Cercopithecinae, however Boussinesq and colleagues (1995) reported the first human case in 1995. The authors recovered a fourth-stage juvenile (J_4) female from the cerebrospinal fluid of a patient from Cameroon harboring *Loa loa*, but who did not exhibit any neurological symptoms. In another study, microfilariae recovered from patients with cerebral filariasis in Zimbabwe identified as *Mansonella perstans* may have been infected instead with *Meningonema* and not the former species. In fact, microfilariae of *Meningonema* have not been confirmed in humans, although they have been found in the peripheral blood of monkeys; therefore, careful examination of blood samples might reveal that *Meningonema* infection is actually a frequent zoonosis in humans (Boussinesq et al., 1995).

Subfamily Lemdaniinae

Filarioids in this subfamily tend to parasitize birds and, less commonly, reptiles and mammals. Adults are characterized by a subterminal anus in both females and males, an absent buccal capsule, and spicules that are similar in size and form, such as in *Eulimdana*, or may be markedly different, as in *Lemdana* or *Makifilaria* (Figure 22). These filarioids develop in Mallophaga lice, such as *Eufilaria bartlettae*, a parasite of the blackbird (*Turdus merula*) and in mosquitoes of the genus *Anopheles*, such as *Saurocitus agamae*, a parasite of the lizard *Agama agama*.

***Wolbachia* Bacteria in Filarioids**

Intracellular bacteria belonging to the genus *Wolbachia* were discovered in filarial nematodes and arthropods in the 1970s with the advent of electron microscopy (Kozek and Figueroa Marroquin, 1977). In arthropods, *Wolbachia pipientis* generally induces alterations in host reproduction, acting as a tool to manipulate pest insects; while in filarioid nematodes, the evidence shows that these bacteria are required for development and reproduction (Stouthamer et al., 1999). *Wolbachia pipientis* bacteria are typically contained in a host-derived vacuole, inhabiting the hypodermic cells and the reproductive tissues of the female filarioid worm. Bacteria have also been recovered in juvenile filarioid stages, but not in male worms. This suggests a vertical mode of transmission through the cytoplasm of the nematode egg, which parallels observations in *Wolbachia* of arthropods. The bacteria may be present in the tissues alone, in small groups of bacteria, or in large groups that fill their cellular environment.

Data generated through electron microscopy and immunohistochemical examinations have helped to elucidate the presence or absence of *Wolbachia pipientis* in filarial species. In the 1990s, PCR amplification and sequencing of the *Wolbachia* genome showed that *Wuchereria bancrofti*, *Litomosoides sigmodontis*, *Mansonella ozzardi*, and all the species examined in the genera *Dirofilaria*, *Onchocerca*, and *Brugia* harbor *Wolbachia pipientis*.

Phylogenetic analysis by comparison of 16S rDNA sequences from *Wolbachia pipientis* have showed that filarial *Wolbachia* are closely related and form a separate group from the *Wolbachia* of arthropods. *Wolbachia* of filariae segregate into 2 clusters (named C and D), which diverge from the A and B clusters that are recognized for arthropods. Within the C and D filarial *Wolbachia* lineages, the bacterial phylogeny is congruent with the nematode phylogeny. However, in the rodent filaria *Acanthocheilonema viteae*, PCR consistently showed no evidence of *Wolbachia*; this was also the case for *A*. *reconditum*, microfilariae of *Mansonella perstans* and *Litomosoides yutajensis* (Onchocerciinae); *Loa loa*, and *Foleyella furcata* (Dirofilariinae); *Ochoterenella* spp. (Waltoneliinae); *Setaria equina*, *S. labiatopapillosa*, *S. tundra* (Setariinae), and *Filaria martis* (Filariinae) (Casiraghi et al., 2004).

Mapping the presence or absence of *Wolbachia pipientis* in different species within the subfamilies of the Onchocercidae and Filariidae will support the trees generated by molecular data of filarioids and will help to elucidate the phylogeny of Filariata.

