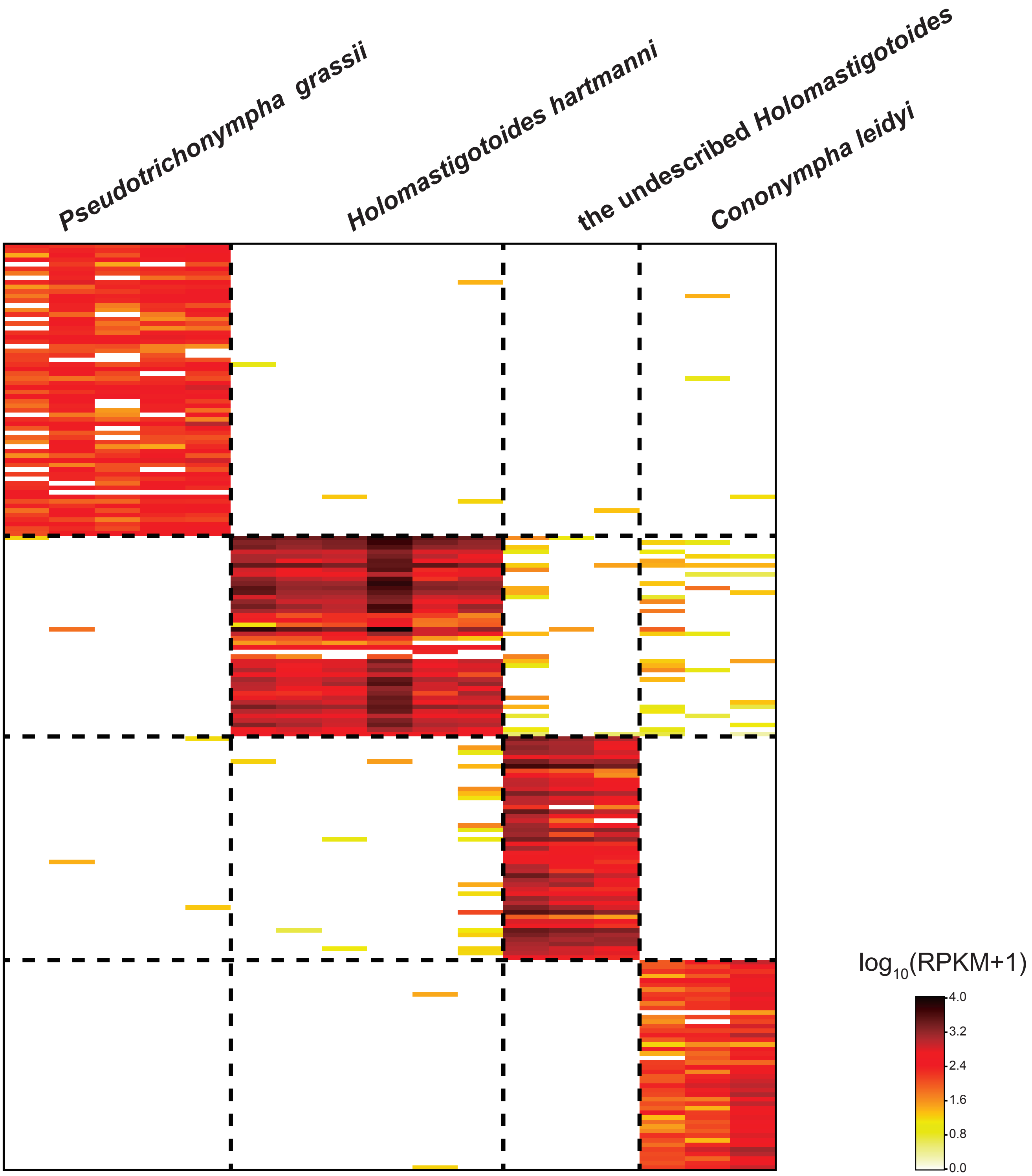
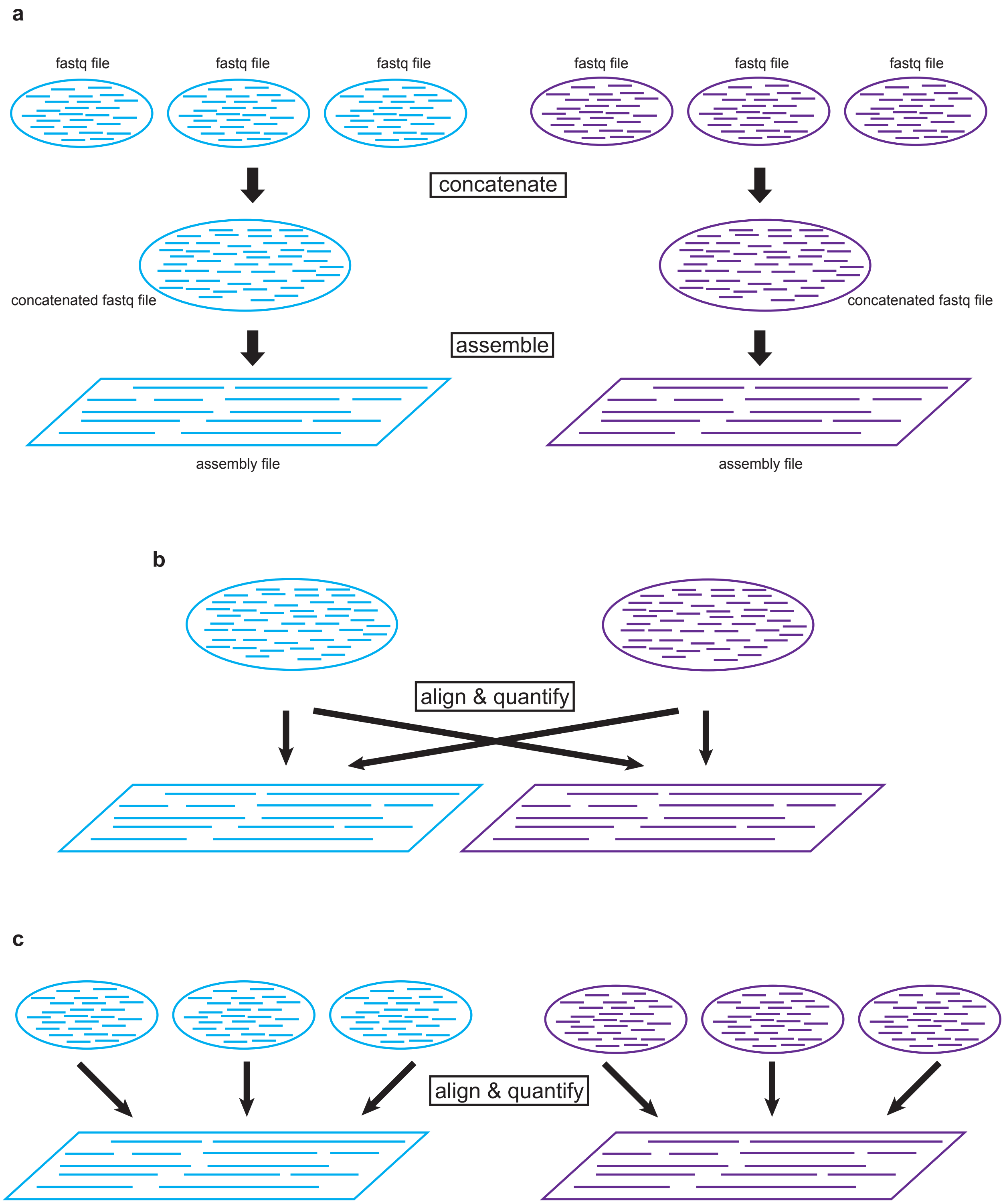


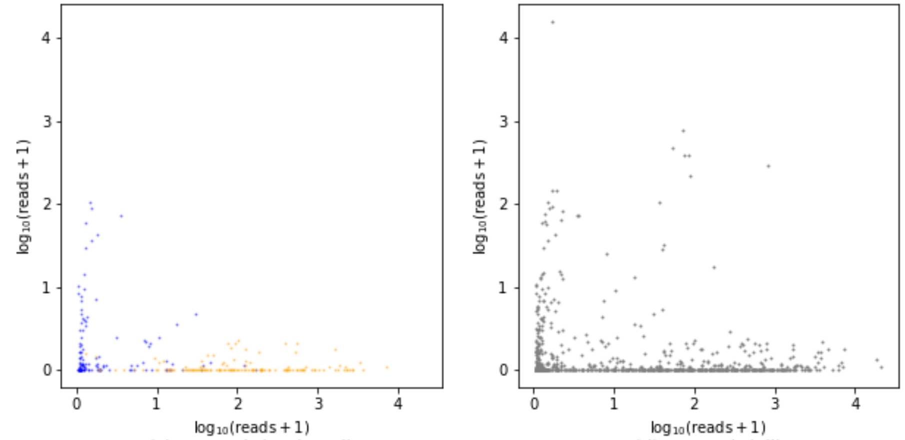
Supplementary Figure 1. Distribution of genes for ribosomal proteins found in concatenated assembly among the libraries from single cells. The assembly was reconstructed from the reads of 17 libraries generated by Miseq. The heat-map represents the logarithm reads per kilobase per million (RPKM) of contigs in each transcriptome. The genes were sorted manually. The sequences of ribosomal proteins in the heatmap and RPKMs are given in Supplementary Table 1.



Supplementary Figure 2. Workflow of bioinformatic analysis of single-cell transcriptomes. The FASTQ file, concatenated FASTQ file, and assembly file derived from same species are colored in same colors. Although we sequenced the transcriptomes of four species and produced 12 FASTQ files in total, six FASTQ files of two species are shown in this Figure for simplicity. **a** Workflow of *de novo* transcriptomes. The reads (FASTQ files) generated from three different single-cells of the same species were concatenated into a single file and assembled. The resultant assembly was treated as a reference in the following analyses. **b** Workflow of the cross-contamination evaluation among the libraries targeting the different species. All the concatenated FASTQ files were mapped to all references. The number of aligned reads was normalized by the size of concatenated FASTQ files. **c** Workflow of the reproducibility evaluation among libraries targeting the same species. Three FASTQ file-generated references were re-aligned with the reference. The number of aligned reads was normalized to Trimmed Mean of M value among libraries.

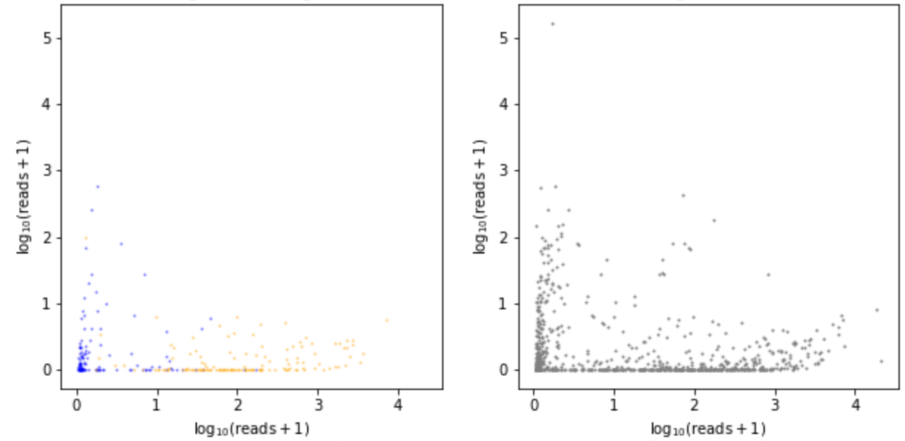
(a) Pg vs Hm (assigned)

(b) Pg vs Hm (all)



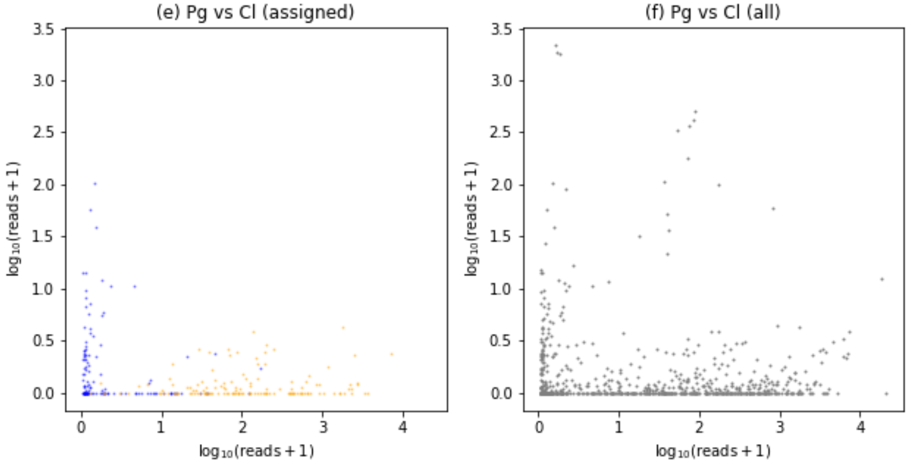
(c) Pg vs Hh (assigned)

(d) Pg vs Hh (all)

