

The structured diversity of specialized gut symbionts of the New World army ants

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Supplementary Figures

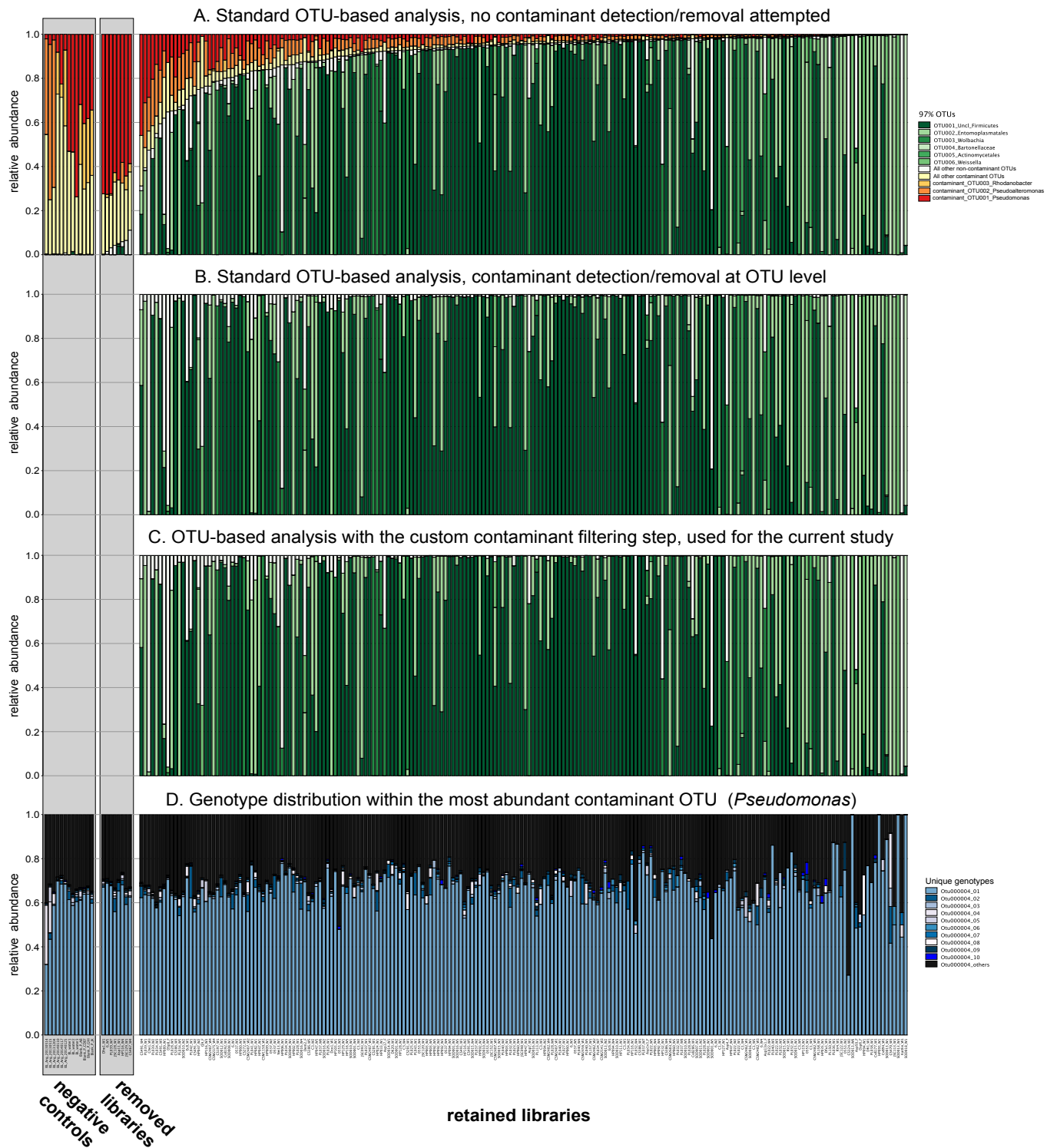
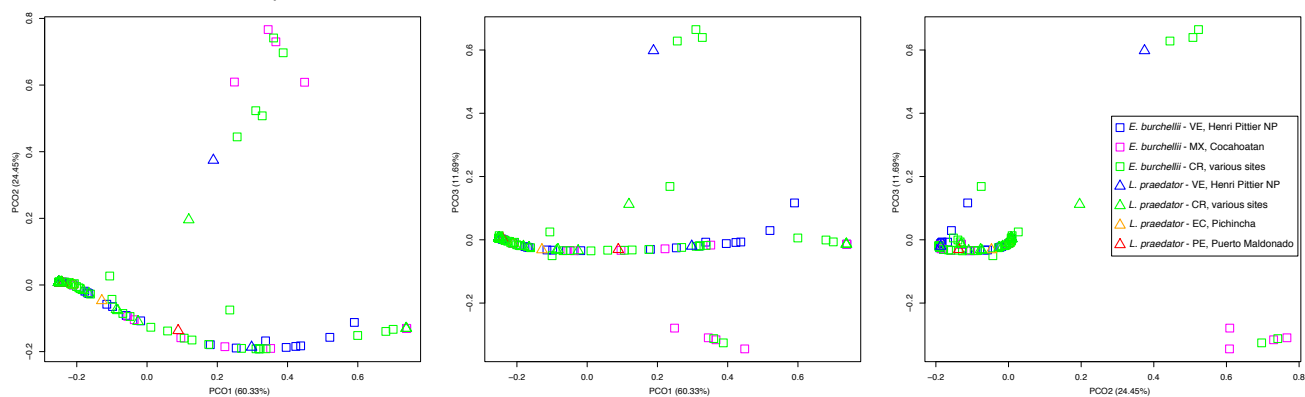
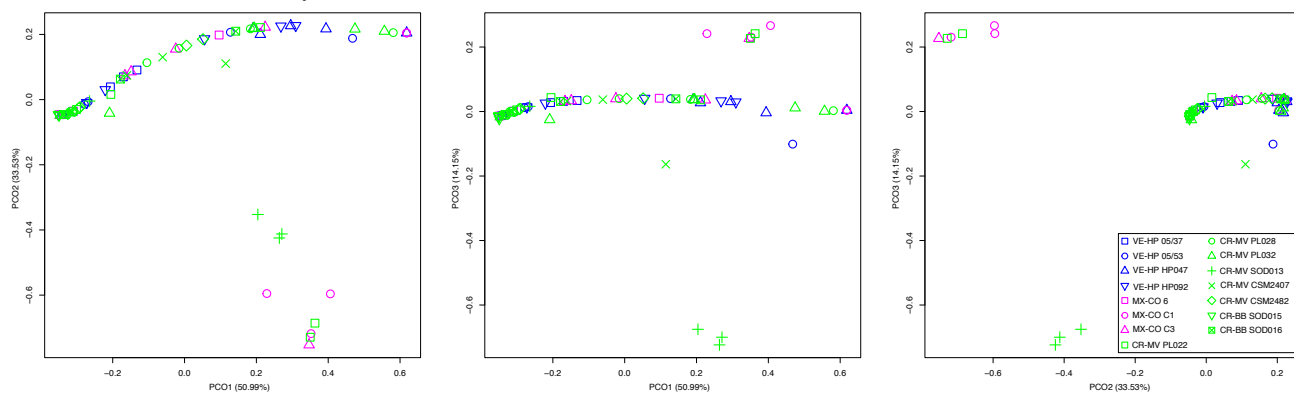


