Supplementary Information

A mesophilic autotrophic ammonia-oxidizing archaeon of the thaumarchaeal group I.1a cultivated from a deep oligotrophic soil horizon

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This file includes:

Supplementary Table S1 to S3

Supplementary reference

Supplementary Figure Legends

Supplementary Figures S1 to S8

Table S1. Primers used for PCR amplification during library construction and real-time quantification.

Target gene	*Application*	*Sequence (5′ to 3′)*	*Position*	*Reference*
16S rRNA				
519F 727R	Archaeal quantification	CAGCMGCCGCGGTAA GCTTTCRTCCCTCACCGT	519–533[a] 712–727[a]	(1, 2)
Bac518F Bac786R	Bacterial quantification	CCAGCAGCCGCGGTAAT CTACCAGGGTATCTAATC	518–534[a] 786–803[a]	(3, 4)
27F	Clone library of bacteria (with1492R)	AGAGTTTGATCMTGGCTCAG	8-27[a]	(2)
20F 1492R	Phylogenetic analysis and clone library of AOA	TTCCGGTTGATCCYGCCRG TACGGYTACCTTGTTACGACTT	2–20[a] 1510–1492[a]	(5) (2)
βAMOF βAMOR	First PCR (β-AOB)	TGGGGRATAACGCAYCGAAAG AGACTCCGATCCGGACTACG	143–163[a] 1296–1315[a]	(6)
CTO189F CTO654R	Second PCR (β-AOB)	GGAGRAAAGYAGGGGATCG CTAGCYTTGTAGTTTCAAACGC	189-207[a] 633-654[a]	(7)
amoA				
AamoAF AamoAR	Phylogenetic analysis and clone library of AOA	STAATGGTCTGGCTTAGACG ACATACAGATGGATGGCCGC	19–38[b] 582–601[b]	(8)
amoA1F amoA2R	PCR (β-AOB)	GGGGTTTCTACTGGTGGT CCCCTCKGSAAAGCCTTCTTC	332–349[c] 820–822[c]	(9)
A189F A682R	PCR (γ-AOB)	GGNGACTGGGACTTCTGG GAASGCNGAGAAGAASGC	172–189[d] 665–682[d]	(10)

[a] Numbering is based on the 16S rRNA gene of *Escherichia coli*.

[b, c, d] Numbering is based on the *amoA* gene of a metagenomic clone from the Sargasso Sea (AACY01435967), *N. europaea* (L08050), and *Nitrosococcus oceani* (AF047705), respectively.

Table S2. Comparison of 16S rRNA and amoA gene of strain MY2 with those of other AOA strains and fosmid clones.

	% identity[a]	
	Strain MY2	
Species or clone	**16S rRNA**	***amoA***
Nitrosopumilus maritimus (11)	92.9	79.5
"*Candidatus* Nitrosoarchaeum koreensis" (12)	92.9	80.5
"*Candidatus* Nitrosoarchaeum limnia" (13)	93.3	81.7
Cenarchaeum symbiosum (14)	93.2	76.1
"*Candidatus* Nitrosotalea devanaterra" (15)	88.9	75.1
"*Candidatus* Nitrososphaera gargensis" (16)	84.3	71.3
Nitrososphaera viennensis (17)	85.4	70.9
"*Candidatus* Nitrosocaldus yellowstonii" (18)	82.8	70.1
54d9 (19)	85.1	71.8

[a] Comparisons are based on 16S rRNA genes (Ca. 1300 bp) and *amoA* genes (Ca. 600 bp).

Table S3. Clusters of orthologous groups (COG) automated classification.

		MY2		*N. maritimus*		*N. koreensis*		*N. gargensis*	
Functional category		ORF (unique ORF)	% of ORF	ORF	% of ORF	ORF	% of ORF	ORF	% of ORF
CELLULAR PROCESSES AND SIGNALING									
D	Cell cycle control and mitosis	14 (11)	0.66	11	0.55	5	0.26	9	0.25
M	Cell wall/membrane/envelop biogenesis	52 (34)	2.44	49	2.45	46	2.37	50	1.40
N	Cell motility	32 (26)	1.50	6	0.30	9	0.46	23	0.64
O	Post-translational modification, protein turnover, chaperone functions	77 (36)	3.61	74	3.71	60	3.08	90	2.52
T	Signal Transduction	76 (70)	3.56	47	2.35	42	2.16	65	1.82
U	Intracellular trafficing and secretion	17 (8)	0.80	11	0.55	8	0.41	10	0.28
V	Defense mechanisms	4 (1)	0.19	13	0.65	14	0.72	15	0.42
Z	Cytoskeleton	1 (0)	0.05	0	0.00	1	0.05	0	0.00
INFORMATION STORAGE AND PROCESSING									
A	RNA processing and modification	2 (2)	0.09	0	0.00	1	0.05	1	0.03
B	Chromatin Structure and dynamics	1 (0)	0.05	0	0.00	1	0.05	2	0.06
J	Tranlsation	134 (29)	6.28	136	6.81	129	6.63	145	4.07
K	Transcription	98 (37)	4.59	76	3.81	89	4.58	118	3.31
L	Replication and repair	69 (35)	3.23	56	2.80	54	2.78	163	4.57
METABOLISM									
C	Energy production and conversion	92 (29)	4.31	100	5.01	86	4.42	118	3.31
E	Amino Acid metabolis and transport	105 (32)	4.92	109	5.46	98	5.04	125	3.51
F	Nucleotide metabolism and transport	45 (10)	2.11	45	2.25	42	2.16	46	1.29
G	Carbohydrate metabolism and transport	44 (16)	2.06	46	2.30	41	2.11	58	1.63
H	Coenzyme metabolis	87 (33)	4.08	95	4.76	75	3.86	82	2.30
I	Lipid metabolism	30 (6)	1.41	30	1.50	27	1.39	37	1.04

