Supplementary materials Raitzz et al.

File S1. SweeP Analyses

In this study, not all results were performed on specific scripts. In some cases, the results were obtained step by step in the MATLAB® command window and in other data of interchange between the various applied software. Below, is included excerpts from scripts with details and the links to the tools used that can assist in understanding the methods.

1) Function to calculate the distance of SWeeP Vectors:

```
pdist()
```

pdist() is a standard function in MATLAB® that calculate the pairwise distance (by default Euclidean distances) between observations (vectors);

2) Excerpt of script to obtain neighbour joining:

```
treeout = seqneighjoin(xdist,'equivar',xnames);
```

Were *xdist* is a pdist vector (MATLAB standart result from a *pdist()* function call) and *xname* is a cell var with the nodes names.

3) Normalization by the mean of ANI matrices:

Here, the normalization was performed for the upper and lower ANI matrices separately (see the script below). First, ANI was converted into dissimilarity matrices and then the lower and upper matrices were divided by their corresponding mean values. It was decided to normalize the mean to guarantee a better correspondence between the matrices in the visualization. This technique allowed us to compare different distance / dissimilarity matrices in the same heat map.

```
% ANI matrix
MANI
% Transforms lower and upper ANI matrices in dissimilarity
matrices
```

```
D1 = 100 - tril(MANI)+tril(MANI)';
D2 = 100 - triu(MANI)+triu(MANI)'
% iani1 - Order for Lower triangular part of the ANI matrix
[~ iani1] = HFclus(distmat2pdist(D1),'Method','Nj');
% iani2 - Order for upper triangular part of the ANI matrix
[~ iani2] = HFclus(distmat2pdist(D2),'Method','Nj');
% HFclus is an 'in house' function used for several purposes;
here it receives a MATLAB pdist vector and returns de order
of the leaves in the corresponding neighbour joining
phylogenetic tree (exploiting the MATLAB bioinformatics
toolbox functions)

% Normalization and matrix reordering for Heat map
Mx = tril(D1)/mean(D1(:))+triu(D2)/mean(D2(:));
Mx = Mx(iani1,iani2);
% Transforms matrix into image
xHeat = mat2im3(Mx);
```

4) Links to all the programs used in this study:

FastQC

https://www.bioinformatics.babraham.ac.uk/projects/fastqc/

Trimmomatic

http://www.usadellab.org/cms/?page=trimmomatic

BBMap

https://sourceforge.net/projects/bbmap/

SPAdes

https://cab.spbu.ru/software/spades/

Prokka

https://github.com/tseemann/prokka

QUAST

http://quast.sourceforge.net/

FastANI

https://github.com/ParBLiSS/FastANI

RAFTS3G

https://sourceforge.net/projects/rafts-g/

SWeeP

https://sourceforge.net/projects/spacedwordsprojection/

Dendroscope

https://software-ab.informatik.uni tuebingen.de/download/dendroscope3/welcome.html

Clustal Omega

https://www.ebi.ac.uk/Tools/msa/clustalo/

IQ-Tree

http://www.iqtree.org/

File S2. *Growth conditions for assessment of nitrogenase activity using the acetylene reduction assay*

Azoarcus olearius DQS-4^T, *Az. communis* Swub3^T, *Az. communis* LMG 5514 (LMG), *Az. indigens* VB32^T, and *Azoarcus* sp. TTM-91 were grown on SM or CVM media, prepared following the composition described in [1]. The SM medium was supplemented with a vitamin solution (1 mg L^{-1} thiamine hydrochloride, 2 mg L^{-1} D-pantothenic acid, 1 mg L^{-1} nicotinic acid, and 0.1 mg L^{-1} biotin) modified from UMS medium [2]. Semi solid medium (SM or ½CVM) was prepared at the moment of use by mixing pre-warmed solid (1.5% agar) and liquid media to reach a final concentration of 0.18% agar. ½CVM is CVM [1] but with half of the amount of bactopeptone and yeast extract *i.e.* 1.5 and 0.5 g L^{-1}, respectively. *Aromatoleum* sp. CIB and *Ar. toluvorans* Td21^T were grown on MNF medium [3] supplemented with 3 mM sodium benzoate as carbon source.