Figure S1. The effects of removal of putative contaminants on microbial community composition across libraries. A) The relative abundance of the dominant OTUs, including those regarded as real ant-associated bacteria and those identified as contaminants, across all sequenced libraries. Analysis has mostly followed Mothur's Standard Operating Procedure – see Supplementary Material for details. We classified as contaminants those OTUs which were not at least 10x more abundant in one of the experimental libraries than in any of the blanks; B) The relative abundance of the dominant OTUs in experimental libraries (analysis as in panel A) after the OTUs regarded as contaminants have been removed. Data is not shown for blanks or for experimental libraries deemed to be heavily contaminated; C) The relative abundance of the dominant OTUs in experimental libraries after analysis that included the custom contaminant filtering step – see Supplementary Material for details. This analysis has been used for the current study; D) The relative abundance of the dominant unique genotypes assigned to the most abundant OTU classified as a contaminant (*Pseudomonas*). In all panels, the same set and order of libraries has been used.

A. All *E. burchellii* and *L. praedator* colonies



B. *E. burchellii* colonies only



C. *L. praedator* colonies only

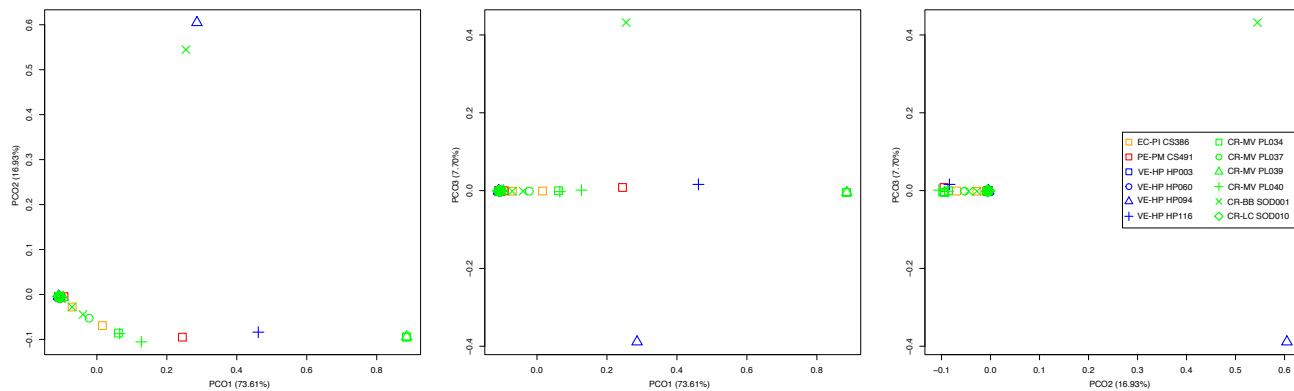


Figure S2. Principal coordinates analyses (PCoA) plots for *Eciton burchellii* and *Labidus praedator* colonies sampled in replicate. The samples and data from Figure 2 were used. Legends from the third (rightmost) plot in a panel refer to all plots in that panel.

