

***Wolbachia* and termite association: present status and future implications**

Inherited symbionts play an important role in the ecology and evolution of many species. One such inherited symbiont, *Wolbachia*, is known to have many interesting and diverse symbiotic associations with its arthropod and nematode hosts, ranging from parasitism to mutualism (Werren *et al.* 2008). The organism is notable for significantly altering the reproductive capabilities of its arthropod hosts and manipulating their cell biology by inducing different phenotypes such as male killing, feminization, parthenogenesis, cytoplasmic incompatibility (sperm–egg incompatibility) and even speciation in certain species (Werren *et al.* 2008). These interactions become more interesting due to the spatial variation and phenotype of *Wolbachia* strains. *Wolbachia* strains that are genotypically very closely related can induce diverse phenotypic effects in different hosts, whereas different strains can induce similar phenotypic effects in the same hosts (Jiggins *et al.* 2002). Therefore, characterization of the *Wolbachia* genotype and its phenotypic effect in different hosts is important for understanding the ecology and evolution of different species. Since *Wolbachia* cannot be cultured outside host cells, traditional microbiological methods cannot be applied to study these bacteria. Currently, they are categorized into eleven different ‘supergroups’ (labelled alphabetically A–K) on the basis of clades formed in gene phylogenies (Lo *et al.* 2002; Bordenstein and Rosengaus 2005; Casiraghi *et al.* 2005; Ros *et al.* 2009).

Termites are a group of social insects usually classified in the taxonomic rank of order Isoptera, and described as ‘ecosystem engineers’ due to their important role in providing soil ecosystem services. They are major detritivores, particularly in the subtropical and tropical regions, and their recycling of wood and other plant matter is of considerable ecological importance (Harris 1971). Their ancient origin (Devonian period), great diversity and considerable ecological, biological and behavioural plasticity suggests that characterization of *Wolbachia* in this group is needed in order to understand the impact of the symbiont on termite reproduction, evolution and speciation (Roy and Harry 2007). The phenotypic effects of *Wolbachia* in Isoptera are still unknown but molecular data concerning these termite symbionts have recently become available. The available literature suggests the occurrence of *Wolbachia* in termite families Termopsidae, Kalotermitidae, Serritermitidae, Rhinotermitidae and Termitidae. Four phylogenetically different *Wolbachia* supergroups have been reported in termites. Twenty termite species have been reported to harbour *Wolbachia*. Out of these, thirteen species (*Kalotermites flavicollis*, *Coptotermites lacteus*, *Coptotermites acinaciformis*, *Cryptotermites secundus*, *Heterotermites* sp., *Nasutitermites takasagoensis*, *Nasutitermites* sp., *Nasutitermites nigriceps*, *Hospitalitermites medioflavus*, *Microcerotermites* sp., *Apilitermites longiceps*, *Labiotermites labralis*, *Microtermes* sp.) have supergroup F *Wolbachia* infection (Casiraghi *et al.* 2005; Lo *et al.* 2002; Lo and Evans 2007; Roy and Harry 2007). Two *Zootermopsis* species (*Zootermopsis nevadensis* and *Zootermopsis angusticollis*) carry supergroup H *Wolbachia* (Bordenstein and Rosengaus 2005). *Cubitermites* sp. *affinis subarquatus* harbour diverse types of *Wolbachia* belonging to the supergroup A and B clade (Roy and Harry 2007), *Incisitermites snyderi* carry supergroup A *Wolbachia* (Baldo *et al.* 2006), while *Wolbachia* from *Serritermites serrifer*, *Neotermites luykxi* and *Neotermites jouteli* belong to a divergent sister clade within supergroup A (Lo and Evans 2007). Termites, like other groups of arthropods, can tolerate *Wolbachia* of more than one supergroup, although individual species can harbour only single infections. This provides some evidence for the horizontal transmission of *Wolbachia*. Infection with different *Wolbachia* supergroups in various termite species can be parsimoniously explained by independent acquisition of these lineages in termites, rather than a single ancient ancestral infection, with subsequent divergence and/or widespread loss (Bordenstein and Rosengaus 2005).

