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Wolbachia endobacterium in wild population of *Aedes albopictus* (Skuse) (Diptera: Culicidae) and phylogeny from Andaman and Nicobar Islands, India

Arun Sivan, A.N. Shriram, Debdutta Bhattacharya & P. Vijayachari

Regional Medical Research Centre (Indian Council of Medical Research), Port Blair, Andaman & Nicobar Islands, India

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Wolbachia endosymbionts are gram-negative, maternally inherited intracellular alpha-proteobacteria, known to infect a wide range of invertebrates which include insects, mites, isopods, crustaceans and filarial nematodes[1]. Maternal inheritances have been detected in 20–75% of the invertebrate species. Among eight major clades (A–H) of *Wolbachia* clades, A, B, E, F, G and H have been detected in insects, arachnids and crustaceans; while C and D have been documented from filarial nematodes[2–3]. Effects of *Wolbachia*-host interactions are manifold, *viz*. mutualistic to pathogenic, parthenogenesis, cytoplasmic incompatibility, selective male killing and feminization[1].

Diversity of *Wolbachia* infection in mosquito genera has been reported from Southeast Asia, Europe and Africa[4]. Information on the association of *Wolbachia* with *Aedes albopictus* is limited. Recently, it is reported that *Wolbachia* clades *w*AlbA and *w*AlbB co-infect *Aedes albopictus*[5–6]. *Aedes albopictus*, the Asian tiger mosquito, native to Asia is an invasive species. Instances of introduction and establishment of *Ae. albopictus* are evident[7] and in recent times the distribution has spread to Africa, Europe and Australia[8]. In addition to being an invasive species, this mosquito is an aggressive day time biter and has been implicated in the transmission of dengue[9] and chikungunya[10].

It has been the premise that *Wolbachia* endosymbionts have a role in the host speciation through reproductive isolation which they cause in the infected hosts. Besides, they do provide an interesting and wide array of evolutionary signals to genetic conflicts[11]. The ability of *Wolbachia* to alter reproductive capabilities of its host enables it to suppress insect populations[12]. Against this background, *Wolbachia* is being explored as an alternative tool for the control of *Ae. aegypti* and *Ae. albopictus*. But before such an approach is explored, it becomes necessary to assess the presence of endosymbiont in *Ae. albopictus*. Hence, a preliminary study was undertaken to screen for the presence of these endosymbionts in the wild population of *Ae. albopictus*, from the Andaman and Nicobar archipelago, endemic for dengue[13] and where sporadic cases of chikungunya are being observed since the first ever upsurge in 2006[14], hitherto reported for wide prevalence of *Aedes* spp[15]. We used polymerase chain reaction for detecting *Wolbachia* among *Ae. albopictus*, and determined its phylogenetic relationship with other known species using the partial genomic nucleotide sequence of *Wolbachia* surface protein (*wsp*) gene.

The study was carried out in three localities, *viz*. Haddo, Old Pahargaon and Bambooflat in South Andaman district. In addition, one locality from Car Nicobar (Nicobar district), located at a distance of about 540 km from Port Blair was also included in the study. This island is geographically isolated and separated by vast stretch of sea. Larval and adult samples of mosquitoes were collected during November 2011. Specimens of mosquitoes were morphologically identified using standard taxonomic keys[16].

DNA extraction was carried out from individual mosquito sample using DNA extraction solution kit (Genie, Bengaluru, India), following manufacturer's instructions and subjected to PCR amplification using *Wolbachia* specific primers to amplify the *wsp* gene (650 bp) using primers *wsp* 81F [5'-TGG TCC AAT AAG TGA TGA AGA AAC-3'] and *wsp* 691R [5'-AAA AAT TAA ACG CTA CTC CA-3']17. Detection of *Wolbachia* species was carried out by PCR using the group specific *wsp* primers for A group; (136A; F5'-TGAAATTTTACCTCTTTTC-3 and 691R; 5-'AAAAATTAAACGC TACTCCA-3') and for B group (81F; 5'-TGG TCCAATAAGTGATGAAG AAAC-3' 522R; 5'-ACCAGCTTTT GCTTGATA-3') following a method reported earlier[18].

