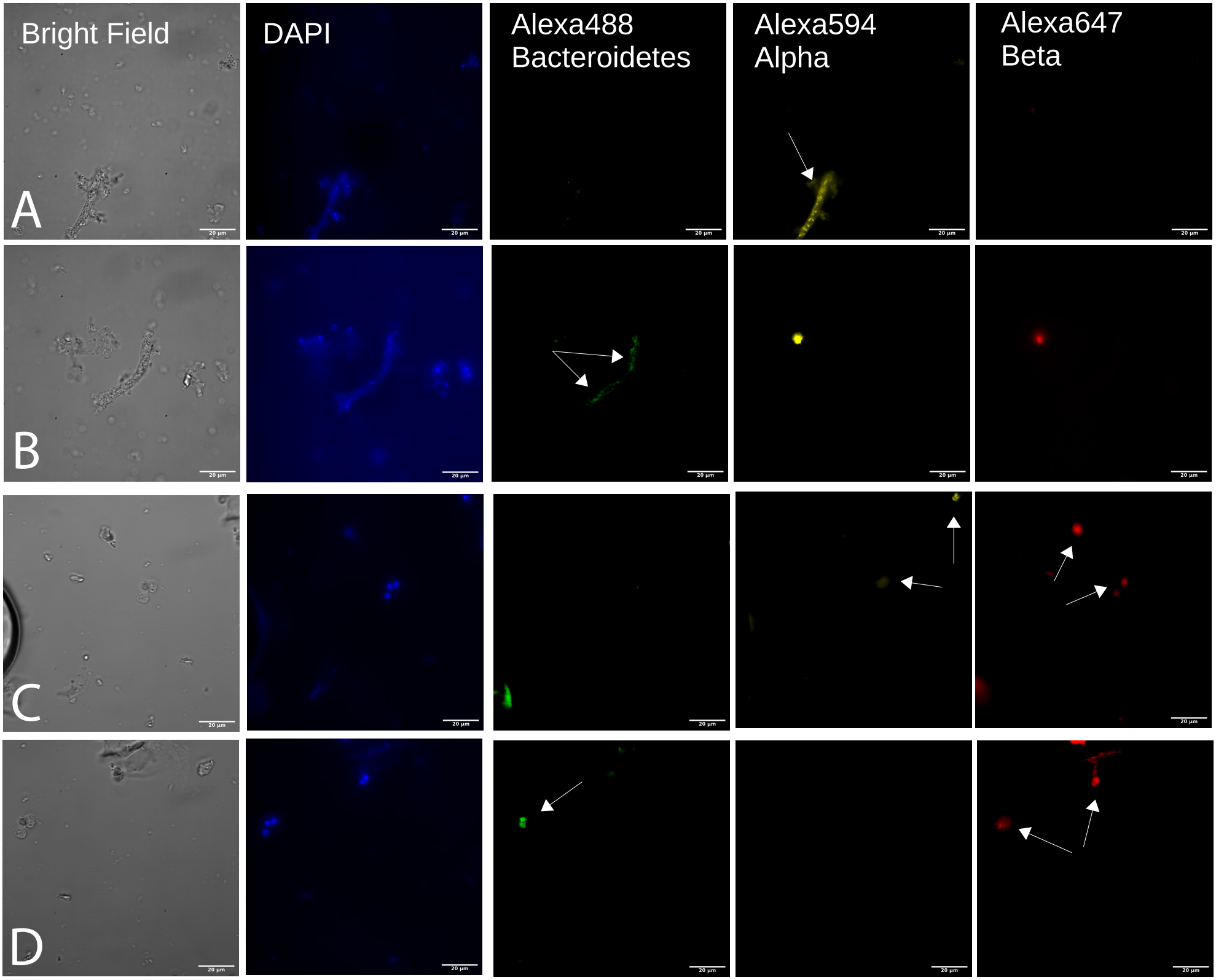
Bright Field
DAPI
Alexa488
Bacteroidetes
Alexa594
Alpha
Alexa647
Beta
A
B
C
D
20 µm

Figure S1: FISH hybridization microscopy of *Nephromyces* and their endosymbionts. Related to Figure 1.