Phylogeny of Filarioid Nematodes

A phylogeny of filarioid nematodes based on morphological characters has been proposed by Anderson and Bain (1976) and Chabaud and Bain (1994). However, due to the convergence of morphological characters among lineages, the phylogenies proposed are not sustainable at all and the proposed evolutionary scenario is weak. The major question regards the classification of the Onchocercidae, and their origin and evolution. Analyses based on molecular characters still are ongoing. A huge amount of sequence data is available for pathogenic filarioids (for example, *Wuchereria bancrofti* and *Brugia malayi*) as well as model filarial parasites (such as *Litomosoides sigmodontis* and *Acanthocheilonema vitae*) than for the remaining filarioids, for which data are scarce. The first phylogenetic analyses were conducted for the Onchocercinae and Dirofilariinae. However, biological material is scarce, impeding broad taxonomic sampling, and the markers used (12S rDNA and coxI genes) are not suitable for resolving the internal nodes which would help elucidate the evolution within the Onchocercidae. Lefoulon and colleagues (2015) proposed a robust phylogenetic hypothesis of the relationships within the Onchocercidae based on 7 loci: 2 mitochondrial and 5 nuclear genes of 48 species belonging to 7 subfamilies. These authors concluded that the tree topology is not congruent with the classic systematic delineations and the present phylogeny neither supports the monophyly of the Dirofilariinae, Onchocercinae, nor Splendidofilariinae (Figure 24) (Lefoulon et al., 2015).

Future studies including other sequence data, the presence or absence of *Wollbachia pipientis*, and more species within the order Filariata are necessary for the elucidation of the phylogeny of this group.

Literature Cited

Anderson, R. C. 2000. Nematode Parasites of Vertebrates, Their Development and Transmission. CAB International, Wallingford, United Kingdom, 650 p.

Anderson, R. C., and O. Bain. 1976. Diplotriaenoidea, Aproctoidea and Filarioidea. *In* R. C. Anderson, A. G. Chabaud, and S. Willmott, eds. Keys to the Nematode Parasites of Vertebrates, Part 3. Commonwealth Agricultural Bureaux, Farnham Royal, United Kingdom, p. 59–116.

Ash, L. R., and M. D. Little. 1964. *Brugia beaveri* sp. n. (Nematoda: Filarioidea) from the raccoon (*Procyon lotor*) in Louisiana. Journal of Parasitology 50: 119–123. doi: 10.2307/3276044

Bain, O. 1966. Diversité et étroite spécificité parasitaire des Filaires de chauves-souris, confondues sous le nom de *Litomosa filaria* (van Beneden, 1872). Bulletin du Muséum national d'histoire naturelle, Paris, 2e série, 38: 928–939.

Bain, O. 1972. Recherches sur le morphogénèse des filaires chez l'hôte intermedédiaire. Annales de parasitologie humaine et comparée 47: 251–303. https://www.parasite-journal.org/articles/parasite/pdf/1972/02/parasite1972472p251.pdf

Bain, O., and A. G. Chabaud. 1988. Un appareil favorisant l'accouplement des filaires: Les renflements de la région antérieure du corps. Annales de Parasitologie humaine et comparée 63: 376–379.

Bain, O., and B. N. Chaniotis. 1975. *Befilaria puertoricensis* n. sp. nouvelle filaire Oswaldofilariinae d'iguanidae aux Caraïbes (Puerto Rico). Bulletin du Muséum national d'histoire naturelle, Paris, 1975, 3e serie, 281, Zoologie, 191: 1–5.

Bain, O., and J. Prod'hon. 1974. Homogénéité des filaires de batraciens des genres *Waltonella*, *Ochoterenella* et *Madochotera*; création des Waltonellinae n. subfam. Annales de Parasitologie (Paris) 49: 721–739.

Bain, O., B. Kouyaté, and M. Baker. 1982. Nouvelles données sur les Oswaldofilariinae (Filarioidea, Nematoda). Bulletin du Muséum national d'histoire naturelle, Paris, 4 serie, 4 section A, 1–2: 61–69.

Bain, O., Y. Mutafchiev, K. Junker, R. Guerrero, et al. 2015. Review of the genus *Mansonella* Faust, 1929 *sensu lato* (Nematoda: Onchocercidae), with descriptions of a new subgenus and a new subspecies. Zootaxa 3918: 151–193. doi: 10.11646/zootaxa.3918.2.1

Bartlett, C. M., and R. C. Anderson. 1987. *Chandlerella bushi* n. sp. and *Splendidofilaria caperata* Hibler, 1964 (Nematoda: Filarioidea) from *Fulica americana* (Gruiformes: Rallidae) in Manitoba, Canada. Canadian Journal of Zoology 65: 2,799–2,802. doi: 10.1139/z87-422

Bartlett, C. M., and R. C. Anderson. 1987. *Lemdana wernaarti* n. sp. and other filarioid nematodes from *Bubo virginianus* and *Asio otus* (Strigiformes) in Ontario, Canada, with a revision of *Lemdana* and a key to avian filarioid genera. Canadian Journal of Zoology 65: 1,100-1,109.

