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Characterization of a Newly Discovered Symbiont of the Whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae)

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***Bemisia tabaci* (Hemiptera: Aleyrodidae) is a species complex containing >28 cryptic species, some of which are important crop pests worldwide. Like many other sap-sucking insects, whiteflies harbor an obligatory symbiont, "*Candidatus* Portiera aleyrodidarum," and a number of secondary symbionts. So far, six genera of secondary symbionts have been identified in *B. tabaci*. In this study, we report and describe the finding of an additional bacterium in the indigenous *B. tabaci* cryptic species China 1 (formerly known as *B. tabaci* biotype ZHJ3). Phylogenetic analysis based on the 16S rRNA and *gltA* genes showed that the bacterium belongs to the *Alphaproteobacteria* subdivision of the *Proteobacteria* and has a close relationship with human pathogens of the genus *Orientia*. Consequently, we temporarily named it *Orientia*-like organism (OLO). OLO was found in six of eight wild populations of *B. tabaci* China 1, with the infection rate ranging from 46.2% to 76.8%. Fluorescence *in situ* hybridization (FISH) of *B. tabaci* China 1 in nymphs and adults revealed that OLOs are confined to the bacteriome and co-occur with "*Ca.* Portiera aleyrodidarum." The vertical transmission of OLO was demonstrated by detection of OLO at the anterior pole end of the oocytes through FISH. Quantitative PCR analysis of population dynamics suggested a complex interaction between "*Ca.* Portiera aleyrodidarum" and OLO. Based on these results, we propose "*Candidatus* Hemipteriphilus asiaticus" for the classification of this symbiont from *B. tabaci*.**

Symbiotic bacteria (symbionts) are ubiquitous in animal hosts and particularly prevalent in arthropods. Among these bacteria, some coevolve with their hosts and are strictly vertically transmitted. These bacteria provide essential nutrients to supplement the asymmetric diet of hosts and are thus called primary symbionts (1). Other symbionts are not necessary for all hosts but may have important effects on host biology and are thus called secondary or facultative symbionts (2). These secondary symbionts may confer resistance to parasitoid wasps, pathogens, and heat stress (2, 3) or manipulate hosts' reproduction (4).

The whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodoidea) is a cryptic species complex containing at least 28 morphologically indistinguishable putative species (referred to here as "species") (5–7), some of which have caused considerable losses to agricultural crops by sucking plant phloem sap and transmitting plant viruses (8). Whiteflies, like other phloem-feeding hemipterans, require bacteria for balancing their diet. "*Candidatus* Portiera aleyrodidarum" (*Oceanospirillales*), the primary symbiont of *B. tabaci*, was recently reported to provide its whitefly host with essential amino acids as well as carotenoids (9). That symbiont is localized in the bacteriome, which comprises a number of bacteriocytes. "*Ca.* Portiera aleyrodidarum" can be vertically transmitted through bacteriocytes during reproduction (10). In addition to "*Ca.* Portiera aleyrodidarum," six genera of secondary symbionts have so far been found in *B. tabaci*, including "*Candidatus* Cardinium hertigii" (*Bacteroidales*) (11), "*Candidatus* Fritschea bemisiae" (*Chlamydiales*) (12), "*Candidatus* Hamiltonella defensa" (*Enterobacteriales*) (13), *Arsenophonus* spp. (*Enterobacteriales*) (13), *Wolbachia* spp. (*Rickettsiales*) (14), and *Rickettsia* spp. (*Rickettsiales*) (10).

In a previous study, we detected a novel *Orientia*-like organism (OLO) in laboratory specimens of *B. tabaci* (15). In this study, we investigated the natural distribution, taxonomic position, *in vivo* localization, and population dynamics of this novel OLO in the *B. tabaci* species China 1 (formerly known as *B. tabaci* biotype ZHJ3), and we describe our findings here. Based on the data, we propose the provisional name "*Candidatus* Hemipteriphilus asiaticus" for this secondary symbiont newly discovered from *B. tabaci*.

MATERIALS AND METHODS

Whitefly rearing and collection. *Bemisia tabaci* China 1 (mitochondrial cytochrome oxidase 1 [*mtCOI*] gene; GenBank accession no. GQ303180) was obtained from Hangzhou, Zhejiang, China, in November 2009 and maintained on cotton [*Gossypium hirsutum* (Malvaceae) cv. Zhe-Mian 1793] in climate chambers at 27 ± 1°C with 14 h of light and 10 h of dark and 70% ± 10% relative humidity (16). Other than the primary symbiont "*Ca.* Portiera aleyrodidarum," *Wolbachia* and OLO were detected in this population (15). All analyses of OLO were done with this laboratory population. Samples of wild *B. tabaci* China 1 were collected from different localities covering 5 provinces of China from June to October 2009 (Table 1). Whiteflies used for molecular analysis were initially immersed in 95% ethanol and subsequently kept at −20°C until DNA extraction.

