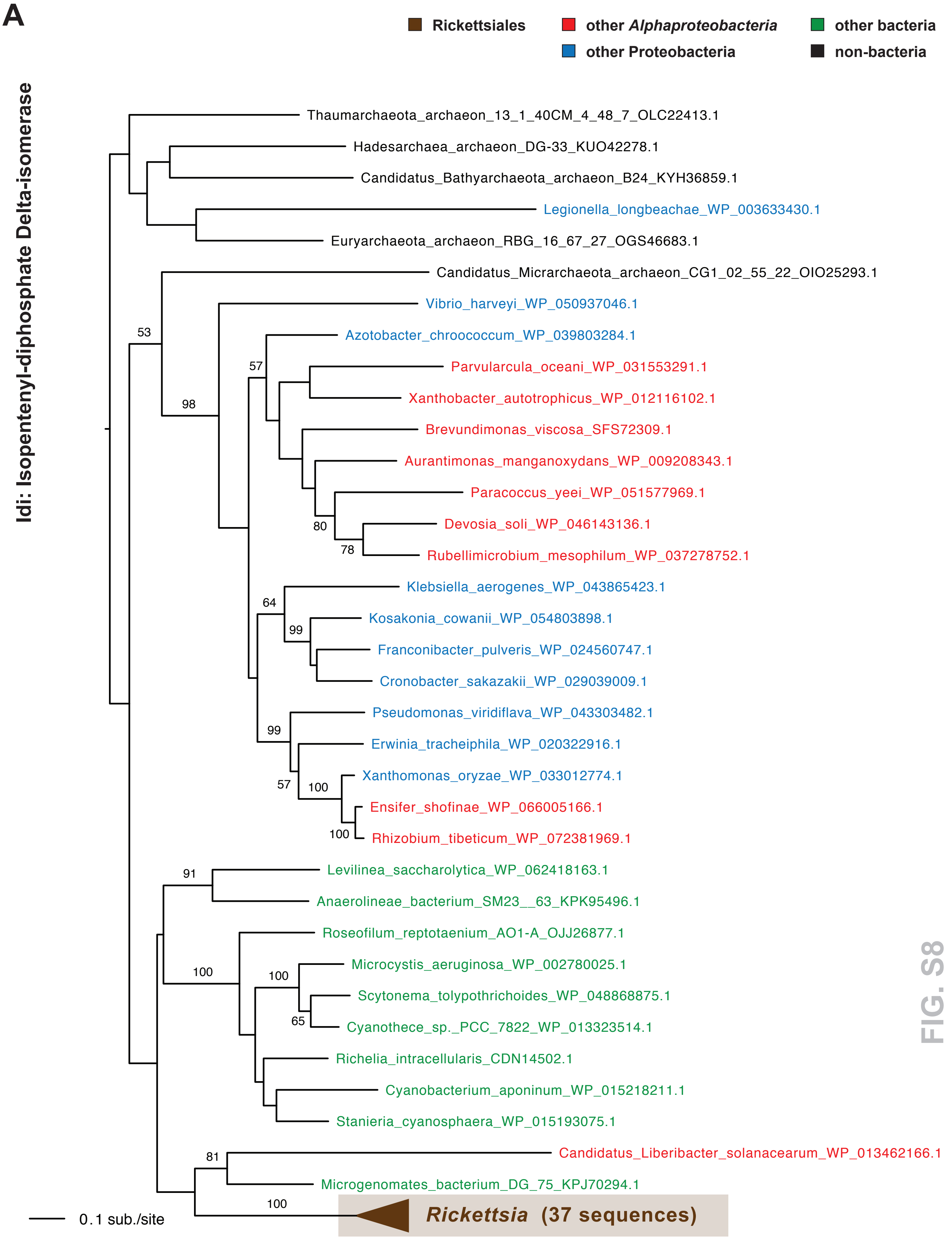
FIG S8. Phylogeny estimation of isopentenyl-diphosphate delta-isomerase (*idi*) and queuosine biosynthesis (*queD,E,C,F,A,H*) genes.

(A-H) Phylogeny estimations of Idi (A), Tgt (B), QueD (C), QueE (D), QueC (E), QueF (F), QueA (G), and QueH (H) proteins. Datasets for each protein were constructed as follows: the rickettsial protein was used in blastp queries against several taxon-specific databases: 1) “Rickettsiales”, 2) “Holosporales”, 3) “*Alphaproteobacteria* (minus Rickettsiales and Holosporales)”, 4) “*Proteobacteria* (minus *Alphaproteobacteria*)”, 5) “Bacteria (minus *Proteobacteria*)”, and 6) “minus Bacteria”. The top 5-10 (query-dependent) subjects from each search resulting in significant (> 40 bits) alignments were all compiled and aligned using MUSCLE v3.8.31 (default parameters) (1). Protein phylogenies were estimated under maximum likelihood with RAxML v8.2.4 (2) using a gamma model of rate heterogeneity and estimation of the proportion of invariant sites. Both the Lee and Gascuel (LG) and Blocks of Amino Acid Substitution Matrix (BLOSUM62) models of amino acid substitution were used, and branch support was assessed with 1,000 pseudo-replications.

REFERENCES

1. **Edgar RC**. 2004. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res **32**:1792–1797.
2. **Stamatakis A**. 2014. RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics **30**:1312–1313.



