

Convergent patterns in the evolution of mealybug symbioses involving different intrabacterial symbionts

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Supplementary Information

Supplementary Materials and Methods

Microscopy

Mealybugs were fixed in 4% paraformaldehyde solution for 4 hours at 4°C, washed twice in 1:1 solution of PBS and ethanol, and stored in this solution at 4°C on the day of sampling for subsequent fluorescence *in situ* hybridization (FISH). FISH was performed on dissected specimens as described earlier (Toenshoff *et al.*, 2012). We employed a general bacterial probe (EUB-338I: 5'-GCT GCC TCC CGT AGG AGT-3') (Amann *et al.*, 1990), a general gammaproteobacterial probe (Gam42a: 5'-GCC TTC CCA CAT CGT TT-3') and an unlabeled competitor probe (Bet42a: 5'-GCC TTC CCA CTT CGT TT-3') (Manz *et al.*, 1992) together with a probe specific to the 16S rRNA of the gammaproteobacterial symbiont of *T. manniapara* (Trabutinella-300: 5'-CAG TGT GGC TGT TTA TCC-3') using 20v/v% formamide in the hybridization buffer. Samples were hybridized for at least 1.5 hours and were analyzed on a Leica Sp8 confocal laser-scanning microscope.

Frozen egg sacs of *T. mannipara* stored at -80°C were dissected and individual insects were fixed under vacuum in 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1M phosphate buffer at pH 7.2 for two days at 4°C. The mealybugs were cut open on the sides to improve the penetration of the fixative. Samples were then washed in the buffer under vacuum for 4h and post-fixed in 2% osmium tetroxide in 0.1M phosphate buffer. They were subsequently dehydrated in an ethanol series, substituted in acetone and embedded in Agar Low Viscosity Resin®. Polymerization was performed at 60°C for two days. Semi-thin sections (0.5µm) were cut on a Leica® EM UC7, mounted on slides and stained

with a solution of 0.25% azure II, 0.25% methylene blue, 0.25% toluidine blue in 0.25% sodium borate. The slides were observed on a Zeiss® Axio plan-2 light microscope. For ultrastructure observation ultrathin sections (70 nm) were stained with 0.5% uranyl acetate and 3% lead citrate prior observations with a Zeiss® Libra 120 transmission electron microscope.

Genome annotation pipeline

The following steps were performed in the ConsPred genome annotation pipeline (Weinmaier *et al.*, unpublished; available at <https://sourceforge.net/p/conspred/>). Coding sequences (CDSs) were predicted based on intrinsic signals by Glimmer (Salzberg *et al.*, 1998), GeneMark (Lukashin & Borodovsky, 1998), Prodigal (Hyatt *et al.*, 2010) and Critica (Badger & Olsen, 1999), as well as based on sequence homology using blastx searches against the non-redundant protein database (nr) of the National Center for Biotechnology Information (NCBI). RNAmmer (Lagesen *et al.*, 2007), tRNA-scan SE (Lowe & Eddy, 1997), and Infernal (Nawrocki & Eddy, 2013) together with the Rfam database (Griffiths-Jones *et al.*, 2003) were applied to identify ribosomal RNAs, tRNAs and non-coding RNAs. CRISPR repeats were inferred by PILER-CR (Edgar, 2007). InterProScan annotated conserved domains in the consensus CDSs (Zdobnov & Apweiler, 2001). Functional assignment was according to the best blastp hits against the UniProt Swiss-Prot and TrEMBL databases (Bairoch *et al.*, 2005). Pathways and representation of main functional categories were predicted by sequence homology searches against the KEGG (Kanehisa & Goto, 2000) and eggNOG (Jensen *et al.*, 2008) databases, respectively.

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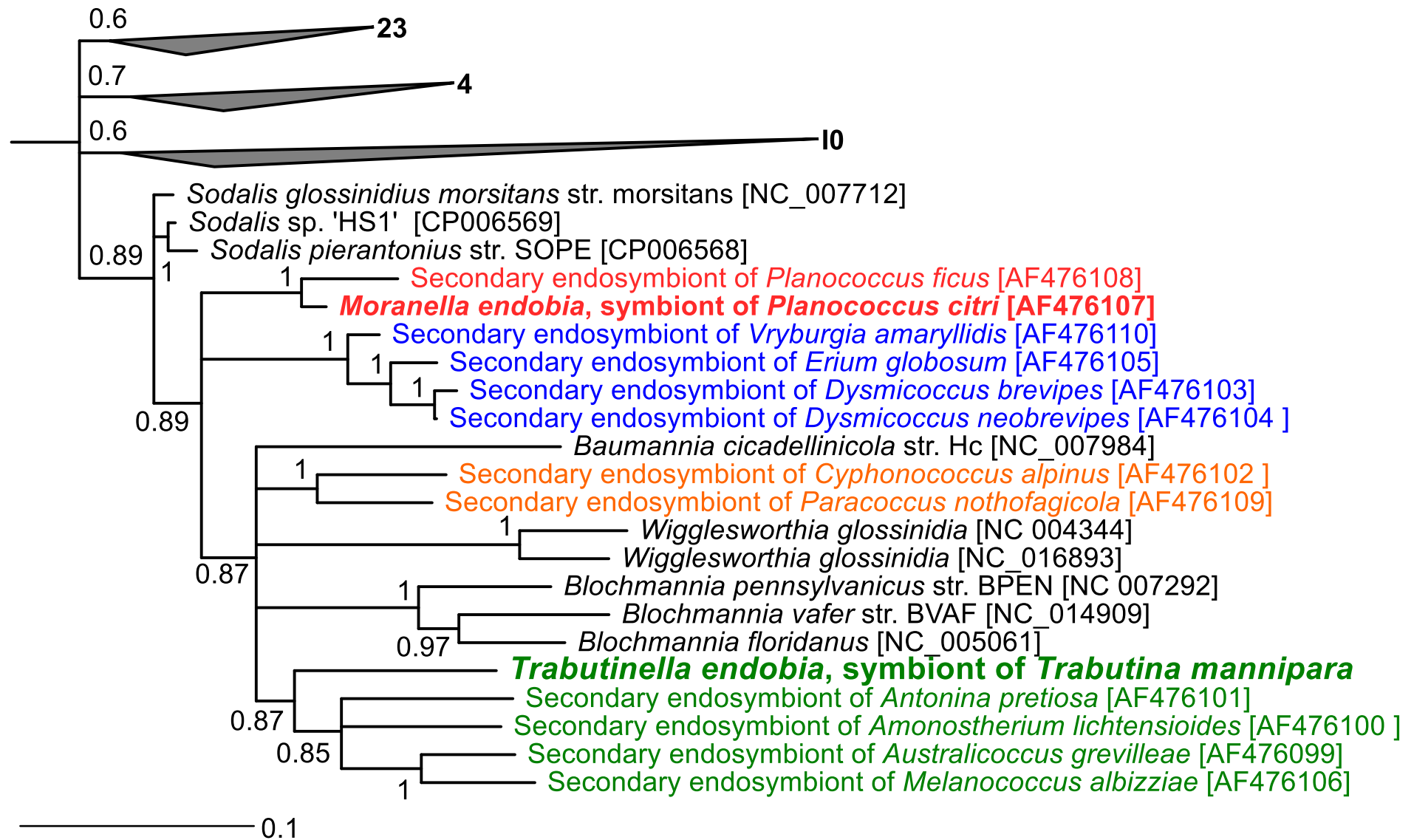


