WITHDRAWN:
The Temporal Dynamics of Three Bacterial Endosymbionts, *Wolbachia*, *Arsenophonus*, And *Rhizobiales*, of the Invasive Yellow Crazy Ant (*Anoplolepisgracilipes*) in Taiwan are associated with Climate Extremes

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Research Article

Keywords: Symbiont composition, coinfection, Wolbachia removal, climate variation

Posted Date: June 28th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-644453/v1

EDITORIAL NOTE:

The full text of this preprint has been withdrawn by the authors while they make corrections to the work. Therefore, the authors do not wish this work to be cited as a reference. Questions should be directed to the corresponding author.

Abstract

Symbiotic interactions have facilitated major evolutionary transitions, making them a key component of the success of life. By altering the host's life-history traits or potential to respond to natural stresses, symbiotic organisms could either exacerbate or ameliorate the effects of environmental pressure on their hosts. These variations are in turn likely to alter the population dynamics of the host species. We examined the temporal dynamics of three bacterial symbionts, *Wolbachia*, *Arsenophonus*, and *Rhizobiales*, in two neighboring yellow crazy ant (*Anoplolepis gracilipes* (Smith)) colonies for three consecutive months (July - September 2019) in southern Taiwan. Coinfections of *Wolbachia* and *Rhizobiales* were consistently detected in all colonies. While the symbiont compositions remained consistent throughout the sampling period at both sites, the coinfection rate of *Wolbachia* and *Rhizobiales* showed a negative tendency with increases in the daily mean temperature and its standard deviation, the diurnal temperature difference, and especially precipitation over time. These relationships might be the key to understanding the temporal effects of coinfection dynamics on possible adaptations and physiological responses in *A. gracilipes*. We then empirically demonstrated the best *Wolbachia* removal efficiency (40%-27%) under high-temperature treatment, and that the spatial prevalence of *Wolbachia* increased with latitude in the Southern Hemisphere. Our work highlights the potential protection against climate extremes provided by symbiont coinfection and how climate affects the microbial ecological community at a fine scale.

Introduction

The yellow crazy ant, *Anoplolepis gracilipes* (Smith), is one of the most widespread, abundant and damaging invasive ants and poses significant threats to local biodiversity and ecosystem sustainability in most of its introduced range [1]. It is widely dispersed throughout inland areas and locations near the Pacific and Indian Oceans, and the speed with which it spreads is almost unrivaled [2]. However, the abundance of *A. gracilipes* varies temporally and spatially, especially in its introduced range, with some populations persisting at relatively low density, some achieving extraordinarily high densities, and some crashing for unknown reasons [3, 4].

Most studies on this species have focused on its ecology or population structure [5, 6] and have revealed its geographical distribution pattern and introduction history [1, 7–8]. The prevalence and genetic diversity of three bacterial endosymbionts, *Wolbachia*, *Arsenophonus*, and *Rhizobiales*, were revealed by Sebastien et al. in nine *A. gracilipes* populations around the Pacific islands, mainland Australia and Christmas Island in the Indian Ocean [3].

Wolbachia are a group of endosymbiotic bacteria that are ubiquitous in ants [9]. Transmission within individuals occurs directly from queen ants to their offspring via reproduction and indirectly via contact with a vector [10]. The bacteria can modify the reproductive patterns of their hosts, leading to cytoplasmic incompatibility and mating between uninfected females and infected males, resulting in no diploid/dead offspring [11], parthenogenesis [12] and feminization [3, 13]. *Arsenophonus* is also a group

of symbiotic bacteria inducing a female-biased sex ratio in the host through male mortality; *Arsenophonus* is known to occur in a broad spectrum of hosts, including major pest species such as whiteflies and *A. gracilipes* [14, 15]. While *Arsenophonus* is a maternally inherited bacterium like *Wolbachia*, it is also horizontally transmitted at high frequency among distantly related host species [15, 16]. There is considerable room on the spectrum between parasitism and mutualism for both of these bacterial groups. Some may behave as obligate mutualists in hematophagous insects, exhibiting a nutritional role [17], or act as facultative mutualists possibly protecting against parasitoid attacks in psyllids [18] and enhancing host reproductive fitness and thermal tolerance [19]. *Rhizobiales* is a noteworthy symbiont of plants and lichens and belongs to a clade of mutualistic bacteria usually found in herbivorous ants [20, 21]. This symbiont can fix atmospheric nitrogen, recycle nitrogenous waste products and supply nitrogen to the host [21, 22]. For example, in the digestive tracts of several *Cephalotes* ant species, the urea-recycling gene of *Rhizobiales* could be found [23].

For most symbiont infections, once an infection has reached fixation in a population, the host and endosymbiont tend to coevolve to minimize fitness costs. Pintureau et al. [24] suggested that *Wolbachia* increases some aspects of fitness in *Trichogramma* under unfavorable climate and reproductive conditions. By increasing the heat tolerance of parasitoids, endosymbionts may influence how the host distribution responds to climate factors [25, 26].

Symbiont coinfections between species are likely to change host fitness and physiological phenomena. A few studies have shown that host fitness is affected by symbiont coinfection. For example, coinfection between *Regiella* and *Spiroplasma* reduces the survival of the host in the pea aphid, *Acyrthosiphon pisum* [27]; moreover, pea aphids harboring both *Hamiltonella* and *Spiroplasma* exhibit decreased fecundity, a prolonged generation time, and a decline in adult weight [28]. However, not all coinfections reduce host fitness. *Wolbachia*- and *Spiroplasma*- infected *Tetranychus truncatus* displays not only increased fecundity and hatchability but also an enhanced detoxification ability [29]. In addition to affecting host fitness, coinfection may also affect other host features. For example, *Drosophila melanogaster* and *Hylyphantes graminicola* coinfected by different symbionts showed an increase in the metabolism of free amino acids and fat [30, 31]. Although coinfection events often occur in insects, the environmental factors that shapes them are unclear.

