Phylogeny and Density Dynamics of *Wolbachia* Infection of the Health Pest *Paederus fuscipes* Curtis (Coleoptera: Staphylinidae)

Chen Ge 1,†, Jiayao Hu 1,†, Zimiao Zhao 1,†, Ary A. Hoffmann 2, Shuojia Ma 1, Li Shen 1, Jie Fang 1, Jianqing Zhu 3, Weidong Yu 1 and Weibin Jiang 1,*

1 Laboratory of Environmental Entomology, College of Life Sciences, Shanghai Normal University, Xuhui, Shanghai 200234, China; gretchen9505@163.com (C.G.); huijiaoy@shnu.edu.cn (J.H.); zhaozimiao818926@163.com (Z.Z.); mashuojia@126.com (S.M.); shenlismile@163.com (L.S.); fangjie2019@163.com (J.F.); ywd@shnu.edu.cn (W.Y.)

2 School of BioSciences, The University of Melbourne, Bio21 Institute, Parkville, VIC 3052, Australia; Ary@unimelb.edu.au

3 Shanghai Zoological Park, Changning, Shanghai 200335, China; zzzjjq@gmail.com

*† These authors contributed equally to this work.

Received: 30 July 2020; Accepted: 8 September 2020; Published: 11 September 2020

Simple Summary: *Wolbachia pipientis* is a maternally inherited endosymbiont of arthropods and filarial nematodes, and was reported to occur in *Paederus fuscipes*, a beetle that causes dermatitis linearis and conjunctivitis in humans when they come in contact with skin. In this study, we report the phylogenetic position and density dynamics of *Wolbachia* in *P. fuscipes*. The phylogeny of *Wolbachia*, based on an analysis of MLST genotyping, showed that *Wolbachia* from *P. fuscipes* belongs to supergroup B. Quantitative PCR indicated that the infection density in adults was higher than in any other life stage (egg, larva or pupa), and that reproductive tissue in adults had the highest infection densities, with similar densities in the sexes. These findings provide a starting point for understanding *Wolbachia* infection dynamics in *P. fuscipes*, and interactions with other components of the microbiota.

Abstract: The maternally inherited obligate intracellular bacteria *Wolbachia* infects the reproductive tissues of a wide range of arthropods and affects host reproduction. *Wolbachia* is a credible biocontrol agent for reducing the impact of diseases associated with arthropod vectors. *Paederus fuscipes* is a small staphylinid beetle that causes dermatitis linearis and conjunctivitis in humans when they come into contact with skin. *Wolbachia* occur in this beetle, but their relatedness to other *Wolbachia*, their infection dynamics, and their potential host effects remain unknown. In this study, we report the phylogenetic position and density dynamics of *Wolbachia* in *P. fuscipes*. The phylogeny of *Wolbachia* based on an analysis of MLST genotyping showed that the bacteria from *P. fuscipes* belong to supergroup B. Quantitative PCR indicated that the infection density in adults was higher than in any other life stage (egg, larva or pupa), and that reproductive tissue in adults had the highest infection densities, with similar densities in the sexes. These findings provide a starting point for understanding the *Wolbachia* infection dynamics in *P. fuscipes*, and interactions with other components of the microbiota.

Keywords: *Paederus fuscipes*; *Wolbachia* infection; phylogeny; density dynamics
1. Introduction

