CHAPTER II

LITERATURE REVIEW

1. Filarial nematodes

Filarial nematodes or filariae are thread-like parasites of humans and various animal species that are transmitted by blood-sucking vectors. They refer to nematodes of the order Spirurida that have been collected into the superfamily Filarioidea (Anderson and Bain, 1976). This superfamily is consisted of two families: Filariidae and Onchocercidae. It is important to note that all the filarial nematodes that cause known diseases in humans are members of the subfamilies, Onchocercinae and Dirofilariinae, of the family Onchocercidae. The family also encompasses additional 6 subfamilies (Waltonellinae, Setariinae, Oswaldofilariinae, Icosiellinae, Splendidofilariinae, and Lemdaninae) (Bain and Chabaud, 1986). Taxonomy of the family Onchocercidae is shown in **Figure 2**.

Superkingdom:	Eukaryota
Kingdom:	Animalia
Subkingdom:	Metazoa
Phylum:	Nematoda
Class:	Chromadorea
Order:	Spirurida
Suborder:	Spirurina
Superfamily:	Filarioidea
Family:	Onchocercidae

Figure 2 Taxonomy of the family Onchocercidae.

Filarial diseases are a major health problem in many tropical and subtropical areas. The major parasites of humans are *Brugia malayi*, *Wuchereria bancrofti* and *Onchocerca volvulus* (**Table 1**). The adult worms inhabit specific tissues where they mate and produce microfilariae, the characteristic tiny, thread-like larvae. The microfilariae infect vector arthropods, in which they mature to infective larvae.

Table 1 Major pathogenic filarial parasites of humans. (Garcia *et al.*, 2001).

Species	Location of Adults	Major pathology	Location of microfilariae	Major vectors
Brugia malayi	Lymphatics	Lymphagitis, elephantiasis	Blood	Species of *Mansonia*, *Coquillettidia*, and *Anopheles* mosquitoes
Wuchereria bancrofti	Lymphatics	Lymphagitis, elephantiasis	Blood	Species of *Culex*, *Anopheles*, *Aedes*, and *Ochlerotatus* mosquitoes
Onchocerca volvulus	Subcutaneous tissues	Loss of vision, dermatitis	Fluid in the subcutaneous nodules, dermal layers of the skin, blood, and eye	*Simulium* spp. (blackflies)

2. Lymphatic filariasis (Elephantiasis)

Lymphatic filariasis, known as elephantiasis, is caused by filarial parasites: *W. bancrofti*, *B. malayi*, and *B. timori* (Ottesen *et al.*, 1997; Fischer *et al.*, 2004). Majority of the disease is affected by *W. bancrofti* accounting for 90% of the cases, and the minority accounting for 10% by *B. malayi*, and 0.67% by *B. timori*. According to worldwide estimation, over 120 million people are infected in 83 countries, including Thailand, and 40 millions of them are seriously debilitated and disfigured by the disease (WHO, 1993; Triteeraprapab and Songtrus, 1999; Triteeraprapab *et al.*, 2001). Lymphatic filariasis is ranked by the World Health Organization (WHO) as the world's second leading cause of permanent and long-term disability, and targeted by WHO to be eliminated as a public health problem by the year 2020 (WHO, 1995; Behbehani, 1998). On the other hand, it is the world's third of the most tropical diseases leading cause of long-term disability, with disease burden estimated at 5.6 million disability adjusted life-years (DALYs: the number of healthy years of life lost due to premature death and disability) (Morel, 2000). Among the pathogens causing lymphatic filariasis, *W. bancrofti* is prevalent in tropical areas worldwide; *B. malayi* is limited to Asia; and *B. timori* is restricted to some islands of Indonesia.