Idi: Isopentenyl-diphosphate Delta-isomerase

FIG. S8

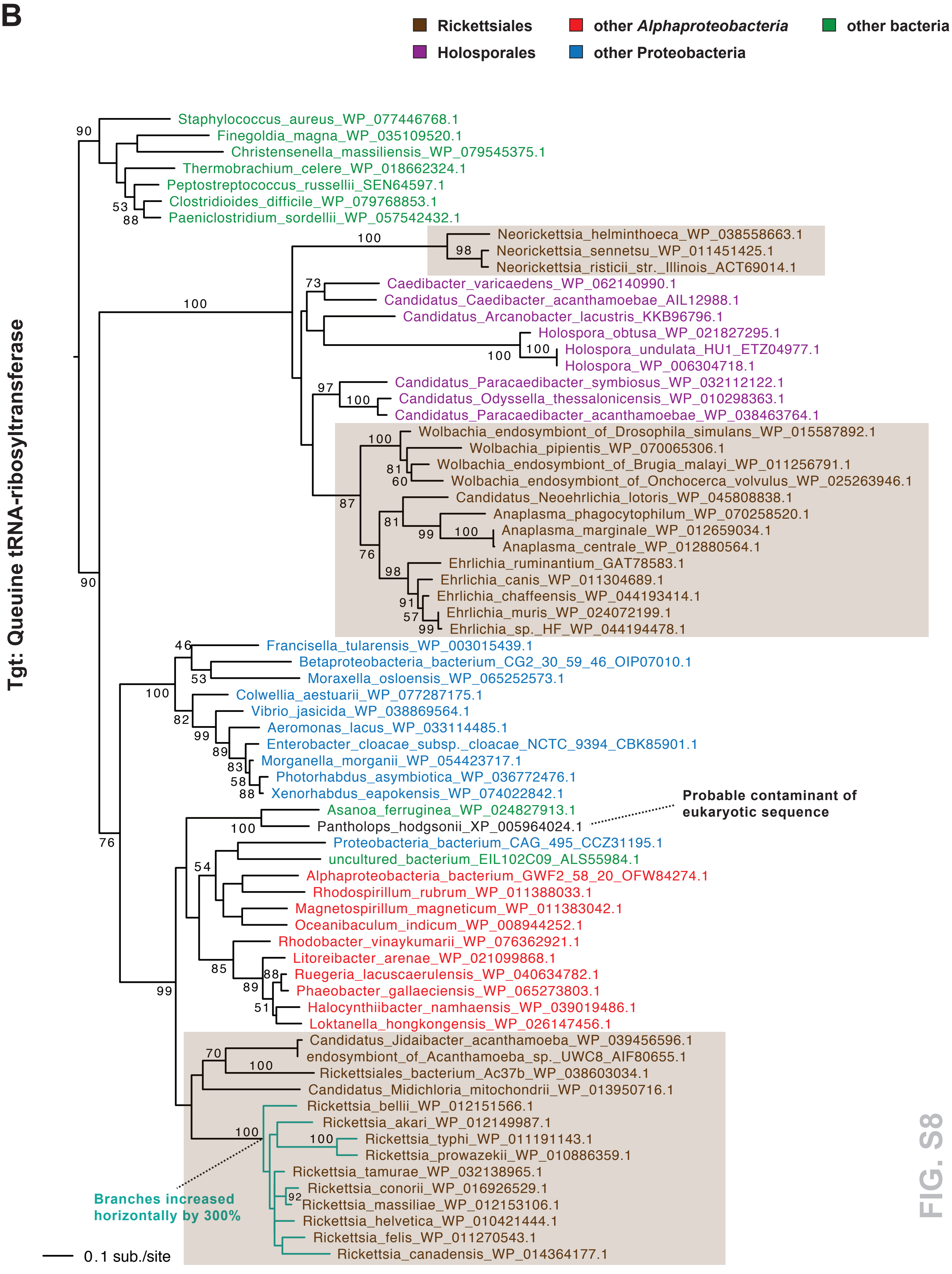

B
🟫 Rickettsiales
🟪 Holosporales
🟥 other *Alphaproteobacteria*
🟦 other Proteobacteria
🟩 other bacteria
Tgt: Queuine tRNA-ribosyltransferase
Staphylococcus_aureus_WP_077446768.1
Finegoldia_magna_WP_035109520.1
Christensenella_massiliensis_WP_079545375.1
Thermobrachium_celere_WP_018662324.1
Peptostreptococcus_russellii_SEN64597.1
Clostridioides_difficile_WP_079768853.1
Paeniclostridium_sordellii_WP_057542432.1
90
53
88
Neorickettsia_helminthoeca_WP_038558663.1
Neorickettsia_sennetsu_WP_011451425.1
Neorickettsia_risticii_str._Illinois_ACT69014.1
100
98
Caedibacter_varicaedens_WP_062140990.1
Candidatus_Caedibacter_acanthamoebae_AIL12988.1
Candidatus_Arcanobacter_lacustris_KKB96796.1
Holospora_obtusa_WP_021827295.1
Holospora_undulata_HU1_ETZ04977.1
Holospora_WP_006304718.1
73
100
100
100
Candidatus_Paracaedibacter_symbiosus_WP_032112122.1
Candidatus_Odyssella_thessalonicensis_WP_010298363.1
Candidatus_Paracaedibacter_acanthamoebae_WP_038463764.1
97
100
Wolbachia_endosymbiont_of_Drosophila_simulans_WP_015587892.1
Wolbachia_pipientis_WP_070065306.1
Wolbachia_endosymbiont_of_Brugia_malayi_WP_011256791.1
Wolbachia_endosymbiont_of_Onchocerca_volvulus_WP_025263946.1
Candidatus_Neoehrlichia_lotoris_WP_045808838.1
Anaplasma_phagocytophilum_WP_070258520.1
Anaplasma_marginale_WP_012659034.1
Anaplasma_centrale_WP_012880564.1
Ehrlichia_ruminantium_GAT78583.1
Ehrlichia_canis_WP_011304689.1
Ehrlichia_chaffeensis_WP_044193414.1
Ehrlichia_muris_WP_024072199.1
Ehrlichia_sp._HF_WP_044194478.1
100
81
60
87
81
99
100
76
98
91
57
99
90
Francisella_tularensis_WP_003015439.1
Betaproteobacteria_bacterium_CG2_30_59_46_OIP07010.1
Moraxella_osloensis_WP_065252573.1
Colwellia_aestuarii_WP_077287175.1
Vibrio_jasicida_WP_038869564.1
Aeromonas_lacus_WP_033114485.1
Enterobacter_cloacae_subsp._cloacae_NCTC_9394_CBK85901.1
Morganella_morganii_WP_054423717.1
Photorhabdus_asymbiotica_WP_036772476.1
Xenorhabdus_eapokensis_WP_074022842.1
46
53
100
82
99
89
83
58
88
Asanoa_ferruginea_WP_024827913.1
Pantholops_hodgsonii_XP_005964024.1
Probable contaminant of eukaryotic sequence
100
Proteobacteria_bacterium_CAG_495_CCZ31195.1
uncultured_bacterium_EIL102C09_ALS55984.1
Alphaproteobacteria_bacterium_GWF2_58_20_OFW84274.1
Rhodospirillum_rubrum_WP_011388033.1
Magnetospirillum_magneticum_WP_011383042.1
Oceanibaculum_indicum_WP_008944252.1
Rhodobacter_vinaykumarii_WP_076362921.1
Litoreibacter_arenae_WP_021099868.1
Ruegeria_lacuscaerulensis_WP_040634782.1
Phaeobacter_gallaeciensis_WP_065273803.1
Halocynthiibacter_namhaensis_WP_039019486.1
Loktanella_hongkongensis_WP_026147456.1
76
54
85
88
89
51
99
Candidatus_Jidaibacter_acanthamoeba_WP_039456596.1
endosymbiont_of_Acanthamoeba_sp._UWC8_AIF80655.1
Rickettsiales_bacterium_Ac37b_WP_038603034.1
Candidatus_Midichloria_mitochondrii_WP_013950716.1
70
100
Rickettsia_bellii_WP_012151566.1
Rickettsia_akari_WP_012149987.1
Rickettsia_typhi_WP_011191143.1
Rickettsia_prowazekii_WP_010886359.1
Rickettsia_tamurae_WP_032138965.1
Rickettsia_conorii_WP_016926529.1
Rickettsia_massiliae_WP_012153106.1
Rickettsia_helvetica_WP_010421444.1
Rickettsia_felis_WP_011270543.1
Rickettsia_canadensis_WP_014364177.1
100
100
92
Branches increased horizontally by 300%
0.1 sub./site