P	Inorganic ion transport and metabolism	58 (24)	2.72	63	3.15	52	2.67	86	2.41
Q	Secondary Structure	12 (7)	0.56	15	0.75	17	0.87	29	0.81
POORLY CHARACTERIZED									
R	General Functional Prediction only	156 (86)	7.31	152	7.61	116	5.96	176	4.94
S	Function Unknown	112 (60)	5.25	86	4.31	72	3.70	121	3.39
COG gene		**1318 (592)**	**62.0**	**1220**	**61.1**	**1085**	**55.8**	**1569**	**44.0**
Total ORFs		**2126**	**100**	**1997**	**100**	**1945**	**100**	**3566**	**100**

1

Supplementary reference

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Supplementary Figure Legends

Fig. S1. Effect of various antibiotics on the ammonia oxidation activity of strain MY2. Activity of ammonia oxidation was determined by nitrite accumulation. Initial inoculum cell density was ca. 2.5×10^6 cells ml^{-1}. Concentration of streptomycin (100 μg ml^{-1}), kanamycin (50 μg ml^{-1}), ampicillin (50 μg ml^{-1}) penicillin-G (50 μg ml^{-1}) gentamycin (20 μg ml^{-1}), mitomycin-C (20 μg ml^{-1}), tetracycline (15 μg ml^{-1}). Control culture was set without antibiotics. The error bars represent the standard deviations from triplicate experiments.

Fig. S2. Phylogenetic analysis of the archaeal 16S rRNA gene sequence (ca. 1.3 kbp) obtained from strain MY2. The archaeal 16S rRNA gene was amplified using primers 20F and 1492R. Branching patterns supported by > 50% bootstrap values (1,000 iterations) according to the neighbor-joining method are denoted by their respective bootstrap values. The cluster groups are shown at the right of the figure based on the origins of the reference sequences. “ThAOA” indicates a thermophilic AOA lineage. The scale bar represents 2% estimated sequence divergence. Enriched or isolated AOAs among the reference sequences are indicated in boldface.

Fig. S3. Effect of different concentrations of allylthiourea (ATU) on the ammonia oxidation by strain MY2. Strain MY2 was grown in AFM with 1 mM ammonia. Ammonia oxidation activity was indicated by nitrite accumulated. The error bars represent the standard deviations from three replicates.

Fig. S**4.** Circular representation of the genome of strain MY2. The outermost circle shows RNA (circle 1), and the gene content is predicted on the reverse and forward strands (circles 2 and 3). The colors of predicted ORFs are based on COG functional categories (see the key for the color designations). Circle 4 shows the GC skew and circle 5 shows the G + C ratio

(values greater or smaller than the average percentage in the overall chromosome are shown in red and green, respectively)

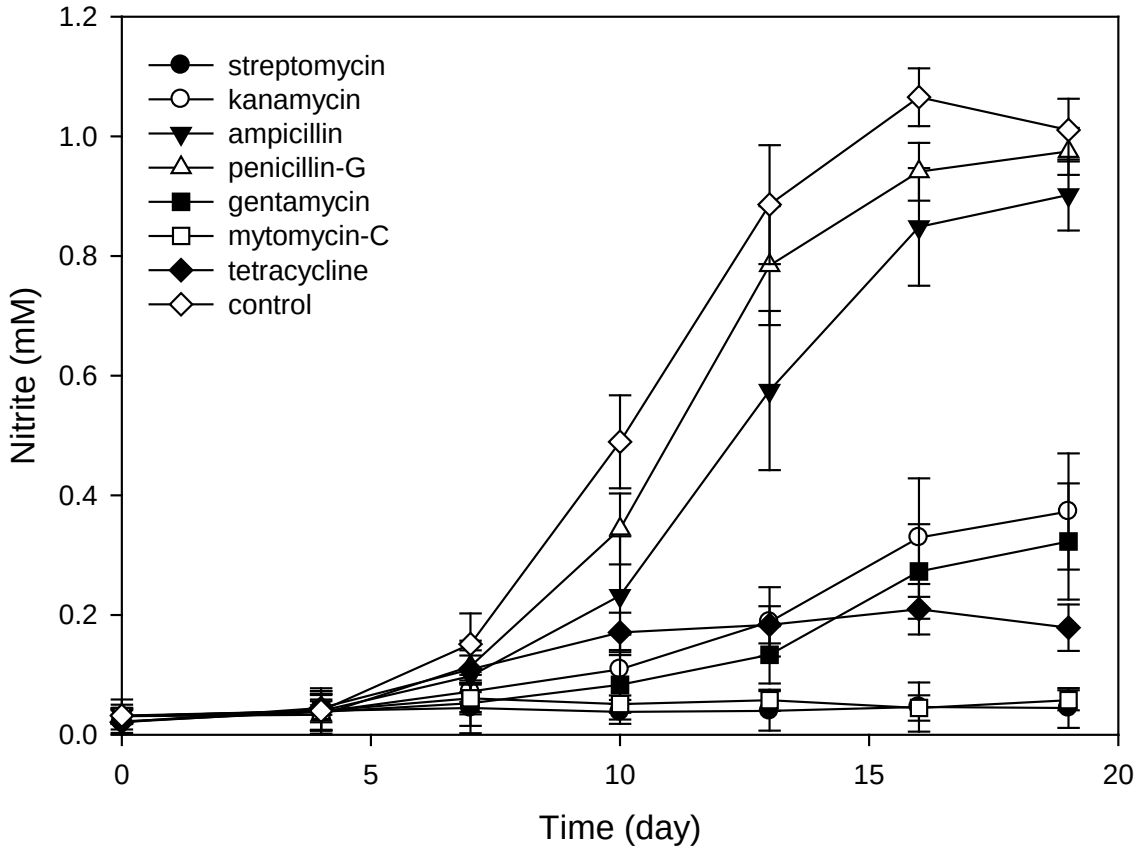
Fig. S5. Dot plot representation of the pairwise alignments of the strain MY2, "*Ca.* N. gargensis", "*Ca.* N. koreensis" and *N. maritimus* genomes. Alignments were performed on the six-frame amino acid translation of the genome sequences using the program in the MUMmer 3.23 package. In all plots, a dot indicates a gene compared, with forward or reverse matches shown in red and blue, respectively.

