Supplemental Material

Characterization of a newly discovered symbiont in the whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae)

Running title: Novel *Orientia* like symbiont in *Bemisia tabaci*

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Section: Invertebrate microbiology

Appendix 1

FISH protocol

Specimens were collected directly into Carnoy's fixative (ethanol: chloroform: glacial acetic acid, 6:3:1) and fixed overnight. After fixation, the samples were decolorized in 6% H_2O_2 in ethanol for 2 h and then hybridized overnight in hybridization buffer (20 mM Tris-HCl (pH 8.0), 0.9 M NaCl, 0.01% sodium dodecyl sulfate, 30% deionized formamide) containing 10 pmol of fluorescent probes. Stained samples were viewed under a Leica TC Sp5 confocal microscope.

Appendix 2

Q-PCR protocol

Quantitative PCRs were finished mainly by the SYBR® Premix Ex Taq™ II and Bio-Rad CFX96™ Real-Time System. Each of the PCR mixtures consisted of 10.0 μl of 2×SYBR® Premix Ex Taq™ II buffer (Takara, Dalian, China), 0.8 μl forward and reverse primer solution (10 μM each), 2.0 μl of DNA sample solution and 6.4 μl of double distilled water. The PCR temperature profile was 2 min at 95 °C, 40 cycles of 5 s at 95 °C and 34 s at 60°C. Standard curves for each of the genes were drawn using standard plasmid samples at concentrations of 10^2, 10^3, 10^4, 10^5, 10^6, 10^7 and 10^8 gene copies per μl of the target gene.

Fig. S1

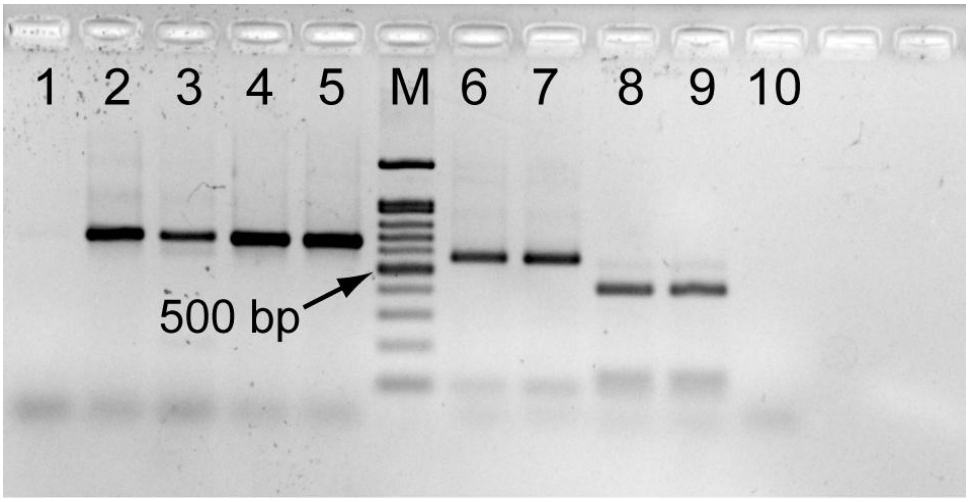

Fig. S1 RFLP pattern of PCR products of 16S rRNA gene of the OLO and *Rickettsia* in *B. tabaci* corresponding to *Hph*I digestion. The different profiles were obtained from two individuals representing each of the OLO and *Rickettsia* in *B. tabaci*. The bands shown on the lower left are primer dimers. Lane 1,10, no template controls; lane 2-3, undigested OLO; lane 4-5, undigested *Rickettsia*; lane 6-7, digested OLO, resulting in fragments of 566 bp and 101 bp, respectively; lane 8-9, digested *Rickettsia*, resulting in fragments of 411 bp, 128 bp, 99 bp and 27 bp (although the three lower bp bands are blurred), respectively; lane M, DNA size markers (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0 and 1.5 kb from bottom to top).

