

Supplemental information to Salcher et al.:

Table S1: Details of the isolation experiments conducted in Lake Zurich, Switzerland during 2010-2013.

Table S2: Genes involved in methylotrophic pathways present in the genomes of 'Ca. Methylopumilus planktonicus' MMS-2-53 and 'Ca. Methylopumilus turicensis' MMS-10A-171.

Figure S1: Relative abundances (in % of DAPI) of microbes affiliated with LD28 ('Ca. Methylopumilus planktonicus', upper panel) and PRD01a001B ('Ca. Methylopumilus turicensis', lower panel) determined in Lake Zurich, Switzerland, during the investigation period 2008-2011.

Figure S2: Physical (A: water temperature, F: spherical irradiance), chemical (B: oxygen content, C: concentration of PO₄-P, D: NO₃-N, E: NH₄-N), and biological (G: concentration of total chlorophyll *a*, H: chlorophyll *a* associated with the cyanobacterium *Planktothrix rubescens*, I: chlorophyll *a* associated with diatoms) parameters determined in Lake Zurich, Switzerland, during the investigation period 2008-2011.

Figure S3: Bootstrapped maximum likelihood tree (GTR-Gamma model) of the 16S rDNA of microbes affiliated with LD28 ('Ca. Methylopumilus planktonicus'). Sequences in bold represent isolated strains from Lake Zurich (see table S1 for abbreviations). The scale bar at the bottom represents 1 % estimated sequence divergence.

Figure S4: Bootstrapped maximum likelihood tree (GTR-Gamma model) of the 16S rDNA of microbes affiliated with PRD01a001B ('Ca. Methylopumilus turicensis'). Sequences in bold represent isolated strains from Lake Zurich (see table S1 for abbreviations). The scale bar at the bottom represents 1 % estimated sequence divergence.

Figure S5: Graphic circular map of genome of 'Ca. Methylopumilus planktonicus' strain MMS-2-53. From outside to the center: Genes on forward strand, genes on reverse strand (both coloured in COG categories), BLASTx comparison to 3 closely related taxa ('Ca. Methylopumilus turicensis' strain MMS-10A-171, HTCC2181 of the marine OM43 lineage, and *Methylothera mobilis* JLW8), GC content, GC skew. COG classification was done with the WebMGA server (Wu et al., 2011) and the genome was displayed with the CGView server (Grant et al. 2008).

Figure S6: Graphic circular map of genome of 'Ca. Methylopumilus turicensis' strain MMS-10A-171. From outside to the center: Genes on forward strand, genes on reverse strand (both coloured in COG categories), BLASTx comparison to 3 closely related taxa ('Ca. Methylopumilus planktonicus' MMS-2-53, HTCC2181 of the marine OM43 lineage, and *Methylothera mobilis* JLW8), GC content, GC skew. COG classification was done with the WebMGA server (Wu et al., 2011) and the genome was displayed with the CGView server (Grant et al. 2008).

Table S1: Details of the isolation experiments conducted in Lake Zurich, Switzerland during 2010-2013.