Fig. S6. Venn diagrams showing the numbers of ORFs shared between the predicted proteins of strain MY2, *N. koreensis* and *N. maritimus*. Shared ORFs from the query genome were considered to be conserved if they had a BLAST match of ≥ 60% of the overall sequence identity and 70% of the length of the query ORF. Numbers in brackets below species names indicate total number of ORFs. The bar graph presents the percentage of COGs of strain MY2.

Fig. S7. Ammonia monooxygenase (*amo*) gene order of strain MY2 compared with ammonia oxidizing bacteria (AOB), close relatives of thaumarchaeal group I.1a (name in red) and I.1b (name in blue).

Fig. S8. Organization of flagella and chemotaxis genes in ammonia oxidizing archaea. Flagellum-associated genes (*flaB, flaG, flaF, flaH, flaI, flaJ*) are shown in light blue. Chemotaxis genes (MCP, methyl-accepting chemotaxis protein; *cheA*, histidine kinase; *cheY*, response regulator; *cheW*, adapter protein; *cheC, cheD, cheR, cheB,* adaptation proteins) are shown in light green. The gene for the transcriptional regulator *TrmB* is shown in white and unknown proteins are shown in grey. Color bars indicate the percentage of identity with MY2.

Fig. S1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penicillin-G
gentamycin
mytomycin-C
tetracycline
control
Nitrite (mM)
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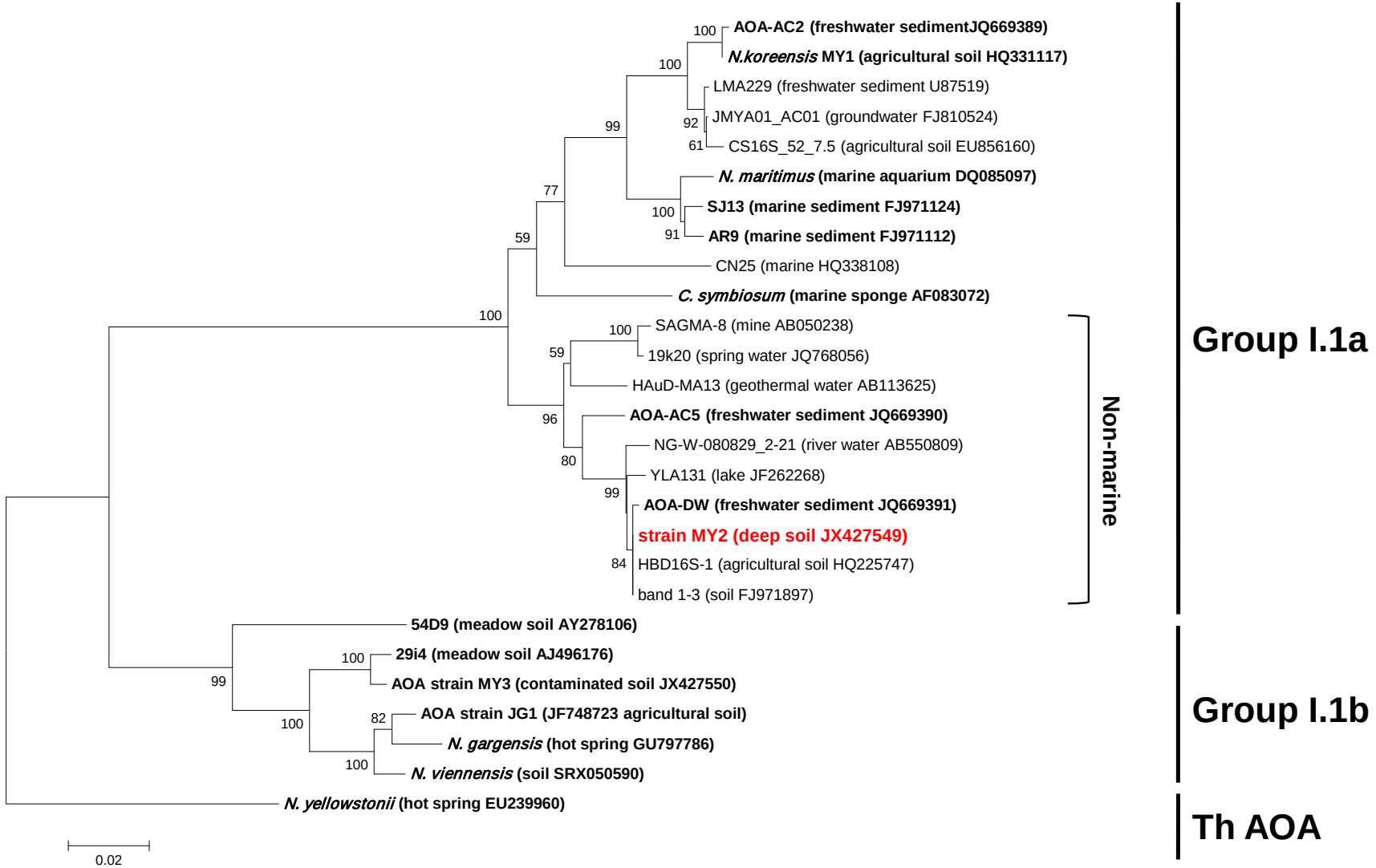
0.6
0.8
1.0
1.2
0
5
10
15
20

