Supporting Information

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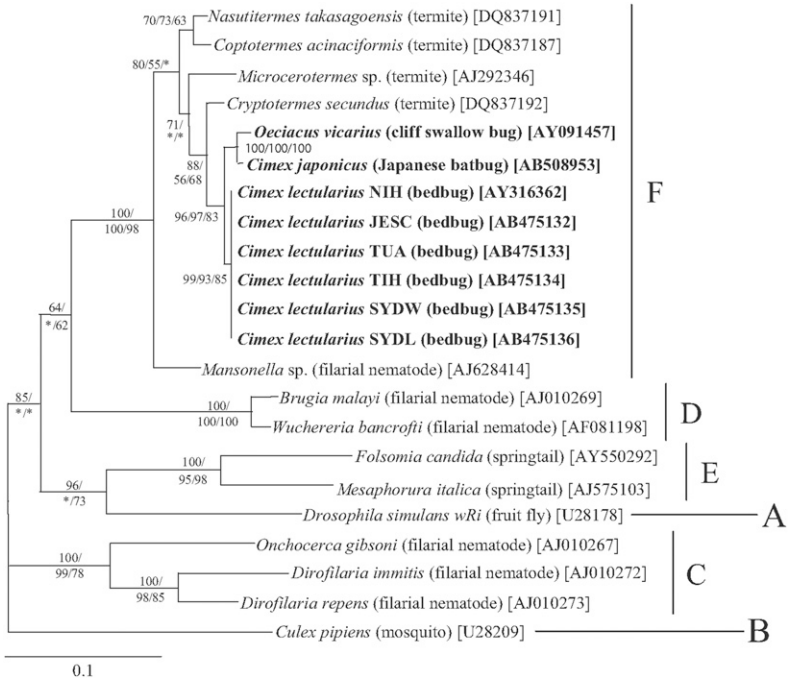

Fig. S1. Phylogenetic placement of *Wolbachia* from *C. lectularius* and allied bugs on the basis of 722 aligned nucleotide sites of ftsZ gene sequences. A Bayesian (BA) phylogeny is shown. Host insect names in italic, accession numbers in brackets, and *Wolbachia* supergroups A–F on the right side are shown. Posterior probabilities for BA analysis and bootstrap probabilities for maximum parsimony (MP) and maximum likelihood (ML) analyses greater than 50% are indicated at the nodes in the order of BA/MP/ML.

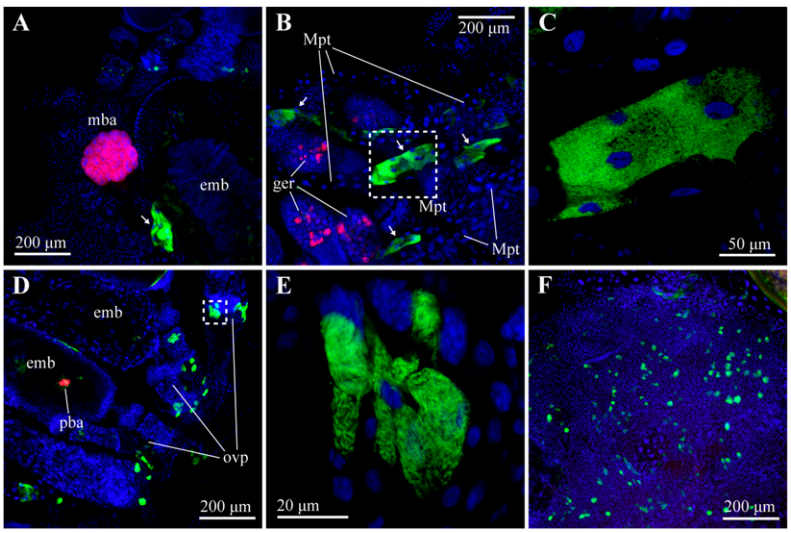

Fig. S2. Localization of γ-proteobacterial symbiont in *C. lectularius*. (*A*) A Malpighian tubule infected with γ-proteobacterial symbiont (arrow) nearby a maternal bacteriome and an embryo. (*B*) Infected regions (arrows) and uninfected regions (Mpt) of Malpighian tubules nearby ovarioles. (*C*) An enlarged image of the infected Malpighian tubule. (*D*) A number of infected cells in ovariole pedicels. (*E*) An enlarged image of infected cells in the ovariole pedicel. (*F*) Infected cells scattered in mesospermalage, a hemocyte-containing female organ into which sperm are introduced via traumatic insemination. Green, red, and blue signals indicate γ-proteobacteria, *Wolbachia* and insect nuclei, respectively. Abbreviations: emb, embryo; ger, germalium; mba, maternal bacteriome; Mpt, Malpighian tubule; pba, embryonic primordial bacteriome; ovp, ovariole pedicel.

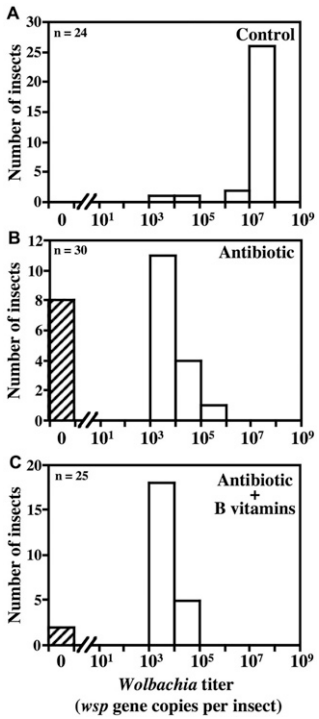

Fig. S3. *Wolbachia* infection titers in adult insects of *C. lectularius* after 8 weeks of experimental treatments. (*A*) Control insects. (*B*) Antibiotic-treated insects fed on a rifampicin-containing blood meal. (*C*) Antibiotic-treated and B vitamin–supplemented insects fed on a rifampicin- and B vitamin–containing blood meal.