Weather extremes driven by climate change are expected to destabilize agricultural ecosystems [1]. To maintain sustainable ant control in ecosystems, an understanding of the extent to which climate change will affect pests and their symbionts is needed. Climate change is expected to drive population asynchrony or coextinction of hosts, symbionts and mutualists, indicating that more research should be focused on these important ecological relationships [2]. Increasing seasonal variability and asymmetric changes in daily maximum and minimum temperatures (diurnal differences) have altered the thermal environment that organisms experience [32]. There are a range of approaches for examining the effects of climate change on changes in the distributions and abundances of hosts and their symbionts [8, 25].

In the current study, we employed both field and laboratory surveys to determine the occurrence of *A. gracilipes*, the prevalence of three bacterial endosymbionts (*Wolbachia*, *Arsenophonus*, and *Rhizobiales*) of *A. gracilipes* in southern Taiwan, and the effect of inhibiting *Wolbachia* infection under elevated temperature conditions. The specific aims of the current study were (1) to examine how the temporal prevalence and dynamics of *A. gracilipes* symbionts in Taiwan may be altered under different temperature and precipitation conditions and (2) to test the effect of heat treatment on the inhibition of *Wolbachia* infection in *A. gracilipes*. Determining how fine-scale climate patterns may affect ant distributions and ant-symbiont relationships should provide important insights for future control programs.

Materials And Methods

Symbiont infection of A. gracilipes **colonies**

Field survey

Two neighboring survey sites of the National Pingtung University of Science and Technology, Pingtung County, Taiwan (Site A: 22°38'56.8"N 120°36'47.0"E; Site B: 22°38'56.8"N 120°36'48.2"E) were selected (Site A and B) and sampled for *A. gracilipes* from June to September 2019. Wooden boxes (size 30 cm x 20.3 cm x 3.5 cm) (Fig. 1) were placed next to tree roots where *A. gracilipes* was frequently observed and covered with fallen leaves to maintain moisture. The boxes were checked once per month, and recovered the entire box back to the laboratory if *A. gracilipes* individuals observed (a new replacement box would be set in the same spot) then surveyed in the plastic boxes to isolate the entire ant colony and determine the infection status of the symbiotic bacteria in their colonies. All sampled *A. gracilipes* colonies were kept in 25°C incubators (DB150, Jor Fai, Hsinchu, Taiwan) with a constant light and dark period of 12 L:12 D.

DNA extraction and symbiont infection of A. gracilipes **colonies**

For each sampled *A. gracilipes* colony, 90 workers were randomly selected; their abdomens were placed in a 1.5 ml centrifuge tube, 100 µl 5% Chelex [33] reagent and 2 µl proteinase K solution were added, a grinding rod was used to grind the mixture, and the mixture was heated at 56°C for 40 minutes and then at 98°C for 10 minutes to extract DNA. Polymerase chain reaction (PCR) was adopted to detect symbiotic bacteria. The reactants included 0.5 µl each of the primers, 1 µl DNA extract, 10 µl 2x Taq, and 8 µl ddH2O, for a total volume of 20 µl. The three pairs of symbiotic bacterial primers were as follows: *Wolbachia* primers *wsp*81F (5-TGGTCCAATAAGTGATGAAGAAAC-3') and *wsp*691R (5'-AAAAATTAAACGCTACTCCA-3'); *Arsenophonus* primers *Ars*F (5'- GGGTTGTAAAGTACTTTCAGTCGT-3') and *Ars*R YTATCTCTAAAGGMTTCGCTGGATG-3'); and *Rhizobiales* primers *Tet*119F (5'-GGGGAA AGATTTATTGGTT-3') and 1513R (5'-TACIGITACCTTGTTACGACT T-3'). PCR was initiated at 95°C for 5 min, followed by 35 cycles at 95°C for 1 min, 40°C for 1 min, and 72°C for 1 min, with a final extension at 72°C for 7 min. The PCR products were obtained by electrophoresis in 1.5% agarose gels and sequenced.

Sequences were aligned using MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms [34] to determine whether the test sample was infected by the three abovementioned endosymbiotic bacteria.

Climate records and correlation analysis with symbiont infection rate dynamics

To reveal the factors that affect symbiont infection, daily maximum, minimum, and mean temperature and precipitation records for every sample were collected from the weather station in Linluo Township, Pingtung County, Taiwan, less than five kilometers from our sampling sites, (Central Weather Bureau (CWB), https://www.cwb.gov.tw/V8/E/W/Town/Town.html?TID=1001307). In the current study, we concentrated on the likely responses of symbionts and their hosts to variable climatic conditions, focusing particularly on factor variability, such as 1) the daily mean temperature and its standard deviation, 2) the diurnal difference (maximum-minimum), 3) precipitation and 4) extreme high temperature (EHT) events such as heatwaves where temperatures are above 31°C as defined by [35], among each sampling period (30 days before each sampling event). We first calculated the difference in the symbiont infection rate in each sampling period and then constructed scatter plots to investigate whether the climate factors or extreme events involving temperature and precipitation affect *A. gracilipes* and the infection dynamics of its three symbionts.

High-temperature treatment to remove symbionts

High-temperature treatment of *A. gracilipes* was initiated in June, two entire colonies freshly collected at Site A and Site B, respectively, were performed in 35℃ incubators with a constant light and dark period of 12 L:12 D and continued for 30 days, at which point the specimens were returned to 25°C for an additional 60 days. Ninety workers were randomly selected every 30 days (thus totally four samples were collected), and immediately frozen at -20℃ for DNA extraction and the symbionts detection with protocols identical to those described earlier. Different infection rates of these symbionts before and after the high-temperature treatment were then calculated.