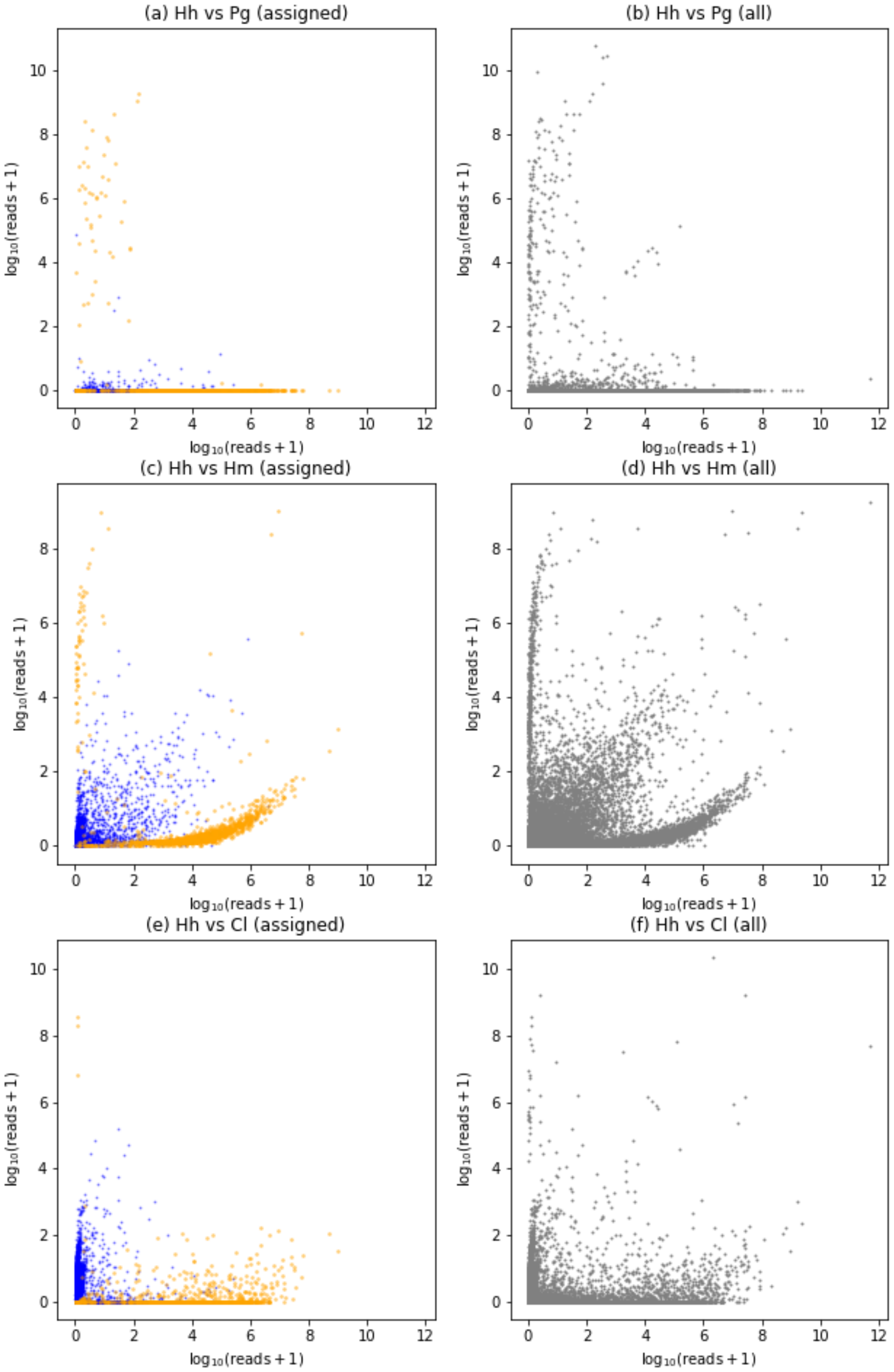


(e) Pg vs Cl (assigned)

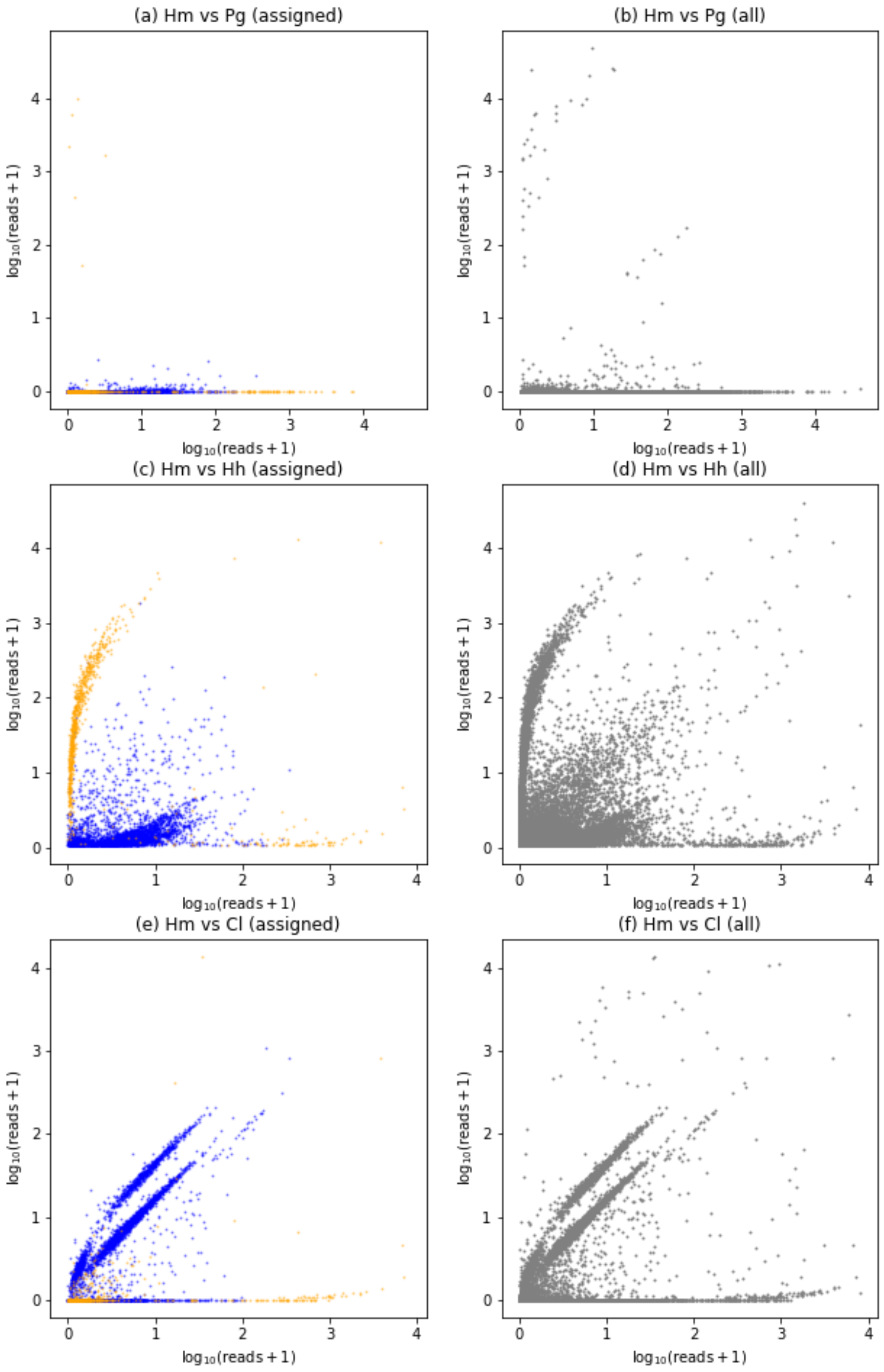
(f) Pg vs Cl (all)



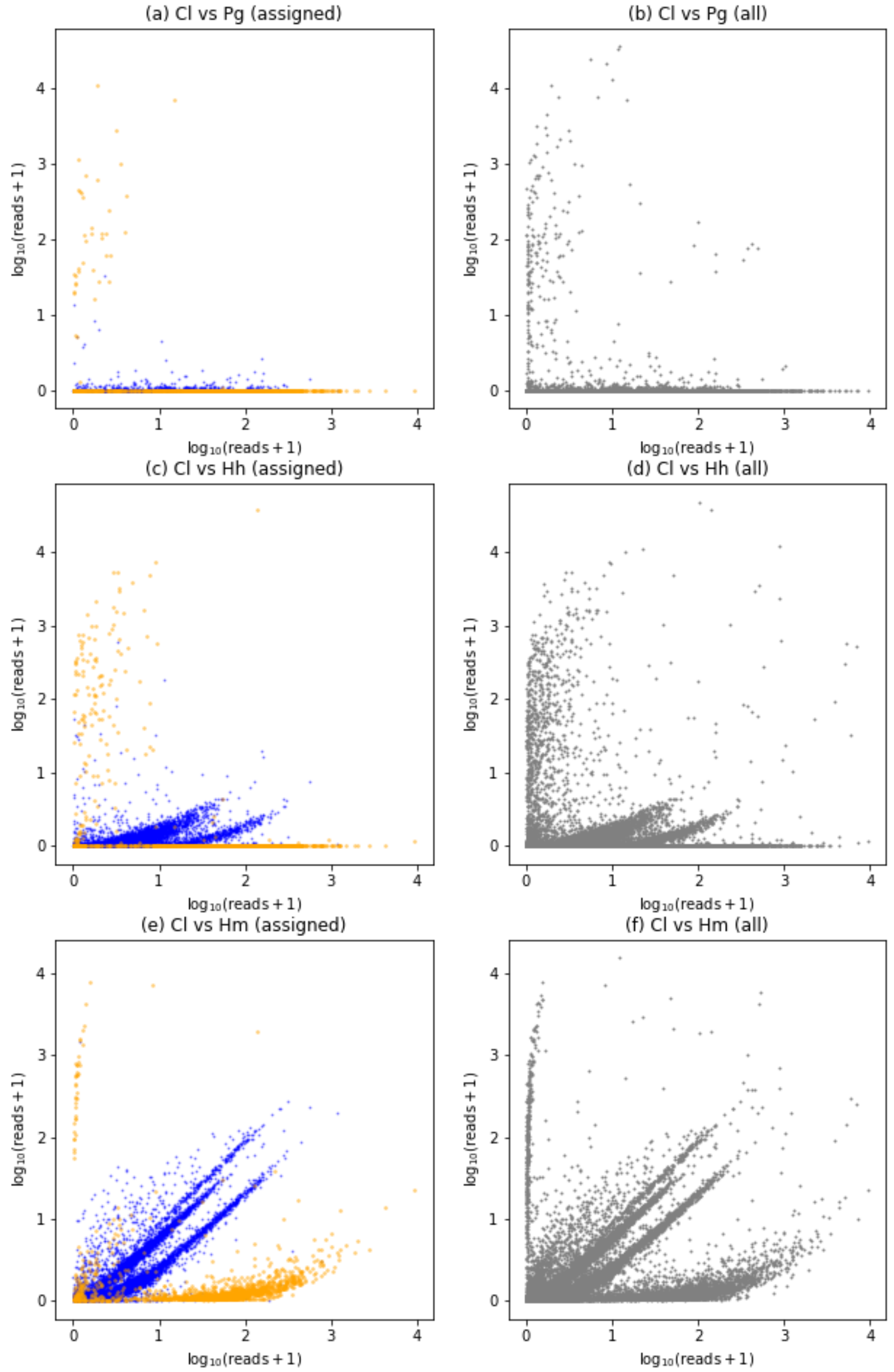
Supplementary Figure 3. Quantification of cross-contaminated contigs between the library of *Pseudotrichonympha grassii* and the other symbiotic protists. The *x* and *y* axes represent $\log_{10}$ of the normalized read counts in the respective libraries. Blue and orange dots represent bacterial and parabasalian contigs, respectively. **a** Pair plot comparison of cross-contaminated contigs assigned to Bacteria or Parabasalia between the library targeting *P. grassii* (Pg) and *Holomastigotoides hartmanni* (Hh). **b** Pair plot comparison of all the cross-contaminated contigs between Pg and Hh libraries. **c** Pair plot comparison of cross-contaminated contigs assigned to Bacteria or Parabasalia between the libraries targeting Pg and *H. minor* (Hm). **d** Pair plot comparison of all the cross-contaminated contigs between Pg and Hm libraries. **e** Pair plot comparison of cross-contaminated contigs assigned to Bacteria or Parabasalia between the library targeting Pg and *Cononympha leidyi* (Cl). **f** Pair plot comparison of all the cross-contaminated contigs between Pg and Cl libraries.