2.1 Life cycle

The infection is transmitted by biting of infected mosquitoes (**Figure 3**). During a blood meal, the infective larvae or third-stage larvae (L3) of lymphatic filarial parasites, penetrate into the bite wound, and pass to the lymphatic vessels and lymph nodes where they develop into an adult stage, mate, and ultimately produce microfilariae. Adult lymphatic filarial parasites have a life span of 5-10 years, while microfilariae can live long for 6-12 months. Millions of the offsprings of the female adults are released into the host's blood circulation, and can infect a biting mosquito. After infection, these microfilariae shed their sheath, penetrate the stomach wall and migrate to the thoracic muscles. Then they undergo metamorphosis into first-stage larvae (L1), and subsequently the mature infective third stage larvae. The infective larvae migrate to the mosquito's proboscis, from which they pass to another human, and the life cycle is re-initiated via the mosquito bites.

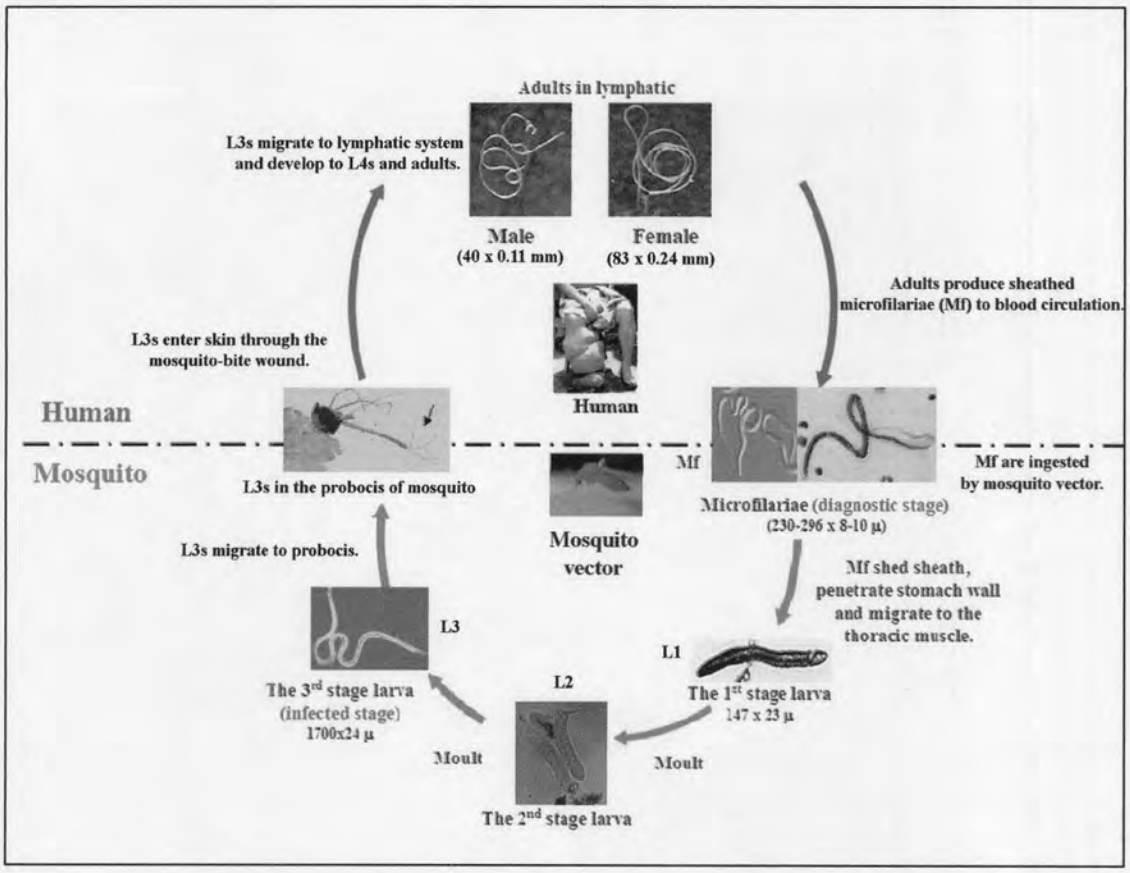

Figure 3 Life cycle of lymphatic filarial parasites.

