

NOVEL SUPERGROUP U WOLBACHIA IN BAT MITES OF THAILAND

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Abstract. *Wolbachia* is an endosymbiont infecting mostly arthropods and is vertically transmitted to its host offspring; however, *Wolbachia* in mites has rarely been reported. We have discovered, for the first time, *Wolbachia* endosymbiont in *Spinturnix* mite infesting thick-thumbed myotis bat (*Myotis rosseti*) in Thailand. *Wolbachia* spp were detected in *Spinturnix* spp by PCR employing species-specific 16S rDNA and *ftsZ* primers. Consensus sequences were then used to construct phylogenetic trees, which indicated *Spinturnix* mite *Wolbachia* spp belonged to a clade separate from existing species, which we named *Wolbachia* Supergroup U. The role of *Wolbachia* spp in *Spinturnix* mites remains to be determined.

Keywords: *Myotis rosseti*, *Spinturnix* spp, *Wolbachia* spp, mite

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INTRODUCTION

Wolbachia is an obligate intracellular bacterium belonging to class Anaplasmataceae, order Rickettsiales, closely related to *Ehrlichia* and *Anaplasma* genera. The presumptive endosymbionts have been described as *Rickettsia*-like and *Wolbachia*-like microbes on the basis of their ultrastructure (Hayes and Burgdorfer, 1981). These symbiotic bacteria are widely found

in many arthropods and filarial nematodes particularly in insects and are transmitted vertically through female host to offspring (Werren, 1998). *Wolbachia* affects host offspring in four ways, namely, killing of males whereby infected males die during larval development thereby increasing the proportion of born females; causing cytoplasmic incompatibility (CI) where *Wolbachia*-infected males are unable to successfully reproduce with uninfected

females; inducing parthenogenesis; and stimulating feminization of infected males, processes which are beneficial to *Wolbachia* bacteria existence at the expense of the hosts (Werren, 1998).

Wolbachia spp have been divided into taxonomic supergroups A to S based on sequence analysis of genes or gene fragment, eg *ftsZ*, *wsp* and 16S rDNA (Socolovschi *et al*, 2012; Lefoulon *et al*, 2020). *Wolbachia* supergroups P and Q were isolated from quill mites infesting migrant birds (Socolovschi *et al*, 2012), and supergroup R and S from cave spider and pseudoscorpion respectively (Wang *et al*, 2016; Lefoulon *et al*, 2020). Recently, Laidoudi *et al* (2020) reported a new *Wolbachia* supergroup T present in bed bug. A number of species of mites are vectors of symbiont arthropods including *Wolbachia* (Chaisiri *et al*, 2015). Here, we report a *Wolbachia* strain belonging to a new supergroup U in mites parasitized the bats from Chachoengsao Province, central Thailand.

MATERIALS AND METHODS

Sample collection

Thick-thumbed myotis bat (*Myotis rosseti*) is a common species present in Chachoengsao Province, central Thailand. Seven bats were trapped with fishing net at Ratchasan District in January 2015 and morphologically identified according to criteria of Duengkae (2007). Mites ($n = 70$) were collected from wings and ventral side of bat body and transported at 4°C within 2-3 days to the Faculty of Science, Mahidol University, Bangkok for species

identification and molecular analysis (Uchikawa *et al*, 1994; Stanyukovich, 1997).

PCR amplification and DNA sequencing

A group of mites ($n = 10$) were cleaned by sequential surface wash for 5 minutes each with 70% ethanol and 10% sodium hypochlorite and finally three times, for 5 minutes each, with sterile distilled water. DNA was extracted using a QIAamp DNA Extraction Kit for Tissue (Qiagen, Hilden, Germany). EHR16SD/EHR16SR (Parola *et al*, 2000) and *ftsZ* (Holden *et al*, 1993) primer pairs were employed for PCR-based detection of family Anaplasmataceae and sequence of 16S rDNA amplicon (Black and Piesman, 1994) for genus identification.

Phylogenetic analysis

Consensus sequence of 16S rDNA (348-350 bp) and *ftsZ* (700-760 bp) amplicon respectively were aligned with corresponding *Wolbachia* nucleotide sequences deposited in GenBank database and neighbor-joining (NJ) method was then used to generate phylogenetic trees employing MEGA7 software (Kumar *et al*, 2016). Branching is considered when bootstrap values are >50% (1,000 pseudoreplicates).