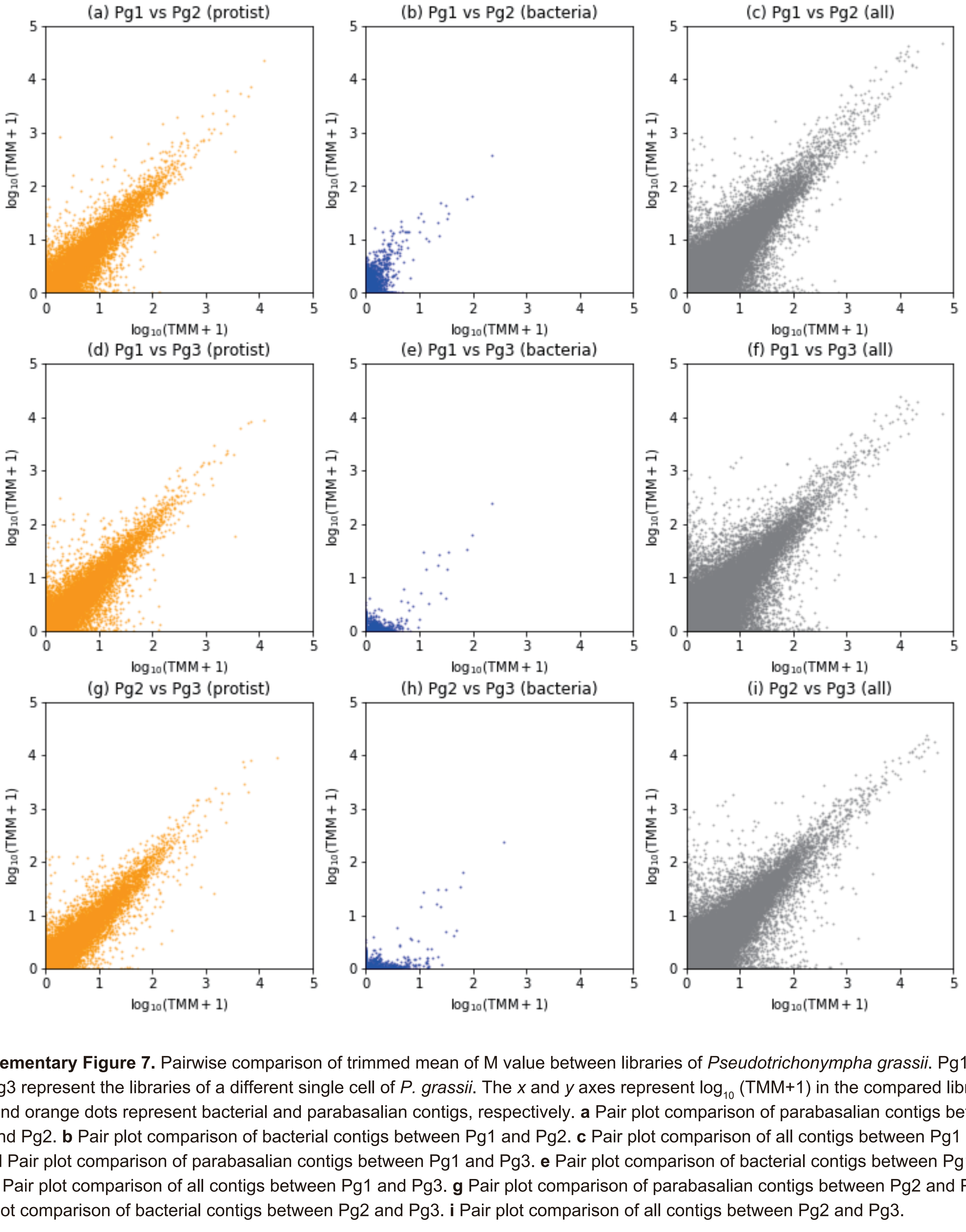
Supplementary Figure 4. Quantification of the abundance of cross-contaminated contigs between the library of *Holomastigotoides hartmanni* and the other symbiotic protists. The detailed descriptions are the same as Fig. S2. **a** Pair plot comparison of cross-contaminated contigs assigned to Bacteria or Parabasalia between libraries targeting *H. hartmanni* (Hh) and *Pseudotrichonympha grassii* (Pg). **b** Pair plot comparison of all the cross-contaminated contigs between Hh and Pg libraries. **c** Pair plot comparison of cross-contaminated contigs assigned to Bacteria or Parabasalia between the libraries targeting Hh and *H. minor* (Hm). **d** Pair plot comparison of all the cross-contaminated contigs between Hh and Hm libraries. **e** Pair plot comparison of cross-contaminated contigs assigned to Bacteria or Parabasalia between the libraries targeting Hh and *Cononympha leidyi* (Cl). **f** Pair plot comparison of all the cross-contaminated contigs between Hh and Cl libraries.



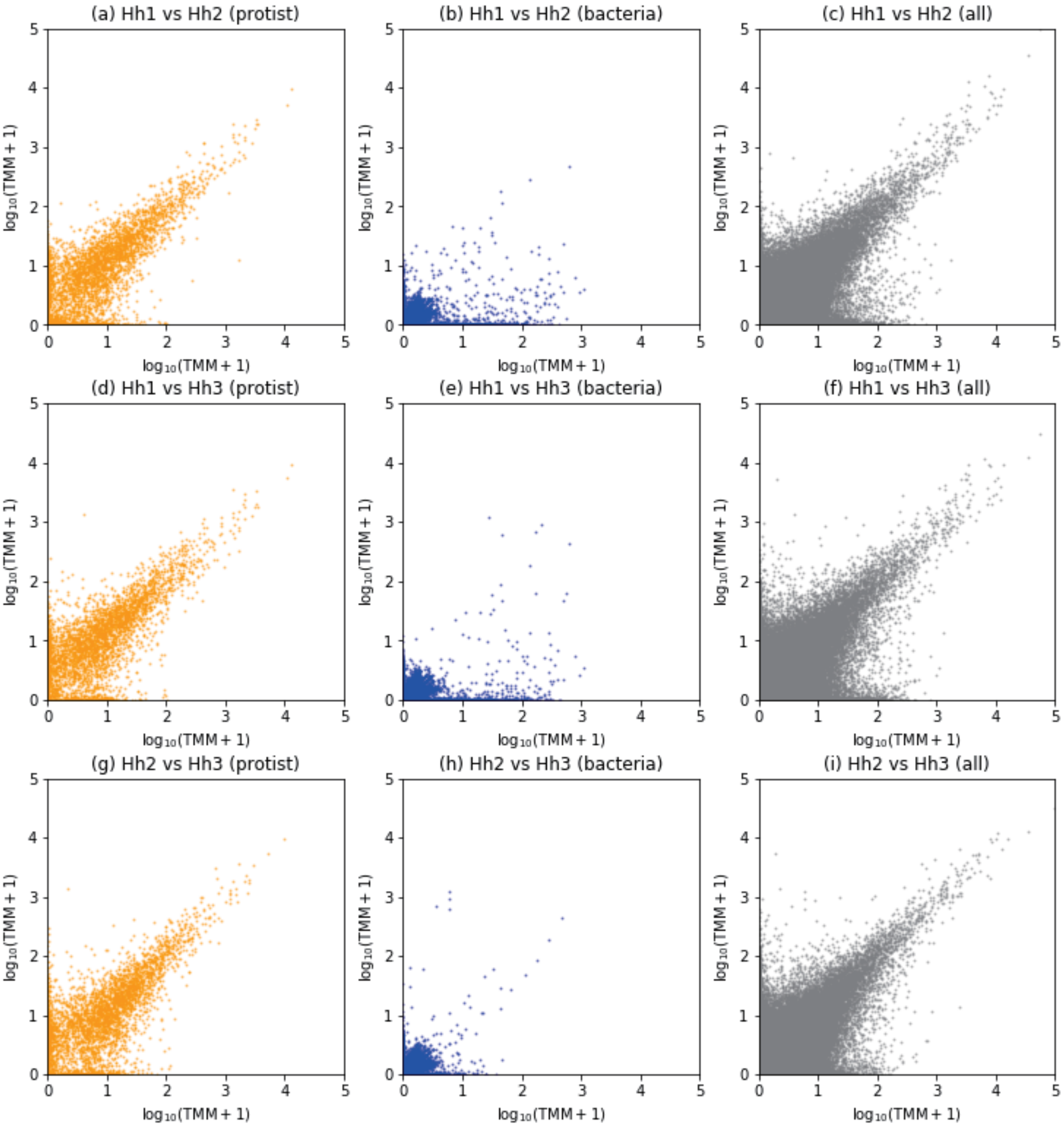
Supplementary Figure 5. Quantification of cross-contaminated contigs between the libraries of *Holomastigotoides minor* and the other symbiotic protists. Detailed descriptions are the same as in Fig.S2. **a** Pair plot comparison of cross-contaminated contigs assigned to Bacteria or Parabasalia between the libraries targeting *H. minor* (Hm) and *Pseudotrichonympha grassii* (Pg). **b** Pair plot comparison of all the cross-contaminated contigs between Hm and Pg libraries. **c** Pair plot comparison of cross-contaminated contigs assigned to Bacteria or Parabasalia between the libraries targeting Hm and *H. hartmanni* (Hh). **d** Pair plot comparison of all the cross-contaminated contigs between Hm and Hh libraries. **e** Pair plot comparison of cross-contaminated contigs assigned to Bacteria or Parabasalia between the libraries targeting Hm and *Cononympha leidyi* (Cl). **f** Pair plot comparison of all the cross-contaminated contigs between Hm and Cl libraries.



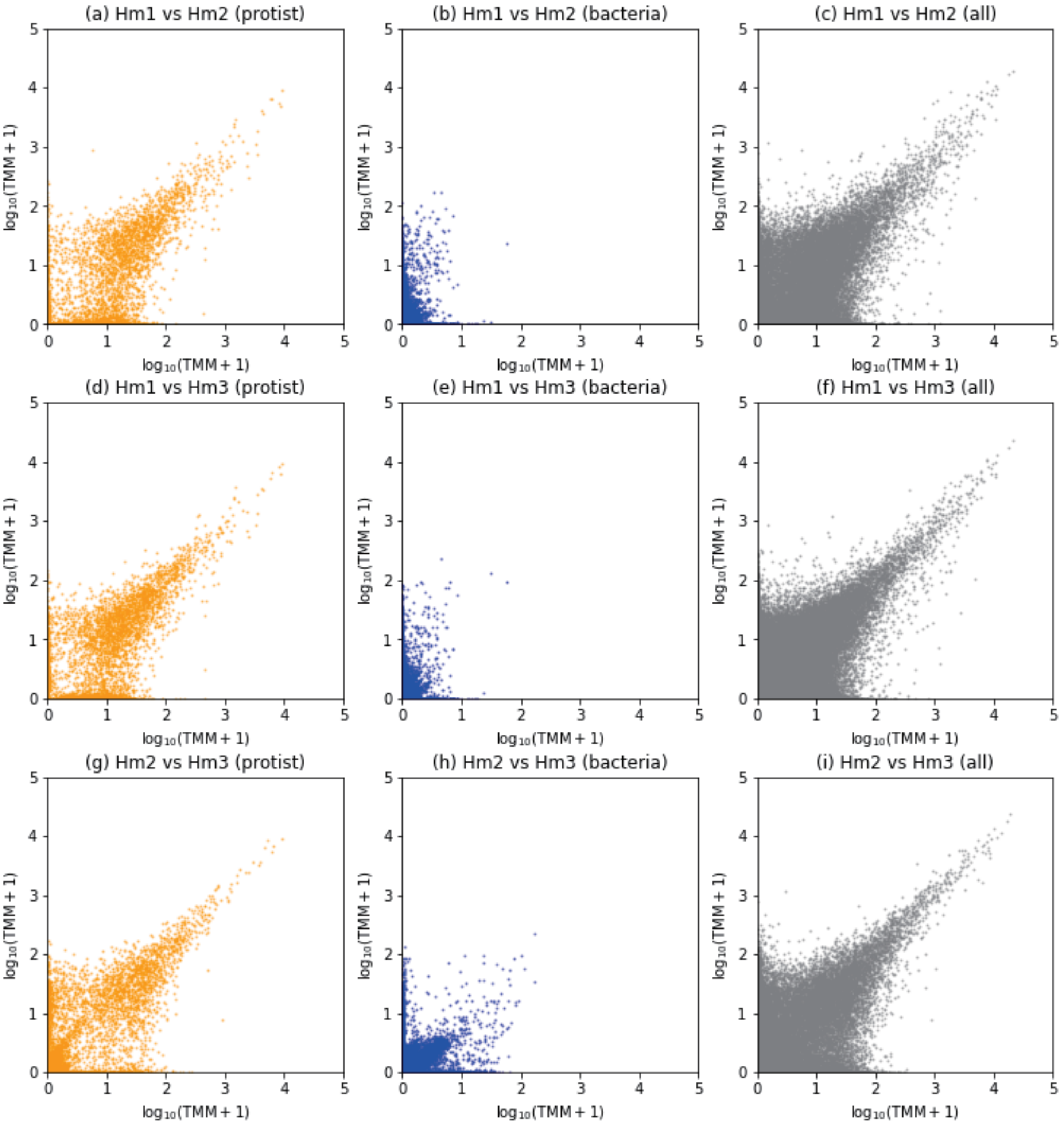
Supplementary Figure 6. Quantification of cross-contaminated contigs between the library of *Cononimpha leidyi* and the symbiotic protists. Detailed descriptions are the same as in Fig. S2. **a** Pair plot comparison of cross-contaminated contigs assigned to Bacteria or Parabasalia between the libraries targeting *C. leidyi* (Cl) and *Pseudotrichonympha grassii* (Pg). **b** Pair plot comparison of all the cross-contaminated contigs between Cl and Pg libraries. **c** Pair plot comparison of cross-contaminated contigs assigned to Bacteria or Parabasalia between the libraries targeting Cl and *Holomastigotoides hartmanni* (Hh). **d** Pair plot comparison of all the cross-contaminated contigs between Cl and Hh libraries. **e** Pair plot comparison of cross-contaminated contigs assigned to Bacteria or Parabasalia between the libraries targeting Cl and *H. minor* (Hm). **f** Pair plot comparison of all the cross-contaminated contigs between Cl and Hm libraries.