Meta-analysis for three symbiont infection rates across the Pacific Ocean region

The infection rates of *Wolbachia*, *Arsenophonus*, and *Rhizobiales* were obtained from Sebastien et al. in which a total of nine *A. gracilipes* populations around the Pacific and Australian regions [3]. We performed a meta-analysis in order to compare the effects of latitude on symbiont infection/coinfection pattern.

Results

Symbiont infection of A. gracilipes **colonies**

Anoplolepis gracilipes was collected in six of the eight sampling boxes, four boxes per site. We did not detect *A. gracilipes* at Site B in June and Site A in September 2019, presumably because of the variation in its abundance. Both of the field colonies (denoted as A and B) were infected by the three symbiotic bacteria *Wolbachia*, *Arsenophonus*, and *Rhizobiales*. The primers *wsp*82F/*wsp*691R for *Wolbachia* amplified a single band of 526 bp. The primers *Tet*119F/*Tet*1513R for *Rhizobiales* amplified a single band of 512 bp. The primers *Ars*F/*Ars*R for *Arsenophonus* amplified a single band of 804 bp. The prevalence of *Wolbachia* ranged from 70.0–76.7% at Site A and from 16.7–26.7% at Site B (Table 1). The prevalence of *Rhizobiales* ranged from 30.0–43.3% at Site A and from 20.0–30.0% at site B (Table 1). The prevalence of *Arsenophonus* ranged from 0–10% at Site A and from 0–6.7% at Site B (Table 1). Among the symbiotic bacteria, *Wolbachia* had the highest infection rate at Site A (76.7%), while its infection rate at Site B was only 16.7% (Table 1). While Site A and B were only 10 meters apart each other, the prevalence of symbiotic bacteria at the two sites appeared to differ. Temporally, all infection rates of three symbionts gradually decreased from July except for the *Wolbachia* infection at Site A.

Table 1. Percentage of sampled ants positive for the bacterial symbionts *Wolbachia*, *Rhizobiales*, and *Arsenophonus* in six colonies in Taiwan

Site	*n*	*Wolbachia*		*Rhizobiales*		*Arsenophonus*	
		Mean	95% CI	Mean	95% CI	Mean	95% CI
A: June	90	73.3	(64.1-82.6)	43.3	(32.9-53.7)	0	(0-0)
A: July	90	70.0	(60.2-79.8)	36.7	(26.4-46.9)	10.0	(3.8-16.2)
A: Aug.	90	76.7	(68.0-85.4)	30.0	(20.7-39.3)	3.3	(0.0-7.1)
B: July	90	26.7	(17.6-35.7)	30.0	(20.7-39.3)	6.7	(1.5-11.9)
B: Aug.	90	23.3	(14.6-32.1)	26.7	(17.3-36.0)	6.7	(1.5-11.9)
B: Sep.	90	16.7	(9.0-24.3)	20.0	(11.3-28.7)	0	(0-0)

n is the total number of individual ant workers sampled from each population. The 95% CI column lists 95% confidence intervals obtained from bootstrap analysis.

Dual and triple infections were observed in multiple individual ant workers (Fig. 2). Coinfection by *Wolbachia* and *Rhizobiales* was present in all six colonies. Coinfection by *Wolbachia* and *Arsenophonus* was found in the July sample from both Site A and B and in the August sample from Site B. Coinfection by *Rhizobiales* and *Arsenophonus* was found in the July sample from Site A and August sample from Site B. One colony found in the July sample from Site A was coinfected by all three bacteria. Although the two study sites were in close proximity, it was obvious that the symbiont compositions remained consistent over time at both sites (Fig. 2; Site A: $G_4 = 21.6$, $P = 0.3$; Site B: $G_4 = 0.07$, $P = 0.07$). The

dynamics of the *Wolbachia* and *Rhizobiales* coinfection rate were the opposite of those of most other symbiont infection rates across months and sites (gray color denoting W + R, Fig. 3).

Climate records and correlation analysis with symbiont infection rate dynamics

The infection rate dynamic of each symbiont presented a positive tendency with increases in the daily average temperature, diurnal temperature difference and frequencies of extreme events, with the most significant effects observed for *Arsenophonus*. However, most infection rate dynamics of each symbiont was negatively correlated with the temperature standard deviation (Fig. 4B).

To understand the different tendencies of the infection rate dynamics of *Rhizobiales* and *Wolbachia* coinfection, all coinfection statuses were presented in relation to a) the daily mean temperature standard deviation, b) the diurnal difference (maximum-minimum temperature), and c) precipitation (Fig. 5). The infection rate dynamics of *Rhizobiales* and *Wolbachia* (purple and yellow symbols) presented negative tendencies with increases in the daily mean temperature standard deviation, the diurnal temperature difference, and precipitation, but positive tendencies (grey triangles) with the temperature variances and precipitation.

High-temperature treatment to remove symbionts

In the high-temperature treatment, the abundances of all symbionts were significantly lower when *A. gracilipes* was reared at 35℃ (Fig. 6). Ants from the two sampling sites had 40%-27% lower *Wolbachia* infection rates (G_3 = 0.74, P = 0.9), 15%-7% lower *Rhizobiales* infection rates (G_3 = 0.06, P = 0.9), and 2%-0% lower *Arsenophonus* infection rates (G_3 = 0.002, P = 0.9) in response to the treatment. Therefore, high-temperature treatment markedly reduced *Wolbachia* infection rates but had little effect on *Arsenophonus* infection rates.