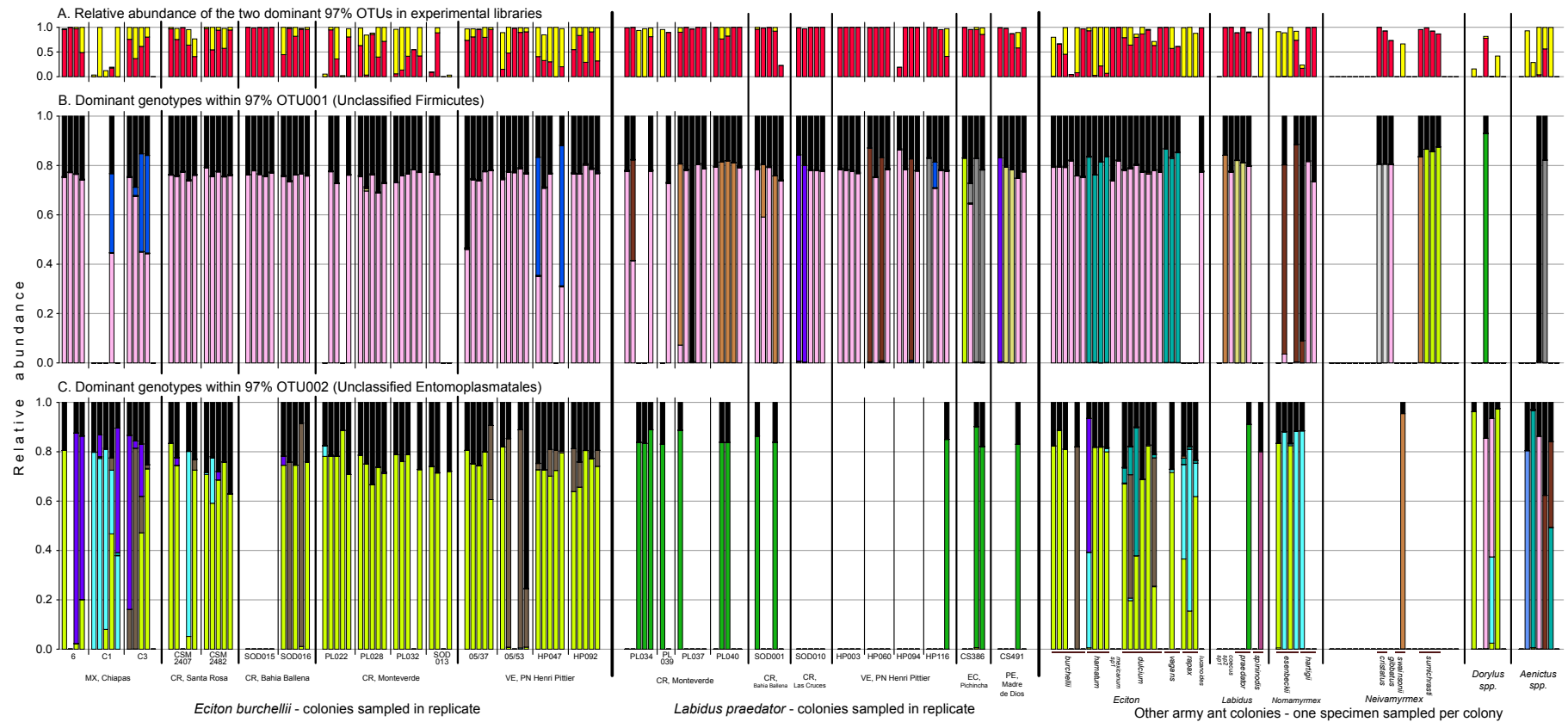


Figure S3. Genotype-level diversity of specialized symbionts of army ants, based on amplicon sequencing of the V4 region of the 16S rRNA gene. A) Relative abundance of 97% OTUs representing the two dominant ant symbionts, Unclassified Firmicutes (red) and Unclassified Entomoplasmatales (yellow), in all experimental libraries. B) Relative abundance of the dominant unique genotypes classified to OTU001 (Unclassified Firmicutes) within that OTU. Colours represent the 12 most abundant unique genotypes in the dataset, and black represents all other (rare) unique genotypes combined. Data are shown only for libraries where OTU001 is represented by at least 100 reads. C) Relative abundance of the dominant unique genotypes classified to OTU002 (Unclassified Entomoplasmatales) within that OTU. Colors represent 12 most abundant unique genotypes in the dataset, and black represents all other (rare) unique genotypes combined. Data shown only for libraries where OTU002 was represented by at least 100 reads