Bartlett, C. M., and R. C. Anderson. 1989. Mallophagan vectors and the avian filarioids: New subspecies of *Pelecitus fulicaeatrae* (Nematoda: Filarioidea) in sympatric North American hosts, with development, epizootiology, and pathogenesis of the parasite in *Fulica americana* (Aves). Canadian Journal of Zoology 67: 2,821–2,833. doi: 10.1139/z89-398

Bartlett, C. M., and O. Bain. 1987. New avian filarioids (Nematoda: Splendidofilariinae): *Dessetfilaria guianensis* gen. n., sp. n., *Andersondilaria africanus* gen. n., sp. n., and *Splendidofilaria chandenieri* sp. n. Proceedings of the Helminthological Society of Washington 54: 1–14.

Bartlett C. M., P. L. Wong and, R. C. Anderson. 1985. *Eulimdana lari* (Yamaguti, 1935) n. comb. (Nematoda: Filarioidea) from *Phalaropus* spp. (Charadriiformes) in Canada and a review of the genus *Eulimdana* Founikoff, 1934. Canadian Journal of Zoology 63: 666–672. doi: 10.1139/z85-096

Blažek, K., J. Dyková, and J. Páv. 1968. The occurrence and pathogenicity of *Setaria cervi* Rud., in the central nervous system of deer. Folia Parasitologica 15: 123–130. https://folia.paru.cas.cz/pdfs/fol/1968/02/04.pdf

Boussinesq, M., O. Bain, A. G. Chabaud, N. Gardon-Wendel, et al. 1995. A new zoonosis of the cerebrospinal fluid of man probably caused by *Meningonema peruzzii*, a filaria of the central nervous system of Cercopithecidae. Parasite 2: 173–176. doi: 10.1051/parasite/1995022173

Bursey, C. R., S. R. Telford, Jr., and S. R. Goldberg. 2003. *Icosiella turgeocauda* n. sp. (Nematoda: Onchocercidae) and *Seuratascaris numidica* (Nematoda: Ascarididae), parasites of the frog, *Rana cancrivora* (Anura: Ranidae), from Luzon, Republic of the Philippines. Journal of Parasitology 89: 342–345. doi: 10.1645/0022-3395(2003)089[0342:ITNSNO]2.0.CO;2

Cano J., M. P. Rebollo, N. Golding, R. L. Pullan, et al. 2014. The global distribution and transmission limits of lymphatic filariasis: Past and present. Parasites and Vectors 7: 466. doi: 10.1186/s13071-014-0466-x

Casiraghi, M., O. Bain, R. Guerrero, C. Martin, et al. 2004. Mapping the presence of *Wolbachia pipientis* on the phylogeny of filarial nematodes: Evidence for symbiont loss during evolution. International Journal for Parasitology 34: 191–203. doi: 10.1016/j.ijpara.2003.10.004

CDC (United States Centers for Disease Control and Prevention). 2021. Neglected tropical diseases. https://www.cdc.gov/globalhealth/ntd/index.html

CDC (United States Centers for Disease Control and Prevention). 2020. Parasites, lymphatic filariasis: Guidance for evaluation and treatment. https://www.cdc.gov/parasites/lymphaticfilariasis/health_professionals/dxtx.html

Chabaud, A. G., and O. Bain. 1994. The evolutionary expansion of the Spirurida. International Journal for Parasitology 24: 1,179–1,201. doi: 10.1016/0020-7519(94)90190-2

Chandratre, G. A., R. Singh, S. Sharma, S. Saharan, et al. 2017. Subcutaneous parafilariasis in buffalo (*Bubalus bubalis*). International Journal of Current Microbiology and Applied Sciences 6: 766–770. doi: 10.20546/ijcmas.2017.604.095.

Dantas-Torres, F., and D. Otranto. 2013. Dirofilariosis in the Americas: A more virulent *Dirofilaria immitis*? Parasites and Vectors 6: 288–297. doi: 10.1186/1756-3305-6-288

Desportes, C. 1942. *Forcipomiya velox* Winn. et *Sycorax silacea* Curtis, vecteurs *d'Icosiella neglecta* (Diesing) filaire commune de la grenouille verte. Annales de parasitology humaine et comparée 19: 53–68.

Desportes, C. 1941. Nouvelles recherches sur la morphologie et sur l'évolution d'*Icosiella neglecta* (Diesing, 1851) filaire commune de la grenouille verte. Annales de parasitology humaine et comparée 18: 46–67.