Meta-analysis for three symbiont infection rates across the Pacific Ocean region

Endosymbiont infections were common in colonies of *A. gracilipes* in Taiwan. Similarly, the prevalence of *Wolbachia* infection in *A. gracilipes* is higher than that of the two other bacterial symbionts in the pantropical distribution of the ant across the Pacific Ocean region (Fig. 6, modified from [3]). Linear regression analysis of the infection rates of *Wolbachia* in the Southern Hemisphere showed a positive correlation with latitude, such that the higher the latitude was, the higher the infection rates of *A. gracilipes* individuals (R^2 = 0.67; P = 0.044). However, the same pattern was not observed in the Northern Hemisphere. The different patterns observed near the equator might be attributed to the different time ranges and trapping approaches used for ant population sampling.

Discussion

In Sebastien et al. [3], ants were collected at different sites throughout the Pacific region, in Australia, and on Christmas Island through hand collections or pitfall traps. The sampling times for the Southern Hemisphere all fell within October-November and late spring to early summer; for sampling in the Northern Hemisphere, especially in the current study, the wooden boxes were sampled from June to September, i.e., during the summer period. Sampling in different seasons might therefore have interfered with the pattern of *Wolbachia* infection in *A. gracilipes* [4, 8]. Furthermore, individual sites had infestations ranging in size from 10 to 50 km^2, and between 2 and 61 ants from each site were screened by Sebastien et al. [3]. In the current study, the wooden boxes were placed next to tree roots, where *A. gracilipes* was frequently observed in each monthly collection, so relatively intact nests, including hundreds of workers, were screened in each sample. Different sampling approaches reflect different scales of symbiont compositions. Because of the high variability of *A. gracilipes* abundance, larger-scale sampling could be a useful approach for understanding symbiont compositions across the different areas [1, 4].

In southern Taiwan, relatively consistent symbiont compositions across three consecutive sampling months (from June to September 2019) at Sites A and B in summer indicated temporal symbiont stability within *A. gracilipes* nests (Fig. 2); thus, the symbiont compositions could serve as an indicator for the nest or reflect possible supercolony relationships, at least at fine geographic scales [36]. To the best of our knowledge, the current study revealed the intra-nest dynamics of the symbionts associated with *A. gracilipes* colonies for the first time; such information is key to understanding the effects of these symbionts on possible adaptations and physiological reactions in *A. gracilipes* [4].

The *Arsenophonus* infection rates observed here appear to be lower (3.3%-10%) than the that (50.8%) observed in Arnhem Land, Australia (Fig. 7). Ant samples in both studies presented coinfections of *Arsenophonus* and *Wolbachia*, although there are 50.8% *Arsenophonus*-infected ants in Arnhem Land, these ants are also infected with *Wolbachia* so singly *Arsenophonus*-infected ant is rare [3]. Coinfection by these two symbionts has been observed in the whitefly species *Bemisia tabaci* and *Trialeurodes vaporariorum*, in which *Arsenophonus* was a relatively dominant symbiont [37]. In that case, both *Arsenophonus* and *Wolbachia* were restricted to bacteriocytes during whole developmental stages, which might contribute to the fitness and heat tolerance of their hosts [26, 37]. A low prevalence of *Arsenophonus* and *Wolbachia* coinfection in *A. gracilipes* in the current study suggests that their coinfection might play a trivia role and that the dominancy of *Wolbachia* is obvious in *A. gracilipes* host (Fig. 7).

Rhizobiales infection in *A. gracilipes*, on the other hand, was observed at a much higher frequency, with a maximum infection rate of 43.3% in Taiwan (both Site A and B) compared to an 11.8% infection rate in Samoa (Fig. 7). In contrast to *Arsenophonus* and *Wolbachia* that are known to be restricted to the bacteriocytes and reproductive systems of their hosts, *Rhizobiales* was confined to the guts of ants and correlated with their herbivorous diets (Russell et al. 2009). While *A. gracilipes* is in general an

omnivorous ant species, this ant has been reported to consume honeydew secreted by aphids, scale insects and other honeydew-producing insects [38, 39]. In the spider mite *Tetranychus truncatus*, coinfection of *Wolbachia* and *Spiroplasma* was found to enhance male fitness by increasing the expression of many genes related to digestion, reproduction, and oxidation-reduction [29]. Similarly, the synthesis of fat and free amino acids in a small spider, *H. graminicola*, was also promoted by coinfection of *Wolbachia* and *Cardinium* [31].

Despite being speculating, the finding of coinfection dynamics of *Rhizobiales* and *Wolbachia* being positively correlated with temperature variances and precipitation may provide some clues on the potential role of coinfection, especially when compared with either single infection status, which presented an opposite tendency (Fig. 5). To the best of our knowledge, this is the first study to demonstrate the possible effects of *Rhizobiales* and *Wolbachia* coinfection on ants' adaptation to climate extremes. In particular, unlike the abovementioned studies in which artificially stimulated and analyzed organisms' reactions in the laboratory conditions, the current study highlighted that climate extremes may favor coinfection. Consistent with *Arsenophonus* displaying the most significant increase tendencies in association with average temperature and the frequency of extreme high-temperature events (Fig. 4), the high-temperature treatment had a relatively weaker effect on the infection rate dynamics of this symbiont than on those of the other two symbionts, indicating the better heat tolerance of individual ants infected by *Arsenophonus*. The better removal efficiency observed for *Wolbachia* than the other two bacteria under the high-temperature treatment also supported the increasing tendency of this symbiont with an increasing distance from the equator (Fig. 4; Fig. 7). The decrease of *Wolbachia* infection of *A. gracilipes* under high-temperature treatment could be attribute to elimination the *Wolbachia*-infected individuals, instead of *Wolbachia* itself from individuals, hence more individuals and colony size should be considered as well in the following study (Li et al. 2014). One limitation of the current study is the low efficiency of the wooden boxes, which influenced the interpretation of the temporal dynamics (Fig. 2). The loss of two sampling datasets (Site A: Sep. and Site B: June) compressed the samples and ranges of the climate variations in the temporal analysis. Although there are constraints on the practical applications and utility of the current findings, the wooden boxes may remain the most suitable approach for sampling relatively intact nest of *A. gracilipes* and also for revealing finer-scale information on the dynamics of symbionts.

