Supplementary Information

for

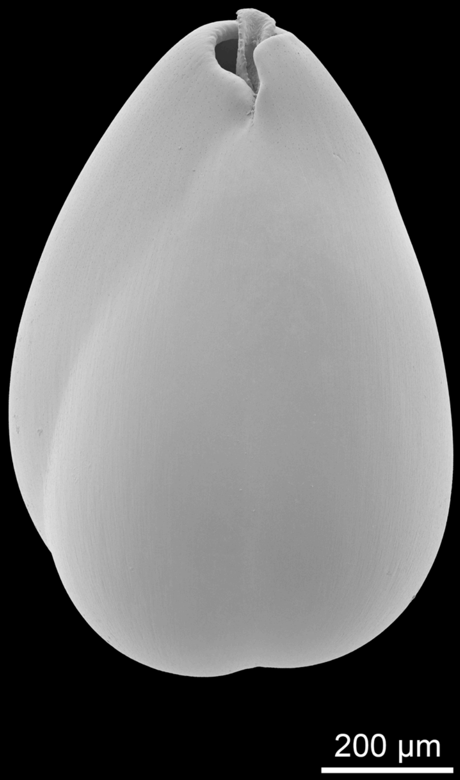
Denitrification in foraminifera has an ancient origin and is complemented by associated bacteria

Christian Woehle, Alexandra-Sophie Roy, Nicolaas Glock, Jan Michels, Tanita Wein, Julia Weissenbach, Dennis Romero, Claas Hiebenthal, Stanislav N. Gorb, Joachim Schönfeld, Tal Dagan

