First report on the presence of natural *Wolbachia* population from major malarial vector mosquitoes *Anopheles culicifacies* s.l., and *Anopheles stephensi* from Tamil Nadu, India

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Abstract

Wolbachia is an alpha-proteobacteria present in several arthropods. The present study focussed on the identification of *Wolbachia* in wild malarial vector mosquitoes. This was achieved by molecular identification of *Wolbachia* from collected mosquitoes. A total of four hundred and eight seven mosquito samples were collected. Morphometric and molecular analysis revealed that they belong to *Anopheles culicifacies* s.l., (48.25%) and *Anopheles stephensi* (51.75%). The presence of *Wolbachia* was identified using *16S* rRNA, *wsp* and *FtsZ* genes, where nested PCR of *16S* rRNA alone was successful and then sequenced. Only seven mosquitoes (1.4%) were positive for *Wolbachia*. *In silico* and restriction digestion of *16S* rRNA gene product using RsaI enzyme showed that the identified *Wolbachia* belongs to supergroup B. The prevalence rate of natural *Wolbachia* was lesser in native malarial vector *An. culicifacies* s.l. and *An. stephensi* was about 1.7% and 1.2%, respectively. This is the first report on the presence of *Wolbachia* in *Anopheles culicifacies* s.l. and *Anopheles stephensi*.

Keywords: Anopheles culicifacies s.l., *Anopheles stephensi*, *Wolbachia*, malarial vector, 16S rRNA, endosymbiont.

Introduction

Wolbachia is an intracellular alpha-proteobacteria found in a wide range of arthropods. It was first discovered by Hertig and Wolbach in 1924 considered to be the abundant endosymbiont found in invertebrates (Hertig & Wolbach 1924) and cause reproductive abnormalities (cytoplasmic incompatibility, male killing, parthenogenesis and feminization) in the host (Werren 1997, Werren et al 2008). This endosymbiotic proteobacteria naturally infect 65% of insect species (Hilgenboecker et al 2008, Werren 1997, Werren et al 1995a) including the family *Culicidae* (de Oliveira et al 2015, Sicard et al 2019). So far *Wolbachia* wildtype is reported in the following mosquito species: *Culex* (*Cx.*) *pipiens* (Hertig & Wolbach 1924), *Cx. quinquefasciatus* (Mahilum et al 2003) *Aedes* (*Ae.*) *albopictus* (Dutton & Sinkins 2004, Sinkins et al 1995), *Ae. aegypti* (Coon et al 2016) and few *Anopheles* species (Baldini et al 2014, Gomes et al 2017).

The natural occurrence of *Wolbachia* in *Anopheles* species has not been extensively studied. The *Anopheles* genera of *Culicidae* consists of 537 species (Harbach 2013), where 41 species are dominant vector species (DVS) responsible for the transmission of malaria (Hay et al 2010). Among the 41 DVS, 19 species/species complex were found within Asian-Pacific region (Sinka et al 2011). As per WHO, 228 million malaria cases occurred worldwide in 2018; where India is one among the twenty countries that carries 85% of the global malarial burden (WHO 2019). In India, *An. baimaii*, *An. fluviatilis*, *An. minimus*, *An. sundaicus*, *An. culicifacies* complex species (A, B, C, D & E) and *An. stephensi* are primary vectors in transmitting malaria, with *An. culicifacies* complex and *An. stephensi* as major contributors (Subbarao et al 2019). *An. culicifaciess. s.l.* is widely distributed in rural, semi-urban and forest areas (Dev & Sharma 2013, Goswami et al 2006, Subbarao et al 2019) and, *An. stephensi* in peri-, semi- and urban areas (Dev & Sharma 2013, Subbarao et al 2019).

In Tamil Nadu, *An. stephensi* (Arjunan et al 2015, Sharma & Hamzakoya 2001, Surendran et al 2019) and *An. culicifacies* (Arjunan et al 2015, Kar et al 1999, Suguna et al 1983) are the main malaria vectors. Interestingly, few reports on natural *Wolbachia* endosymbionts in major malarial vector *Anopheles* species are reported including *An. gambiae*, *An. coluzzii* (Baldini et al 2014, Gomes et al 2017), *An. arabiensis* (Baldini et al 2018) and *An. moucheti* (Ayala et al 2019, Jeffries et al 2018). The present study aimed at the investigation of

Wolbachia infection in wild mosquitoes population collected from different geographical locations in Tamilnadu.

Materials and Methods

Mosquito collection and taxonomy

Mosquito samples were collected from the 5 different locations along the foothills of the Western Ghats, Southern India (Fig.1). Adults were collected using nets and aspirators. Mosquitoes were identified initially by taxonomic keys (Christophers 1933, Das et al 1990) and later verified by DNA barcoding.

DNA extraction and species identification

DNA isolation from individual mosquito was carried out using the Qiagen Blood and Tissue kit (Qiagen) with slight modification. The initial lysis step post homogenization in PBS was carried-out with proteinase K and lysis buffer at 56°C for 3 hr. Genomic DNA extracted was subjected to *COI* (cytochrome C oxidase subunit I) gene amplification using primers reported by Folmer and colleagues (1994) for identification of the mosquito species.

Wolbachia detection by PCR

For *Wolbachia* detection, three different sets of primers targeting conserved genes namely *16S* rRNA gene (Werren & Windsor 2000), *Wolbachia* surface protein (*wsp*) gene (Zhou et al 1998) and *FtsZ* cell cycle gene (Werren & Jaenike 1995) were used for screening. In addition, for low infection detection, a nested PCR using internal primers targeting 412 bp of *16S* rRNA gene was used (Shaw et al 2016). Multilocus strain typing (MLST) of *Wolbachia* was done by targeting five conserved genes *gatB*, *coxA*, *hcpA*, *ftsZ* and *fbpA* as described earlier (Baldo et al 2006). The primer details are given in Supplementary Table 1.

Molecular phylogenetic studies

The *Wolbachia* positive samples were further sequenced. The sequencing was carried out from the PCR products of *16S* rRNA (nested PCR) and *COI* from *Wolbachia* and *Anopheles* respectively and, Sanger sequenced (Barcode Biosciences Pvt. Ltd, Bangalore). The contig assembly was done using MEGA7. The assembled sequences were submitted to GenBank, ENA

Database and accession numbers were obtained. Additional mosquito and *Wolbachia* sp. sequences were collected from GenBank to clarify the interspecies relationship. The phylogenetic tree was constructed by maximum likelihood (ML) analysis in MEGA7 software. The tree inference options were set as follows: Heuristic Method Nearest-Neighbor-Interchange (NNI) with the very strong branch swap filter with 1000 bootstrap replicates, gaps were treated as missing. The number of restriction sites for RsaI was studied using multiple sequence alignment (MSA, Clustal Omega) to find out the supergroups of *Wolbachia* strains reported from this study.

Results:

Mosquito samples were collected from five different study sites **(**Fig.1**)**. Four hundred and eighty-seven mosquito samples belong to the genus *Anopheles* was screened for the presence of *Wolbachia*. The overall study shows *An. stephensi* (51.75%) population was higher in comparison to *An. culicifacies*. The population of *An. stephensi* was higher in Srivilliputtur (70%), while *An. culicifacies* was higher in Cumbum (69.79%) (Table 1).

