Supporting Information

Gomes et al. 10.1073/pnas.1716181114

SI Materials and Methods

Mosquito Collection. Mosquitoes were collected in the villages of Dangassa (12.14°N, 8.20°W) and Kenieroba (12.11°N, 8.33°W), southwest of Bamako, Mali, along the Niger River in May–January 2010–2011, October–November 2015, and July–August 2016 (Fig. 1). Houses were sprayed with pyrethrum, and female mosquitoes were captured and desiccated using Eppendorf tubes with silica gel.

Homogenization of Samples for *Plasmodium* Detection by Anti-CSP ELISA and Genomic DNA Extraction. Head-thorax segments were dissected and homogenates were prepared as described (42). Briefly, samples were homogenized with a plastic pestle in 50 μ L of blocking buffer (5% casein, 50 mM EDTA, PBS pH 7.4) (BB-EDTA), supplemented with 5% Nonidet P-40 (BB-Nonidet P-40). The pestle was rinsed by adding 200 μ L of BB-EDTA, bringing the final homogenate volume to 250 μ L. *Plasmodium* infection was detected using 50 μ L of the homogenate to detect the *P. falciparum* CSP using an anti-sporozoite monoclonal antibody with an ELISA kit (BEI Resources) with samples done in duplicate (100 μ L of total homogenate). The rest of the homogenate (150 μ L) was immediately frozen in dry ice and stored at -80 °C.

Genomic DNA Extraction and Quantitation of P. falciparum and Wolbachia Infection in Field-Collected Mosquitoes. Genomic DNA was extracted from 150 µL of homogenate. The homogenates were incubated with 3 mg/mL proteinase K at 56 °C for 1 h, and genomic DNA (gDNA) was purified using phenol/chloroform extraction, followed by two chloroform extractions. The aqueous layer was transferred to a new tube, 0.1 volumes of 0.3 M sodium acetate and 0.7 volumes of isopropanol were added, and DNA was precipitated by centrifugation (16,000 \times g for 15 min). The pellet was gently washed twice with 100 μ L of 70% ethanol (16,000 × g for 5 min) and resuspended in 200 µL of Tris-EDTA buffer. To further purify the DNA, samples were reprecipitated (with 0.1 volumes of 0.3 M sodium acetate and 0.7 volumes of isopropanol) and gently washed twice with 70% ethanol. DNA pellets were air-dried for 30-60 min and resuspended in 30 µL of TE. Wolbachia was detected by PCR (forward: 5'-CATACCTATTCGAAGGGATAG-3', reverse: 5'-AGCTTCGAGTGAAACCAATTC-3') (25) using the following amplification conditions: 10 min at 95 °C, followed by 45 cycles of 45 s at 95 °C, 45 s at 60 °C and 1 min at 72 °C, followed by 10 min at 72 °C. Nested PCR was used to detect Wolbachia using Wolbachia-specific primers targeting 16S rRNA (forward: 5'-GAAGGGATAGGGTCGGTTCG-3', reverse: 5'-CAATTCC-CATGGCGTGACG-3') under the following conditions: 15 min at 95 °C, followed by 35 cycles of 15 s at 95 °C, 25 s at 66 °C, and 1 min at 72 °C, followed by 5 min at 72 °C (23). PCRs were performed using Taq DNA Polymerase, native kits (ThermoFisher) with 500 nM forward and reverse primers. The specificity of the PCRs was confirmed by Sanger sequencing.