Date	water temp	med	# wells inoc	# cells /well	# + wells	# pure cult	# Meth	V _{pos}	V _{pure}	V _{Meth}	label	Acc. No.	Meth affil
19/08/2010	19	1	288	1	74	19	-	29.7	6.8	-	MMS-1	-	-
14/10/2010	15.1	1	95	1	10	6	1	11.1	6.5	1.1	MMS-2	LN681407	M. pla.
11/05/2011	16.7	1	95	1.4	-	-	-	-	-	-	MMS-3	-	-
25/05/2011	13.2	1	95	1.4	40	2	1	40.3	1.6	0.8	MMS-4	LN681408	M. pla.
07/06/2011	19.1	1	95	1.4	8	-	-	6.5	-	-	MMS-5	-	-
22/06/2011	19.7	1	95	1.4	11	-	-	9.1	-	-	MMS-6	-	-
06/07/2011	21.5	1	95	1.4	42	-	-	43.0	-	-	MMS-7	-	-
19/07/2011	21.0	1	95	1.4	32	1	-	30.3	0.8	-	MMS-8	-	-
04/08/2011	20.6	2	95	1.4	26	-	-	23.6	-	-	MMS-9	-	-
17/08/2011	21.1	3	95	1.4	39	-	-	38.9	-	-	MMS-10	-	-
24/08/2011	24.7	3	95	1.4	25	-	-	22.5	-	-	MMS-11	-	-
08/09/2011	20.9	3	95	1.4	56	1	1	65.6	0.8	0.8	MMS-12	LN681409	M. pla.
21/09/2011	19.4	3	167	3.2	94	4	4	25.5	0.8	0.8	MMS-13	LN681410-13	M. pla.
05/10/2011	19.4	3	119	2.8	79	7	3	38.8	2.2	0.9	MMS-14	LN681414-16	M. pla.
18/10/2011	15.2	3	119	2.8	88	24	17	47.9	8.0	5.5	MMS-15	LN681417-33	M. pla.
03/11/2011	12.7	3	119	2.8	67	3	2	29.5	0.9	0.6	MMS-16	LN681434-35	M. pla.
16/11/2011	11.3	3	119	2.8	94	18	16	55.6	5.8	5.1	MMS-17	LN681436-51	M. pla.
30/11/2011	9.3	3	119	2.8	21	15	13	6.9	4.8	4.1	MMS-18	LN681452-64	M. pla.
14/12/2011	7.4	3	192	2.8	63	18	18	26.9	5.8	5.8	MMS-19	LN681465-82	M. pla.
10/05/2012	12.1	4	191	2.4	28	25	-	6.7	6.5	-	MMS-20	-	-
25/05/2012	13.5	4	168	2.0	18	17	-	5.7	5.3	-	MMS-21	-	-
01/06/2012	13.5	4	144	2.7	-	-	-	-	-	-	MMS-22	-	-
01/06/2012	13.5	5	144	1.7	-	-	-	-	-	-	MMS-F3	-	-
20/06/2012	17.8	6	24	2.3	10	10	-	23.1	23.1	-	MMS-5H	-	-
20/06/2012	17.8	4	72	2.3	2	2	-	1.2	1.2	-	MMS-3A	-	-
20/06/2012	17.8	4	72	2.3	4	3	-	2.5	1.8	-	MMS-4A	-	-
20/06/2012	17.8	4	72	2.3	1	1	-	0.6	0.6	-	MMS-5A2	-	-
20/06/2012	17.8	5	24	2.3	1	1	-	1.8	1.8	-	MMS-2FG3	-	-
20/06/2012	17.8	5	48	2.3	4	3	-	3.7	2.8	-	MMS-2FG3b	-	-
14/08/2012	23.2	4	96	2	5	5	-	2.7	2.7	-	MMS-7A	-	-
14/08/2012	23.2	4	120	2	14	13	-	6.2	5.7	-	MMS-8A	-	-
14/08/2012	23.2	4	167	1.9	4	3	-	1.3	1.0	-	MMS-9A	-	-
14/08/2012	23.2	4	192	1.8	40	39	12	13.4	13.0	7.4	MMS-10A	LN681522-33	M. tur.
15/08/2012	23.2	4	238	1.8	-	-	-	-	-	-	MMS-11A	-	-
31/08/2012	23.2	4	288	2.4	10	8	3	1.5	1.2	0.43	MMS-12A	LN681483-85	M. pla.
12/11/2012	11.5	7	240	2.3	185	6	-	63.1	1.3	-	MMS-12Awh	-	-
12/11/2012	11.5	7	240	2.3	171	58	1	53.4	12.1	0.2	MMS-14A	LN681486	M. pla.
22/05/2013	13.2	8	120	1.6	20	11	-	11.4	6.0	-	MMS-IA	-	-
22/05/2013	13.2	9	120	1.6	40	22	-	25.3	13.3	-	MMS-IB	-	-
23/05/2013	13.2	9	168	2.0	128	95	2	71.8	44.5	0.6	MMS-IIA	LN681487-88	M. pla.
23/05/2013	13.2	8	168	2.0	92	49	-	39.7	17.2	-	MMS-IIB	-	-
03/06/2013	12.3	10	264	1.7	264	191	-	100	74.4	-	MMS-III	-	-
13/06/2013	15.9	10	288	1.8	74	47	-	17.0	10.2	-	MMS-IV	-	-
15/07/2013	20.1	11	144	1.8	14	6	1	5.8	2.4	0.4	MMS-VA	LN681489	M. pla.
15/07/2013	20.1	12	144	1.8	51	30	19	25.0	13.4	8.1	MMS-VB	LN681490-508	M. pla.
14/08/2013	24.6	12	264	1.7	28	25	13	6.5	5.8	3.0	MMS-VI	LN681509-21	M. pla.
SUM			6507		2077	788	127						
MEAN				2.0				24.8	8.7	2.7			

Abbreviations: water temp, water temperature, med, medium; # wells inoc, number of wells inoculated; # cells/well, number of cells inoculated per well; # + wells, number of wells with cells growing; # pure cult, number of pure cultures; # Meth, number of cultures affiliated with *Methylophilaceae*; V_{pos}, total viability; V_{pure}, viability of pure cultures; V_{meth}, viability of isolates affiliated with *Methylophilaceae*; label, labeling scheme for pure cultures; Acc No, accession numbers; Meth affil, affiliation of isolated *Methylophilaceae*, M. pla., '*Candidatus Methylophilum planktonicum*' (LD28); M. tur., '*Candidatus Methylophilum turicensis*' (PRD01a001B).

Media:

- 1: filtered and autoclaved lake water (LW) + minimal carbon medium (MC)#1
- 2: LW + MC#1 + Vitamins (V)
- 3: LW + MC#1 + V + amino acids (AA)
- 4: LW + 10x MC#2 + V + AA
- 5: artificial lake water (ALW) + TES + 10x MC#2 + medium 4 (1:1)
- 6: ALW + 10x MC#2
- 7: LW + 10x MC#2 + MC#3 + V + AA
- 8: LW + MC#2 + MC#3 + inorganic basal medium (IBM) + stock4 + V + 10xAA
- 9: LW + pyruvate (50 μ M) + urea (0.5 μ M) + MC#4 + V + 10xAA
- 10: LW+ V + 10xAA
- 11: LW + MC#2 + pyruvate (50 μ M) + urea (0.5 μ M) + V + 10xAA
- 12: LW + pyruvate (50 μ M) + urea (0.5 μ M) + V + 10xAA

Minimal carbon medium (MC)#1: 1 μ M NH₄Cl, 0.1 μ M K₂HPO₄, 11.1 μ M D-glucose, 13.3 μ M D-ribose, 22.7 μ M pyruvate, 21.7 μ M glycerol, 9 μ M N-acetylglucosamine, 0.1 μ M glutamate, 0.1 μ M glutamine, 0.05 μ M arginine, 0.1 μ M methionine, 0.1 μ M glycine

MC#2: 1 μ M NH₄Cl, 0.1 μ M K₂HPO₄, 55.5 μ M D-glucose, 66.6 μ M D-ribose, 217.2 μ M formate, 217.2 μ M ethanol, 84.7 μ M succinate, 131.5 μ M glycolate, 108.6 μ M glycerol, 45.2 μ M N-acetylglucosamine

MC#3: 62.1 μ M putrescine, 39.3 μ M spermidine, 66.6 μ M D-xylose, 66.6 μ M arabinose