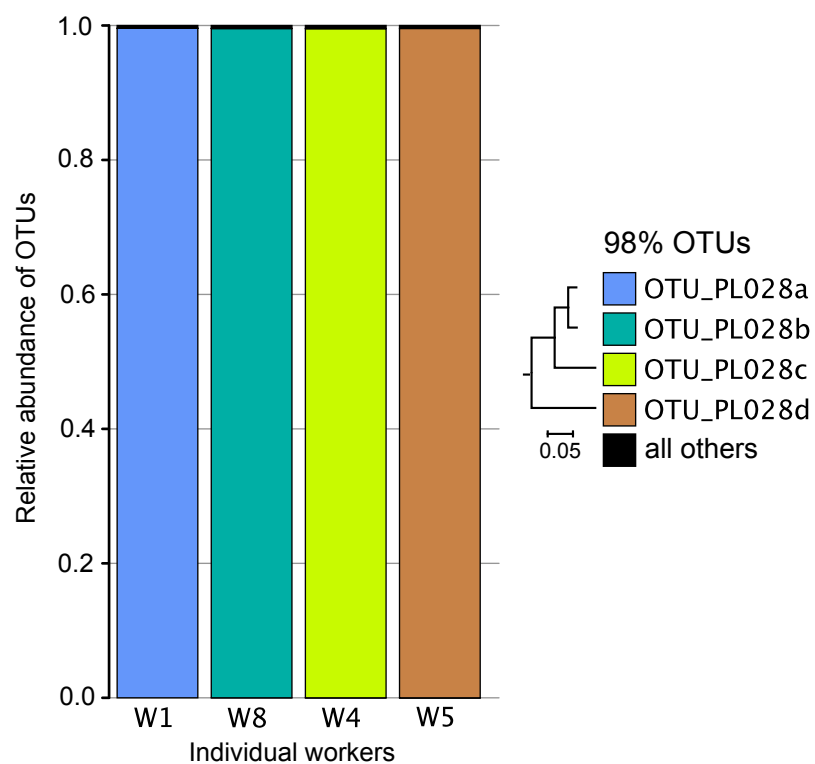


Figure S4. Strain-level diversity of Unclassified Firmicutes in four individual workers from *Eciton burchellii* colony PL028, based on 454 pyrosequencing of the *rplB* amplicon. Colors represent distinct 98% OTUs (symbiont strains).