Wolbachia Phylogenetic Analysis and MLST Typing. For phylogenetic analysis, Rickettsiales-specific primers targeting a variable region of the 16S rRNA were used [forward: 5'-CAGACGGGTGAG-TAATG(C/T)ATAG-3', reverse: 5'-TATCACTGGCAGTTTC-CTTAAAG-3'] under the following conditions: 10 min at 95 °C, followed by 45 cycles of 45 s at 95 °C, 45 s at 64 °C and 1 min at 72 °C, followed by 5 min at 72 °C (25). PCRs were performed using Taq DNA Polymerase, native kits (ThermoFisher) with 500 nM forward and reverse primers. Following Sanger sequencing, downstream analysis was performed using the Geneious R10 software

platform (Biomatters). Sequenced PCR products were blasted against the National Center for Biotechnology Information (NCBI) nucleotide database. The top 500 results were downloaded and manually inspected to remove redundancy. Reference *Rickettsia* sequences were added and sequence alignment was performed using the MUSCLE algorithm. A phylogenetic tree was reconstructed using a Tamura-Nei genetic distance model. For MLST typing, primer sequences and PCR conditions were used as instructed by the *Wolbachia* MLST Database (26). PCRs were performed using Taq DNA Polymerase, native kits (ThermoFisher) with 500 nM forward and reverse primers.

Wolbachia and Plasmodium Detection and by gPCR. Wolbachia levels within mosquito genomic DNA samples were analyzed by SYBR green qRT-PCR. Wolbachia- and Plasmodium-specific primers against 16S (forward: 5'-CATACCTATTCGAAGGGATAG-3', reverse: 5'-TTGCGGGACTTAACCCAACA-3') and 28S (forward: 5'-GTGGCCTATCGATCCTTTA- 3', reverse: 5'-GCG-TCCCAATGATAGGAAGA-3') rRNA were used, respectively. The amount of gDNA template was normalized based on the levels of A. gambiae S7 rRNA gene in each sample (forward: 5'-GCGTCCCAATGATAGGAAGA-3', reverse: 5'-GCTGCAA-ACTTCGGCTATTC-3'). The PCRs were performed under the following conditions: 15 min at 95 °C, followed by 45 cycles of 15 s at 94 °C, 20 s at 60 °C and 30 s at 72 °C, followed by 5 min at 72 °C. PCRs were performed using DyNAmo HS SYBR Green qPCR Kit (ThermoFisher) with 300 nM forward and reverse primers using a CFX96 system (Bio-Rad). gDNA isolated from females of the NIH A. gambiae G3 colony, that is not infected with Wolbachia, was used as positive and negative control for the qPCR assays. Amplification of the A. gambiae S7 gene was confirmed, as well as the lack of Wolbachia amplification. Cycles threshold (Ct) values beyond 40 cycles were considered as negative, and the limit of detection was set at a point where the correlation between technical replicates was very high (R^2 = 0.9978, *P* < 0.0001).

Establishment of *Wolbachia*-Infected *A. coluzzii* Colony. Females were collected from Dangassa and allowed to lay eggs in individual containers. Eggs were allowed to hatch and mothers were genotyped as either *A. gambiae* (S molecular form) or *A. coluzzii* (M molecular form), as previously described (43). *A. coluzzii* larvae from 23 females were pooled and reared. Upon emergence, adult mosquitoes were mixed for three generations with *A. coluzzii* males from a colony kept at the Laboratory of Malaria and Vector Research, NIH. Mosquitoes were treated with Penicillin-Streptomycin (Sigma-Aldrich), because some bacteria from the microbiota, such as *Asaia*, have been reported to reduce vertical transmission of *Wolbachia* anophelines (44). Briefly, mosquitoes received 100 units/mL Penicillin and 100 µg/mL Streptomycin in water for 2 d before and 2 d after blood feeding. Sucrose was provided as a dry sugar cube, and mosquitoes were allowed to lay eggs in a wet filter paper.

