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Distribution and recombination of *Wolbachia* endosymbionts in Korean coleopteran insects

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Abstract

Background: *Wolbachia* are among the most prevalent endosymbiotic bacteria and induce reproductive anomalies in various invertebrate taxa. The bacterium has huge impacts on host reproductive biology, immunity, evolution, and molecular machinery. However, broad-scale surveys of *Wolbachia* infections at the order scale, including the order Coleoptera, are limited. In this study, we investigated the *Wolbachia* infection frequency in 201 Coleopteran insects collected in Korea.

Results: A total of 26 species (12.8%) belonging to 11 families harbored *Wolbachia*. The phylogenetic trees of based on partial 16S rRNA gene sequences and partial *Wolbachia* surface protein (*wsp*) gene sequences were largely incongruent to that of their hosts. This result confirms that *Wolbachia* evolved independently from their hosts,

Conclusion: Phylogenetic trees suggest that complex horizontal gene transfer and recombination events occurred within and between divergent *Wolbachia* subgroups.

Keywords: Horizontal gene transfer, Recombination, *Wolbachia*

Background

Wolbachia are highly prevalent endosymbiotic bacteria known to induce reproductive anomalies, such as cytoplasmic incompatibility, feminization, male-killing, and parthenogenesis, in various arthropod species, nematodes, isopods, and mites (Stouthamer et al. 1997, Werren and Windsor 2000; Zug and Hammerstein 2012). Bacteria in the genus have major impacts on host reproductive biology, immunity, evolution, and molecular machinery. Despite the controversy over the taxonomic status, the prevailing view is that *Wolbachia* in various hosts should be considered a single species divided into 14 supergroups (Glowska et al. 2015; Lindsey et al. 2016; Lo et al. 2002; Ramírez-Puebla et al. 2016). The genus has been a focus of research owing to its potential to control host populations, especially mosquito populations, by artificially infecting cytoplasmic incompatibility-inducing strains (Bourtzis et al. 2014; Werren 2008; Xi et al. 2006). Recent success in field trials has provided a new and safe approach for the control of *Aedes aegypti,* a vector of fatal viruses, such as dengue, Zika, and chikungunya (O'Neill 2016; O'Neill et al. 2018). Similar approaches for control are not necessarily limited to mosquito species but can be applied to virtually any host pest (Rostami et al. 2016).

Despite its ecological and evolutionary importance and application, in Korea, infection surveys of the bacterium have been limited to specific taxa (Choi et al. 2015; Jeong et al. 2009a and b; Jeong et al. 2012; Park et al. 2016). Since the order, Coleoptera is the largest group of insects and includes pest species affecting economic activity in Korea (see Moon and Lee 2015), we determined to investigate the *Wolbachia* infection frequency in the insects collected in Korea.

To our knowledge, this is the first intensive survey of *Wolbachia* infection at the order level in Korea.

Results and discussion

Wolbachia infection frequency

In the survey, 26 out of 201 coleopteran species were infected with *Wolbachia* and the genes are annotated (Tables 1 and 2, and Additional file 1: Table S1). Among 27 families, we detected *Wolbachia* in 10. For 18 families, we

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Table 1 Infection frequency at the family level

Number	Family	No. species	Infected	% infection
1	Carabidae	24	2	8.3
2	Dytiscidae	4	0	0.0
3	Hydrophilidae	2	0	0.0
4	Histeridae	2	0	0.0
5	Silphidae	2	0	0.0
6	Staphylinidae	2	0	0.0
7	Lucanidae	4	0	0.0
8	Scarabaeidae	26	0	0.0
9	Psephenidae	1	1	100.0
10	Buprestidae	4	0	0.0
11	Melyridae	1	0	0.0
12	Nitidulidae	1	0	0.0
13	Languriidae	2	1	50.0
14	Byturidae	1	1	100.0
15	Endomychidae	1	1	100.0
16	Tenebrionidae	14	0	0.0
17	Coccinellidae	13	0	0.0
18	Oedemeridae	7	1	14.3
19	Stenotrachelidae	1	0	0.0
20	Meloidae	1	0	0.0
21	Anthicidae	2	0	0.0
22	Cerambycidae	21	2	9.5
23	Chrysomelidae	39	10	25.6
24	Athribidae	1	0	0.0
25	Attelabidae	9	4	44.4
26	Apionidae	1	1	100.0
27	Curculionidae	15	2	13.3
	Total	201	26	12.9

