Supplementary Materials
to **"An update on clostridial phylogeny: Gram-negative spore-formers and other misplaced clostridia"** by **Natalya Yutin** and **Michael Y. Galperin**

Phylogenetic tree construction

The phylogenetic tree in Fig. 1 and Fig. S1 was built from a concatenated alignment of 50 ribosomal proteins (L1-L7, L10, L11, L13-L24, L27-L29, L31-L36, S2-S20) with a total of 6,164 unambiguously aligned positions, essentially as described in (Yutin *et al.*, 2012). The ribosomal protein sequences used in (Yutin *et al.*, 2012) were supplemented with those from additional species, extracted from the RefSeq database (Pruitt *et al.*, 2012) and aligned using MUSCLE (Edgar, 2004). Positions including gaps in more than one-third of the sequences and positions with low information content were removed prior to tree computation, as described in Yutin *et al*. (Yutin *et al.*, 2008), which resulted in 6,164 unambiguously aligned positions. Preliminary maximum-likelihood trees were constructed from these alignments using the FastTree program (Price *et al.*, 2010) with default parameters (JTT evolutionary model, discrete gamma model with 20 rate categories). The preliminary tree and the alignment were then used in ProtTest (Darriba *et al.*, 2011) to determine the best substitution matrix. The final maximum-likelihood tree was constructed using TreeFinder [1,000 replicates, Search Depth 2 (Jobb *et al.*, 2004)], with the LG+G substitution matrix (which was found to be the best for a given alignment). The Expected-Likelihood Weights (ELW) of 1,000 local rearrangements were used as confidence values of TreeFinder tree branches.

The maximum-likelihood phylogenetic trees for RpoB (Fig. S2) and GyrB (Fig. S3) protein sequences have been constructed in the same way, from the alignments containing 1,154 and 631 unambiguously aligned positions, respectively. The only difference was the use of the LG+G+F substitution matrix, which was selected by ProTest as the best for both RpoB and GyrB alignments.

References

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Yutin, N., Makarova, K.S., Mekhedov, S.L., Wolf, Y.I., and Koonin, E.V. (2008) The deep archaeal roots of eukaryotes. *Mol Biol Evol* **25**: 1619-1630.