Experimental Infection of Mosquitoes with *P. falciparum*. Mosquito females were artificially infected by membrane feeding with *P. falciparum* NF54 gametocyte cultures. Gametocytogenesis was induced as previously described (45) and 14- to 16-d mature gametocyte cultures (stages IV and V) were used to feed mosquitoes using membrane feeders at 37 °C for 30 min. *Wolbachia* and *Plasmodium* infection levels were analyzed by qPCR using dissected midgut or head-thorax gDNA samples 8–10 or 18–21 d after feeding, respectively. Midgut gDNA was extracted using

the DNEasy Blood and Tissue Kit (Qiagen) following lysozyme and proteinase K treatment. Head-thorax gDNA was extracted from dissected segments. Samples were homogenized in 50 μ L of TE and incubated at 65 °C for 30 min. Samples were incubated with 20 mg/mL lysozyme at 37 °C for 1.5 h, and 1.3 mg/mL proteinase K at 56 °C for 1.5 h. Following the addition of 0.85 M

potassium acetate, samples were incubated on ice for 30 min and centrifuged $16,000 \times g$ for 15 min. The supernatant was transferred, incubated at room temperature for 5 min with 0.7 volumes of isopropanol and centrifuged $16,000 \times g$ for 15 min. The pellet was washed twice with 100 µL of 70% ethanol, air dried for 30–60 min, and resuspended in 20 µL of TE.



Fig. S1. Wolbachia levels in field-collected mosquitoes. A. gambiae s.l. females were collected in the villages of Dangassa (12.14°N, 8.20°W) and Kenieroba (12.11°N, 8.33°W), southwest of Bamako, Mali, in October–November 2015, and Wolbachia levels were determined by qRT-PCR. The amount of gDNA template was normalized based on the levels of A. gambiae S7 rRNA gene in each sample. Sample value distributions were compared using the Mann–Whitney test and are indicated by the red line (n = number of samples, ****P < 0.0001).