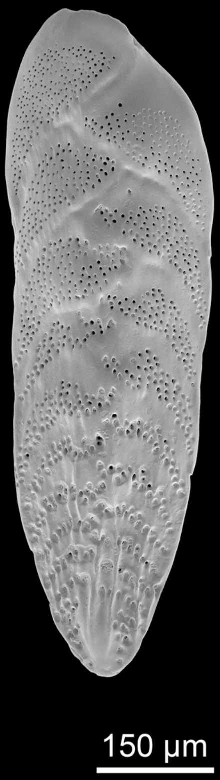
Supplementary Figures S1 – S4

Titles: Dataset 1 – 8

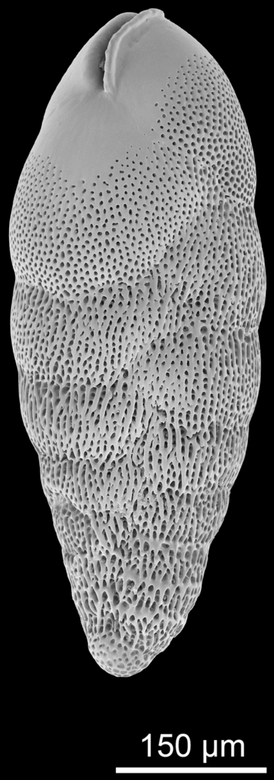
Globobulimina pacifica



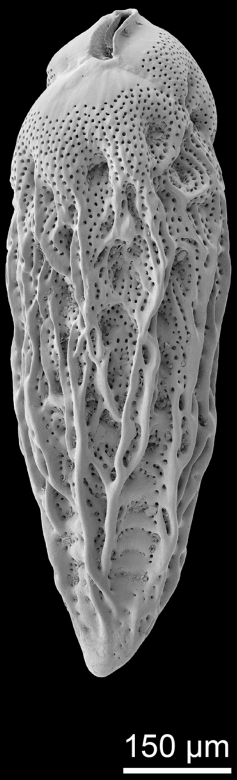