Prior to the nitrogenase activity assay, *Az. olearius*, DQS-4^T, *Az. communis* Swub3^T, *Az. communis* LMG 5514 (LMG), *Az. indigens* VB32^T, and *Azoarcus* sp. TTM-91 were streaked from glycerol stocks into CVM amended with 20 mM NH_4Cl (CVM+N) and incubated at 30°C for 3–5 days. Isolated colonies were then inoculated into 3 mL of liquid CVM+N (in 30 mL plastic universal tubes) and incubated at 30°C and 120 rpm. Once the culture reached saturation, a further inoculation (1/100) in liquid medium was performed before the inoculation into semi-solid medium (SM amended with 0.5 mM sodium glutamate for *Az. olearius*, DQS-4^T, *Az. communis* Swub3^T, and *Az. communis* LMG 5514, or ½CVM for *Az. indigens* VB32^T, and *Azoarcus* sp. TTM-91). The semi-solid medium (3 mL) was set in a 7 mL glass vial (Fisherbrand™ #14873562). Liquid cultures (15–60 μL), pre-grown as above, were used for inoculation into the center-bottom of vials of semi-solid media which was aimed at achieving approximately the same number of cells (OD_{600nm} = 0.01 approximately) across the different inocula. Cells were incubated for 18–24 hours, or until the bacterial pellicle reached the meniscus. Once the pellicle was formed, the plastic cap from the vial was replaced by a suba-seal rubber stopper (n° 21) and the substrate, acetylene (10% of the gas phase), for the nitrogenase activity assay was injected. A similar pre-inoculation procedure was used to assay the nitrogenase activity in *Aromatoleum* sp. CIB and *Ar. toluvorans* Td21^T except that MNF medium was supplemented with 3 mM sodium benzoate as carbon source and 5 mM KNO_3 as nitrogen source. The second re-inoculation into liquid medium was performed on a higher volume, 20 mL of medium in a 100 mL Erlenmeyer flask at 30°C and 120 rpm. Cells were then washed in MNF + 3 mM sodium benzoate to remove excess nitrate and then re-inoculated into fresh medium without nitrate to an OD_{600nm} of approximately 0.01. Ten mL cells (OD_{600nm} of 0.01), were transferred to 20 mL glass universal tubes, sealed with suba-seal rubber stopper n°41 and incubated for 24 hours at 30°C and 120 rpm before injection of the acetylene (10% of the gas phase). *Ar. diolicum* 22Lin^T was grown on UMS medium supplemented with 30 mM malate as carbon source and 20 mM NH_4Cl. Prior to the acetylene reduction assay, 50 mL cells were collected by centrifugation (4000 rpm, 8 min) and re-suspended to an initial O.D600 of 0.4 (20 mL in an 100 mL Pyrex Erlenmeyer flask) in UMS plus 30 mM malate without added ammonium. The flask was stoppered with a suba-seal (nº 37) and the internal flask atmosphere was adjusted to approximately 1% O2. After 6 hours incubation, the acetylene reduction assay was performed as above.

References

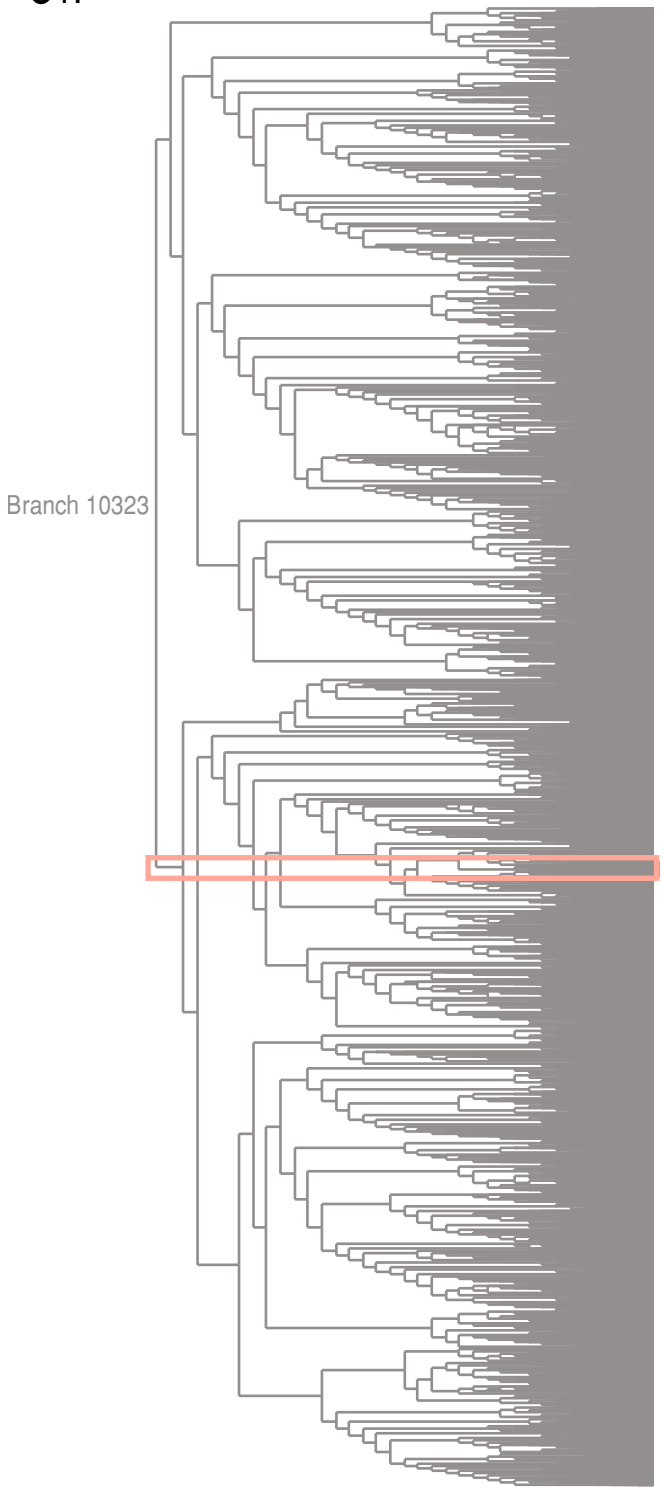
1. Krause, A.; Julich, H.; Mankar, M.; Reinhold-Hurek, B. The regulatory network controlling ethanol-induced expression of alcohol dehydrogenase in the endophyte *Azoarcus* sp. strain BH72. *Mol. Plant-Microbe Interact.* **2017**, 30, 778-785, doi:MPMI-01-17-0013-R.