<u></u>	1 10	20	30	40	50	60	70
wAnga Mali	³⁹ ACACAGGTGTTG	CATGGCTGTCGTC	AGCTCGTGT	CGTGAGAT <mark>-</mark> G	TTGGGTTAAG	P7 FCCCGCAACGA	I07 GCG <mark>C</mark> A
wAnga VK5_STP			AGCTCGTGTG		TTGGGTTAAG	ICCCGCAACGA	GCGCA
wRi	ACACAGGTGTTG	CATGGCTGTCGTC	AGCTCGTGTG	GTGAGAT-G	TTGGGTTAAG	ICCCGCAACGA	GCGCA
wAnga VK5 3.1a	ACACAAGTGTTG	CATGGCTGTCGTC	AGCTCGTGTG	GTGAGAT-G	TTGGGTTAAG	FCCCGCAACGA	GCGCA
wNo _	ACACAAGTGTTG	CATGGCTGTCGTC	AGCTCGTGTG	CGTGAGAT <mark>-</mark> G	TTGGGTTAAG	FCCCGCAACGA	GCGCA
wPip	AC ACAAGTGTTG	CATGGCTGTCGTC	AGCTCGTGTG	CGTGAGAT <mark>-</mark> G	TTGGGTTAAG	FCCCGCAACGA	GCGCA
Rickettsia japonica	ACACAGGIGIIG		AGCICGIGIG	GIGAGAI-G	TIGGGIIAAG	ICCCGCAACGA	GCGCA
Apaplasma phagocytophilum			AGCTCGTGTG		TTCCCTTAAC		GCGCA
Anapiasina phagocytophilum	80	90	100	110	120	130	140
	117	127	137 1	**** 147	157	167	177
wAnga Mali	ΑСССТСАТССТТ	A <mark>GTTACC</mark> Á <mark>T</mark> CAGG	TCATĠCTGG	G <mark>G</mark> ACT <mark>T</mark> TAAG	GAAACTGCCA	зтб <mark>а</mark> та́а <mark>аст</mark> б	GAGGA
wAnga VK5_STP	ACCCTCATCCTT	AGTTACCATCAGG	TAATGCTGG	GACTTTAAG	GAAACTGCCA	JTG <mark>A</mark> TAA <mark>ACT</mark> G	GAGGA
WMel	ACCELCATE				GAAACTGCCA		GAGGA
	ACCCTCATCCTT	AGTTGCTATCAGG			GAAACTGCCA		GAGGA
wNo	ACCCTCATCCTT	AGTTGCCACCAGG	TAATGCTGA	TACTTTAAG	GAAACTGCCA	GTGATAAGCTG	GAGGA
wPip	ACCCTCATCCTT	AGTTGCCATCAGO	TAATGCTGA	TACTTTAAG	GAAACTGCCA	GTGATAAGCTG	GAGGA
Rickettsia japonica	ACCCTCATTCTT	A <mark>T</mark> TT <mark>G</mark> C <mark>C</mark> AGC <mark>G</mark> GG	T <mark>A</mark> A <mark>T</mark> GC <mark>C</mark> GG	G <mark>A</mark> ACT <mark>A</mark> TAAG	AAAACTGCCG	GTG <mark>A</mark> TAA <mark>G</mark> C <mark>C</mark> G	GAGGA
Ehrlichia chaffeensis	ACCCTCATTCTT	A <mark>GTTACC</mark> AAC <mark>A</mark> GG	T <mark>A</mark> ATGCTGG	GCACTCTAAG	GAAACTGCCA	GTG <mark>A</mark> TAA <mark>ACT</mark> G	GAGGA
Anaplasma phagocytophilum	ACCCTCATCCTT	A <mark>GTTGCCAGCG</mark> GG	TTAAGCCGG	G <mark>C</mark> ACT <mark>T</mark> TAAG	GAAACTGCCA	STG <mark>G</mark> TAA <mark>A</mark> C <mark>T</mark> G	GAGGA
	150	160	170	180	190	200	210
wAnga Mali	AGGTGGGGATGA	TGTCAAGTCATCA		rggagtgggg			GCTAC
wAnga VK5 STP	AGGTGGGGA <mark>T</mark> GA	TGTCAAGTCA <mark>T</mark> CA	GGCCCTTA	TGG <mark>AG</mark> TGGGC	TACACACGTG	CTACAATGG <mark>TG</mark>	GCTAC
wMel	AGGTGGGGATGA	TGTCAAGTCA <mark>T</mark> CA	GGCCCTTA	<mark>FG</mark> G <mark>AG</mark> TGGGC	TACACACGTG	CTACAATGG <mark>TG</mark>	GCTAC
wRi	AGGTGGGGATGA	TGTCAAGTCA <mark>T</mark> CA	TGGCCCTTA	TGG <mark>AG</mark> TGGGC	TACACACGTG	CTACAATGG <mark>TG</mark>	GCTAC
