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Diversity and *Wolbachia* infection in rice insect pests from Thailand

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Abstract

Wolbachia is the genus of an endosymbiont that is infected in many arthropod species. *Wolbachia* plays a role in the reproductive system as a reproductive manipulator of its hosts. In Thailand, *Wolbachia* has been detected in many insects but a little is known about insect pests from rice fields. This research project is the investigation of these endosymbionts in insects from rice fields. Rice fields in Phetchaburi and Nakhon Ratchasima provinces in Thailand have not yet been reported to use a high level of insecticides; as a result, these areas still have a high diversity of insects. Samples were collected from October 2018 to September 2019. From this study, there were 418 individuals of insect pests in 7 orders (Hymenoptera, Lepidoptera, Coleoptera, Orthoptera, Diptera, Isoptera and Homoptera), 12 families in total of 17 species including snout weevil, ants, white grub, pyralid stem borer, termites, rice root aphid, mealybug, rice seedling fly, flea beetles, leaf beetle, wireworms, citrus locust, Bombay locust, Oriental migratory locust, Cyrtacanthacris locust, Brown planthoppers and White backed planthoppers which collected from rice fields. These rice insect pests have been collected from three different regions of Thailand. In addition, the number of rice insect pests collected by hand sampling, swing and pitfall trap has the significant positive correlation with the relative moisture in the air. The detection of *Wolbachia* infection in rice insect pests was determined by polymerase chain reaction using *wsp* primers to confirm the infection status of *Wolbachia* and their genetic diversity. *Wolbachia* was detected in mealybug, brown planthoppers and white backed planthoppers from collected specimens in Thailand. These findings indicated that *Wolbachia* are distributed throughout the rice field insect populations from Thailand.

Keywords: infection, *Wolbachia*, diversity, rice, insect pests

Introduction

Wolbachia is the genus of gram-negative, maternally inherited bacteria that infect a diverse range of insects. These bacteria normally infect the reproductive organs of their hosts. *Wolbachia* infection and diversity have been reported in many groups of insects, for examples, Orthoptera, Lepidoptera, Hymenoptera and Diptera (Tagami and Miura, 2004 [13]; Wiwatanaratanabutr and Kittayapong, 2009 [18]; Rattan *et al.*, 2011 [9]; Jeong *et al.*, 2012 [7]; Wiwatanaratanabutr, 2013a [16], 2013b [17]; Carjaval *et al.*, 2018 [5]; Bagheri *et al*., 2019 [2]; Bi and Wang, 2020 [4]). The binomial model data revealed that *Wolbachia* infection in insects are estimated to be 66% and the infection frequency within a species may be 10% - 90% (Hilgenboecker *et al.,* 2008 [6]). *Wolbachia* can be transmitted from infected females to their offspring via the egg. They have evolved to manipulate host reproduction resulting in different abnormal reproductive traits such as cytoplasmic incompatibility or CI (Wiwatanaratanabutr, 2013a [16]). These traits support the distribution of *Wolbachia* in their hosts by providing a fitness advantage to females, thereby increasing their number in the hosts. The evolutionary relationships of *Wolbachia* in insect hosts was mostly determined by the small ribosomal subunit gene (16S rDNA), the bacterial cell cycle gene (*ftsZ*) and the gene encoding an outer surface protein (*wsp*) (O'Neill *et al.*, 1992 [8]; Werren *et al.*, 1995 [14]; Zhou *et al.*, 1998 [19]). Analysis of these genes shows that *Wolbachia* belongs to a monophyletic clade in the alpha-proteobacterial group (Ricci *et al.,* 2002 [11]) that can be classified into many supergroups such as A and B which are the most widely distributed in insects (Augustinos *et al*., 2011 [1]).

Thailand is one of the Southeast Asian countries where the rice fields infested with many insect pests. Therefore, research on geographical survey and control strategies for these insects with their endosymbionts is a high priority for pest management. The central region of Thailand is the major rice producing area following by Northeast, West, North and South, respectively. The evaluation of present status of those insect pests with *Wolbachia* infection in rice fields should be an important component of surveillance programs for rice pests. However, information regarding *Wolbachia* infection in these insects from rice fields in Thailand have nearly never been investigated at present. Here, we present data of *Wolbachia* infection status from these insect pests in rice fields from different geographical populations in Thailand using PCR-based detection.