FIG. S8

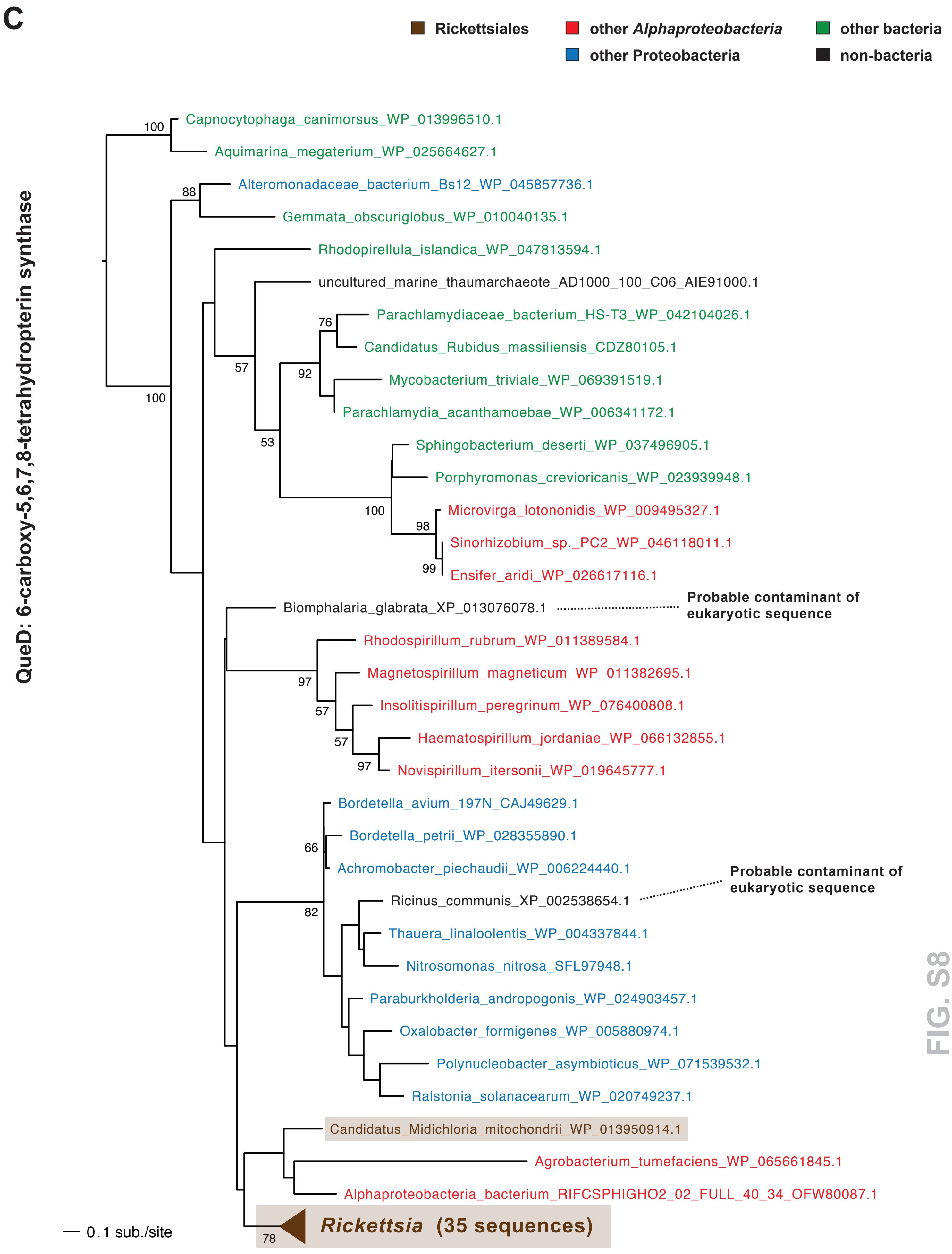

C
🟫 Rickettsiales
🟥 other *Alphaproteobacteria*
🟩 other bacteria
🟦 other Proteobacteria
⬛ non-bacteria
QueD: 6-carboxy-5,6,7,8-tetrahydropterin synthase
Capnocytophaga_canimorsus_WP_013996510.1
Aquimarina_megaterium_WP_025664627.1
Alteromonadaceae_bacterium_Bs12_WP_045857736.1
Gemmata_obscuriglobus_WP_010040135.1
Rhodopirellula_islandica_WP_047813594.1
uncultured_marine_thaumarchaeote_AD1000_100_C06_AIE91000.1
Parachlamydiaceae_bacterium_HS-T3_WP_042104026.1
Candidatus_Rubidus_massiliensis_CDZ80105.1
Mycobacterium_triviale_WP_069391519.1
Parachlamydia_acanthamoebae_WP_006341172.1
Sphingobacterium_deserti_WP_037496905.1
Porphyromonas_crevioricanis_WP_023939948.1
Microvirga_lotononidis_WP_009495327.1
Sinorhizobium_sp._PC2_WP_046118011.1
Ensifer_aridi_WP_026617116.1
Biomphalaria_glabrata_XP_013076078.1
Probable contaminant of eukaryotic sequence
Rhodospirillum_rubrum_WP_011389584.1
Magnetospirillum_magneticum_WP_011382695.1
Insolitispirillum_peregrinum_WP_076400808.1
Haematospirillum_jordaniae_WP_066132855.1
Novispirillum_itersonii_WP_019645777.1
Bordetella_avium_197N_CAJ49629.1
Bordetella_petrii_WP_028355890.1
Achromobacter_piechaudii_WP_006224440.1
Ricinus_communis_XP_002538654.1
Probable contaminant of eukaryotic sequence
Thauera_linaloolentis_WP_004337844.1
Nitrosomonas_nitrosa_SFL97948.1
Paraburkholderia_andropogonis_WP_024903457.1
Oxalobacter_formigenes_WP_005880974.1
Polynucleobacter_asymbioticus_WP_071539532.1
Ralstonia_solanacearum_WP_020749237.1
Candidatus_Midichloria_mitochondrii_WP_013950914.1
Agrobacterium_tumefaciens_WP_065661845.1
Alphaproteobacteria_bacterium_RIFCSPHIGHO2_02_FULL_40_34_OFW80087.1
Rickettsia (35 sequences)
100
88
57
76
92
100
53
100
98
99
97
57
57
97
66
82
78
0.1 sub./site