RESULTS

Mite samples and *Wolbachia* detection rate

All mites ($n = 70$) collected were identified to the genus *Spinturnix* (data not shown). The seven pools of mites ($n = 10$ per pool) showed positive results for presence of family Anaplasmataceae, genus *Wolbachia* (data not shown).

Phylogenetic analysis and identity matrix

Seven different partial sequences of *Wolbachia* 16S rDNA amplicons from the study (Genbank accession nos. KP165041-KP165047) showed 97.1-98.0 and 94.5-98.0% identity to *Wolbachia* from *Brugia malayi* (AF051145) and *Brugia pahangi* (AJ012646) respectively. On the other hand, partial *ftsZ* sequences (GenBank accession nos. MW145493-MW145499) showed 91.7-92.6 and 91.9-92.8% identity to *Wolbachia* from *Microcerotermes* sp (AJ292346) and *Kaloterme flavicollis* (AJ292345) respectively.

Phylogenetic analysis of amplicons from both 16S rDNA and *ftsZ* demonstrated *Wolbachia* spp infecting the *Spinturnix* mites clearly forming a clade (named *Wolbachia* supergroup U) different from other known *Wolbachia* supergroups (Fig 1). Estimation of evolutionary divergence among 16S rDNA and *ftsZ* sequences of *Wolbachia* specimens from *Spinturnix* revealed 99.7-100 and 97.1-100% DNA sequence identity (Table 1).

DISCUSSION

Wolbachia is a common endosymbiotic bacterium associated with filarial nematodes and arthropods including insects, mites, and pseudoscorpion (Rowley *et al*, 2004; Lefoulon *et al*, 2020). It is genetically diverse and to date it has been classified in monophyletic lineage groups up to supergroup T (Laidoudi *et al*, 2020).

As *Wolbachia* can alter reproduction and fitness of their hosts, this bacterium

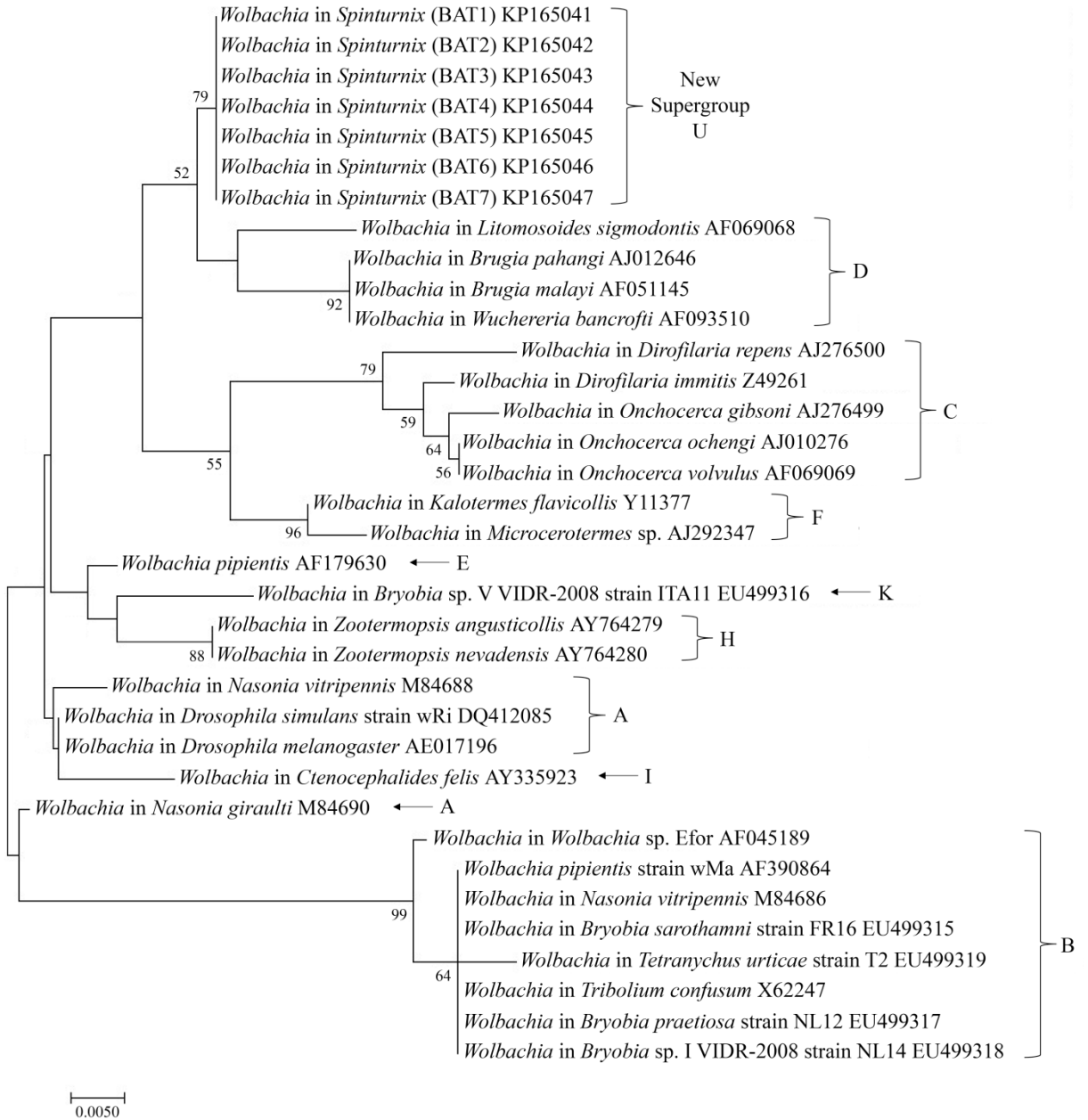
has become the subject of growing attention as a potential biocontrol agent of pests or vectors (Ahantarig and Kittayapong, 2011).