In this study, the diversity and infection status of the

Wolbachia endosymbiont in rice insect pests from 5 provinces in two different geographic regions of Thailand was determined. Study of the infection status and transmission mechanism of *Wolbachia* has been very helpful in utilization of insect natural enemies for insect pest control.

The objectives of the present study were 1) to investigate the diversity and distribution of collected insect pests from rice fields in two different geographic regions of Thailand, and 2) to examine the *Wolbachia* infection status in collected insect pests from rice fields in two different geographic regions of Thailand.

Materials and Methods

Collection of specimen and identification

Insect pests were collected from rice fields in five provinces from three different geographic regions throughout Thailand including Central, Northeast and West during October 2018 and September 2019. Collections were made by using hand sampling, sweep nets, aspirators, swing and pitfall trap at each study site. All insect specimens were frozen on dry ice and transported to the laboratory in Bangkok. Individuals were identified to species using the morphological keys (Reissig *et al.*, 1985 [10]; Wilson and Claridge, 1991 [15]; Barrion and Litsinger, 1994 [3]). Species that could not be distinguished on the morphology were classified to the genus level. Only adult specimens were used and tested for *Wolbachia* infection to ensure accurate identification. Specimens that could not be processed immediately were stored at - 20 ºC for later use.

DNA preparation

Genomic DNA from rice insect pests was extracted and examined for the presence of *Wolbachia* using *wsp* primers (Zhou *et al.*, 1998 [19]). Whole body or adult abdomen of each sample was ground in 100 µl of STE lysis buffer (100 mM NaCl, 1 mM EDTA [pH 8.0], 10 mM Tris-HCl [pH 8.0]) using sterile plastic pestle in a 1.5 ml centrifuge tube according to the method of O'Neill *et al.* (1992) using proteinase K. The homogenate was heated at 95°C for 10 min and then centrifuged at 1,400 g for 1 min. Extracted DNA was stored at -20 ºC until used in 50 µl of elution buffer, concentration determined using a Nanodrop spectrophotometer (Thermo Scientific, Singapore). One microliter of the supernatant was used as template DNA to PCR-screen for examining *Wolbachia* infection.

Polymerase chain reaction (PCR) amplification

The universal mitochondrial eukaryotic 12S rRNA primers (12SAI and 12SBI, O'Neill *et al.*, 1992) were used to check for the quality of the DNA extraction. Specimens that were negative for 12S rRNA gene of host insects were excluded from the data. The *wsp* genetic markers was used to detect *Wolbachia* infection using *wsp* gene primers (Zhou *et al.*, 1998 [19]). The correct size of amplified DNA (PCR product) was around 600 bp. PCR amplification was done on a Hybaid OmniGene thermal cycler using 20 µl reaction mixture volumes. Each reaction contained buffer (10 mM Tris–HCl [pH 8.3], 50 mM KCl, 1.5 mM $MgCl_2$), dNTPs (10 mM each), 20 µM forward and reverse primers and 1 unit of *Taq* DNA polymerase (Promega). The PCR thermal program was a cycle of initial denaturation (94°C) for 3 min, followed by 94°C for 1 min, 55°C for 1 min and 72°C for 1 min in total of 35 cycles, and final extension at 72°C for 10 min was used in this study. DNA extract of *Wolbachia*-infected *Aedes albopictus* was used as a positive screening control for *Wolbachia*. Double-distilled water (ddH_2O) was used as a negative control to check for contamination. Ten microliters of each PCR product were run on a 1% agarose gel with a 1-kb ladder then stained with ethidium bromide and visualized under a UV transilluminator to determine size and the presence of amplified DNA. Specimens yielding a product of the expected size (600 bp) were scored as positive for *Wolbachia* infection.