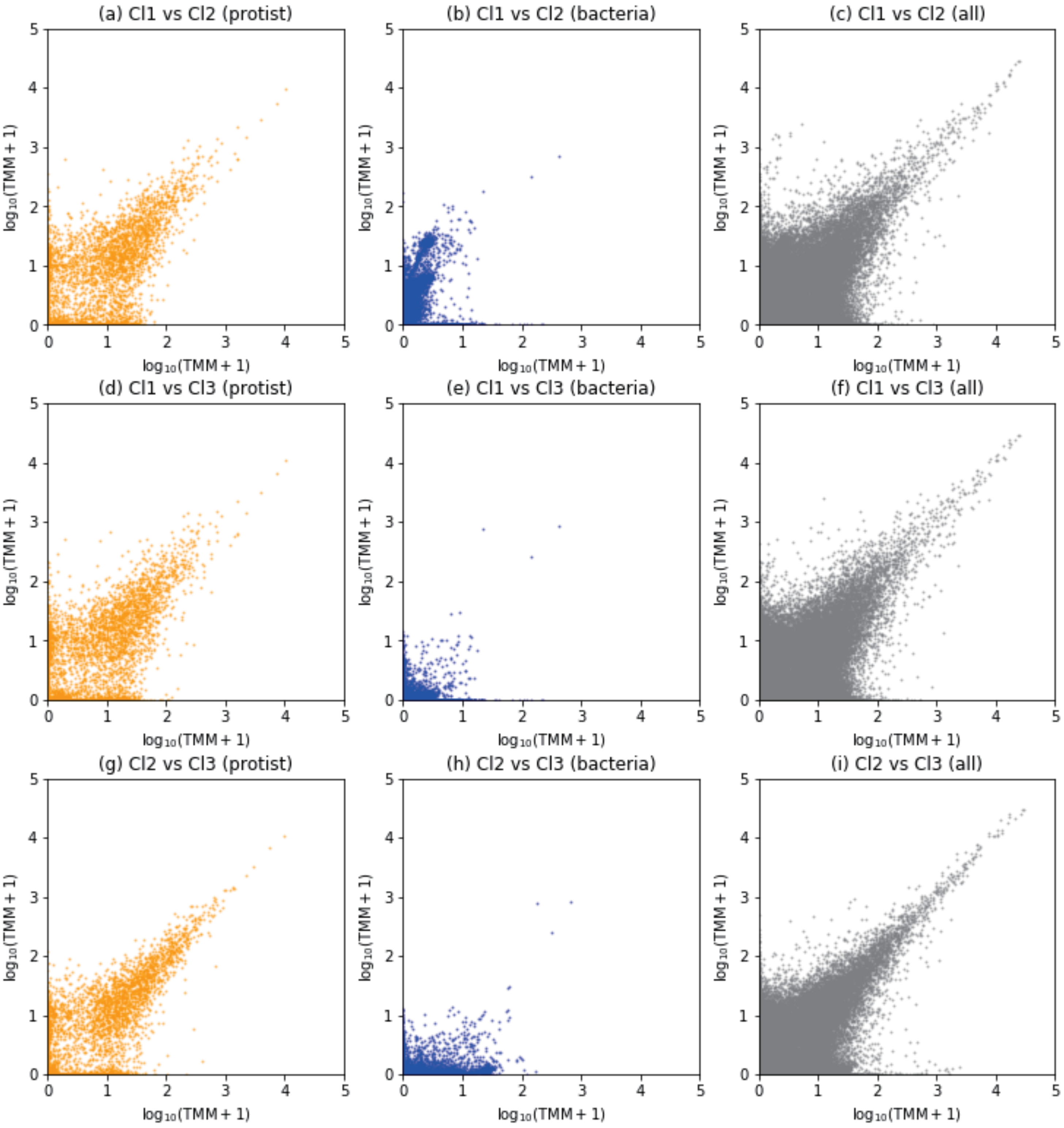
Supplementary Figure 7. Pairwise comparison of trimmed mean of M value between libraries of *Pseudotrichonympha grassii*. Pg1, Pg2, and Pg3 represent the libraries of a different single cell of *P. grassii*. The *x* and *y* axes represent $\log_{10}$ (TMM+1) in the compared libraries. Blue and orange dots represent bacterial and parabasalian contigs, respectively. **a** Pair plot comparison of parabasalian contigs between Pg1 and Pg2. **b** Pair plot comparison of bacterial contigs between Pg1 and Pg2. **c** Pair plot comparison of all contigs between Pg1 and Pg2. **d** Pair plot comparison of parabasalian contigs between Pg1 and Pg3. **e** Pair plot comparison of bacterial contigs between Pg1 and Pg3. **f** Pair plot comparison of all contigs between Pg1 and Pg3. **g** Pair plot comparison of parabasalian contigs between Pg2 and Pg3. **h** Pair plot comparison of bacterial contigs between Pg2 and Pg3. **i** Pair plot comparison of all contigs between Pg2 and Pg3.



Supplementary Figure S8. Pairwise comparison of trimmed mean of M value between libraries of *Holomastigotoides hartmanni*. Hh1, Hh2, and Hh3 represent the libraries of a different single cell of H. hartmanni. Detailed descriptions are the same as in Fig. S6. **a** Pair plot comparison of parabasalian contigs between Hh1 and Hh2. **b** Pair plot comparison of bacterial contigs between Hh1 and Hh2. **c** Pair plot comparison of all contigs between Hh1 and Hh2. **d** Pair plot comparison of parabasalian contigs between Hh1 and Hh3. **e** Pair plot comparison of bacterial contigs between Hh1 and Hh3. **f** Pair plot comparison of all contigs between Hh1 and Hh3. **g** Pair plot comparison of parabasalian contigs between Hh2 and Hh3. **h** Pair plot comparison of bacterial contigs between Hh2 and Hh3. **i** Pair plot comparison of all contigs between Hh2 and Hh3.

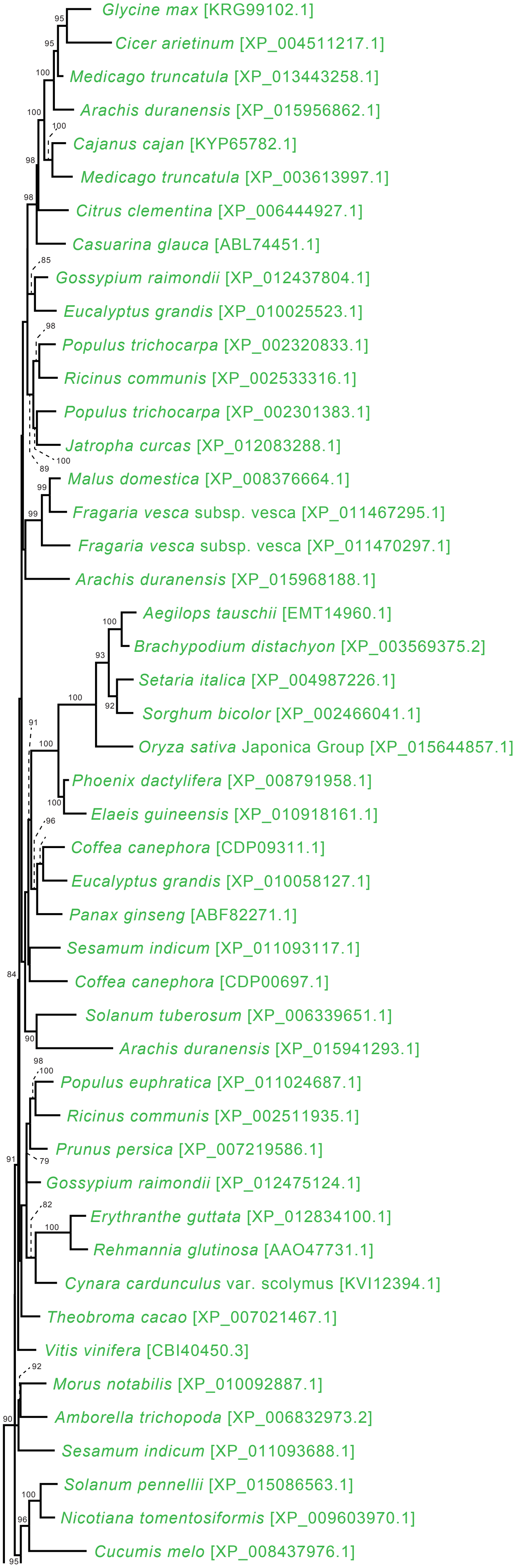


Supplementary Figure 9. Pairwise comparison of trimmed mean of M value between libraries of *Holomastigotoides minor*. Hm1, Hm2, and Hm3 represent the libraries of a different single cell of H. minor. Detailed descriptions are the same as Fig. S6. **a** Pair plot comparison of parabasalian contigs between Hm1 and Hm2. **b** Pair plot comparison of bacterial contigs between Hm1 and Hm2. **c** Pair plot comparison of all contigs between Hm1 and Hm2. **d** Pair plot comparison of parabasalian contigs between Hm1 and Hm3. **e** Pair plot comparison of bacterial contigs between Hm1 and Hm3. **f** Pair plot comparison of all contigs between Hm1 and Hm3. **g** Pair plot comparison of parabasalian contigs between Hm2 and Hm3. **h** Pair plot comparison of bacterial contigs between Hm2 and Hm3. **i** Pair plot comparison of all contigs between Hm2 and Hm3.

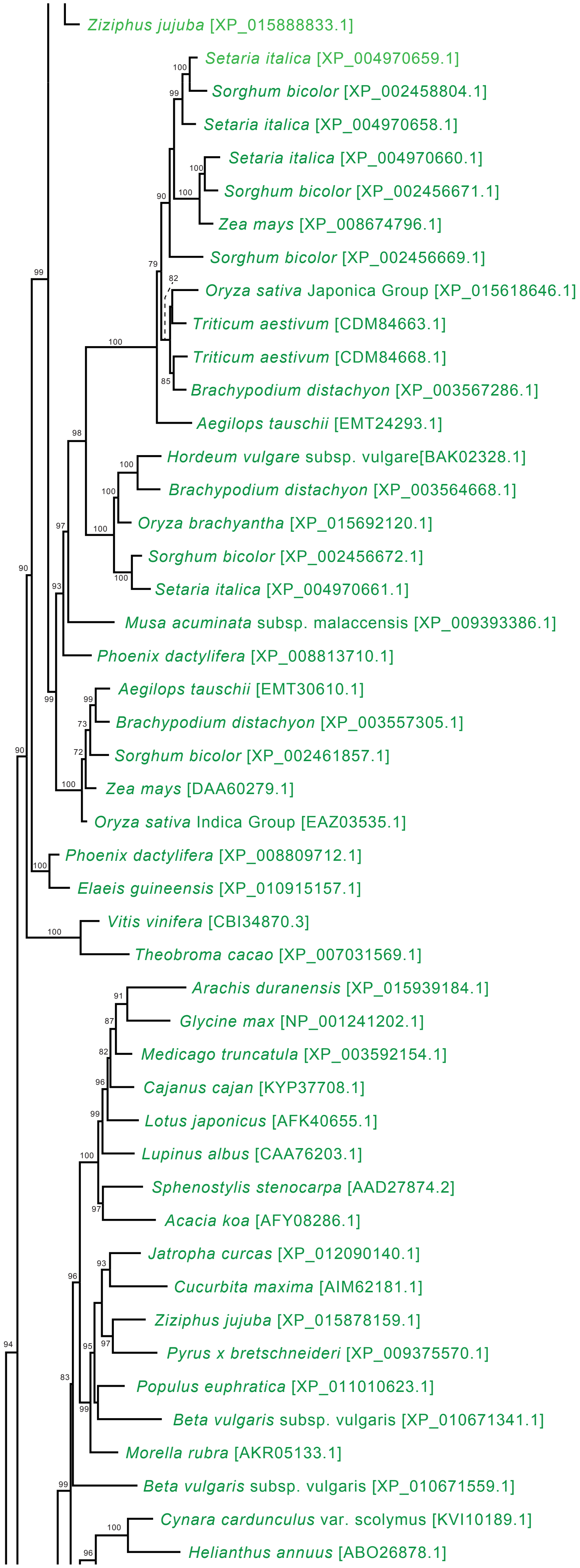


Supplementary Figure 10. Pairwise comparison of trimmed mean of M value between libraries of *Cononympha leidyi*. Cl1, Cl2, and Cl3 represent the libraries of a different single cell of *S. leidyi*. Detailed descriptions are the same as in Fig. S6. **a** Pair plot comparison of parabasalian contigs between Cl1 and Cl2. **b** Pair plot comparison of bacterial contigs between Cl1 and Cl2. **c** Pair plot comparison of all contigs between Cl1 and Cl2. **d** Pair plot comparison of parabasalian contigs between Cl1 and Cl3. **e** Pair plot comparison of bacterial contigs between Cl1 and Cl3. **f** Pair plot comparison of all contigs between Cl1 and Cl3. **g** Pair plot comparison of parabasalian contigs between Cl2 and Cl3. **h** Pair plot comparison of bacterial contigs between Cl2 and Cl3. **i** Pair plot comparison of all contigs between Cl2 and Cl3.

