A REVIEW

RESEARCH ON *Wolbachia* ENDOSYMBIONT (RICKETTSIALES: ANAPLASMATACEAE) ASSOCIATED WITH INSECTS IN MALAYSIA: TRENDS AND PROSPECTS

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ABSTRACT

Wolbachia is a gram-negative bacterium from the family Anaplasmataceae and can be found in almost 70% of arthropods. This bacterium can alter the reproductive system of its hosts through four known effects such as Cytoplasmic Incompatibility (CI), male-killing, feminization and Parthenogenesis Inducing (PI). Besides, this bacterium has also been proven to block the transmission of dengue and chikungunya viruses from its mosquito hosts to human. Due to its ability to manipulate the reproductive system of the host and block virus transmission to human, *Wolbachia* gains new interest to be studied. This review discusses the ascending trends and prospects of *Wolbachia* research in medical, entomological, and agricultural aspects in Malaysia. *Wolbachia* data from the first record until the current one was discussed. The positive developments and interests of *Wolbachia* research are reviewed for the future references of epidemiologists and agriculturists in Malaysia.

Keywords: *Wolbachia*, Malaysia, medical, entomology, agriculture, research.

ABSTRAK

Wolbachia merupakan bakteria gram-negatif daripada famili Anaplasmataceae dan ditemui dalam hampir 70% Artropoda. Bakteria ini dapat mengubahsuai sistem pembiakan perumahnya melalui empat kesan yang diketahui seperti ketidaksesuaian sitoplasma (CI), kematian jantan, feminisasi dan penggalakan partenogenesis (PI). Selain itu, bakteria ini terbukti dapat menyekat penularan virus denggi dan chikungunya daripada perumah nyamuk kepada manusia. Disebabkan oleh kemampuan *Wolbachia* memanipulasi sistem pembiakan perumah dan menyekat penularan virus kepada manusia, kajian terhadap *Wolbachia* semakin mendapat perhatian. Dalam ulasan ini, kami membincangkan peningkatan trend dan prospek penyelidikan *Wolbachia* dalam aspek perubatan, entomologi dan pertanian di Malaysia. Data *Wolbachia* daripada rekod pertama sehingga masakini dibincangkan di dalam ulasan ini. Perkembangan positif dan kepentingan penyelidikan *Wolbachia* diulas untuk rujukan pada masa hadapan bagi ahli epidemiologi dan pakar pertanian di Malaysia.

Kata kunci: *Wolbachia*, Malaysia, perubatan, entomologi, pertanian, penyelidikan.

INTRODUCTION

Wolbachia Hertig & Wolbach is a genus of intracellular bacterium that widely infects up to 70% of arthropods including insects, mites, crustaceans, and nematodes (Saridaki & Bourtzis 2009). This gram-negative bacterium was first discovered by Marshall Hertig and Simeon Burt Wolbach in *Culex pipiens* L. (Diptera: Culicidae) (Hertig & Wolbach 1924) and was described later as species *Wolbachia pipientis* Hertig (Hertig 1936).

According to Casiraghi et al. (2005), this α-proteobacteria is classified into 16 supergroups; A to Q, based on genetic distances, excluding the G supergroup which is the combination of A and B. Molecular markers involved in the classification of supergroups include *16S* rRNA, *gltA*, *groEL*, *coxA*, *ftsZ* and *wsp* (Wang et al. 2016). Supergroups A and B are monophyletic and represent the most common group that infects arthropods. Supergroups C and D are known to be infecting filarial nematodes. *Wolbachia* of the supergroups A-D are the most studied due to its prevalence in the host. Supergroups E-Q infect a variety of hosts such as aphids, fleas, mites, nematodes, springtails, termites, and ticks (Glowska et al. 2015).

Even though *Wolbachia* was described as one single species, genotyping approaches have successfully shown its highly diverse status. The genotyping of *Wolbachia* include strain typing by using multi-locus sequence typing (MLST) of genes *gatB*, *coxA*, *hcpA*, *fbpA*, and *ftsZ*; and *wsp* typing of the hypervariable regions (Baldo et al. 2006). Identification of *Wolbachia* strains and typing were executed by submitting the sequence regions into *Wolbachia* MLST Databases (https://pubmlst.org/wolbachia/) to compare and characterize the strains.

Currently, various studies have been conducted to detect, characterize, and study the effect of this bacteria on the biological aspects of the hosts (Glaser & Meola 2010; Hu et al. 2020; Schneider et al. 2018). The infection by *Wolbachia* has resulted in reproductive manipulation (Figure 1) of the host such as Cytoplasmic Incompatibility (CI), male-killing, feminization, and Parthenogenesis-Inducing (PI). Different *Wolbachia* strains also may result in the differents effect of host manipulation (Sarakatsanou et al. 2011).

Cytoplasmic Incompatibility (CI) occurs during the mating process of the hosts with different *Wolbachia* strains (bidirectional) or only the male host is infected with *Wolbachia* (unidirectional) which results in no offspring (Alam et al. 2011; Telschow et al. 2007). Male-killing occurs during the formation of embryo development and results in producing all-female offspring (Fialho & Stevens 2000). The feminization of males occurs during the formation of the male embryo; the infected male embryos are converted into all-females; hence the progenies are females infected with *Wolbachia* (Negri et al. 2006). Parthenogenesis-Inducing

(PI) occurred when an infected female produces all-female progenies without fertilization (Weeks & Breeuwer 2001).