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The utility of *Wolbachia* endosymbiosis to assess transitional steps between parasitism and mutualism in obligate intracellular bacteria has been well recognized (Lo *et al.* 2002, 2007; Bordenstein and Rosengaus 2005; Fenn and Blaxter 2006). Within the genus, there are four genetically defined lineages that can be discretely clustered into clades of mutualists that enhance filarial nematode fertility or viability (supergroups C and D) and clades of mostly reproductive parasites that distort sexual reproduction of their arthropod hosts to enhance their own transmission through the matriline (supergroups A and B) (Bandi *et al.* 1998; Bordenstein *et al.* 2009). Supergroups E, H, I, J and K are found in various arthropods but their host effects are currently unknown. Supergroup G is reported to occur in spiders but has been suggested for temporary removal from phylogenetic analysis due to insufficient and inconsistent data (Baldo and Werren 2007). Supergroup F is interesting and has been found to infect both arthropods as well as members of the filarial nematode genus *Mansonella* but the phenotypic effects induced by these groups are still unknown.

Despite attempts to reconstruct the ancestry of mutualism and parasitism in this bacterium, only one large-scale phylogenomic dataset has been performed and claims to position the root between the arthropod A and B parasites, and the nematode C and D mutualists (Fenn *et al.* 2006). However, a recent study by Bordenstein *et al.* (2009) demonstrated that exploiting an amino acid mixture model and excluding mutationally saturated genes are crucial aspects of correctly accounting for the systematic error associated with the exceedingly long branch lengths between the last common ancestor of the ingroup and that of the outgroup genera. Even with the most taxonomically rich, phylogenomic dataset of the *Wolbachia* clade, careful gene selection and heterogeneous inference models, long-branch attraction (LBA) artifacts preclude an accurate rooting of the *Wolbachia* tree. The findings suggest that all past reconstructions using the Anaplasmataceae outgroups are fatally flawed due to LBA artifacts. The study also suggested that the evolutionary relationships of *Wolbachia* parasites and mutualists remain both unresolved and unresolvable until more suitable outgroup taxa or more taxonomic characters from full genome sequences of all the major *Wolbachia* supergroups become available. Despite this, unrooted phylogenetic trees are still helpful in understanding the supergroup affiliations of *Wolbachia* strains. A phylogenetic tree was constructed to take a collective look at different termite *Wolbachia*, by retrieving *ftsZ* gene sequences for *Wolbachia* of termites and different supergroup hosts from the GenBank database. Phylogeny suggests the possibility of horizontal transfer events for termite *Wolbachia* of supergroups A, B and F. However, supergroup H seems to be restricted only to *Zootermopsis* spp. (figure 1). In terms of geography, so far, *Wolbachia* have been identified from termite host species present in Europe, North America, Africa, South-East Asia and Australia. A strict geographical congruence between *Wolbachia* from termite species is not observed in phylogeny, and country-wise relatedness is not observed for termite *Wolbachia*, with distantly related hosts from different countries sharing closely related strains (figure 1).

There could be many implications of the association between *Wolbachia* and termites. *Wolbachia* are found to be mutualistic for nematodes by providing them with nutritional benefits. For many insects, the nutritional importance of microorganisms is profound, particularly for the provision of vitamins and utilization of cellulose and other plant cell wall components. Do *Wolbachia* provide similar benefits to termites for growth and reproduction as in the case of nematodes? In this respect, association between supergroup F *Wolbachia* and their termite hosts can be an interesting aspect to study as similar supergroup *Wolbachia* have been detected in the filarial nematode of the genus *Mansonella*. Most of the supergroups A and B *Wolbachia* comprise parasitic bacteria that are known to manipulate the reproductive biology of their hosts for their own survival. Studies on the parasitic impact of *Wolbachia* on individual termite hosts can provide important information about termite behaviour. Supergroup H *Wolbachia* are known to occur in only two Pacific dampwood termites (*Zootermopsis* spp) located in the Cascade and Sierra Nevada Mountains of the United States (Bordenstein and Rosengaus 2005). Is supergroup H endemic to these specific regions and restricted to these two termite species only? What would be role of supergroup H *Wolbachia* in host biology? These can be interesting issues for future investigations.