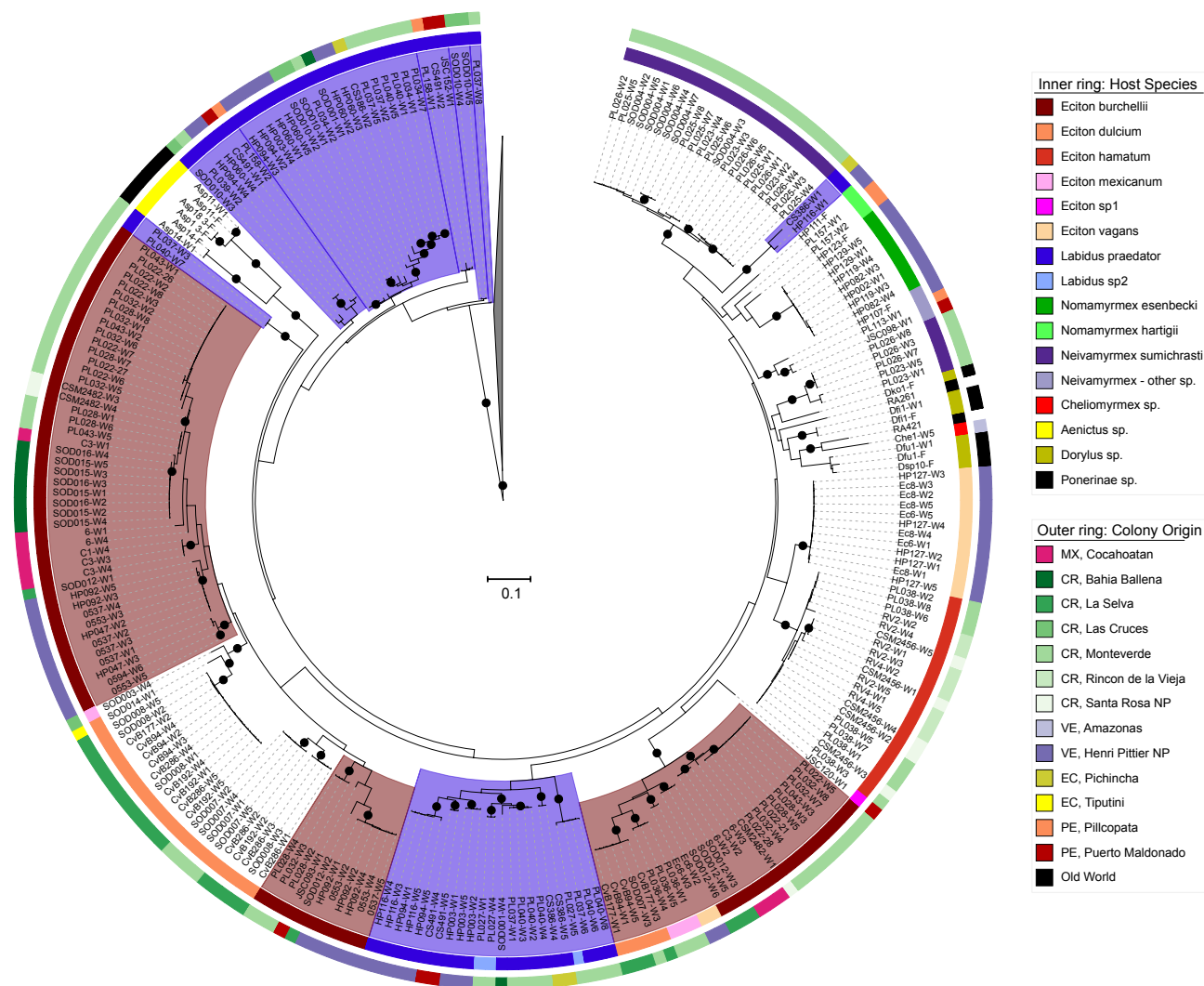