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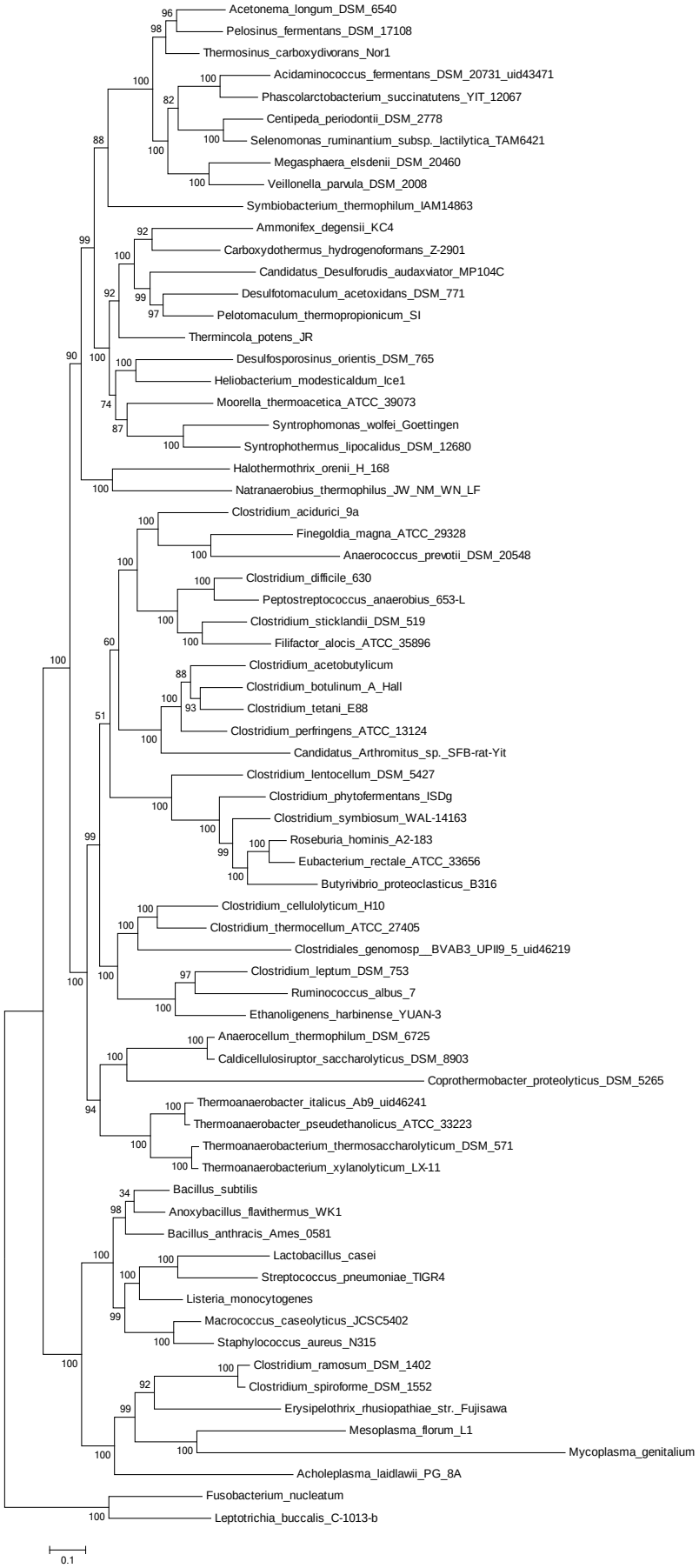
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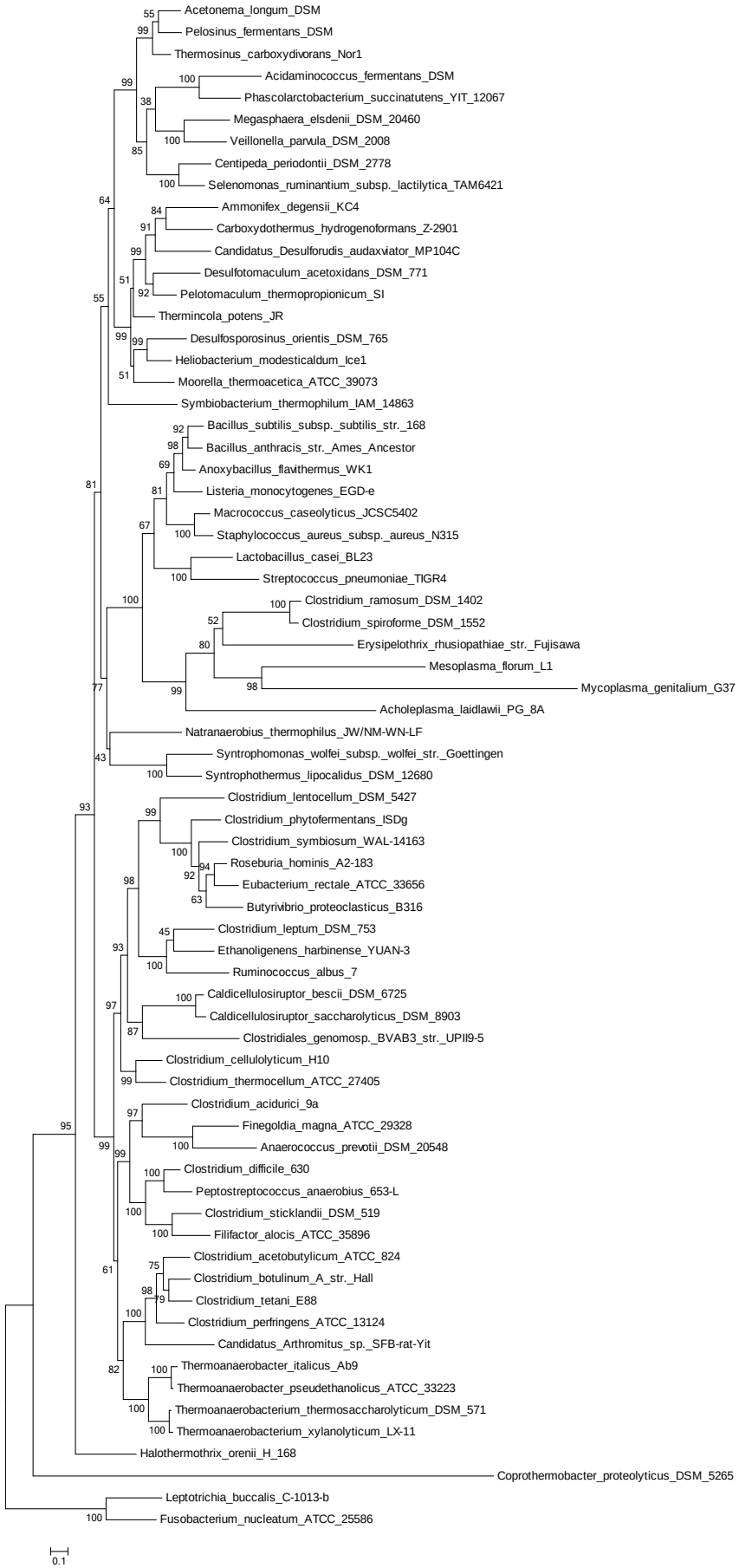