Wolbachia have not yet been detected in vertebrates and no direct effect on vertebrate hosts has been reported, but their indirect effects have been positively correlated in disease-transmitting nematodes. *Wolbachia*-associated molecules have been found to be contributing to the pathogenesis of heartworm disease in dogs (McCall *et al.* 2004). *Wolbachia* associated with these disease-causing filarial worms seem to play an inordinately prominent role in these diseases. A large part of the pathogenicity of filarial

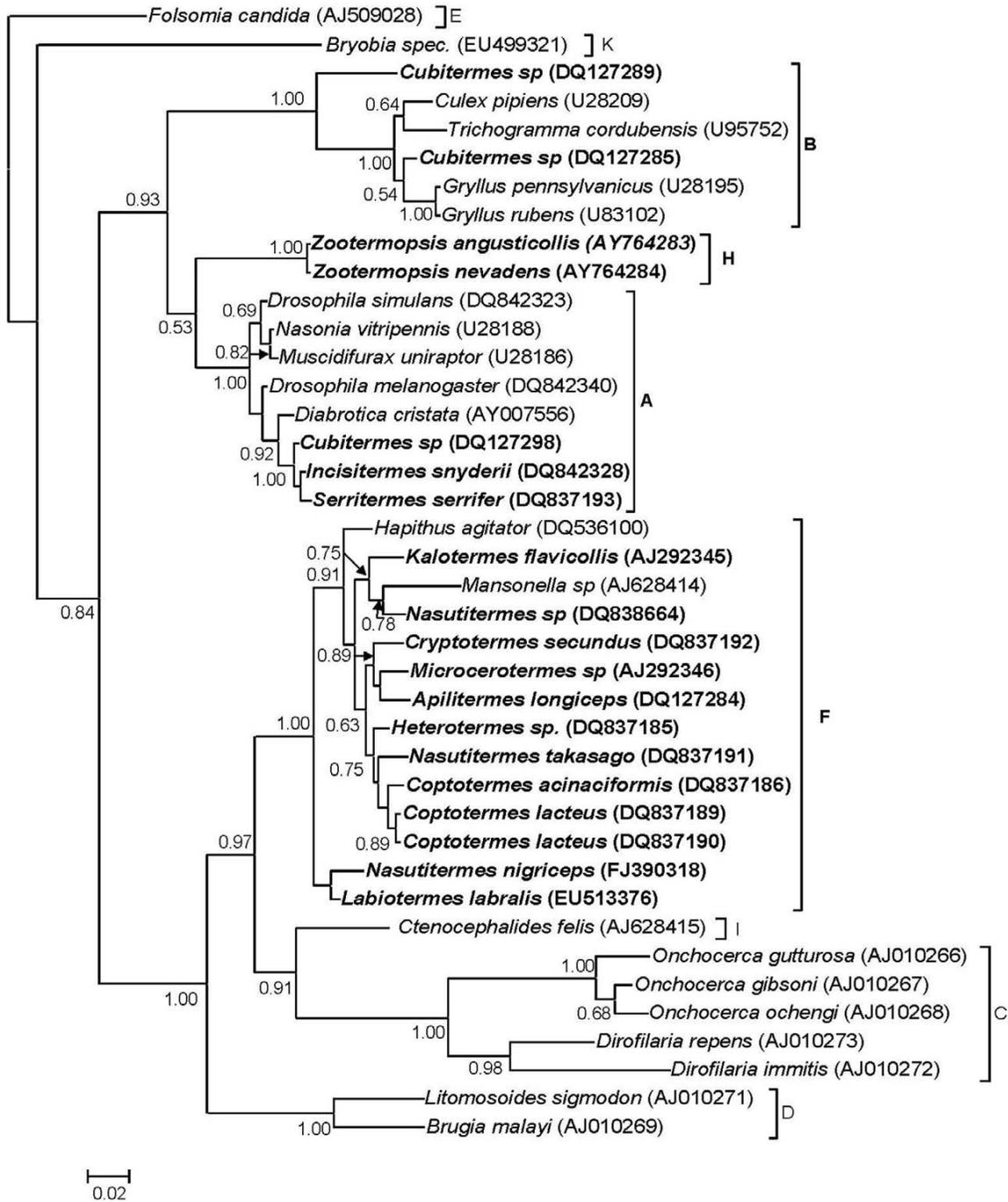


Figure 1. Unrooted phylogenetic relationships between *Wolbachia* from termites (bold) and those infecting other organisms representing different supergroups from GenBank, based on *ftsZ* alignment (563 bp). A phylogenetic tree was constructed using Bayesian inference and the neighbour-joining methods for the dataset. For Bayesian inference of phylogeny, the program MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001) was used. Topology was inferred using the program MrBayes, with GTR+G as a model for nucleotide substitution. Levels of confidence for each node are shown in the form of posterior probabilities. Accession numbers for each strain are shown after the name of each species in parentheses. *Wolbachia* supergroups are shown to the right of the host species' names. Supergroup G was not included in the analysis and supergroup J sequence for the *ftsZ* gene is not available in the database. Scale bar represents substitutions per site.

nematodes is due to the host immune response toward their *Wolbachia*. Elimination of *Wolbachia* from filarial nematodes generally results in either death or sterility (Hoerauf *et al.* 2003). Consequently, current strategies for the control of diseases caused by filarial nematodes include elimination of *Wolbachia* via the simple antibiotic doxycycline rather than far more toxic anti-nematode medications (Taylor *et al.* 2005). About 10% of the estimated 4000 termite species are economically significant as pests that can cause serious structural damage to buildings, crops or plantation forests. Functional studies of the termite–*Wolbachia* association on similar lines as that in nematodes may be able to provide solutions to the control of termites that act as pests affecting human belongings.