Bolivina spissa



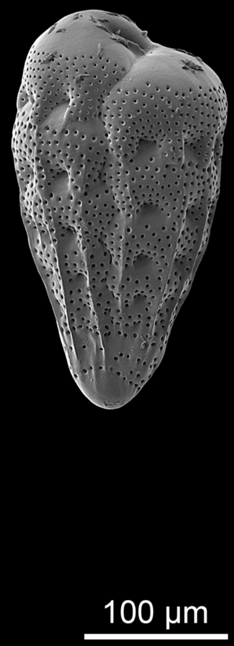
Bolivina seminuda



Bolivina plicata

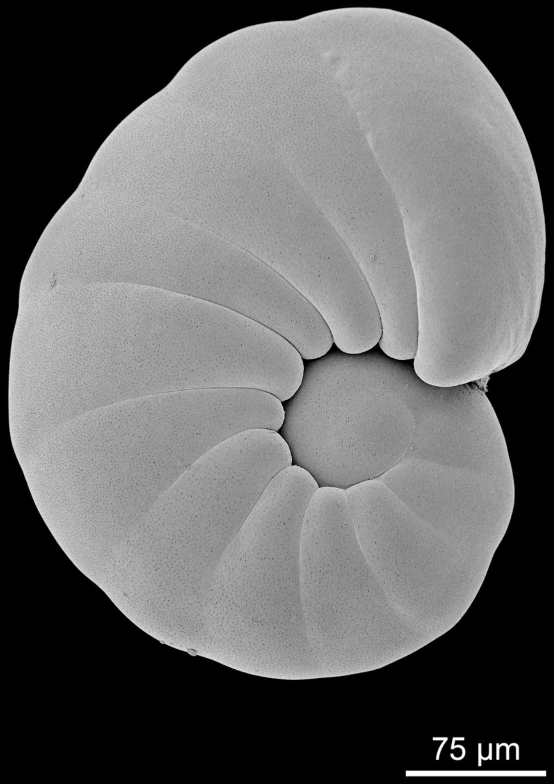


Bolivina costata

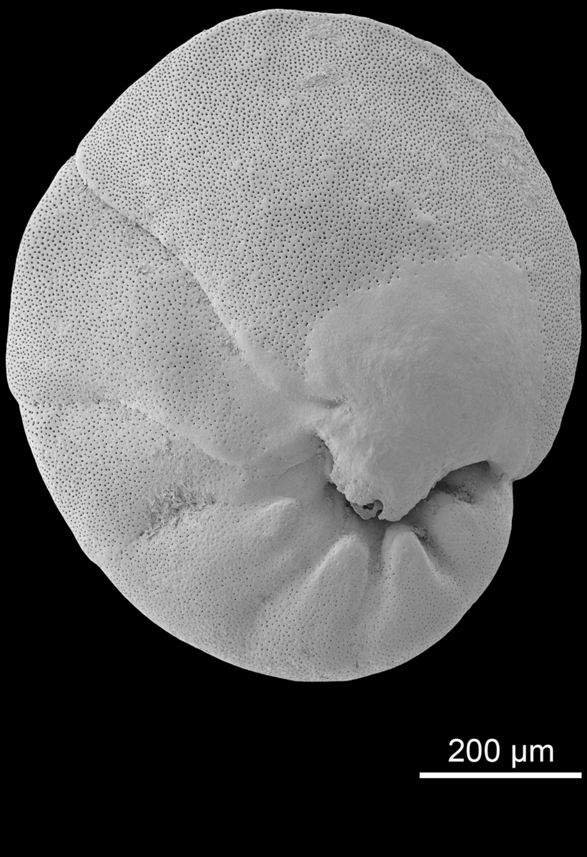