Figure S1. MrBayes tree based on 16S rRNA sequences showing the relation of ‘*Candidatus Trabutinella endobia*’ to other symbionts of mealybugs. *Trabutinella* groups with secondary symbionts of other mealybugs within the tribe Trabutinini. Selected members of non-enterobacterial *Gammaproteobacteria* were used as outgroups (NC_000907, NC_003902, AB194327, NC_002516, NC_002505).

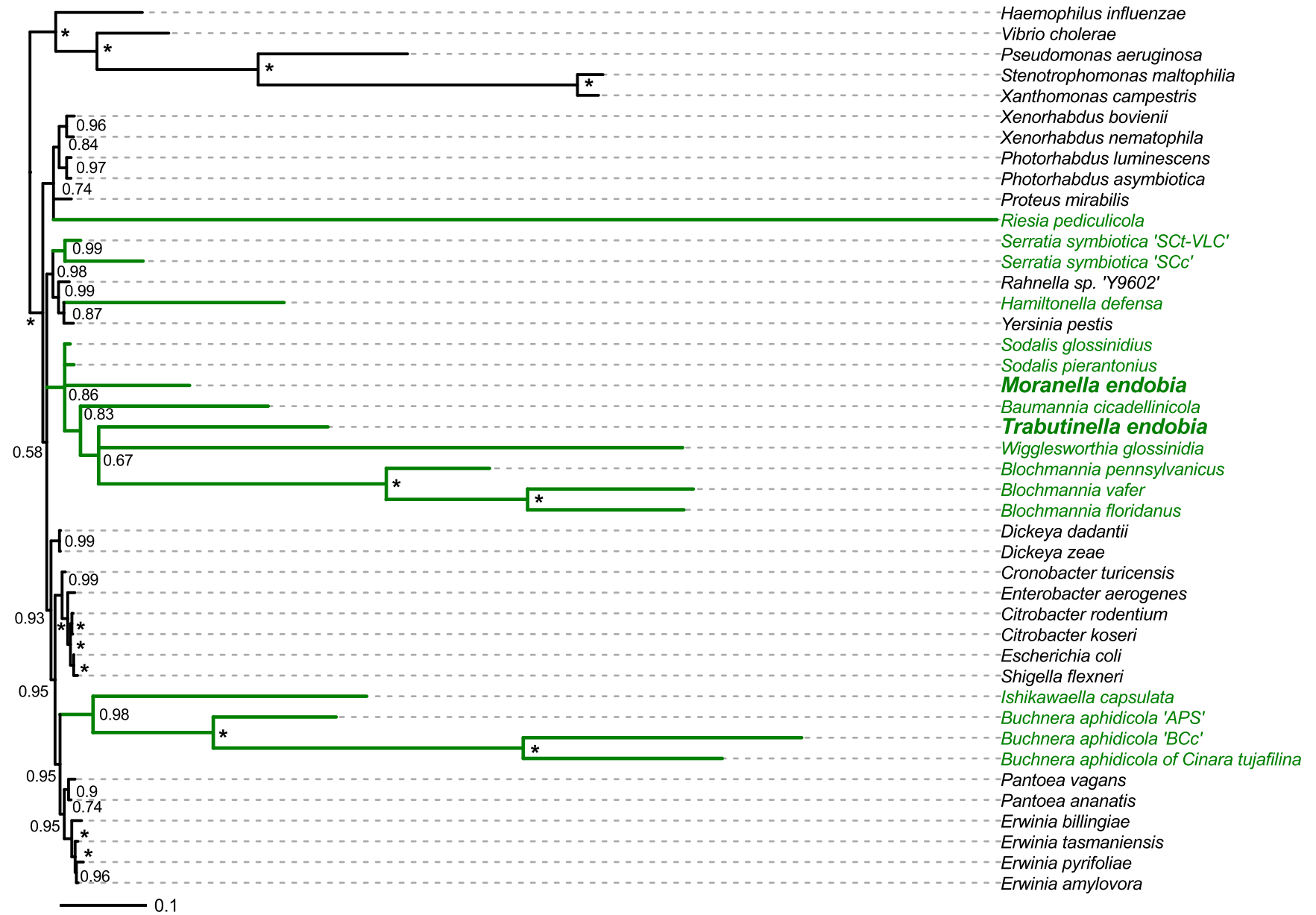


Figure S2. Phylobayes tree showing the relation of '*Candidatus Trabutinnella endobia*' to free-living bacteria and other insect symbionts. Insect symbiont lineages are colored in green. The analysis is based on a concatenated set of 34 ribosomal proteins. Posterior probabilities are shown on the internal nodes; asterisks imply posterior probabilities equal to 1. Nodes with a support of 0.5 or below are collapsed.