FIG. S8

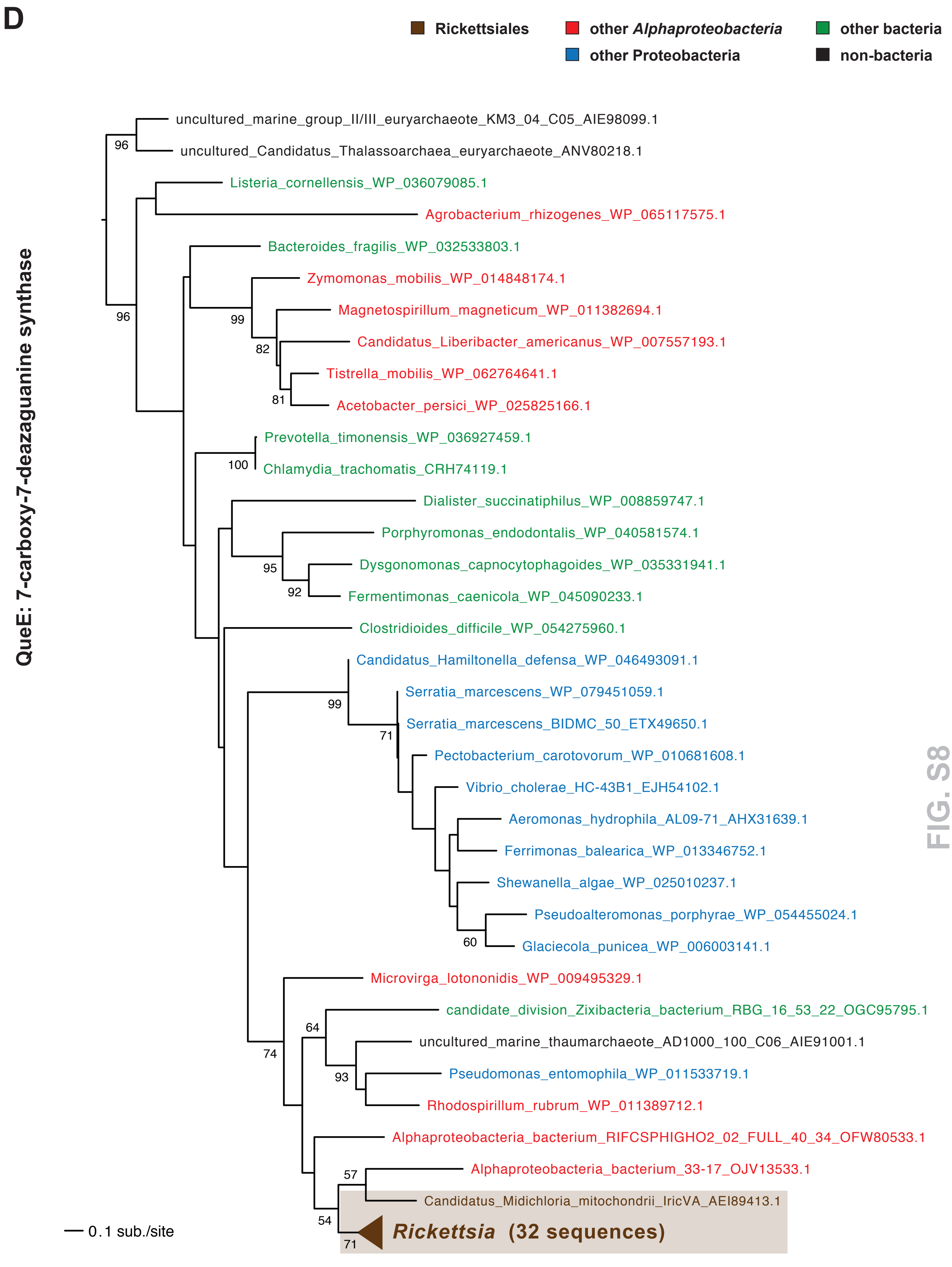

D
QueE: 7-carboxy-7-deazaguanine synthase
🟫 Rickettsiales
🟥 other *Alphaproteobacteria*
🟩 other bacteria
🟦 other Proteobacteria
⬛ non-bacteria
uncultured_marine_group_II/III_euryarchaeote_KM3_04_C05_AIE98099.1
uncultured_Candidatus_Thalassoarchaea_euryarchaeote_ANV80218.1
96
Listeria_cornellensis_WP_036079085.1
Agrobacterium_rhizogenes_WP_065117575.1
Bacteroides_fragilis_WP_032533803.1
Zymomonas_mobilis_WP_014848174.1
Magnetospirillum_magneticum_WP_011382694.1
Candidatus_Liberibacter_americanus_WP_007557193.1
Tistrella_mobilis_WP_062764641.1
Acetobacter_persici_WP_025825166.1
96
99
82
81
Prevotella_timonensis_WP_036927459.1
Chlamydia_trachomatis_CRH74119.1
100
Dialister_succinatiphilus_WP_008859747.1
Porphyromonas_endodontalis_WP_040581574.1
Dysgonomonas_capnocytophagoides_WP_035331941.1
Fermentimonas_caenicola_WP_045090233.1
95
92
Clostridioides_difficile_WP_054275960.1