Uvigerina striata

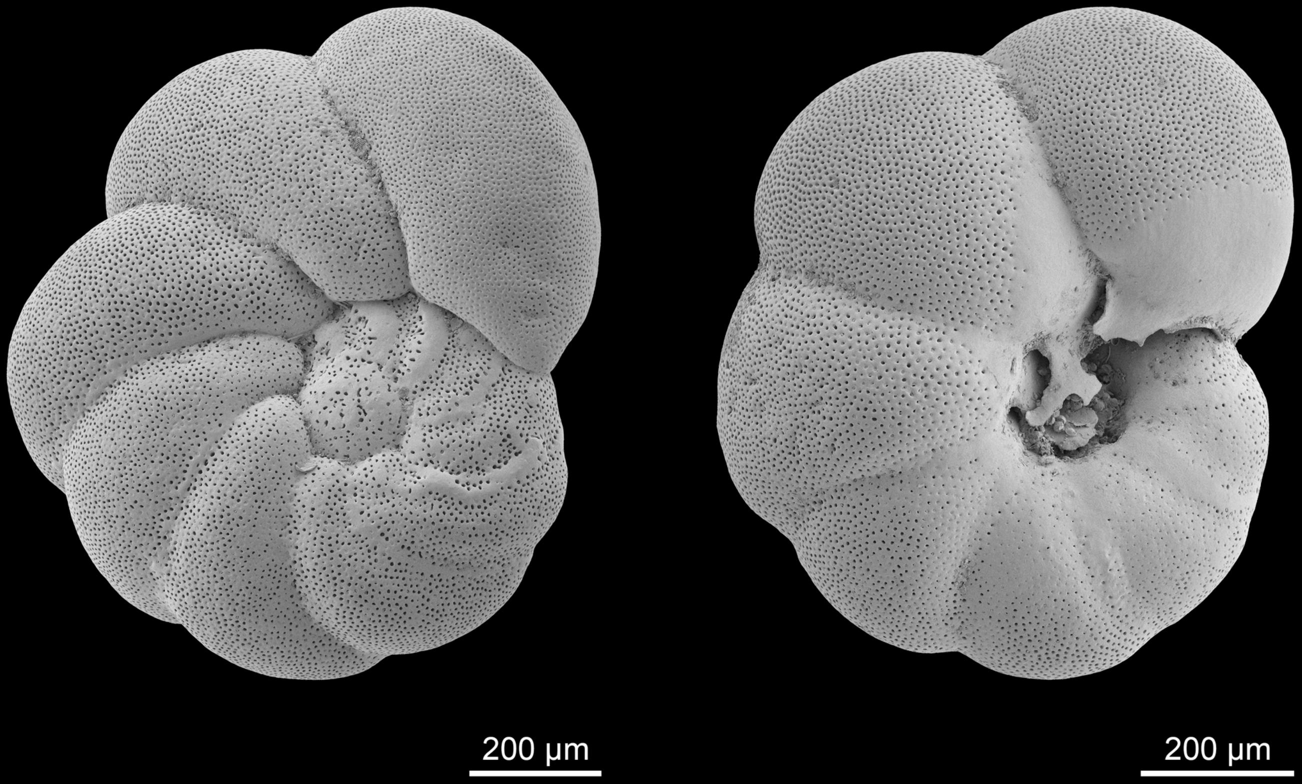
Nonionella auris



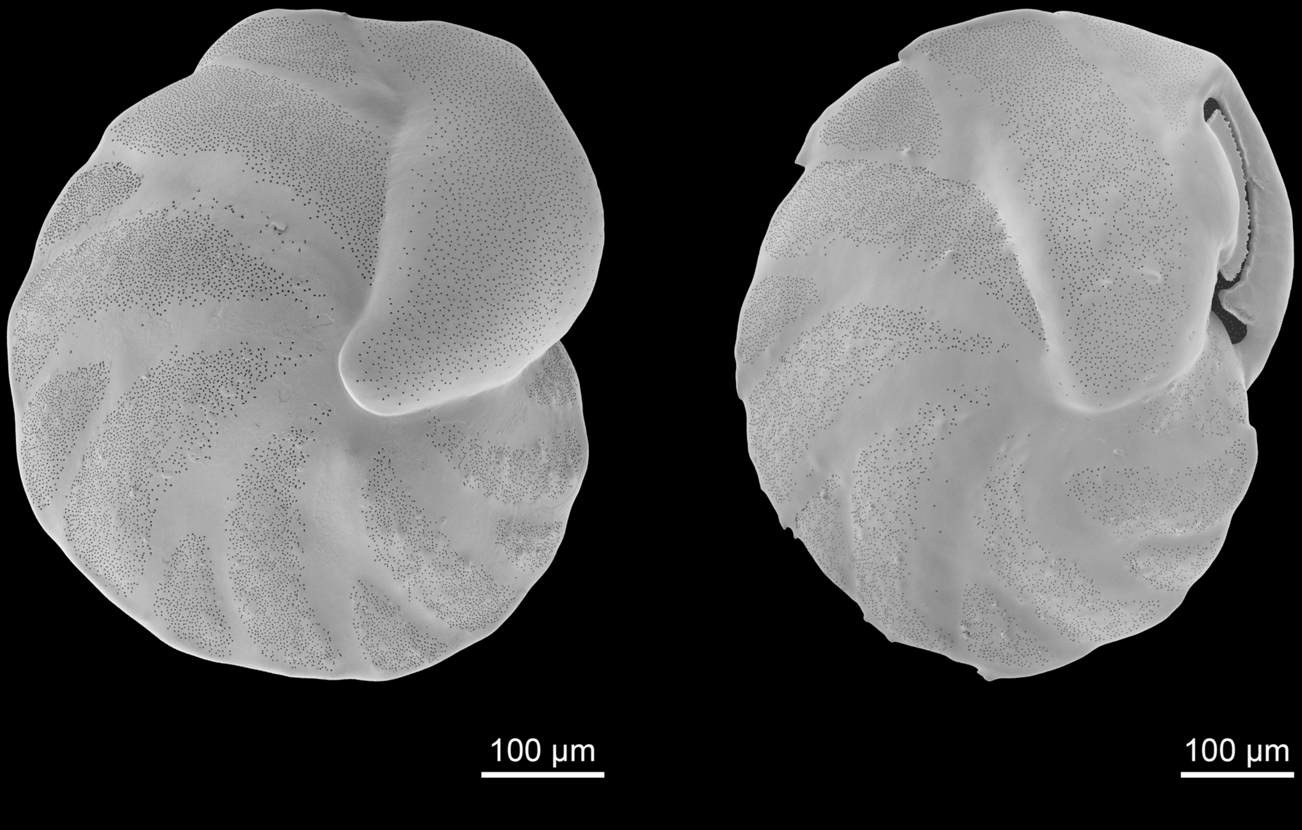
Cancris carmenensis



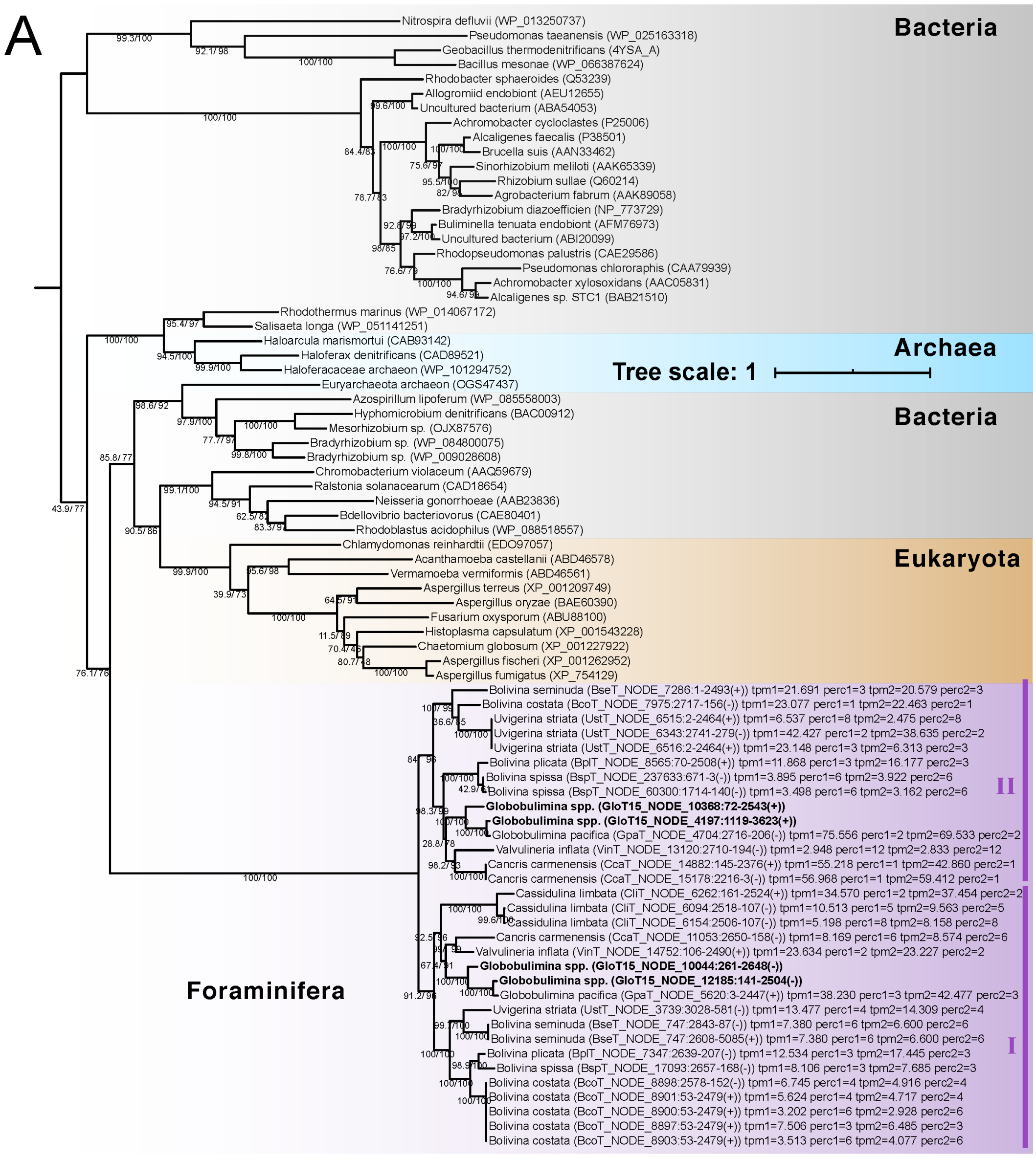