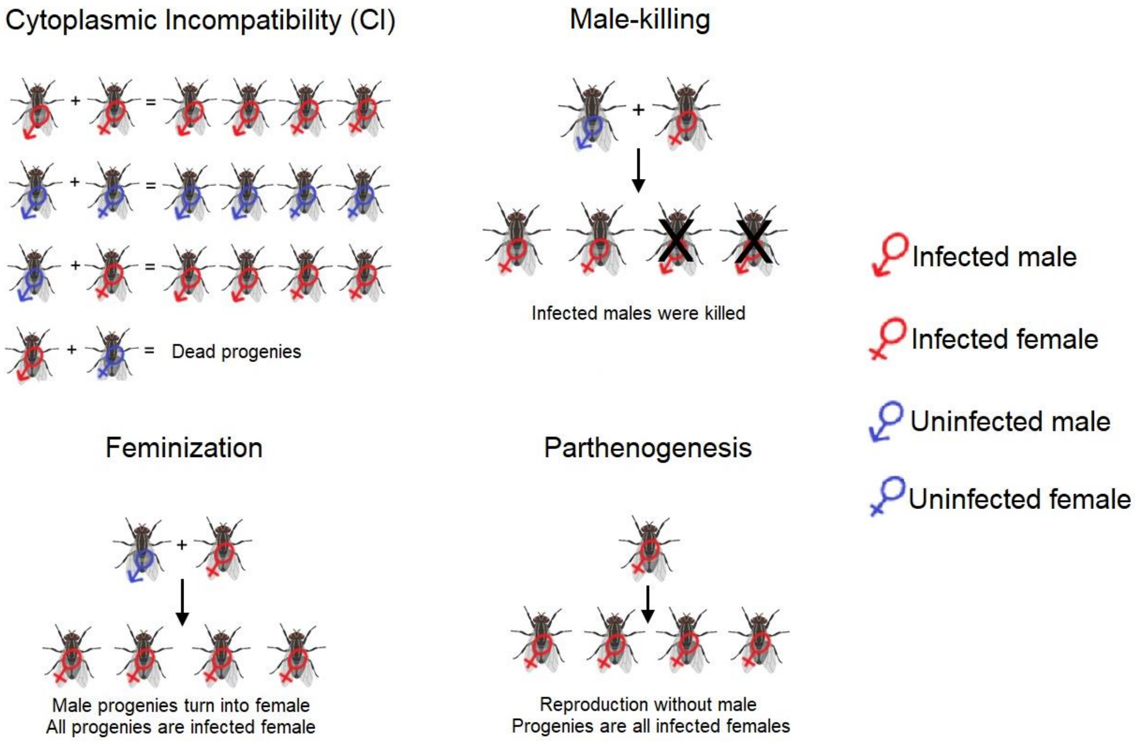

Figure 1. Four reproductive manipulation strategies of *Wolbachia* infection towards its host

The mechanism of reproductive alteration enables this maternally inherited bacteria to be among the most successful inherited endosymbionts in arthropods. The mode of inheritance is exclusively through vertical transmission from maternal to the offspring (Hong et al. 2002). However, there are a few cases of horizontally inherited *Wolbachia* recorded such as in ants, *Acromyrex* spp. (Tolley & Sapountzis 2019), whitefly, *Bemisia tabaci* (Gennadius) (Li et al. 2017), lepidopteran species (Ahmed et al. 2016), and wasp, *Eretmocerus* sp. (Ahmed et al. 2015). Horizontal transmission of *Wolbachia* requires intimate interaction between former and future hosts for successful transmission. For example, firstly, through parasitoid-host relationships, i.e. the transmission of *Wolbachia* from infected parasitoid *Eretmocerus* sp. into its host *B. tabaci* (Ahmed et al. 2015). Secondly, through plant-mediation, the infected *B. tabaci* that feeds on cotton leaves will transfer *Wolbachia* onto the leaves and transmit to the newly non-infected *B. tabaci* (Li et al. 2017). Thirdly, through hemolymph contact between the infected and non-infected terrestrial isopods (Rigaud & Juchault 1995). *Wolbachia* is known to be able to survive in the extracellular environment for up to 50 days before infecting a new host's cells (Li et al. 2017; Rasgon & Gamston 2006). Another method of transmission is through microinjections, which require an established infection from somatic cells into the germlines (Xi et al. 2005). This method has been widely used for introducing *Wolbachia* into mosquito germlines for combating dengue (Walker et al. 2011).

CURRENT TREND

There is a lack of studies on *Wolbachia* infecting insects available from Malaysia. However, the number of studies is ascending by year. A total of 25 research papers of *Wolbachia* studies in Malaysia have been published since 2003; 13 in medical entomology prospect; and 12 in agricultural entomology and *Wolbachia* diversity prospect. Figure 2 shows the increasing number of *Wolbachia* research in the medical entomology, *Wolbachia* diversity, and agricultural entomology fields. It is important to note that research on this bacterium has been gaining much interest since 2013 until the present. The ability of *Wolbachia* to manipulate the reproductive system of the host by favoring maternal inheritance and drive the evolution of host mitochondrial DNA by causing low variability makes this endosymbiont bacterium an appealing subject to investigate. The effects of the infection of *Wolbachia* can provide a positive outcome in the medical entomology and agricultural entomology studies.

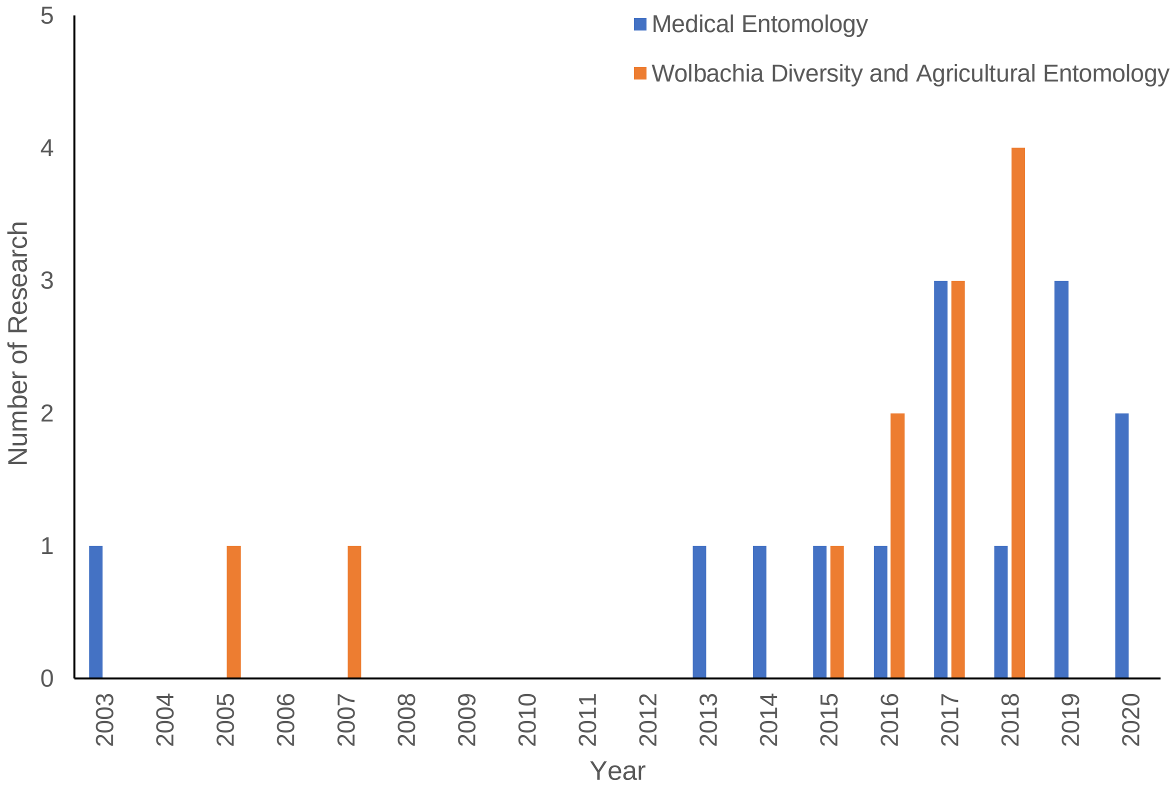

Figure 2. Trend of *Wolbachia* research in Malaysia for medical, agricultural, and diversity study based on published papers