To identify the presence of *Wolbachia* endosymbiont in mosquito, the genes *wsp* and *FtsZ* genes (Supplementary Table 1) were amplified; however, no positive results were obtained. MLST by standard primers and protocols did not yield any positive results in all the samples tested (data not shown). Interestingly, nested *16S* PCR amplification targeting the inner region of the *16S* rDNA gene results in positive outcome indicating the presence of *Wolbachia* endosymbiont in *Anopheles* mosquitoes. Out of 487 samples only seven samples i.e., 1.4% were positive for *Wolbachia* endosymbiont, where 3 are from *An. culicifacies* (MN268747, MN268748, MN268749) and 4 from *An. stephensi* (MN268743, MN268744, MN268746 and MN268750) (Table 2). MSA of *Wolbachia* strains reported the presence of four restriction sites (GTAC) for RsaI in agarose gel electrophoresis (data not shown) and *in silico* (Fig.2), indicating the isolates belong to supergroup B.

The phylogenetic analysis showed that all the *Wolbachia* strains reported from this study (Table 3) were grouped under Supergroup B and non-monophyletic (Fig.3). The *Wolbachia* isolates reported from this study showed high sequence similarity within (99.05% to 99.76%)

and also with the reference sequences used in the study. The isolate TS3 (MN268749) formed a separate clade (Clade I) within supergroup B with three other strains reported from *Drosophila simulans* (NC021084.1) and *Drosophila mauritiana* (NZ_CP034334 and NZ_CP034335) supported with 65% bootstrap value (Fig.3). It also shares 98.55% sequence similarity with all the three reference sequences (Supplementary Table 2) and diverse (0.005±0.005; Supplementary Table 3). The sequence of TS4 forms a distinct clade (Clade II) supported by 64% bootstrap value with *Wolbachia* sequence reported from *Aedes albopictus* (CAGB01000162) and *Culex decens* (MK026556 and MK026557) (Fig.3) and, share 99.04% and 98.99% sequence similarity respectively (Supplementary Table 2). No genetic divergence was observed in Clade II (Supplementary Table 3). Rest of the isolates forms a separate clade (Clade III, Fig.3). They shared high similarity ranging from 99.28-98.57%. *Wolbachia* sequence from *Chrysomya megacephala* (NZ_CP021120.1) (Supplementary Table 2) and were less diverse (0.003±0.002) compared to within group Clade I (Supplementary Table 3). The genetic diversity within group of supergroup B was less (0.005±0.003) and there was no significant diversity observed within supergroup A (Supplementary Table 3). The genetic diversity between supergroup A and supergroup B was 0.02±0.01 (Supplementary Table 4a).

All the seven *Wolbachia* positive mosquitoes were amplified using insect *cytochrome C oxidase I* (*COI*) gene (Table 4) and confirmed at species level. The phylogenetic analysis shows there were two clades *An. stephensi* and *An. culicifacies* and, both were supported by 99% bootstrap value (Fig.4). Among the seven *Wolbachia* positive samples, four belongs to *An. stephensi* (LR736010, LR736012, LR736013 and LR736014) and three belongs to *An. culicifacies* s.l. (LR736007 - LR736009) (Table 2). *An. stephensi* showed highest similarity with KX467337, MH538704, KF406680 and NC_028223 (Fig.4) with a high in-group diversity of 0.052±0.01 (Supplementary Table 5). Interestingly, LR736012 has shown to be divergent from other *An. stephensi* reported. *An. culicifacies* showed high similarity with other *An. culicifacies* strains (KR732656, KJ010898, DQ424962, KJ010896) and supported with 92% bootstrap value (Fig.4) and has low diversity (0.035±0.007) within the analyzed group (Supplementary Table 5). The diversity between *An. stephensi* and *An. culicifacies* was high (0.16±0.03, Supplementary Table 6).

Discussion:

The prevalence of *Wolbachia* has been reported in several arthropods including the orders *Coleoptera*, *Diptera*, *Hemiptera*, *Homoptera*, *Hymenoptera*, *Lepidoptera* and *Orthoptera* (de Oliveira et al 2015, Werren et al 1995a). Among the *Diptera*, *Wolbachia* is been reported in several *Culicidae* including *Aedes*, *Culex* and *Coquillettidia* (de Oliveira et al 2015, Ricci et al 2002). Novel *Wolbachia* infection in *Anopheles* species was least reported (de Oliveira et al 2015, Kittayapong et al 2000, Ricci et al 2002). In this study, we report the occurrence of natural *Wolbachia* endosymbiont in wild *An. stephensi* and *An. culicifacies* for the first time.

Diversity of isolated *Wolbachia*:

Wolbachia was identified using *16S* rRNA gene (O'Neill et al 1992, Rousset et al 1992); however fine-scale phylogeny was not possible due to low evolutionary divergence of *16S* rRNA, thus *FtsZ* (Werren et al 1995b) and *wsp* (Zhou et al 1998) genes were used. Several studies reported that *FtsZ* and *wsp* was unsuccessful (Baldini et al 2014, Marcon et al 2011, Wong et al 2020) and, our results are similar where amplification using *wsp* and *Fts*Z primers yielded no positive results. Nested PCR amplification of the inner region of *16S* rRNA was used in case of low-intensity *Wolbachia* infection (Shaw et al 2016) and proved to beneficial in identifying *Wolbachia* infection in *Anopheles* mosquitoes (Baldini et al 2018, Niang et al 2018, Shaw et al 2016, Wong et al 2020); similarly in our study we detected *Wolbachia* from *An. stephensi* and *An. culicifacies.*

Baldo and colleagues (2006) proposed genotyping *Wolbachia* using MLST (*gatB*, *coxA*, *hcpA*, *ftsZ* and *fbpA*). In some cases, MLST in *Wolbachia* were not successful (Baldo et al 2006) possibly due to primer sequence divergence (Gomes et al 2017) and low infection densities (Jeffries et al 2018). Bleidorn and Gerth (2018) have pointed out MLST loci are not suitable markers to study either genome-wide divergence rate or strain identification. However in our study, MLST amplification was unsuccessful and, it might be due to low infection density or primer sequence divergence or both.

Werren and colleagues (1995b) have reported *16S* rRNA can be used to distinguish between A and B supergroup due to the absence of RsaI restriction site in *Wolbachia* belonging

to group A. Interestingly, Pourali and colleagues (2009) have shown the presence of RsaI restriction site in supergroup A; however they have shown there was more RsaI site in B than A. We have performed *in silico* RsaI restriction site search in *16S* rRNA gene on reported *Wolbachia* supergroup A (EU096232, NC_002978.6, NC_012416, KP089991), supergroup B (NC_021984, CAGB01000162, MH967031) and our isolates; supergroup A has two restriction sites for all strains except KP089991 (*Wolbachia* from *An. coluzzii* (Buck et al 2016)) that has three restriction sites, which is possible due to recombination; our isolates and supergroup B shows four restriction sites (Fig.2).

The *16S* rDNA phylogeny shows all the *Wolbachia* isolates from this study belongs to supergroup B and it's non-monophyletic. Similar to our observation, recent reports (Gomes et al 2017, Sawasdichai et al 2019, Wong et al 2020) show supergroup B is polyphyletic. Though our samples are in separate clades, the genetic diversity within the group (Supplementary Table 3) and clades of supergroup B was less (Supplementary Table 4b). This shows that the nucleotide substitution or recombination was minimum in *16S* rRNA gene.

Diversity of *Anopheles* mosquitoes and *Wolbachia* prevalence:

The genus *Anopheles* belongs to the family *Culicidae* that comprises of 465 species further divided into seven subgenera. *Anopheles* is one among the subgenera consists of 182 species (Harbach 2004, Harbach 2013). The molecular phylogeny of *Anopheles* was limited to lower level classification respective to malarial vector and, morphologically defined groups found to be monophyletic (Harbach 2004). Similarly in our study, *An. culicifacies* and *An. stephensi* was first identified morphologically; later identified by *COI* gene amplification and, observed to be monophyletic within their respective species.

