Multilocus Sequence Typing Analysis. To determine the supergroup of *Wolbachia* infecting *Di*, multilocus sequence typing (MLST) analysis was conducted (13). DNA was extracted from one male and one female as described in the analysis of 16S rRNA gene. Five ubiquitous genes (*gatB, coxA, hcpA, fsZ, and fbpA*) were amplified by using specific primers (13) under the following conditions: an initial cycle of 94 °C for 2 min; 37 cycles of 94 °C for 30 s, 54 °C (59 °C for *fsZ*) for 45 s, and 72 °C for 1 min 30 s; and a final cycle of 72 °C for 10 min. The 20-μL PCR mixture contained 10× ExTaq Buffer (TaKaRa Bio), 0.2 mM dNTP mixture, 0.5 μM each primer, 0.5 U of TaKaRa ExTaq polymerase, and 2.0 μL of DNA. PCR products were purified with NucleoSpin Gel and PCR Clean-up (Macherey-Nagel) and were sent to FASMAC (Atsugi, Japan) for sequencing. Obtained sequences were aligned by using CLUSTAL X (37). The phylogenetic tree was constructed by the maximum likelihood method for concatenated MLST sequences by using MEGA6 software (38); sequences from other *Wolbachia* representatives were included. The nucleotide substitution model GTR + G was selected by using jModelTest 2.1.10. Nodal support was evaluated with 1,000 bootstrap resamplings (40). The sequence data were deposited in the DDBJ/EMBL/GenBank database (accession nos. LC164018–LC164022).

Phylogenetic Tree Construction. The full-length 16S rRNA gene sequences of the OTUs that belonged to *Anaplasma* in Fig. 5 were aligned using jModelTest 2.1.10. The phylogenetic tree was constructed using neighbor-joining method with 100 bootstrap replications. *Wolbachia* supergroups B, D, and F were distributed within Cluster 1. Supergroups A and E were distributed within cluster 2. The major species, S004255337 (*Wolbachia* endosymbiont of *C. zealandica*) in male and female *B. longissima*, belonged to cluster 1 (Fig. S2).

Presence of the Previously Unidentified Alphaproteobacterial Endosymbiont in *B. longissima* from Different Locations. Presence of the bacterial endosymbiont was tested as described in the methods section under “Confirmation of the elimination of bacterial endosymbionts by the antibiotic treatment” for the specimens from the following locations: Australia, Papua New Guinea, Indonesia (Sumba), and East Timor. Details of the specimens have been described previously (12). A total of 29 specimens that belong to the Pacific clade and two specimens that belong to the Asian clade were analyzed. DNA extracted from the thorax muscle was used (12) and a single band was detected in all specimens belonging to the Pacific clade (Fig. S3), indicating that all were infected with the previously unidentified alphaproteobacterial endosymbiont.

Fig. S1. Maximum likelihood phylogenetic tree of the concatenated MLST data (2,073 or 2,079 bp). Letters next to the species names indicate the supergroups. The ID codes and the sequence type numbers are obtained from the MLST database. Maximum likelihood bootstrap values based on 1,000 replicates (>50%) are given at the nodes.
Fig. S2. Phylogenetic distance tree of Anaplasma obtained from OTUs of male and female *Brontispa longissima* collected in Dili, East Timor (Asian clade). Circle colors indicate the top five numbers of OTUs in Fig. 5. The number at each branch indicates the bootstrap values (>50%) (100 replications). The phylogenetic tree was constructed in MEGA6 using neighbor-joining method, and based on the full length 16S rRNA sequences of the mapped OTUs that belonged to *Anaplasma* in Fig. 5.
Fig. S3. Detection of the previously unidentified bacterial endosymbiont in B. longissima from different locations: 1–3, Australia; 4–6, Papua New Guinea; 7–9, Indonesia (Sumba); 10 and 11, East Timor (Puno); 12–14, East Timor (Home); 15–17, East Timor (Raca); 18 and 19, East Timor (Dili). Specimens in 1–17 belong to the Pacific clade; specimens in 18 and 19, to the Asian clade.