Fig. S2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CS16S_52_7.5 (agricultural soil EU856160)
N. maritimus (marine aquarium DQ085097)
SJ13 (marine sediment FJ971124)
AR9 (marine sediment FJ971112)
CN25 (marine HQ338108)
C. symbiosum (marine sponge AF083072)
SAGMA-8 (mine AB050238)
19k20 (spring water JQ768056)
HAuD-MA13 (geothermal water AB113625)
AOA-AC5 (freshwater sediment JQ669390)
NG-W-080829_2-21 (river water AB550809)
YLA131 (lake JF262268)
AOA-DW (freshwater sediment JQ669391)
strain MY2 (deep soil JX427549)
HBD16S-1 (agricultural soil HQ225747)
band 1-3 (soil FJ971897)
54D9 (meadow soil AY278106)
29i4 (meadow soil AJ496176)
AOA strain MY3 (contaminated soil JX427550)
AOA strain JG1 (JF748723 agricultural soil)
N. gargensis (hot spring GU797786)
N. viennensis (soil SRX050590)
N. yellowstonii (hot spring EU239960)
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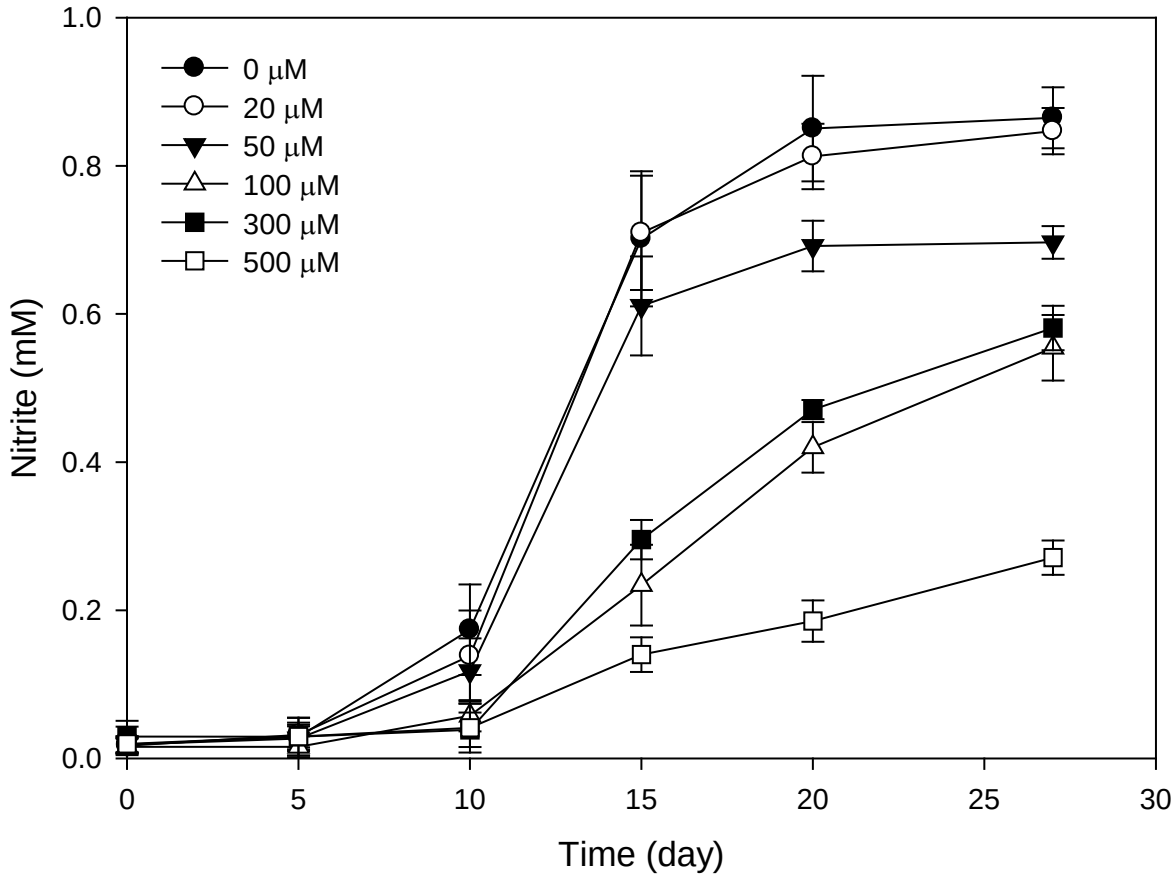
Non-marine
Group I.1a
Group I.1b
Th AOA

Fig. S3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0.4
0.2
0.0
Nitrite (mM)
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10
15
20
25
30
Time (day)
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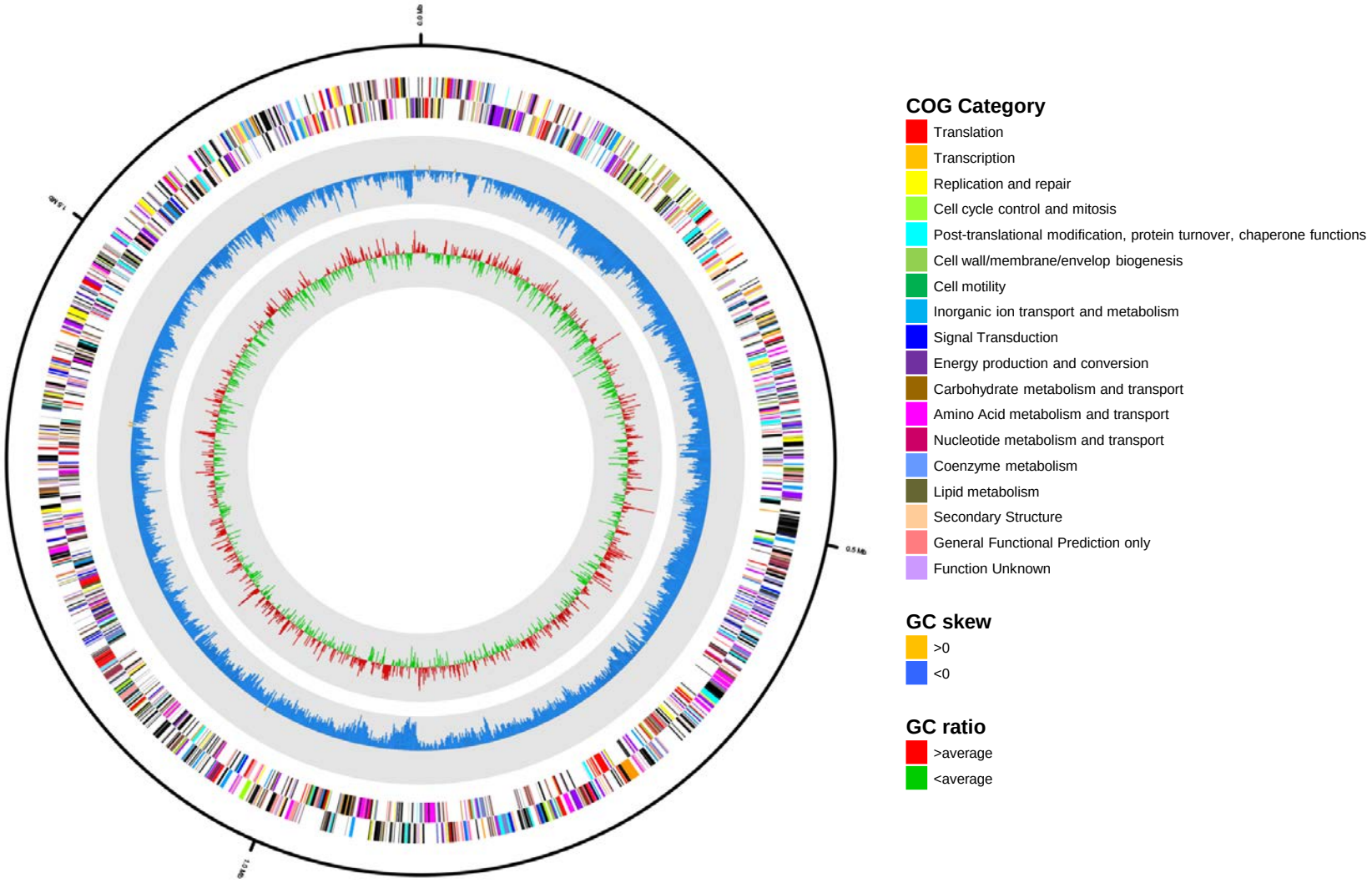
20 μM
50 μM
100 μM
300 μM
500 μM

