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Molecular identification of native *Wolbachia pipientis* in *Anopheles minimus* in a low-malaria transmission area of Umphang Valley along the Thailand-Myanmar border

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Abstract

Background: *Wolbachia*, obligate intracellular bacteria, infect the majority of arthropods, including many mosquito species of medical importance. Some *Wolbachia* strains interfere with the development of *Plasmodium* parasites in female *Anopheles*, a major vector of malaria. The use of *Wolbachia* as a means to block malaria transmission is an emerging vector control strategy in highly endemic areas. Hence, identification of native *Wolbachia* strains in areas where malaria transmission is low may uncover a particular *Wolbachia* strain capable of *Plasmodium* interference. This study aims to identify native *Wolbachia* strains in female *Anopheles* spp. that are predominant in a low-malaria transmission area in mainland Southeast Asia.

Methods: Following a 2-year survey of malaria vectors in Umphang Valley of Tak Province, Thailand, DNA extracts of female *An. minimus, An. peditaeniatus,* and *An. maculatus* were subjected to amplification of the conserved region of the 16S rRNA-encoding gene. The DNA sequences of the amplicons were phylogenetically compared with those of known *Wolbachia* strains.

Results: Among three *Anopheles* spp., amplification was detected in only the DNA samples from *An. minimus*. The DNA sequencing of amplicons revealed 100% similarity to *Wolbachia pipientis*, confirming the specificity of amplification. The *Wolbachia*-positive *An. minimus* samples were devoid of *Plasmodium* 18S rRNA amplification. The phylogenetic trees indicate a close relationship with *Wolbachia* strains in subgroup B.

Conclusion: To the best of our knowledge, the data presented herein provide the first molecular evidence of a *Wolbachia* strain in *An. minimus*, hereinafter named *w*Anmi, in a low-malaria transmission area in the Umphang Valley of western Thailand. Further biological characterization is required to examine its potential for malaria transmission control in the field.

Keywords: Wolbachia, Anopheles, Malaria, 16S rRNA, PCR, Phylogenetics

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