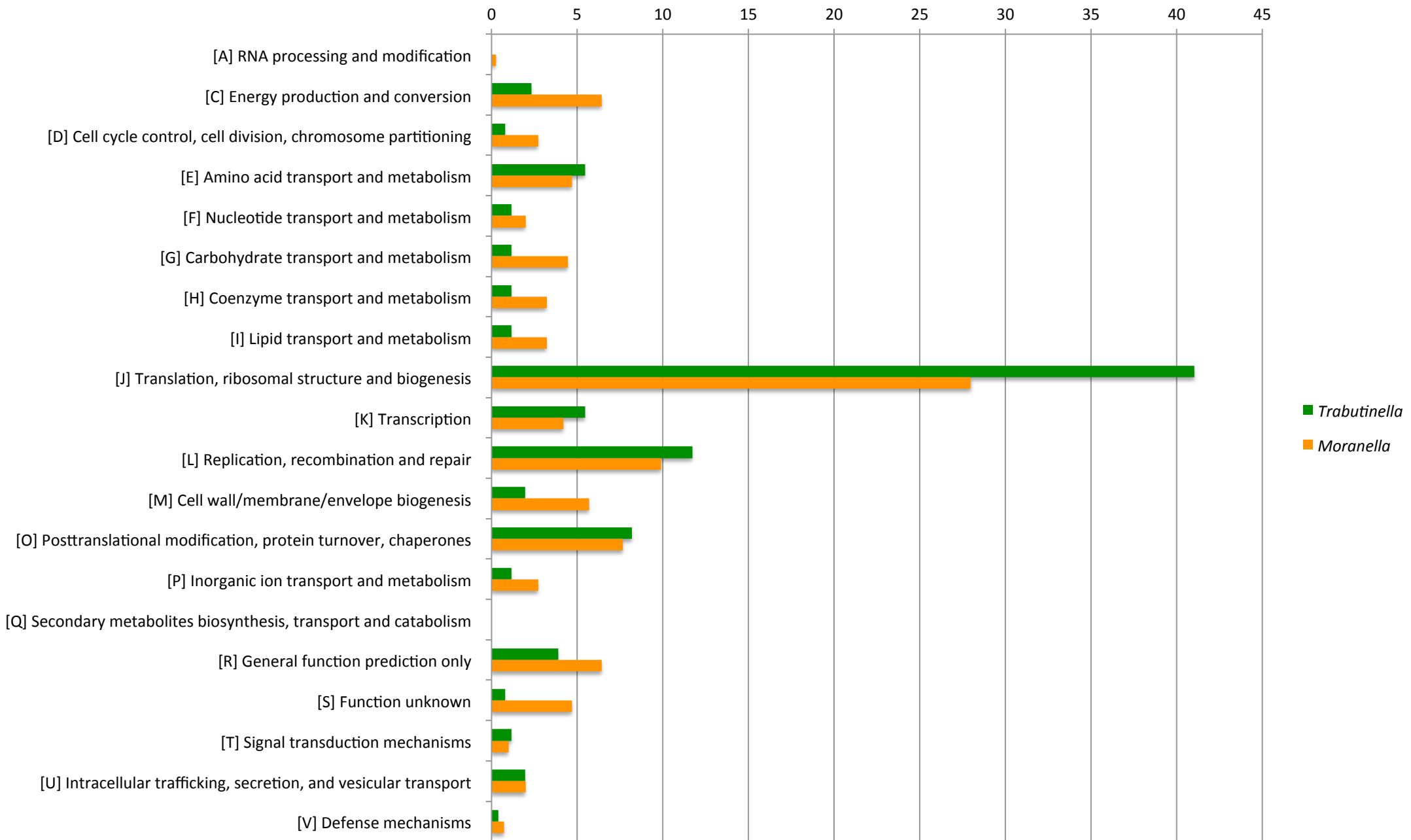


Figure S3. Percental distribution of genes among main functional categories according to the EggNOG classification in *Trabutinnella* versus *Moranella*