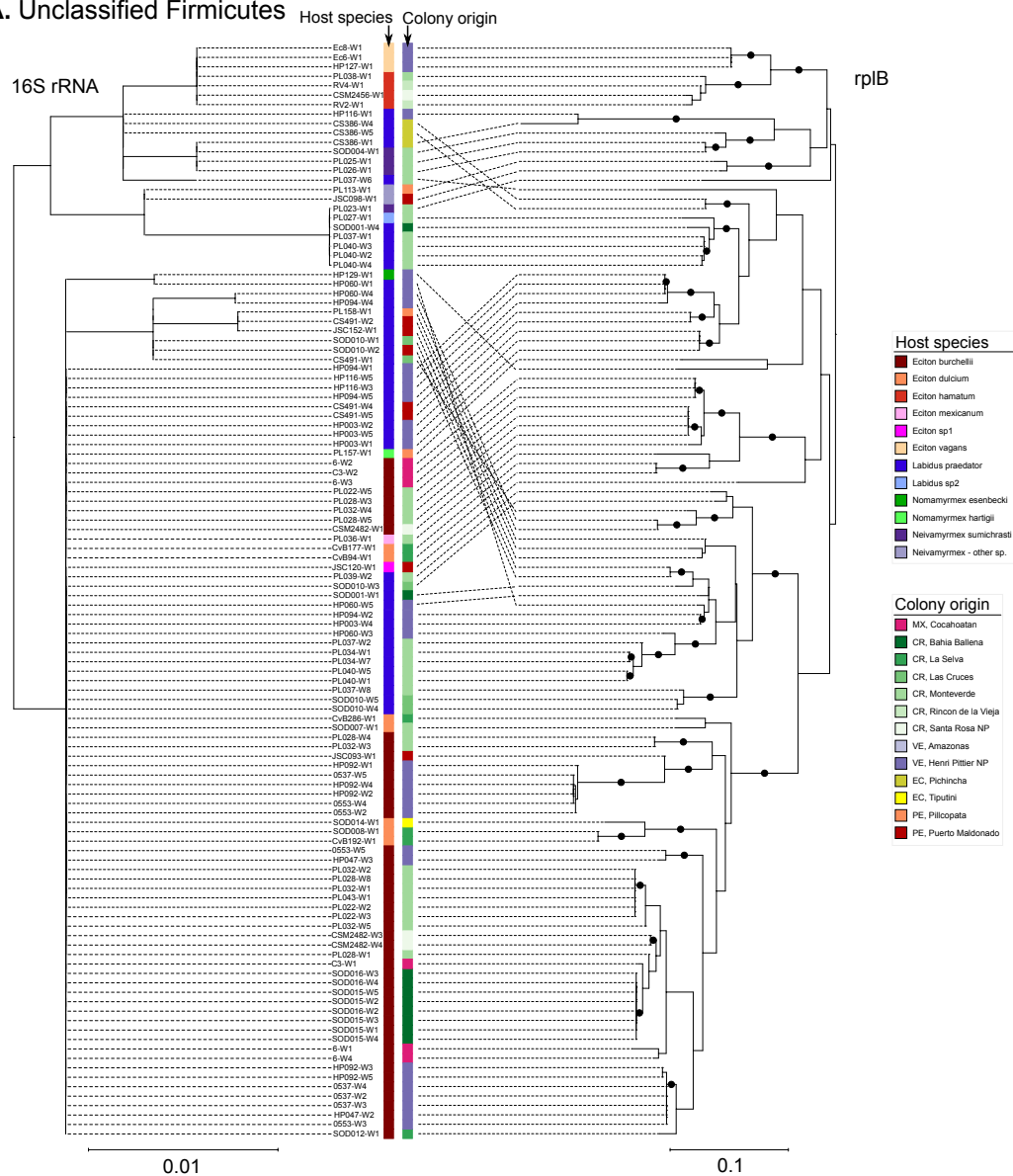