2. Pini, F.; East, A.K.; Appia-Ayme, C.; Tomek, J.; Karunakaran, R.; Mendoza-Suarez, M.; Edwards, A.; Terpolilli, J.; Rowoth, J.; Downie, J.A.; et al. Bacterial biosensors for in vivo

spatiotemporal mapping of root secretion. *Plant Physiol.* **2017**, 174, 1289-1306, doi:10.1104/pp.16.01302

3. Fernández, H.; Prandoni, N.; Fernández-Pascual, M.; Fajardo, S.; Morcillo, C.; Díaz, E.; Carmona, M. Azoarcus sp. CIB, an anaerobic biodegrader of aromatic compounds shows an endophytic lifestyle. *PLoS One* **2014**, *9*, e110771, doi:10.1371/journal.pone.0110771

S1.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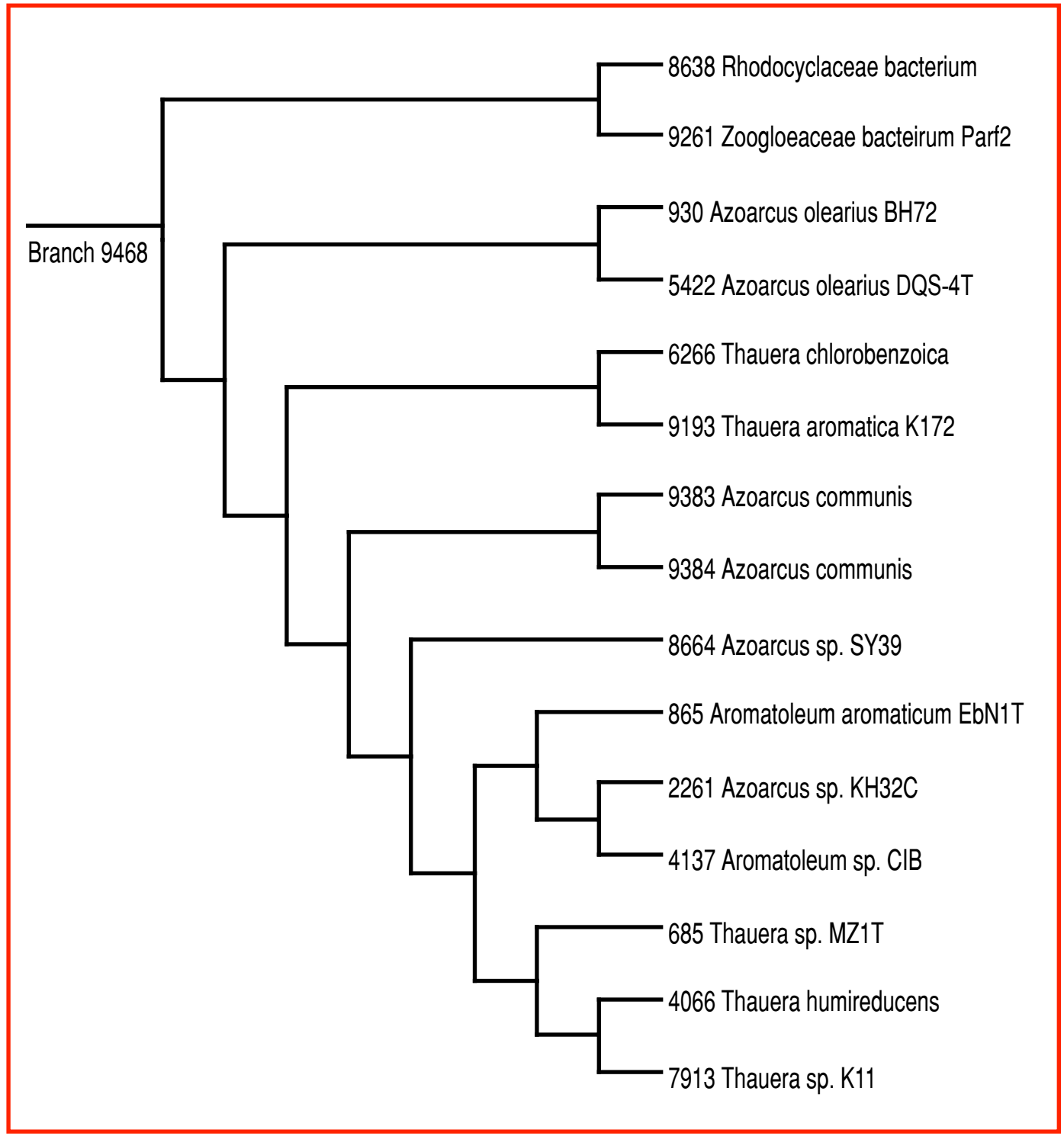
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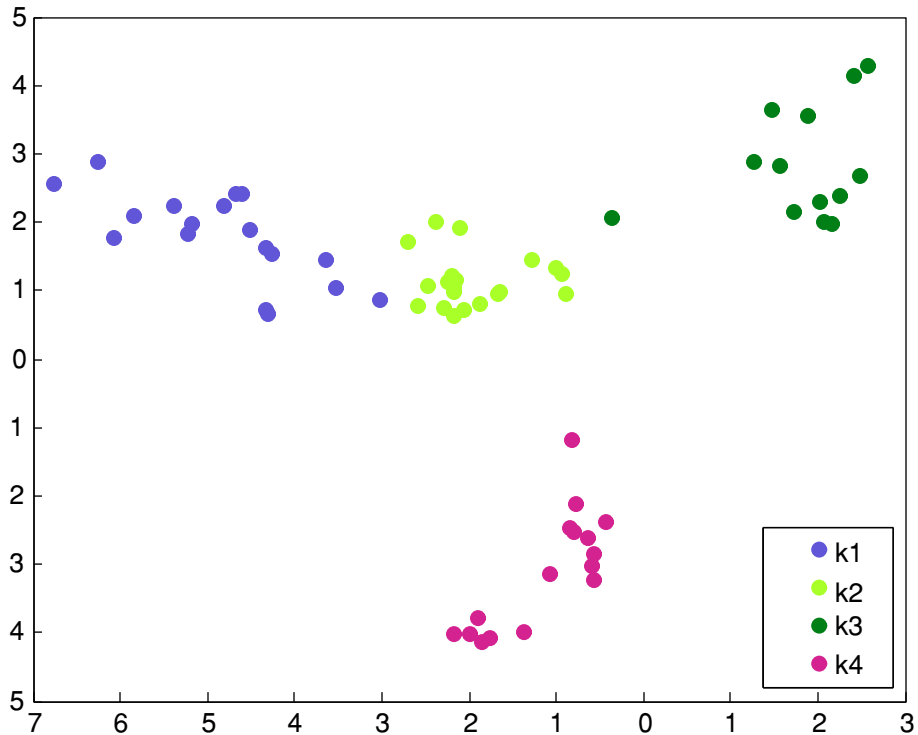
6266 Thauera chlorobenzoica
9193 Thauera aromatica K172
9383 Azoarcus communis
9384 Azoarcus communis
8664 Azoarcus sp. SY39
865 Aromatoleum aromaticum EbN1T
2261 Azoarcus sp. KH32C
4137 Aromatoleum sp. CIB
685 Thauera sp. MZ1T
4066 Thauera humireducens
7913 Thauera sp. K11