Here, we report, for the first time a new supergroup *Wolbachia*, named U, from *Spinturnix* mites infesting bats in Thailand, based on analysis of partial sequences of 16S rDNA and *ftsZ*. *Wolbachia* has been reported as an endosymbiont of mites in Families Dermanyssoidea and Phytoseioide, Order Mesostigmatas (Chaisiri *et al*, 2015). Our findings suggest *Spinturnix* mites are involved in transmission of supergroup U *Wolbachia* via thick-thumbed myotis bat and possibly other bat species as it is one of the most diverse groups of mammals, consisting of at least 138 species in Thailand (Bumrungsri *et al*, 2006; Soisook *et al*, 2013). The role of superfamily U *Wolbachia* in *Spinturnix* mite is unknown. Further extensive studies of *Wolbachia* endosymbionts in bat mites should provide a clearer understanding of their abundance and distribution in Thailand, which should be useful in future planning of appropriate strategies for biocontrol of mites in the country.

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A



B

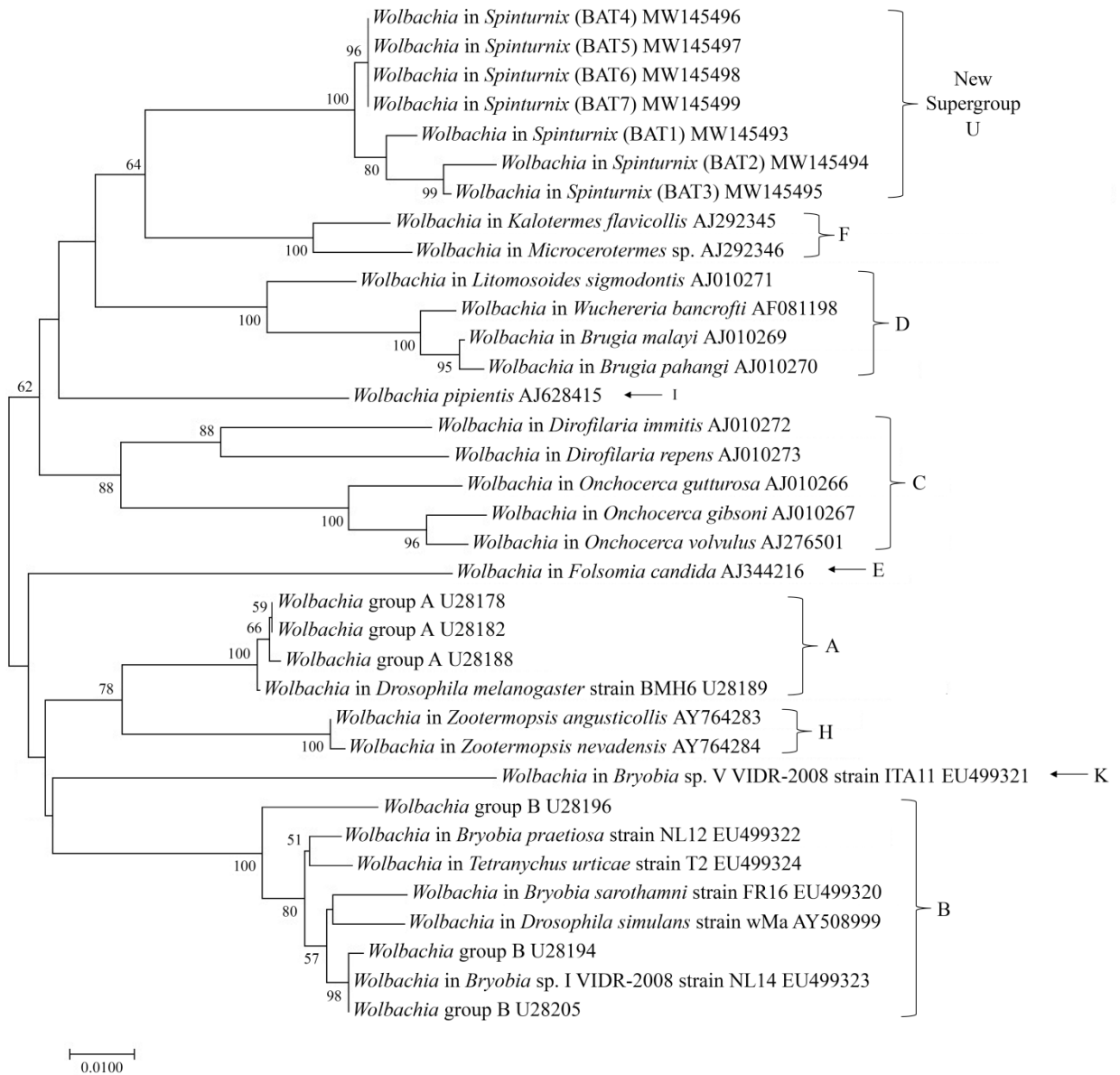


Fig 1 - Neighbor-joining phylogenetic analysis of *Wolbachia* from *Spinturnix* mites infesting bats (BAT1-BAT7) based on 16S rDNA (A) and *ftsZ* (B) partial sequences

Note: Only bootstrap values (1,000 replicates) >50 are indicated at branch node. Scale bars represent the genetic variation for the length of the scale.