The current study employed fine-scale (ant nests) and consecutive sampling to investigate the temporal dynamics of three symbionts of *A. gracilipes* in the field during the summer season. Both temperature variations and precipitation strongly affected the dynamics of the symbionts. The increasing coinfection of *Rhizobiales* and *Wolbachia* with more precipitation revealed by our analyses indicates that the contributions of ant-symbiont and symbiont-symbiont relationships may be profound in shaping host's response to environmental factors. Different spatial and temporal sampling provide datasets that are important to develop hypotheses regarding the key traits that limit species distributions and define the prevalence of host-symbiont relationships (Wu et al. 2017). Such hypotheses can help guide future experimental work with the aim of understanding how climate change, such as climate seasonality/extremes, may affect these species at finer scales. We also used a method potentially

suitable for decreasing symbiont infections in ant colonies however, longer-term assessments are needed to determine the main driver of the coinfection dynamics of *Rhizobiales* and *Wolbachia* in local colonies of *A. gracilipes*, along with phenological studies of *A. gracilipes* and improvement of sampling efficiency.

Declarations

Acknowledgements

We are grateful to the scientists who conducted and published their symbionts studies of *A. gracilipes*. This work was supported by the Ministry of Science and Technology, Taiwan through grants (MOST 106-2313-B-020-014-MY2 and MOST 108-2313-B-020-010-MY3).

Compliance with Ethical Standards

The authors declare that they have no conflicts of interest. This work has not been published before, completely, in part, or in another form, and is not under consideration for publication elsewhere.

Declarations

To be used for life science journals + articles with biological applications

Funding: This work was supported by the Ministry of Science and Technology, Taiwan through grants (MOST 106-2313-B-020-014-MY2 and MOST 108-2313-B-020-010-MY3).

Conflicts of interest/Competing interests: The authors declare that they have no known competing financial interests or personal relationships that can influence this work.

Availability of data and material: Yes.

Code availability: Not applicable.

Authors' contributions: Hsiao, Y. T.: Methodology, Validation, Formal analysis, Investigation, Data curation, Writing - original draft. **Lai, C. T.**: Validation, Formal analysis, Suggestion for experimental design and data analysis. **Wu L. H.**: Conceptualization, Methodology, Data curation, Supervision, Project administration, Funding acquisition.

Ethics approval: Not applicable.

Consent to participate: Not applicable.

Consent for publication: The authors give our consent for the publication of identifiable details, which can include photograph(s) within the text (“Material”) to be published in the Microbial Ecology.

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Figures

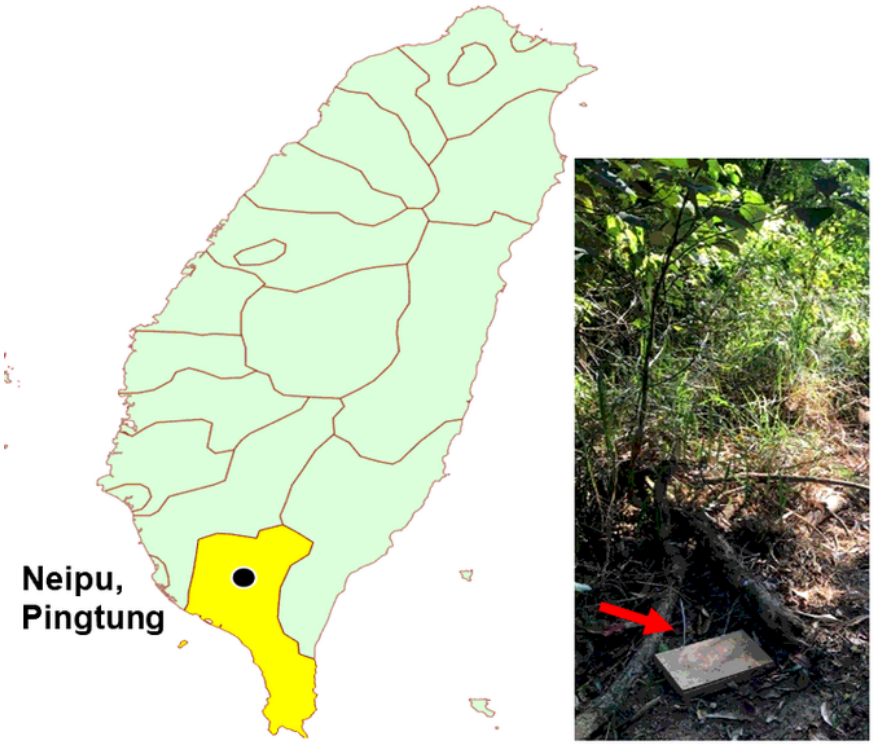

Figure 1

Location of sampling sites and photograph of a collection wooden box. Red arrow represents the position of wooden box.