S2a.



S2b.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Thauera sp. 63
Thauera humireducens SgZ-1
Thauera butanivorans NBRC 103042
Thauera linaloolentis 47Lol_1
Thauera linaloolentis 47Lol_2
Thauera phenolivorans ZV1C
Thauera aromatica K172
Thauera chlorobenzoica 3CB1
Thauera sp. UPWRP
Thauera phenylacetica B4P
Thauera sp. SWB20
Thauera aminoaromatica S2
Thauera sp. MZ1T
k3
Thauera
Root
Aromatoleum anaerobium LuFRes1T
Aromatoleum bremense PbN1T
Aromatoleum aromaticum EbN1T
Aromatoleum aromaticum pCyN1
Aromatoleum buckelii U120T
Aromatoleum sp. PA01
EbN1T group
Azoarcus sp. KH32C
Aromatoleum diolicum 22Lin2T
Aromatoleum sp. DN11
Aromatoleum toluvorans Td21T
Aromatoleum petrolei Ton1T
Aromatoleum sp. CIB
Aromatoleum tolulyticum Tol-4T
Aromatoleum toluolicum T
Aromatoleum evansii KB740T
Aromatoleum toluclasticum MF63T
CIB group
k4
Aromatoleum
Azoarcus sp. TTM-91
Azoarcus indigens VB32T_2
Azoarcus indigens VB32T_1
Azoarcus sp. DD4
Azoarcus olearius BH72
Azoarcus olearius DQS-4T
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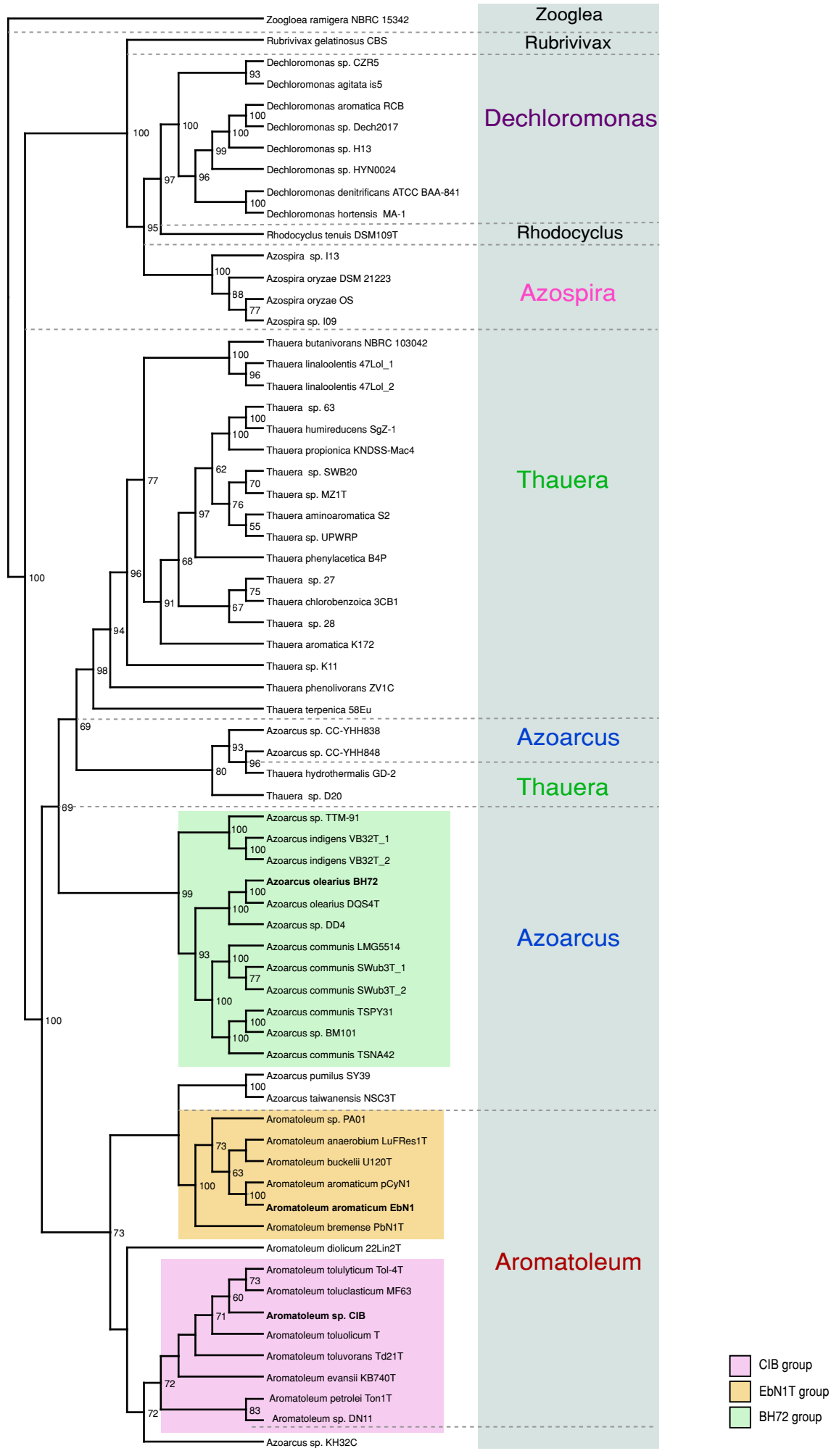
Azoarcus communis SWub3T_2
Azoarcus communis SWub3T_1
Azoarcus sp. TSNA42
Azoarcus sp. BM101
Azoarcus sp. TSPY31
BH72 group
Azoarcus sp. CC-YHH838
Azoarcus sp. CC-YHH848
k2
Azoarcus
Thauera sp. D20
Thauera hydrothermalis GD-2
Thauera
Azoarcus taiwanensis NSC3T
Azoarcus pumilus SY39T
Azoarcus
Zoogloea ramigera NBRC15342
Zoogloea
Rhodocyclus tenuis DSM109T
Rhodocyclus
Rubrivivax gelatinosus CBS
Rubrivivax
Azospira sp. I13
Azospira sp. I09
Azospira oryzae DSM 21223
Azospira oryzae PS
Azospira
Dechloromonas denitrificans ATCC BAA-841
Dechloromonas hortensis MA-1
Dechloromonas sp. H13
Dechloromonas aromatica RCB
Dechloromonas sp. HYN0024
Dechloromonas agitata is5
Dechloromonas sp. Dech2017
Dechloromonas sp. CZR5
k1
Dechloromonas
PCA k3
PCA k4
EbN1T group
CIB group
PCA k2
BH72 group
PCA k1