2.2 Clinical manifestations of lymphatic filariasis

There is a wide range of clinical manifestations of longstanding infection with lymphatic filarial parasites (Figueredo-Silva *et al.*, 2002). Generally, lymphatic filariasis consists of asymptomatic microfilaremia. Other patients who carry the adult worms may be amicrofilaraemic and asymptomatic, or have acute lymphangitis and the chronic manifestations of the diseases (haematuria, hydrocele, chylocele, chyluria, lymphedema and elephantiasis). Additional manifestations of the filarial infection are tropical pulmonary eosinophilia (TPE) syndrome, and drug-induced adverse reactions (Dreyer *et al.*, 1998).

2.2.1 Asymptomatic microfilaraemic state

In areas where lymphatic filariasis is endemic, the vast majority of infected individuals have a few overt clinical manifestations of filariasis, despite the presence of large numbers of circulating microfilariae in the peripheral blood. It has now been clearly indicated that, although they may be clinically asymptomatic, almost all patients with *W. bancrofti* or *B. malayi* microfilaraemia have some degree of subclinical disease. Subclinical forms of the disease recognized in microfilaraemic individuals are haematuria and/or proteinuria that reflect low-grade renal damage (Dreyer *et al.*, 1992). The renal abnormalities are found ~40% of the microfilaraemic patients. Another form is from the observations on microfilaraemic patients using lymphoscintigraphy to visualize the functional anatomy of lymphatic vessels. Although clinically asymptomatic, they have markedly abnormal, dilated and tortuous lymphatic vessels, and obviously atypical patterns of lymphatic flow (Freedman *et al.*, 1994; Dissanayake *et al.*, 1995; Suresh *et al.*, 1997).

2.2.2 Acute manifestations of lymphatic filariasis

The acute clinical manifestations of filariasis are characterized by recurrent attacks of fever associated with inflammation of the lymph nodes (adenitis) and/or lymph vessels (lymphangitis) termed adenolymphangitis (ADL). In *W. bancrofti* infection (bancroftian filariasis), in addition to the lymph nodes in the inguinal, axillaries and epitrochlear regions, the lymphatic system of the male genitalia is frequently affected, leading to funiculitis, epididymitis or orchitis, or to a combination of these (Pani *et al.*, 1995). In brugian filariasis (*Brugia* infection), the affected lymph nodes are mostly positioned in the inguinal and axillaries, with inflammation along the course of the distal lymphatic vessels (Pani *et al.*, 1990).

The acute clinical course of filariasis may last for several days or up to 4-6 weeks with a fulminating episode, and may result in prolonged inability to work (Gyapong *et al.*, 1996). The acute episodes are characterized by local pain, tenderness, warmth and lymphadenitis and/or lymphangitis. Other commonly associated findings include fever, oedema, constitutional complaints, and localized or ulcerated abscesses especially in areas where *Brugia* is endemic.

In endemic areas, two distinct types of acute ADL episodes are recognized: (a) ADL caused directly by the parasite infection itself; and (b) ADL secondary to bacterial or fungal infection. The former ADL is termed acute filarial lymphangitis (AFL). The most common presentation is that of a cord-like structure associated with retrograde lymphangitis in the lower or upper limps. Lymphangitis is frequently accompanied by mild fever, headache, and malaise. In the scrotal area or the breast it may present as a painful palpable nodule. Recurrence of these attacks at the same sites is common (Shenoy *et al.*, 1995; Pani *et al.*, 1995). The latter form of ADL is the most common form. It is usually recognized as a syndrome with a clinical picture that can include high fever, chills, myalgia and headache. Recent evidences have suggested that bacterial or fungal super infections of limbs with compromised lymphatic function play the primary role in triggering episodes of ADL (Montestruc *et al.*, 1960; Olszewski *et al.*, 1993), which themselves actually cause or exacerbate the chronic obstruction changes in the lymphatic of affected patients. The acute process usually starts in the skin and then spreads along the lymphatic vessels to the lymph nodes (Olszewki *et al.*, 1993). Based on the observations, this form of acute ADL is termed acute dermatolymphangioadenitis (ADLA) (Olszewki *et al.*, 1993).