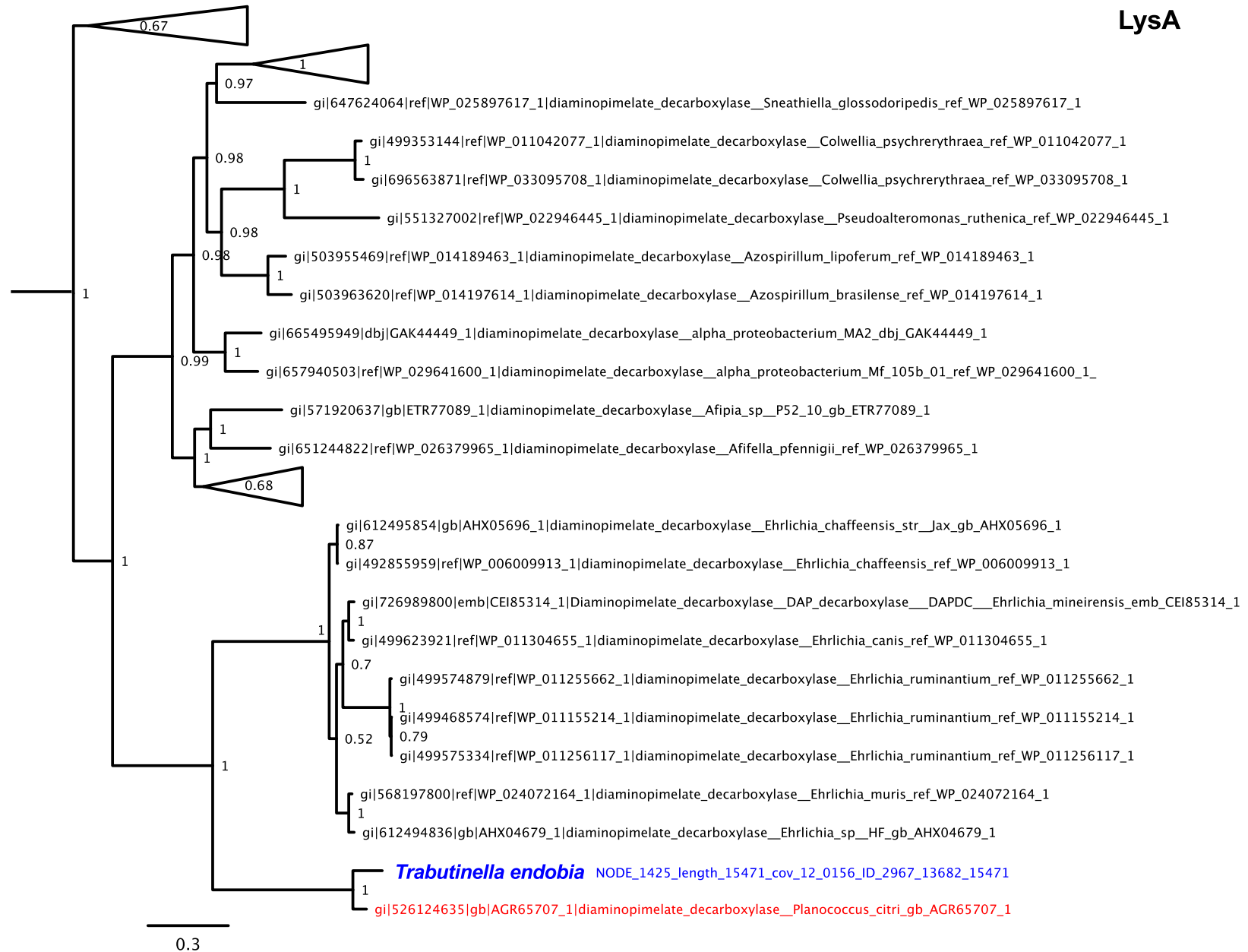


Figure S4 (A-I) MrBayes trees showing the phylogenetic relationships of the products of *lysA*, *dapF*, *cysK*, *ribA*, *ribD*, *bioA*, *bioB*, *bioD* and *tmsI*, horizontally acquired genes in the genome of *Trabutina mannipara*.