DNA extraction, cloning, and sequencing. For all samples, total DNA was extracted from individual adult specimens following the method of Frohlich et al. (17). The bacterial 16S rRNA gene was amplified using the universal primers 27F and 1494R (18). The bacterial citrate synthase-encoding gene (*gltA*) was amplified using the primers *glt*AF3 (5′-ACATG CAGACCATGAGCAGA-3′) and *glt*AR11 (5′-CATTTCATTCCATTGT GCCATC-3′) (19). The *groEL* gene was amplified using the newly

Received 1 October 2012 Accepted 31 October 2012
Published ahead of print 9 November 2012
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Supplemental material for this article may be found at http://dx.doi.org/10.1128/AEM.03030-12.

doi:10.1128/AEM.03030-12

TABLE 1 Detection of *Rickettsia* and OLO in Chinese *B. tabaci* China 1 populations

Whitefly population	Locality in China	Latitude	Longitude	Host plant	Collection date	Whiteflies tested			GenBank accession no. of bacterium 16S rRNA	GenBank accession no. of whitefly *mtCOI* gene[a]
						Total no.	% with OLO	% with *Rickettsia*		
JGS-JX	Jinggangshan, Jiangxi	26°74′N	114°30′E	*Ipomoea batatas* (sweet potato)	Oct. 2009	24	58.3		JX042437	HM137328
ZY1-GZ	Zunyi, Guizhou	27°39′N	107°70′E	*Ipomoea batatas* (sweet potato)	Jul. 2009	27	77.8		JX042441	HM137355
ZY2-GZ	Zunyi, Guizhou	27°39′N	107°70′E	*Solanum melongena* (eggplant)	Jul. 2009	30	76.7		JX042440	
BB1-CQ	Beibei, Chongqing	29°46′N	106°22′E	*Glycine max* (soybean)	Jul. 2009	27	74.1		JX042434	HM137341
BB2-CQ	Beibei, Chongqing	29°81′N	106°41′E	*Ipomoea batatas* (sweet potato)	Jul. 2009	27	55.6		JX042435	HM137350
CD-SC	Chengdu, Sichuan	30°44′N	103°54′E	*Glycine max* (soybean)	Jul. 2009	26	46.2		JX042436	HM137315
LS-SC	Leshan, Sichuan	29°36′N	103°45′E	*Glycine max* (soybean)	Jul. 2009	28		3.6	JX042438	HM137316
MY-SC	Mianyang, Sichuan	31°39′N	104°51′E	*Ipomoea batatas* (sweet potato)	Jul. 2009	12		25.0	JX042439	HM137318

[a] GenBank accession numbers of *mtCOI* genes were all obtained from reference 6.

designed primers OR-*groEL*-F (5′-CACCWAAAATTACTAAAGATGG-3′) and OR-*groEL*-R (5′-TAGAARTCCATWCCKCCCATWC-3′). All PCR analyses were conducted with *Taq* polymerase (TaKaRa, Dalian, China) in a PTC-200 thermocycler (Bio-Rad, CA). The cycling conditions were an initial denaturation at 94°C for 3 min, followed by 34 cycles of 94°C for 30 s, 52°C for 30 s, and 72°C for 2 min and a final extension of 72°C for 10 min. The PCR products were cloned into the pMD-18T plasmid vector (TaKaRa, Dalian, China) and sequenced in an ABI 3730 DNA analyzer.

Diagnostic PCR and RFLP analysis. To detect the presence of OLO in whitefly populations, primers OLO-F (5′-GCTCAGAACGAACGCTRKC-3′) and OLO-R (5′-TTCGCCACTGGTGTTCCTC-3′) were developed to amplify a product of about 670 bp from OLO's 16S rRNA gene. The PCR procedures were 94°C for 4 min, followed by 35 cycles of 94°C for 30 s, 60°C for 1 min, and 72°C for 2 min. An endonuclease, HphI, was found to digest the PCR products into different bands and distinguish OLO and *Rickettsia* of *B. tabaci*. The DNA cloning and sequencing methods described above were used to confirm the restriction fragment length polymorphism (RFLP) results. The quality of DNA samples was confirmed by PCR amplification of the partial mitochondrial cytochrome oxidase 1 (*mtCOI*) gene of the whitefly. Primers used to amplify the *mtCOI* gene fragment were C1-J-2195 and L2-N-3014 (17). All PCRs included sterile water as a negative control and a positive control.