2.2.3 Chronic manifestations of lymphatic filariasis

The chronic signs of lymphatic filariasis rarely develop before the age of 15 years, and only a small proportion of the filarial-infected population is affected. However, immigrants from areas where filariasis is not endemic tend to develop elephantiasis more often, and much sooner (sometimes within 2-3 years) than do the local population of endemic areas (Partono, 1987). In bancroftian filariasis, the incidence of the major signs of chronic disease: hydrocele, chyluria, lymphoedema, and elephantiasis may differ from one area to another. The most common are hydrocele, and swelling of the testis, followed by elephantiasis of the entire lower limb, the scrotum, the entire arm, the vulva, and the breast, in descending of frequency (Pani *et al.*, 1995; 1990). In brugian filariasis, the leg below the knee is characteristically affected, and sometimes the arm below the elbow. Genital involvement has not been reported, except in areas where brugian filariasis occurs together with *W. bancrofti*

Lymphoscintigraphic studies have shown that lymphoedema is not always the results of occlusion of lymphatic channels, but can also happen when there is extensive collateralization. Skin changes such as skin fold thickening, hyperkeratosis, hypo- or hypertrichosis, pachydermia, pigmentary changes, chronic ulceration, epidermal and sub-epidermal nodules, and clinical intertrigo may also be seen in chronic infection (Burri *et al.*, 1996).

2.2.4 Tropical pulmonary eosinophilia

The usual presenting features are cough, dyspnea, wheezing similar to bronchial asthma (Spry and Kumaraswami, 1982). Microfilaraemia are almost never present in the blood, but remnants of microfilariae surrounded by aggregates of eosinophils are sometimes found in the liver, spleen, lymph nodes or lungs (Spry and Kumaraswami, 1982). Eosinophilia, and increased levels of IgE and of anti-filarial antibodies are commonly found (Ottesen and Nutman, 1992). TPE is characterized by immunological hyper responsiveness of the human hosts to the parasite, especially to the microfilariae (Ottesen *et al.*, 1979). The eosinophils found in bronchoalveolar lavage studies are activated, and have been shown to release oxygen radicals and other pro-inflammatory molecules (Rom *et al.*, 1990).

2.2.5 Clinical manifestations of adverse reactions to treatment

When patients with lymphatic filariasis are treated with antifilarial drugs, such as DEC or ivermectin, they develop characteristic adverse reactions. Adverse reactions following treatment of lymphatic filariasis are common, and frequently severe. They are generally caused not by direct drug toxicity but by host inflammatory responses to dying microfilariae (Ottesen, 1987). The adverse reactions can be systematic and local reactions (Babu *et al.*, 2006). Systematic reactions are headache, body ache, dizziness, decreased appetite, malaise, nausea, urticaria, vomiting, and sometimes bronchial asthma (McLaughlin *et al.*, 2003). Local reactions are lymphadenitis, funiculitis, epididymitis, orchitis, and lymphangitis, abscess formation, ulceration, and transient lymphoedema. Systematic reactions and fever are positively associated with microfilaraemia, and the density of microfilariae (Turner *et al.*, 1994; Haarbrink *et al.*, 1999). They occur early during the treatment, and generally do not last for more than 3 days. Local reactions occur mainly in patients who have histories

of adenolymphangitis, and tend to occur later and may last longer. Mild adverse reactions will disappear spontaneously, and usually are not necessary to interrupt with treatment. However, patients with severe adverse reactions require hospitalization.

3. Pathogenesis of lymphatic filariasis

In general, the microfilaraemic patients can remain asymptomatic for undetermined period of time, or progress into the chronic disease. The pathogenesis of lymphatic filariasis results from a complex interplay of factors related to adult worms (pathogenic potential and worm burden), host immune responses (resistance or tolerance), and secondary bacterial and fungal infections (Freedman, 1998; Dreyer *et al.*, 2000).