Results and Discussion

A total number of 418 insect pest individuals from 12 families in 7 orders including Hymenoptera, Lepidoptera, Coleoptera, Orthoptera, Diptera, Isoptera and Homoptera which consists of 17 species including *Hypomeces squamosus* Fabricius (snout weevil), *Pheidole* sp. (ants), *Lachnosterna* sp. (white grub), *Euzophera perticella* (stem borer), *Odontotermes takensis* (termites), *Tetraneura nigriabdominalis* (rice root aphid), *Cataenococcus* sp. (mealybug), *Atherigona oryzae* (Malloch) (rice seedling fly), *Chaetocnema basalis* Baly (flea beetles), *Monolepta signata* Olivier (leaf beetle), *Melanotus* sp. (wireworms), *Chondracris rosea* (citrus locust), *Patanga succincta* (Linnaeus) (Bombay locust), *Locusta migratoria* (Oriental migratory locust), *Cyrtacanthacris tatarica* (Linnaeus) (Cyrtacanthacris locust), *Nilaparvata lugens* (Brown planthoppers), and *Sogatella furcifera* (White backed planthoppers) were collected during the field surveys from five provinces in three geographic regions (Central, Northeast and West) throughout Thailand (Fig 1) and screened for the presence of *Wolbachia*.

The five provinces include Saraburi, Nakhon Nayok, Nakhon Ratchasima and Prachinburi which located in the Central and Northeast region of Thailand, and Phetchaburi province that located in the West region of Thailand. Based on PCR results, *Wolbachia* was detected in three insect pest species including *Cataenococcus* sp. (mealybug), *Nilaparvata lugens* (Brown planthoppers), and *Sogatella furcifera* (white backed planthoppers). Most of *Wolbachia*-infected individuals were collected from the Central, followed by the Northeast and the West, respectively (Table 1).

The number of all insects collected in the Central were significantly higher than those collected in the other two regions. In total, 94 of 418 specimens (22.49 %) or 3 of 17 species (17.65 %) were positive for *Wolbachia* infection. All specimens were collected from rice agroecosystems including paddies with growing rice, paddies with ratooning rice, paddies with dense grass, field drainage with dense grass and stream with dense grass.

In addition, the number of rice insect pests collected in this study has the significant positive correlation with the relative moisture in the air. The insect pest species name, family, locations (province), no. of samples tested, no. of positive samples, and geographic regions are listed in Table 1.

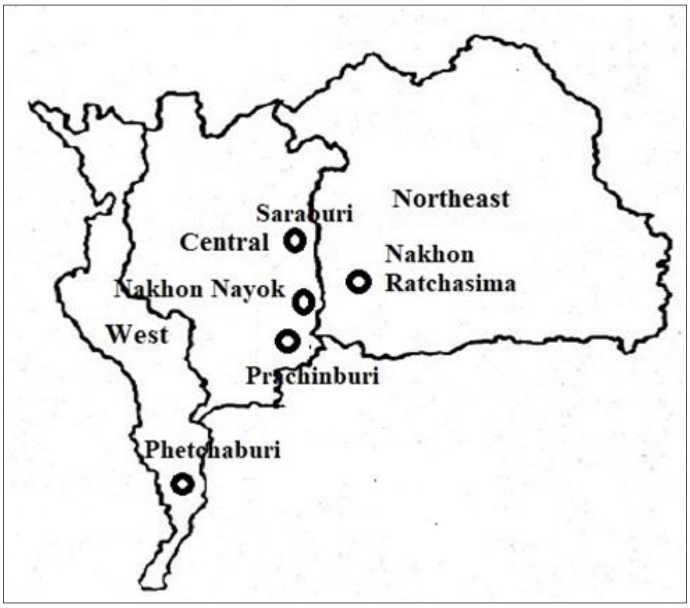

Fig 1: Geographic distribution of the five provinces surveyed from three different geographic regions including the Central and the Northeast regions which include four provinces - Saraburi, Nakhon Nayok, Nakhon Ratchasima and Prachinburi, and the West region that include one province - Phetchaburi of Thailand.