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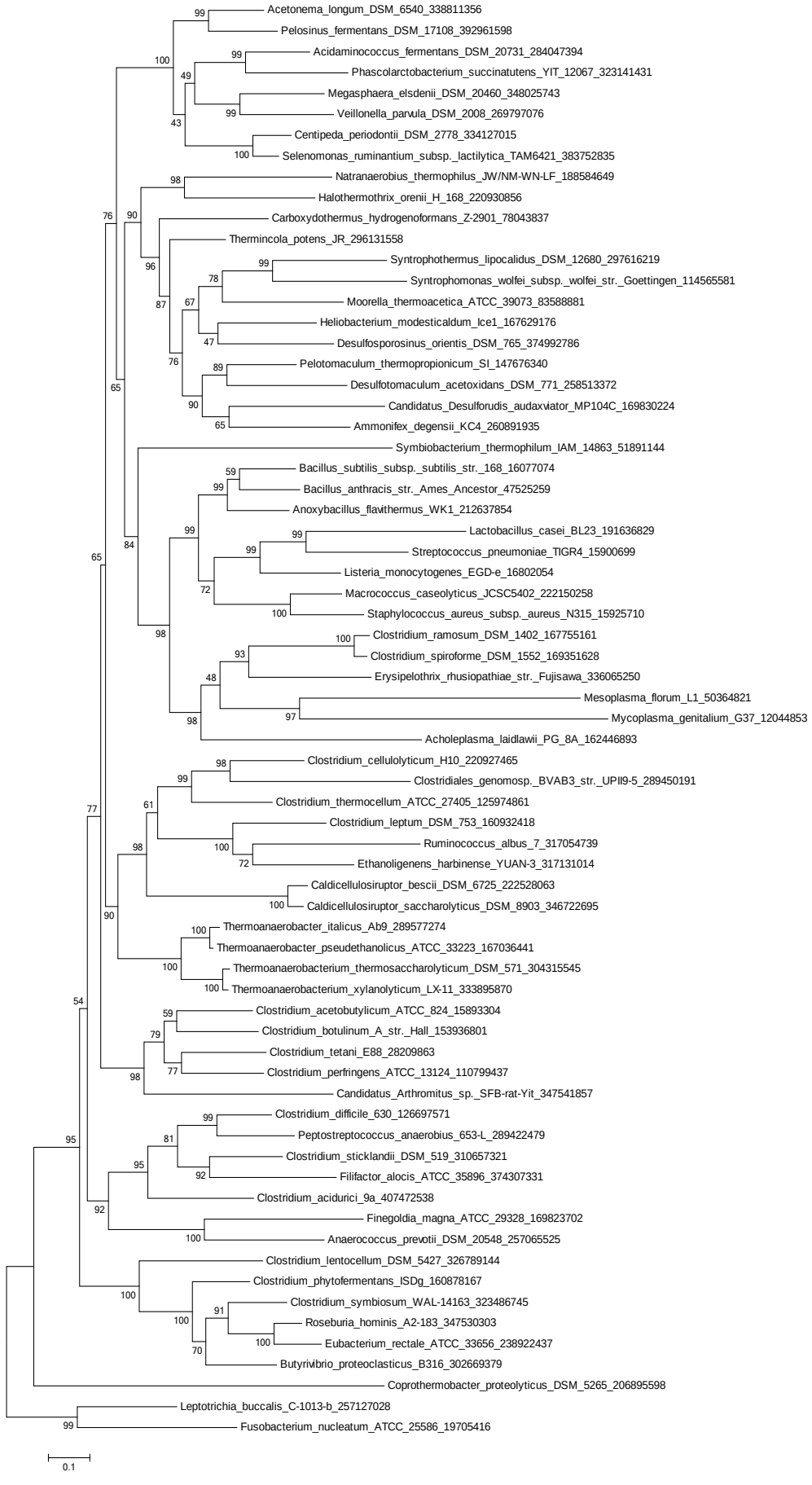
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