wAnga VK5_3.1a	AGGTGGGGATGA	I GTCAAGTCAICA	GGCCTTTA	GGAGTGGGC	TACACACGTG		TCTAC
WNO	AGGIGGGGAIGA						TCTAC
Rickettsia iaponica	AGGTGGGGGACGA	GTCAAGTCATCA	TGGCCCTTA	GGGTTGGGC	TACACACGCGTG		TTTAC
Ehrlichia chaffeensis	AGGTGGGGGATGA	TGTCAAGTCAGCA	GGCCCTTA	TAGGGTGGGC	TACACACGTG	TACAATGGCA	ACTAC
Anaplasma phagocytophilum	AGGTGGGGATGA	T GTCAAGTCA <mark>G</mark> CA	CGGCCCTTA	IGGGG TGGGC	TACACACGTG	TACAATGGTG	ACTAC
	220	230	240	250	260	270	280
	257	266	276	285	295	305	315
wAnga Mali	A <mark>ATG</mark> GG <mark>CT</mark> GC <mark>A</mark> A	AGT-CGC <mark>AAGGCT</mark>	GAGCTAATCO	C <mark>T-</mark> TAAAAG <mark>C</mark>	CATCTCAGTT	CGGATTGTACT	CTGCA
wAnga VK5_STP	AATGGGCTGCAA	AGT-CGCGAGGCT	AAGCTAATCO	CT TAAAAG <mark>C</mark>	CATCTCAGTT	LGGATTGT <mark>A</mark> CT	CTGCA
wMel	AATGGGCTGCAA	AGT-CGCGAGGCT	AAGCTAATCO		CATCTCAGTT		CTGCA
	AATGGGCTGCAA		AAGCTAATCO				CTGCA
wNo	AATGGGTTGCAA	GTGCGCAAGCCT	AAGCTAATCO		CATCTCAGTT	GGATTGTACT	CTGCA
wPip	AATGGGCTGCAA	GGTGCGCAAGCCT	AAGCTAATCO	C-TAAAAGA	CATCTCAGTT	CGGATTGTACT	CTGCA
Rickettsia japonica	A <mark>GAG</mark> GG <mark>AA</mark> GC <mark>A</mark> A	GAC-GGC <mark>GACGT</mark> G	GAGCAAATCO	C-TAAAAG <mark>A</mark>	CATCTCAGTT	CGGATTGTTCT	CTGCA
Ehrlichia chaffeensis	A <mark>ATA</mark> GG <mark>TC</mark> GC <mark>G</mark> A	GAT-CGC <mark>A</mark> AGATT	TAGCTAATCO	A-TAAAAG	TGTCTCAGTT	LGGATTGT <mark>T</mark> CT	CTGCA
Anaplasma phagocytophilum	AATAGGTTGCAA	IGT-CGCAAGGCT	GAGCTAATCO	G-TAAAAG	CATCTCAGTTO	GGATTGTCCT	CTGCA
	290	300	310	320	330	340	349
wAnga Mali	ACTCGAG <mark>TG</mark> CAT	GAAGT <mark>T</mark> GGAATCG	CTAGTAATCO	GTGGATCAGC		GAATACGTTCT	CGGG
wAnga VK5_STP	ACTCGAG <mark>TG</mark> CAT	GAAGTTGGAATCG	CTAGTAATCO	GTGGATCAGC		JAATACG TTCT	CGGG
wMeI	ACTCGAG <mark>TG</mark> CAT	GAAGT <mark>T</mark> GGAATCG	GCTAGTAATCO	G <mark>T</mark> GGATCA <mark>G</mark> C	A <mark>C</mark> GCC <mark>A</mark> CGGT	GAATACG <mark>T</mark> TCT	CGGG
WRI	ACTCGAG <mark>TG</mark> CAT	GAAGT	CTAGTAATCO	GTGGATCAGC	ACGCCACGGT	JAATACGTTCT	CGGG
wAnga VK5_3.1a	ACTCGAGTACAT					JAATACG-TCT	CGGG
wNO	ACTEGAGTACAT	GAAGT	CTAGTAATCO			SAATACGTTCT	CGGG
Rickettsia japonica	ACTCGAGAGCAT	GAAGT	CTAGTAATCO	GGGATCAC	ATGCCCCGCGGT	GAATACGTTCT	CGGG
Ehrlichia chaffeensis	ACTCGAGAGCAT	GAAGTCGGAATCG	CTAGTAATCO	GTGGATCATC	ATGCCACGGT	GAATACG TTCT	CGGG
Anaplasma phagocytophilum	ACTCGAG <mark>GG</mark> CAT	GAAGTCGGAATCG	CTAGTAATCO	GTGGATCAGC	ATGCCACGGT	JAATACG TTCT	CGGG