MC#4: 30 μ M NH₄Cl, 50 μ M oxaloacetate, 50 μ M taurine, 1 μ M betaine, 40 μ M CaCl₂

Amino acid solution (AA): 0.5 μ M isoleucine, leucine, lysine, methionine, alanine, phenylalanine, threonine, tryptophane, valine, arginine, histidine, asparagine, aspartate, cysteine, proline, serine, tyrosine, 2 μ M glutamine, glutamate, glycine

Vitamine solution (V): 0.593 μ M thiamine, 0.08 μ M niacin, 0.000074 μ M cobalamine, 0.005 μ M para-amino benzoic acid, 0.074 μ M pyridoxine, 0.081 μ M pantothenic acid, 0.004 μ M biotin, 0.004 μ M folic acid, 0.555 μ M myo-inositol

Artificial lake water (ALW, Zotina et al. 2003): 2 mM NaHCO₃, 30 μ M KNO₃, 200 μ M MgSO₄, 40 μ M CaCl₂, 160 μ M CaSO₄, 30 μ M NH₄Cl, 0.322 μ M K₂HPO₄

TES (trace element solution): 12 μ M FeCl₃, 16 μ M H₃BO₃, 1 μ M MnCl₂, 0.1 μ M ZnSO₄, 0.04 μ M CuSO₄, 0.04 μ M CoCl₂, 0.03 μ M NaMoO₄, 0.4 μ M NiCl₂,

Inorganic basal medium (IBM, Hahn et al. 2003): 304 μ M MgSO₄, 182 μ M Ca(NO₃)₂, 190 μ M NaHCO₃, 20 μ M KCl, 16 μ M K₂HP₄, 17 μ M Na₂EDTA-Fe, 0.1 ml TES

References:

Hahn M, Lünsdorf H, Wu Q, Schauer M, Höfle M, Boenigk J *et al* (2003). Isolation of novel ultramicrobacteria classified as *Actinobacteria* from five freshwater habitats in Europe and Asia. *Appl Environ Microbiol* **69**: 1442-1451.

Zotina T, Köster O, Jüttner F (2003). Photoheterotrophy and light-dependent uptake of organic and organic nitrogenous compounds by *Planktothrix rubescens* under low irradiance. *Freshwater Biol* **48**: 1859-1872.

Table S2: Genes involved in methylotrophic pathways present in the genomes of ‘Ca. Methylopumilus planktonicus’ MMS-2-53 (Acc. No. LN827929) and ‘Ca. Methylopumilus turicensis’ MMS-10A-171 (Acc. No LN794158).

Pathway	Gene	Protein/function	‘Ca. M. planktonicus’ MMS-2-53	‘Ca. M. turicensis’ MMS-10A-171
Methanol oxidation	<i>xoxF</i>	Homologue of MDH large subunit	BN1208_1208	BN1209_1575
	<i>xoxJ</i>	unknown	BN1208_1207	BN1209_1574
	<i>xoxG</i>	cytochrome c, electron acceptor from MDH	BN1208_1206	BN1209_1572, BN1209_1573
	<i>mxar</i>	unknown	BN1208_0638	BN1209_0567
	<i>mxas</i>	unknown	BN1208_0637	BN1209_0566
	<i>mxaa</i>	Ca ²⁺ insertion into MDH	BN1208_0636	BN1209_0565
	<i>mxac</i>	Ca ²⁺ insertion into MDH	BN1208_0635	BN1209_0564
	<i>mxak</i>	Ca ²⁺ insertion into MDH	BN1208_0634	BN1209_0563
	<i>mxal</i>	Ca ²⁺ insertion into MDH	BN1208_0633	BN1209_0562
	<i>mxad</i>	unknown	BN1208_0373	BN1209_0453
	<i>pqqB</i>	PQQ biosynthesis	BN1208_0720	BN1209_0756
	<i>pqqC</i>	PQQ biosynthesis	BN1208_0721	BN1209_0757
	<i>pqqD</i>	PQQ biosynthesis	BN1208_0722	BN1209_0758
	<i>pqqE</i>	PQQ biosynthesis	BN1208_0723	BN1209_0759
	<i>pqqF</i>	PQQ biosynthesis	BN1208_1061	BN1209_1323
<i>pqqG</i>	PQQ biosynthesis	BN1208_1060	BN1209_1322	
H ₄ F-linked form-aldehyde oxidation	<i>foID</i>	Methylenetetrahydrofolate dehydrogenase (NADP ⁺) (EC 1.5.1.5) / Methylenetetrahydrofolate cyclohydrolase (EC 3.5.4.9)	BN1208_0070	BN1209_0056
	<i>fhs</i>	Formate-tetrahydrofolate ligase	BN1208_0733	-
	<i>metF</i>	5,10-methylenetetrahydrofolate reductase	BN1208_0111	BN1209_0102, BN1209_0103
	<i>ygfZ</i>	glycine cleavage T protein (aminomethyl transferase)	BN1208_0632	BN1209_0560
H ₄ MPT-linked formaldehyde oxidation	<i>fhcA</i>	formyltransferase/hydrolase complex subunit A	-	BN1209_0742
	<i>fhcD</i>	formyltransferase/hydrolase complex subunit D	-	BN1209_0741
	<i>fhcC</i>	formyltransferase/hydrolase complex subunit C	-	BN1209_0740
	<i>mptG</i>	beta-ribofuranosylamino-benzene 5-phosphate synthase	-	BN1209_0744
	<i>mtdB</i>	methylene H ₄ MPT dehydrogenase	-	BN1209_0745
	<i>orfY</i>	unknown	-	BN1209_0746
	<i>mch</i>	methenyl H ₄ MPT cyclohydrolase	-	BN1209_0747
	<i>orf5</i>	biosynthesis of H ₄ MPT	-	BN1209_0748
	<i>orf7</i>	unknown	-	BN1209_0749
	<i>fae</i>	formaldehyde activating enzyme	-	BN1209_1682
	<i>orf17</i>	unknown	-	BN1209_0773
	<i>orf1</i>	unknown	-	BN1209_0774
	<i>orf9</i>	biosynthesis of H ₄ MPT	-	BN1209_0775
	<i>pabB</i>	para-aminobenzoate synthase component I	-	BN1209_0776, BN1209_1662
	<i>orf21</i>	biosynthesis of H ₄ MPT	-	BN1209_0777
	<i>pcbD</i>	unknown	-	BN1209_0778
	<i>orf22</i>	biosynthesis of H ₄ MPT	-	BN1209_0842
	<i>orf19</i>	biosynthesis of H ₄ MPT	-	BN1209_0841
	<i>orf20</i>	biosynthesis of H ₄ MPT	-	BN1209_0840
<i>afp</i>	dihydromethanopterin reductase	-	BN1209_0839	