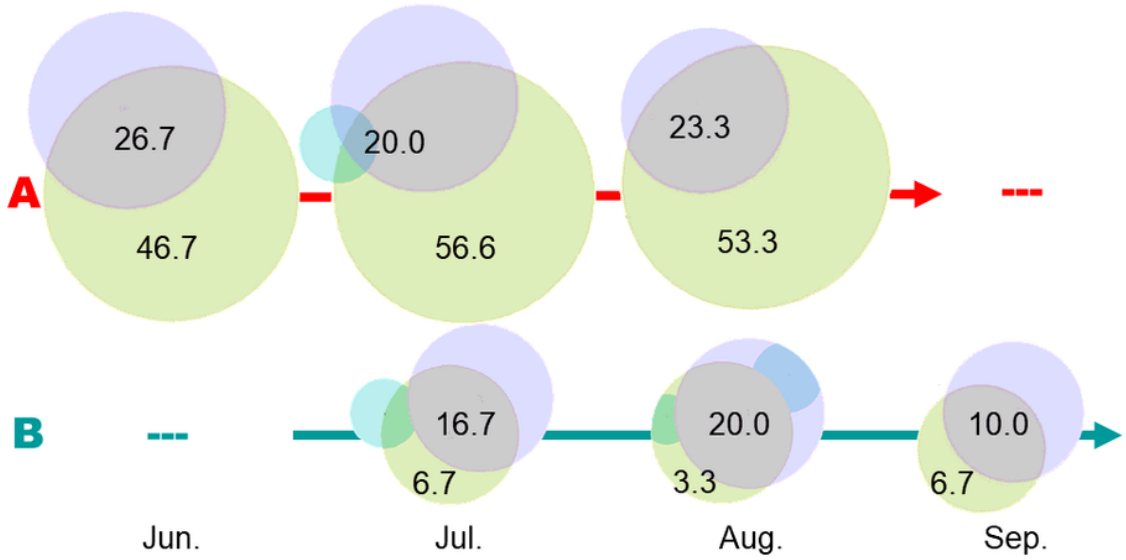

Figure 2

The infection rates of three symbionts detected in Anoplolepis gracilipes collected from both sites in different months. The colors represent different symbionts. The area of the circle represents the infection rate by a single or multiple bacteria, the solid line represents the captured population of A. gracilipes, and the dotted line represents records not captured. See Table 1 for more details. The green color represents the infection rate of Arsenophonus, the purple color represents the infection rate of Rhizobiales, and the yellow color represents the infection rate of Wolbachia.

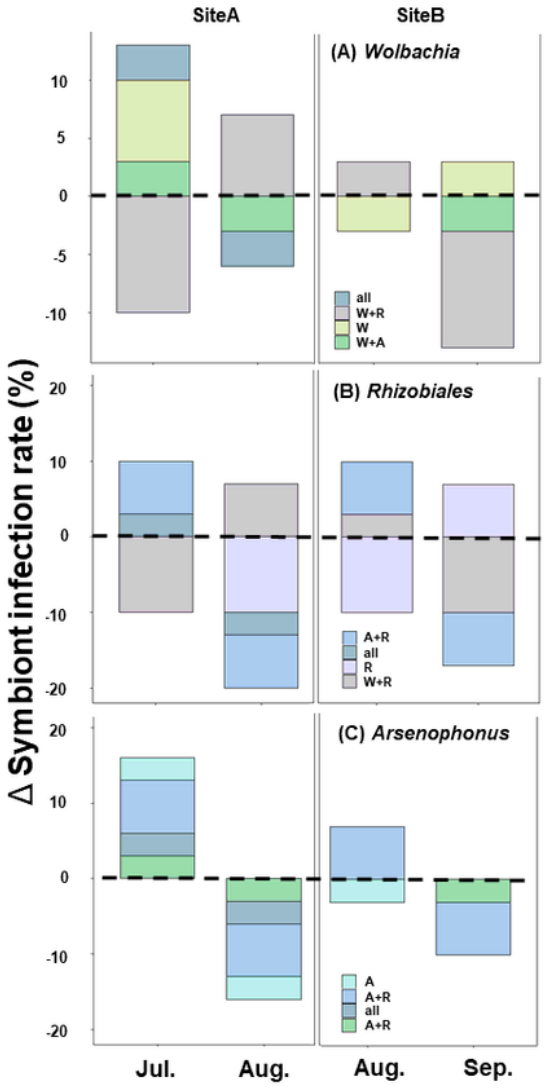

Figure 3

The dynamics (Δ) of the infection rate of symbionts among months at each site. (A) The coinfection rate of Wolbachia or Wolbachia with the other symbiont. (B) The infection rate of Rhizobiales or Rhizobiales with the other symbiont. (C) The infection rate of Arsenphonus or Arsenphonus with the other symbiont. Different colors represent ants infected by a single or multiple bacteria.