To till date, the prevalence of *Wolbachia* has been reported in 20 species of wild *Anopheles* mosquitoes. Baldini and colleagues (2014) for the first time reported *Wolbachia* in wild *An. gambiae* and *An. coluzzii* from Burkina Faso. Later *Wolbachia* was reported in other wild *Anopheles* mosquitoes including *An. gambiae* (different from Burkina Faso (Gomes et al 2017)), *An. arabiensis* (Baldini et al 2018), *An. moucheti*, (Ayala et al 2019, Jeffries et al 2018), *An. funestus* (Niang et al 2018), *An. melas* (Jeffries et al 2019), *An. nili*, *An. coustani* (Ayala et al

2019), *An. maculatus* (*s.s.*), *An. sawadwongporni*, *An. pseudowillmori*, *An. dirus* (*s.s.*), *An. baimaii* (Sawasdichai et al 2019), *An. balabacensis*, *An. latens*, *An. introlatus*, *An. macarthuri*, *An. barbirostris*, *An. hyrcanus* and *An. sinensis* (Wong et al 2020) with our report on additional two species of *Anopheles*, totalling 22 species.

The natural prevalence rate is nil or low in *Anopheles* in comparison to other mosquitoes (Kittayapong et al 2000, Rasgon & Scott 2004, Wong et al 2020). Within *Anopheles* species, the natural *Wolbachia* infection is variable. *An. arabiensis* (Baldini et al 2018, Jeffries et al 2018), *An. coluzzii* (Jeffries et al 2018), *An. funestus* (Niang et al 2018), *An. melas* (Jeffries et al 2019), *An. maculatus* (*s.s.*), *An. sawadwongporni*, *An. pseudowillmori*, *An. dirus* (*s.s.*), *An. baimaii* (Sawasdichai et al 2019), *An. balabacensis*, *An. introlatus*, *An. macarthuri* (Wong et al 2020) shows lesser natural prevalence rate. Higher prevalence rate has been reported from an unknown *Anopheles* species from Sub-Saharan Africa (Jeffries et al 2018), *An. moucheti* (Ayala et al 2019, Jeffries et al 2018), *An. latens*, *An. hyrcanus* and *An. barbirostris* (Wong et al 2020). *An. gambiae* shows variable prevalence rate (Buck et al 2016, Gomes et al 2017, Jeffries et al 2018) and, possibly spatial population dynamics (Buck et al 2016) may play a role in it. We have lesser prevalence of *Wolbachia* in *An. stephensi* (1.19%) and *An. culicifacies* (1.70%) in comparison to all other reported species.

The less prevalence rate of *Wolbachia* in *Anopheles* mosquitoes has raised several questions. Maternal transmission of *Wolbachia* was observed in natural population of *An. gambiae*, *An. coluzzii* and *An. arabiensis* (Baldini et al 2014, Buck et al 2016, Gomes et al 2017, Shaw et al 2016). Experimental evidence points out horizontal transfer of *Wolbachia* in *An. gambiae* (Hughes et al 2014, Hughes et al 2011, Hughes et al 2012), *An. stephensi* (Bian et al 2013, Hughes et al 2014, Joshi et al 2017) and *An. coluzzii* (Shaw et al 2016) is possible; however some results in transient infection as in *An. gambiae* (Hughes et al 2011) than permanent (maternal transmission) as seen in *An. stephensi* (Bian et al 2013, Joshi et al 2017). Evidence suggests that native microbiome in *Anopheles* mosquitoes impedes the vertical transmission of *Wolbachia* (Hughes et al 2014, Jeffries et al 2018, Straub et al 2020). *Asaia*, an acetic acid bacterium inhibits *Wolbachia* maternal transmission (Hughes et al 2014). *Variovorax*, a beta-proteobacteria has been observed in *Wolbachia* negative *An. coluzzii* (Straub et al 2020),

which warrants further research as a competitor to *Wolbachia*. The different *Wolbachia* strains can also differ in their interaction with the host (Hughes et al 2012) probably a reason for lesser prevalence rate. Taken together, prevalence of *Wolbachia* in *Anopheles* mosquito might be subjected to 1) native microbiota interference and 2) *Wolbachia*-host interaction. Since we studied on *Anopheles*, it is possible that native microbiota could interfere in *Wolbachia* colonizing this species leading to lesser prevalence rate; further research will be carried out in the future.

Lateral gene transfer (LGT) of *Wolbachia* genome was observed in *Callosobruchus chinensis* (Kondo et al 2002, Nikoh et al 2008), *Onchocerca volvulus* (Fenn et al 2006) and, *D. ananassae* (Hotopp et al 2007). Incase of mosquitoes, LGT has been observed in *Ae. aegypti*, *Ae. mascarensis* (Klasson et al 2009) and *An. gambiae* (Korochkina et al 2006). Salivary gland surface (*SGS*) genes from *An. gambiae* and *Ae. aegypti* genome is said to be transferred from *Wolbachia* via LGT (Korochkina et al 2006), where these genes are particularly found in female salivary gland; it's expression increase with age, after blood feeding and, facilitates *Plasmodium* sporozoite invasion (King et al 2011, Korochkina et al 2006). However, there is no study on SGS relationship with *Wolbachia* infection. Endogenous bornavirus-like nucleoprotein, a functional protein homologous to Borna virus *nucleoprotein* integrated into *Ictidomys tridecemlineatus* ~8.5 MYA (Suzuki et al 2014) inhibits exogenous Borna virus *in vivo* (Fujino et al 2014). Similarly we hypothesis, *SGS* gene might interfere with *Wolbachia* infection in *Anopheles* mosquitoes leads to less prevalence rate which needs further research.

Chrostek and Gerth (2019) pointed out that true symbiosis has to be established by demonstrating intercellular bacterial cells and intraovarian transmission. To till date, the available techniques are limited and thus addressing the above said factors in wild mosquitoes is quite a challenge, but the future might hold a better way to prove the above said factors in elucidating true symbiosis of *Wolbachia* in wild mosquitoes.

Conclusion:

The current study has shown *Wolbachia* for the first time in *Anopheles* mosquitoes namely *An. culicifacies* and *An. stephensi* from Tamil Nadu, India. Nested *16S* rRNA PCR amplification is

helpful in identification than *wsp*, *FtsZ* and MLST loci genes. The prevalence is lesser compared to other mosquitoes, which may be due to inhibition by native microbiota, host and *Wolbachia* interaction, and/or inhibition by endogenous gene product that result from LGT; these factors will be analyzed in our future research.

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Author contribution:

Concept, study design and methodology: SGS and APA. Performed lab experiments: TM and APA. Phylogenetic design and analysis: TM, SGS and APA. Pre-draft written: TM, SGS and APA. Critical revision of final version: SGS and APA. All authors read and approved the final manuscript.