Pathway	Gene	Protein/function	'Ca. M. planktonicus' MMS-2-53	'Ca. M. turicensis' MMS-10A-171
Formate oxidation	<i>fdhA</i>	formate dehydrogenase alpha subunit	BN1208_1192	BN1209_0677
	<i>fdhB</i>	formate dehydrogenase beta subunit	BN1208_1193	BN1209_0676, BN1209_1191
	<i>fdhC</i>	formate dehydrogenase gamma subunit	BN1208_1194	BN1209_0675
	<i>fdhD</i>	formate dehydrogenase accessory protein	BN1208_1191	BN1209_0678
	<i>fdhE</i>	formate dehydrogenase delta subunit	BN1208_1190	BN1209_0679
Ribulose monophosphate (RuMP) cycle for formaldehyde assimilation/oxidation	<i>hps</i>	3-hexulose-6-phosphate synthase	BN1208_0845	BN1209_0202, BN1209_0771
	<i>hpi</i>	6-phospho-3-hexuloisomerase	BN1208_0844	BN1209_0772
	<i>tal</i>	transaldolase	BN1208_0846, BN1208_0901	BN1209_0770
	<i>pgi</i>	glucose 6-phosphate isomerase	BN1208_0816	BN1209_0914
	<i>zwf</i>	glucose 6-phosphate dehydrogenase	BN1208_0649	BN1209_0580
	<i>pgl</i>	6-phosphogluconolactonase	BN1208_0650	BN1209_0581
	<i>gndA</i>	6-phosphogluconolactonase	BN1208_0651	BN1209_0582
	<i>gndB</i>	6-phosphogluconate dehydrogenase (NADP)	-	BN1209_0428
	<i>edd</i>	6-phosphogluconate dehydratase	BN1208_1051	BN1209_1306
	<i>eda</i>	2-dehydro-3-deoxy-phosphogluconate aldolase	BN1208_1050	BN1209_1305
	<i>tkt</i>	transketolase	BN1208_1157	BN1209_1513
	<i>rpe</i>	ribulose-phosphate 3-epimerase	BN1208_1297	BN1209_1663
	<i>ppi</i>	ribose-5-phosphate isomerase	BN1208_0100, BN1208_0677	BN1209_0087, BN1209_0663

Figure S1: Relative abundances (in % of DAPI) of microbes affiliated with LD28 ('*Cand. Methylopumilus planktonicus*', upper panel) and PRD01a001B ('*Cand. Methylopumilus turicensis*', lower panel) determined in Lake Zurich, Switzerland, during the investigation period 2008-2011.