Eberhard, M. L. 1979. Studies on the *Onchocerca* (Nematoda: Filarioidea) found in cattle in the United States, I: Systematics of *O. gutturosa* and *O. lienalis* with a description of *O. stilesi* sp. n. Journal of Parasitology 65: 379–388.

Esslinger, J. H. 1986. Redescription of *Foleyellides striatus* (Ochoterena and Caballero, 1932) (Nematoda: Filarioidea) from a Mexican frog, *Rana montezumae*, with reinstatement of the genus *Foleyellides* Caballero, 1935. Proceeding of the Helminthological Society of Washington 53: 218–223. http://science.peru.edu/COPA/ProcHelmSocWash_V53_N2_1986I.pdf

Guerrero, R., C. Martin, S. L. Gardner, and O. Bain. 2002. New and known species of *Litomosoides* (Nematoda: Filarioidea): Important adult and larval characters and taxonomic changes. Comparative Parasitology 69: 177–195. doi: 10.1654/1525-2647(2002)069[0177:NAKSOL]2.0.CO;2

Hernandez-Rodriguez, S., P. Gutiérrez-Palomino, and F. Martinez-Gomez. 1986. *Aprocta intraorbitalis* n. sp. parasite de la pie bleue à calotte noire *Cyanopica cyanus* (Passeriformes, Corvidae). Annales de Parasitologie humaine et comparée 61: 65–69. doi: 10.3347/kjp.2017.55.6.667

Keppner, E. J. 1969. *Filaria taxideae* n. sp. (Filarioidea: Filariidae) from the badger, *Taxidea taxus taxus* from Wyoming. Transactions of the American Microscopical Society 88: 581–588.

Keppner, E. J. 1971. The pathology of *Filaria taxideae* (Filarioidea: Filariidae) infection in the badger. Journal of Wildlife Diseases 7: 317–323.

Kozek, W. J., and M. Figueroa Marroquin. 1977. Intracytoplasmatic bacteria in *Onchocerca volvulus*. American Journal of Tropical Medicine and Hygiene 26: 663–678. doi: 10.4269/ajtmh.1977.26.663

Lefoulon, E., O. Bain, J. Bourret, K. Junker, et al. 2015. Shaking the tree: Multi-locus sequence typing usurps current onchocercid (Filarial Nematode) phylogeny. PLoS Neglected

Tropical Diseases 9: e0004233. doi: 10.1371/journal.pntd.0004233

Martin, C., O. Bain, N. Jouvenet, V. Raharimanga, et al. 2006. First report of *Litomosa* spp. (Nematoda: Filarioidea) from Malagasy bats; review of the genus and relationships between species. Parasite 13: 3–10. doi: 10.1051/parasite/2006131003

McLaren, D. J. 1972. Ultrastructural studies on microfilaria (Nematoda: Filarioidea). Parasitology 65: 317–332. doi: 10.1017/s0031182000045108

Mutinda, M., M. Otiende, F. Gakuya, L. Kariuki, et al. 2012. Putative filariosis outbreak in white and black rhinoceros at Meru National Park in Kenya. Parasites and Vectors 5: 206–211. doi: 10.1186/1756-3305-5-206

Nevill, E. M. 1979. The experimental transmission of *Parafilaria bovicola* to cattle in South Africa using *Musca* species (subgenus *Eumusca*) as intermediate host. Onderstepoort Journal of Veterinary Research 46: 51–57.

Notarnicola, J., and G. T. Navone. 2002. A new species, *Litomosoides odilae* n. sp. (Nematoda: Onchocercidae) from *Oligoryzomys nigripes* (Rodentia: Muridae) in the rainforest of Misiones, Argentina. Journal of Parasitology 88: 967–71. doi: 10.1645/0022-3395(2002)088[0967:ANSLON]2.0.CO;2

Notarnicola, J., and G. T. Navone. 2003. Systematic and distribution of *Orihelia anticlava* (Molin, 1858) (Nematoda, Onchocercidae) from dasypodids of South America. Acta Parasitologica 48: 103–110.