examined fewer than 4 species. Among families for which at least 5 species were examined, Attelabidae showed the highest infection frequency (4 out of 9 species), followed by Oedemeridae (1 out of 7 species), Chrysomelidae (10 out of 39 species), and Curculionidae (2 out of 15 species) (Table 1). On the other extreme, infection was not detected in any Carabidae samples. Recent two in-depth reviews show that the *Wolbachia* infection frequency in beetle species is about 38% and 27% respectively (Kajtoch et al. 2019; Kajtoch and Kotásková 2018). In our analysis, only 12.9% of beetle species harbored the bacterium. This discrepancy may be explained by a difference in the sampling method. Since we examined a single specimen per species, partial infections within populations were not resolved. Further tests should include multiple specimens for each species. All gene sequences named after the host insect species were deposited at GenBank and the accession numbers are in Table 2 and Additional file 1: Table S1. There have been studies on the *Wolbachia* infection status in various coleopteran insects (Werren et al. 1995; Kajtoch and Kotásková 2018). Incongruence in infection frequency from them may be caused by geographical variation and taxonomic composition.

Table 2 Annotation of *Wolbachia* from beetle species

Genus	Family	16 s	*wsp*
Colpodes buchanani	Carabidae	sCbu	wCbu
Dicranoncus femoralis	Carabidae	sDfe	wDfe
Eubrianax granicollis	Psephenidae	sEgr	wEgr
Anadastus praeustus	Languriidae	sApr	wApr1
	Languriidae		wApr2
	Languriidae		wApr3
	Languriidae		wApr4
	Languriidae		wApr5
Byturus aestivus	Byturidae	sBae	wBae
Ancylopus pictus	Endomychidae	sApi	wApi
Oedemeronia lucidicolis	Oedemeridae	sOlu	wOlu
Pogonocherus seminiveus	Cerambycidae	sPse	wPse
Rhaphuma diminuta	Cerambycidae	sRdi	wRdi
Aulacophora indica	Chrysomelidae	sAin	wAin
Basilepta pallidula	Chrysomelidae	sBpa	wBpa
Monolepta shirozui	Chrysomelidae	sMsh	wMsh
Lema diversa	Chrysomelidae	sLdi	wLdi
Medythia nigrobilineata	Chrysomelidae	sMni	wMni
Smaragdina semiaurantiaca	Chrysomelidae	sSse	wSse1
Aulacophora nigripennis	Chrysomelidae	sAni	wAni1
Aspidomorpha transparipennis	Chrysomelidae	sAtr	wAtr
Gallerucida bifasciata	Chrysomelidae	sGbi	wGbi
Agelasa nigriceps	Chrysomelidae	sAnig	wAnig
	Chrysomelidae		wSse2
	Chrysomelidae		wAni2
Euops lespedezae	Attelabidae	sEle	wEle
Paracycnotrachelus longiceps	Attelabidae	sPlo	wPlo
Cycnotrachelus coloratus	Attelabidae	sCco	wCco
Byctiscus venustus	Attelabidae	sBve	wBve
Apion collare	Brenthidae	sAco	wAco
Baris dispilota	Culculionidae	sBdi	wBdi
Lixus maculatus	Culculionidae	sLma1	wLma1

Phylogeny of *Wolbachia*

Phylogenetic trees based on 16S rRNA and *wsp* were largely incongruent (Fig. 1). This result confirms that *Wolbachia* evolved independently from their hosts, as indicated by Kajtoch and Kotásková (2018).