S3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Dechloromonas hortensis MA-1
Rhodocyclus tenuis DSM109T
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Thauera propionica KNDSS-Mac4
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Thauera sp. MZ1T
Thauera aminoaromatica S2
Thauera sp. UPWRP
Thauera phenylacetica B4P
Thauera sp. 27
Thauera chlorobenzoica 3CB1
Thauera sp. 28
Thauera aromatica K172
Thauera sp. K11
Thauera phenolivorans ZV1C
Thauera terpenica 58Eu
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Thauera hydrothermalis GD-2
Thauera sp. D20
Azoarcus sp. TTM-91
Azoarcus indigens VB32T_1
Azoarcus indigens VB32T_2
Azoarcus olearius BH72
Azoarcus olearius DQS4T
Azoarcus sp. DD4
Azoarcus communis LMG5514
Azoarcus communis SWub3T_1
Azoarcus communis SWub3T_2
Azoarcus communis TSPY31
Azoarcus sp. BM101
Azoarcus communis TSNA42
Azoarcus pumilus SY39
Azoarcus taiwanensis NSC3T
Aromatoleum sp. PA01
Aromatoleum anaerobium LuFRes1T
Aromatoleum buckelii U120T
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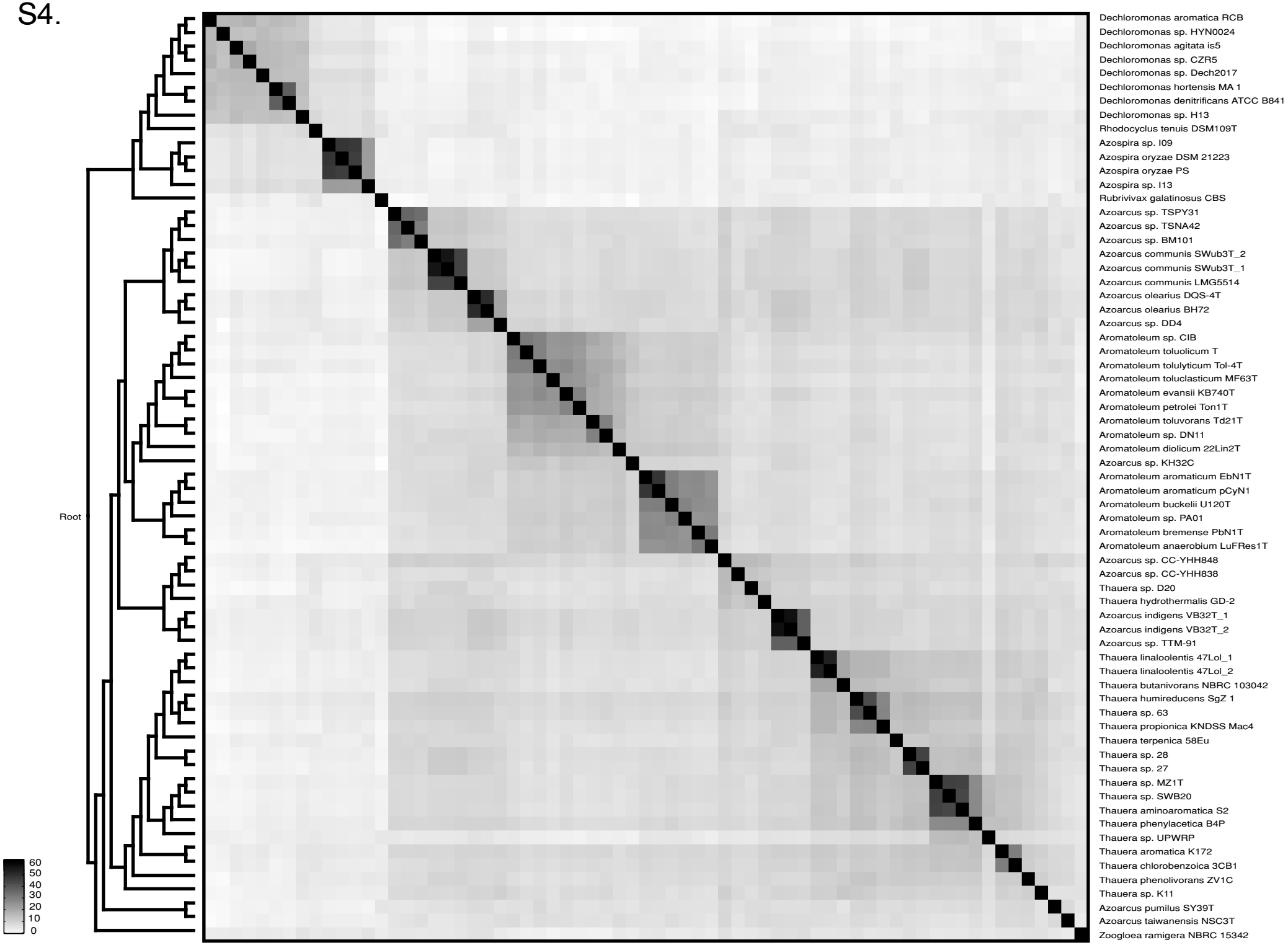
Aromatoleum diolicum 22Lin2T
Aromatoleum tolulyticum Tol-4T
Aromatoleum toluclasticum MF63
Aromatoleum sp. CIB
Aromatoleum toluolicum T
Aromatoleum toluvorans Td21T
Aromatoleum evansii KB740T
Aromatoleum petrolei Ton1T
Aromatoleum sp. DN11
Azoarcus sp. KH32C
Zooglea
Rubrivivax
Dechloromonas
Rhodocyclus
Azospira
Thauera
Azoarcus
Thauera
Azoarcus
Aromatoleum
CIB group
EbN1T group
BH72 group