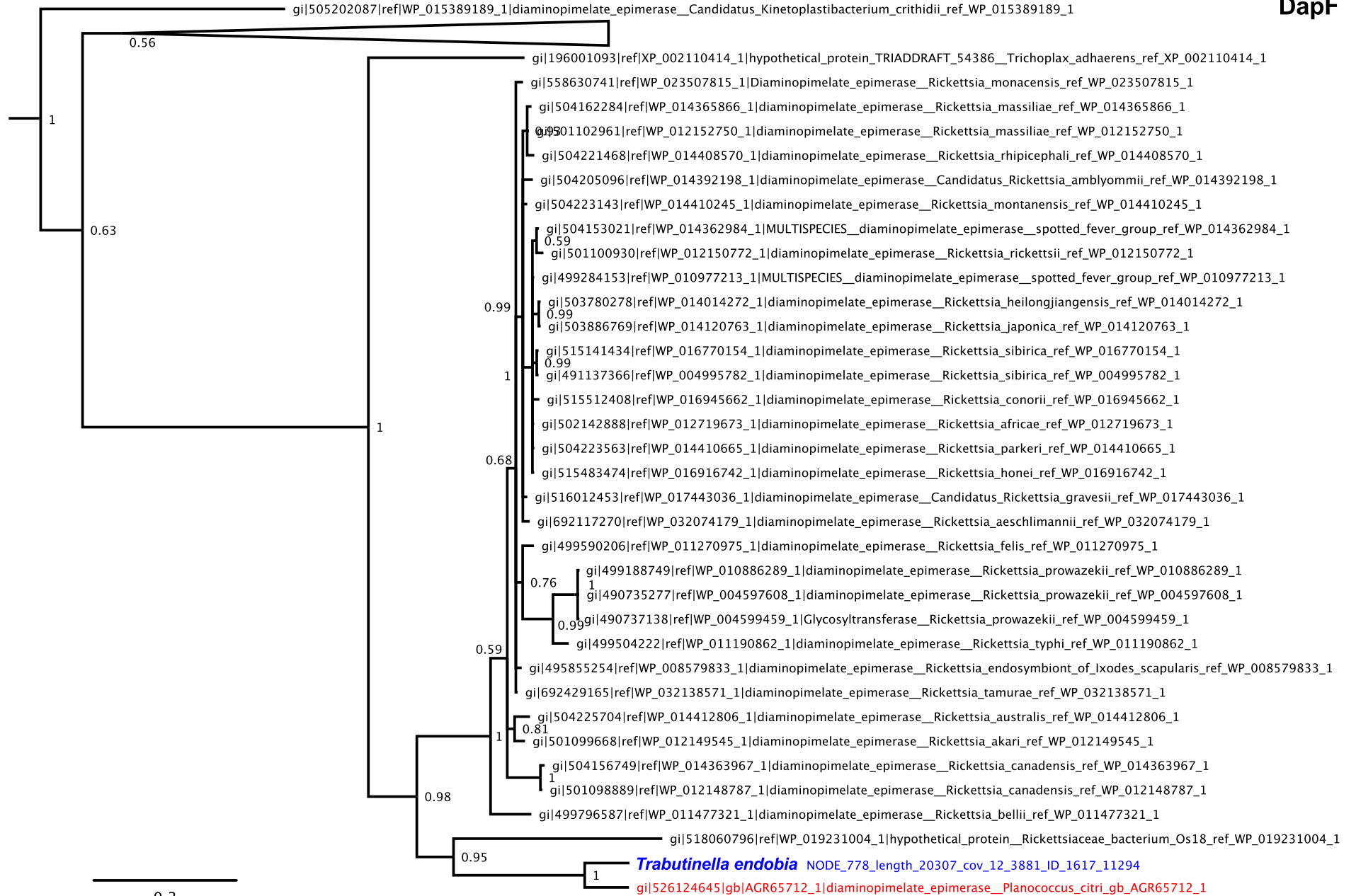


Figure S4B

CysK

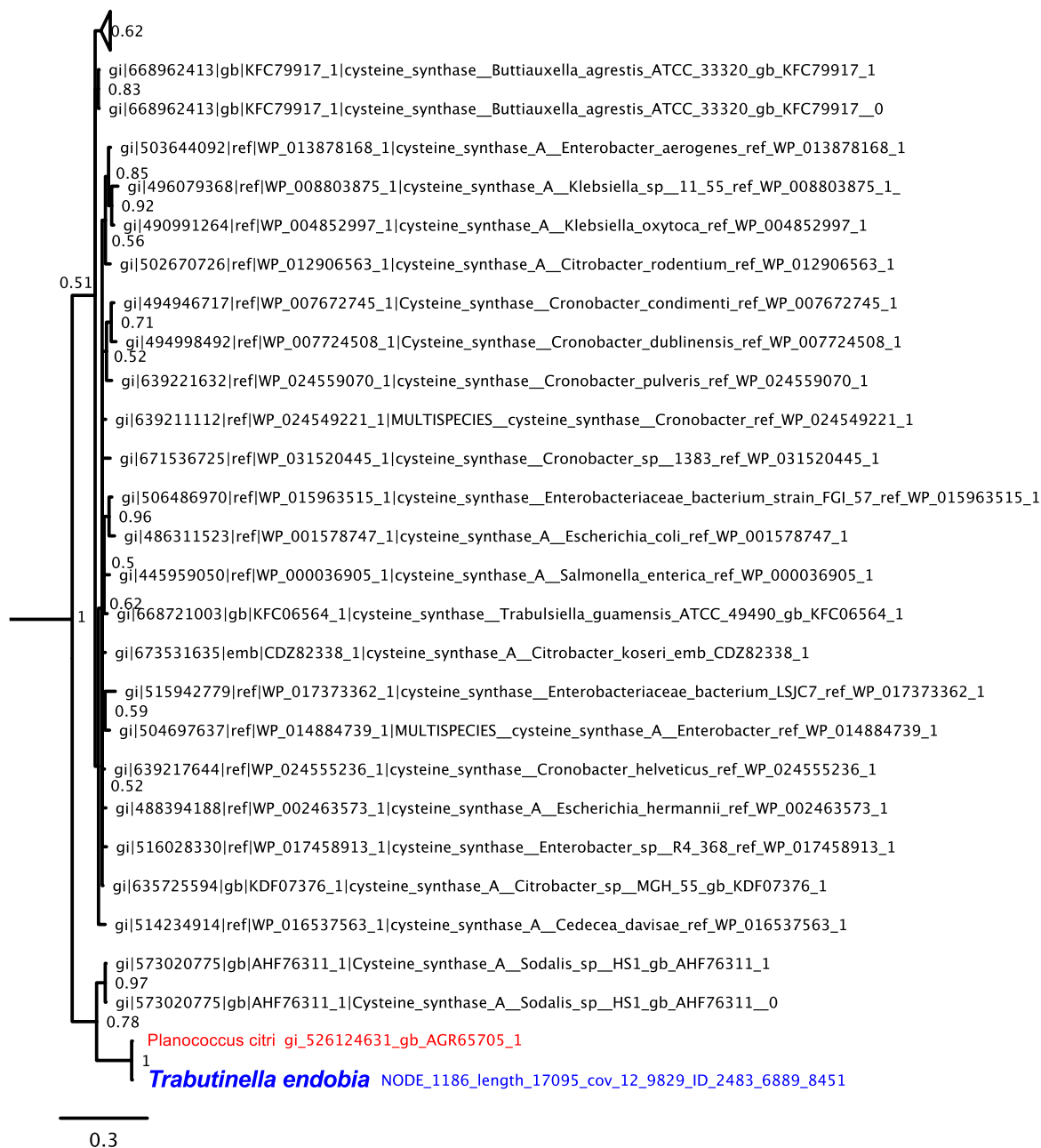