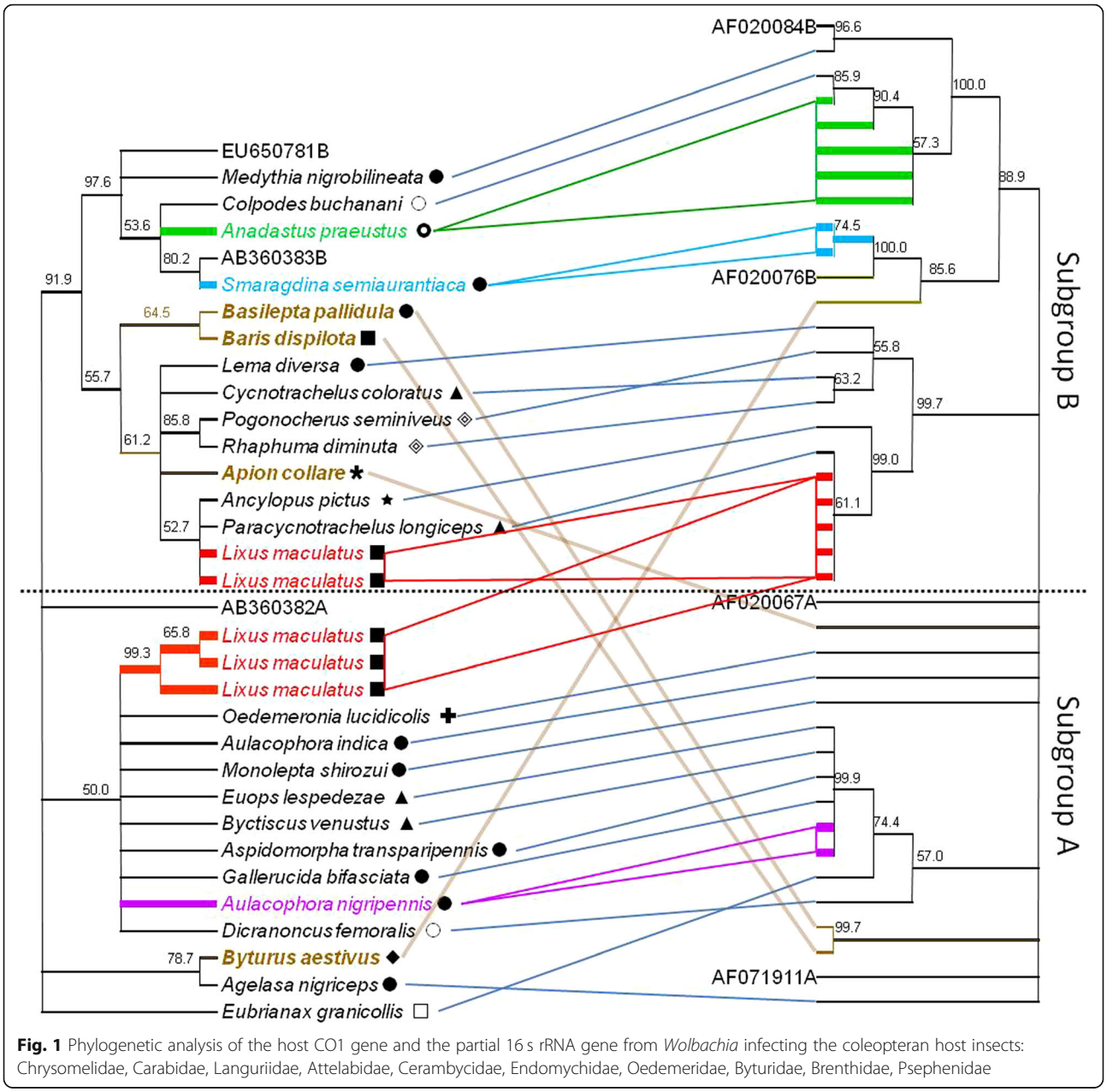

Fig. 1 Phylogenetic analysis of the host CO1 gene and the partial 16 s rRNA gene from *Wolbachia* infecting the coleopteran host insects: Chrysomelidae, Carabidae, Languriidae, Attelabidae, Cerambycidae, Endomychidae, Oedemeridae, Byturidae, Brenthidae, Psephenidae