S4.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Dechloromonas sp. H13
Rhodocyclus tenuis DSM109T
Azospira sp. I09
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Aromatoleum sp. CIB
Aromatoleum toluolicum T
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Thauera sp. 63
Thauera propionica KNDSS Mac4
Thauera terpenica 58Eu
Thauera sp. 28
Thauera sp. 27
Thauera sp. MZ1T
Thauera sp. SWB20
Thauera aminoaromatica S2
Thauera phenylacetica B4P
Thauera sp. UPWRP
Thauera aromatica K172
Thauera chlorobenzoica 3CB1
Thauera phenolivorans ZV1C
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50
40
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20
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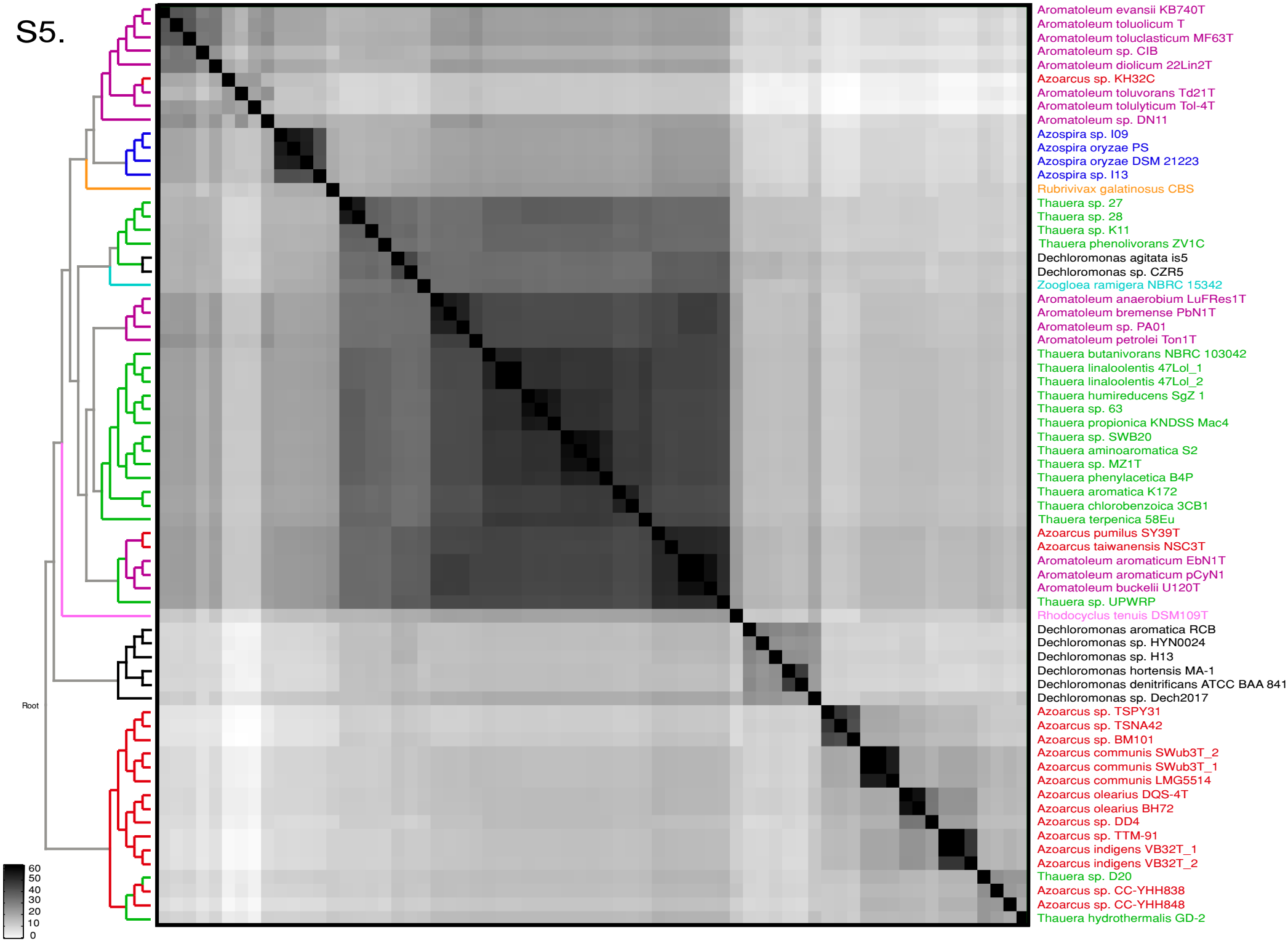