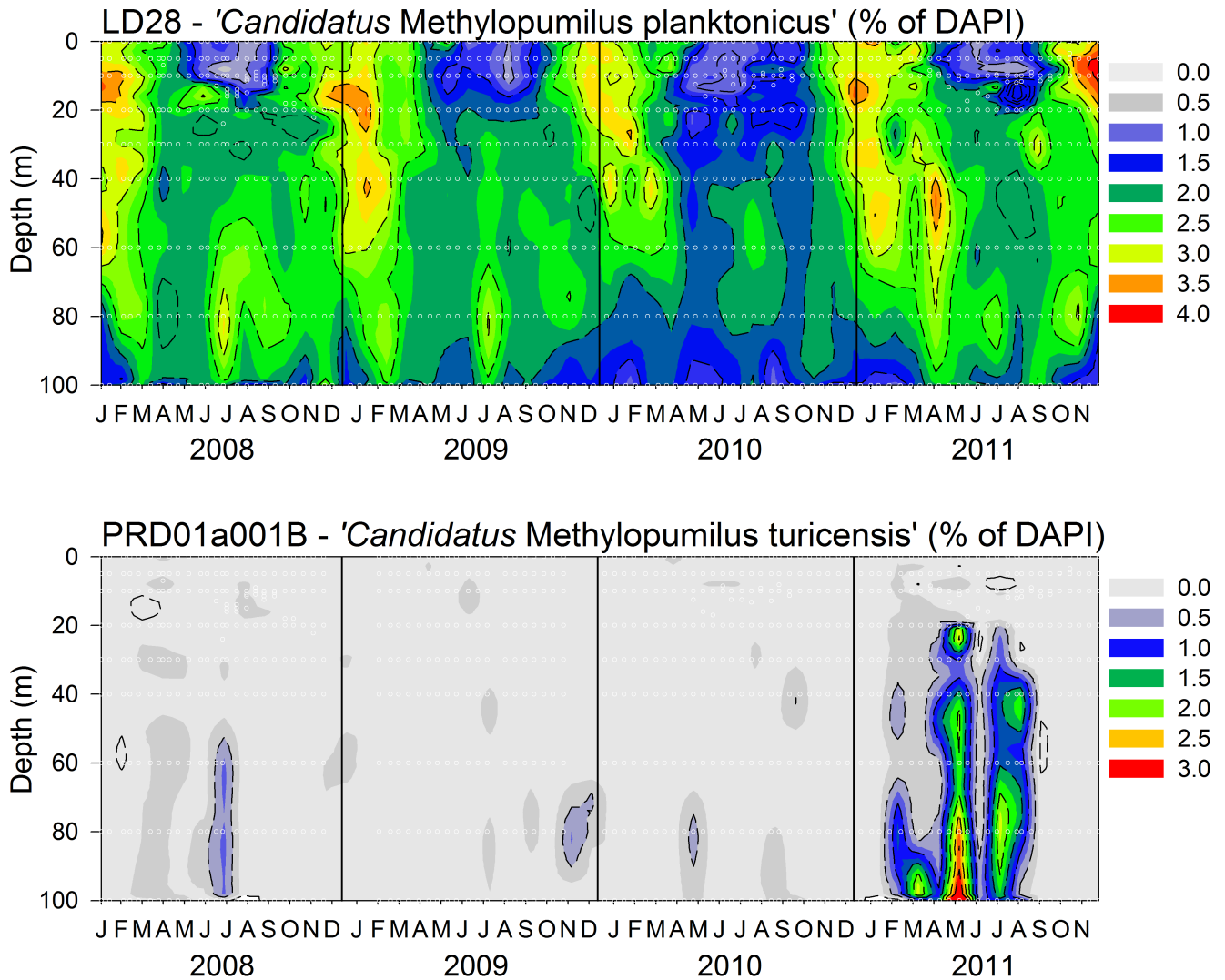


Figure S2: Physical, chemical, and biological parameters determined in Lake Zurich, Switzerland, during the investigation period 2008-2011.