Interestingly, some species, such as *Lixus maculates,* were superinfected with 5 strains of *Wolbachia*. These strains likely diverged after a single infection, as evidenced by the monophyletic clustering of each gene (Fig. 2). However, *Anadastus praeustus*, *Smaragdina semiaurantiaca*, and *Aulacophora nigripennis* were superinfected based only on *wsp* gene diversity. For *Wolbachia*-infecting *Basilepta pallidula*, *Baris dispilota*, *Apion collare*, and *Byturus aestivus,* we detected incongruence between 16S rRNA and *wsp* phylogenies, as observed for *Byturus unicolor* in this study (Fig. 2). This finding indicates that the genes evolved independently.

The *Wolbachia* strain in *Anadastus praeustus* showed five *wsp* alleles. This can potentially be explained by synonymous substitutions in 16S rRNA and *wsp*, rather than by recombination (data not shown). *Wolbachia* strains infecting *Lixus maculates* told a different story. The 16S rRNA gene of *Wolbachia*-infecting *Lixus maculatus* could be assigned to two main subgroups (three in subgroup A and two in B). However, the *wsp* sequences exhibited high similarity and were all assigned to subgroup A (Fig. 2). This implies that strains in the two subgroups infected the host and accumulated synonymous substitutions after recombination. Under the assumption that the 16S rRNA