Valvulineria inflata

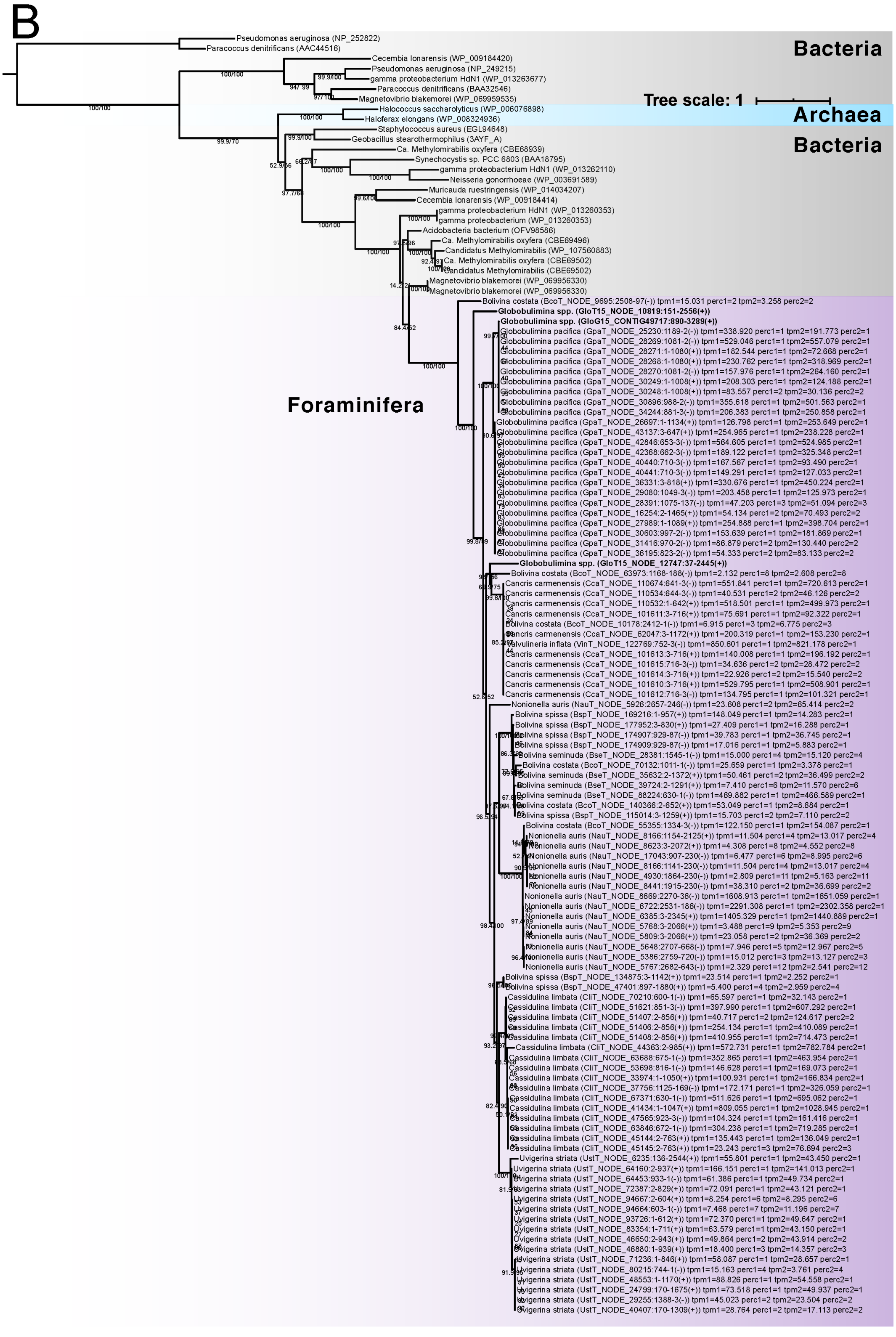


Cassidulina limbata



Suppl. Figure S1. Scanning electron micrographs of sampled species. Detailed views of the graphs shown in Figure 1A.