Fig. S4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0.5 Mb
1.0 Mb
1.5 Mb
COG Category
Translation
Transcription
Replication and repair
Cell cycle control and mitosis
Post-translational modification, protein turnover, chaperone functions
Cell wall/membrane/envelop biogenesis
Cell motility
Inorganic ion transport and metabolism
Signal Transduction
Energy production and conversion
Carbohydrate metabolism and transport
Amino Acid metabolism and transport
Nucleotide metabolism and transport
Coenzyme metabolism
Lipid metabolism
Secondary Structure
General Functional Prediction only
Function Unknown
GC skew
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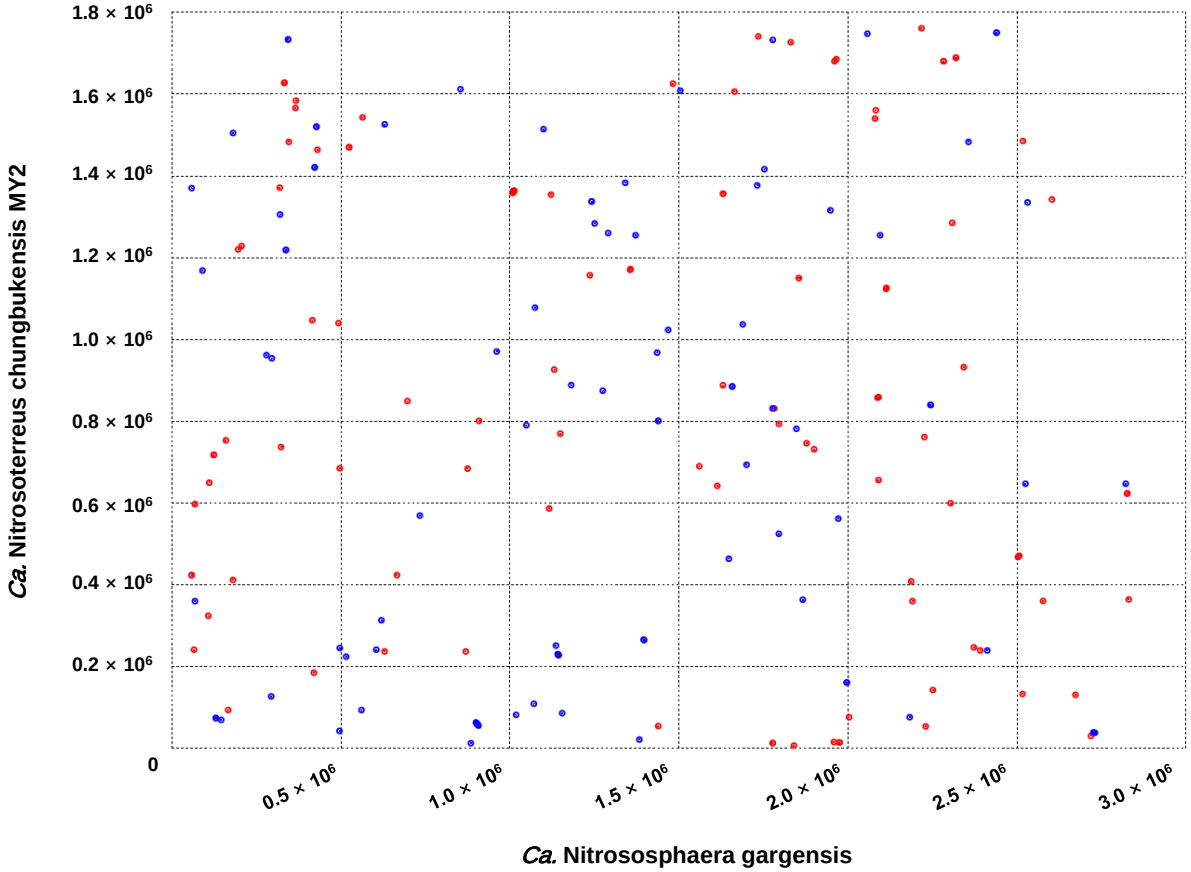
<0
GC ratio
>average
<average