Wolbachia pipientis is the most widespread endosymbiotic bacterium of insects and other arthropods, infecting perhaps two-thirds of present-day insect species, as well as about 40% of terrestrial arthropod species [1]. The transmission of Wolbachia is predominantly vertical and secondarily horizontal [2]. It can induce a number of reproductive manipulations in its host, including cytoplasmic incompatibility [3], thelytokous parthenogenesis [4], feminization of genetic males [5] and male killing [6]. Wolbachia may generate positive fitness effects on numerous hosts, such as filarial nematodes, fruit flies, bedbugs and wasps [7–10], and decrease host transmission of dengue [11], malaria [12], West Nile virus [13] and other pathogens [14]. It is considered as a novel method for controlling mosquito- and vector-borne human diseases [15]. The vector control approaches include population suppression [16–18] and population replacement strategies [19]. The population suppression approaches involve rearing and releasing large numbers of male mosquitoes that cannot produce viable offspring when they mate with wild females. By contrast, population replacement approaches involve the release of both male and female mosquitoes that carry a heritable factor that cannot produce offspring when they mate with wild females. Wolbachia infections have been reported in various Coleoptera families, such as Buprestidae, Chrysomelidae, Curculionidae, Dytiscidae, Gyriidae, Haliplidae, Hydraenidae, Noteridae, Staphilinidae and Tenebrionidae, but usually only with a limited coverage of species [20–26]. Paederus fuscipes Curtis is a widespread beetle, with a distribution from the British Isles in the east, across Central Asia to Japan, and southeast to Australia. Although P. fuscipes preys on several agricultural pests and represents an important beneficial insect [27], it can also adversely affect human health, because its vesicant hemolymph can cause dermatitis linearis and conjunctivitis if it comes into contact with human skin [28–30]. P. fuscipes neither bite nor sting, but can cause dermatitis linearis and conjunctivitis by accidental brushing or crushing of the insects over an exposed area of the human skin. The symptoms are due to a toxic substance named pederin released from their hemolymph [31]. P. fuscipes was originally examined with respect to Wolbachia infection by Yun et al. [26], and its infection status was recently confirmed by Maleki-Ravasan et al. [24]. Yun et al. [26] found the indirect horizontal transmission of Wolbachia between rove beetles and their predator spiders, while Maleki-Ravasan et al. [24] provided an estimate of Wolbachia prevalence (76%, 95/125) in P. fuscipes in Iran. However, little is known about other aspects of this infection, including its tissue distribution patterns and density dynamics in P. fuscipes.

In this study, we characterized the Wolbachia in P. fuscipes by MLST genotyping. Furthermore, we measured Wolbachia density across all the developmental stages, body parts and tissues of P. fuscipes with qPCR. The Wolbachia spatiotemporal infection density in beetles may help to indicate the likely effects of Wolbachia on this host.

2. Materials and Methods

2.1. Samples and DNA Extraction

A laboratory stock of P. fuscipes was established from 33 adult beetles (18 females and 15 males) collected in Nanyang, Henan province, China, in May 2019. They were fed separately under greenhouse conditions at 25 °C, 60% relative humidity and a photoperiod of 16 h of light and 8 h of darkness. To establish isofemale lines, beetle pairs were kept in a fixed order in perforated plastic boxes, as described by Kellner and Dettner [40], with some leaves for shelter, and a small dish containing moistened cotton in which to lay the eggs. The females were fed with pork liver powder and honey.
(50 µg for one beetle per day) and were allowed to lay eggs seven days later. The eggs were isolated for hatching, and the isofemale line was established using resulting sibling larvae.

DNA was isolated from different developmental stages of the F1 generation (egg, larva, pupa and adult) and parental samples. Nine rove beetles were tested per developmental stage, except for the eggs, which were tested in nine groups of three eggs (n = 27 in total). The tissues (gut and reproductive tissue) and body parts (head, thorax, and abdomen without the gut and gonads) were dissected from other adult beetles (9 males and 9 females). Each tissue sample was dissected from a beetle. The method of dissection was carried out following Kador et al. [41]. The DNA was isolated from the dissected body parts and tissues using a QIAamp DNA Mini kit (Qiagen, Hilden, Germany) following the manufacturer’s instructions [42].

2.2. Wolbachia Screening and Multilocus Sequence Typing

To screen for the presence of Wolbachia, a region of 870 bp in length was amplified from all the samples using general Wolbachia primers for 16S rRNA [43] (Table 1). The PCR reactions followed the published protocols [44]. The characterization of Wolbachia strains was performed by sequencing multiple loci recommended by the Wolbachia MLST database (http://pubmlst.org/Wolbachia) [45,46] (Table 1). The MLST typing included sequencing fragments from five Wolbachia genes: gatB, coxA, hcpA, ftsZ and fbpA.