B
Bacteria
Tree scale: 1
Archaea
Bacteria
Foraminifera
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Cecembia lonarensis (WP_009184420)
Pseudomonas aeruginosa (NP_249215)
gamma proteobacterium HdN1 (WP_013263677)
Paracoccus denitrificans (BAA32546)
Magnetovibrio blakemorei (WP_069959535)
Halococcus saccharolyticus (WP_006076898)
Haloferax elongans (WP_008324936)
Staphylococcus aureus (EGL94648)
Geobacillus stearothermophilus (3AYF_A)
Ca. Methylomirabilis oxyfera (CBE68939)
Synechocystis sp. PCC 6803 (BAA18795)
gamma proteobacterium HdN1 (WP_013262110)
Neisseria gonorrhoeae (WP_003691589)
Muricauda ruestringensis (WP_014034207)
Cecembia lonarensis (WP_009184414)
gamma proteobacterium HdN1 (WP_013260353)
gamma proteobacterium (WP_013260353)
Acidobacteria bacterium (OFV98586)
Ca. Methylomirabilis oxyfera (CBE69496)
Candidatus Methylomirabilis (WP_107560883)
Ca. Methylomirabilis oxyfera (CBE69502)
Candidatus Methylomirabilis (CBE69502)
Magnetovibrio blakemorei (WP_069956330)
Magnetovibrio blakemorei (WP_069956330)
100/100
100/100
94/ 99
99.9/100
97/100
100/100
99.9/70
99.9/100
52.9/66
66.2/87
100/100
100/100
97.7/68
99.6/100
100/100
100/100
97.5/96
100/100
100/100
92.4/97
100/100
14.2/21
100/100
84.4/62
100/100
100/100
99.8/89
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100/100
86.3/92
67.8/63
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100/100
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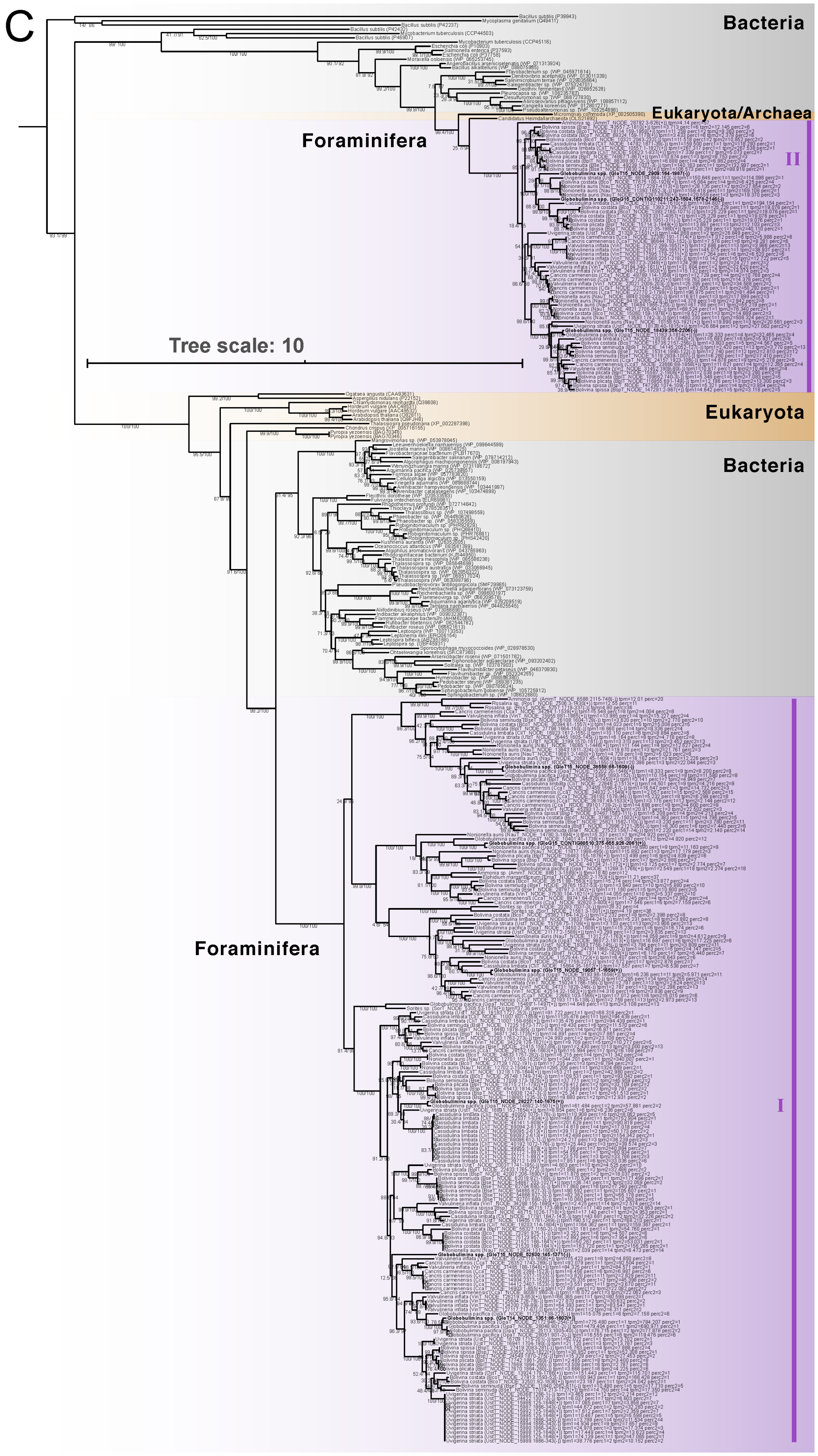
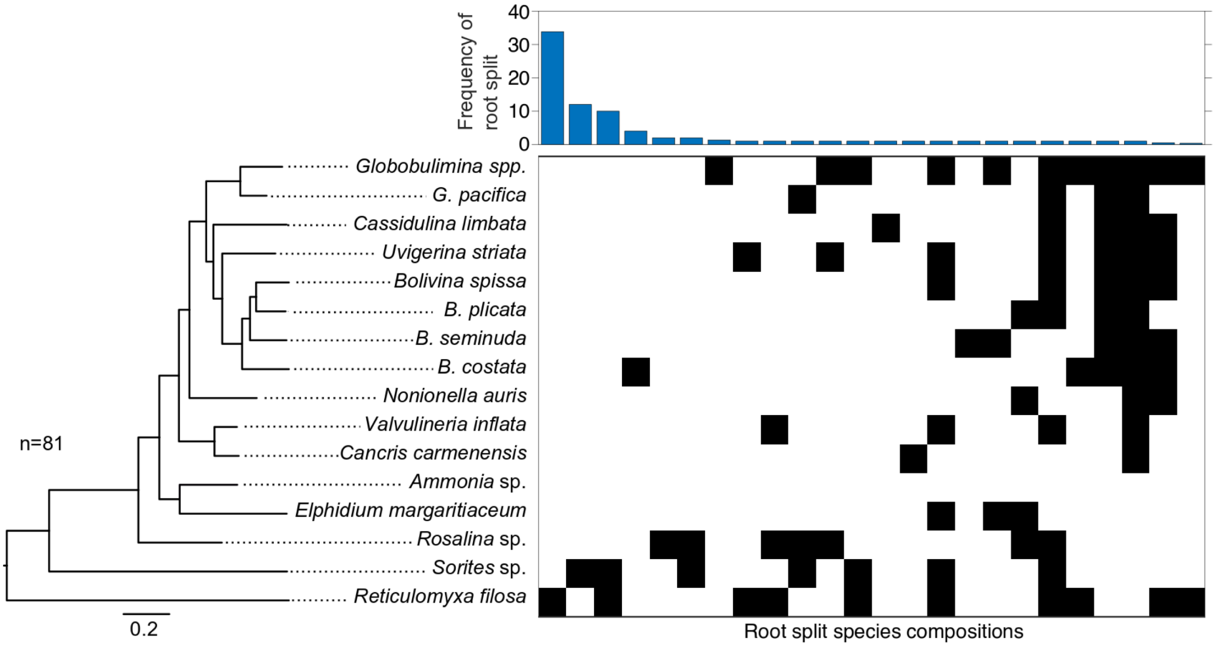
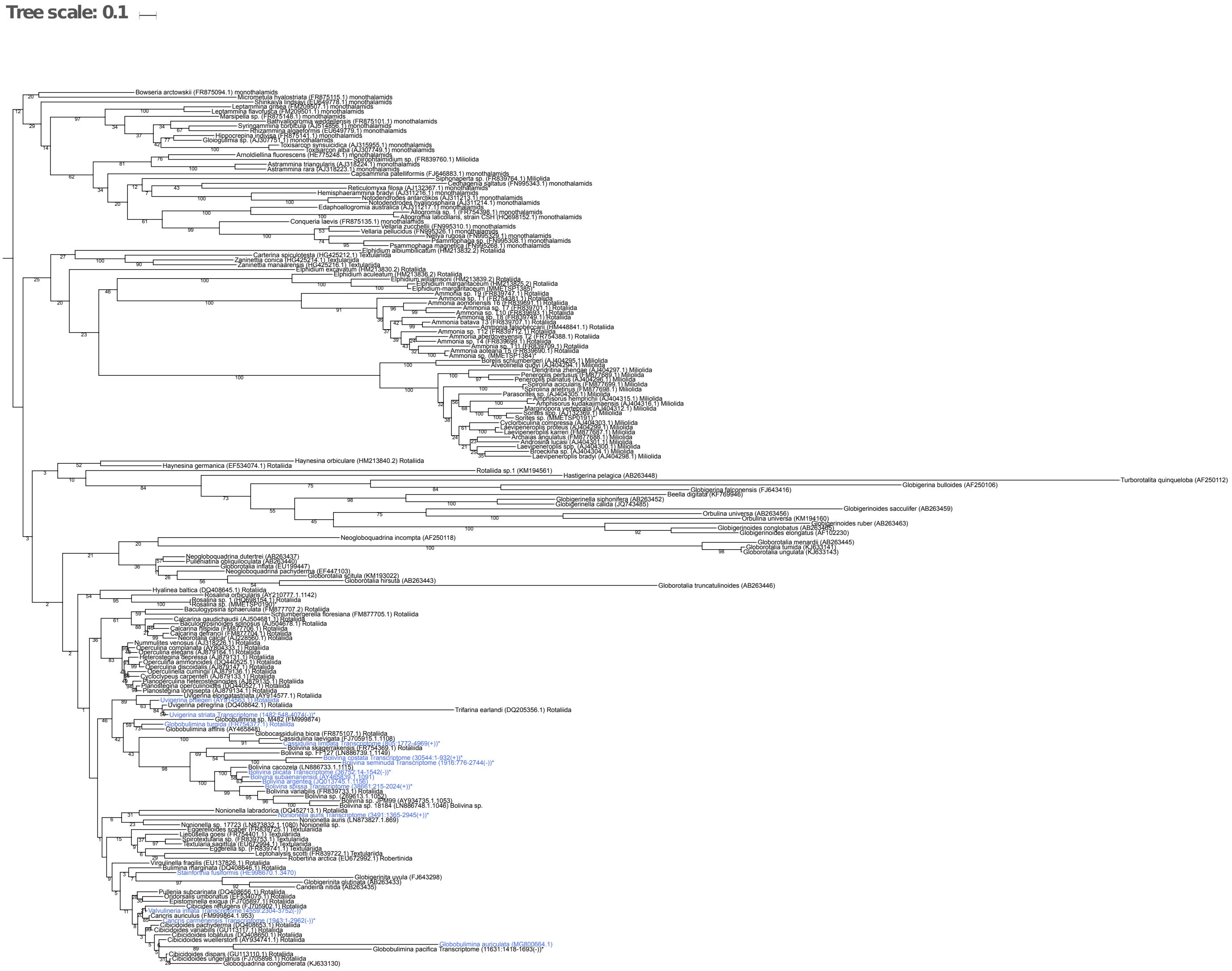
50.1/81
100/100
91.9/95