The same field of view was imaged under brightfield, 457 nm (DAPI), 488 (bacteroidetes probe), 594 nm (alphaproteobacterial probe), and 647 nm (betaproteobacterial probe). *Nephromyces* cells are indicated by white arrows, whereas florescence not indicated by white arrows is from autofluorescence of uric acid crystals in the renal sac. A) Hybridization of the alphaproteobacteria probe. Row B) Hybridization of the bacteroidetes probe. Row C) Hybridization of the alphaproteobacteria and betaproteobacterial probes in the same field of view localized to different *Nephromyces* cells. Row D) Hybridization of the bacteroidetes and betaproteobacterial probes in the same field of view localized to different *Nephromyces* cells. Photos taken on an Olympus BX51 upright compound microscope at 100x magnification images edited in imageJ.

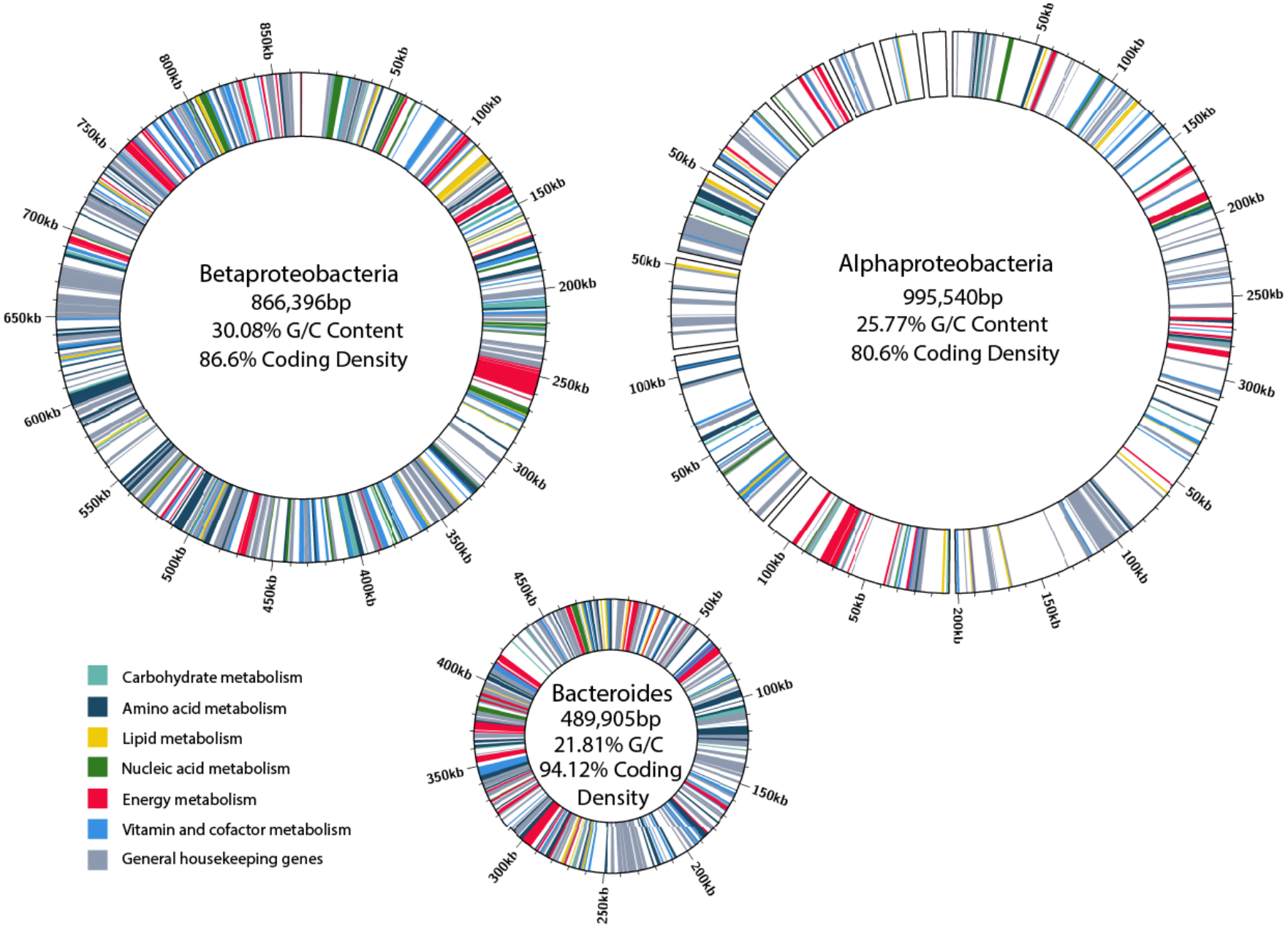
Betaproteobacteria
866,396bp
30.08% G/C Content
86.6% Coding Density
Alphaproteobacteria
995,540bp
25.77% G/C Content
80.6% Coding Density
Bacteroides
489,905bp
21.81% G/C
94.12% Coding
Density
Carbohydrate metabolism
Amino acid metabolism
Lipid metabolism
Nucleic acid metabolism
Energy metabolism
Vitamin and cofactor metabolism
General housekeeping genes