The MLST data were aligned with a homologous sequence of a wide range of arthropods retrieved from the Wolbachia MLST database (http://pubmlst.org/Wolbachia) as well as from the NCBI database (Supplementary Materials Table S1). These sequences were aligned with manual correction using Bioedit v. 7.0 [47]. The best-fit partitioning scheme and corresponding nucleotide substitution models for the concatenated matrix were selected by PartitionFinder v2.1.1 [48] using the Bayesian Information Criterion (BIC). The GTR+R model is the best-fit substitution model for five partitions. The concatenated supermatrix was analyzed with maximum likelihood (ML) inference using IQtree 1.4.2 [49]. IQtree is an efficient software for phylogenomic inference. A combination of hill-climbing approaches and stochastic perturbation method can be time-efficiently implemented. To assess nodal support, we performed 1000 ultrafast bootstrap replicates and an SH-aLRT test with 1000 replicates. The UFBoot is largely unbiased compared to standard or alternative bootstrap strategies, and SH-aLRT is conservative [50–52]. Only nodes with support values of UFBoot ≥ 80 and SH-aLRT ≥ 75 were considered robust.
Table 1. Primer sequences and amplicon lengths of PCR products of target genes.

| Gene/Region | Primers | Sequence (5′–3′) | Amplicon Length | Annealing Temperature | Reference |
|-------------|---------|-----------------|-----------------|-----------------------|-----------|
| 16S rRNA    | 16S_F   | TTTAGCGCTGTATGGTATAA | 870 bp         | 55 °C                 | [43]      |
|             | 16S_R   | GATAAGGTATTTTTGATCTG |               |                       |           |
| gatB        | gatB_F1 | GATTTGCGCTGCTATGGTATAA | 471 bp         | 54 °C                 | [46]      |
|             | gatB_R1 | AGATAGGTATGATTTTCATGTT |               |                       |           |
| coxA        | coxA_F1 | TGTTTAGCCTGCTATGGTATAA | 487 bp         | 54 °C                 | [46]      |
|             | coxA_R1 | GAAATAGGTATGATTTTCATGTT |               |                       |           |
| hcpA        | hcpA_F1 | GAAATAGGTATGATTTTCATGTT | 515 bp         | 54 °C                 | [46]      |
|             | hcpA_R1 | GAAATAGGTATGATTTTCATGTT |               |                       |           |
| ftsZ        | ftsZ_F1 | ATYTGGGCGCAATATAAARGATGTCR | 524 bp         | 54 °C                 | [46]      |
|             | ftsZ_R1 | AGYATGGGCGCAATATAAARGATGTCR |               |                       |           |
| fbpA        | fbpA_F1 | GCTTGCTCCRCTTGGYWTGAT | 509 bp         | 59 °C                 | [46]      |
|             | fbpA_R1 | GCTTGCTCCRCTTGGYWTGAT |               |                       |           |
| wsp         | wsp1_F1 | TGGTATGGTGTTGGTGACG | 158 bp         | 50 °C                 | [53]      |
|             | wsp1_R1 | TGGTATGGTGTTGGTGACG |               |                       |           |
| RPS3        | RPS3_F  | CCCAGAATCATATATCC | 191 bp         | 50 °C                 | [54]      |
|             | RPS3_R  | CCCAGAATCATATATCC |               |                       |           |
2.3. qPCR and Statistical Analyses

To measure the infection dynamics of Wolbachia across all tested developmental stages, body parts and tissues of P. fuscipes, qPCR was performed in triplicate for each sample using Platinum SYBR Green (Invitrogen) referring to the manufacturer’s protocol. qPCR reactions were performed in a total volume of 20 µL, comprising 10 µL of 2X Platinum SYBR Green, 0.4 µL (5 µM) of each primer and 1 µL (final 5 ng) template DNA. Following Ali et al. [53], the relative Wolbachia density was calculated as the ratio of Cq values between the Wolbachia surface protein gene (wsp) and the host’s ribosomal protein S3 gene (RPS3), which is synonymous with the number of Wolbachia per host cells, because both genes occur as a single copy per haploid genome. The short fragment length (158 bp) of the Wolbachia targeted primer pair (wsp1-F1-wsp1-R1) was used and normalized with a 191bp fragment length of the reference gene (RPS3-F, RPS3-R; Table 1) [53,54]. Relative expression levels were calculated using the 2^-ΔΔCt method [55]. The temperature profile of the qPCR was 94 °C for 4 min, 40 cycles of 95 °C for 30 s, 50 °C for 30 s, and 72 °C for 45 s with fluorescence acquisition of 72 °C at the end of each cycle, then a melting curve analysis after the final cycle. Assays were conducted as three technical replicates.