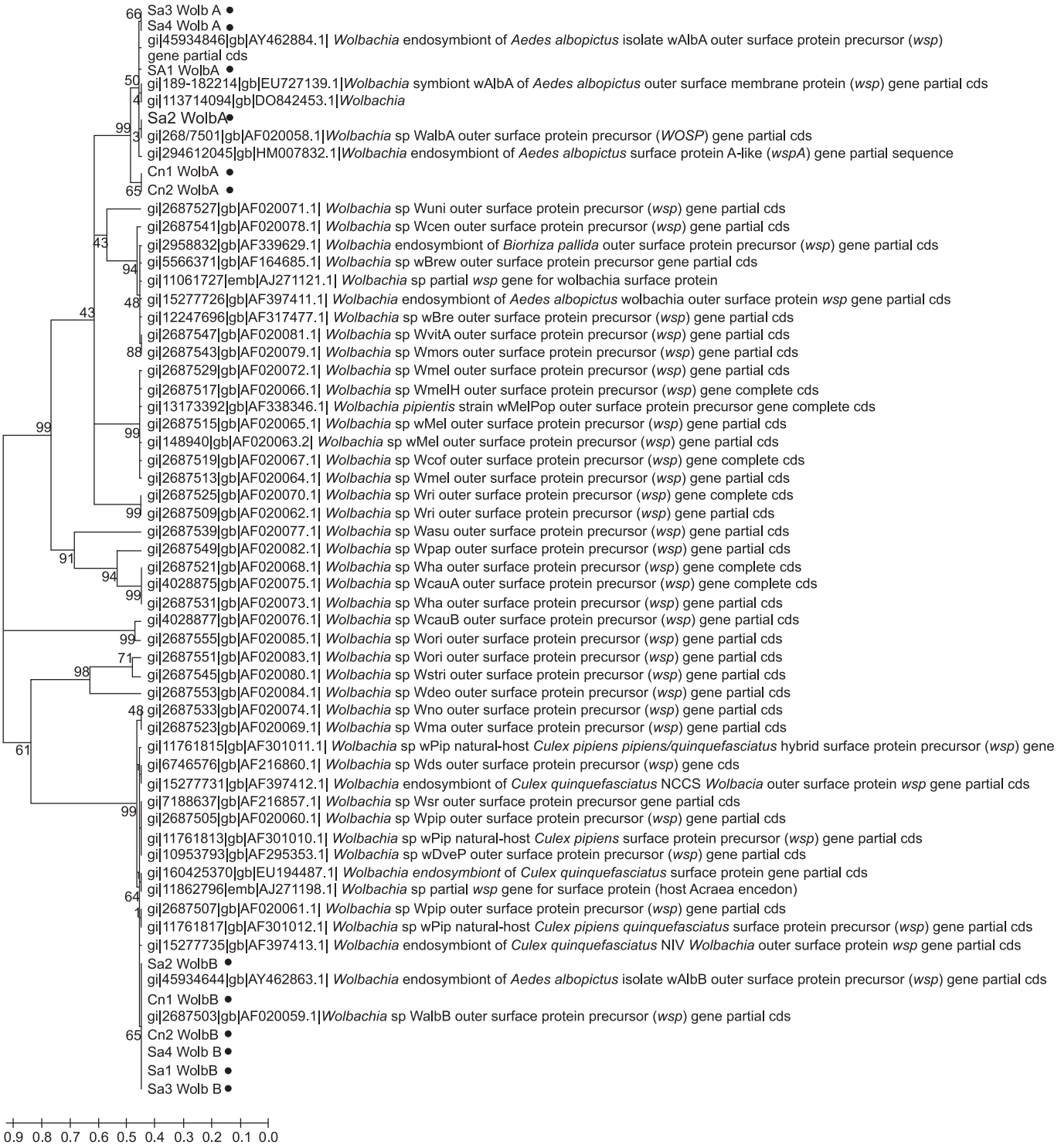

Fig. 1: Phylogenetic tree based on *wsp* gene sequences of *Wolbachia* sp (650 bp), constructed from MEGA4 distances and the neighbour-joining algorithm isolated from *Aedes albopictus* collected from Andaman island. The numbers near the nodes indicate percentage of 1000 bootstrap replicates. The scale bar indicates genetic distance. The GenBank accession numbers are also mentioned. The Andaman & Nicobar Islands isolates are indicated with black dots.