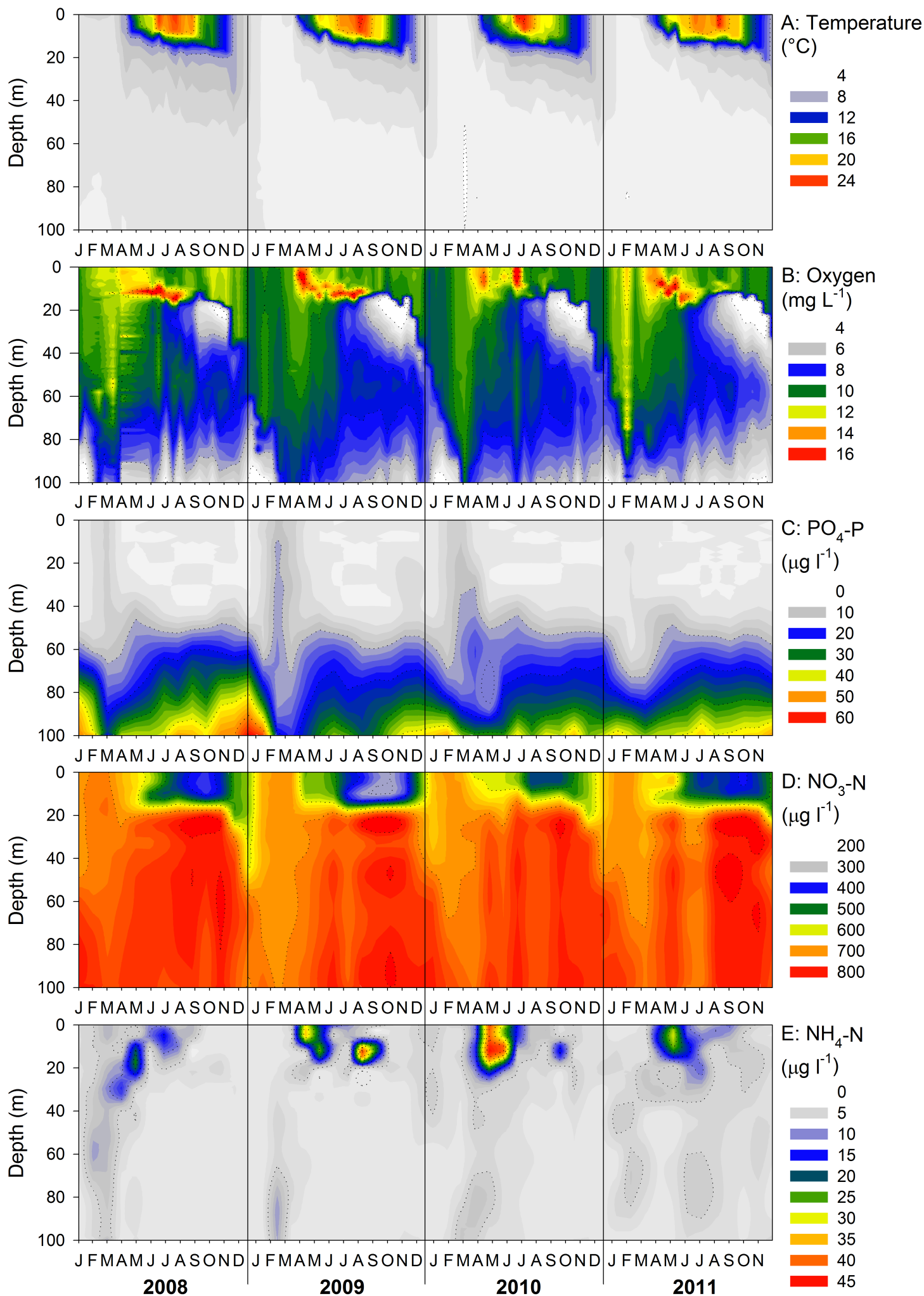


Figure S2 continued

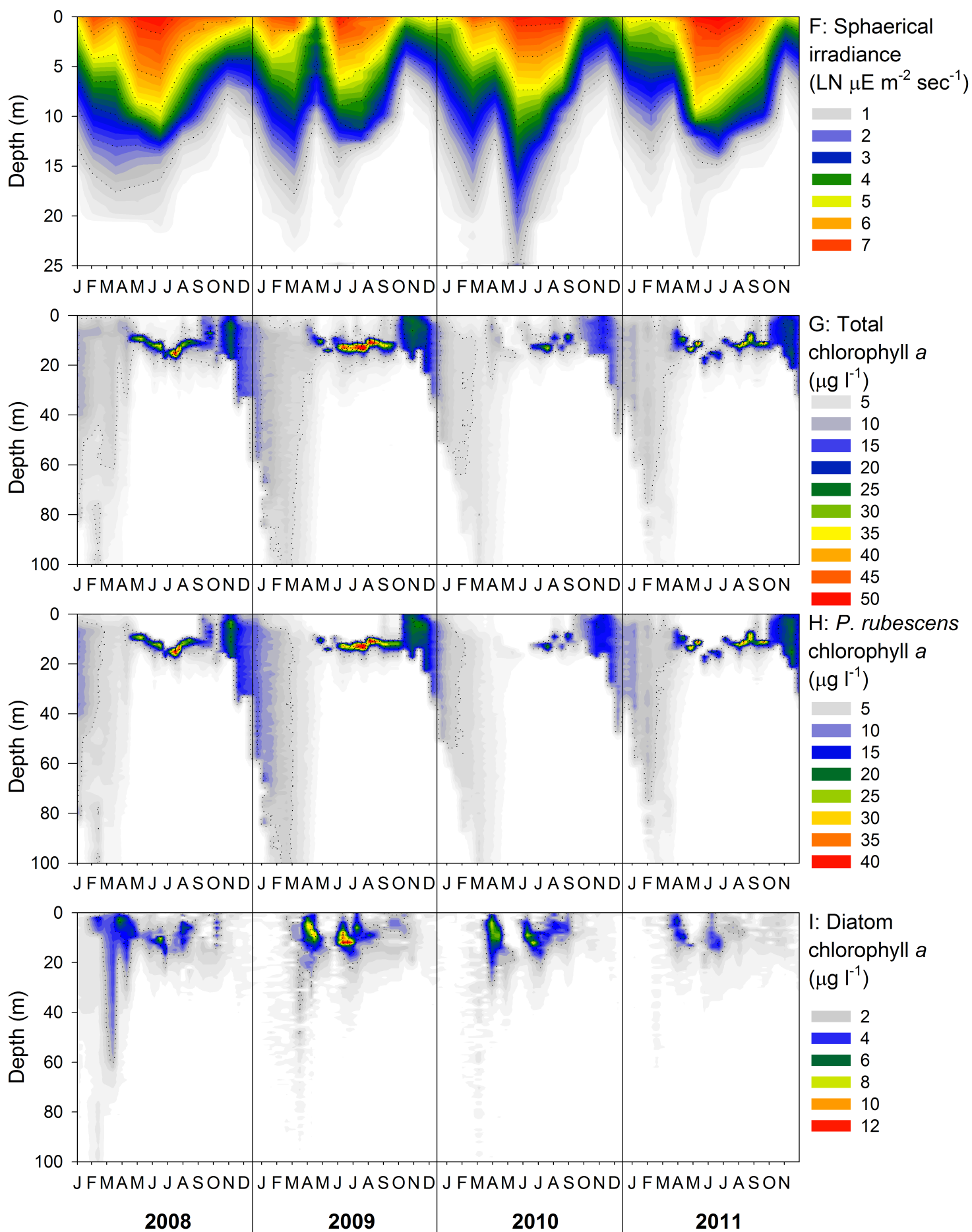


Figure S3: Bootstrapped maximum likelihood tree (GTR-Gamma model) of the 16S rDNA of microbes affiliated with LD28 (*Ca. Methylopumilus planktonicus*). Sequences in bold represent isolated strains from Lake Zurich (see table S1 for abbreviations). The scale bar at the bottom represents 1 % estimated sequence divergence.