Figure S4C

RibA

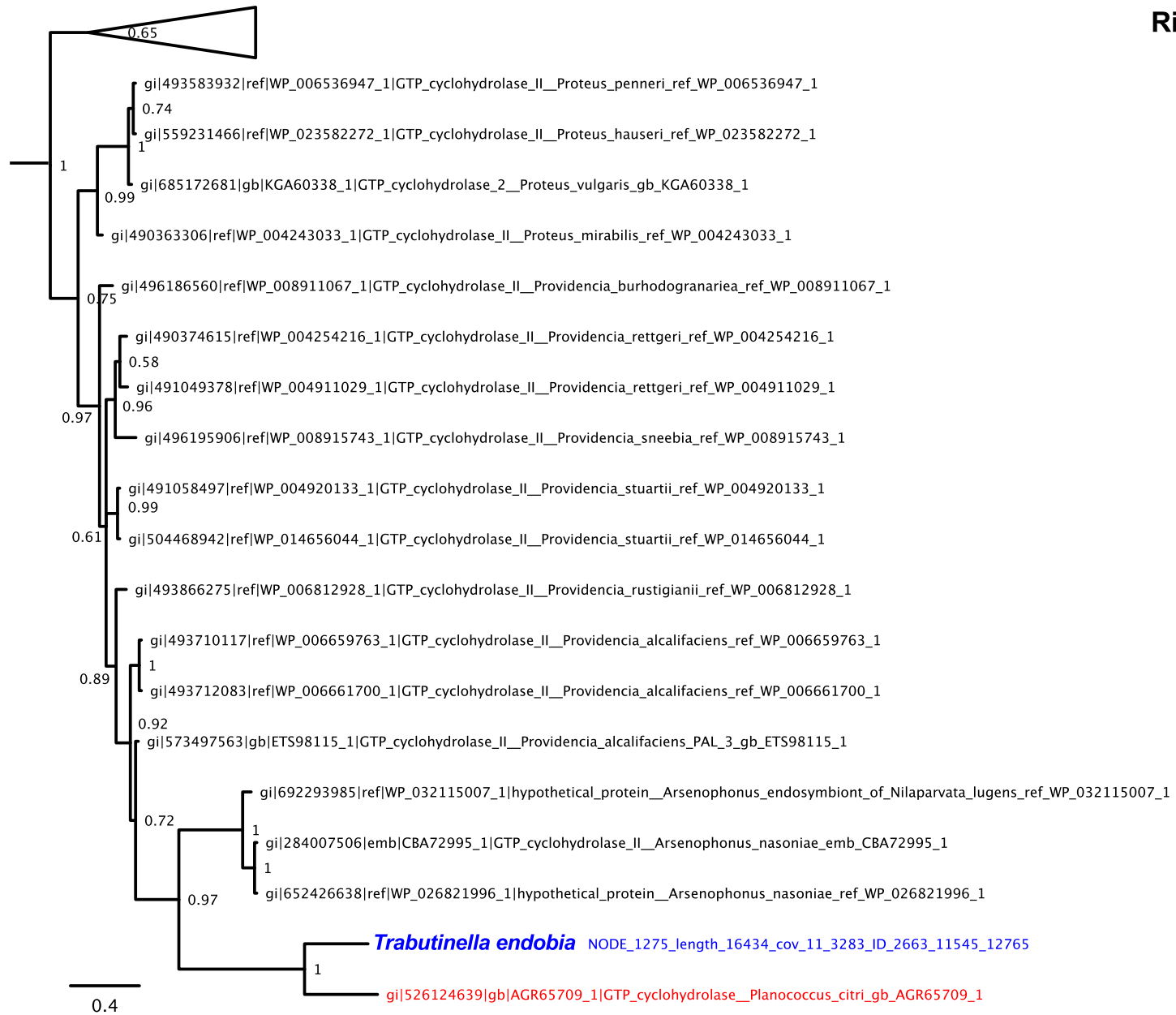


Figure S4D

RibD

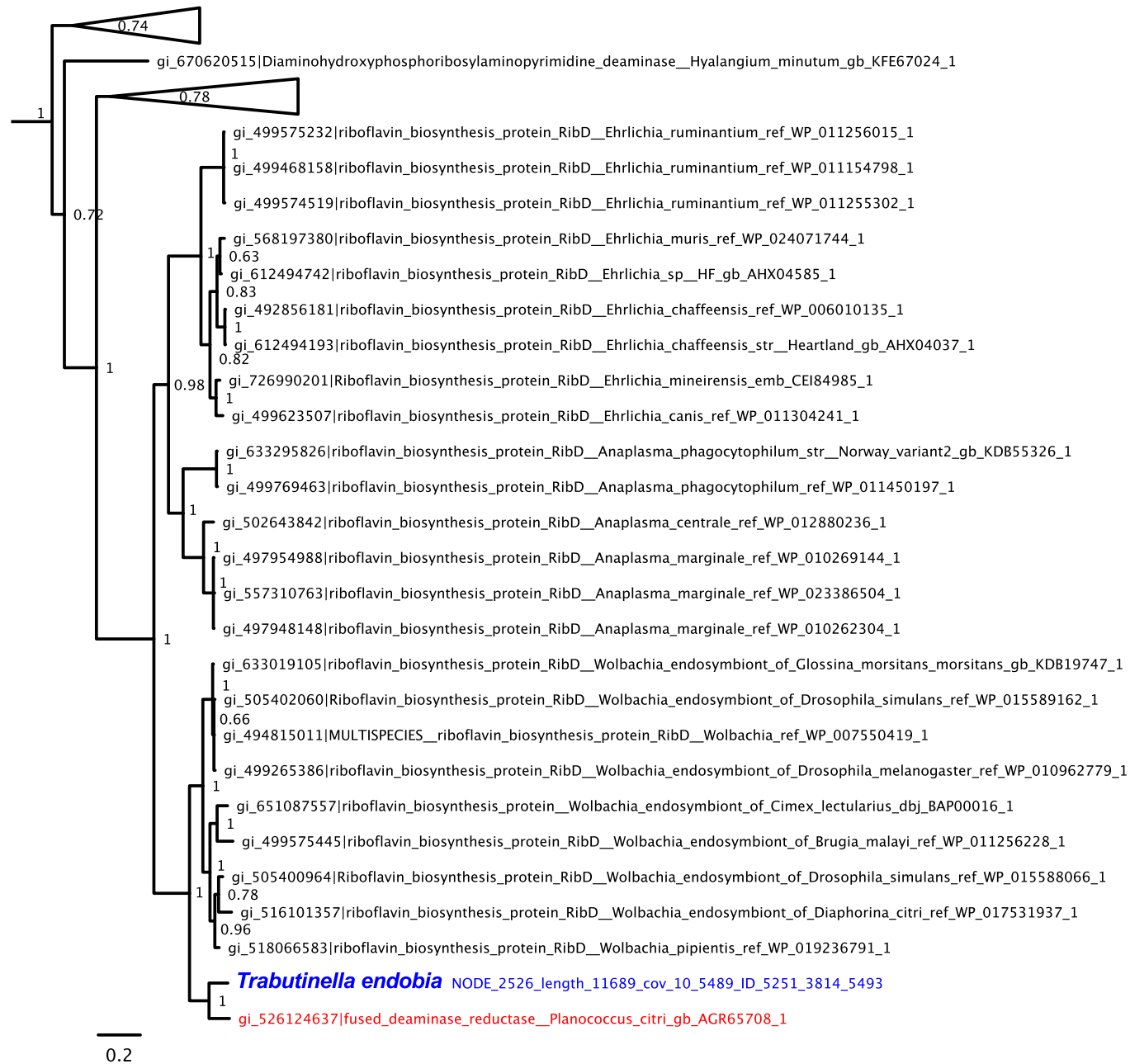