FISH. We followed the method of Sakurai et al. (20) and Gottlieb et al. (10) for the fluorescence *in situ* hybridization (FISH) experiments, with slight modifications (for details, see Appendix S1 in the supplemental material). The probe BTP1-Cy3 (5′-Cy3-TGTCAGTGTCAGCCCAGAAG-3′) was used to target the 16S rRNA of the primary symbiont "*Ca.* Portiera aleyrodidarum" (10). On the basis of the sequence of OLO 16S rRNA, a probe, OLO-Cy5 (5′-Cy5-CTCACCCGTTTGCCACTAAT-3′), was designed using Primer3 software (http://fokker.wi.mit.edu/primer3/). The specificity of the detection was confirmed using the following controls: a no-probe control, an unlabeled competitive suppression control, and OLO-free whiteflies (samples of the *B. tabaci* Mediterranean species [5]).

Molecular phylogenetic analysis. DNA sequences were aligned using the Clustal W (version 1.6) program in MEGA (version 5.05) software (21). The alignments were then inspected and corrected manually. A pairwise distance matrix for aligned 16S rRNA genes was constructed by Kimura's two-parameter method (22). Phylogenetic trees were constructed with the Bayesian inference by the program MrBayes (version 3.1) (23). The best-fit substitution model for each aligned sequence was selected with the jModelTest (version 0.1.1) program using the Bayesian information criterion (24). The Bayesian trees were constructed with a TIM3+G model for the 16S rRNA gene, an HKY+G model for the *gltA* gene, and a GTR+G model for the *groEL* gene. For these gene data, 1 million generations were run; 10,000 trees were obtained, and the first 25% of these were discarded as burn-in.

Quantitative PCR. Bacterial density was quantified by the SYBR green and Bio-Rad CFX96 real-time system (for details, see Appendix S2 in the supplemental material). "*Ca.* Portiera aleyrodidarum" was quantified in terms of the number of 16S rRNA gene copies using primers Port73-F (5′-GTGGGGAATAACGTACGG-3′) and Port266-R (5′-CTCAGTCCCAGTGTGGCTG-3′) (25). OLO was quantified in terms of the number of 16S rRNA gene copies using newly designed primers OLO-16S-F (5′-TAGTGGCAAACGGGTGAGTA-3′) and OLO-16S-R (5′-GCTCATCCATCAGCGATAAA-3′). For normalization, the *B. tabaci* β-actin gene was also quantified as an internal standard using primers Actin-F (5′-TCTTCCAGCCATCCTTCTTG-3′) and Actin-R (5′-CGGTGATTTCCTTCTGCATT-3′) (26). For analysis of the densities of the two symbionts in female and male whiteflies at different times of development, a three-factor analysis of variance (ANOVA) was performed using Data Processing System (DPS) statistical software (27). Means were compared using a least-significant-difference (LSD) test ($P < 0.05$).

Nucleotide sequence accession numbers. The 16S rRNA gene, *gltA* gene, and *groEL* gene sequences obtained in this study were deposited in the GenBank database under accession numbers JX042442, JX042443, and JX042444, respectively. The GenBank accession numbers for the 16S rRNA gene sequences of OLO from field-collected whiteflies are JX042434 to JX042441.

RESULTS

Distribution of *Rickettsia* and OLO in *B. tabaci* species China 1. OLO was detected in six out of the eight China 1 field populations collected in south China in 2009, and 52.2% (105/201) of the adult whiteflies examined were positive (Table 1). OLO was found in whiteflies collected on three host plants (sweet potato, soybean, and eggplant) in five provinces. *Rickettsia* was detected in only 1.8% (4/201) of adult China 1 whiteflies, belonging to two whitefly populations collected on sweet potato and soybean in Sichuan (Table 1). Digestion of OLO 16S rRNA amplicons by HphI produced one bright band and one blurred band with sizes of 566 and 101 bp, respectively, a pattern that is consistent with that of the predicted RFLP for OLO (see Fig. S1 in the supplemental material). Digestion of the 16S rRNA amplicons of *Rickettsia* by HphI consistently produced four bands, the largest of which (411 bp)