We checked for the normality and homoscedasticity of the data prior to using parametric statistical tests. We compared Wolbachia infection densities among the different developmental stages, body parts and tissues of P. fuscipes by ANOVA followed by a multiple comparison test (Tukey’s posthoc test). We used t-tests to compare Wolbachia densities between males and females. All analyses were conducted using SPSS statistics version 21.0 for Windows (SPSS Inc, Chicago, IL, USA).

3. Results

All rove beetles examined by diagnostic PCR for 16SrRNA were Wolbachia-infected. All individuals appeared to have a single infection based on unambiguous electropherograms. The sequence typing of these individuals produced new alleles for the hcpA and coxA loci, with ftsZ, fbpA and gatB matching existing alleles in the database. The strain identified by the Wolbachia MLST database has the designation ST-540. The phylogenetic trees for concatenated alignment were constructed and showed that ST-540 belonged to supergroup B (Figure 1). The most closely related strain was a male-killing ST-540. The phylogenetic trees for concatenated alignment were constructed and showed that ST-540 existing alleles in the database. The strain identified by the these individuals produced new alleles for the hcpA appeared to have a single infection based on unambiguous electropherograms. The sequence typing of

Figure 1. Maximum-likelihood phylogenetic tree of Wolbachia MLST sequences from P. fuscipes and additional ST sequences from a wide range of host species. The phylogeny is inferred by IQTREE. Numbers beside nodes are IQTREE ultrafast bootstrap and SH-aLRT values. The affiliation to the respective supergroup (A, B, D, F) is indicated.
The *Wolbachia* infection densities analyzed through qPCR with the specific *wsp* gene along with an endogenous control gene (RPS3) were found to vary significantly (*F*\(_{3,32}\) = 16.023, *p* < 0.01) across the developmental stages. The infection density in adults was significantly higher than in any other life stage (Figure 2). Moreover, the *Wolbachia* infection density significantly varied between host body parts and tissues, both in females (*F*\(_{4,40}\) = 79.783, *p* < 0.01; Figure 3) and males (*F*\(_{4,40}\) = 68.353, *p* < 0.01; Figure 3), with significantly high infection densities in reproductive tissues and lower densities in the gut (Figure 3). However, the relative *Wolbachia* densities between females and males for body parts and tissues were not significantly different. The densities of *Wolbachia* are therefore substantially influenced by developmental stage and tissues, but not gender.

![Figure 2](image2.png)

**Figure 2.** Relative *Wolbachia* density was measured across different developmental stages. Nine biological replicates were tested for each development stage. This would include both the individuals used from larvae to adults, and the egg pools. Each data point represents the average of three technical replicates. The bars represent mean ± standard error (*n* = 9) and the different letters above the scatter dot plot indicate a significant difference between developmental stages (*p* < 0.05).

![Figure 3](image3.png)

**Figure 3.** Relative *Wolbachia* density was measured across different body parts and tissues. Nine *P. fuscipes* were tested per treatment. Each data point represents the average of three technical replicates. The bars represent mean ± standard error (*n* = 9) and the different letters above the scatter dot plot indicate significant difference between developmental stages (*p* < 0.05). Uppercase letters represent female while male is represented by lowercase letters.
4. Discussion

Based on phylogenetic reconstructions, Wolbachia species exist in 17 supergroups designated by the letters A–R, with supergroup G being controversial [57–59]. The Wolbachia infections in Coleoptera characterized so far belong to supergroups A, B or F. In total, 12% of Coleopteran species tested to date harbored Wolbachia from supergroup A, another 12% harbored Wolbachia from supergroup B and only three species harbored Wolbachia from supergroup F [22]. In this study, Wolbachia infections screened from all tested samples of P. fuscipes were positive and belonged to supergroup B. The Wolbachia from the B supergroup in Coleoptera may affect beetle hosts in several ways. They have been shown to induce cytoplasmic incompatibility in Altica lythri from Central Europe [21], Callosobruchus chinensis from Japan [60] and Conotrachelus nenuphar from the USA [61]. Additionally, they have been suspected as inducing parthenogenesis in Aramigus conirostris from South America [62], and male killing in Adalia bipunctata from Russia [63].