3.1 Subclinical lymphangiectasia caused by living adult lymphatic filarial parasites

The pathology in investigated lymphatic vessels consists of distinct histological features related to the presence of both live and dead parasites (Jungmann *et al.*, 1991; 1992). In endemic areas, the most common change in patients who carry living adult worms is subclinical lymphangiectasia (Dreyer *et al.*, 1999; Dreyer *et al.*, 2002). In such pathology, lymphatic vessels that contain living adult worms are dilated, without any inflammatory responses in the wall. Lymphatic dilatation with none of inflammatory reactions can be observed in nude mice (a mutant mouse strain that lacks a thymus gland and T lymphocytes) or severe combined immunodeficient mice (SCID mice: mice genetically engineered to lack T and B lymphocytes) infected with *Brugia* species (Vincent *et al.*, 1984; Nelson *et al.*, 1991), and can be reversed in nude mice by removing or killing the adult worms (Vickery *et al.*, 1991). The data suggested that factors related to the parasites themselves, rather than being those immunologically

mediated, contribute to lymphangiectasia. Since it can cause lymphatic dysfunction, lymphangiectasia is a major risk factor for development of chronic lymphatic disease.

3.2 Acute filarial lymphangitis is triggered by the death of adult lymphatic filarial parasites

Acute filarial lymphangitis (AFL) is designated an acute condition that presents as a restricted inflammatory nodule or cord in a lymphatic or a lymph node of an extremity, a breast (in woman), or the scrotum (in men). AFL is caused by death of adult lymphatic filarial parasites, either spontaneously or as a result of treatment with a macrofilaricidal drug (Figueredo-Silva *et al.*, 1996). It should be noted that not all natural or drug-triggered AFL episodes cause clinical illness. In many patients, granulomatous reaction develops around dead parasites in the lymphatics without any clinical outcome, and is only detected, incidentally, during physical examinations (Olszewski *et al.*, 1993). However, in other patients, AFL accompanied by local pain, swelling, and tenderness develops corresponding to the granulomatous inflammatory reaction around dying or degenerating parasites.

The studies of human biopsy specimens suggest that AFL is an acute inflammatory process that is triggered by products released from dying or disintegrating parasites, and that neither living nor completely calcified dead parasites cause the acute inflammatory changes (Lichtenberg, 1957; Cooray, 1960; Galindo *et al.*, 1962; Jungmann *et al.*, 1991; 1992).

Ultrasonographic and histopathological studies have documented an episode of AFL triggered by treatment with diethylcarbamazine (DEC) that the acute attack in this case occurs in a body site, where previously living parasites were killed by the drug (Dreyer *et al.*, 1995; Figueredo-Silva *et al.*, 1996; Noroes *et al.*, 1997). Because of the high prevalence of living adult *W. bancrofti* in the lymphatics of the spermatic

cord (Noroes *et al.*, 1996a; 1996b; Dreyer *et al.*, 1996), treatment-triggered acute filariasis is particularly common in the scrotal area. The local reactions observed under histopathological investigations of biopsy samples from patients infected with *W. bancrofti* reveal mild infiltration of inflammatory cells in an early phase following parasite death by DEC treatment (Figueredo-Silva *et al.*, 2002). In the later phase, the lymphatic vessel is occluded by granulomatous inflammatory reaction around dead parasites, with variable numbers of eosinophils, lymphocytes, plasma cells and large macrophages. Rarely, neutrophils may filtrate in the center of the granuloma. The systematic responses are characterized by significantly elevated levels of TNF-α levels, and a positive correlation between its levels and the severity of the AFL (Das *et al.*, 1996).

The simultaneous death of many adult worms resulting AFL is a risk factor for development of some types of the chronic manifestations, such as hydrocele, chylocele and chyluria (Dreyer *et al.*, 2000).

3.3 Acute dermatolymphangioadenitis by secondary bacterial and fungal infections

Dilatation of the lymphatic vessels induced by the presence of the adult parasite finally leads to lymphatic dysfunction, and accumulation of protein-rich fluid in the tissues. The lower limbs, in particular, become predisposed to recurrent bacterial infections. Trauma, interdigital fungal infections, and onchomycosis provide entry sites for bacteria, which multiply rapidly, and cause a reticular lymphangitis of the small collecting vessels (ADLA) (Jungmann *et al.*, 1992). Bacteria that are generally regarded as commensal or saprophytic have been isolated numerous times from the blood or tissue fluids during the acute attacks (Montestruc *et al.*, 1960; Olszewski *et al.*, 1997; Dreyer *et al.*, 1999). Early investigations emphasized the

etiological role of streptococcal infections. Recurrent bacterial infections are an important co-factor in the progression to lymphedema and elephantiasis.