Reference:

Arjunan NK, Kadarkarai M, Kumar S, Pari M, Thiyagarajan N, et al. 2015. Factors influencing the spatial distribution of Anopheles larvae in Coimbatore District, Tamil Nadu, India. *Acta tropica* 152: 121-30

Ayala D, Akone-Ella O, Rahola N, Kengne P, Ngangue MF, et al. 2019. Natural Wolbachia infections are common in the major malaria vectors in Central Africa. *Evolutionary Applications* 12: 1583-94

Baldini F, Rouge J, Kreppel K, Mkandawile G, Mapua SA, et al. 2018. First report of natural Wolbachia infection in the malaria mosquito Anopheles arabiensis in Tanzania. *Parasites & vectors* 11: 635

Baldini F, Segata N, Pompon J, Marcenac P, Shaw WR, et al. 2014. Evidence of natural Wolbachia infections in field populations of Anopheles gambiae. *Nature communications* 5: 3985

Baldo L, Dunning Hotopp JC, Jolley KA, Bordenstein SR, Biber SA, et al. 2006. Multilocus sequence typing system for the endosymbiont Wolbachia pipientis. *Applied and environmental microbiology* 72: 7098-110

Bian G, Joshi D, Dong Y, Lu P, Zhou G, et al. 2013. Wolbachia invades Anopheles stephensi populations and induces refractoriness to Plasmodium infection. *Science* 340: 748-51

Bleidorn C, Gerth M. 2018. A critical re-evaluation of multilocus sequence typing (MLST) efforts in Wolbachia. *FEMS Microbiol Ecol* 94

Buck M, Nilsson LK, Brunius C, Dabiré RK, Hopkins R, Terenius O. 2016. Bacterial associations reveal spatial population dynamics in Anopheles gambiae mosquitoes. *Sci Rep* 6: 22806

Christophers SR. 1933. *The fauna of British India, including Ceylon and Burma. Diptera.Vol. IV. Family Culicidae. Tribe Anophelini*. London: Taylor and Francis. vi [vii], 317pp. + 3 pls pp.

Chrostek E, Gerth M. 2019. Is Anopheles gambiae a Natural Host of Wolbachia? *mBio* 10

Coon KL, Brown MR, Strand MR. 2016. Mosquitoes host communities of bacteria that are essential for development but vary greatly between local habitats. *Molecular ecology* 25: 5806-26

Das BP, Rajagopal R, Akiyama J. 1990. Pictorial key to the species of Indian anopheline mosquitoes. Pictorial key to the species of Indian anopheline mosquitoes. *Journal of Pure and Applied Zoology* 2: 131-62

de Oliveira CD, Goncalves DS, Baton LA, Shimabukuro PH, Carvalho FD, Moreira LA. 2015. Broader prevalence of Wolbachia in insects including potential human disease vectors. *Bulletin of entomological research* 105: 305-15

Dev V, Sharma VP. 2013. The dominant mosquito vectors of human malaria in India In *Anopheles mosquitoes - New insights into malaria vectors*, ed. S Manguin: IntechOpen

Dutton TJ, Sinkins SP. 2004. Strain-specific quantification of Wolbachia density in Aedes albopictus and effects of larval rearing conditions. *Insect molecular biology* 13: 317-22

Fenn K, Conlon C, Jones M, Quail MA, Holroyd NE, et al. 2006. Phylogenetic Relationships of the Wolbachia of Nematodes and Arthropods. *PLoS pathogens* 2: e94

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular marine biology and biotechnology* 3: 294-9

Fujino K, Horie M, Honda T, Merriman DK, Tomonaga K. 2014. Inhibition of Borna disease virus replication by an endogenous bornavirus-like element in the ground squirrel genome. *Proceedings of the National Academy of Sciences* 111: 13175-80

Gomes FM, Hixson BL, Tyner MDW, Ramirez JL, Canepa GE, et al. 2017. Effect of naturally occurring Wolbachia in Anopheles gambiae s.l. mosquitoes from Mali on Plasmodium falciparum malaria transmission. *Proceedings of the National Academy of Sciences of the United States of America* 114: 12566-71

Goswami G, Singh OP, Nanda N, Raghavendra K, Gakhar SK, Subbarao SK. 2006. Identification of all members of the anopheles culicifacies complex using allele-specific polymerase chain reaction assays. *The American journal of tropical medicine and hygiene* 75: 454-60

Harbach RE. 2004. The classification of genus Anopheles (Diptera: Culicidae): a working hypothesis of phylogenetic relationships. *Bulletin of entomological research* 94: 537-53

Harbach RE. 2013. The Phylogeny and Classification of Anopheles In *Anopheles mosquitoes - New insights into malaria vectors*, ed. S Manguin: IntechOpen

Hay SI, Sinka ME, Okara RM, Kabaria CW, Mbithi PM, et al. 2010. Developing global maps of the dominant anopheles vectors of human malaria. *PLoS medicine* 7: e1000209

Hertig M, Wolbach SB. 1924. Studies on Rickettsia-Like Micro-Organisms in Insects. *The Journal of medical research* 44: 329-74

Hilgenboecker K, Hammerstein P, Schlattmann P, Telschow A, Werren JH. 2008. How many species are infected with Wolbachia?--A statistical analysis of current data. *FEMS microbiology letters* 281: 215-20

Hotopp JCD, Clark ME, Oliveira DCSG, Foster JM, Fischer P, et al. 2007. Widespread Lateral Gene Transfer from Intracellular Bacteria to Multicellular Eukaryotes. *Science* 317: 1753-56

Hughes GL, Dodson BL, Johnson RM, Murdock CC, Tsujimoto H, et al. 2014. Native microbiome impedes vertical transmission of Wolbachia in Anopheles mosquitoes. *Proceedings of the National Academy of Sciences of the United States of America* 111: 12498-503

Hughes GL, Koga R, Xue P, Fukatsu T, Rasgon JL. 2011. Wolbachia infections are virulent and inhibit the human malaria parasite Plasmodium falciparum in Anopheles gambiae. *PLoS pathogens* 7: e1002043

Hughes GL, Vega-Rodriguez J, Xue P, Rasgon JL. 2012. Wolbachia strain wAlbB enhances infection by the rodent malaria parasite Plasmodium berghei in Anopheles gambiae mosquitoes. *Applied and environmental microbiology* 78: 1491-5

Jeffries CL, Cansado-Utrilla C, Stica C, Walker T. 2019. High density Novel *Wolbachia* strains in *Anopheles* species from Guinea. *bioRxiv*: 772855

Jeffries CL, Lawrence GG, Golovko G, Kristan M, Orsborne J, et al. 2018. Novel Wolbachia strains in Anopheles malaria vectors from Sub-Saharan Africa. *Wellcome open research* 3: 113

Joshi D, Pan X, McFadden MJ, Bevins D, Liang X, et al. 2017. The maternally inheritable Wolbachia wAlbB induces refractoriness to Plasmodium berghei in Anopheles stephensi. *Frontiers in microbiology* 8

Kar I, Subbarao SK, Eapen A, Ravindran J, Satyanarayana TS, et al. 1999. Evidence for a new malaria vector species, species E, within the Anopheles culicifacies complex (Diptera: Culicidae). *Journal of medical entomology* 36: 595-600

King JG, Vernick KD, Hillyer JF. 2011. Members of the salivary gland surface protein (SGS) family are major immunogenic components of mosquito saliva. *The Journal of biological chemistry* 286: 40824-34

Kittayapong P, Baisley KJ, Baimai V, O'Neill SL. 2000. Distribution and diversity of Wolbachia infections in Southeast Asian mosquitoes (Diptera: Culicidae). *Journal of medical entomology* 37: 340-5

Klasson L, Kambris Z, Cook PE, Walker T, Sinkins SP. 2009. Horizontal gene transfer between Wolbachia and the mosquito Aedes aegypti. *BMC Genomics* 10: 33

Kondo N, Nikoh N, Ijichi N, Shimada M, Fukatsu T. 2002. Genome fragment of Wolbachia endosymbiont transferred to X chromosome of host insect. *Proceedings of the National Academy of Sciences of the United States of America* 99: 14280-5