Figure S4E

BioA



Figure S4F

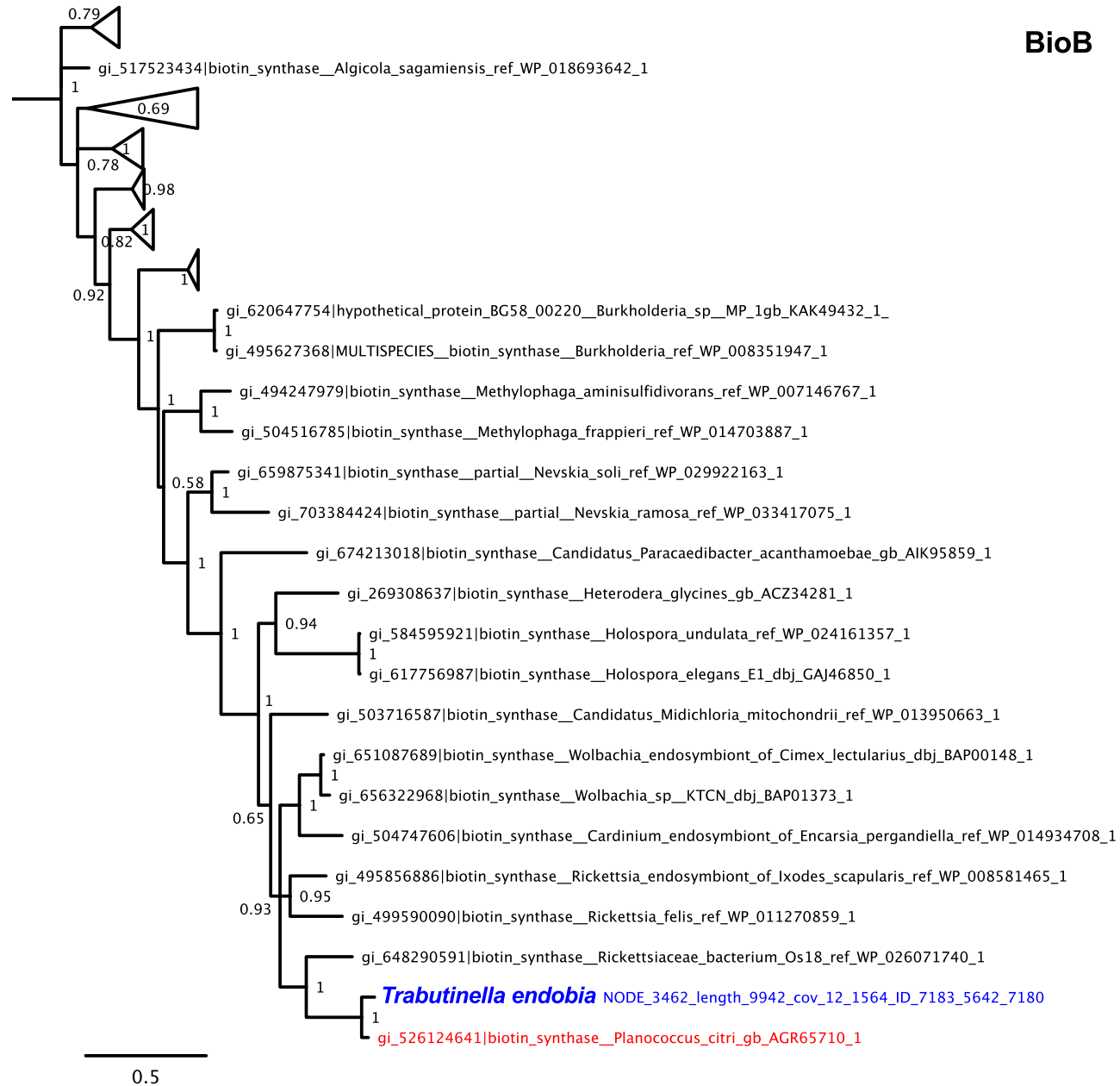


Figure S4G