4. *Wolbachia* of arthropods and filarial nematodes

Wolbachia is a genus of the class Alphaproteobacteria belonging to the order Rickettsiales (**Figure 4**). These gram-negative intracellular bacteria are found widespread in arthropods as well as in filarial nematodes (Werren, 1997; Bandi *et al.*, 1998). On the basis of 16S rDNA gene and groESL operon sequence analysis, it is organized into the family Anaplasmataceae, which also includes all the species of the genera *Ehrlichia*, *Anaplasma*, *Cowdria*, and *Neorickettsia* (Dumler *et al.*, 2001). In contrast to members of the family Rickettsiaceae, which grow in the cytoplasm or nucleus of their eukaryotic host cells, members of the Anaplasmataceae replicate while enclosed in a eukaryotic host cell membrane-derived vacuole.

Kingdom:	Bacteria
Phylum:	Proteobacteria
Class:	Alphaproteobacteria
Order:	Rickettsiales
Family:	Anaplasmataceae
Genus:	*Wolbachia*

Figure 4 Taxonomy of the genus *Wolbachia*.

Wolbachia cannot grow in a cell-free medium. Traditional methods for bacterial species and strain determination, which largely depend on pure culture of bacterial isolates, have not been used in the genus *Wolbachia*. In the absence of a formal nomenclatural system, the *Wolbachia* community currently refers to the different lineages as supergroups (Bandi *et al.*, 2003). In addition, the species name, *W. pipientis*, remains single until new data are generated in different research areas (*e.g.* comparative genomics, molecular phylogenetics, and screening for *Wolbachia* in new hosts). The DNA-sequence-based methods, including phylogenetic analysis based on 16S rDNA, dnaA, ftsZ, gltA, groEL and wsp genes have been employed (Bandi *et al.*, 1998; Bordenstein and Rosengaus, 2005). At present, eight (denoted A through H) taxonomic supergroups are described for the genus *Wolbachia* by their places in molecular phylogenies. These eight supergroups are labeled alphabetically and include A and B found in various arthropods, C and D restricted to filarial nematodes, E containing *Wolbachia* from springtails (*Folsomia candida*), and F containing *Wolbachia* from termites (*Kalotermes flavicollis* and *Microcerotermes* spp.), weevils (*Rhinocyllus conicus*), and the filarial nematode *Mansonella ozzardi* (Werren *et al.*, 1995; Bandi *et al.*, 1998; Vandekerchove *et al.*, 1999; Lo *et al.*, 2002) (**Figure 5**). The more recently proposed supergroups G and H are comprised of *Wolbachia* from Australian spiders (G), and the Pacific dampwood termites (*Zootermopsis angusticollis* and *Z. nevadensis*) (H) (Rowley *et al.*, 2004; Bordenstein and Rosengaus, 2005).