Korochkina S, Barreau C, Pradel G, Jeffery E, Li J, et al. 2006. A mosquito-specific protein family includes candidate receptors for malaria sporozoite invasion of salivary glands. *Cellular Microbiology* 8: 163-75

Mahilum MM, Storch V, Becker N. 2003. Molecular and electron microscopic identification of Wolbachia in Culex pipiens complex populations from the Upper Rhine Valley, Germany, and Cebu City, Philippines. *Journal of the American Mosquito Control Association* 19: 206-10

Marcon HS, Coscrato VE, Selivon D, Perondini AL, Marino CL. 2011. Variations in the sensitivity of different primers for detecting Wolbachia in Anastrepha (diptera: tephritidae). *Brazilian journal of microbiology : [publication of the Brazilian Society for Microbiology]* 42: 778-85

Niang EHA, Bassene H, Makoundou P, Fenollar F, Weill M, Mediannikov O. 2018. First report of natural Wolbachia infection in wild Anopheles funestus population in Senegal. *Malaria journal* 17: 408

Nikoh N, Tanaka K, Shibata F, Kondo N, Hizume M, et al. 2008. Wolbachia genome integrated in an insect chromosome: Evolution and fate of laterally transferred endosymbiont genes. *Genome Research* 18: 272-80

O'Neill SL, Giordano R, Colbert AM, Karr TL, Robertson HM. 1992. 16S rRNA phylogenetic analysis of the bacterial endosymbionts associated with cytoplasmic incompatibility in insects. *Proceedings of the National Academy of Sciences of the United States of America* 89: 2699-702

Pourali P, Roayaei Ardakani M, Jolodar A, Razi Jalali MH. 2009. PCR screening of the Wolbachia in some arthropods and nematodes in Khuzestan province. *Iranian Journal of Veterinary Research* 10: 216-22

Rasgon JL, Scott TW. 2004. An initial survey for Wolbachia (Rickettsiales: Rickettsiaceae) infections in selected California mosquitoes (Diptera: Culicidae). *Journal of medical entomology* 41: 255-7

Ricci I, Cancrini G, Gabrielli S, D'Amelio S, Favi G. 2002. Searching for Wolbachia (Rickettsiales: Rickettsiaceae) in mosquitoes (Diptera: Culicidae): large polymerase chain reaction survey and new identifications. *Journal of medical entomology* 39: 562-7

Rousset F, Vautrin D, Solignac M. 1992. Molecular identification of Wolbachia, the agent of cytoplasmic incompatibility in Drosophila simulans, and variability in relation with host mitochondrial types. *Proceedings. Biological sciences* 247: 163-8

Sawasdichai S, Chaumeau V, Dah T, Kulabkeeree T, Kajeechiwa L, et al. 2019. Detection of diverse Wolbachia 16S rRNA sequences at low titers from malaria vectors in Kayin state, Myanmar. *Wellcome open research* 4: 11

Sharma SK, Hamzakoya KK. 2001. Geographical Spread of Anopheles stephensi Vector of Urban Malaria, and Aedes aegypti, Vector of Dengue/DHF, in the Arabian Sea Islands of Lakshadweep, India. *Dengue Bulletin* 25: 88-91

Shaw WR, Marcenac P, Childs LM, Buckee CO, Baldini F, et al. 2016. Wolbachia infections in natural Anopheles populations affect egg laying and negatively correlate with Plasmodium development. *Nature communications* 7: 11772

Sicard M, Bonneau M, Weill M. 2019. Wolbachia prevalence, diversity, and ability to induce cytoplasmic incompatibility in mosquitoes. *Current opinion in insect science* 34: 12-20

Sinka ME, Bangs MJ, Manguin S, Chareonviriyaphap T, Patil AP, et al. 2011. The dominant Anopheles vectors of human malaria in the Asia-Pacific region: occurrence data, distribution maps and bionomic precis. *Parasites & vectors* 4: 89

Sinkins SP, Braig HR, O'Neill SL. 1995. Wolbachia pipientis: bacterial density and unidirectional cytoplasmic incompatibility between infected populations of Aedes albopictus. *Experimental parasitology* 81: 284-91

Straub TJ, Shaw WR, Marcenac P, Sawadogo SP, Dabire RK, et al. 2020. The Anopheles coluzzii microbiome and its interaction with the intracellular parasite Wolbachia. *Sci Rep* 10: 13847

Subbarao SK, Nanda N, Rahi M, Raghavendra K. 2019. Biology and bionomics of malaria vectors in India: existing information and what more needs to be known for strategizing elimination of malaria. *Malaria journal* 18: 396

Suguna SG, Tewari SC, Mani TR, Hiriyan J, Reuben R. 1983. Anopheles culicifacies species complex in Thenpennaiyar riverine tract, Tamil Nadu. *The Indian journal of medical research* 77: 455-9

Surendran SN, Sivabalakrishnan K, Sivasingham A, Jayadas TTP, Karvannan K, et al. 2019. Anthropogenic factors driving recent range expansion of the malaria vector Anopheles stephensi. *Frontiers in public health* 7: 53

Suzuki Y, Kobayashi Y, Horie M, Tomonaga K. 2014. Origin of an endogenous bornavirus-like nucleoprotein element in thirteen-lined ground squirrels. *Genes & genetic systems* 89: 143-8

Werren JH. 1997. Biology of Wolbachia. *Annual review of entomology* 42: 587-609

Werren JH, Baldo L, Clark ME. 2008. Wolbachia: master manipulators of invertebrate biology. *Nature reviews. Microbiology* 6: 741-51

Werren JH, Jaenike J. 1995. Wolbachia and cytoplasmic incompatibility in mycophagous Drosophila and their relatives. *Heredity* 75 (Pt 3): 320-6

Werren JH, Windsor D, Guo LR. 1995a. Distribution of *Wolbachia* among neotropical arthropods. *Proc Royal Soc Lond B* 262: 197-204

Werren JH, Windsor DM. 2000. Wolbachia infection frequencies in insects: evidence of a global equilibrium? *Proceedings. Biological sciences* 267: 1277-85

Werren JH, Zhang W, Guo LR. 1995b. Evolution and phylogeny of Wolbachia: reproductive parasites of arthropods. *Proceedings. Biological sciences* 261: 55-63

WHO. 2019. *World malaria report 2019*. pp. 232. Geneva: World Health Organization.

Wong ML, Liew JWK, Wong WK, Pramasivan S, Mohamed Hassan N, et al. 2020. Natural Wolbachia infection in field-collected Anopheles and other mosquito species from Malaysia. *Parasites & vectors* 13: 414

Zhou W, Rousset F, O'Neil S. 1998. Phylogeny and PCR-based classification of Wolbachia strains using wsp gene sequences. *Proceedings. Biological sciences* 265: 509-15

Figure legends:

Fig.1: Study site from Tamil Nadu

The map shows the study site at 5 different locations along the foothills of the Western Ghats, Southern India. The five locations were Coimbatore, Pollachi, Palani, Cumbum, Sirvilliputhur and Tenkasi. *An. stephensi* and *An. culicifacies* was populated at all the sites (red circle). The presence of *Wolbachia* was marked in yellow star for *An. stephensi* and green diamond for *An. culicifacies*.

Fig.2: Multiple sequence alignment of *Wolbachia 16S* rRNA sequence

The *Wolbachia 16S* rRNA sequence was aligned using Clustal O at EMBL-EBI server and edited in JalView. The restriction site of RsaI was highlighted in black. The sequences used are known *Wolbachia* supergroup A (EU096232, NC_002978.6, NC_012416, KP089991), supergroup B (NC_021984, CAGB01000162, MH967031), outgroup *Ricketssia* (CP003319, NR_074459.2) and our isolates. The restriction sites show our isolates belong to supergroup B with four restriction sites. Supergroup A consists of two restriction sites and, *Ricketssia* consists of a single restriction site.