Supplementary Figure 11



Supplementary Figure 11(continued)



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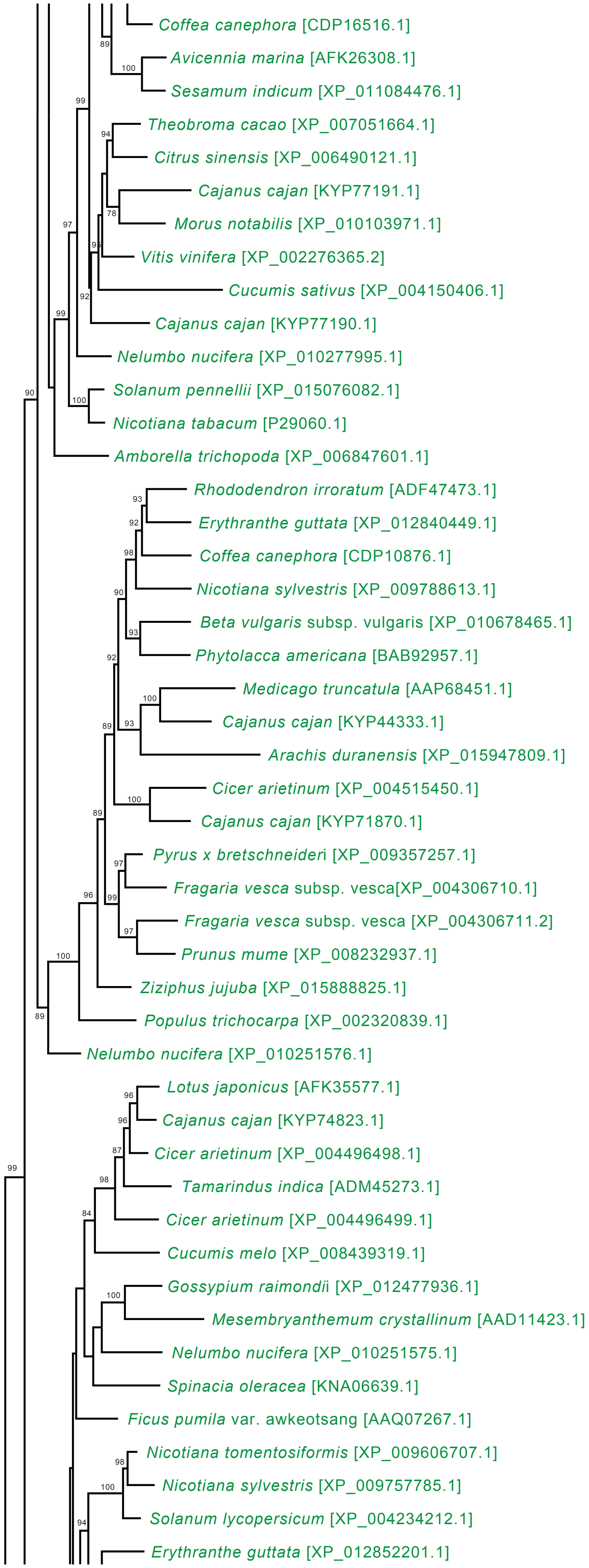

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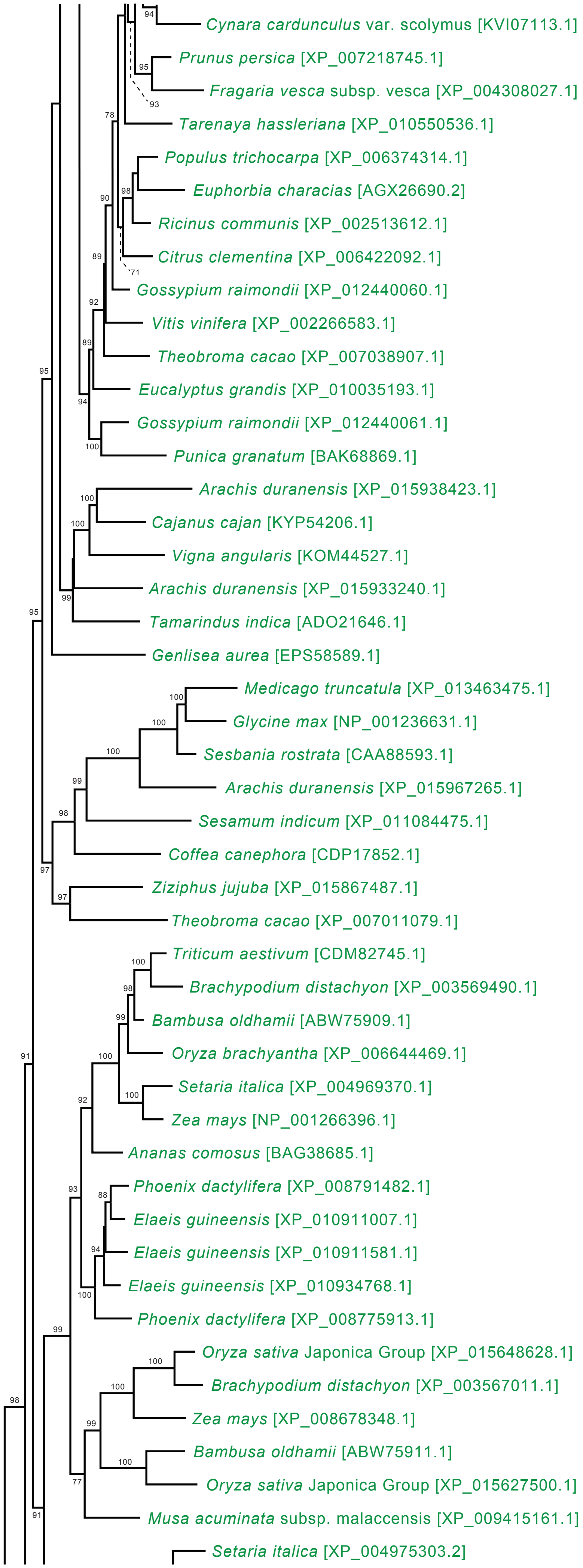

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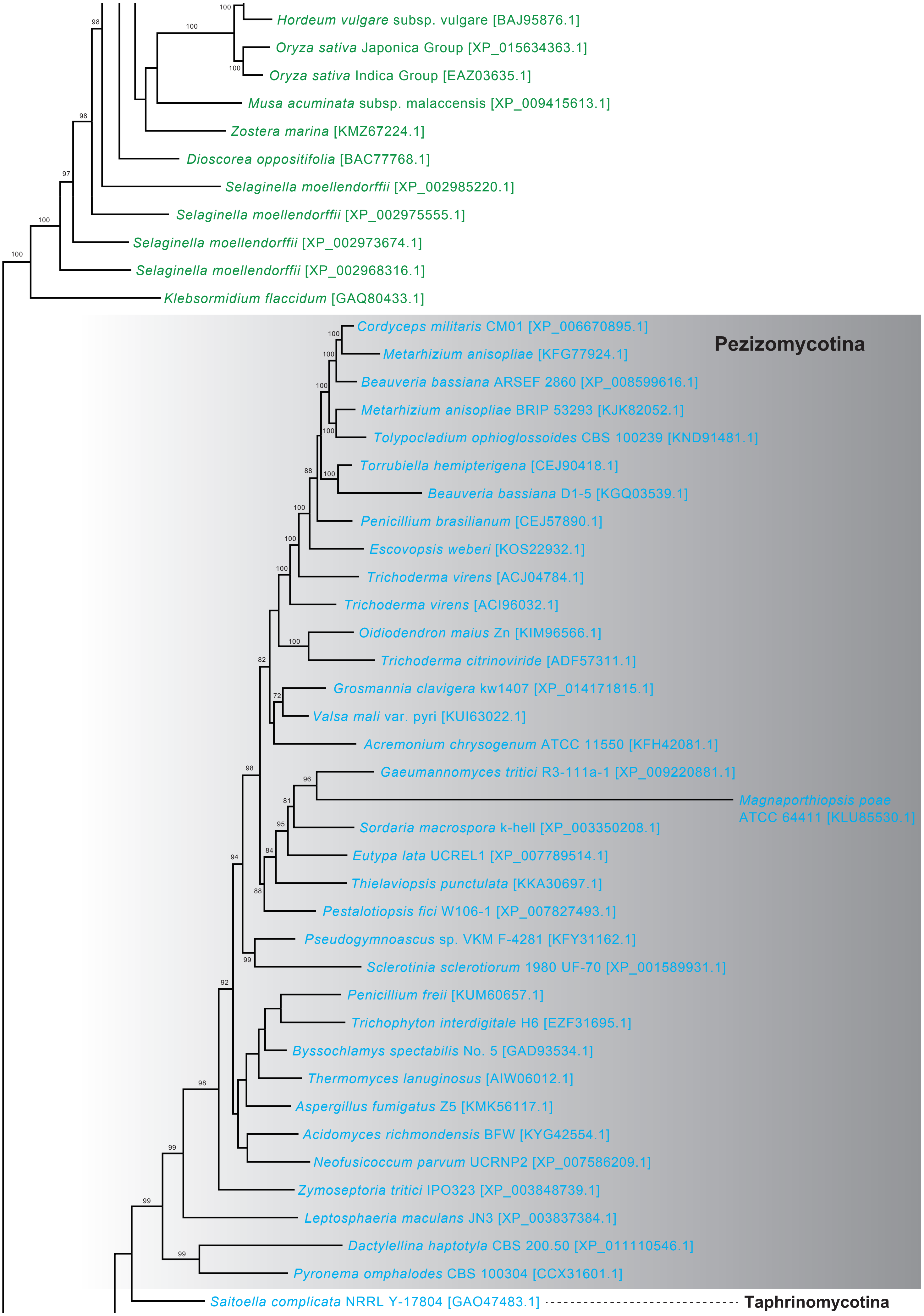

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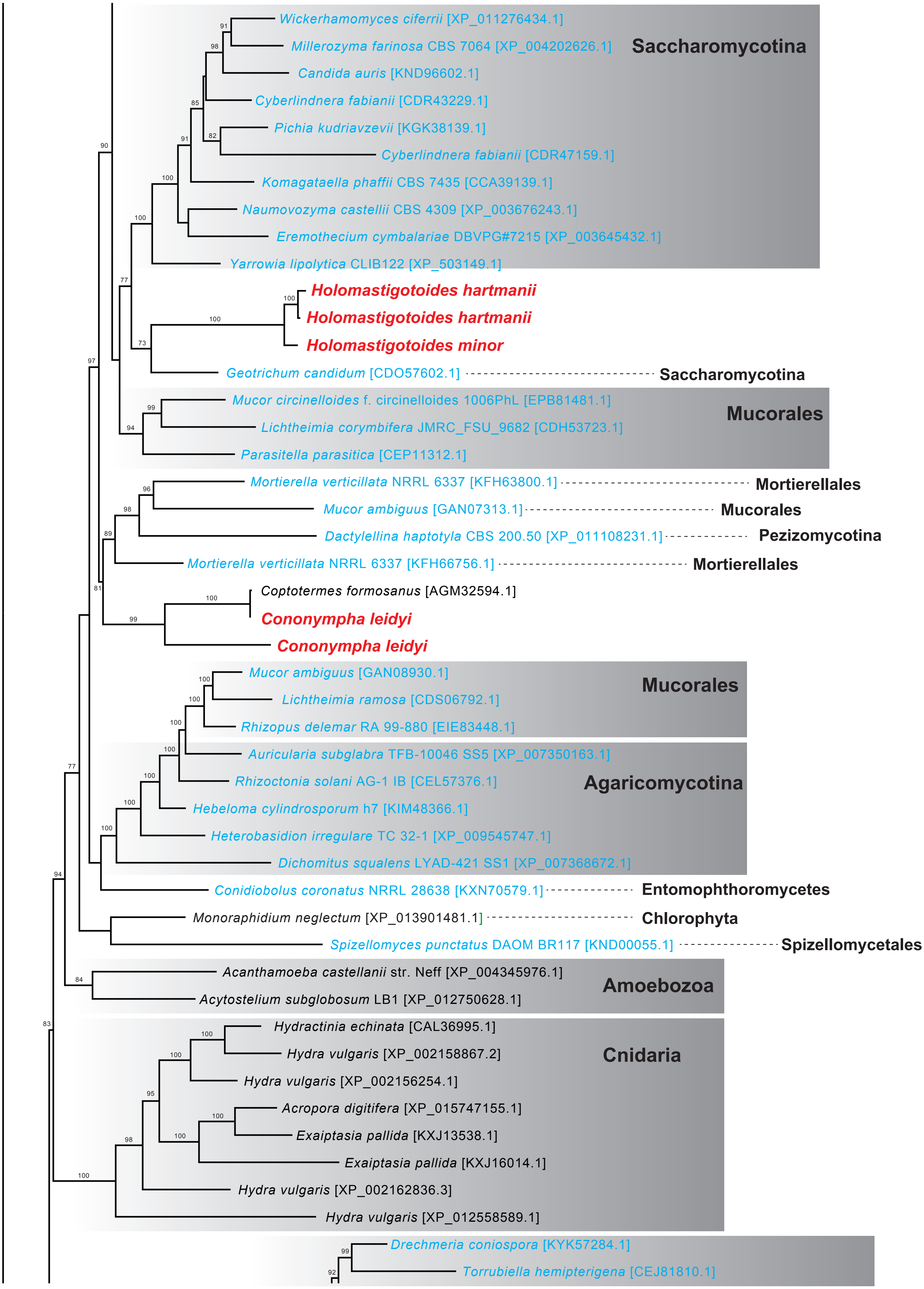