Candidatus_Hamiltonella_defensa_WP_046493091.1
Serratia_marcescens_WP_079451059.1
Serratia_marcescens_BIDMC_50_ETX49650.1
99
71
Pectobacterium_carotovorum_WP_010681608.1
Vibrio_cholerae_HC-43B1_EJH54102.1
Aeromonas_hydrophila_AL09-71_AHX31639.1
Ferrimonas_balearica_WP_013346752.1
Shewanella_algae_WP_025010237.1
Pseudoalteromonas_porphyrae_WP_054455024.1
Glaciecola_punicea_WP_006003141.1
60
Microvirga_lotononidis_WP_009495329.1
candidate_division_Zixibacteria_bacterium_RBG_16_53_22_OGC95795.1
uncultured_marine_thaumarchaeote_AD1000_100_C06_AIE91001.1
Pseudomonas_entomophila_WP_011533719.1
Rhodospirillum_rubrum_WP_011389712.1
64
74
93
Alphaproteobacteria_bacterium_RIFCSPHIGHO2_02_FULL_40_34_OFW80533.1
Alphaproteobacteria_bacterium_33-17_OJV13533.1
Candidatus_Midichloria_mitochondrii_IricVA_AEI89413.1
57
54
71
Rickettsia (32 sequences)
0.1 sub./site

FIG. S8

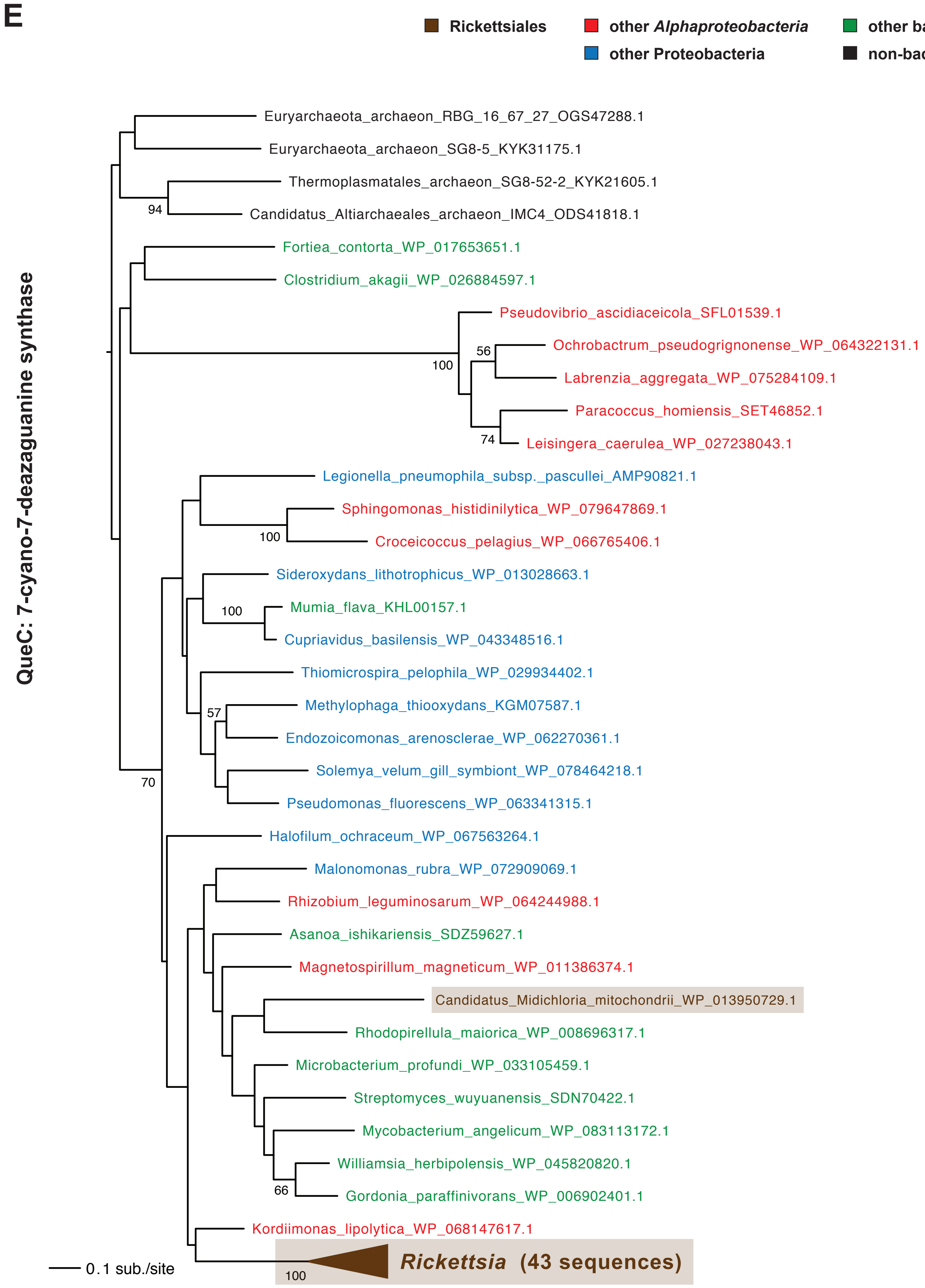
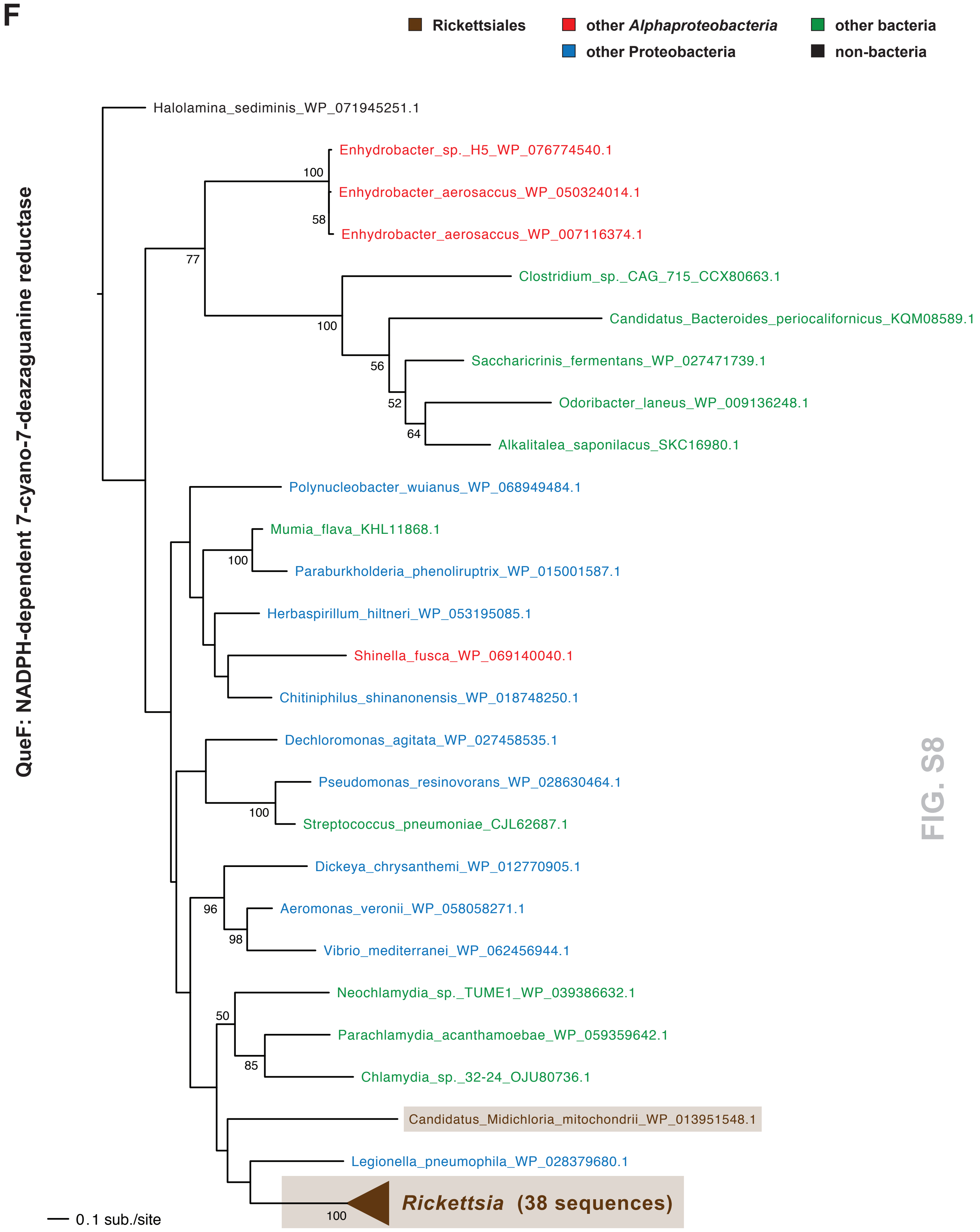