Fig.3: Molecular phylogenetic analysis of *Wolbachia*

The phylogeny was inferred from the nucleotide dataset of *16S* rRNA gene by using the ML method. The sequences from this study were represented in red font. The tree with the highest log likelihood (-290.81) is shown. The analysis involved 44 nucleotide sequences. There were a total of 120 positions in the final dataset. Scale bar 0.02 represents nucleotides substitution per position.

Fig.4: Molecular phylogenetic analysis of *Anopheles* mosquitoes

The phylogeny was inferred from the nucleotide dataset of *COI* gene by using the ML method. The sequences from this study were represented as green diamond. The tree with the highest log likelihood (-1429.44) is shown. The analysis involved 19 nucleotide sequences. There were a total of 396 positions in the final dataset. Scale bar 0.05 represents nucleotides substitution per position.

Table 1: Collection of mosquito samples from different locations

Place	***An. culicifacies***		***An. stephensi***	
	Collected (%*)	***Wolbachia* positive (%#)**	**Collected (%*)**	***Wolbachia* positive (%#)**
Coimabtore	38 (45.24)	1 (2.63)	46 (54.76)	1 (2.17)
Pollachi	48 (38.71)	1 (2.08)	76 (61.29)	0
Cumbum	67 (69.79)	1 (1.49)	29 (30.21)	1 (3.44)
Srivillputtur	21 (30.00)	1 (4.76)	49 (70.00)	0
Tenkasi	61 (53.98)	0	52 (46.02)	1 (1.92)
Total	235 (48.25)	4 (1.70)	252 (51.75)	3 (1.19)

*Percentage from total mosquitoes collected
#Percentage of *Wolbachia* positive within species

Table 2: The host and the *Wolbachia* endosymbiont isolate ID and sequence accession number

Host	**Isolate ID**	**Mosquito sequence Accession No.**	***Wolbachia* sequence Accession No.**
Anopheles culicifacies	TS1	LR736007	MN268747
Anopheles culicifacies	TS2	LR736008	MN268748
Anopheles culicifacies	TS3	LR736009	MN268749
Anopheles stephensi	TS4	LR736010	MN268750
Anopheles stephensi	AE4	LR736012	MN268743
Anopheles stephensi	AE5	LR736013	MN268744
Anopheles stephensi	AE6	LR736014	MN268746

Table 3: Sequences used for *Wolbachia* phylogeny

S. No	Accession No	Source organism	Isolate /Strain Id	Used sequence	Year of isolation	Country
1.	CAGB01000162	*Aedes albopictus*	wAlbB	Whole genome	2011	France
2.	CP003319	*Rickettsia massiliae*	AZT80	Complete genome	2012	USA
3.	EU096232	*Drosophila*	EW-p	16S rRNA partial sequence	2007	South Korea
4.	KP089991	*Anopheles coluzzii*	VK5_8.1.1	16S rRNA partial sequence	2012	Burkina Faso
5.	MH596693	*Anopheles arabiensis*	isolate 3	16S rRNA partial sequence	2018	Tanzania
6.	MH596694	*Anopheles arabiensis*	isolate 7	16S rRNA partial sequence	2018	Tanzania
7.	MH596695	*Anopheles arabiensis*	isolate 13	16S rRNA partial sequence	2018	Tanzania
8.	MH596696	*Anopheles arabiensis*	isolate 15	16S rRNA partial sequence	2018	Tanzania
9.	MH596697	*Anopheles arabiensis*	isolate 19	16S rRNA partial sequence	2018	Tanzania
10.	MH596698	*Anopheles arabiensis*	isolate 40	16S rRNA partial sequence	2018	Tanzania
11.	MH596699	*Anopheles arabiensis*	isolate 55	16S rRNA partial sequence	2018	Tanzania
12.	MH596700	*Anopheles arabiensis*	isolate 63	16S rRNA partial sequence	2018	Tanzania
13.	MH596701	*Anopheles arabiensis*	isolate 137	16S rRNA partial sequence	2018	Tanzania
14.	MH596702	*Anopheles arabiensis*	isolate 138	16S rRNA partial sequence	2018	Tanzania
15.	MH596703	*Anopheles arabiensis*	isolate 140	16S rRNA partial sequence	2018	Tanzania
16.	MK026554	*Aedeomyia madagascarica*	TSA-AMAD-1	16S rRNA partial sequence	2016	Madagascar
17.	MK026555	*Culex antennatus*	TSA-CANT-1	16S rRNA partial sequence	2016	Madagascar
18.	MK026556	*Culex decens*	TSA-CDEC-1	16S rRNA partial sequence	2016	Madagascar
19.	MK026557	*Culex decens*	TSA-CDEC-2	16S rRNA partial sequence	2016	Madagascar
20.	MK026558	*Culex duttoni*	TSA-CDUT-1	16S rRNA partial sequence	2016	Madagascar
21.	MK026559	*Mansonia uniformis*	TSA-MUNI-1	16S rRNA partial sequence	2016	Madagascar
22.	MK026560	*Uranotaenia sp.*	ANI-USP1-1	16S rRNA partial sequence	2016	Madagascar
23.	MK026561	*Uranotaenia sp.*	TSA-USP1-1	16S rRNA partial sequence	2016	Madagascar
24.	MK026562	*Uranotaenia sp.*	TSA-USP2-1	16S rRNA partial sequence	2016	Madagascar
25.	MK026563	*Uranotaenia sp.*	TSA-USP2-2	16S rRNA partial sequence	2016	Madagascar
26.	MN268743	*Anopheles stephensi*	AE4	16S rRNA partial sequence	2017	India*
27.	MN268744	*Anopheles stephensi*	AE5	16S rRNA partial sequence	2017	India*