The amplified products were sequenced (Genetic Analyzer 3130, ABI) following the manufacturer's protocol (ABI, USA). The phylogenetic analysis of the *wsp* gene sequence was BLAST analyzed at NCBI. Multiple sequence alignment was done with closely related sequences using CLUSTAL W programme at EBI site. The phylogenetic

trees were constructed using Kimura-2-distances and the neighbour-joining algorithms in the MEGA 4.0 software.

A total of 57 mosquito specimens were collected from the South Andaman, *viz*. Bambooflat (12), Old Pahargaon (13), Haddo (24) and Car Nicobar (8), respectively. All the mosquito samples collected from South Andaman and Car Nicobar Islands were found positive for the presence of the *Wolbachia* species. Further, PCR assay using species-specific primers revealed that all the *Ae*. *albopictus* were co-infected with both the species of *Wolbachia*, *Wolbachia* type A and B. The neighbour-joining phylogenetic analysis of the *wsp* gene of *Wolbachia* sequence study showed close relationship with the known sequences of *wsp* gene obtained from the NCBI database (Fig. 1). The phylogeny also revealed grouping of *w*AlbA and *w*AlbB with other sequences belonging to A and B group, respectively (Fig. 1). The *wsp* gene sequence from the present study showed 99% nucleotide sequence homology with *Wolbachia* sp in *Ae*. *albopictus* from different geographical locations of India, USA, China, France, United Kingdom and Taiwan.

Association of *Wolbachia* endosymbiont with *Culex* and *Aedes* genera include several medically important species, which have been implicated in the transmission of CHIKV, DENV, JEV and *Wuchereria bancrofti* in India[6]. Understanding the phylogeny of *Wolbachia* endosymbiont, based on the fast evolving gene *wsp*, has been well established[19]. Therefore, based on the understanding of *wsp* gene phylogeny, two distinct types of *Wolbachia* species have been identified and associated with *Ae*. *albopictus,* i.e. one belonging to the Pip group of B super group while the other to the AlbA group of A super group[18]. In the present study, both the reported strains (*w*AlbA and *w*AlbB) inhabited the same host *Ae*. *albopictus*. Further, 100% infection of both *w*AlbA and *w*AlbB was observed in the vector mosquitoes collected from South Andaman district and geographically isolated Car Nicobar Islands, which has shown evidence of antibodies to dengue and chikungunya viruses (personal communication, NVBDCP, A & N Islands).

It has been reported that in the Thailand population of *Ae. albopictus*, the field prevalence of co-infection of these endosymbionts was observed to be 99.4%[5]. On the other hand, it has been hypothesized that the reported *w*AlbA in Indian population of *Ae*. *albopictus* represents a novel strain of *Wolbachia* sp[17]. Co-infection of *w*AlbA and *w*AlbB has not been observed in other mosquito genera, except *Aedes* and *Toxorhynchites*[6]. Further, phylogenetic analysis of the 16S rRNA gene sequences have indicated a 2% sequence divergence and separate *Wolbachia* into two distinct groups, those that infect *Culex* group of mosquitoes belong to the B group, while those which reside in *Aedes* are members of the A group[20].

In conclusion, the current study showed that *Wolbachia* endosymbionts (*w*AlbA and *w*AlbB) occurred in the population of *Ae*. *albopictus* in the Andaman and Nicobar archipelago. These observations are central to the understanding of the actual prevalence of these endosymbionts in *Ae*. *albopictus*, with larger mosquito samples from a wider area. Improved understanding of the prevalence pattern of these endosymbionts, their role in vector biology and competence for transmitting the viruses, are warranted for developing alternative control tools.

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Correspondence to: Dr A.N. Shriram, Regional Medical Research Centre (ICMR), Post Bag No.13, Port Blair–744 101, Andaman & Nicobar Islands, India.
E-mail: anshriram@gmail.com; shriraman@icmr.org.in

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