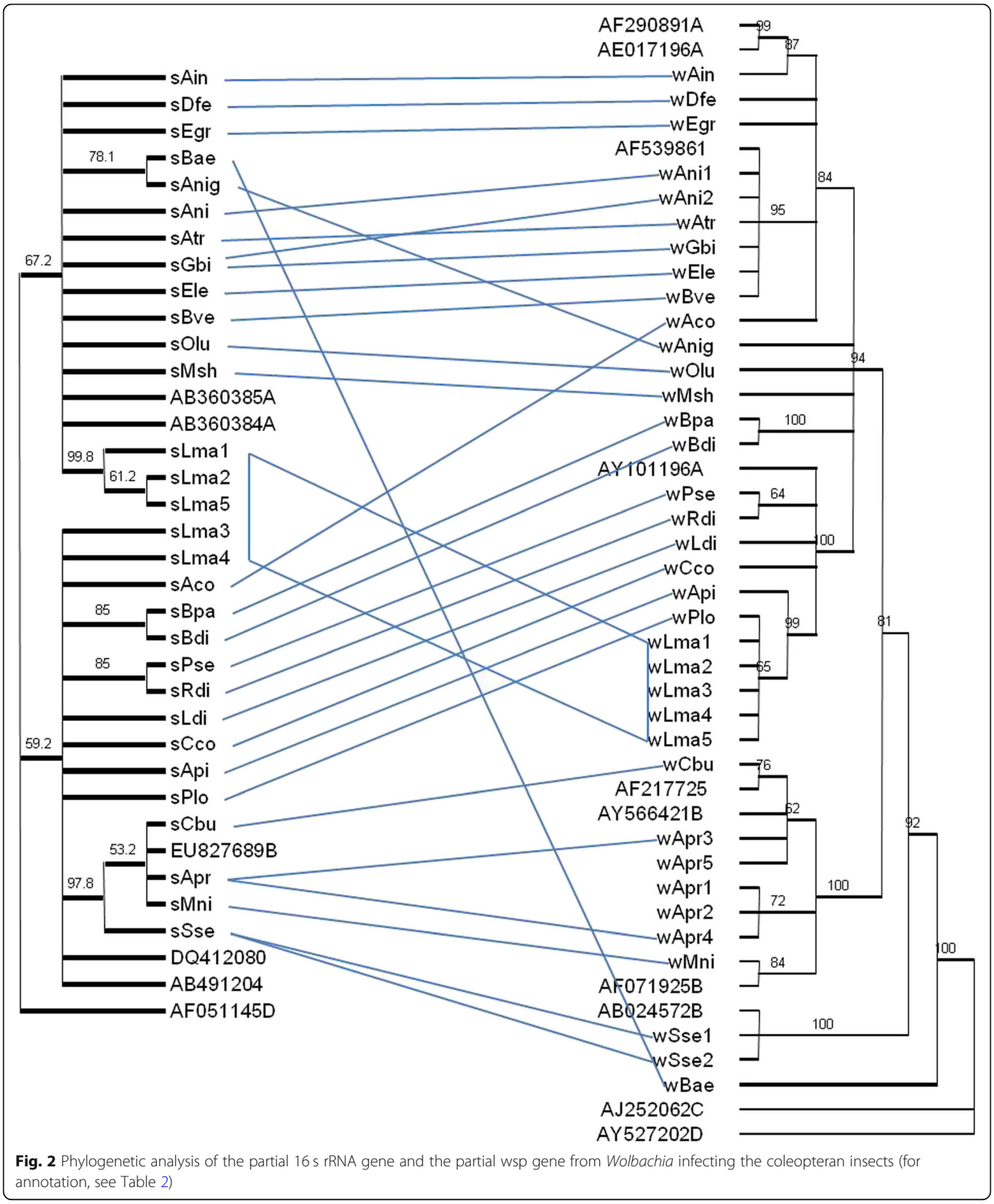

Fig. 2 Phylogenetic analysis of the partial 16 s rRNA gene and the partial wsp gene from *Wolbachia* infecting the coleopteran insects (for annotation, see Table 2)

and *wsp* phylogenies are strictly congruent, strains that acquired *wsp* of subgroup B were selected against. Since the two genes are functional, this suggests that the strain with *wsp* classified as subgroup A has deleterious mutations in essential genes other than *wsp*. Another less likely explanation is that these strains were simply lost due to drift with no sex ratio drive; however, this does not explain how only particular strains were affected. Previously *Wolbachia* was

suspected to induce parthenogenesis and oocyte formation in the host species (Chen et al. 2012; Rodriguero et al. 2010; Son et al. 2008). However, we were unable to infer the effect of infection on hosts' life history due to the lack of general biology of the Korean coleopteran insects.

Conclusions

In this study, 12.8% of Korean beetle species are infected with *Wolbachia*. Phylogenetic trees suggest that complex horizontal gene transfer and recombination events occurred within and between divergent *Wolbachia* subgroups resulting in largely incongruent phylogenetic relationships.

Materials and methods

Study materials

Coleopteran species were mostly collected by light trapping at night between May and August 2008 in various locations in South Korea (Additional file 1: Table S1). They were brought into the laboratory and identified carefully. Voucher specimens were deposited at the NAAS repository. Genomic DNA was extracted from a leg of the specimens using the DNeasy Mini Kit (Qiagen, Hilden, Germany) or the AccuPrep® Genomic DNA Extraction Kit (Bioneer, Daejeon, Korea) and stored at − 80 °C until use.

Determination of *Wolbachia* infection

All PCRs were performed using a total volume of 20 μl in AccuPower® PCR PreMix (Bioneer) and a thermocycler (Biometra, Tubingen, Germany).

The quality of genomic DNA was determined by PCR using a universal arthropod 28S rRNA primer set (forward: TAC CGT GAG GGA AAG TTG AAA and reverse: AGA CTC CTT GGT CCG TGT TT) or a universal *CO1* primer set (forward: GGT CAA CAA ATC ATA AAG ATA TTG G and reverse: TAA ACT TCA GGG TGA CCA AAA AAT CA) (Jeong et al. 2009a, 2009b). The temperature profile for 28S rRNA diagnostic PCR was 2 min of pre-denaturation at 94 °C, followed by 38 cycles of 30 s at 94 °C, 50 s at 58 °C, 90 s at 72 °C, and a final extension for 10 min at 72 °C.

A *Wolbachia*-diagnostic marker (forward: CAT ACC TAT TCG AAG GGA TAG and reverse: AGC TTC GAG TGA AAC CAA TTC) was used to determine the infection status of specimens. Genomic DNA of *Wolbachia*-infected *Cadra cautella* (Pyralidae: Lepidoptera) was used as a positive control. The temperature profile for *Wolbachia*-specific diagnostic PCR was 2 min of pre-denaturation at 94 °C, followed by 38 cycles of 30 s at 94 °C, 45 s at 55 °C, 90 s at 72 °C, and a final extension for 10 min at 72 °C.