C
Bacteria
Eukaryota/Archaea
Foraminifera
II
Tree scale: 10
Eukaryota
Bacteria
Foraminifera
I

Suppl. Figure S2. Complete phylogenies of NirK, Nor and Nrt homologs. Phylogenetics trees of the foraminiferal denitrification proteins A) NirK B) Nor and C) Nrt that survived the cutoff together with homologs from public databases. Transcriptome labels contain transcription values of the two replicates (tpm1 & tpm2) as well as their percentile expression rank within corresponding transcriptomes. The labels contain as well the transcript id, region and orientation for individual open reading frames used. Numbers of the branches represent statistical support by ultrafast bootstrap and SH-like approximate likelihood ratio test results (following the '/') each with 1000 replicates. Branch support values of zero are omitted. If only one value is shown it represents the SH-like approximate likelihood ratio test. The background colour coding indicates different taxonomic groups. *Gobobulimina* spp. homologs from Woehle et al. 2018 are shown in bold. Further phylogenetic (sub-)clades, resembling those from Woehle et al. 2018, are highlighted by purple bars. The Nor tree was rooted using two cbb3 oxidases as outgroup, while for the Nrt tree representatives of DHA14 and ACS family of MFS transporters as outgroup. The NirK tree was rooted using the MAD method.



Suppl. Figure S3. Foraminifera rooting based on single-protein trees. A Maximum-likelihood phylogenetic reconstruction of foraminifera species based on 81 eukaryotic protein marker sequences is shown on the left. Parametric bootstrap support is 1000/1000 at all the branches. Right of the phylogeny a split representation of root splits determined via the MAD approach for the 81 single gene trees is shown. Each column represents a root. Root branches are reported as separation of two groups (black and white boxes) indicating for the species found on either side of the root split. The bar graph (top) reports the single gene tree count supporting the corresponding root splits. These were ranked by frequency.



Suppl. Figure S4. Uncollapsed 18S phylogeny of foraminifera. Species shown to denitrify experimentally are highlighted in blue. The only exception is *Stainforthia fusiformis*, where denitrification activity has been shown for an unspecified species of the same genus. Species also shown in Figure 2 or Figure S1 are highlighted by asterisks. Bootstrap support values with 1000 replicates are shown at the branches. The trees were rooted by the clade of monothalamids containing *R. filosa*. The phylogeny corresponds to the one shown in Figure 3.

Dataset 1. Sequencing statistics for metagenomics & transcriptomics.

Dataset 2. List of foraminiferal homologs of denitrification proteins remaining after applying cutoffs.

Dataset 3. List of foraminiferal homologs of denitrification proteins removed by cutoffs.

Dataset 4. List of marker proteins used for phylogenetic reconstructions.

Dataset 5. 18S sequences including metadata for phylogenetic reconstructions.

Dataset 6. Proportions of family rank classification of foraminifera-associated microbial communities.

Dataset 7. High quality genomic bins of foraminifera-associated species communities.

Dataset 8. Metabolic classification of Desulfobacteracea genomes.