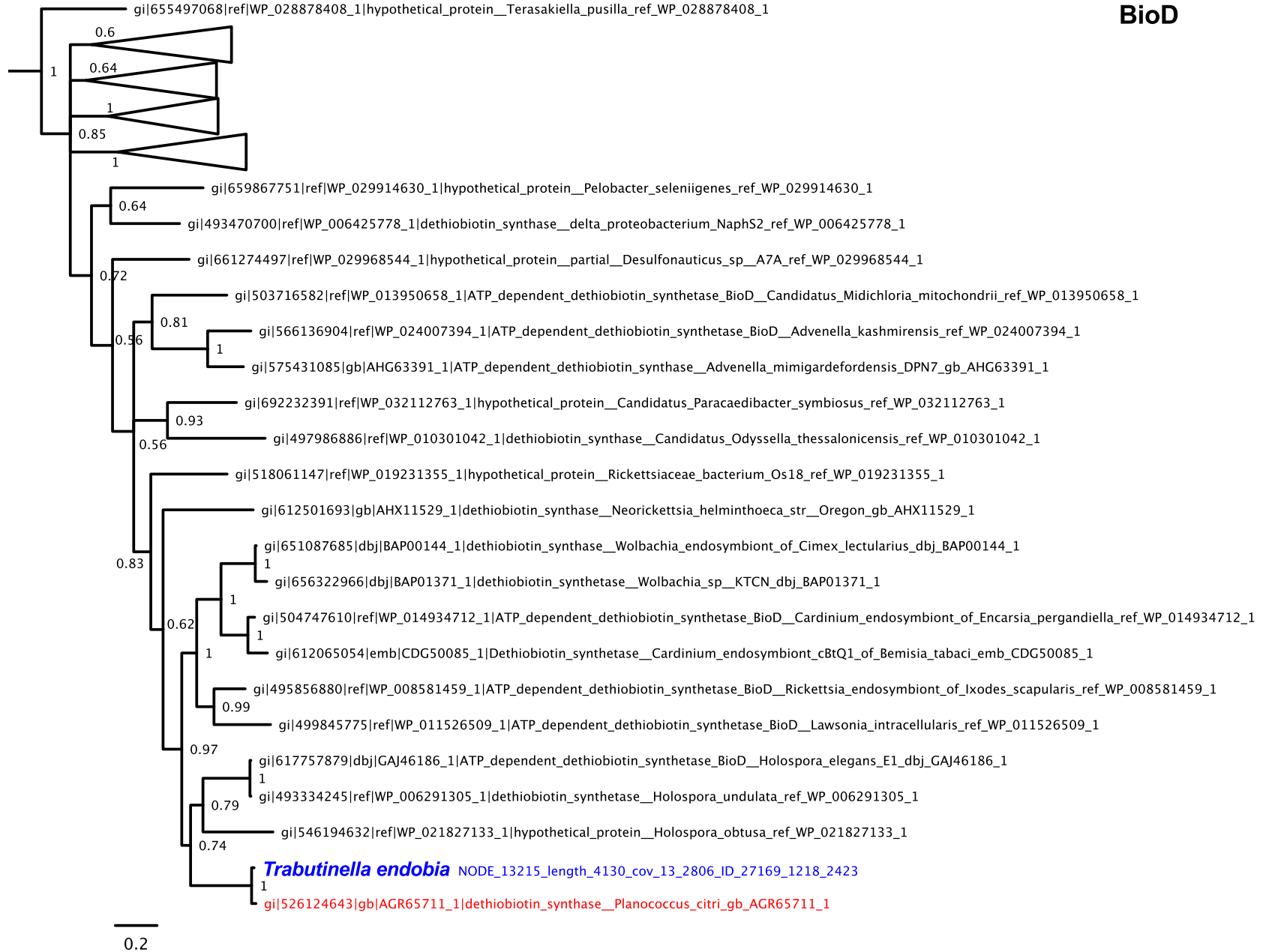


Figure S4H

Tms1

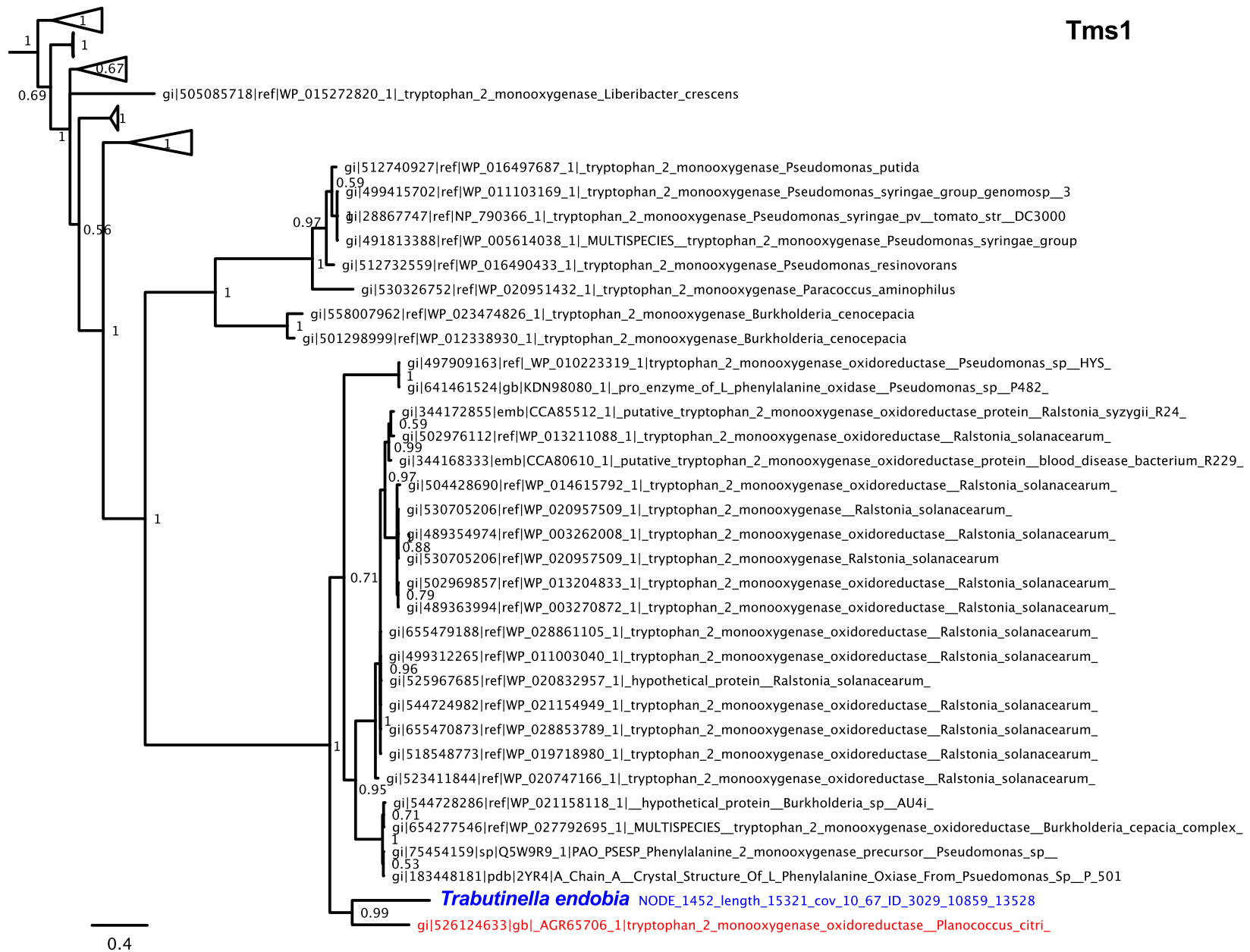


Figure S4I