Fig. S5 (A)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1.2 × 10⁶
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0.6 × 10⁶
0.4 × 10⁶
0.2 × 10⁶
0
Ca. Nitrosoterreus chungbukensis MY2
0.5 × 10⁶
1.0 × 10⁶
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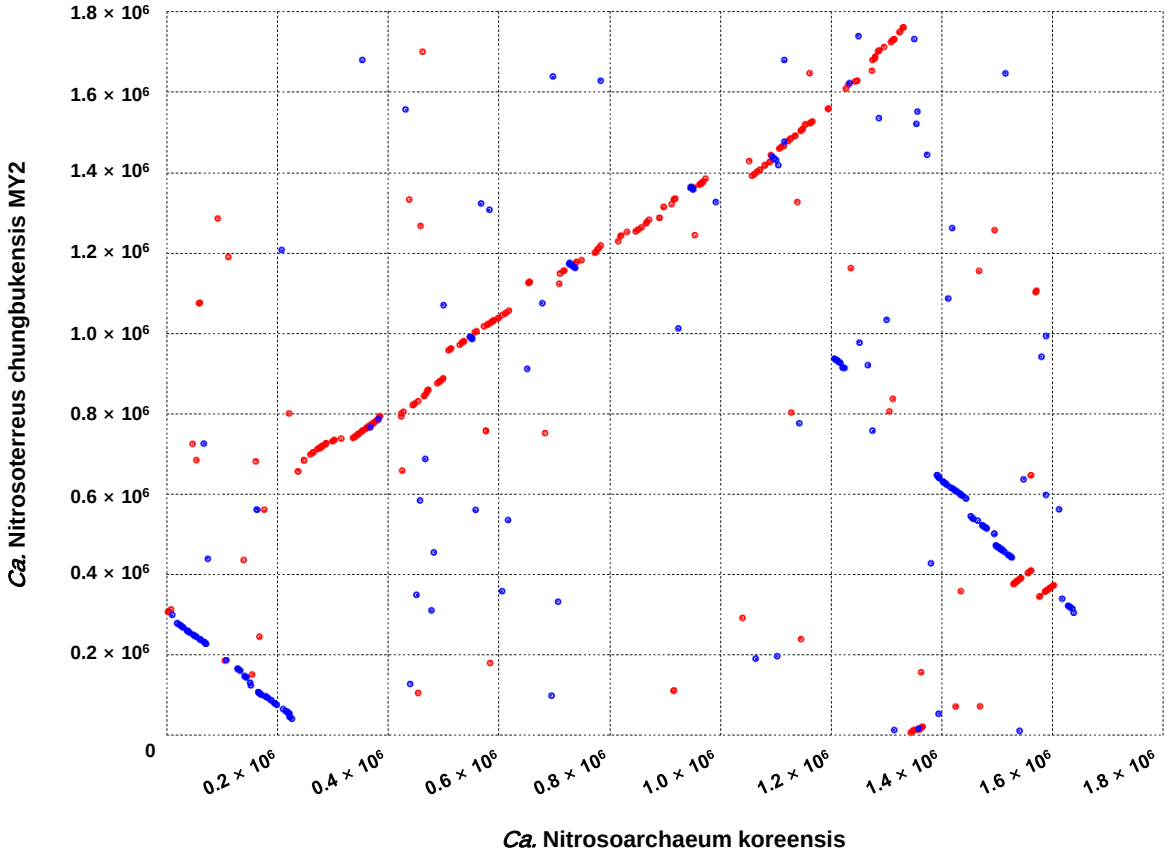
2.5 × 10⁶
3.0 × 10⁶
Ca. Nitrososphaera gargensis

Fig. S5 (B)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1.4 × 10⁶
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0.4 × 10⁶
0.2 × 10⁶
0
0.2 × 10⁶
0.4 × 10⁶
0.6 × 10⁶
0.8 × 10⁶
1.0 × 10⁶
1.2 × 10⁶
1.4 × 10⁶
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1.8 × 10⁶
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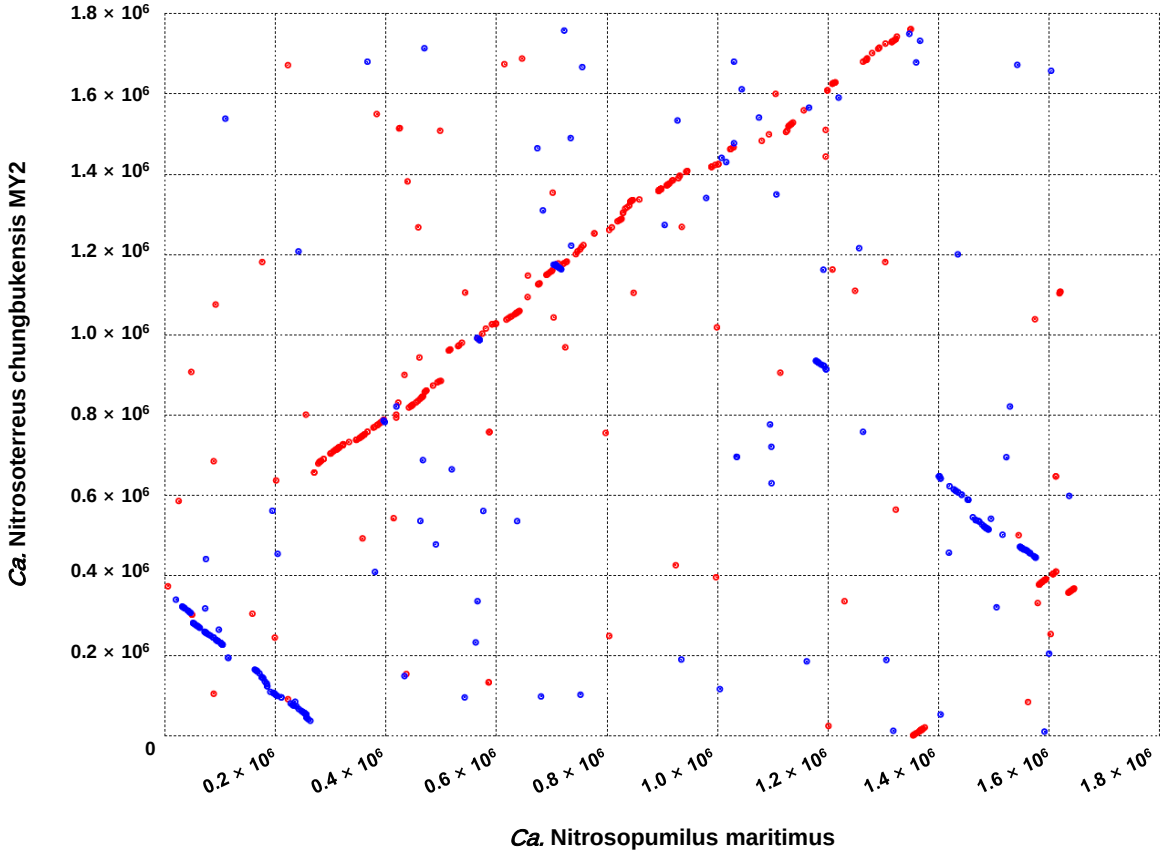
Ca. Nitrosoarchaeum koreensis