Table 1 PCR-based distribution of *Wolbachia* in 17 rice insect pest species from 12 families in 7 orders in total of 418 specimens collected from three different geographic regions (C = the Central, NE = the Northeast and W = the West) from Thailand. The three species (94 specimens) in bold are found positive for *Wolbachia*.

Species name	Family	Location (Province)	Geographic regions	No. of samples tested	No. of positive samples
Sogatella furcifera	**Delphacidae**	**Phetchaburi** **Nakhon Nayok** **Nakhon Ratchasima**	**W** **C** **NE**	**14** **25** **19**	**14** **25** **19**
Nilaparvata lugens	**Delphacidae**	**Nakhon Nayok**	**C**	**34**	**34**
		Phetchaburi	**W**	**23**	**23**
		Saraburi	**C**	**28**	**28**
Pheidole sp.	Formicidae	Nakhon Nayok	C	31	0
		Nakhon Ratchasima	NE	27	0
Odontotermes takensis	Termitidae	Prachinburi	C	43	0
Atherigona oryzae	Muscidae	Phetchaburi	W	6	0
		Saraburi	C	8	0
Chaetocnema basalis	Chrysomelidae	Phetchaburi	W	2	0
		Nakhon Ratchasima	NE	6	0
Monolepta signata	Chrysomelidae	Nakhon Nayok	C	4	0
		Phetchaburi	W	1	0
Hypomeces squamosus	Curculionidae	Nakhon Ratchasima	NE	12	0
		Saraburi	C	4	0
Lachnosterna sp.	Scarabaeidae	Nakhon Nayok	C	7	0
Melanotus sp.	Elateridae	Phetchaburi Saraburi Nakho Nayok	W C C	2 3 4	0 0 0
Tetraneura nigriabdominalis	Aphididae	Saraburi Nakhon Nayok Nakhon Ratchasima	C C NE	14 18 11	0 0 0
Chondracris rosea	Acrididae	Nakhon Nayok	C	4	0
Patanga succincta	Acrididae	Saraburi	C	4	0
		Nakhon Nayok	C	2	0
Locusta migratoria	Acrididae	Nakhon Ratchasima	NE	6	0
		Saraburi	C	3	0
Cyrtacanthacris tatarica	Acrididae	Phetchaburi	W	7	0
Cataenococcus sp.	Pseudococcidae	Nakhon Nayok	C	16	16
		Phetchaburi	W	6	6
		Nakhon Ratchasima	NE	13	13
Euzophera perticella	Pyralidae	Saraburi	C	8	0
		Phetchaburi	W	3	0

The field investigation has indicated that 22.49 % (94 of 418 specimens) or 17.65 % (3 of 17 species) of rice insect pests were infected with *Wolbachia*. *Wolbachia* infections have been reported in many insect species but only a few in insect pests from rice fields. Most insect pest species collected in this study were in the family Delphacidae which have been known to be important pests in rice field. Most individuals were collected from paddies with growing rice, paddies with ratooning rice, and paddies with dense grass which have been reported on the outbreak of these insect pests in many provinces throughout Thailand. The abundance of those species in Thailand in the present study may have resulted primarily from the changes in temperature, habitats, cropping systems, the evolution of bacterial strains, host species and time.

This percentage might undoubtedly increase if more specimens were tested, and different methods of DNA extraction or PCR were used. This study indicates that the widespread distribution of *Wolbachia* was unequal among these rice insect pest genera and geographic regions of Thailand. Species of *Nilaparvata* and *Sogatella* positive for *Wolbachia* included many important pest species involved in the rice agriculture of Thailand. The distribution of all species here was mostly collected in the Central region where is the main source of rice production in Thailand.

Most of *Wolbachia*-infected individuals were collected from the Central region where is the major area for rice agriculture of Thailand. In this region, temperature, terrains, and habitats are quite different from the other regions of Thailand. The Central and the Northeast are the major rice-growing areas followed by the North and the South, respectively. Interestingly, most of the *Wolbachia*-uninfected species are determined as minor pests of rice and are not listed to be of economic importance in Thailand (Roffey, 1979 [12]). These results may imply that climate differences might influence survival of the insects as well as the infections of *Wolbachia*.

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