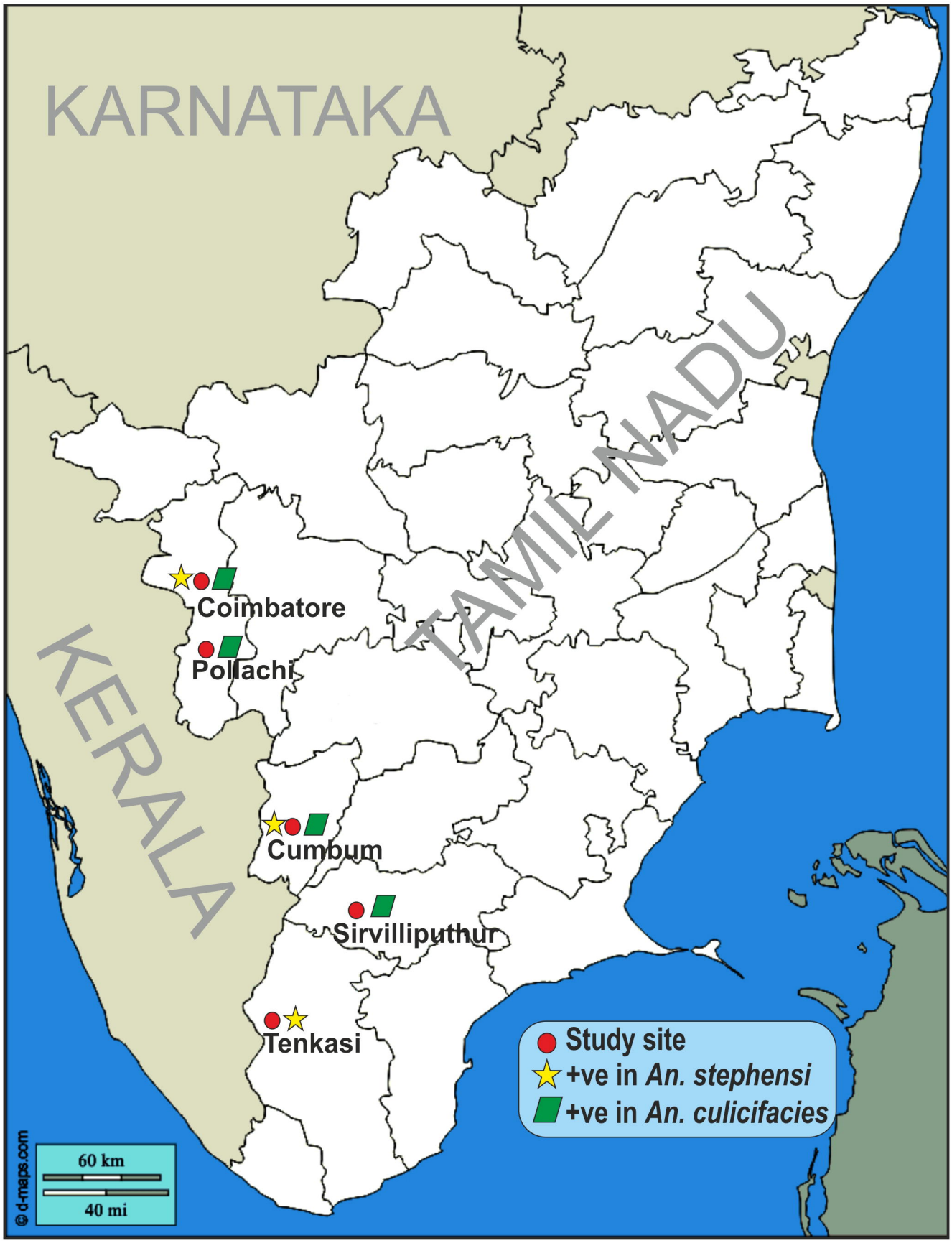
28.	MN268746	*Anopheles stephensi*	AE6	16S rRNA partial sequence	2017	India*
29.	MN268747	*Anopheles culicifacies*	TS1	16S rRNA partial sequence	2017	India*
30.	MN268748	*Anopheles culicifacies*	TS2	16S rRNA partial sequence	2017	India*
31.	MN268749	*Anopheles culicifacies*	TS3	16S rRNA partial sequence	2017	India*
32.	MN268750	*Anopheles stephensi*	TS4	16S rRNA partial sequence	2017	India*
33.	NC_002978	*Drosophila melanogaster*	wMel	Complete genome	2003	USA
34.	NC_012416	*Drosophila simulans*	wRi	Complete genome	2009	Sweden
35.	NC_021084	*Drosophila simulans*	wNo	Complete genome	2012	Sweden
36.	NR_074459	*Rickettsia japonica*	YH	16S rRNA partial sequence	2013	USA
37.	NZ_CP021120	*Chrysomyamegacephala*	wMeg	Complete genome	2015	Brazil
38.	NZ_CP034334	*Drosophila mauritiana*	wMau	Complete genome	2018	USA
39.	NZ_CP034335	*Drosophila mauritiana*	wMau	Complete genome	2018	USA
40.	NZ_CP041215	*Carposina sasakii*	wCauA	Complete genome	2017	China
41.	NZ_CP042444	*Drosophila melanogaster*	wMel_I23	Complete genome	2019	USA
42.	NZ_CP042445	*Drosophila melanogaster*	wMel_ZH26	Complete genome	2019	USA
43.	NZ_CP042446	*Drosophila melanogaster*	wMel_N25	Complete genome	2019	USA
44.	NZ_CP042904	*Drosophila ananassae*	W2.1	Complete genome	2019	USA

*this study

Table 4: Sequences used for *Anopheles* mosquito phylogeny

S.No.	Accession No.	Source organism	Isolate/Strain Id	Used sequence	Year of isolation	Country
1.	LR736007	*Anopheles culicifacies*	TS1	COI gene, partial cds; mitochondrial	2017	India*
2.	LR736008	*Anopheles culicifacies*	TS2	COI gene, partial cds; mitochondrial	2017	India*
3.	LR736009	*Anopheles culicifacies*	TS3	COI gene, partial cds; mitochondrial	2017	India*
4.	LR736010	*Anopheles stephensi*	TS4	COI gene, partial cds; mitochondrial	2017	India*
5.	LR736012	*Anopheles stephensi*	AE4	COI gene, partial cds; mitochondrial	2017	India*
6.	LR736013	*Anopheles stephensi*	AE5	COI gene, partial cds; mitochondrial	2017	India*
7.	LR736014	*Anopheles stephensi*	AE6	COI gene, partial cds; mitochondrial	2017	India*
8.	NC_028223	*Anopheles stephensi*	ASTEP20150811V3	Mitochondrion, complete genome	2015	USA
9.	MH538704	*Anopheles stephensi*	voucher BUZOOT	COI gene, partial cds; mitochondrial	2018	India
10.	KX467337	*Anopheles stephensi*	voucher MOSQ02-16	COX1 gene, partial cds; mitochondrial		India
11.	KF406680	*Anopheles stephensi*	voucher NIBGE DIP-00281	COI gene, partial cds; mitochondrial	2007	Pakistan
12.	NC_028216	*Anopheles culicifacies*	voucher ACUL20150811V4	Mitochondrion, complete genome	2015	USA
13.	KR732656	*Anopheles culicifacies*	isolate B	Mitochondrion, complete genome	2015	China
14.	KJ010898	*Anopheles culicifacies*	voucher UNB-04	CO1 gene, partial cds; mitochondrial	2013	UK
15.	DQ424962	*Anopheles culicifacies*	-	COI) gene, partial cds; mitochondrial	2006	India
16.	KR817729	*Anopheles culicifacies*	voucher BUZOO-M-Ac	COI gene, partial cds; mitochondrial	2015	India
17.	KJ010896	*Anopheles culicifacies*	voucher F04	COI gene, partial cds; mitochondrial	2013	Srilanka
18.	KJ010892	*Anopheles culicifacies*	voucher H06	CO1 gene, partial cds; mitochondrial	2013	Srilanka
19.	NC_035159	*Aedes aegypti*	strain LVP_AGWG	Mitochondrion, complete genome	2017	USA