Fig. S2

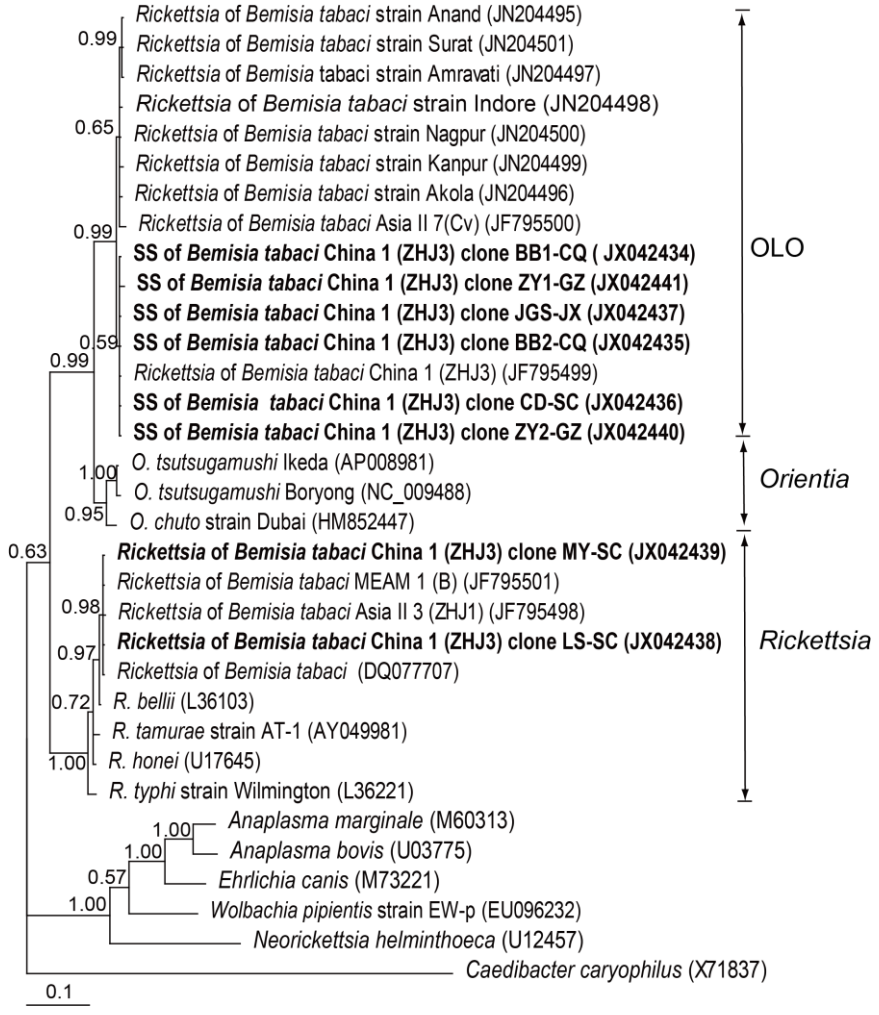

Fig. S2 Phylogenetic analysis of the OLO identified from different *Bemisia tabaci* populations based on bacterial 16S rRNA gene sequences (644 sites). The tree was constructed using a TPM3 + G substitution model for Bayesian analysis. Bayesian posterior probabilities (>0.50) are shown at the nodes. The names and sequence accession numbers are shown in parentheses. Sequences obtained in this study are shown in bold.