Figure S2: Bacterial Endosymbiont Genomes. Related to Figure 2 & 3.

Circos diagram of bacterial endosymbionts from *Nephromyces*, showing overview of major functional categories (as predicted by KEGG), GC content, size, and coding density. Genomes are scaled relative to each other. The Alphaproteobacteria has not been assembled into a single molecule, and we show the size distribution of the 11 contigs making up this assembly.

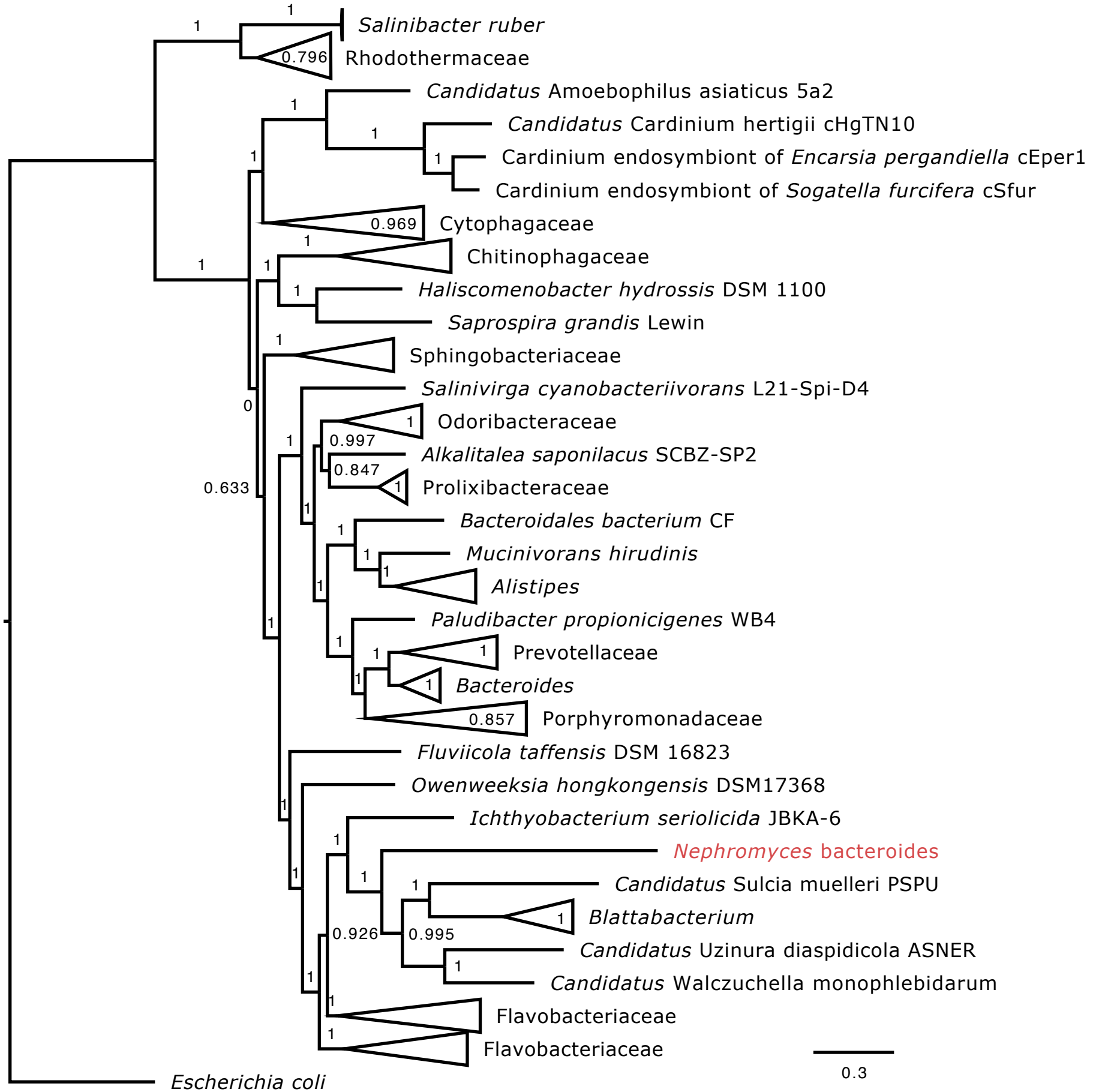
Salinibacter ruber
Rhodothermaceae
Candidatus Amoebophilus asiaticus 5a2
Candidatus Cardinium hertigii cHgTN10
Cardinium endosymbiont of Encarsia pergandiella cEper1
Cardinium endosymbiont of Sogatella furcifera cSfur
Cytophagaceae
Chitinophagaceae
Haliscomenobacter hydrossis DSM 1100
Saprospira grandis Lewin
Sphingobacteriaceae
Salinivirga cyanobacteriivorans L21-Spi-D4
Odoribacteraceae
Alkalitalea saponilacus SCBZ-SP2
Prolixibacteraceae
Bacteroidales bacterium CF
Mucinivorans hirudinis
Alistipes
Paludibacter propionicigenes WB4
Prevotellaceae
Bacteroides
Porphyromonadaceae
Fluviicola taffensis DSM 16823
Owenweeksia hongkongensis DSM17368
Ichthyobacterium seriolicida JBKA-6
Nephromyces bacteroides
Candidatus Sulcia muelleri PSPU
Blattabacterium
Candidatus Uzinura diaspidicola ASNER
Candidatus Walczuchella monophlebidarum
Flavobacteriaceae
Flavobacteriaceae
Escherichia coli
0.3

Figure S3: **Betaproteobacteria Phylogeny. Related to Figure 4.**

Maximum likelihood tree of 203 genes and 471 genomes from betaproteobacteria analysed in the GToTree pipeline with proportion of 1000 bootstrap replicates indicated at each node. *Escherichia coli* was used as an outgroup.