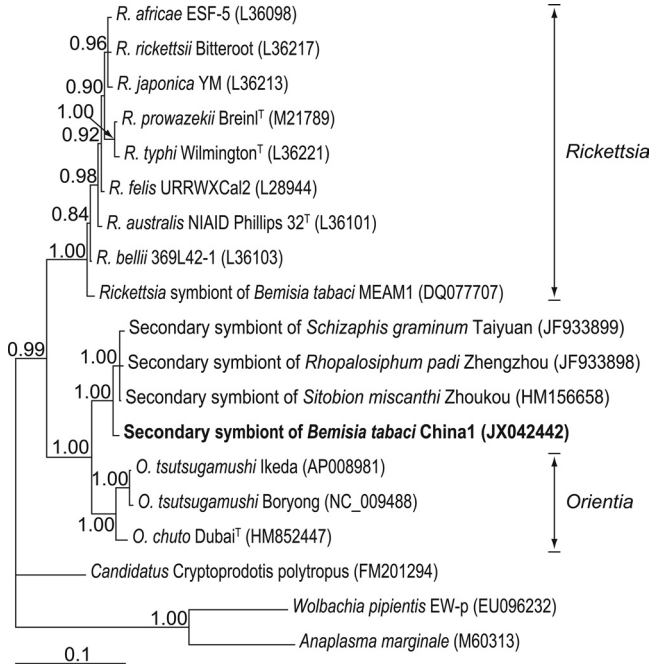

FIG 1 Phylogenetic position of the OLO identified from *Bemisia tabaci* putative species China 1 based on bacterial 16S rRNA gene sequences (1,063 sites). The tree was constructed using a TIM3+G substitution model for Bayesian analysis. Bayesian posterior probabilities (>0.50) are shown at the nodes. The names and sequence GenBank accession numbers (in parentheses) are shown. The sequence obtained in this study is shown in bold.

was bright, while the others (128 bp, 99 bp, and 27 bp) were blurred (see Fig. S1 in the supplemental material).

Phylogenetic analysis and taxonomic position of OLO. A 1,426-bp sequence of the OLO 16S rRNA gene was obtained using the combination of universal primers with the OLO primers OLO-F and OLO-R. This 16S rRNA sequence showed the highest sequence similarity (98.8%, on average) to sequences from the grain aphid *Sitobion miscanthi* L-type symbionts (SMLS) by a BLAST search of the sequences in GenBank. The next BLAST-hit sequence was from one causative agent of scrub typhus, *Orientia chuto* (GenBank accession no. HM852447), which had a sequence similarity of 95%. A 91.3% sequence similarity was obtained between the 16S rRNA genes of OLO and the *Rickettsia* symbiont of *B. tabaci* (GenBank accession no. DQ077707). The A+T content of the OLO 16S rRNA gene was 48.5%.

Phylogenetic analysis of the 16S rRNA sequence of OLO using Bayesian methods placed this bacterium near the *Orientia* spp. in the *Rickettsiales* order. Phylogenetic analyses consistently gave very strong support (1.00 support values) for a single clade corresponding to OLO in the family *Rickettsiaceae* together with symbionts of *Sitobion miscanthi*. This clade was distinct from *Rickettsia*, which is the known secondary symbiont nearest OLO in *B. tabaci* (Fig. 1; see Fig. S2 in the supplemental material). Additional phylogenetic analyses based on the *gltA* gene and *groEL* gene confirmed the results of the 16S rRNA gene analyses (Fig. 2).

***In situ* hybridization of OLO in *B. tabaci*.** Localization of "*Ca.* Portiera aleyrodidarum" and OLO was studied in oocytes, nymphs, and adults of *B. tabaci* using fluorescently modified specific probes targeting the 16 rRNA genes of these bacteria. "*Ca.* Portiera aleyrodidarum" was detected exclusively inside the bacteriocytes at all developmental stages (Fig. 3A to D). OLO was strictly located in the bacteriocytes among the abundant "*Ca.* Portiera aleyrodidarum" organisms and was never detected in any other host organs (Fig. 3A to D). The signals of the bacteriocytes in the female adult whitefly were relatively stronger than those in the male whitefly (Fig. 3C and D). The presence of OLO at the anterior pole of the oocytes indicated vertical transmission of these symbionts (Fig. 3A). The specificity of the detected signals was con-