Fig. S2. Alignment of a conserved region of the 16S rRNA gene using *Wolbachia*-specific primers (W16S-Spec). Asterisks denotes the nucleotide residues that encode an Rsal restriction site, a signature feature of *Wolbachia* supergroup B.

	1	10	20	30	40	50	60	
hcpA wAnga Mali	KQGLP-	DPELNPRL	RSAIFAAR	ENLPKDKID	AIKNAAGN	AGENYDEIQ	/ E G H G P S G T A L T	VΗ
hcpA Wolbachia	KQGLP	DPELNPRL	RSAIFAAR	KENLPKDKID	AIKNAAGN	AGENYEEIQY	ΥΕGΥGΡSGTTLΓ	VΗ
hcpA Anaplasma phagocytophilum	R S G S P -	VPELNPNL	RSALASAK	A F NLPKDRIE A	AIRSAQGNE	ADDSYEEITY	Y E G Y G	VН
hcpA Rickettsia japonica	KTGSSN	NPENNPRL	RNALTAAR	SQNLPKERID	AINSA ND S S	SNTENYTEIRY	EGY ASNGIAII	VE
hcpA Ehrlichia chaffeensis	KQGLP-	DPEFNSRL	RSALAAK	KENLPKDRID	AIKSATGN	QTDNYEEVV	/ E G Y G P G N I A L M	Q
	70		80	90	100	110	120	130
hcpA wAnga Mali	ALTNNF	RNRTASEVR	FIFSRRGG	ΙΔΕΤGSVNYL	FDHVGLIVY	K T E G I – N F D I	DLFDYGAELEVL	ΝV
hcpA Wolbachia	ALTNNF	<u>R N R T A S E V R</u>	YIFSRKGG	<u> </u>	FDHVGLIV	K T E GM – N F DI	DLFNYGIELEVL	ΝV
hcpA Anaplasma phagocytophilum	ALSNNF	RNRTAGELR	HIFTRHGG	K <u>L G E R G S I S Y I</u>	<u>_ F D H V G L I V </u>	GAAQVG SFD \	/ IFDEATSLGAL	DL
hcpA Rickettsia japonica	ALTDNK	<u>NRTAAEVR</u>	SSFTKYGG	S L G E T G S V N Y I	<u>FNHCGVIQ</u>	PI-NLASNE		DΙ
hcpA Ehrlichia chaffeensis	TLTNNF	RNRTAAELR	HALSKYNG	<	FNHVGVIAY	<pre>/KASSIDSFDS</pre>	SLENTALELHAL	DV
		140	149					
hcpA wAnga Mali	E E	NSSEGLYI	ITCEV					
hcpA Wolbachia	<u>EE</u>	NGSEGLYI	ITCEV					
hcpA Anaplasma phagocytophilum	EEHD	NGEEKEYH	VTCQV					
hcpA Rickettsia japonica	<u> S</u> D	DTTHTIY-	$ \top D$					
hcpA Ehrlichia chaffeensis	EEIIQ	DTQEKIYY	VICNV					

Fig. S3. Sequence alignment of the deduced amino acid sequence of the region of the hcpA gene from Wolbachia and other non-Wolbachia preteobacterium. These sequences are used in the MLST scheme, a universal genotyping tool for Wolbachia.

NAS PNAS

fbpA wAnga Mali fbpA Wolbachia fbpA Anaplasma phagocytophilum fbpA Ehrlichia chaffeensis

fbpA wAnga Mali fbpA Wolbachia fbpA Anaplasma phagocytophilum fbpA Ehrlichia chaffeensis

fbpA wAnga Mali fbpA Wolbachia fbpA Anaplasma phagocytophilum fbpA Ehrlichia chaffeensis