*this study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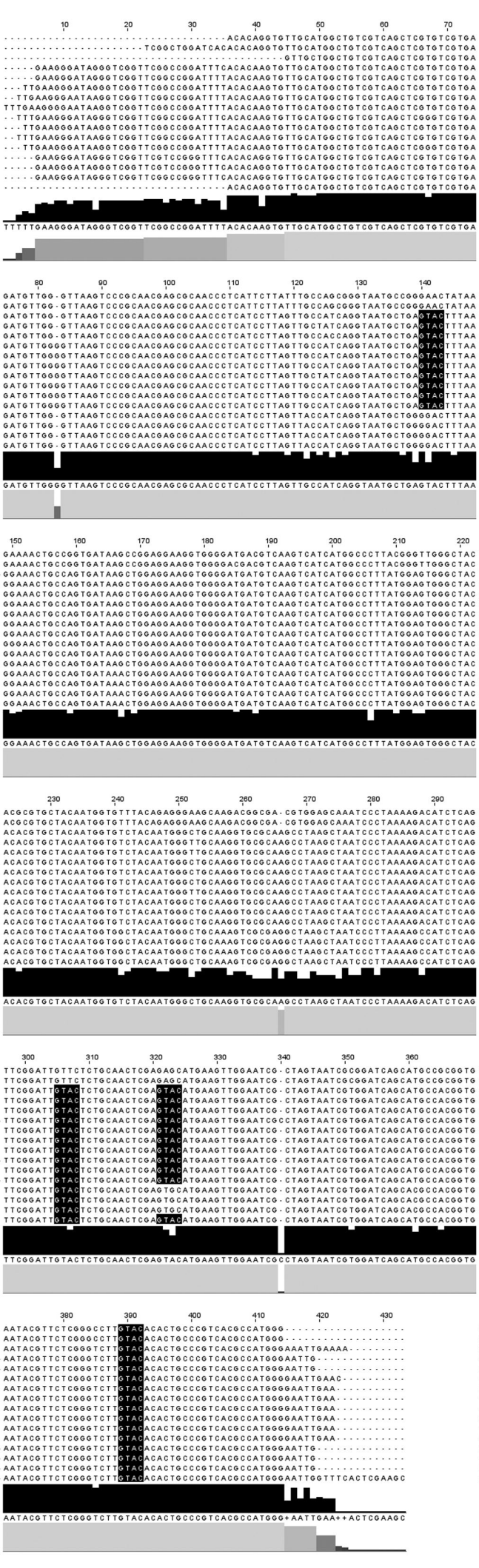
60 km
40 mi
© d-maps.com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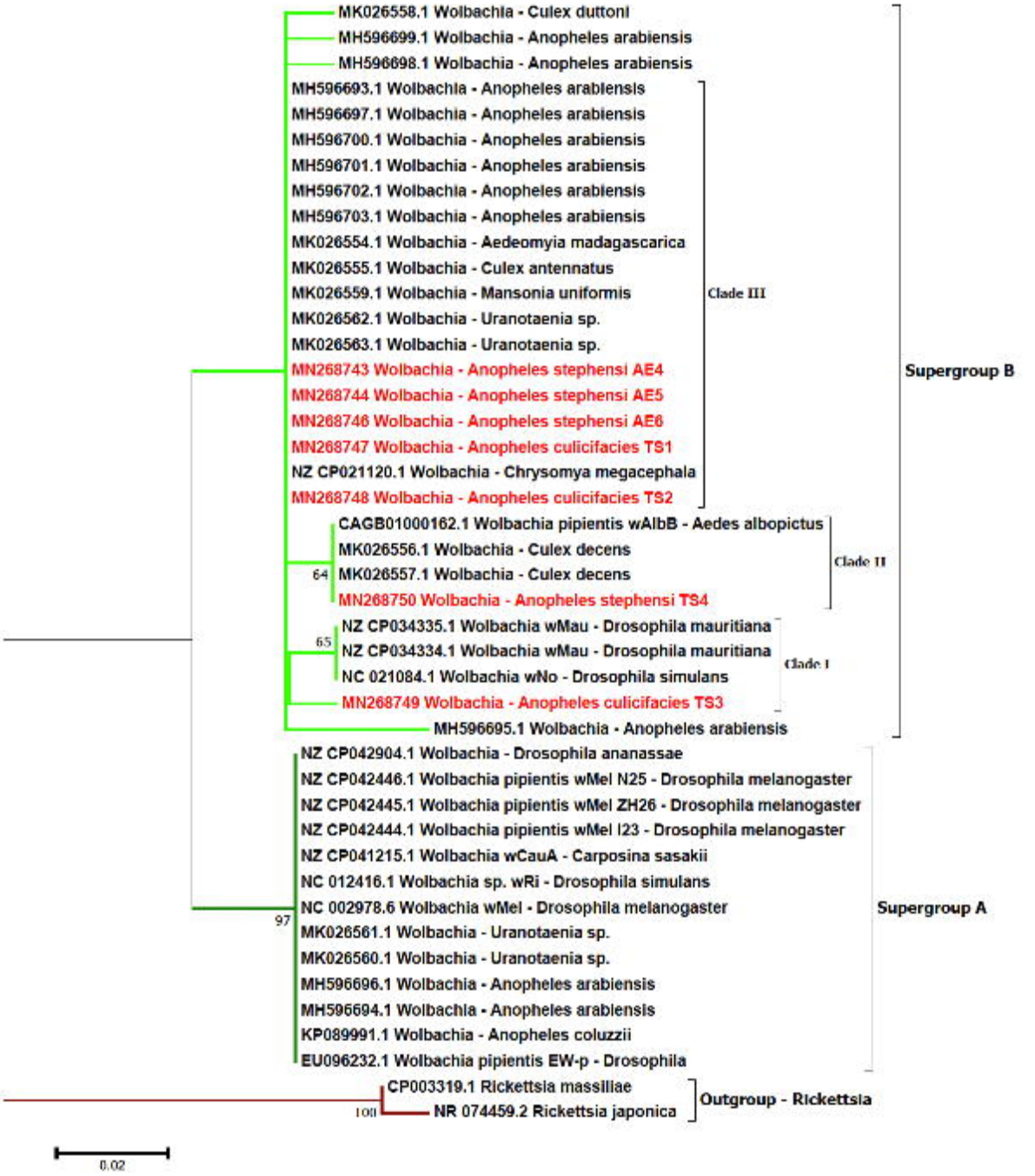
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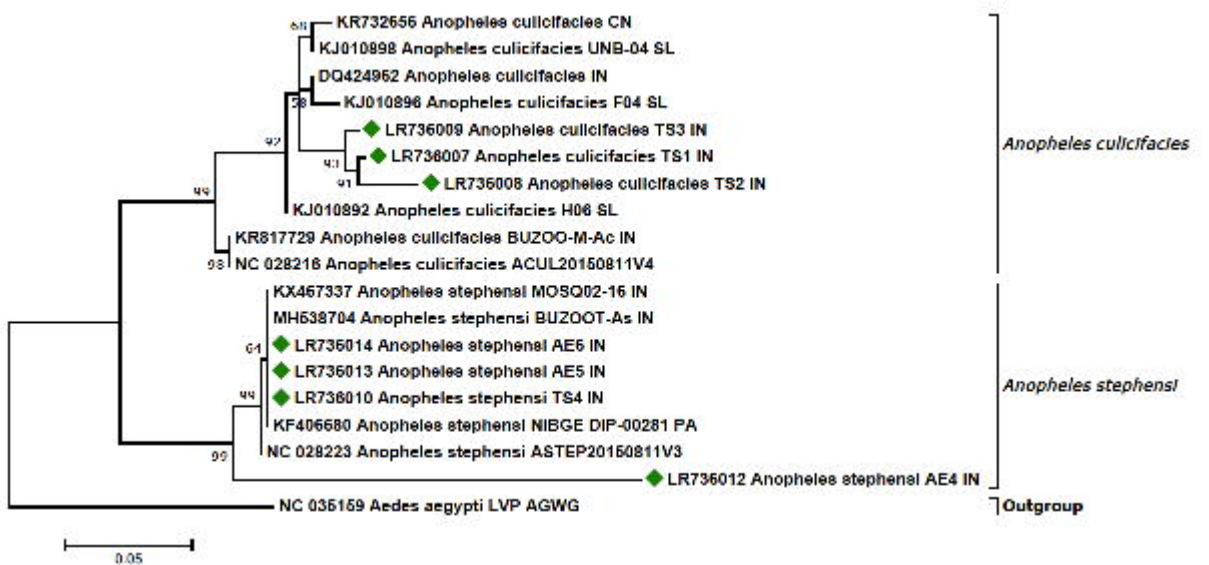
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