MEDICAL ENTOMOLOGY PROSPECT

The first study of *Wolbachia* in Malaysia was conducted for medical entomology purposes to determine the *Wolbachia* infection in 12 new- and old-world *Aedes albopictus* (Skuse) populations (Armbruster et al. 2003). The mosquito sample from Malaysia was positively infected by two strains of *Wolbachia*, which were *w*albA and *w*AlbB, resulting in low variability in mitochondrial DNA. *Wolbachia* infected *Ae. albopictus* is presumed to cause the low variability in mtDNA, and in part of the host mitochondrial DNA evolution through genetic drift during colonization. The evolutionary of the *Ae. albopictus* provides information on the adaptability and survivability of these insect vectors (Tsetsarkin et al. 2014).

Joanne et al. (2014) experimented *Wolbachia* removal technique in *Ae. albopictus* of Kuala Lumpur population through 1.25 mg/mL dose of tetracycline treatment on adult mosquitoes. *Aedes albopictus* is a naturally infected mosquito with *Wolbachia*, thus making it difficult to find the non-infected wild population of *Ae. albopictus*. In this study, *Ae. albopictus* was chosen to understand the ability of *Wolbachia* to block the transmission of dengue and chikungunya virus from mosquitoes to humans and the biology behind this mechanism. The removal of *Wolbachia* in *Ae. albopictus* helps future study in providing *Wolbachia* negative mosquito for studying susceptibility of mosquito with different dengue serotypes.

The detection of *Wolbachia* in *Ae. albopictus* has been conducted in five localities in Peninsular Malaysia (Afizah et al. 2015). Results showed that adults sampled from all populations were positively infected by *w*AlbA and *w*AlbB strains using the *wsp* marker. Female *Ae. albopictus* was superinfected by both strains while the male species were infected with either the superinfection of A and B or a single infection of B strain. For pooled mosquitoes, 96.05% and 95.40% of females and males were positive with the *Wolbachia* infection, respectively. The wild population of *Ae. albopictus* are known to be naturally infected with *Wolbachia* and the mode of infection is as described in the introduction section. The high prevalence of *Wolbachia* in wild *Ae. albopictus* provides initial guideline data in developing potential strategies in combating dengue transmission in Peninsular Malaysia.

Realizing the potentials of *Wolbachia*, more studies have been conducted to detect the prevalence of *Wolbachia* in *Aedes* spp. in several localities in Malaysia. For example, Teo et al. (2017) reported that 25% of both *Ae. albopictus* and *Ae. aegypti* (L.) larvae from four localities in Kuala Lumpur were infected with *Wolbachia*. This study record as a novel finding as wild *Ae. aegypti* is never been recorded to harbour *Wolbachia*. A study by Ahmad et al. (2017) reports a high prevalence of *Wolbachia* infection from eight wild populations of *Ae. albopictus.* The study also reveals *Wolbachia* infection does not interfere with the Chikungunya virus (CHIKV) infection in the mosquitoes. The presence of *Wolbachia* in the *Ae. albopictus* does not inhibit the replication of CHIKV in the midguts and salivary glands of the mosquito making *Ae. albopictus* as a competent vector for CHIKV. A study by Joanne et al. (2017) also revealed that *Wolbachia* infection in *Ae. albopictus* does not affect the replication rate of four dengue virus (DENV) serotypes (DENV-1, DENV-2, DENV-3, and DENV-4) in the midgut and salivary glands of the species. The *Ae. albopictus* that is positively infected with *Wolbachia* is susceptible and capable of transmitting the DENV, makes it a competent secondary vector for DENV.

The detection of *Wolbachia* associated with insects in Malaysia was mainly conducted through Polymerase Chain Reaction (PCR) either by using *wsp*, *ftsZ*, *12S*rRNA, or *16S* rRNA markers. Loop-Mediated Isothermal Application (LAMP) has been used as an alternative method demonstrating higher sensitivity, which detects 45.6% of *Wolbachia* compared to 31% by PCR in seven mosquito species (Noor-Shazleen-Husnie et al. 2018).

Johari (2019) showed that 2.2% of larvae and 1.9% of adult mosquitoes were positive for all four DENV serotypes in the Klang Valley. Off all the collected *Aedes* species, 57.3% of larvae and 100% of adult *Ae. albopictus* were positive with *Wolbachia* strains *w*AlbA and *w*AlbB while *Ae. aegypti* were found negatively infected with *Wolbachia*. The high prevalence of *Wolbachia* in *Ae. albopictus* does not interfere with the infection of DENV in the species.

Nazni et al. (2019) have released artificially infected *Ae. aegypti* with *w*AlbB strain into six dengue hotspots in Kuala Lumpur for the establishment of *Wolbachia* strain in the urban population. Results showed that the infection was successfully established and maintained in the population after two years of release with a rate of > 90%. A decline in dengue cases has been observed in the released sites. Generally, *w*Mel strain is released into the wild population in other countries for the establishment of the strains (Hoffmann et al. 2011), however, *w*AlbB strain was chosen to be released in Malaysia due to the tropical weather. The *w*Mel strain is susceptible to the high temperature, thus it will reduce the infection densities, hence make it unable to maintain in the population. Compared to the *w*AlbB strain that was proven to be less susceptible to the high temperature (Ulrich et al. 2016).

Besides mosquitoes, studies on *Wolbachia* were also conducted in other insect vectors. Tay (2013) has successfully detected *Wolbachia* in cat flea, *Ctenocephalides felis* (Bouché), a well-known vector for *Bartonella* disease in humans. A high prevalence of *Wolbachia* (62.1%) was detected from *C. felis* and high carriage rates of *Rickettsia felis* (Bouyer) and *Bartonella* spp. were recorded in positive *Wolbachia* specimens. Khoo et al. (2016) conducted a study on bacteria pools in the *Haemaphysalis* spp. ticks of domesticated animals from two villages of indigenous communities in Perak. Results showed 392 bacterial genera were detected in three ticks species (*Haemaphysalis wellingtoni*, *H. hystricis*, and *H. bispinosa*) and *Wolbachia* was found in the 19 specimens out of 62 ticks collected (prevalence = 30.65%). The high abundance of bacteria in the ticks detected from the domesticated animals are linked to the potential agents for tick-borne diseases, for example, *R. felis* causing the flea-borne spotted fever in humans. Hence, more studies should be conducted to examine the physiology or disease transmission between the bacteria and ticks for more information on the microbiome of ticks in Malaysia.