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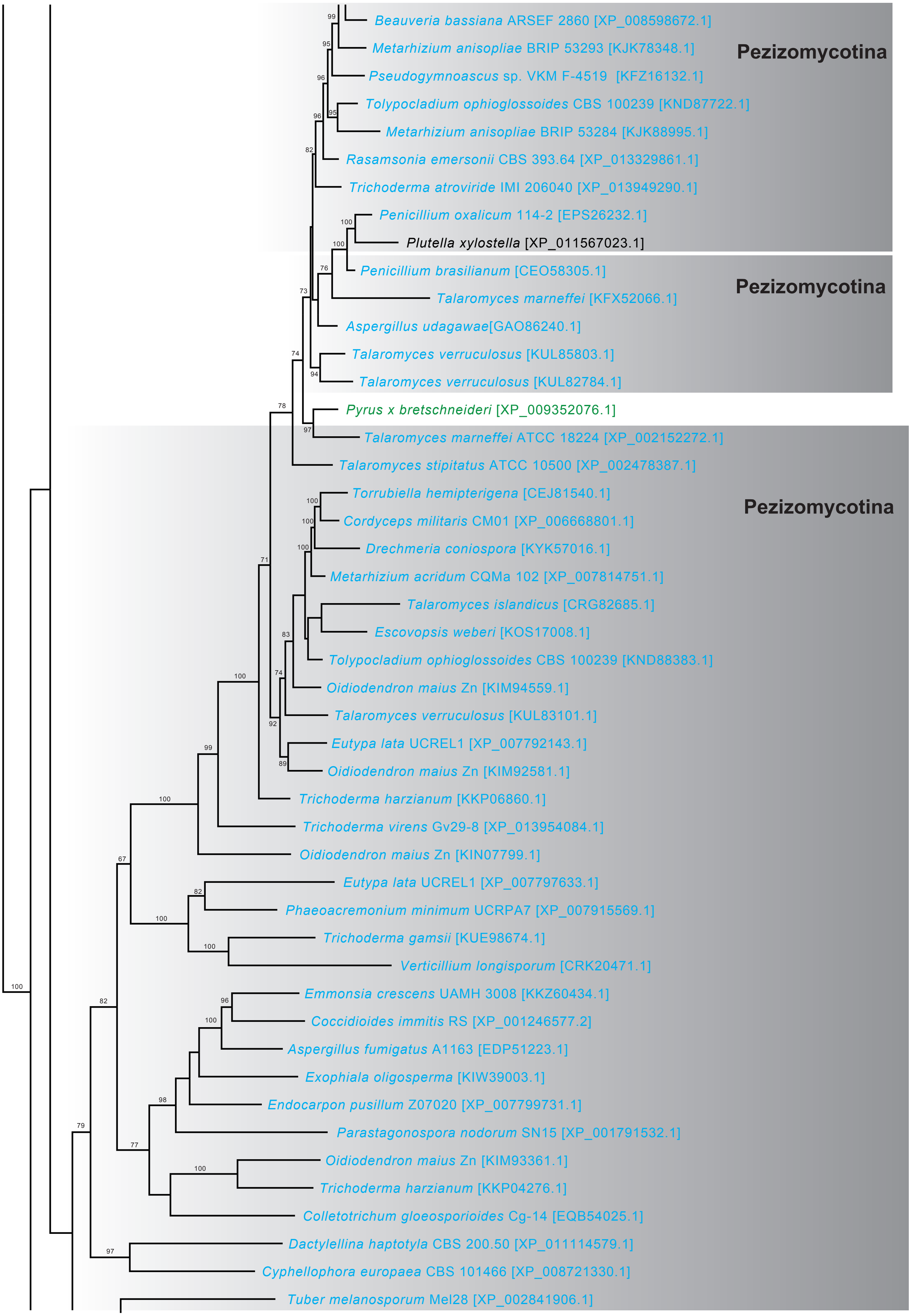

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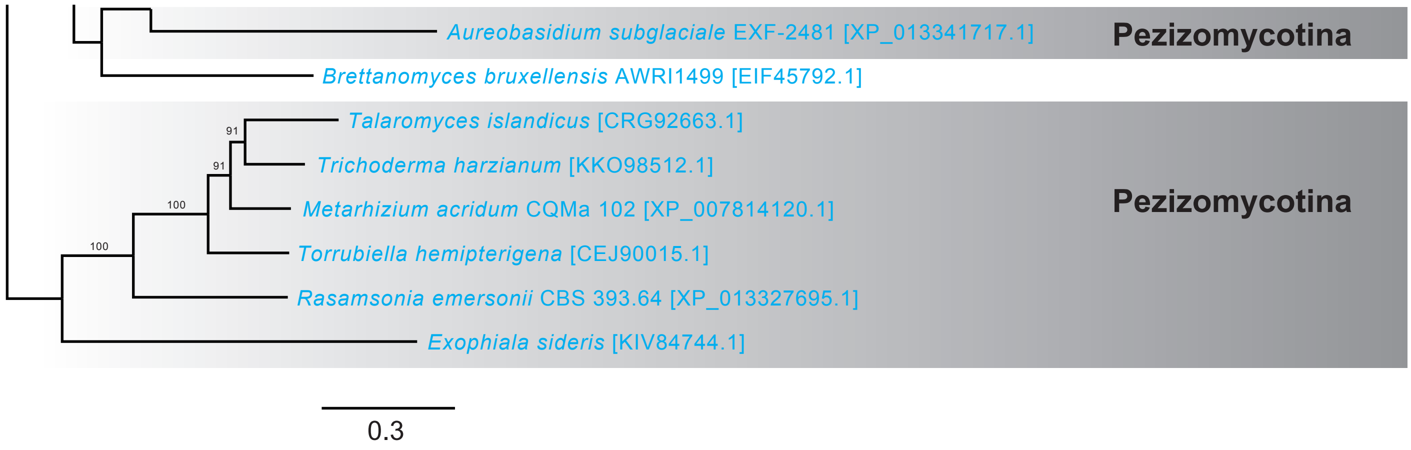
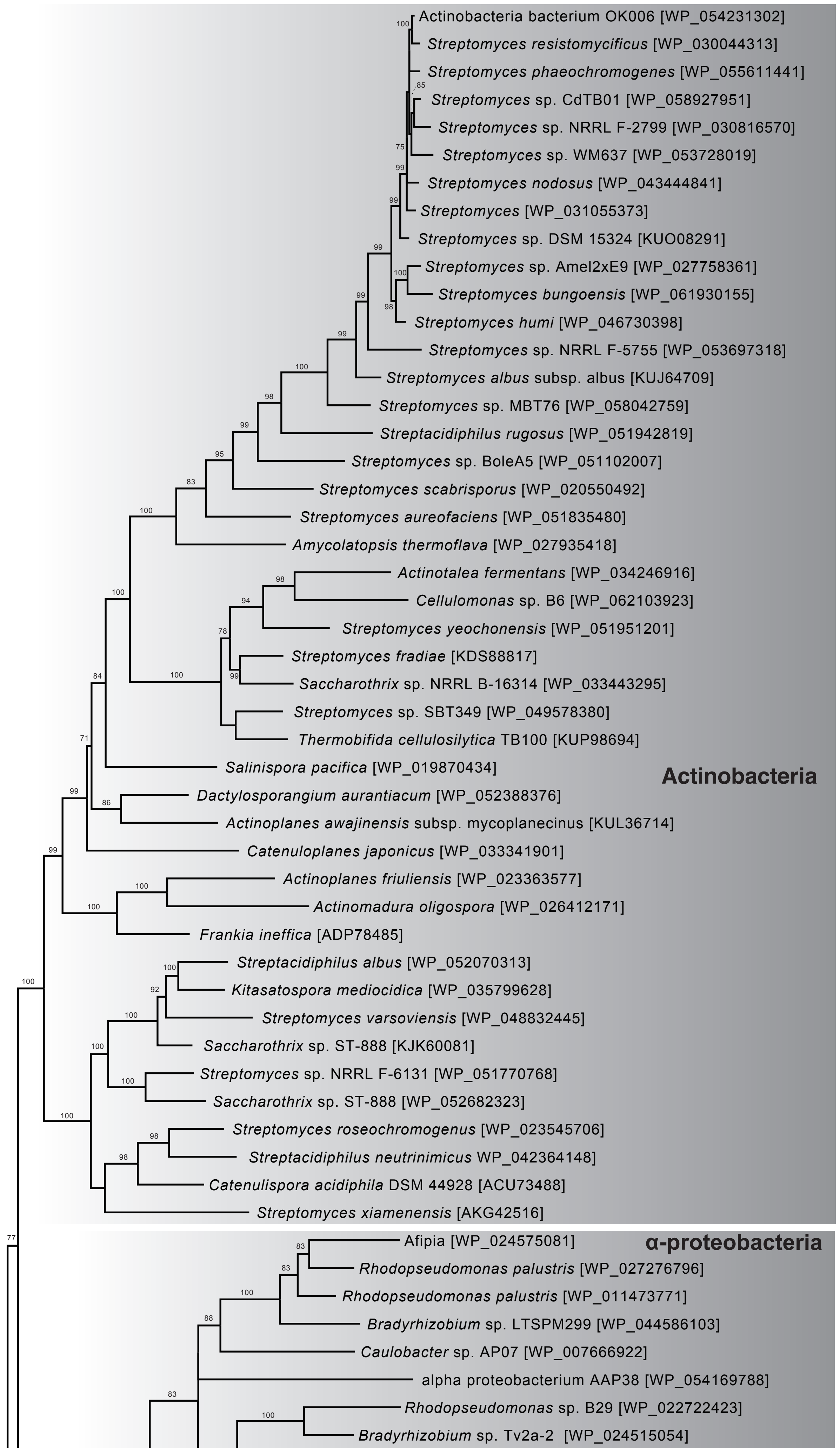
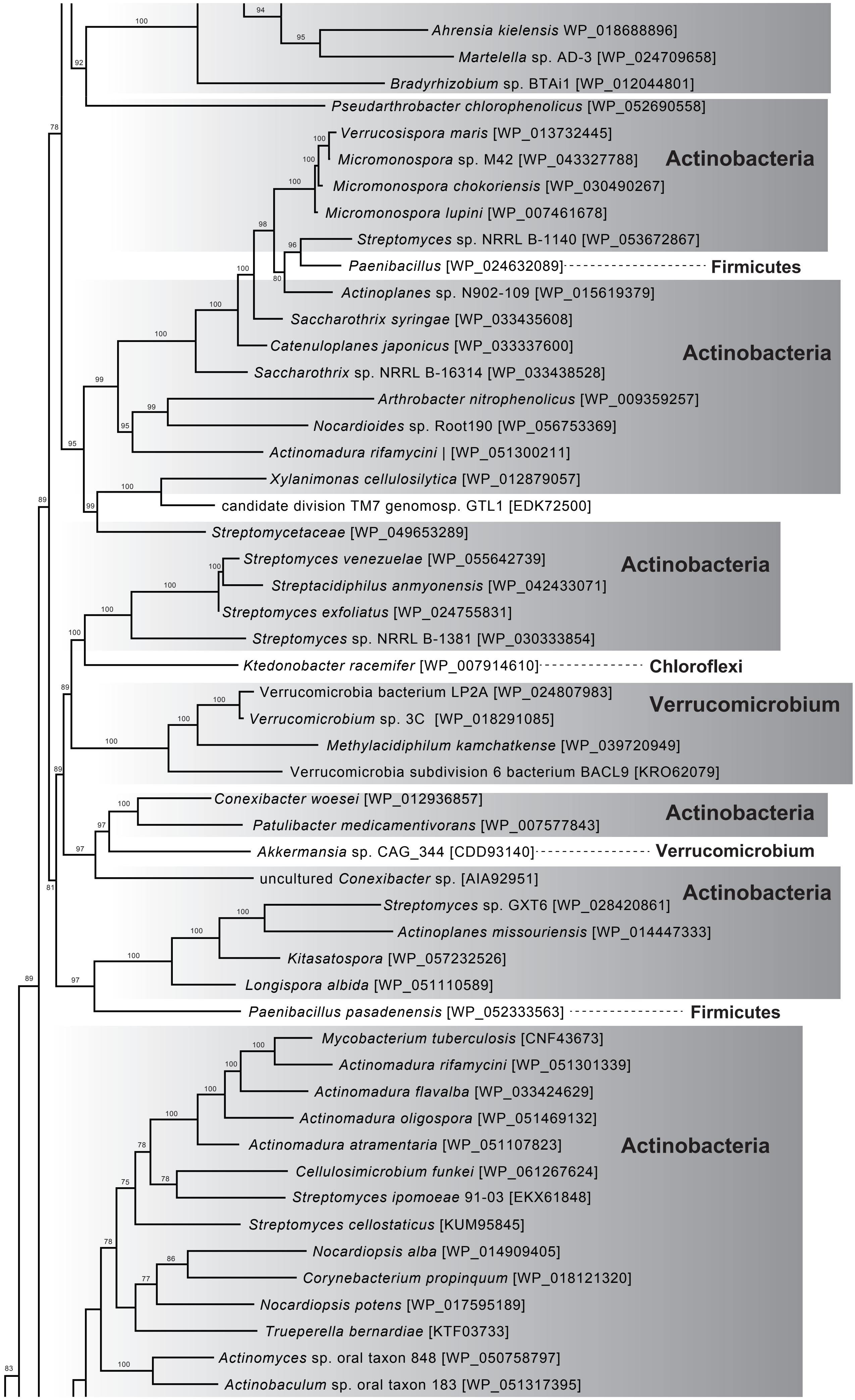


Supplementary Figure 11. Unrooted maximum-likelihood (ML) tree of ***chitinase*****.** 357 *chitinase* sequences with 253 amino acid positions were submitted to maximum likelihood inference. The species belonging to land plant and fungi are colored in green and blue, respectively. The sequences from *Coptotermes formosanus* symbionts are indicated by red font. The accession number of each operational taxonomic unit is indicated in parentheses. Only the bootstrap probabilities greater than 70 are given above the node. Other details are the same as in the legend for Fig. 5b.