We provided a quantitative analysis of Wolbachia infection densities across different development stages, body parts and tissues of P. fuscipes by qPCR. All individuals were Wolbachia positive, suggesting accurate Wolbachia vertical transmission by a parent to its offspring. Wolbachia density in adults was higher than in any other life stage (eggs, larvae and pupae) while the infection density in pupae was lowest (Figure 2). While there is a statistical difference, this difference may not equate to any biological differences. The Wolbachia density dynamics for the life stages in P. fuscipes were in accordance with those for three other Coleopteran species, Tribolium confusum [31], Octodonta nipae [53], and Brontispa longissima [64]. Wolbachia may be subject to the differential control of proliferation during the development of hosts [31]. The high Wolbachia density in adults and in eggs may be caused by functional associations with those host tissues. Since Wolbachia are primarily vertically transmitted from mothers to offspring through the egg cytoplasm, Wolbachia density is expected to be higher in the reproductive tissues of adults and in eggs [31,53,64]. Many studies have reported that Wolbachia display a strong tropism for the germline so as to ensure vertical transmission, particularly after rare horizontal transfer, as discussed for Drosophila [34,65,66].

Kellner and Dettner [67] noted that pederin is synthesized in about 90% of the females, and can be transferred to their offspring. The discovery of the pederin biosynthetic gene cluster led to the finding that the endosymbiotic Gram-negative bacteria, identified as closely related to Pseudomonas aeruginosa, were the producers of these compounds [68,69]. Kador et al. [41] found that Pseudomonas-like endosymbionts are located inside a structure of the female genitalia of P. riparius, based on FISH investigations. The Pseudomonas-like endosymbionts distributed in the female genitalia of Paederus species produce pederin as a defensive compound against insect and arachnid predators, and this does not apparently decrease the fitness of their hosts [70]. Maleki-Ravasan et al. [24] reported that the coinfection rates of both Pseudomonas-like endosymbionts and Wolbachia were 70.59% in females and 17.57% in males. Perhaps Wolbachia and Pseudomonas may interact with each other and with their Paederus beetles. It is unclear whether the Pseudomonas regulates the population of Wolbachia via pederin or not. Hence, the co-occurrence of Wolbachia and Pseudomonas in rove beetles may imply that Wolbachia is adapted to cope with adverse conditions triggered by Pseudomonas [71]. The nature of such potential interactions needs further investigation, and the effect of Wolbachia on reproduction in rove beetles also needs to be examined.

5. Conclusions

This study demonstrated that Wolbachia from P. fuscipes belonged to supergroup B, based on an analysis of MLST genotyping. The infection density in adults was higher than in any other life stage, and the reproductive tissues in adults had the highest infection densities, with similar densities between the sexes. These findings provide a starting point for understanding Wolbachia infection dynamics in P. fuscipes and interactions with other components of the microbiota, and could be a potential area for future research.
Supplementary Materials: The following are available online at http://www.mdpi.com/2075-4450/11/9/625/s1, Table S1: Accession number of various host lines used in this study.

Author Contributions: Conceptualization, W.J.; methodology, C.G. and Z.Z.; analysis, J.F., S.M. and L.S.; resources, W.Y., J.Z. and J.H.; writing—original draft preparation, W.J.; writing—review and editing, A.A.H.; supervision, W.J. All authors have read and agreed to the published version of the manuscript.

Funding: This study was financially supported by grants from National Natural Science Foundation of China (No.31401997), Shanghai Natural Science Foundation (20ZR1440800) and Shanghai Municipal Human Resources and Social Security Bureau (No.2019112).

Acknowledgments: Authors are thankful to anonymous reviewers for their valuable feedback and suggestions on a previous draft of this manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

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Author/s: 
Ge, C; Hu, J; Zhao, Z; Hoffmann, AA; Ma, S; Shen, L; Fang, J; Zhu, J; Yu, W; Jiang, W

Title: 
Phylogeny and Density Dynamics of Wolbachia Infection of the Health Pest Paederus fuscipes Curtis (Coleoptera: Staphylinidae)

Date: 
2020-09-11

Citation: 
Ge, C., Hu, J., Zhao, Z., Hoffmann, A. A., Ma, S., Shen, L., Fang, J., Zhu, J., Yu, W. & Jiang, W. (2020). Phylogeny and Density Dynamics of Wolbachia Infection of the Health Pest Paederus fuscipes Curtis (Coleoptera: Staphylinidae). Insects, 11 (9), https://doi.org/10.3390/insects11090625.

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