Fig. S5 (C)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0.2 × 10⁶
0.4 × 10⁶
0.6 × 10⁶
0.8 × 10⁶
1.0 × 10⁶
1.2 × 10⁶
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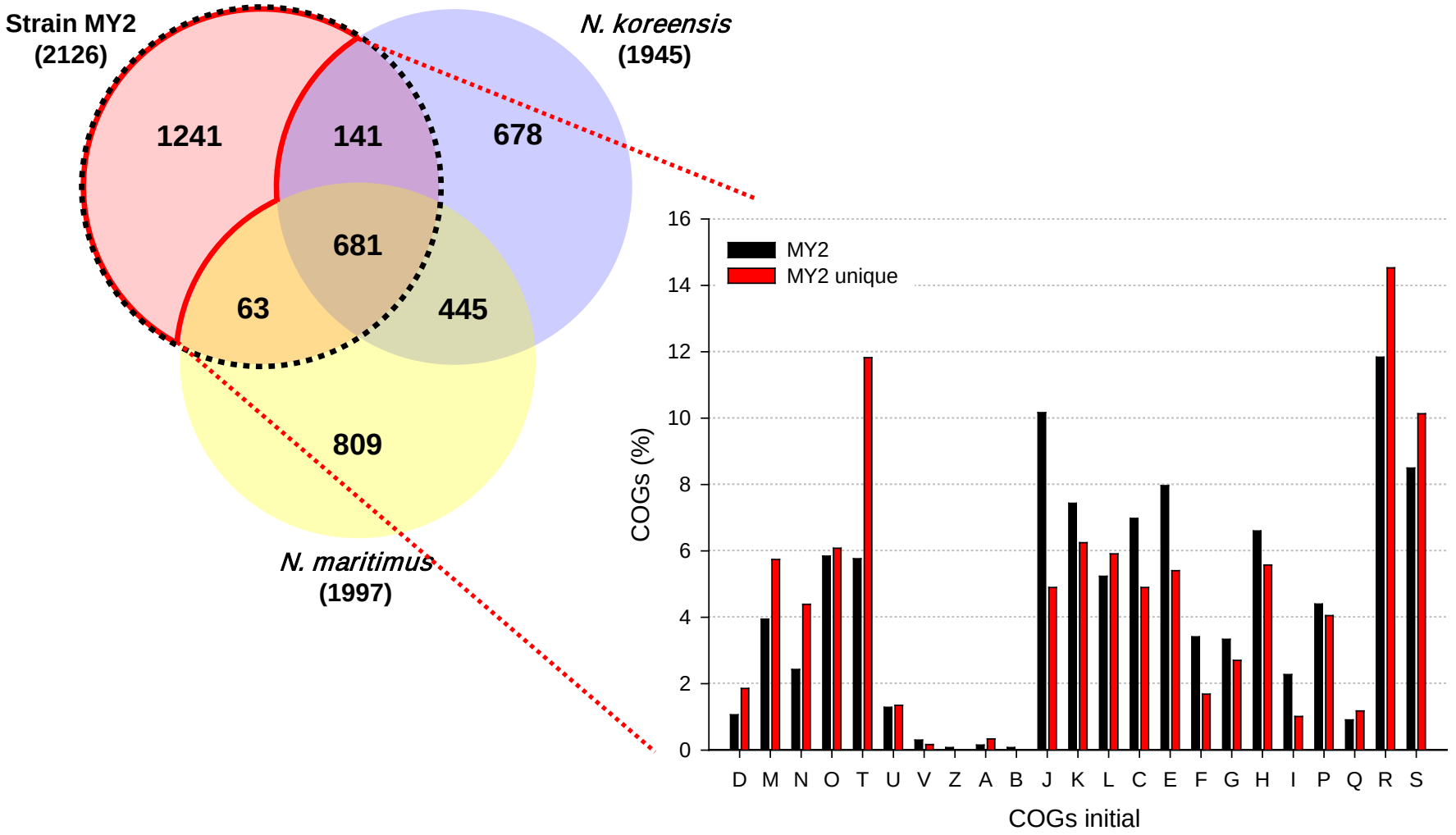
Ca. Nitrosopumilus maritimus