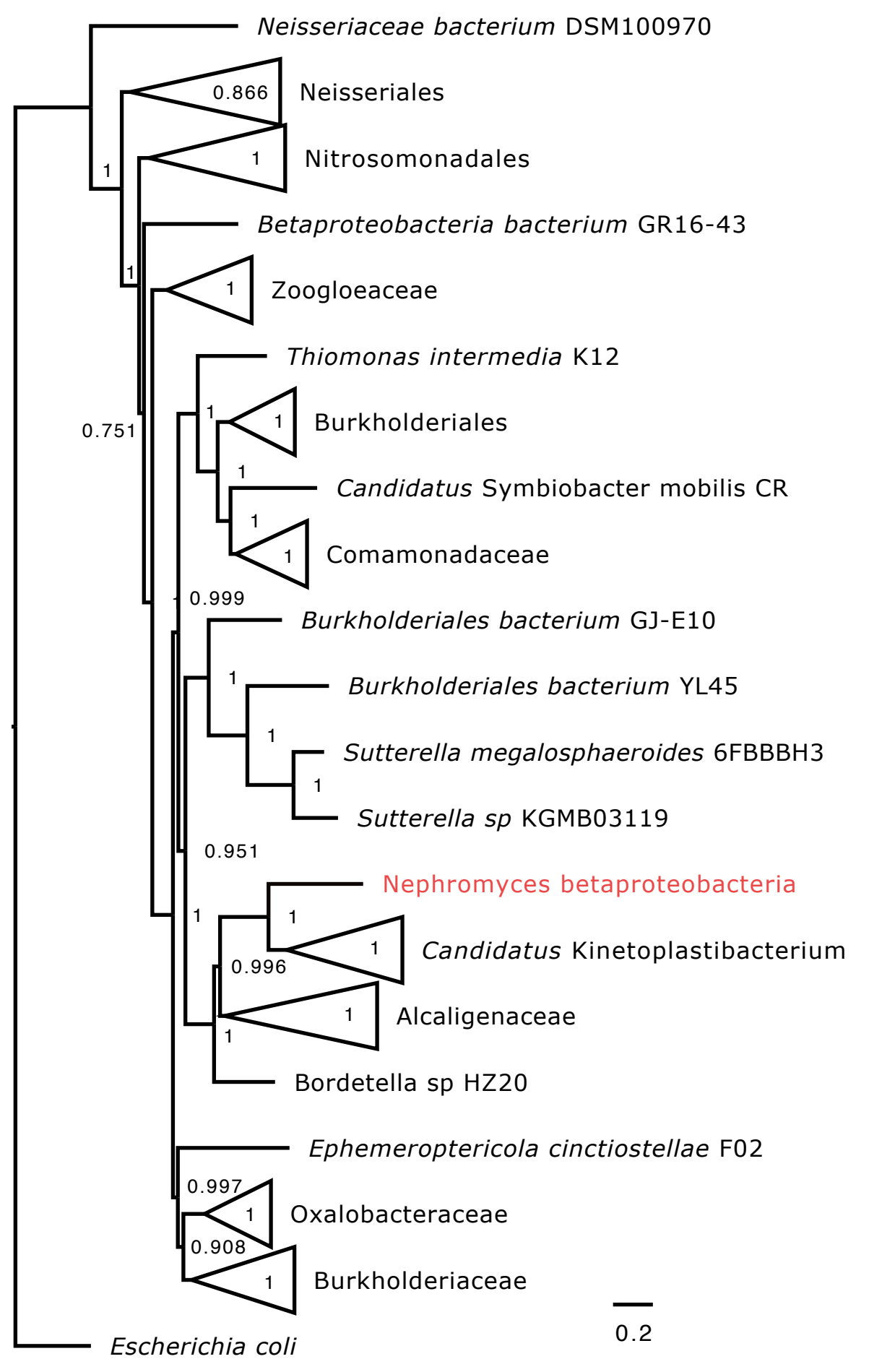
Neisseriaceae bacterium DSM100970
0.866
Neisseriales
1
Nitrosomonadales
1
Betaproteobacteria bacterium GR16-43
1
Zoogloeaceae
Thiomonas intermedia K12
1
1
Burkholderiales
0.751
1
Candidatus Symbiobacter mobilis CR
1
1
Comamonadaceae
0.999
Burkholderiales bacterium GJ-E10
1
Burkholderiales bacterium YL45
1
Sutterella megalosphaeroides 6FBBBH3
1
Sutterella sp KGMB03119
0.951
Nephromyces betaproteobacteria
1
1
1
Candidatus Kinetoplastibacterium
0.996
1
Alcaligenaceae
1
Bordetella sp HZ20
Ephemeroptericola cinctiostellae F02
0.997
1
Oxalobacteraceae
0.908
1
Burkholderiaceae
0.2
Escherichia coli

Figure S4: **Bacteroidetes Phylogeny. Related to Figure 4.**

Maximum likelihood tree of 90 genes and 388 genomes from Bacteroidetes from the GToTree pipeline with bootstrap values. *Escherichia coli* was used as an outgroup.