Supplementary Figure 12



Supplementary Figure 12(continued)



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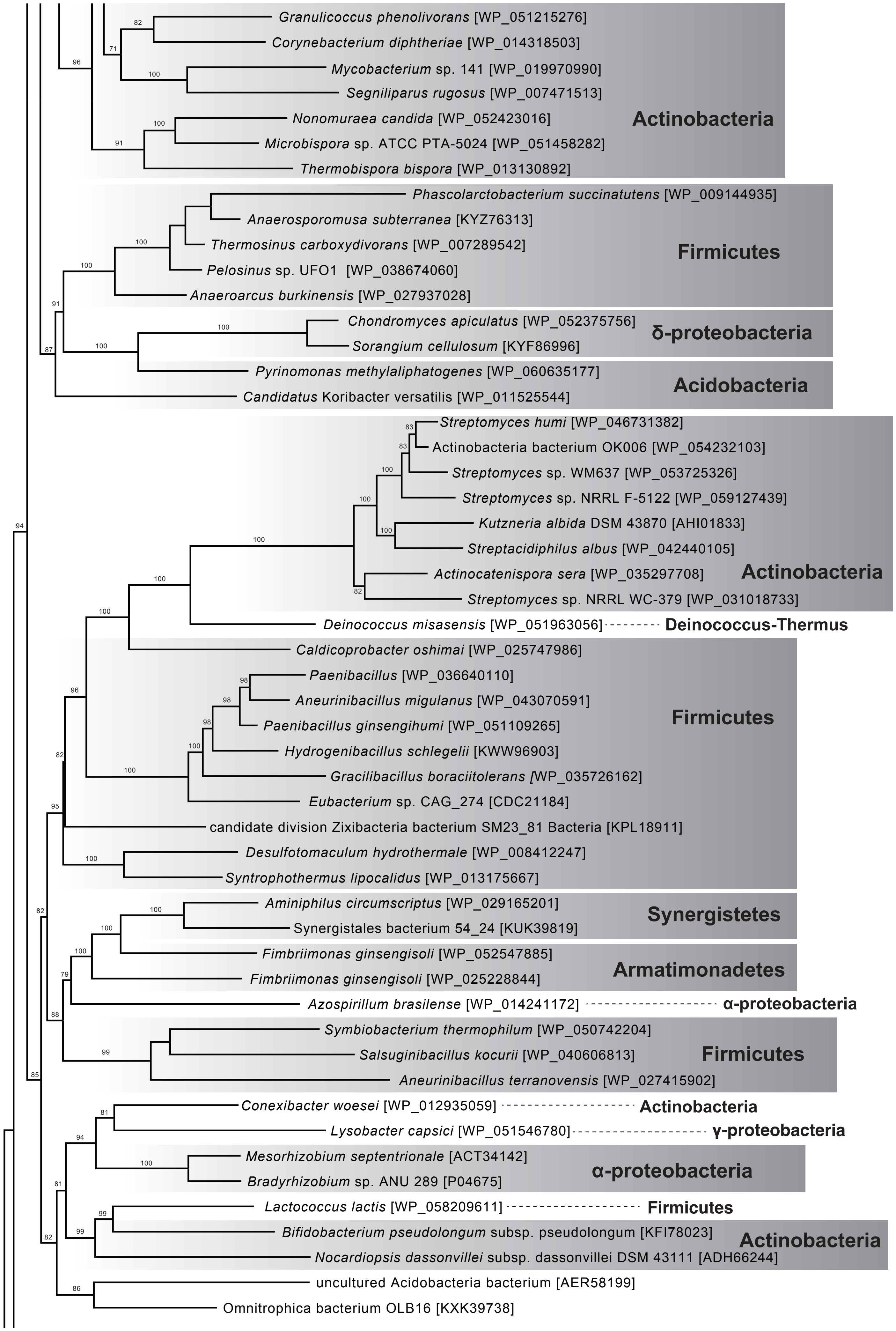

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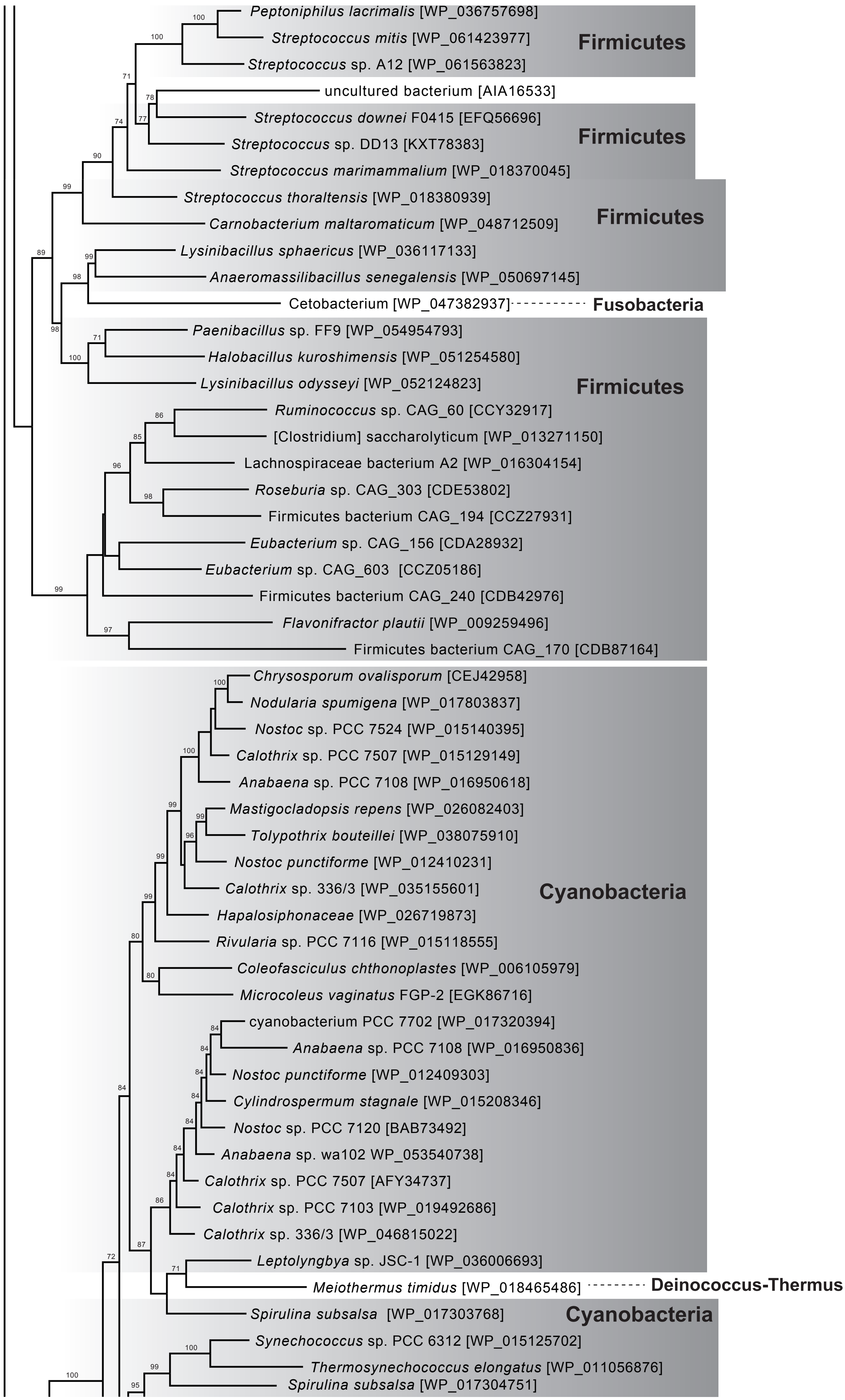

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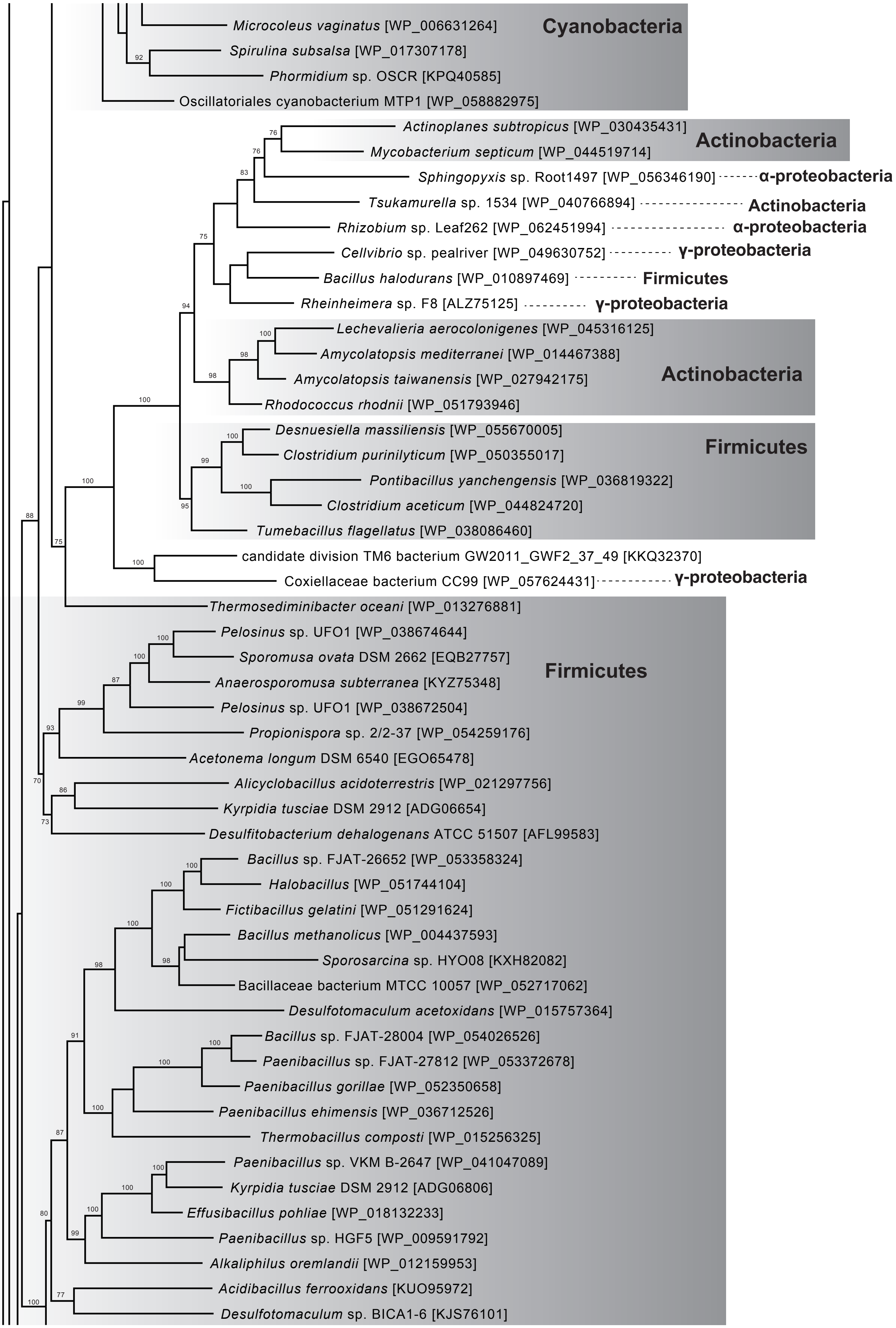

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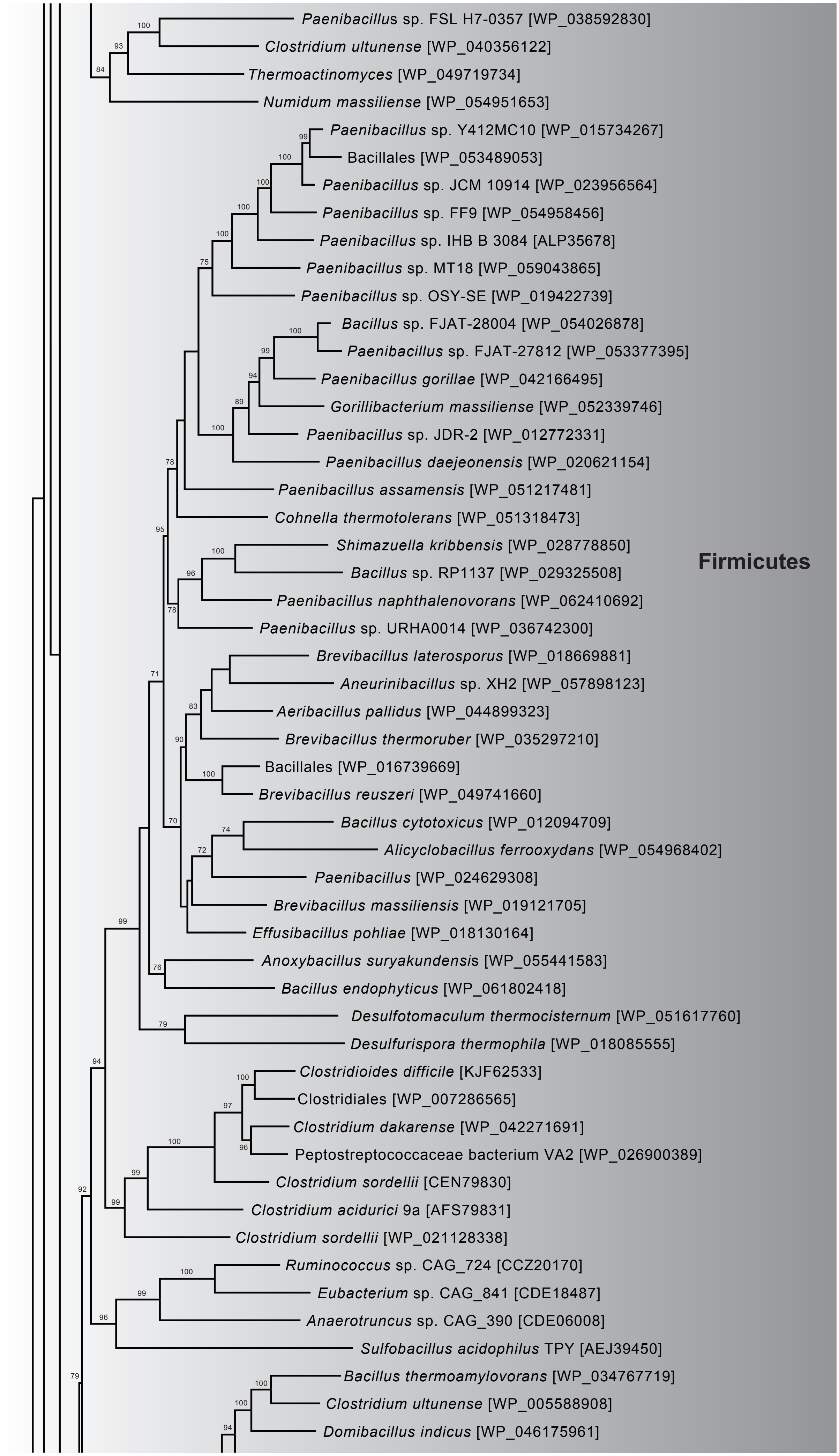