Table 1
 Pairwise comparison of 16S rDNA and *ftsZ* partial DNA sequences of *Wolbachia* in *Spinturnix* from bats, Chachoengsao Province, Thailand

Type of DNA sequence	Percent identity						
	BAT1	BAT2	BAT3	BAT4	BAT5	BAT6	BAT7
16S rDNA gene							
1. <i>Wolbachia</i> in <i>Spinturnix</i> of BAT1	100						
2. <i>Wolbachia</i> in <i>Spinturnix</i> of BAT2	99.7	100					
3. <i>Wolbachia</i> in <i>Spinturnix</i> of BAT3	100	99.7	100				
4. <i>Wolbachia</i> in <i>Spinturnix</i> of BAT4	100	99.7	100	100			
5. <i>Wolbachia</i> in <i>Spinturnix</i> of BAT5	100	99.7	100	100	100		
6. <i>Wolbachia</i> in <i>Spinturnix</i> of BAT6	100	99.7	100	100	100	100	
7. <i>Wolbachia</i> in <i>Spinturnix</i> of BAT7	100	99.7	100	100	100	100	100
<i>ftsZ</i> gene							
1. <i>Wolbachia</i> in <i>Spinturnix</i> of BAT1	100						
2. <i>Wolbachia</i> in <i>Spinturnix</i> of BAT2	98.5	100					
3. <i>Wolbachia</i> in <i>Spinturnix</i> of BAT3	99.1	99.4	100				
4. <i>Wolbachia</i> in <i>Spinturnix</i> of BAT4	98.6	97.4	98.0	100			
5. <i>Wolbachia</i> in <i>Spinturnix</i> of BAT5	98.6	97.4	98.0	100	100		
6. <i>Wolbachia</i> in <i>Spinturnix</i> of BAT6	98.3	97.1	97.7	99.7	99.7	100	
7. <i>Wolbachia</i> in <i>Spinturnix</i> of BAT7	99.1	97.9	98.5	99.5	99.5	99.2	100

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