E
Rickettsiales
other Alphaproteobacteria
other bacteria
other Proteobacteria
non-bacteria
QueC: 7-cyano-7-deazaguanine synthase
Euryarchaeota_archaeon_RBG_16_67_27_OGS47288.1
Euryarchaeota_archaeon_SG8-5_KYK31175.1
Thermoplasmatales_archaeon_SG8-52-2_KYK21605.1
94
Candidatus_Altiarchaeales_archaeon_IMC4_ODS41818.1
Fortiea_contorta_WP_017653651.1
Clostridium_akagii_WP_026884597.1
Pseudovibrio_ascidiaceicola_SFL01539.1
56
Ochrobactrum_pseudogrignonense_WP_064322131.1
100
Labrenzia_aggregata_WP_075284109.1
Paracoccus_homiensis_SET46852.1
74
Leisingera_caerulea_WP_027238043.1
Legionella_pneumophila_subsp._pascullei_AMP90821.1
Sphingomonas_histidinilytica_WP_079647869.1
100
Croceicoccus_pelagius_WP_066765406.1
Sideroxydans_lithotrophicus_WP_013028663.1
100
Mumia_flava_KHL00157.1
Cupriavidus_basilensis_WP_043348516.1
Thiomicrospira_pelophila_WP_029934402.1
57
Methylophaga_thiooxydans_KGM07587.1
Endozoicomonas_arenosclerae_WP_062270361.1
Solemya_velum_gill_symbiont_WP_078464218.1
70
Pseudomonas_fluorescens_WP_063341315.1
Halofilum_ochraceum_WP_067563264.1
Malonomonas_rubra_WP_072909069.1
Rhizobium_leguminosarum_WP_064244988.1
Asanoa_ishikariensis_SDZ59627.1
Magnetospirillum_magneticum_WP_011386374.1
Candidatus_Midichloria_mitochondrii_WP_013950729.1
Rhodopirellula_maiorica_WP_008696317.1
Microbacterium_profundi_WP_033105459.1
Streptomyces_wuyuanensis_SDN70422.1
Mycobacterium_angelicum_WP_083113172.1
Williamsia_herbipolensis_WP_045820820.1
66
Gordonia_paraffinivorans_WP_006902401.1
Kordiimonas_lipolytica_WP_068147617.1
0.1 sub./site
100
Rickettsia (43 sequences)