Notarnicola, J., O. Bain, and G. T. Navone. 2000. Two new species of *Litomosoides* (Nematoda: Filarioidea) in sigmodontines (Rodentia: Muridae) from Rio de La Plata marshland Argentina. Journal of Parasitology 86: 1,318–1,325. doi: 10.1645/0022-3395(2000)086[1318:TNSOLN]2.0.CO;2

Notarnicola, J., F. A. Jiménez-Ruiz, and S. L. Gardner. 2010. *Litomosoides* (Nemata: Filarioidea) of bats from Bolivia with records for three known species and the description of a new species. Journal of Parasitology 96: 775–782. doi: 10.1645/GE-2371.1

Notarnicola, J., F. A. Jiménez-Ruiz, and S. L. Gardner. 2007. A new species of *Dipetalonema* (Filarioidea: Onchocercidae) from *Ateles chamek* from the Beni of Bolivia. Journal of Parasitology 93: 661–667. doi: 10.1645/GE-962R1.1

Orihel, T. C., and J. H. Esslinger. 1973. *Meningonema peruzzii* gen. et sp. n. (Nematoda: Filarioidea) from the central nervous system of African monkeys. Journal of Parasitology 59: 437–441. doi: 10.2307/3278768

Osipov, A. N. 1966. Life cycle of *Setaria altaica* (Rajewskaja, 1928), a parasite of the brain of Siberian deer. Doklady Akademii Nauk SSSR 168: 247–248.

Pereira, F. B., S. Lima Sousa, and O. Bain. 2010. *Oswaldofilaria chabaudi* n. sp. (Nematoda: Onchocercidae) from a South American tropidurid lizard (Squamata: Iguania) with an update on Oswaldofilariinae. Parasite 17: 307–318. doi: 10.1051/parasite/2010174307

Petit, G., O. Bain, A. F. Gomes, and L. Touratier. 1983. *Piratuboides huambensis* n. sp., filaire Oswaldofilariinae parasite de lézards en Afrique australe. Bulletin de Muséum national d'histoire naturelle, Paris, 4 série, 5, section A, 3: 743–747.

Purnomo, and M. J. Bangs. 1996. *Icosiella intani* n. sp. (Filarioidea: Onchocercidae), a parasite of *Rana cancrivora* from South Kalimatan, Indonesia. Journal of the Helminthological Society of Washington 63: 47–50. http://science.peru.edu/COPA/JHelmSocWash_V63_N1_1996I.pdf

Round, M. C. A. 1964. New species of *Stephanofilaria* in skin lesion from the black rhino (*Diceros bicornis*). Journal of Helminthology 38: 87–96. doi: 10.1017/S0022149X00033630

Shin, J., K.-S. Ahn, G.-H. Suh, H.-J. Kim, et al. 2017. First blindness cases of horses infected with *Setaria digitata* (Nematoda: Filarioidea) in the Republic of Korea. Korean Journal of Parasitology 55: 667–671. doi: 10.3347/kjp.2017.55.6.667

Souza Lima, S., B. Marun, P. V. Alves, and O. Bain. 2012. *Ochoterenella esslingeri* n. sp. (Nematoda: Onchocercidae: Waltonellinae) from *Bokermannohyla luctuosa* (Anura: Hylidae) in Minas Gerais, Brazil, with notes on *Paraochoterenella* Purnomo & Bangs, 1999. Parasite 19: 341–350. doi: 10.1051/parasite/2012194341

Stouthamer, R., J. A. Breeuwer, and G. D. Hurst. 1999. *Wolbachia pipientis*: Microbial manipulator of arthropod reproduction. Annual Review of Microbiology 53: 71-102. doi: 10.1146/annurev.micro.53.1.71

Ta-Tang, T. H., J. L. Crainey, R. J. Post, S. L. B. Luz, et al. 2018. Mansonellosis: Current perspectives. Research and Reports in Tropical Medicine 9: 9–24. doi: 10.2147/RRTM.S125750

Vanderhoeven, E., J. Notarnicola, and I. Agostini. 2017. First record of *Dipetalonema robini* Petit, Bain & Roussilhon 1985 (Nematoda: Onchocercidae) parasitizing *Sapajus nigritus* in northeastern Argentina. Mastozoología Neotropical 24: 483–488.

WHO (World Health Organization). 2022. Onchocerciasis. https://www.who.int/news-room/fact-sheets/detail/onchocerciasis

Yonder, S., and J. Pandey. 2023. Filarial hydrocele. StatPearls [Internet]. https://www.ncbi.nlm.nih.gov/books/NBK560776/

Supplemental Reading

Klion, A., and T. B. Nutman. 2011. Loiasis and *Mansonella* Infections. *In* R. Guerrant, D. H. Walker and P. F. Weller, eds. Tropical Infectious Diseases: Principles, Pathogens and Practice, 3rd edition. Saunders Elsevier, Philadelphia, Pennsylvania, United States, p. 735.