KLNSSNSLHÅKDLTSDQALTASVKDALRLGCAAVGFTYPGSAKCEDMME BARETHAFAKSIVGL KLNSSNSLHSKSLTSDQAVTASVKDALRLGCAAVGFTYPGSAKCEDMTE BARKTAAFAKSIVGT KLNSSNSLHSKSLTSDQAVTASVKDALRLGCAAVGFTYPGSAKCEDMTE BARKTAAFAKSIVGT KLNSSNSLHSKSTPTOVITASVKDALRLGCVALGLTYPGSESFBSMVREVKELTHFAKMCGL KLNSSTLLSPKNSFPDQVVTSSVKDALRLGCSALGITYPGSESFBSMVREVKELTHFAKMCGL KLNSSTLLSPKNSFPDQVVTSSVKDALRLGCSALGITYPGSESFBSMVREVKELTHFAKMCGL AVVLWSYPRGEGISKEGETAVDVTAYAAHTAALLGANTIKVKLPACHLEKEKTEFAENINSLSKR AVVVWSYPRGEGISKEGETAVDVTAYAAHTAALLGANTIKVKLPACHLEKEKTEFAENINSLSKR AVVVWSYPRGEGISKEGETAVDVTAYAAHTAALLGANTIKVKLPTSNIERDST-PYDITSLKQR 100 100 100 120 AVVLWSYPRGEGISKEGETAVDVTAYAAHTAALLGANTIKVKLPTSNIERDST-PYDITSLKQR 120 140 143 14YLKKSCFAGKRIV UAYVKKSCCFAGKRIV

Fig. S4. Sequence alignment of the deduced amino acid sequence of the region of the *fbpA* gene from *Wolbachia* and other non-*Wolbachia* preteobacterium. These sequences are used in the MLST scheme, a universal genotyping tool for *Wolbachia*.

ĠMSLTKMPLĖVWSVLLTAEMLIVALPVLAĞAITMLLTDRNIGTTEEDPAĞGGDPVLEQHLEWEE GMSLMKMPLEVWSVLLTAEMLIVALPVLAGAITMLLTDRNIGTAEEDPAGGGDDVLEQHLEWEE GMGLFKMPLEVWSILVTAELILAMPVLGGAITMLLTDRNEGTTEEKPDGGGDPVLEQHLEWEE GMTLMKMPLEVWTILLTSEMLIVSIDVLGGAITMLLTDRNEGTGEENPAGGGDPILEQHLEWEE GMTLLKMPLEVWTILLTSEMLIVTIPVLGGAVTMLLTDRNEGTSEEDPAGGGDPLLEQHLEWEE CoxA wAnga Mali CoxA Wolbachia CoxA Rickettsia japonica extraction CoxA Anaplasma phagocytophilum CoxA Ehrlichia chaffeensis GHP EVYVILEPAFGIISQVVSTFSNRPVFGHTGMIYAMIGIAVFGFMVWAHHMFTVGLGEDAAI GHPEVYVILFPAFGIISQVVSTFSNRPVFGYTGMVYAMIGIAAFGFMVWAHHMFTVGLSADAAI GHPEVYVIFPAFGIVSQVISTFSRRPVFGYTGMVYAMIGIAAFGFMVWAHHMFTVGLSADAAI GHPEVYVIFPAFGIVSQVISTFSRKPVFGYLGMVFALVGIAVGAVVWAHHMFTVGLSALVT GHPEVYIFFPAFGISQVISTFSHKAVFGYLGMVFALVGIAAVGAVVWAHHMFTVGLSALIT GHPEVYIFFPAFGISQVISTFSHKAVFGYLGMVLALVGIAAVGAVVWAHHMFTVGLSALIMT CoxA wAnga Mali CoxA Wolbachia CoxA Rickettsia japonica extraction CoxA Anaplasma phagocytophilum CoxA Ehrlichia chaffeensis TMLIGVÍTGVKVF CoxA wAnga Mali FFSTTTIFIGVITGVKVFR CoxA Wolbachia A G TM L L A V P T G L K L F S V T TM L L G V L T G V K V F S CoxA Rickettsia japonica extraction Т CoxA Anaplasma phagocytophilum CoxA Ehrlichia chaffeensis <u>v v s</u> YFSVTTMLIGVLTGVKVFS

Fig. S5. Sequence alignment of the deduced amino acid sequence of the region of the coxA gene from Wolbachia and other non-Wolbachia preteobacterium. These sequences are used in the MLST scheme, a universal genotyping tool for Wolbachia.