The presence of *Wolbachia* in termites and their effect on human health have not been studied yet. Termites are valuable sources of proteins, fats and essential amino acids, and are therefore used in the diets of both primates and modern humans around the world (Harris 1971). Their high protein content makes them to be used as an important component of the diet of pregnant women. They can also be used to enhance lactation in women and improve the health of children. Termites are successfully used in Indian folk medicine for the treatment of diseases. Certain tribes in the southern and eastern parts of India have been using termites for the treatment of asthma, a disease likely to be worsened by viral infection (Solavan *et al.* 2004; Wilsanand 2005). *Wolbachia* have been found to provide resistance to insect hosts (*Drosophila*) against disease-causing viruses (Hedges *et al.* 2009). Termites have been reported to produce certain antimicrobial peptides. Do *Wolbachia* have role in the production of these peptides? The interesting prospect here would be to test the role of *Wolbachia* in termites with respect to human health, an aspect that has never been explored.

Advances in genomics are helping us to understand insect–microbe interactions. Parallel sequencing technologies and bioinformatics tools for analysis represent tremendous opportunities for the elucidation of complementary metabolic capabilities and the total gene pool of *Wolbachia* in termites. The genomic information of termite *Wolbachia* could further help to understand the exact interactions in detail. *Wolbachia* genomes (*wMel*, *wBm*, *wPip* and *wRi*) have been completely sequenced, several other partial genomes have been published and sequencing of several other genomes is under way. Genomic information of termite *Wolbachia* and comparative studies with available genomes can throw light on the nature of their association with termites, their contribution to the ecology of termites in the context of physiology, and interactions with the wider environment.

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