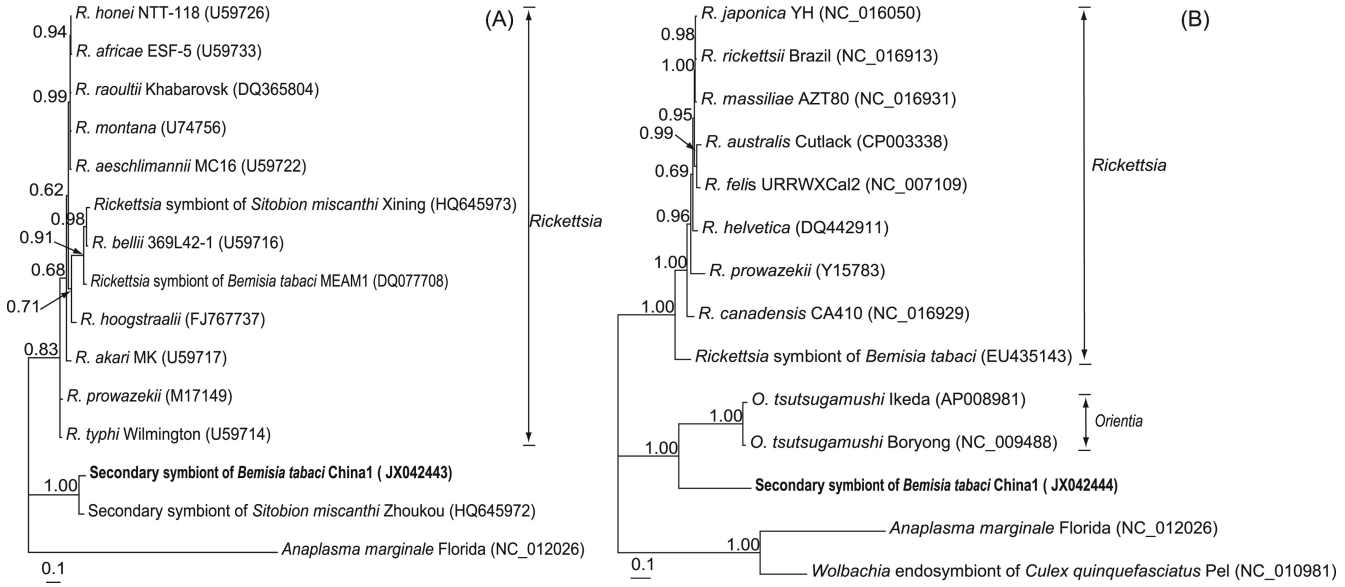

FIG 2 Phylogenetic position of the OLO identified from *Bemisia tabaci* putative species China 1. (A) A Bayesian tree based on bacterial *gltA* gene sequences (495 sites); (B) a Bayesian tree based on bacterial *groEL* gene sequences (1,519 sites). The Bayesian trees were constructed using an HKY+G substitution model for the *gltA* gene and a GTR+G substitution model for the *groEL* gene. Bayesian posterior probabilities (>0.50) are shown at the nodes. The names and sequence GenBank accession numbers (in parentheses) are shown. Sequences obtained in this study are shown in bold.