Figure S5. Unconstrained ML phylogeny of Unclassified Firmicutes based on ribosomal protein L2 (*rplB*) gene. This tree is included as a counterpart to the phylogeny in Fig. 3B, constructed using the same data [573bp sequence of *rplB* gene from 252 ant workers], yet with imposed constraints described in that figure's legend. Annotation details are the same as for Fig. 3B.

A. Unclassified Firmicutes



B. Entomoplasmatales

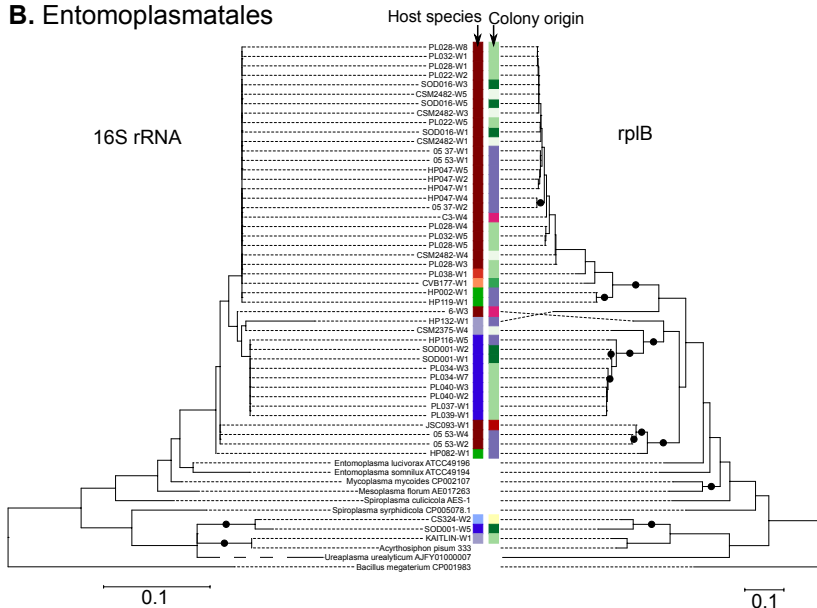


Figure S6. *rplB*-genotype level diversity within 16S rRNA genotypes of the two specialized symbionts of army ants. ML phylogenies based on 252-253bp sequence of 16S rRNA and 573-579bp sequence of *rplB* have been constructed for all samples for which unambiguous *rplB* sequence was obtained, and 16S rRNA amplicon data indicated the presence of a single dominant strain of a given symbiont. Black dots represent bootstrap support values of 95% and over.