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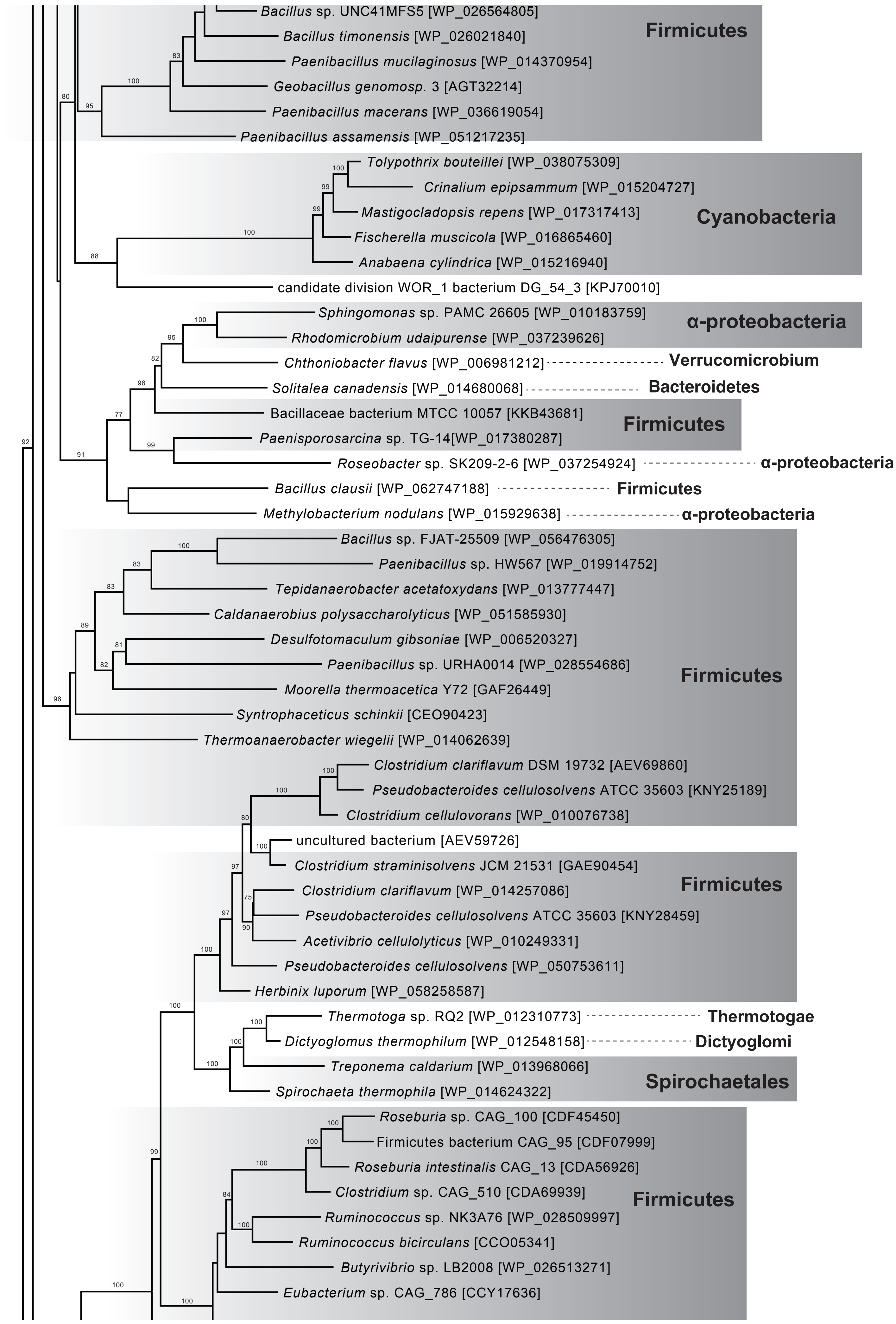

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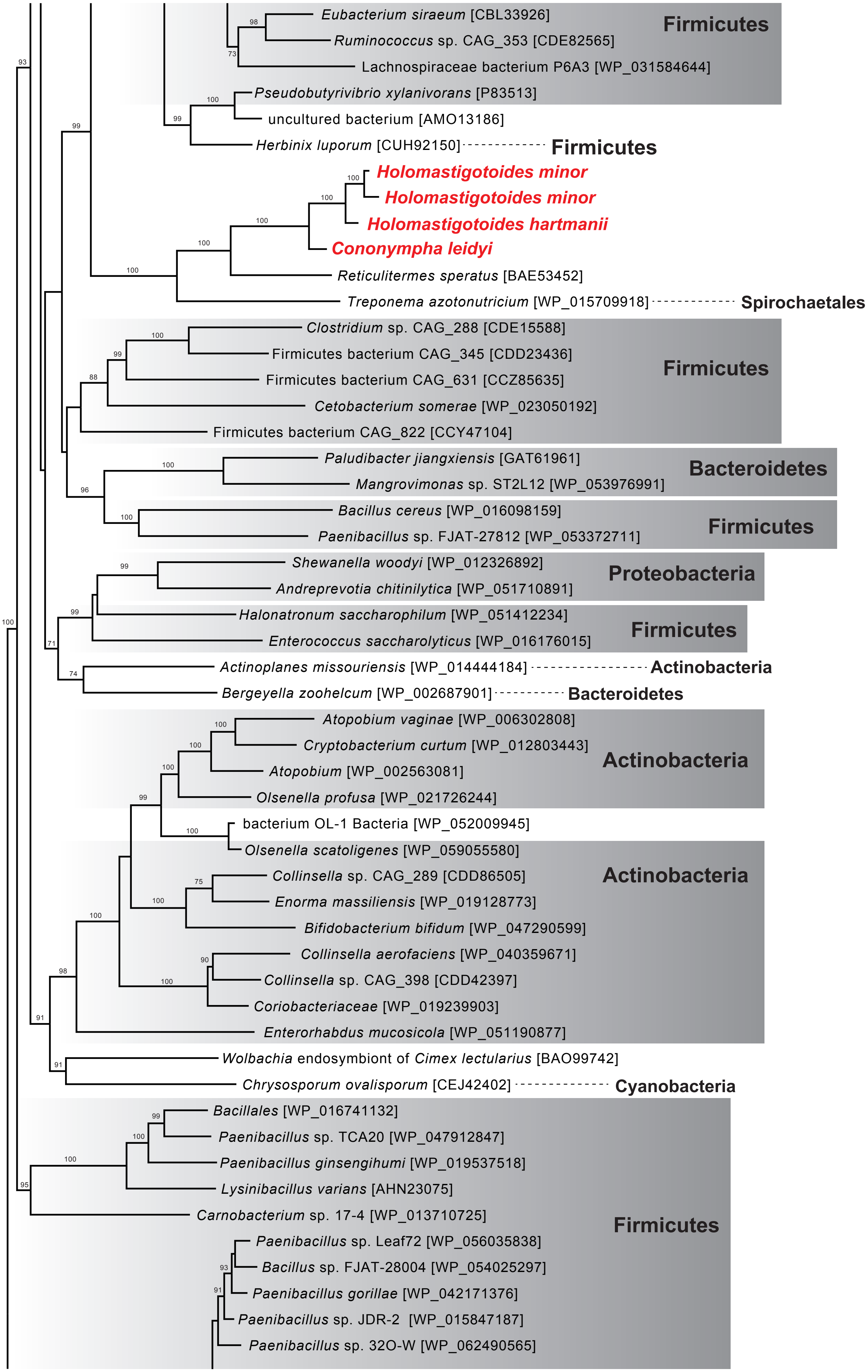

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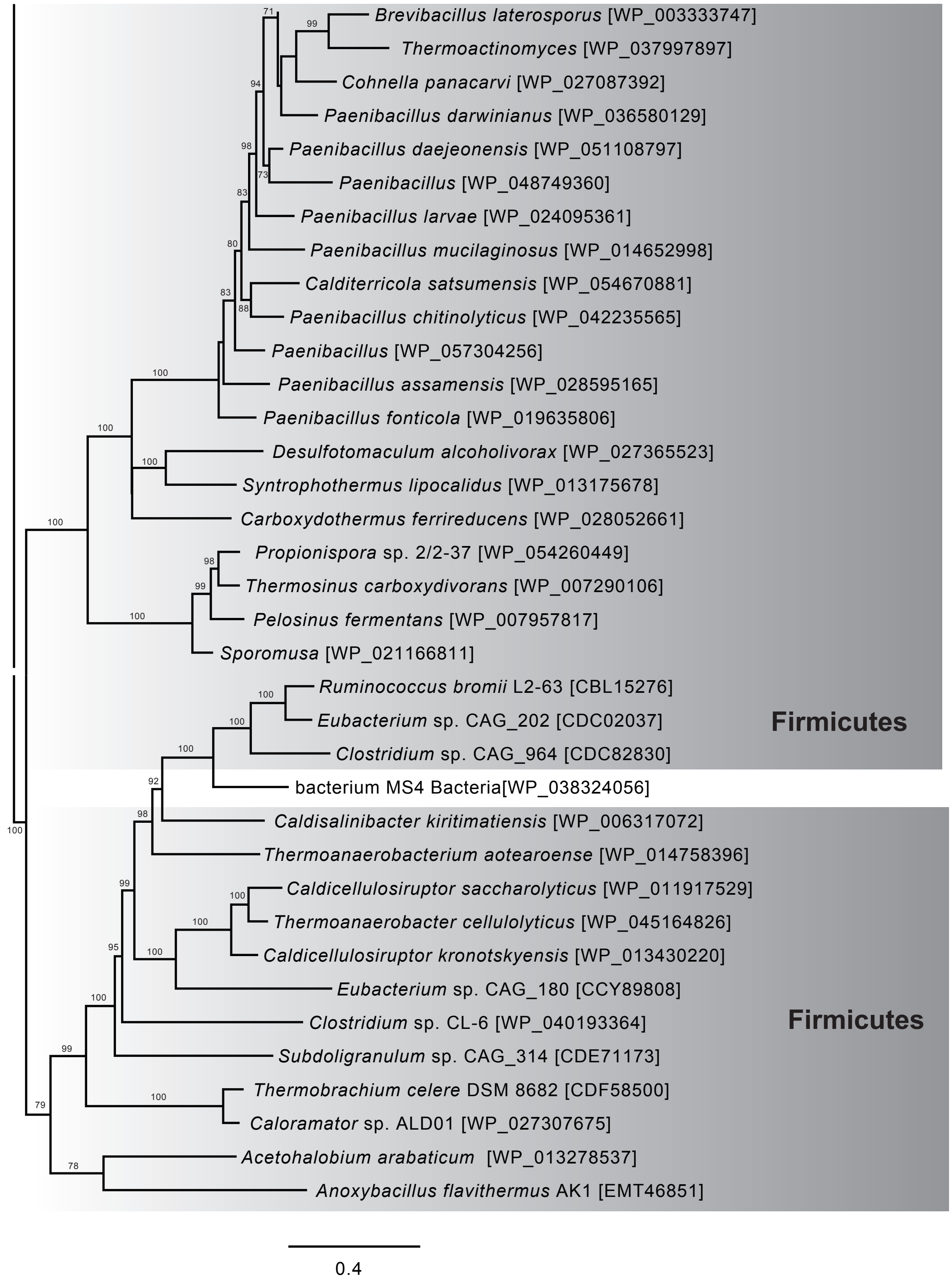

Supplementary Figure 12 Maximum likelihood (ML) tree of *nodB* gene. 453 sequences with 183 amino acid positions were analyzed by the ML method. The sequences from *Coptotermes formosanus* symbionts are colored in red. The accession number of each operational taxonomic unit is indicated in parentheses. Only the bootstrap probabilities greater than 70 are given above the node. Other details are the same as in the legend for Fig. 5c.