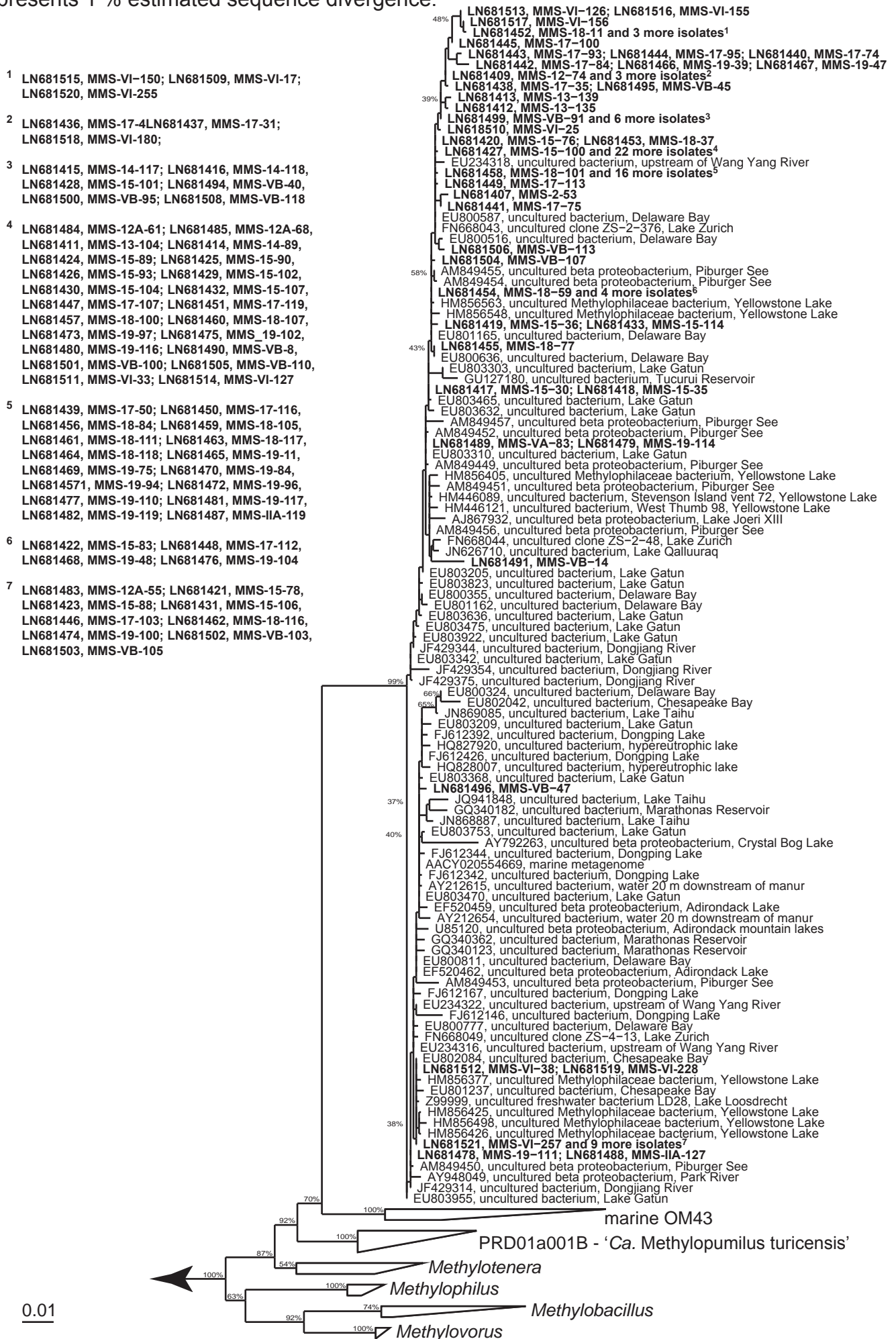


Figure S4: Bootstrapped maximum likelihood tree (GTR-Gamma model) of the 16S rDNA of microbes affiliated with PRD01a001B ('*Ca. Methylopusillus turicensis*'). Sequences in bold represent isolated strains from Lake Zurich (see table S1 for abbreviations). The scale bar at the bottom represents 1 % estimated sequence divergence.