In 2018, the detection of *Wolbachia* in tropical bed bug (*Cimex hemipterus* (Fabricius)) from 10 states in Peninsular Malaysia was conducted by using four different pairs of primers (Hassan et al. 2019). However, all samples showed negative results, thus proving that the tropical bed bug is free from *Wolbachia* infection. The latest study of *Wolbachia* was recorded on *Onchocerca borneensis* and *Malayfilaria sofiani* showed that the *Wolbachia* in *O. borneensis* belongs to supergroup C, while *Wolbachia* in *M. sofiani* belongs to supergroup D (Uni et al. 2020).

Wolbachia studies in Malaysia, particularly in terms of medical entomology research prospect are mainly focussed on the infection in *Aedes* mosquitoes that harbour DENV. Dengue is considered a health threat due to its ascending trend of infection in Malaysia. In 2014, Malaysia was hit with 250% of infections (108,698 cases) (Pang & Loh 2016). At the early stage, the mosquitoes control regimes such as fogging and insecticidal treatment approaches were implemented but failed due to the limited effectiveness and high cost (Pang & Loh 2016). Following the successful establishment of *Wolbachia* in the *Aedes* population to suppress dengue transmission in Cairns, Australia conducted by Hoffmann et al. (2011), the vector control management since then has shifted to the implementation of *Wolbachia* infection in *Aedes* mosquitoes as an alternative approach in affected regions such as Vietnam (Nguyen et al. 2015), Indonesia (Anders et al. 2018), Singapore (National Environment Agency 2019), and Brazil (Callaway 2016).

The effect of Cytoplasmic Incompatibility (CI) from the *Wolbachia* infection can be exploited into two approaches which are the reduction of mosquitoes' population via Sterile Insect Technique (SIT) and Incompatible Insect Technique (IIT) programmes to control dengue cases. The infection of *Wolbachia* resulted in the sterilization of males due to the modification of paternal chromosomes during spermatogenesis (Tram & Sullivan 2002). Hence, the mating between infected males and uninfected wild females (unidirectional CI) or females with different *Wolbachia* strains (bidirectional CI) was unsuccessful due to asynchronous mitosis which resulted in embryonic lethality (Flores & O'Neill 2018). *Wolbachia* has also been proven to cause pathogen blocking and restricted virus prevalence and intensity in the tissues of their hosts (Caragata et al. 2016; Kambris et al. 2009). Even though the mechanisms of pathogen-blocking are still unclear, it was suggested that *Wolbachia* may stimulate and increases the broad-spectrum immune responses such as antimicrobial peptides towards the infected hosts (Caragata et al. 2016; Kambris et al. 2009). A study by Dutra et al. (2016) found that *Wolbachia*-infected *Ae. aegypti* mosquitoes were resistant towards to Zika virus (ZIKV) as no ZIKV particles were contained from the saliva of the infected mosquitoes. Caragata et al. (2019) also discussed that the pathogen blocking capability in *Wolbachia*-infected *Ae. aegypti* is not affected by ZIKV and DENV co-infection and suggested that *Wolbachia*-infected *Ae. aegypti* is a promising method for controlling mosquito-borne diseases with a complex pattern of arbovirus transmission. In addition, the introduction of the virulent *Wolbachia* strain such as *w*MelPo*p* into the mosquito population can reduce the average age and titre levels of the DENV in mosquito individuals, thus leading to a reduced risk of dengue infection due to mosquito bites (Walker et al. 2011).

Wolbachia DIVERSITY AND AGRICULTURAL ENTOMOLOGY PROSPECT

The first *Wolbachia* detection in insects was conducted on the population of butterfly species, *Hypolimnas bolina* (L.) (Charlat et al. 2005). The sample was collected in Sabah which showed positive for *Wolbachia* infection *w*Bol1 strain. This strain was known to cause a male-killing effect in the population of *H. bolina* in Fiji and Japan (Dyson 2002; Mitsuhashi et al. 2004).

Detection of *Wolbachia* was conducted on termites from 44 populations in several countries includes Malaysia (Lo & Evans 2007) by using *16S* and *ftsZ* gene markers. Two populations were from Sabah and Sarawak with three different worker species [*Heterotermes tenuior* (Snyder), *Heterotermes* sp. and *Longipeditermes longipes* (Haviland)]. A total of 73% of specimens were recorded to be not infected with *Wolbachia*. The study aimed to dispute Bandi et al. (1997) result in high *Wolbachia* prevalence in termites (100% *Wolbachia* infection). *Wolbachia* are commonly known to enhance maternal transmission and distorting the reproductive system of the arthropods. The low prevalence of *Wolbachia* in the worker termites needs to be extensively studied to understand the role of *Wolbachia* and the parthenogenetically produced offspring of the queen.

Ahmed et al. (2016) revealed that four lepidopteran species collected from East Malaysia namely *Nacaduba angusta* (Druce), *Anthene emolus* (Godart), *Surendra vivarna* Horsfield, and *H. bolina* (L.) were infected with *Wolbachia*. Surprisingly, *Wolbachia* strains from these samples were also detected from other insect samples from other geographical regions. For example, species *A. emolus* was infected with strain ST37 together with wasp species from America, the Netherlands, and France [*Polistes dominulus* (Christ) and *Tetrastichus coeruleus* (Nees)]. The results suggested that horizontal transfer of *Wolbachia* may occur during ecological contacts such as the sharing of food sources and natural enemies between arthropods.

Wolbachia also has been detected in five important agricultural Opiinae parasitoid wasps of starfruits in Malaysia [*Fopius arisanus* (Sonan), *Fopius vandenboschi* (Fullaway), *Psyttalia* sp., *Psyttalia fletcheri* (Silvestri), and *Psyttalia incisi* (Silvestri)] (Mohammed et al.

2015). A subsequent study by Mohammed et al. (2017a) revealed *Wolbachia* from five Opiinae species belonged to the supergroup A with the addition of samples and localities. Mohammed et al. (2017b) proved that *F. vandenboschi* has three *Wolbachia* variants (wspHap3, wspHap5 and wspHap6), while *F. arisanus* has two variants (wspHap2 and wspHap1). These results were later proven in Mohammed et al. (2018a) whence *F. arisanus* has two variants (Type 1 and Type 2) using three different markers (*groEL*, *gltA*, and *wsp*). *Wolbachia* was estimated to infect Opiinae wasps associated with starfruits in Peninsular Malaysia more or less 0.138- 0.11 million years ago (mya) involving at least two independent infections and two secondary losses (Mohammed et al. 2017b). Despite the close biological interaction between hosts and parasitoids, three species of *Bactrocera* fruit flies were found to be negatively infected with *Wolbachia*, contrary with their parasitoid's species (*F. arisanus, F. vandenboschi, Diachasmimorpha longicaudata* (Ashmead), *Psyttalia sp.1, Psyttalia sp.2, P. fletcheri and P. incisi*) that proved to be infected with *Wolbachia* (Mohammed et al. 2017b).