FIG. S8



QueF: NADPH-dependent 7-cyano-7-deazaguanine reductase

FIG. S8

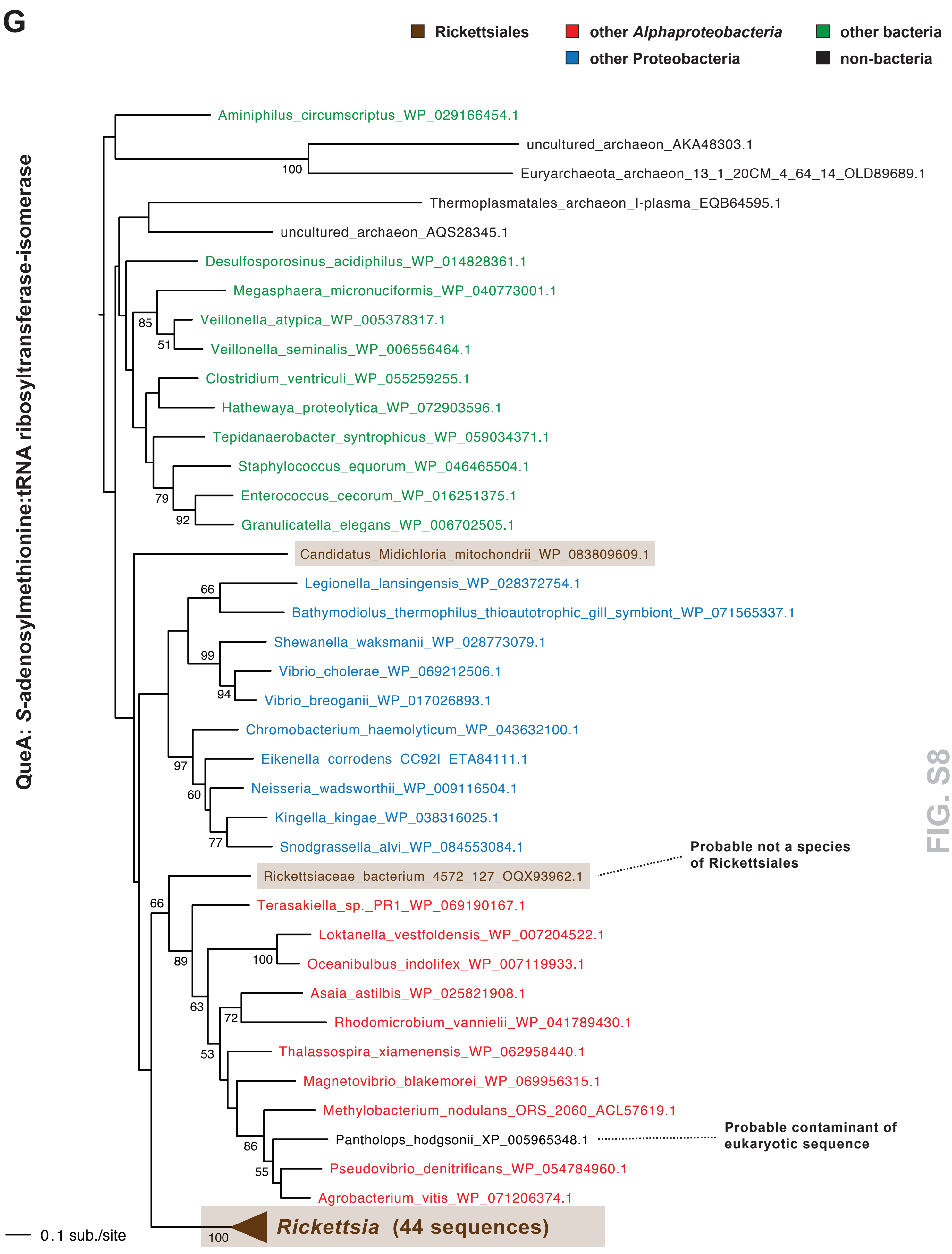

G
🟫 Rickettsiales
🟥 other *Alphaproteobacteria*
🟩 other bacteria
🟦 other Proteobacteria
⬛ non-bacteria
QueA: *S*-adenosylmethionine:tRNA ribosyltransferase-isomerase
Aminiphilus_circumscriptus_WP_029166454.1
uncultured_archaeon_AKA48303.1
100
Euryarchaeota_archaeon_13_1_20CM_4_64_14_OLD89689.1
Thermoplasmatales_archaeon_I-plasma_EQB64595.1
uncultured_archaeon_AQS28345.1
Desulfosporosinus_acidiphilus_WP_014828361.1
Megasphaera_micronuciformis_WP_040773001.1
85
Veillonella_atypica_WP_005378317.1
51
Veillonella_seminalis_WP_006556464.1
Clostridium_ventriculi_WP_055259255.1
Hathewaya_proteolytica_WP_072903596.1
Tepidanaerobacter_syntrophicus_WP_059034371.1
Staphylococcus_equorum_WP_046465504.1
79
Enterococcus_cecorum_WP_016251375.1
92
Granulicatella_elegans_WP_006702505.1
Candidatus_Midichloria_mitochondrii_WP_083809609.1
66
Legionella_lansingensis_WP_028372754.1
Bathymodiolus_thermophilus_thioautotrophic_gill_symbiont_WP_071565337.1
Shewanella_waksmanii_WP_028773079.1
99
Vibrio_cholerae_WP_069212506.1
94
Vibrio_breoganii_WP_017026893.1
Chromobacterium_haemolyticum_WP_043632100.1
97
Eikenella_corrodens_CC92I_ETA84111.1
60
Neisseria_wadsworthii_WP_009116504.1
Kingella_kingae_WP_038316025.1
77
Snodgrassella_alvi_WP_084553084.1
Rickettsiaceae_bacterium_4572_127_OQX93962.1
Probable not a species of Rickettsiales
66
Terasakiella_sp._PR1_WP_069190167.1
89
Loktanella_vestfoldensis_WP_007204522.1
100
Oceanibulbus_indolifex_WP_007119933.1
63
Asaia_astilbis_WP_025821908.1
72
Rhodomicrobium_vannielii_WP_041789430.1
53
Thalassospira_xiamenensis_WP_062958440.1
Magnetovibrio_blakemorei_WP_069956315.1
Methylobacterium_nodulans_ORS_2060_ACL57619.1
86
Pantholops_hodgsonii_XP_005965348.1
Probable contaminant of eukaryotic sequence
55
Pseudovibrio_denitrificans_WP_054784960.1
Agrobacterium_vitis_WP_071206374.1
100
Rickettsia (44 sequences)
0.1 sub./site