Fig. S6


Strain MY2
(2126)
N. koreensis
(1945)
N. maritimus
(1997)
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141
678
681
63
445
809
MY2
MY2 unique
COGs (%)
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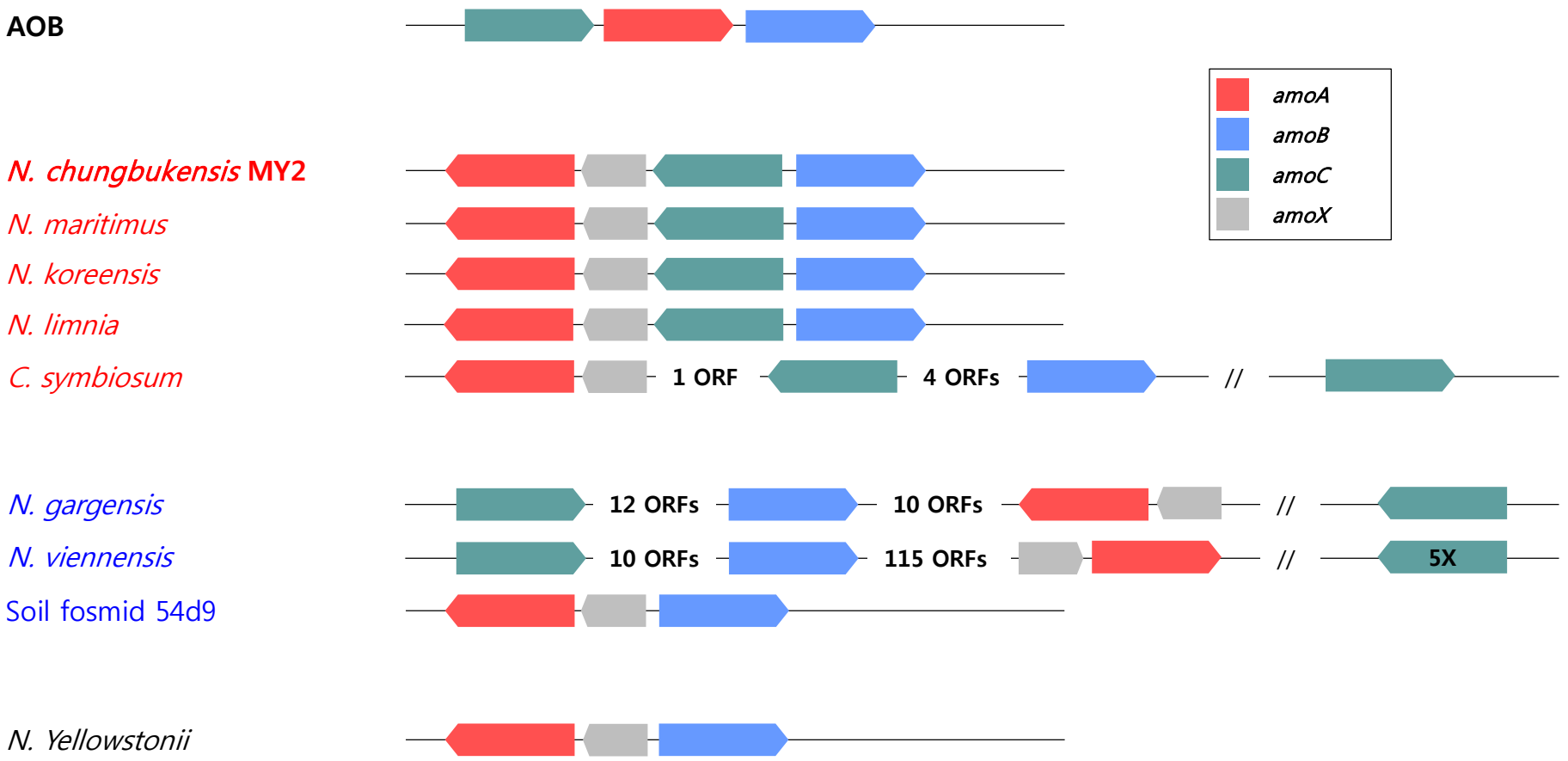
2
4
6
8
10
12
14
16
D M N O T U V Z A B J K L C E F G H I P Q R S
COGs initial

Fig. S7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N. maritimus
N. koreensis
N. limnia
C. symbiosum
1 ORF
4 ORFs
//
N. gargensis
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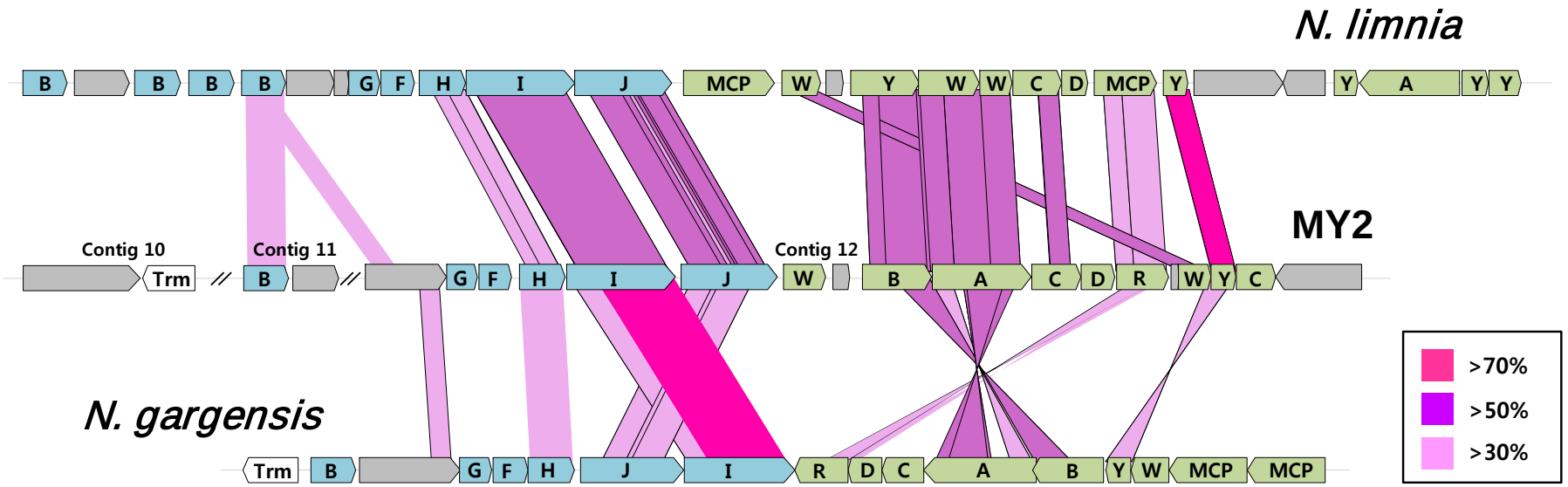
//
N. viennensis
10 ORFs
115 ORFs
//
5X
Soil fosmid 54d9
N. Yellowstonii

Fig. S8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MCP
W
Y
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Trm
B
G
F
H
J
I
R
D
C
A
B
Y
W
MCP
MCP
>70%
>50%
>30%