A study on the solanum fruit fly, *Bactrocera latifrons* (Hendel) that was collected from ripe chilies, *Capsicum annuum* (L.) in the garden of the University of Malaya was conducted by Yong et al. (2017). Results showed that *Wolbachia* can be detected in almost all stages of the life cycle of *B. latifrons* (percentages of positively infected with *Wolbachia*; 98.61% in the larvae, 98.18% in the pupae, 97.59% in adult males, and 99.8% in adult females).

The screening of *Wolbachia* was conducted on the coleopteran palm pest, *Brontispa longissima* Gestro which was collected from five countries, including Malaysia (Ali et al. 2018). The study evaluated the genomic of *Wolbachia* and mtDNA of *B. longissima*. The Malaysian *Wolbachia* samples showed a significant genetic divergence among and between other populations with *wsp* 0.74%, *coxA* 0.25%, *fbpA* 0.24%, *gatB* 2.01%, and *hcpA* 3.80%. This result rendered that the beetle from Malaysia's population into Haplotype 1 along with Hainan and Taiwan's population.The high prevalence of *Wolbachia* in *B. longissima* also suggests a reduced mtDNA diversity in the species population. A study by Wan et al. (2018) attempted to detect *Wolbachia* infection in Red Palm Weevil (RPW), *Rhynchophorus ferrugineus* (Olivier). Female RPW samples were collected from three localities in Peninsular Malaysia and screened by using the *wsp* gene marker. Results showed that all 55 collected samples were not infected with *Wolbachia*. *Wolbachia* is known to affect the genetic structure and diversity of the hosts (Ali et al. 2018). The absence of *Wolbachia* in RPW provides baseline information in exploring the robust methods in controlling and management of the pest.

Apart from detection, characterization, and genomic composition of *Wolbachia* in insect's population, a further study was conducted to establish a novel *Wolbachia* synthetic peptide, Wolfar, into a potential biopesticide (Mohammed et al. 2018b). The peptide was synthesized from conserved region *wsp* and tested on cricket, *Acheta domesticus* (L.). Results showed there are regulations in the prostaglandin of the crickets, which indicated that WolFar was able to trigger the immune system of *A. domesticus*, thus potential to be applied as a biopesticide. Further studies should be conducted to formulate Wolfar for the means of disrupting the immune system of the agricultural pests.

At the moment, the *Wolbachia* research trend in terms of entomological and agricultural fields in Malaysia is focusing on the diversity and the biological aspects of *Wolbachia* infections in crop pests and beneficial insects. Several studies on discovering the prevalence of *Wolbachia* and strain diversity have been conducted worldwide. However, these studies are limited in Malaysia. Researchers are committed to exploring the rate of *Wolbachia* infection and strain diversity due to the ability of this maternally inherited endosymbiont to hitchhike in the mitochondrial DNA. Hence, promoting species speciation of infected hosts (Bordenstein 2003; Brucker & Bordenstein 2012) through the processes of reproductive isolation and the selective sweep of mtDNA (Miller et al. 2010). These studies are significantly important as the evolutionary and transmission of *Wolbachia* could also reflect its hosts mitochondrial DNA diversity and evolution. Most of the studies in agricultural field were conducted to understand the roles of *Wolbachia* in altering the reproductive system in infected insects. The effects of CI and parthenogenesis can be used to suppress or even better to eradicate the population of crop pests and to increase the population of beneficial insects. For example, Zabalou et al. (2004) had successfully established *Wolbachia*-infected lines of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) donated by the infected cherry fruit fly, *Rhagoletis cerasi* (L.) and found that the crossing between the infected males and uninfected females of *C. capitata* later showed promising results of 100% on egg mortality. Some parasitoids species also depend on *Wolbachia* infection to keep producing female offspring and directly increasing the rate of parasitization for controlling pests. According to Meyer and Hoy (2007), an encyrtid endoparasitoid for the Asian citrus psyllid, *Diaphorina citri* Kuwayama namely *Diaphorencyrtus aligarhensis* (Shafee, Alam and Agarwal) was found to be naturally infected with *Wolbachia* and had shown the parthenogenesis effect as only female offspring were produced. However, after being treated with tetracycline in lab condition, male offspring started to emerge from the population and proven to be free from *Wolbachia* infection. Hence, this endosymbiont has the potential to be suggested, developed, and applied as an alternative strategy in the Integrated Pest Management (IPM) due to its environmentally friendly bio-control agent properties compared to the chemical control strategies.

FUTURE

Based on the current trend of *Wolbachia*-insect associated research in Malaysia, the number of studies expected to be increasing in the future. Currently, the establishment of *Wolbachia* in the urban population of *Ae. aegypti* has shown promising success (Nazni et al. 2019). More research needs to be conducted to introduce this bacterium into other mosquito-vector populations, such as *Anopheles* spp. Persistence monitoring of dengue, chikungunya, and other arboviruses cases in mosquitoes carrying *Wolbachia* can provide an indicator for the success of this programme.

As for the agricultural field, the prevalence and effects of natural *Wolbachia* infection in economically important insects such as pests and beneficial insects should be widely characterized. The full understanding regarding this matter can be useful in manipulating the targeted insect population in agriculture for developing an Integrated Pest Management (IPM) programme that may reduce the dependency on chemical control for managing pest infestation.

CONCLUSION

Currently, there is an ascending trend of *Wolbachia*-insect associated research in Malaysia, but it is still limited and insufficient. The study of *Wolbachia* in Malaysia can be classified as new and recently gaining interest due to its ability to block virus diseases such as dengue and zika. The number of *Wolbachia* strains from Malaysian arthropods' will be increased as well as the characterization of the strain. There is also a need for in-depth studies on the effects of *Wolbachia* infections to fully understand its roles towards the insect populations in Malaysia. Furthermore, full genome sequencing of *Wolbachia* will be widely used for assisting the characterization of the strain, and indirectly aiding the study of the evolutionary of *Wolbachia* in Malaysia.

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REFERENCES

Afizah, A.N., Roziah, A., Nazni, W.A. & Lee, H.L. 2015. Detection of *Wolbachia* from field collected *Aedes albopictus* Skuse in Malaysia. *Indian Journal of Medical Research* 142(2): 205-210.