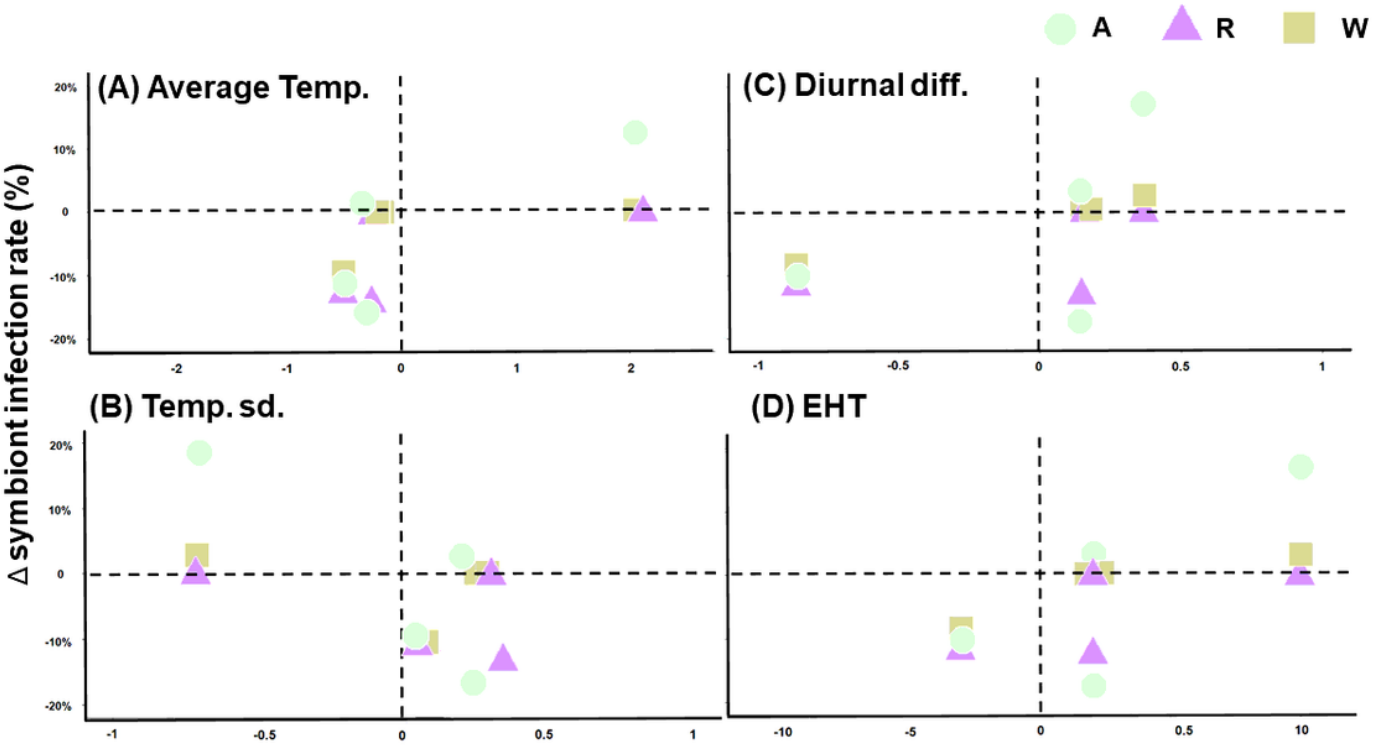

Figure 4

The dynamics (Δ) of the infection rate of symbionts under different environmental factors: (A) Average temperature (Average Temp.), (B) temperature standard deviation (Temp. sd.), (C) diurnal difference (Diurnal diff.), and (D) extreme high temperature (number of days with an average temperature exceeding 31°C in 30 days, EHT). The dotted line divides the graph into four quadrants. The green circles represent the infection rate dynamics of Arsenophonus, the purple triangles represent the infection rate dynamics of Rhizobiales, and the yellow squares represent the infection rate dynamics of Wolbachia.

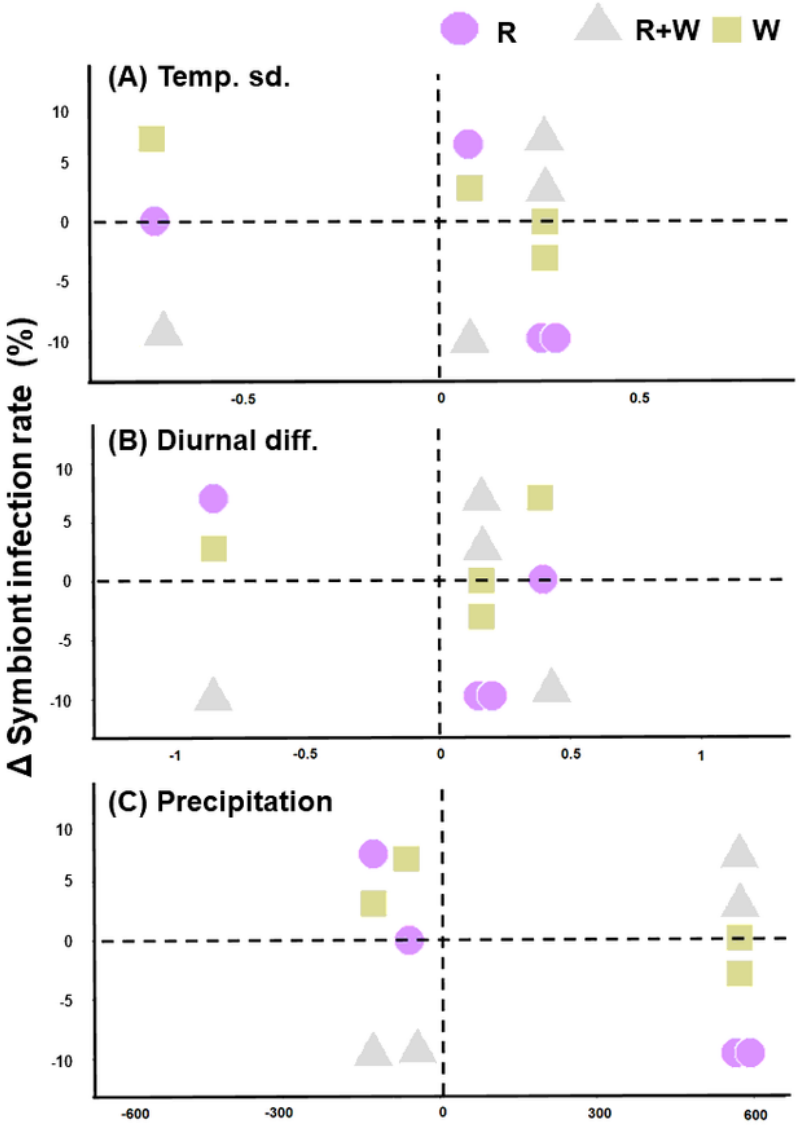

Figure 5

The dynamics (Δ) of Rhizobiales and Wolbachia infection rates and their coinfection rate in relation to different environmental factors. (A) Temperature standard deviation (Temp. sd.), (B) diurnal difference (Diurnal diff.), and (C) precipitation. The purple circles represent the infection rate dynamics of Rhizobiales, the gray triangles represent the infection rate dynamics of Rhizobiales and Wolbachia under coinfection, and the yellow squares represent the infection rate dynamics of Wolbachia.