S5.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Thauera sp. 27
Thauera sp. 28
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Dechloromonas agitata is5
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Zoogloea ramigera NBRC 15342
Aromatoleum anaerobium LuFRes1T
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Thauera linaloolentis 47Lol_1
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Azoarcus sp. DD4
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Thauera sp. D20
Azoarcus sp. CC-YHH838
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Root
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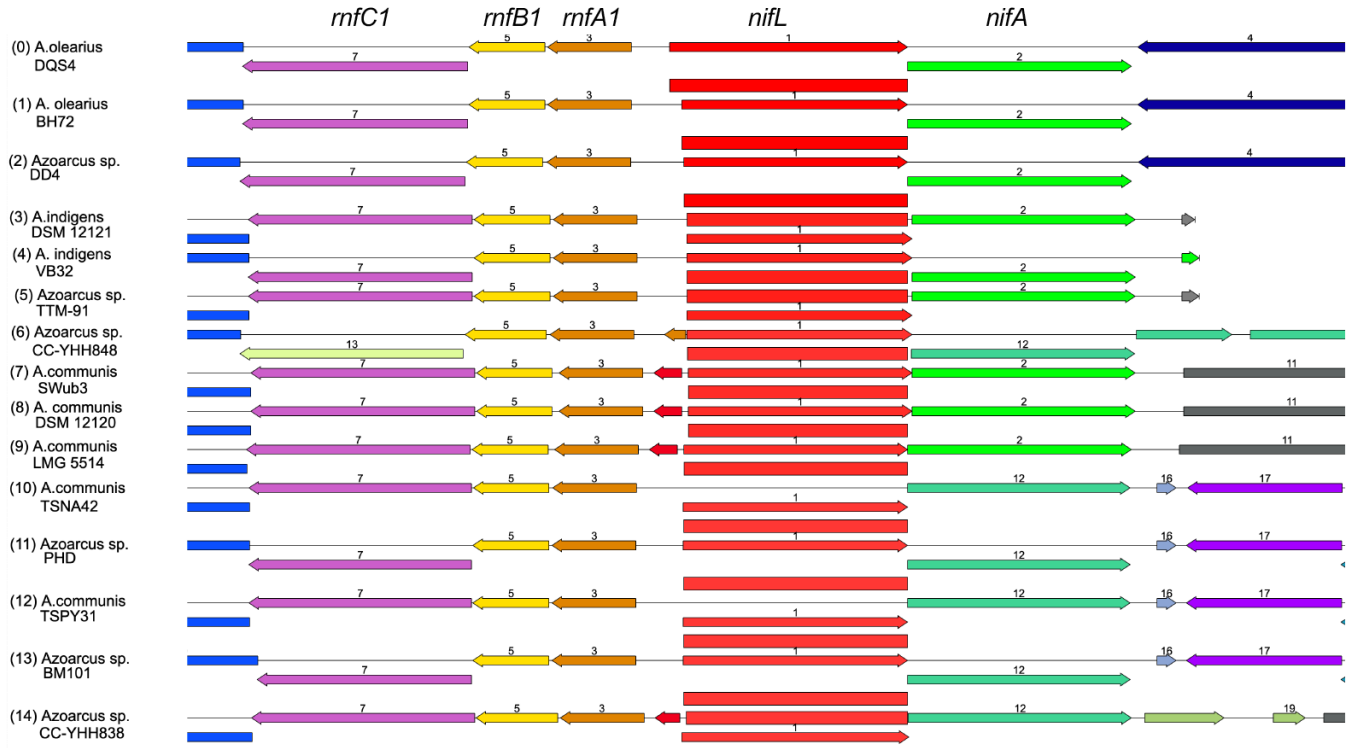
40
30
20
10
0