Fig. S3

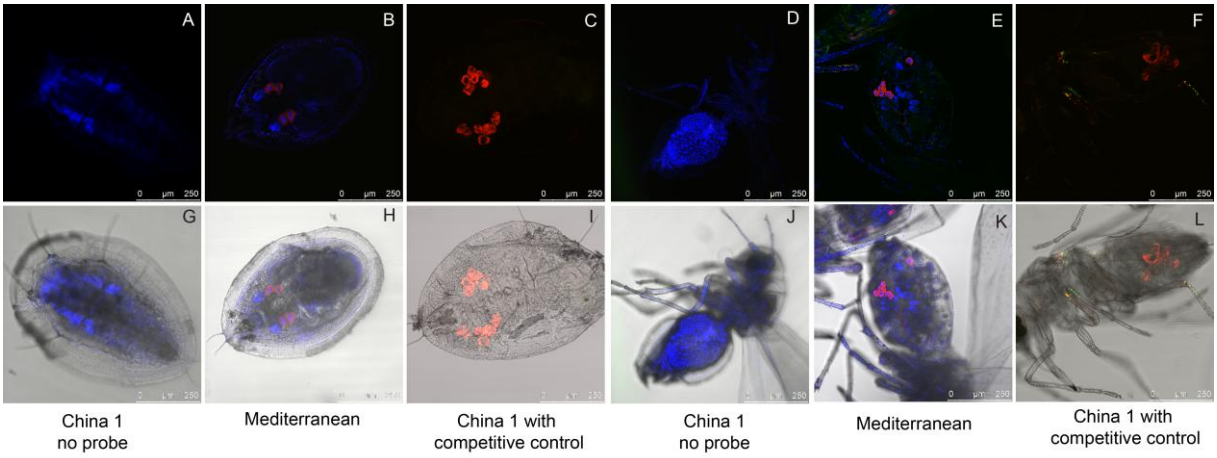

Fig. S3 FISH controls. China 1: no probe control; Mediterranean: OLO-free control; China 1 with competitive control. A-F: Overlay of channels of DAPI (blue), "*Ca.* Portiera aleyrodidarum" (red) and OLO (green); G-L: Overlay of channels of DAPI, "*Ca.* Portiera aleyrodidarum", OLO and white light. Competitive controls lack DAPI for the break of DAPI detector. Signals on legs and wings are chitin autofluoresence.

Table S1 Statistics of three-factor ANOVA of effects of development time, host sex and symbionts on symbiont density

Source of variation	Bacterial densities in terms of 16S rRNA gene copies per insect			Bacterial densities in terms of 16S rRNA gene copies per β–actin gene copy		
	df	*F*	*P*	df	*F*	*P*
Time	2	0.716	0.493	2	2.402	0.099
Sex	1	33.179	<0.001	1	24.151	<0.001
Symbiont	1	0.274	0.602	1	0.960	0.331
Time × sex	2	0.214	0.808	2	1.193	0.311
Time × symbiont	2	2.255	0.114	2	1.742	0.184
Sex × symbiont	1	0.037	0.848	1	0.338	0.563
Time × sex × symbiont	2	1.533	0.224	2	1.085	0.345
Error	58			58		
Total	69			69		

75 **Table S2** AT contents of 16S rRNA gene of OLO, the primary and secondary symbiont of various insects (*B. tabaci* included), and
76 free-living bacteria representing the Alpha-subclass of the *Proteobacteria*.

Symbiont	Host insect	AT content (%)	GenBank accession no.
OLO of *Bemisia tabaci* China 1	*Bemisia tabaci* (whitefly)	48.5	JX042442
Primary symbiont of various insects			
Portiera aleyrodidarum	*Bemisia tabaci* (whitefly)	52.3	JN204485
Buchnera aphidicola	*Baizongia pistaciae* (aphid)	51.8	NC_004545
Wigglesworthia glossinidia	*Glossina brevipalpis* (tsetse fly)	51.3	NC_004344
Secondary symbiont of various insects			
Arsenophonus symbiont	*Bemisia tabaci* (whitefly)	45.8	JN204476
Arsenophonus symbiont	*Stomaphis quercus* (aphid)	46.2	FJ655543
Cardinium symbiont	*Bemisia tabaci* (whitefly)	50.8	JN204480
Cardinium symbiont	*Aspidiotus nerii* (buckler scale)	52.2	GQ455437
Hamiltonella defensa	*Acyrthosiphon pisum* (aphid)	46.2	AY907546
Regiella insecticola	*Sitobion avenae* (aphid)	46.3	FJ357498
Rickettsia symbiont	*Bemisia tabaci* (whitefly)	49.5	DQ077707
Rickettsia symbiont	*Curculio hilgendorfi* (weevil)	48.7	AB604668
Serratia symbiotica	*Cinara tujafilina* (aphid)	45.5	EU348323
Wolbachia symbiont	*Bemisia tabaci* (whitefly)	53.0	JN204502
Wolbachia pipientis	*Drosophila sp*. (fruit fly)	52.2	EU096232
Free living bacteria			
Acetobacter aceti		44.6	D30768
Agrobacterium tumefaciens		45.2	D01256
Caulobacter crescentus		44.4	AJ227756
Rhodospirillum rubrum		43.2	D30778

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