Ahmad, N.A., Vythilingam, I., Lim, Y.A., Zabari, N.Z.A.M. & Lee, H.L. 2017. Detection of *Wolbachia* in *Aedes albopictus* and their effects on chikungunya virus. *The American Journal of Tropical Medicine and Hygiene* 96(1): 148-156.

Ahmed, M.Z., Breinholt, J.W. & Kawahara, A.Y. 2016. Evidence for common horizontal transmission of *Wolbachia* among butterflies and moths. *BMC Evolutionary Biology* 16(1): 118.

Ahmed, M.Z., Li, S.J., Xue, X., Yin, X.J., Ren, S.X., Jiggins, F.M., Greeff, J.M. & Qiu, B.L. 2015. The intracellular bacterium *Wolbachia* uses parasitoid wasps as phoretic vectors for efficient horizontal transmission. *PLoS Pathogen* 11(2): e1004672.

Alam, U., Medlock, J., Brelsfoard, C., Pais, R., Lohs, C., Balmand, S., Carnogursky, J., Heddi, A., Takac, P., Galvani, A. & Aksoy, S., 2011. *Wolbachia* symbiont infections induce strong cytoplasmic incompatibility in the tsetse fly *Glossina morsitans*. *PLoS Pathogen 7*(12): p.e1002415.

Ali, H., Muhammad, A., Bala, N.S., Wang, G., Chen, Z., Peng, Z. & Hou, Y. 2018. Genomic evaluations of *Wolbachia* and mtDNA in the population of coconut hispine beetle, *Brontispa longissima* (Coleoptera: Chrysomelidae). *Molecular Phylogenetics and Evolution* 127: 1000-1009.

Anders, K.L., Indriani, C., Ahmad, R.A., Tantowijoyo, W., Arguni, E., Andari, B., Jewell, N.P., Rances, E., O'Neill, S.L., Simmons, C.P. & Utarini, A. 2018. The AWED trial (Applying *Wolbachia* to Eliminate Dengue) to assess the efficacy of *Wolbachia*-infected mosquito deployments to reduce dengue incidence in Yogyakarta, Indonesia: study protocol for a cluster randomised controlled trial. *Trials* 19(1): 302.

Armbruster, P., Damsky, Jr. W. E., Giordano, R., Birungi, J., Munstermann, L.E. & Conn, J. E. 2003. Infection of New-and Old-World *Aedes albopictus* (Diptera: Culicidae) by the intracellular parasite *Wolbachia*: Implications for host mitochondrial DNA evolution. *Journal of Medical Entomology* 40(3): 356-360.

Baldo, L., Bordenstein, S., Wernegreen, J.J. & Werren, J.H. 2006. Widespread recombination throughout *Wolbachia* genomes. *Molecular Biology and Evolution* 23(2): 437-449.

Bandi, C., Sironi, M., Nalepa, C.A., Corona, S. & Sacchi, L. 1997. Phylogenetically distant intracellular symbionts in termites. *Parassitologia* 39: 71–75.

Bordenstein, S.R. 2003. Symbiosis and the origin of species. In Bourtzis, K. & Thomas, A.M. (eds.) *Insect Symbiosis*. Boca Raton: CRC Press, 283–304.

Brucker, R.M. & Bordenstein, S.R. 2012. Speciation by symbiosis. *Trends in Ecology & Evolution* 27: 443–51.

Callaway, E. 2016. Rio fights Zika with biggest release yet of bacteria-infected mosquitoes. *Nature* 539: 17–18.

Caragata, E.P., Rezende, F.O., Simoes, T.C. & Moreira, L.A. 2016. Diet-Induced Nutritional Stress and Pathogen Inter-ference in *Wolbachia*-Infected *Aedes aegypti*. *PLoS Neglected Tropical Diseases.* 10(11): e0005158.

Caragata, E.P., Rocha, M.N., Pereira, T.N., Mansur, S.B., Dutra, H.L. & Moreira, L.A. 2019. Pathogen blocking in *Wolbachia*-infected *Aedes aegypti* is not affected by Zika and dengue virus co-infection. *PLoS Neglected Tropical Diseases* 13(5): e0007443.

Casiraghi, M., Bordenstein, S.R., Baldo, L., Lo, N., Beninati, T., Wernegreen, J.J., Werren, J.H. & Bandi, C. 2005. Phylogeny of *Wolbachia pipientis* based on gltA, groEL and ftsZ gene sequences: clustering of arthropod and nematode symbionts in the F supergroup, and evidence for further diversity in the *Wolbachia* tree. *Microbiology* 151(12): 4015-4022.

Charlat, S., Hornett, E.A., Dyson, E.A., Ho, P.P., Loc, N.T., Schilthuizen, M., Davies, N., Roderick, G.K. & Hurst, G.D. 2005. Prevalence and penetrance variation of male-killing *Wolbachia* across Indo-Pacific populations of the butterfly *Hypolimnas bolina*. *Molecular Ecology* 14(11): 3525-3530.

Dutra, H.L.C., Rocha, M.N., Dias, F.B.S., Mansur, S.B., Caragata, E.P. & Moreira, L.A. 2016. *Wolbachia* blocks currently circulating Zika virus isolates in Brazilian *Aedes aegypti* mosquitoes. *Cell Host & Microbe* 19(6): 771-774.

Dyson, E.A. 2002. Inherited parasites in the butterfly *Hypolimnas bolina* (Lepidoptera: Nymphalidae). PhD disertation. University College London.

Fialho, R.F. & Stevens, L. 2000. Male-killing *Wolbachia* in a flour beetle. *Proceedings of the Royal Society B: Biological Sciences* 267(1451): 1469-1473.

Flores, H.A. & O'Neill, S.L. 2018. Controlling vector-borne diseases by releasing modified mosquitoes. *Nature Reviews Microbiology* 16(8): 508-518.

Glaser, R.L. & Meola, M.A. 2010. The native Wolbachia endosymbionts of *Drosophila melanogaster* and *Culex quinquefasciatus* increase host resistance to West Nile virus infection. *PloS One* 5(8): e11977.

Glowska, E., Dragun-Damian, A., Dabert, M. & Gerth, M., 2015. New *Wolbachia* supergroups detected in quill mites (Acari: Syringophilidae). *Infection, Genetics and Evolution* 30: 140-146.

Hassan, N.H.M., Ismail, D.E., Masran, S.N.A.S. & Ab Majid, A.H. 2019. Molecular detection and screening of *Wolbachia* infections in tropical bed bugs *Cimex hemipterus* (Hemiptera: Cimicidae) from Peninsular Malaysia Populations. *The Malaysian Journal of Microbiology* 15(2): 166-172.

Hertig, M., & Wolbach, S.B. 1924. Studies on rickettsia-like micro-organisms in insects. *The Journal of Medical Research* 44(3): 329-374.