S6.

A.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rnfB1
rnfA1
nifL
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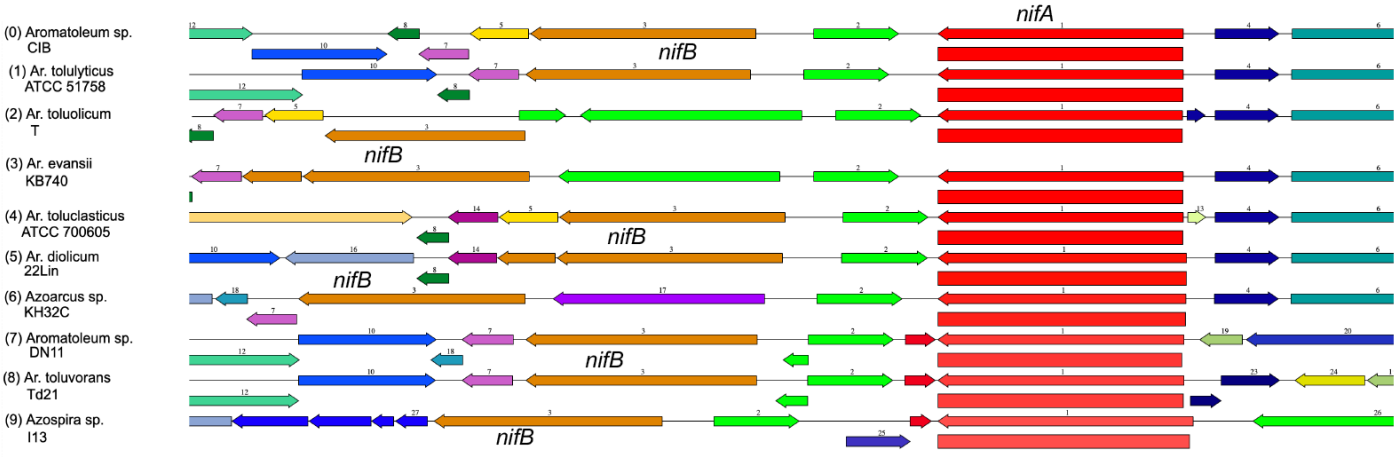
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(5) Ar. diolicum 22Lin
(6) Azoarcus sp. KH32C
(7) Aromatoleum sp. DN11
(8) Ar. toluvorans Td21
(9) Azospira sp. I13