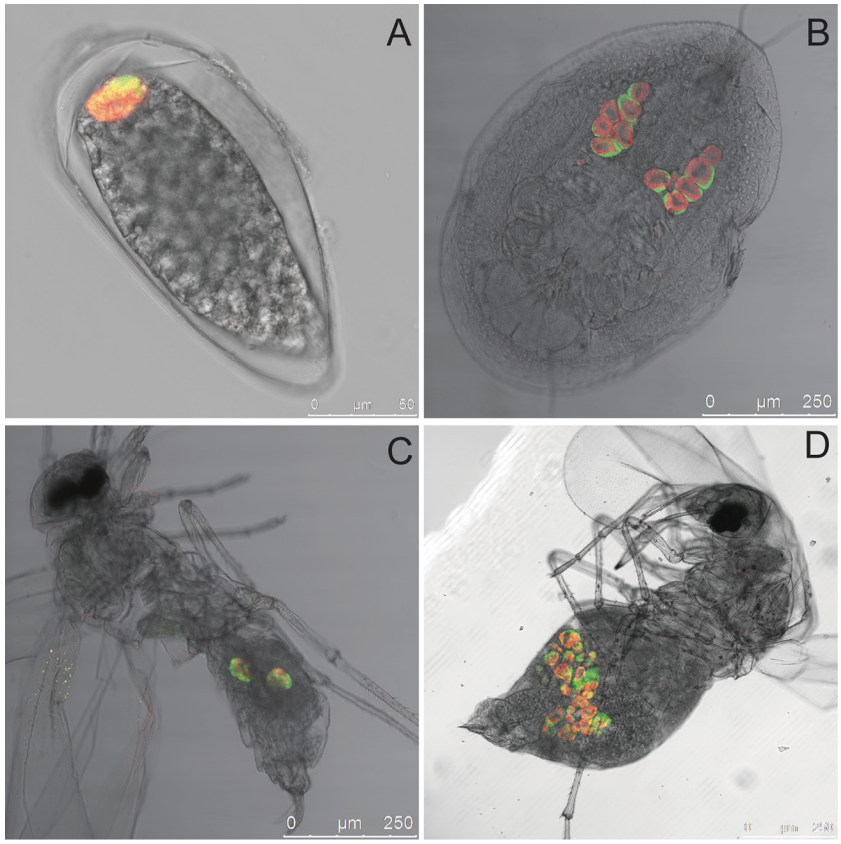

FIG 3 Whole-mount FISH of *B. tabaci* oocytes, nymphs, and adults using a "*Ca.* Portiera aleyrodidarum"-specific probe (red) and an OLO-specific probe (green). (A to D) Overlay of "*Ca.* Portiera aleyrodidarum" and OLO channels of oocytes, nymphs, male adults, and female adults, respectively, on a bright-field channel.

firmed by the no-probe control, competitive control, and OLO-free whitefly control (see Fig. S3 in the supplemental material).

Population dynamics of *"Ca.* Portiera aleyrodidarum" and OLO in whitefly. The data in Fig. 4A indicate that when expressed as the number of 16S rRNA gene copies per insect, the densities for both "*Ca.* Portiera aleyrodidarum" and OLO remained relatively constant during the immature stages of the hosts (i.e., <25 days) but then substantially increased in females as the hosts reached adulthood (25 days and after). The data in Fig. 4B indicate that when expressed as the number of 16S rRNA gene copies per β-

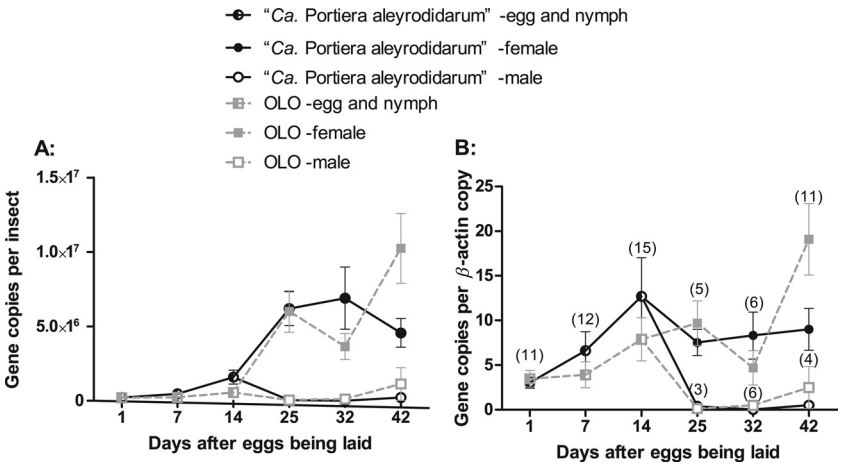

FIG 4 Infection dynamics of "*Ca.* Portiera aleyrodidarum" and OLO in the developmental course of the host whitefly. (A) "*Ca.* Portiera aleyrodidarum" and OLO titers in terms of the number of 16S rRNA gene copies per insect; (B) "*Ca.* Portiera aleyrodidarum" and OLO titers in terms of the number of 16S rRNA gene copies per β-actin gene copy. Whitefly sexes are indistinguishable before the adult phase (earlier than day 25 after hatching). Means and standard errors of the means are shown. Sample sizes are shown in parentheses above the bars.

actin gene copy, the densities for both "*Ca.* Portiera aleyrodidarum" and OLO did not vary greatly as the hosts developed. Because the sex of whiteflies is indistinguishable in the egg and nymph stages, only data for adult whiteflies were analyzed using a three-factor ANOVA. The results of ANOVA indicate that, of the three factors considered, host sex was the most influential, and there were complex two-factor and three-factor interactions (see Table S1 in the supplemental material). Although each of these multiple-factor interactions was not significant alone, their combined effects could affect the significance between the mean values when the means were subsequently compared by the LSD test.