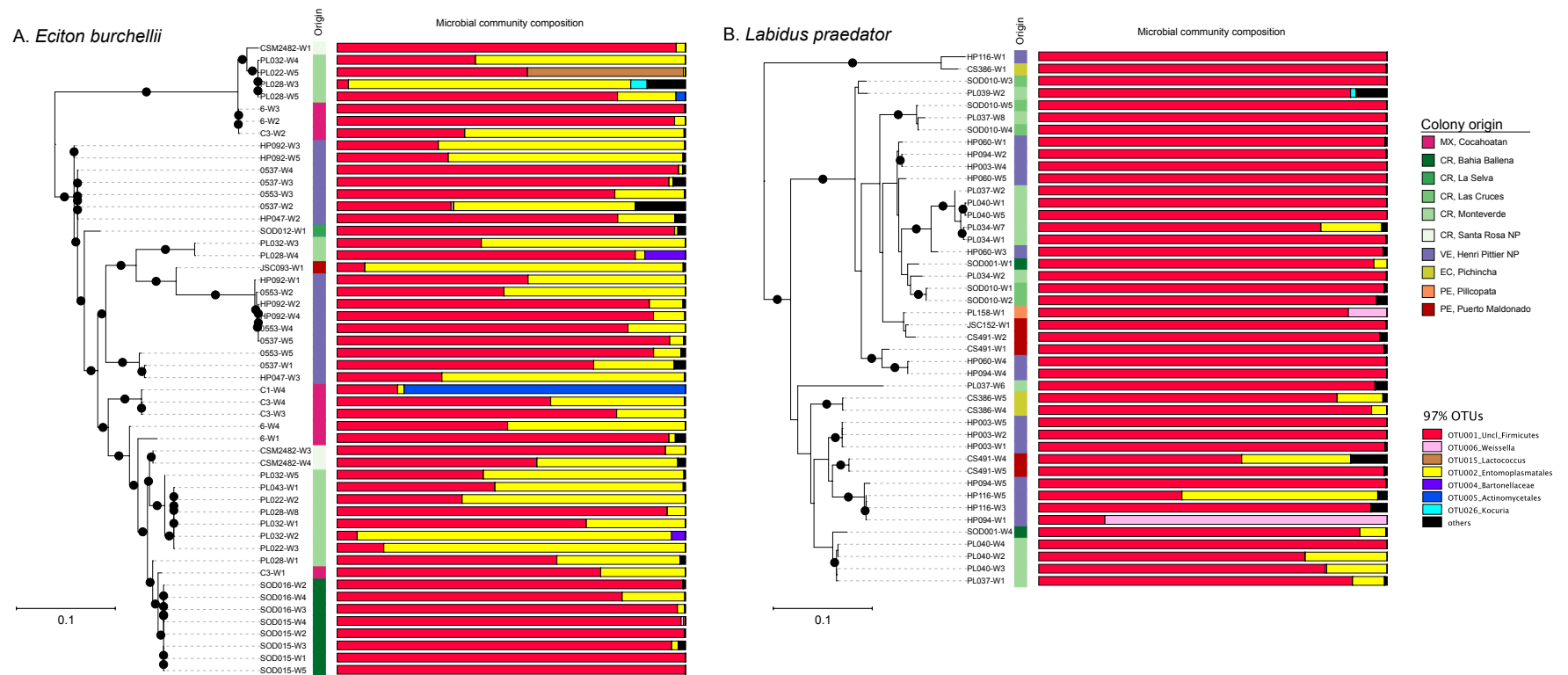


Figure S7. The relationship between *rplB* genotypes of Unclassified Firmicutes and the microbial community composition in two army ant species. ML phylogenies based on a 573bp sequence of ribosomal protein L2 (*rplB*) gene (as in Fig. 3B) and the microbial community composition data based on 16S rRNA amplicon sequencing (as in Figs. 1 and 2) are constructed for all workers of a given species with both types of data generated. Bootstrap support values of 95% or more are represented by black dots on branches.