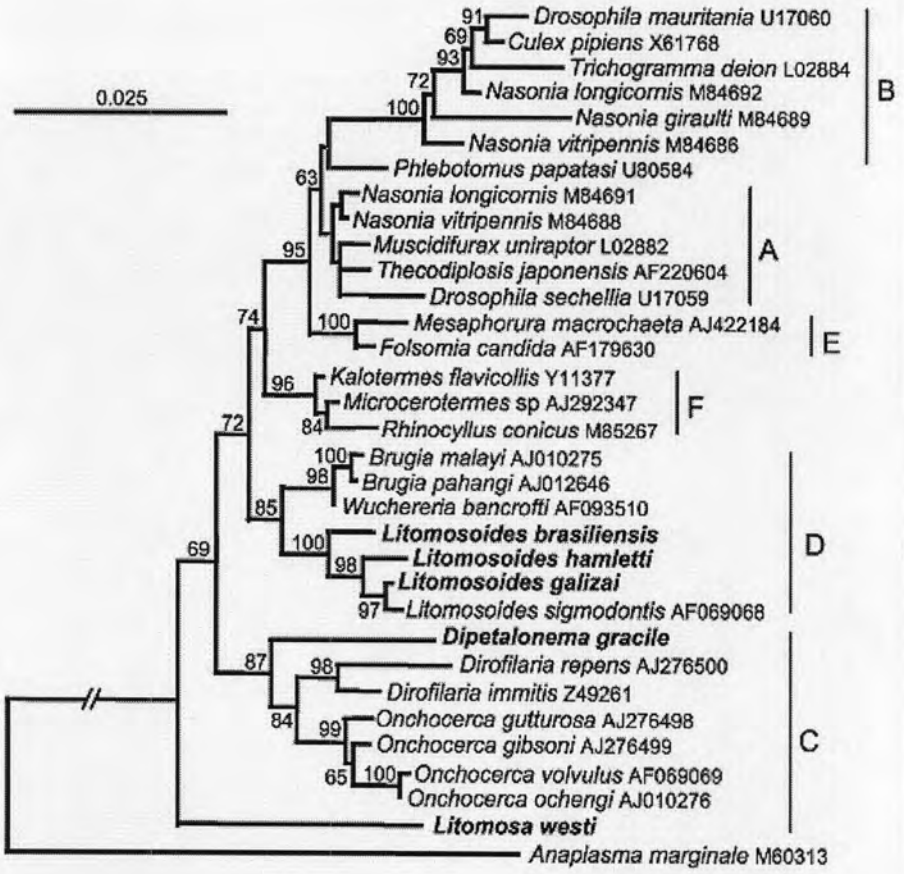

Figure 5 Phylogeny of *Wolbachia* based on 16S rDNA gene sequences (Casiraghi *et al.*, 2004). Representatives of *Wolbachia* supergroups A–F are shown. The supergroup F also includes the filarial nematode *Mansonella ozzardi* (Lo *et al.*, 2002).

4.1 *Wolbachia* of arthropods

In 1924, intracellular bacteria were firstly reported, as rickettsia-like microorganisms, within the ovaries and testes of the mosquito *Culex pipiens* by Hertig and Wolbach (Werren *et al.*, 1997; Stouthamer *et al.*, 1999). They were subsequently named *Wolbachia pipientis* (Werren *et al.*, 1997; Stouthamer *et al.*, 1999). Phylogenies based on 16S rDNA sequences have confirmed that morphological similarities to the Rickettsiae are based on phylogenetic relatedness (O'Neill *et al.*, 1992; Rousset *et al.*, 1992; Stouthamer *et al.*, 1993). It has been estimated that these bacteria infect at least 20% of all insect species (Haine and Cook, 2005). *Wolbachia* have also been found commonly in isopods (Rousset *et al.*, 1992) and mites (Jeyaprakash and Hoy, 2000). *Wolbachia* infecting the reproductive tissues of arthropods are transmitted maternally from infected females to their progeny via the egg cytoplasm, and have evolved to manipulate host reproduction.

Research interest in *Wolbachia* is initially sparked when it is discovered that they cause several kinds of reproductive changes in arthropod reproduction (Werren and O'Neill, 1997). These reproductive manipulations include (1) inducing embryonic lethality in insect embryos that result when uninfected females are mated to infected males (cytoplasmic incompatibility) (Sinkins *et al.*, 2004; McGraw and O'Neill, 2004; Mercot and Charlat, 2004), (2) inducing parthenogenesis in infected insects (the ability of infected unfertilized insect eggs to successfully develop into functional female adults) (Stouthamer *et al.*, 1993; Huigens *et al.*, 2004), and (3) overriding chromosomal sex determination in crustaceans to convert infected genetic males into functional phenotypic females (feminization of genetic males) (Rigaud, 1997; Moreau and Rigaud, 2003; Cordaux *et al.*, 2004). Each of these reproductive effects enhances transmission of *Wolbachia* to the arthropod population which is not infected with *Wolbachia* (Werren and O'Neill, 1997).