Positive PCR samples for 28S rRNA and/or CO1 and the *Wolbachia* diagnostic marker were scored as infected. Additionally, when a sample was negative for 28S rRNA and/or CO1 but positive for the *Wolbachia* marker, the species was scored as infected.

Genotyping of *Wolbachia*

Multilocus sequence typing (MLST) is widely used for genotyping and assignment to supergroups in *Wolbachia* (Baldo et al. 2006; but see Bleidorn and Gerth 2017). The MLST was not adopted since this study was a basic survey on *Wolbachia* from Korean Coleopteran insects. Instead, we chose two conventionally used genes. For samples identified as infected, PCR was performed with genomic DNA to obtain the partial 16S rRNA gene sequence and partial *Wolbachia* surface protein (*wsp*) gene sequence. For *Wolbachia,* nested PCR was performed following the methods described by Weeks et al. (2003). For the initial PCR, the 16S rDNA primer set was used (27f and 1513r) (Weisburg et al., 1991). The temperature profile was 4 min of pre-denaturation at 94 °C, followed by 35 cycles of 1 min at 94 °C, 1 min at 52 °C, and 1 min at 72 °C, and a final extension for 5 min at 72 °C. Then, *Wolbachia*-specific PCR was performed using the *Wolbachia*-specific 16S rDNA primer set (176f and 1012r) taken from O'Neill et al. (1992). One microliter of initial PCR product was diluted 10-fold and used as template DNA. The temperature profile for PCR was 4 min of pre-denaturation at 94 °C, followed by 35 cycles of 1 min at 94 °C, 1 min at 50 °C, and 1 min at 72 °C, and a final extension for 5 min at 72 °C. The partial *wsp* gene of the bacterium was obtained using the following primer set: 81f, TGG TCC AAT AAG TGA TGA AGA AAC; 691r, AAA AAT TAA ACG CTA CTC CA (Zhou et al. 1998). The temperature profile was 5 min of pre-denaturation at 94 °C, followed by 35 cycles of 1 min at 94 °C, 1 min at 55 °C, and 1 min at 72 °C, and a final extension for 5 min at 72 °C.

Phylogenetic analyses

A multiple sequence alignment of all partial 16S rRNA genes and *wsp* genes from *Wolbachia* was generated using ClustalW embedded in MEGA (ver. 6, Tamura et al. 2013). The protein-coding *wsp* gene sequences were translated into amino acid sequences before alignment. These sequences were then reverse-translated into DNA sequences.

The alignments were manually adjusted. All gaps were manually removed, resulting in 765 bp of the partial 16S rRNA gene, of which 31 sites were parsimony-informative, and 509 bp of *wsp,* of which 172 sites were parsimony-informative. The HKY + I and TVM + I + G models of evolution were used to estimate phylogenies for 16S rRNA and the *wsp*, respectively, the best-fit models were determined by log-likelihood ratio tests using ModelTest (ver. 3.7, Posada and Crandall 1998).

Likelihood analyses were performed using PAUP* (ver. 4.0b, Swofford 2002) by a heuristic search with 10,000 random sequence replicates and the tree-bisection-reconnection branch swapping algorithm. Nodal support was estimated using 500 bootstrap replicates with 10 random addition sequence replicates per bootstrap replicate.

The phylogenetic trees were visualized and manually edited using TreeGraph 2 (Müller and Müller 2004).

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s41610-019-0143-2.

Additional file 1. Information on the beetle insect samples used for this study with infection status.

Abbreviations

COI: Cytochrome oxidase I; PCR: Polymerase chain reaction; rDNA: Ribosomal DNA; rRNA: Ribosomal RNA; *wsp*: *Wolbachia* surface protein

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Authors' contributions

GJ designed the study. TMH and HCP collected the samples in the field. GJ, SP, and PN analyzed the data and wrote the manuscript. SSK reviewed the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The sequence data can freely be obtained from NCBI. The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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