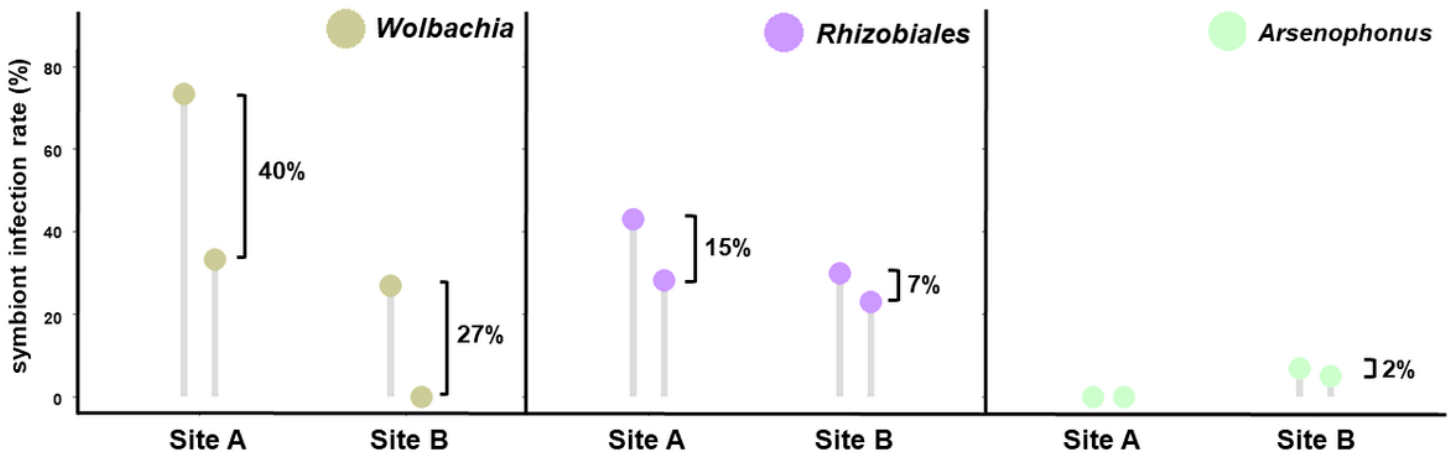

Figure 6

Variations in the symbiont infection rates of Anoplolepis gracilipes after high-temperature (35°C) treatment for 30 days: Arsenophonus (green), Rhizobiales (purple), and Wolbachia (yellow). For each site, the first circle represents the symbiont infection rate before the high-temperature treatment, and the second circle represents the symbiont infection rate after high-temperature treatment. Ninety A. gracilipes individuals were sampled and tested for each circle.

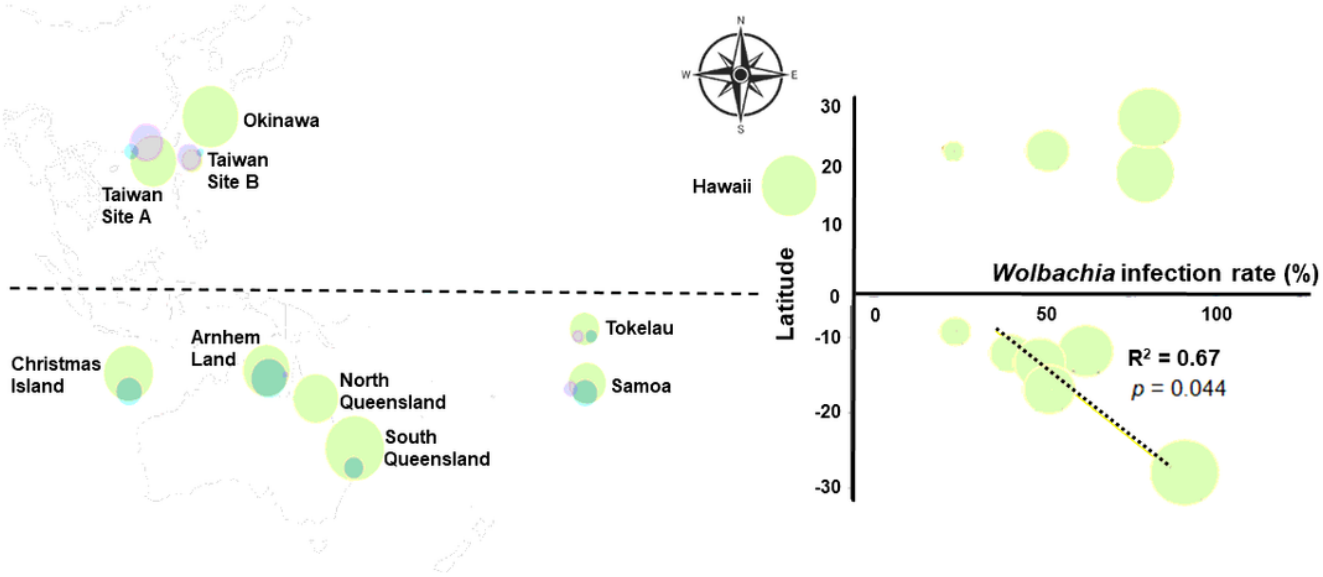

Figure 7

The distribution and percentage of the symbionts Arsenophonus (green), Rhizobiales (purple), and Wolbachia (yellow) in all colonies. The size of the circle represents the infection rate. The distribution of symbionts from Okinawa to South Queensland (from north to south). Except for the sampling sites in Taiwan, the sites were modified from Sebastien et al. [3]. Linear regression analysis of the infection rate of Wolbachia between the Northern and Southern Hemispheres. In the Southern Hemisphere, the Wolbachia infection rate and latitude have a positive correlation (R2 = 0.67; P = 0.044), and the shaded area represents the confidence interval (CI) for the regression line.