Hertig, M. 1936. The rickettsia, *Wolbachia pipientis* (gen. et sp. n.) and associated inclusions of the mosquito, *Culex pipiens*. *Parasitology* 28(4): 453-486.

Hoffmann, A.A., Montgomery, B.L., Popovici, J., Iturbe-Ormaetxe, I., Johnson, P.H., Muzzi, F., Greenfield, M., Durkan, M., Leong, Y. S., Dong, Y. & Cook, H. 2011. Successful establishment of *Wolbachia* in *Aedes* populations to suppress dengue transmission. *Nature* 476: 454–457.

Hong, X.Y., Gotoh, T. & Nagata, T. 2002. Vertical transmission of *Wolbachia* in *Tetranychus kanzawai* Kishida and *Panonychus mori* Yokoyama (Acari: Tetranychidae). *Heredity* 88(3): 190-196.

Hu, Y., Xi, Z., Liu, X., Wang, J., Guo, Y., Ren, D., Wu, H., Wang, X., Chen, B. & Liu, Q., 2020. Identification and molecular characterization of *Wolbachia* strains in natural populations of *Aedes albopictus* in China. *Parasites & Vectors* 13(1): 28.

Joanne, S., Vythilingam, I., Teoh, B.T., Leong, C.S., Tan, K.K., Wong, M.L., Yugavathy, N. & AbuBakar, S. 2017. Vector competence of Malaysian *Aedes albopictus* with and without *Wolbachia* to four dengue virus serotypes. *Tropical Medicine & International Health* 22(9): 1154-1165.

Joanne, S., Vythilingam, I., Yugavathy, N. & Doss, J.I. 2014. Modified technique of *Wolbachia* removal from Malaysian *Aedes albopictus*. *Asian Pacific Journal of Tropical Biomedicine* 4(7): 557-560.

Johari, N.A.B. 2019. Epidemiology of Dengue in Malaysia and the Dynamics of Dengue Virus-Vector-Host Interactions. PhD dissertation. International Medical University.

Kambris, Z., Cook, P.E., Phuc, H.K. & Sinkins, S.P. 2009. Immune activation by life-shortening *Wolbachia* and reduced filarial competence in mosquitoes. *Science* 326(5949): 134–6.

Khoo, J.J., Chen, F., Kho, K.L., Shanizza, A.I.A., Lim, F.S., Tan, K.K., Chang, L.Y. & AbuBakar, S. 2016. Bacterial community in *Haemaphysalis* ticks of domesticated animals from the Orang Asli communities in Malaysia. *Ticks and Tick-Borne Diseases* 7(5): 929-937.

Li, S.J., Ahmed, M.Z., Lv, N., Shi, P.Q., Wang, X.M., Huang, J.L. & Qiu, B.L. 2017. Plant mediated horizontal transmission of *Wolbachia* between whiteflies. *The ISME Journal* 11(4): 1019-1028.

Lo, N. & Evans, T.A. 2007. Phylogenetic diversity of the intracellular symbiont *Wolbachia* in termites. *Molecular Phylogenetics and Evolution* 44(1): 461-466.

Meyer, J.M. & Hoy, M.A. 2007. *Wolbachia*-associated thelytoky in *Diaphorencyrtus aligarhensis* (Hymenoptera: Encyrtidae), a parasitoid of the Asian citrus psyllid. *Florida Entomologist* 90(4): 776-779.

Miller, W.J., Ehrman, L. & Schneider, D. 2010. Infectious speciation revisited: impact of symbiont-depletion on female fitness and mating behaviour of *Drosophila paulistorum. PLoS Pathogens* 6: e1001214.

Mitsuhashi, W., Fukuda, H., Nicho, K. & Murakami, R. 2004. Male-killing *Wolbachia* in the butterfly *Hypolimnas bolina*. *Entomologia Experimentalis et Applicata* 112: 57–64.

Mohammed, M.A., Aman-Zuki, A., Yusof, S., Othman, N.W., Md-Zain, B.M. & Yaakop, S. 2018a. Classification of endosymbiont *Wolbachia* (Rickettsiales: Anaplasmataceae) in opiine wasps (Hymenoptera: Braconidae). *AIP Conference Proceedings* 1940(1): 020039.

Mohammed, M.A., Aman-Zuki, A., Othman, N.W., Tagami, Y. & Yaakop, S. 2018b. The role of a novel *Wolbachia* (Rickettsiales: Anaplasmataceae) synthetic peptide, WolFar, in regulating prostaglandin levels in the hemolymph of *Acheta domesticus* (Orthoptera: Gryllidae). *Turkish Journal of Zoology* 42(4): 422-431.

Mohammed, M.A., Aman-Zuki, A., Yusof, S., Md-Zain, B.M. & Yaakop, S. 2017a. Prevalence and evolutionary history of endosymbiont *Wolbachia* (Rickettsiales: Anaplasmataceae) in parasitoids (Hymenoptera: Braconidae) associated with *Bactrocera* fruit flies (Diptera: Tephritidae) infesting carambola. *Entomological Science* 20(1): 382-395.

Mohammed, M.A., Aman-Zuki, A., Yusof, S., Md-Zain, B.M., Othman, N.W. & Yaakop, S. 2017b. Detection of *Wolbachia* endobacteria in parasitoids associated with starfruit cultivations in Malaysia. *International Journal on Advanced Science, Engineering and Information Technology* 5(1): 17-21.

Mohammed, M.A., Aman-Zuki, A., Yusof, S. & Yaakop, S. 2015. Isolation and characterization of *Wolbachia* (Rickettsiales: Rickettsiaceace) from several economic importance parasitoids (Hymenoptera: Braconidae). *International Journal of Bioscience, Biochemistry and Bioinformatics* 5(4): 256-263.

National Environment Agency. 2019. Project Wolbachia Singapore. https://www.nea.gov.sg/corporate-functions/resources/research/wolbachia-aedes-mosquito-suppression-strategy [10 April 2020].

Nazni, W.A., Hoffmann, A.A., Noor, A.A., Cheong, Y.L., Mancini, M.V., Golding, N., Kamarul, G.M., Arif, M.A., Thohir, H., NurSyamimi, H. & Zatil, A.M.Z. 2019. Establishment of *Wolbachia* strain *w*AlbB in Malaysian populations of *Aedes aegypti* for dengue control. *Current Biology* 29(24): 4241-4248.

Negri, I., Pellecchia, M., Mazzoglio, P.J., Patetta, A. & Alma, A. 2006. Feminizing *Wolbachia* in *Zyginidia pullula* (Insecta, Hemiptera), a leafhopper with an XX/X0 sex-determination system. *Proceedings of the Royal Society B: Biological Sciences* 273(1599): 2409-2416.