The densities of both "*Ca.* Portiera aleyrodidarum" and OLO in female whiteflies were significantly higher than those in males when expressed as either the number of 16S rRNA gene copies per insect or the number of 16S rRNA gene copies per β-actin gene copy (Fig. 4; see Table S1 in the supplemental material). Of the various multiple-factor interactions, the time-symbiont interaction had the largest effects (see Table S1 in the supplemental material). The LSD tests indicate that while at days 25 and 32 there were no significant differences in density between the two bacteria, at day 42 the density of OLO was significantly higher than that of "*Ca.* Portiera aleyrodidarum" when expressed as either the number of 16S rRNA gene copies per insect ($P = 0.028$) or the number of 16S rRNA gene copies per β-actin gene ($P = 0.024$).

DISCUSSION

Recently, an *Orientia*-like organism (OLO) was detected by primers designed for *Rickettsia* 16S rRNA genes from two native species (Asia II 7 and China 1) of the whitefly *B. tabaci* species complex in China (15). The current study was initiated to further characterize that symbiont and determine its phylogenetic status. On the basis of the nearly complete 16S rRNA gene, partial *gltA* gene, and *groEL* gene, Bayesian phylogenetic analyses demonstrated that this OLO belongs to the *Rickettsiales* order within the *Alphaproteobacteria* subdivision of the *Proteobacteria* (Fig. 1 and 2). 16S rRNA genes from OLO and SMLS, which are highly similar (98.8%, on average), formed a distinct and robust monophyletic clade in the *Rickettsiaceae* family and as a sister group of *Rickettsia* and *Orientia*. The base composition of 16S rRNA genes from representatives of the *Alphaproteobacteria* and arthropod symbionts is shown in Table S2 in the supplemental material. Primary symbionts of various insects exhibit high A+T contents (over 51%), while free-living bacteria have much lower percentages of A+T contents (from 43.2% to 45.2%) (28). The A+T content of the OLO 16S rRNA gene (48.5%) places it in the group of secondary symbionts, whose A+T contents vary between those of primary symbionts and those of free-living bacteria.

We followed the guidelines of Tindall et al. (29) to characterize the phylogenetic affiliation of OLO and further clarify its taxonomic status. When identifying bacteria by 16S rRNA gene analysis, a <97% similarity of a sequence to all others is acceptable for classifying a microorganism to a new species, and a new genus name should be given when the similarity between bacterial isolates is ≤95% (29), though additional data are needed as complementary evidence. The 16S rRNA gene of OLO exhibits 94.98% sequence similarity with that of the previously formally described organism *O. chuto*, and thus, OLO may be considered a new genus.

In this study, OLO was detected in six out of the eight *B. tabaci* China 1 field populations collected in China. What seems to be the same bacterium has also been found from whiteflies in India. Recently, Singh et al. (30) reported that of the 14 whitefly populations belonging to the Asia I and Asia II genetic groups of *B. tabaci* from different locations in north India, OLO was present in 7. Apart from whiteflies, bacteria belonging to the same genus as OLO have so far been reported only in genera of the family Aphididae (19, 31).

The presence of OLO in oocytes, as demonstrated by whole-mount FISH, suggests vertical transovarial transmission of that bacterium (Fig. 3A). In addition, OLO was found to be strictly localized in the bacteriocytes together with "*Ca.* Portiera aleyrodidarum" at all *B. tabaci* developmental stages. Its distribution was similar to that of "*Ca.* Hamiltonella defensa" and *Arsenophonus* (32). This phenomenon is possibly related to the particular mechanism of intact migration of the bacteriocyte into the ovaries in the whitefly, through which OLO could escape host immune responses (33). Because OLO is strictly limited to the whitefly bacteriocytes and is unlikely to go into the hemolymph and stylet, OLO is probably not horizontally transmitted.

When the population dynamics and density of "*Ca.* Portiera aleyrodidarum" and OLO during host development were monitored using quantitative PCR, "*Ca.* Portiera aleyrodidarum" proliferation was found to be correlated with the reproductively active stage of the whitefly (Fig. 4A). This coincidence of the symbiont with its host is similar to that of the primary symbiont *Buchnera* with its aphid hosts and probably reflects the biological role of providing missing essential amino acids for the insect hosts (1, 20). The density of OLO exhibited a pattern of dynamics similar to that of "*Ca.* Portiera aleyrodidarum"; however, OLO's density outstripped that of "*Ca.* Portiera aleyrodidarum" in the late developmental stage of the adults (Fig. 4). Because both "*Ca.* Portiera aleyrodidarum" and OLO are confined in the same bacteriocytes (Fig. 3), interspecies interactions may be expected.