Fig. S6. Effect of naturally occurring *Wolbachia* infection on *Plasmodium* sporozoite infection in field-collected *A. coluzzii*. *Wolbachia* and *Plasmodium* levels in field-collected *A. coluzzii* were determined by qRT-PCR. (*A*) Correlation between *Wolbachia* and *Plasmodium* levels. The relative abundance of coinfected mosquitoes (W^+Pf^+) is indicated. (*B*) Levels of *Plasmodium* sporozoite infection in *Wolbachia*-infected and uninfected females. Each data point represents the level of *Plasmodium* infection in a single mosquito, and medians are indicated by the line. Sample value distributions were compared using the Mann–Whitney test. Pie charts represent the prevalence of *Plasmodium* sporozoite infection in *Wolbachia*-infected and uninfected samples. The prevalence was compared using the χ^2 test (n = number of samples). **P < 0.01.

Table S1.	Number of mosqu	itoes collected in 2	010 from six Malia	an villages and t	he number of
samples po	ositive for anti-CSP	ELISAs from head	-thorax mosquito	homogenates	

Location	Plasmodium +	Total	Plasmodium prevalence
Kenieroba	46	3,788	1.21
Fourda	5	851	0.58
Dangassa	89	3,280	2.71
Dangassa-somonosso	6	475	1.26
Niaganabougou	51	4,812	1.06
Niaganabougou-somonosso	8	1,815	0.44

Table S2. Primer sequences used for PCR amplifications

PNAS PNAS

Primer	Forward primer	Reverse primer
W16S-Spec	CATACCTATTCGAAGGGATAG	AGCTTCGAGTGAAACCAATTC
W16S-Nested	GAAGGGATAGGGTCGGTTCG	CAATTCCCATGGCGTGACG
W16S-WE	CAGACGGGTGAGTAATGYATAG	TATCACTGGCAGTTTCCTTAAAG
W16S-qPCR	CATACCTATTCGAAGGGATAG	TTGCGGGACTTAACCCAACA
gatB	GAKTTAAAYCGYGCAGGBGTT	TGGYAAYTCRGGYAAAGATGA
coxA	TTGGRGCRATYAACTTTATAG	CTAAAGACTTTKACRCCAGT
hcpA	GAAATARCAGTTGCTGCAAA	GAAAGTYRAGCAAGYTCTG
ftsZ	ATYATGGARCATATAAARGATAG	TCRAGYAATGGATTRGATAT
fbpA	GCTGCTCCRCTTGGYWTGAT	CCRCCAGARAAAAYYACTATTC
57	AGAACCAGCAGACCACCATC	GCTGCAAACTTCGGCTATTC
P285	GTGGCCTATCGATCCTTTA	GCGTCCCAATGATAGGAAGA

Table S3.	wAnga-Mali sequence homology to specific regions of the coxA, hcpA, and	nd
fbpA gene	IS I I I I I I I I I I I I I I I I I I	

Gene	Wolbachias, %	A. phagocytophilum, %	R. japonica, %	E. chaffeensis, %
hcpA	91	55	46	56
fbpA	93	59	_	65
coxA	93	73	70	74

These sequences are used to classify Wolbachia strains using the multilocus sequence typing (MLST) scheme, a universal genotyping tool for *Wolbachia* (*coxA*, cytochrome *c* oxidase, subunit I; *fbpA*, fructose-bisphosphate aldolase; *hcpA*, conserved hypothetical protein;). Different *Wolbachia* strains that infect other hosts have the highest nucleotide sequenced homology to these genes in *wAnga-Mali* [*Wolbachia* from *Megalothorax incertus* for *hcpA* (91%); *Brugia pahangi* for *fbpA* (93%); and *Thalassaphorura houtanensis* for *coxA* (93%)]. The sequence homology to other Rickettsiales is lower and is indicated in the table. Sequence comparisons were done at the amino acid levels (blastx) using BLAST/NCBI/NIH.