Nguyen, T.H., Le Nguyen, H., Nguyen, T.Y., Vu, S.N., Tran, N.D., Le, T.N., Vien, Q.M. et al. 2015. Field evaluation of the establishment potential of *w*MelPop *Wolbachia* in Australia and Vietnam for dengue control. *Parasites & Vectors* 8(1): 563.

Noor-Shazleen-Husnie, M.M., Emelia, O., Ahmad-Firdaus, M.S., Zainol-Ariffin, P. & Aishah-Hani, A. 2018. Detection of *Wolbachia* in wild mosquito populations from selected areas in Peninsular Malaysia by loop-mediated isothermal amplification (LAMP) technique. *Tropical Biomedicine* 35(2): 330-46.

Pang, E.L. & Loh, H.S. 2016. Current perspectives on dengue episode in Malaysia. *Asian Pacific of Journal Tropical Medicine* 9(4): 395-401.

Rasgon, J.L., Gamston, C.E. & Ren, X. 2006. Survival of *Wolbachia pipientis* in cell-free medium. *Applied and Environmental Microbiology* 72(11): 6934-6937.

Rigaud, T. & Juchault, P. 1995. Success and failure of horizontal transfers of feminizing *Wolbachia* endosymbionts in woodlice. *Journal of Evolutionary Biology* 8: 249–255.

Sarakatsanou, A., Diamantidis, A.D., Papanastasiou, S.A., Bourtzis, K. & Papadopoulos, N.T. 2011. Effects of Wolbachia on fitness of the Mediterranean fruit fly (Diptera: Tephritidae). *Journal of Applied Entomology* 135(7): 554-563.

Saridaki, A. & Bourtzis, K. 2009. *Wolbachia*-induced reproductive parasitism and applications. *Entomologia Hellenica* 18: 3-16.

Schneider, D.I., Parker, A.G., Abd-alla, A.M. & Miller, W.J. 2018. High-sensitivity detection of cryptic *Wolbachia* in the African tsetse fly (*Glossina* spp.). *BMC Microbiology* 18(1): 140.

Tay, S.T. 2013. *Wolbachia* endosymbionts, *Rickettsia felis* and *Bartonella* species, in *Ctenocephalides felis* fleas in a tropical region. *Journal of Vector Ecology* 38(1): 200-202.

Telschow, A., Flor, M., Kobayashi, Y., Hammerstein, P. & Werren, J.H. 2007. *Wolbachia*-induced unidirectional cytoplasmic incompatibility and speciation: Mainland-island model. *PLoS One* 2(8): e701.

Teo, C.H.J., Lim, P., Voon, K. & Mak, J.W. 2017. Detection of dengue viruses and *Wolbachia* in *Aedes aegypti* and *Aedes albopictus* larvae from four urban localities in Kuala Lumpur, Malaysia. *Tropical Biomedicine* 34(3): 583-597.

Tolley, S.J.A.I. & Sapountzis, P. 2019. *Wolbachia* horizontal transmission events in ants: What do we know and what can we learn? *Frontiers in Microbiology* 10(296): 1-9.

Tram, U. & Sullivan, W. 2002. Role of delayed nuclear envelope breakdown and mitosis in *Wolbachia*-induced cytoplasmic incompatibility. *Science* 296(5570): 1124-1126.

Tsetsarkin, K.A., Chen, R., Yun, R., Rossi, S.L., Plante, K.S., Guerbois, M., Forrester, N., Perng, G.C., Sreekumar, E., Leal, G. & Huang, J. 2014. Multi-peaked adaptive landscape for chikungunya virus evolution predicts continued fitness optimization in *Aedes albopictu* s mosquitoes. *Nature Communications* 5(1): 1-14.

Ulrich, J.N., Beier, J.C., Devine, G.J. & Hugo, L.E. 2016. Heat sensitivity of *w*Mel *Wolbachia* during *Aedes aegypti* development. *PLoS Neglected Tropical Diseases* 10(7): e0004873.

Uni, S., Udin, A.S.M., Agatsuma, T., Junker, K., Saijuntha, W., Bunchom, N., Fukuda, M., Martin, C., Lefoulon, E., Labat, A. & Khan, F.A.A. 2020. Description, molecular characteristics and *Wolbachia* endosymbionts of *Onchocerca borneensis* Uni, Mat Udin & Takaoka n. sp. (Nematoda: Filarioidea) from the Bornean bearded pig *Sus barbatus* Müller (Cetartiodactyla: Suidae) of Sarawak, Malaysia. *Parasite Vector* 13(1): 50.

Walker, T.J.P.H., Johnson, P.H., Moreira, L.A., Iturbe-Ormaetxe, I., Frentiu, F.D., McMeniman, C.J., Leong, Y.S., Dong, Y., Axford, J., Kriesner, P. & Lloyd, A.L. 2011. The *w*Mel *Wolbachia* strain blocks dengue and invades caged *Aedes aegypti* populations. *Nature* 476(7361): 450-453.

Wan, N.A.W.M.N., Othman, N.W., Yaakop, S. & Nor, N.S.M. 2018. Morphology and histology of reproductive organ and first screening of *Wolbachia* in the ovary of red palm weevil, *Rhynchophorus ferrugineus* (Coleoptera: Dryophthoridae). *Serangga* 23(2): 183-193.

Wang, G.H., Jia, L.Y., Xiao, J.H. & Huang, D.W. 2016. Discovery of a new *Wolbachia* supergroup in cave spider species and the lateral transfer of phage WO among distant hosts. *Infection, Genetics and Evolution* 41: 1-7.

Weeks, A.R. & Breeuwer, J.A.J. 2001. *Wolbachia*–induced parthenogenesis in a genus of phytophagous mites. *Proceedings of the Royal Society B: Biological Sciences* 268(1482): 2245-2251.

Xi, Z., Dean, J.L., Khoo, C. & Dobson, S.L. 2005. Generation of a novel *Wolbachia* infection in *Aedes albopictus* (Asian tiger mosquito) via embryonic microinjection. *Insect Biochemistry and Molecular Biology* 35(8): 903-910.

Yong, H.S., Song, S.L., Chua, K.O. & Lim, P.E. 2017. Predominance of *Wolbachia* endosymbiont in the microbiota across life stages of *Bactrocera latifrons* (Insecta: Tephritidae). *Meta Gene* 14:6-11.

Zabalou, S., Riegler, M., Theodorakopoulou, M., Stauffer, C., Savakis, C. & Bourtzis, K. 2004. *Wolbachia*-induced cytoplasmic incompatibility as a means for insect pest population control. *Proceedings of the National Academy of Sciences* 101(42): 15042-15045.