In this study, "*Ca.* Portiera aleyrodidarum" and OLO were detected in larger quantities in adult female whiteflies than in adult males. FISH analyses revealed that the number of bacteriocytes in the female whiteflies was greater than that in the males (Fig. 3C and D). The densities of both "*Ca.* Portiera aleyrodidarum" and OLO were higher in the females than in the males (Fig. 4; Table S1 in the supplemental material). This difference between female and male individuals could be explained, at least in part, by the biology of whiteflies. *Bemisia tabaci* has haplodiploid sex determination: fertilized (diploid) eggs develop into females, and unfertilized (haploid) eggs develop into males (34). Therefore, the higher density in female whiteflies may help the maintenance and transmission of symbionts.

After its first report from MEAM1 whiteflies in 2006, *Rickettsia* has so far been reported from three other *B. tabaci* species, MED, Asia II 3 (formerly known as ZHJ1), and Indian Ocean (formerly known as MS) (10, 15, 35, 36). In this study, although the sample size was small, we also found *Rickettsia* in wild populations of *B. tabaci* China 1 (Table 1) and thus added another species of the *B. tabaci* complex to the host range of *Rickettsia*.

Proposal of "*Candidatus* Hemipteriphilus asiaticus" gen. nov., sp. nov. Though the function of OLO is largely unknown, the data collected permit the assignment of OLO as a new genus. The arguments for this proposal include the facts that (i) the highest sequence similarity between OLO and its closely related bacteria is 94.98%, lower than the 95% cutoff value for separating different genera; (ii) the sequences of the OLO 16S rRNA gene

(though partial) from field-collected whiteflies showed nearly 100% similarity with each other, suggesting that OLO has its own well-defined identity; (iii) *Orientia* spp. are mainly found in *Leptotrombidium* spp. (mites) (37), whereas OLO and its relatives, SMLS (the *Sitobion miscanthi* L-type symbiont), are endosymbionts of Hemipteran insects; and (iv) *Orientia* spp. are harbored in the salivary glands of mites and are transmitted to humans during larval feeding (38), whereas OLO and SMLS have not been detected in the salivary glands of their host insects.

Based on its distinct phylogeny and biological traits, we propose to name the newly discovered bacterium associated with *B. tabaci* "*Candidatus* Hemipteriphilus asiaticus." The genus name *Hemipteriphilus* (He.mi.pte′ri.phi.lus, N.L. neut. plur. n.) is derived from the systematic name of the organism's host order, *Hemiptera*, N.L. masc. n., and *philus* (from Gr. philos, friendly to), meaning the friend of Hemiptera. The species name refers to the place, Asia, where this bacterium was found (a.si′a.ti.cus. L. masc. adj. *asiaticus*, Asiatic, belonging to Asia). "*Ca.* Hemipteriphilus asiaticus" gen. nov., sp. nov. belongs to the phylum *Proteobacteria* of bacteria, to the class *Alphaproteobacteria*, to the order *Rickettsiales*, and to the family *Rickettsiaceae* (39). "*Ca.* Hemipteriphilus asiaticus" is a Gram-negative and transovarialy transmitted intracellular symbiont of arthropods. The genus is assigned mainly on the basis of the 16S rRNA gene sequence (GenBank accession no. JX042442), the *gltA* gene sequence (GenBank accession no. JX042443), and the *groEL* gene sequence (GenBank accession no. JX042444).

The bacterium found in the population of the whitefly *B. tabaci* putative species China 1 from Hangzhou, Zhejiang Province, China, is proposed as the type strain. The G+C content of the 1,426-bp OLO 16S rRNA gene is 51.5%. The level of similarity based on the 16S rRNA gene sequence between "*Ca.* Hemipteriphilus asiaticus" and the aphid symbiont SMLS (98.8% on average) places them within the genus "*Candidatus* Hemipteriphilus." So far, "*Candidatus* Hemipteriphilus" has been detected only from Asian populations of Hemiptera (15, 19, 30, 31).

ACKNOWLEDGMENTS

This work was supported by the National Basic Research Program of China (2009CB119203) and the National Natural Science Foundation of China (31101437).

We thank Hans G. Trüper, Rheinische Friedrich-Wilhelms-Universität, Germany, for advice on naming the newly found bacterium and Qi-Yi Tang for help with the statistical analysis. We also thank Gen-Hong Yan, Hua Zhao, Jia Wang, and Jian Hu for assistance in collecting the whitefly samples; Fabrice Vavre, Helene Delatte, Jun-Min Li, Qiong Rao, Wen-Wu Zhou, and Yun-Lin Su for providing valuable suggestions; and Yun-Qin Li for technical assistance with confocal microscopy.

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