FIG. S8

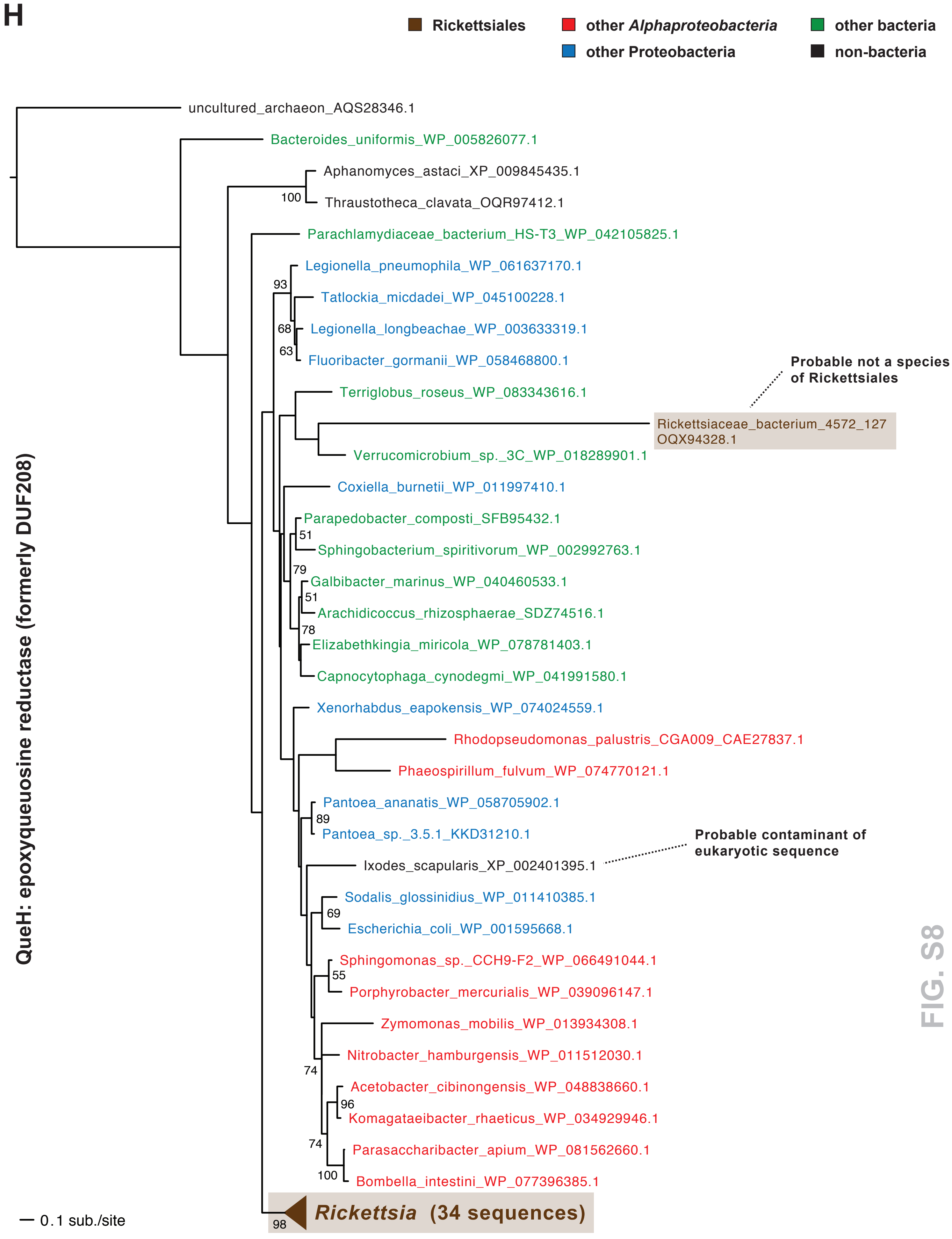

H
🟫 Rickettsiales
🟥 other *Alphaproteobacteria*
🟩 other bacteria
🟦 other Proteobacteria
⬛ non-bacteria
QueH: epoxyqueuosine reductase (formerly DUF208)
uncultured_archaeon_AQS28346.1
Bacteroides_uniformis_WP_005826077.1
Aphanomyces_astaci_XP_009845435.1
100
Thraustotheca_clavata_OQR97412.1
Parachlamydiaceae_bacterium_HS-T3_WP_042105825.1
Legionella_pneumophila_WP_061637170.1
93
Tatlockia_micdadei_WP_045100228.1
68
Legionella_longbeachae_WP_003633319.1
63
Fluoribacter_gormanii_WP_058468800.1
Terriglobus_roseus_WP_083343616.1
Probable not a species of Rickettsiales
Rickettsiaceae_bacterium_4572_127 OQX94328.1
Verrucomicrobium_sp._3C_WP_018289901.1
Coxiella_burnetii_WP_011997410.1
Parapedobacter_composti_SFB95432.1
51
Sphingobacterium_spiritivorum_WP_002992763.1
79
Galbibacter_marinus_WP_040460533.1
51
Arachidicoccus_rhizosphaerae_SDZ74516.1
78
Elizabethkingia_miricola_WP_078781403.1
Capnocytophaga_cynodegmi_WP_041991580.1
Xenorhabdus_eapokensis_WP_074024559.1
Rhodopseudomonas_palustris_CGA009_CAE27837.1
Phaeospirillum_fulvum_WP_074770121.1
Pantoea_ananatis_WP_058705902.1
89
Pantoea_sp._3.5.1_KKD31210.1
Probable contaminant of eukaryotic sequence
Ixodes_scapularis_XP_002401395.1
Sodalis_glossinidius_WP_011410385.1
69
Escherichia_coli_WP_001595668.1
Sphingomonas_sp._CCH9-F2_WP_066491044.1
55
Porphyrobacter_mercurialis_WP_039096147.1
Zymomonas_mobilis_WP_013934308.1
Nitrobacter_hamburgensis_WP_011512030.1
74
Acetobacter_cibinongensis_WP_048838660.1
96
Komagataeibacter_rhaeticus_WP_034929946.1
74
Parasaccharibacter_apium_WP_081562660.1
100
Bombella_intestini_WP_077396385.1
Rickettsia (34 sequences)
98
0.1 sub./site

FIG. S8