It has been proposed that the reproductive abnormalities induced by *Wolbachia* are of interest to applied biologists, who are looking for novel means to genetically manipulated populations of insect pests that are important for economic and health reasons (Beard *et al.*, 1993). For instance, in control of transmission of vector-borne diseases, this approach aims to express foreign anti-parasitic or anti-viral gene products in *Wolbachia* harbored by insects. Parasitoids used in biological control of insects may be more effective when infected with parthenogenesis *Wolbachia* (Stouthamer, 1993). *Wolbachia* and its hosts also are ideal candidates for the study of mechanisms of host-parasite relationship, the evolution of infectious diseases, specifically host resistance, parasite virulence, and transmission dynamics (McGraw and O'Neill, 1999).

4.2 *Wolbachia* of filarial nematodes

4.2.1 *Wolbachia*-nematode mutualistic relationships

In arthropods, *Wolbachia* act as a reproductive parasite in most of the known cases (Werren, 1997; Stouthamer *et al.*, 1999). However, filarial nematode *Wolbachia* behave differently from arthropod *Wolbachia*. There are evolutionary aspects as well as experimental studies suggest that the association between *Wolbachia* and filarial nematodes is obligatory mutualistic (Bandi *et al.*, 2001; Fenn and Blaxter, 2004b; Fenn and Blaxter, 2006). This term is described the association between species living together that neither species can survive under natural conditions without the other. The phylogeny of filarial nematode *Wolbachia* is in the main congruent with that of the filarial nematode hosts (Bandi *et al.*, 1998). In another words, the bacterial phylogeny splits at the same time as the filarial nematode phylogeny. It is an evidence of a close relationship between filarial nematode *Wolbachia*, and their hosts with a stable and long association. In addition, in filarial species positive for *Wolbachia*, the

prevalence of the infection appears 100% (Bandi *et al.*, 2001). There is also no evidence for multiple infections. The phylogenetic patterns and the distribution of filarial nematode *Wolbachia* appear more comparable to those generally observed in obligatory bacteria (Taylor *et al.*, 2005). Nevertheless, as reviewed above, some of the species within the *Wolbachia*-positive genus or the *Wolbachia*-positive subfamilies lack bacteria. For example, *O. flexuosa* has no *Wolbachia*, whereas all other *Onchocerca* species do. Similarly, *Litomosoides yutajensis* is found negative for the bacteria, whereas other members of genus *Litomosoides* harbor them. These indicate that the filarial nematodes might not be absolutely dependent on their intracellular bacterial partners in a long-term phylogenetic sense (Fenn and Blaxter, 2004b).

The information available on the evolutionary aspects and distribution of *Wolbachia* are in general agreement that the relationship between filarial nematodes and filarial nematode *Wolbachia* are likely dependent. *Wolbachia* have not been cultured outside their host cells. In addition, there is an experimental study implying that their habitation is species specific. Filarial nematode *Wolbachia* can be transferred from a naturally infected species, *L. sigmodontis* to a naturally uninfected one, *A. viteae.* However, the level of *Wolbachia* in *A. viteae* reduces along the time that the filarial nematodes are cultured in the Mongolian gerbils (*Meriones unguiculatus*), and *Wolbachia* cannot transmit to the filarial progeny (Hartmann *et al.*, 2003). The dependence indicates that *Wolbachia* should need some benefits from their filarial nematode hosts. On the other hands, *Wolbachia* could benefit their hosts some essentials. Investigation using antibiotics, such as tetracycline, which is known to be effective against *Rickettsiae*, have provided direct evidence for the existence of this dependence. However, the underlying molecular mechanism is largely unknown. The antibiotic showed detrimental effects on filarial nematodes which harbor *Wolbachia*, and no effects on filarial nematodes which do not have *Wolbachia* (*e.g. A. viteae*) (Hoerauf *et al.*, 1999; Bandi *et al.*, 1999).