Table S1. The insects used in this study and their infection with the *Wolbachia* symbiont and the γ-proteobacterial symbiont

	Insect origin and description	*Wolbachia* infection rate*	γ-proteobacterial infection rate*	*Wolbachia* 16S rRNA gene	*Wolbachia* ftsZ gene	γ-proteobacterial 16S rRNA gene	Insect elongation factor 1α gene
The common bedbug *Cimex lectularius*	JESC: A laboratory strain of unknown origin maintained on mice at the Japan Environmental Sanitation Center for over 10 years. Provided by A. Muto.[¶]	100%[†] (46/46)	0%[†] (0/46)	AB475122	AB475132	—	AB475141
	TUA: Insects collected in 2005 and 2006 from a colony at a quail coop in the Tokyo University of Agriculture, Japan. Provided by T. Ishikawa.[¶]	100%[†] (48/48)	96%[†] (46/48)	AB475123	AB475133	AB475137	AB475142
	TIH: A laboratory strain maintained on mice at the Toyama Institute of Health for 7 years since collected at Toyama, Japan. Provided by T. Yamauchi.[¶]	100%[‡] (3/3)	67%[‡] (2/3)	AB475124	AB475134	AB475138	—
	SYDW: Insects collected in 2007 at Sydney, Australia. Provided by S.L. Doggett.[¶]	100%[‡] (4/4)	100%[‡] (4/4)	AB475125	AB475135	AB475139	—
	SYDL: A laboratory strain maintained on rats at the Institute of Clinical Pathology and Medical Research (ICPMR) for 4 years since collected at Sydney, Australia. Provided by S.L. Doggett.[¶]	100%[‡] (4/4)	100%[‡] (4/4)	AB475126	AB475136	AB475140	—
	Total	100% (105/105)	53% (56/105)				
The Japanese batbug *Cimex japonicus*	KTCH: Insects collected in 2008 from a colony of the bat *Vespertilio sinensis* at Kutchan, Hokkaido, Japan. Provided by D. Fukui.[¶]	100%[§] (5/5)	0%[§] (0/5)	AB508951	AB508953	—	—

*Infection rates estimated by diagnostic PCR.
[†]First-instar nymphs were inspected.
[‡]Adult insects were inspected.
[§]Developmental stages were uncertain.
[¶]Affiliations: A. Muto, Japan Environmental Sanitation Center, Kawasaki, Japan; T. Ishikawa, Tokyo University of Agriculture, Setagaya, Japan; T. Yamauchi, Toyama Institute of Health, Imizu, Japan; S. L. Doggett, Institute of Clinical Pathology and Medical Research, Westmead, Australia; D. Fukui, Forestry and Forest Products Research Institute, Sapporo, Japan.

Table S2. Summary of cloning and genotyping of 16S rRNA gene clones from adult insects of *C. lectularius*

Strain	Sex and ID	Tissue	Number of genotyped clones	16S rRNA genotypes*	
				Wolbachia	γ-proteobacterium
TUA	Male 1	Bacteriome	12	11	1
TUA	Male 2	Bacteriome	15	15	0
TUA	Female 1	Bacteriome	15	15	0
TIH	Male 1	Bacteriome	5	5	0
TIH	Male 2	Bacteriome	6	6	0
TIH	Female 1	Bacteriome	8	8	0
JESC	Male 1	Bacteriome	16	16	0
JESC	Male 2	Bacteriome	11	11	0
JESC	Male 3	Bacteriome	14	14	0
JESC	Female 1	Bacteriome	10	10	0
JESC	Female 2	Bacteriome	10	10	0
SYDW	Male 1	Abdomen	8	3	5
SYDW	Male 2	Abdomen	7	3	4
SYDW	Female 1	Abdomen	8	3	5
SYDW	Female 2	Abdomen	7	1	6
SYDL	Male 1	Abdomen	8	3	5
SYDL	Male 2	Abdomen	8	0	8
SYDL	Female 1	Abdomen	6	0	6
SYDL	Female 2	Abdomen	6	3	3
		Total	180	137	43

*Sequencing data and restriction fragment length polymorphism data were combined.

Table S3. List of B vitamins supplemented to the blood meal for *Wolbachia*-eliminated insects

Compounds*	Final concentration*
Thiamine	100 μg/mL
Riboflavin	20 μg/mL
Nicotinic acid	100 μg/mL
Pantothenic acid	100 μg/mL
Pyridoxine	100 μg/mL
D-biotin	1 μg/mL
Folic acid	30 μg/mL
Cobalamin	1 μg/mL
Choline chloride	185 μg/mL
Meso-inositol	118 μg/mL

*Prescription according to a previous study on the kissing bug *Rhodnius prolixus* (1)

1. Lake P, Friend WG (1968) The use of artificial diets to determine some of the effects of *Nocardia rhodonii* on the development of *Rhodonius prolixus*. *J Insect Physiol* 14:543–562.