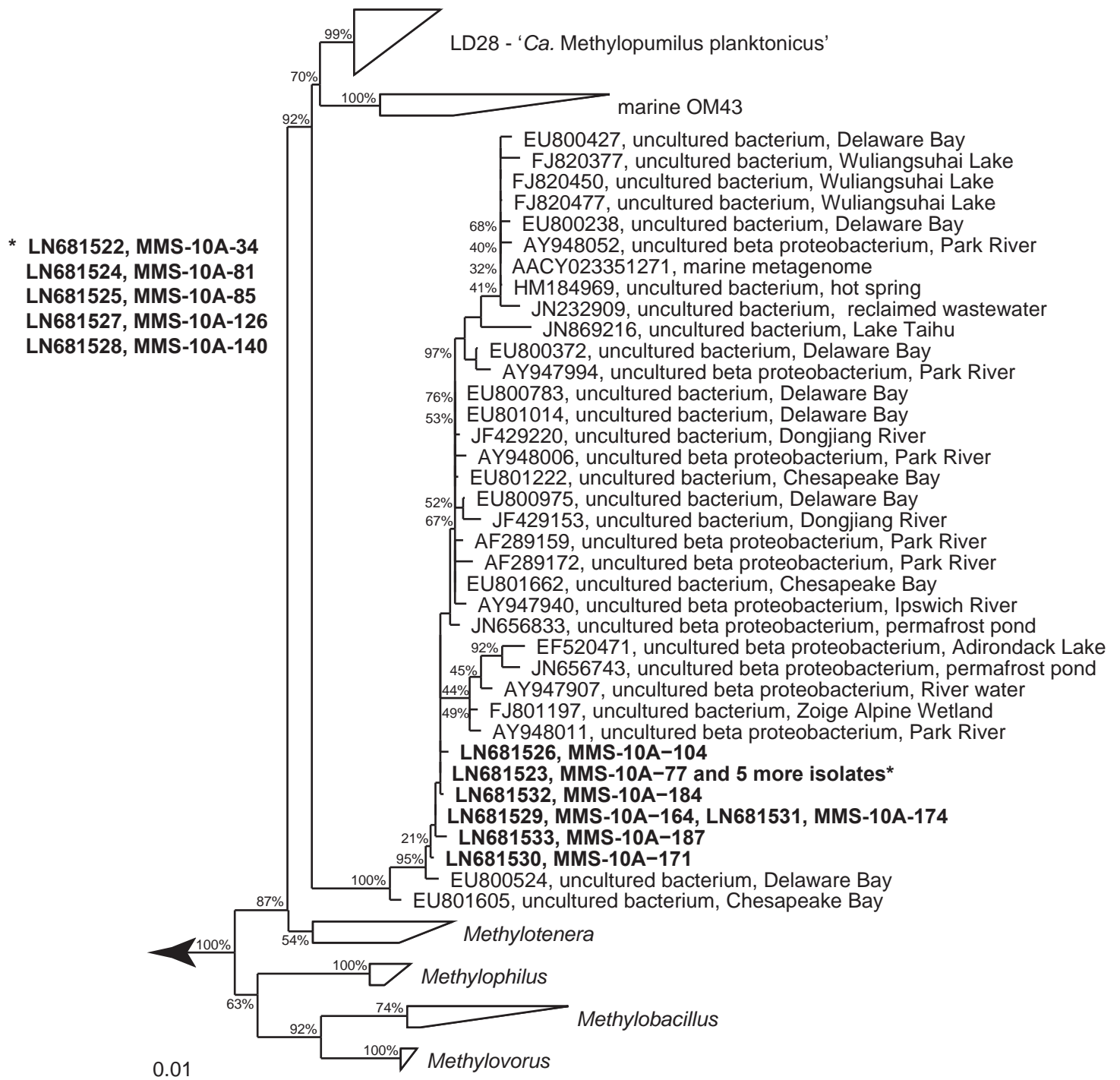


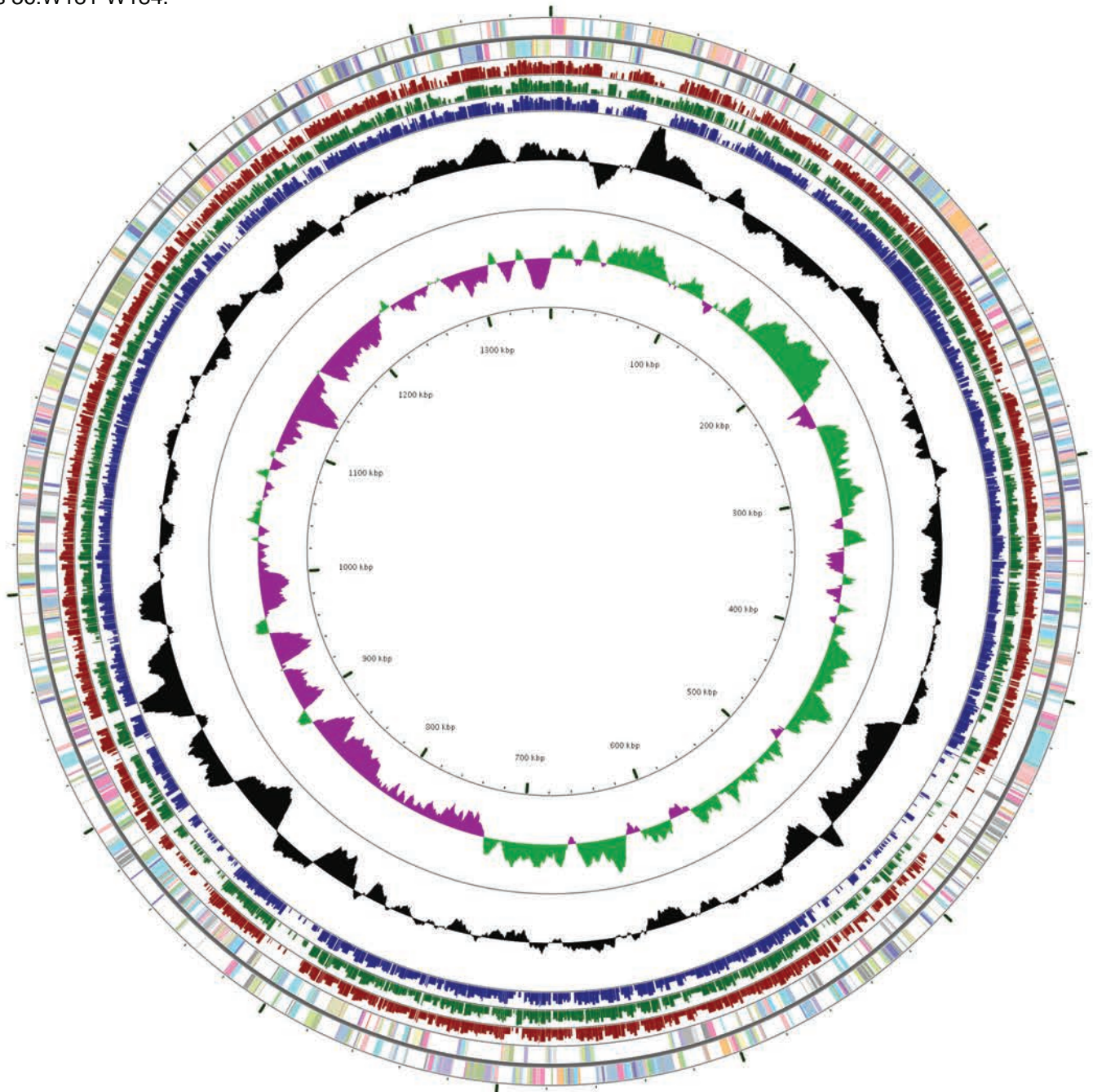
Fig. S5: Graphic circular genome map of 'Ca. Methylopusillus planktonicus' strain MMS-2-53. From outside to the center: Genes on forward strand, genes on reverse strand (both coloured in COG categories), BLASTx comparison to 3 closely related taxa ('Ca. Methylopusillus turicensis' MMS-10A-171, HTCC2181 of the marine OM43 lineage, and *Methylopusillus mobilis* JLW8), GC content, GC skew.

COG classification was done with the WebMGA server (Wu et al., 2011) and the genome was displayed with the CGView server (Grant et al. 2008).

References:

Wu S, Zhu Z, Fu L, Niu B, Li W (2011). WebMGA: a customizable web server for fast metagenomic sequence analysis. BMC Genomics 12: 444.

Grant JR, Stothard P (2008) The CGView Server: a comparative genomics tool for circular genomes. Nucleic Acids Res 36:W181-W184.



COG categories:

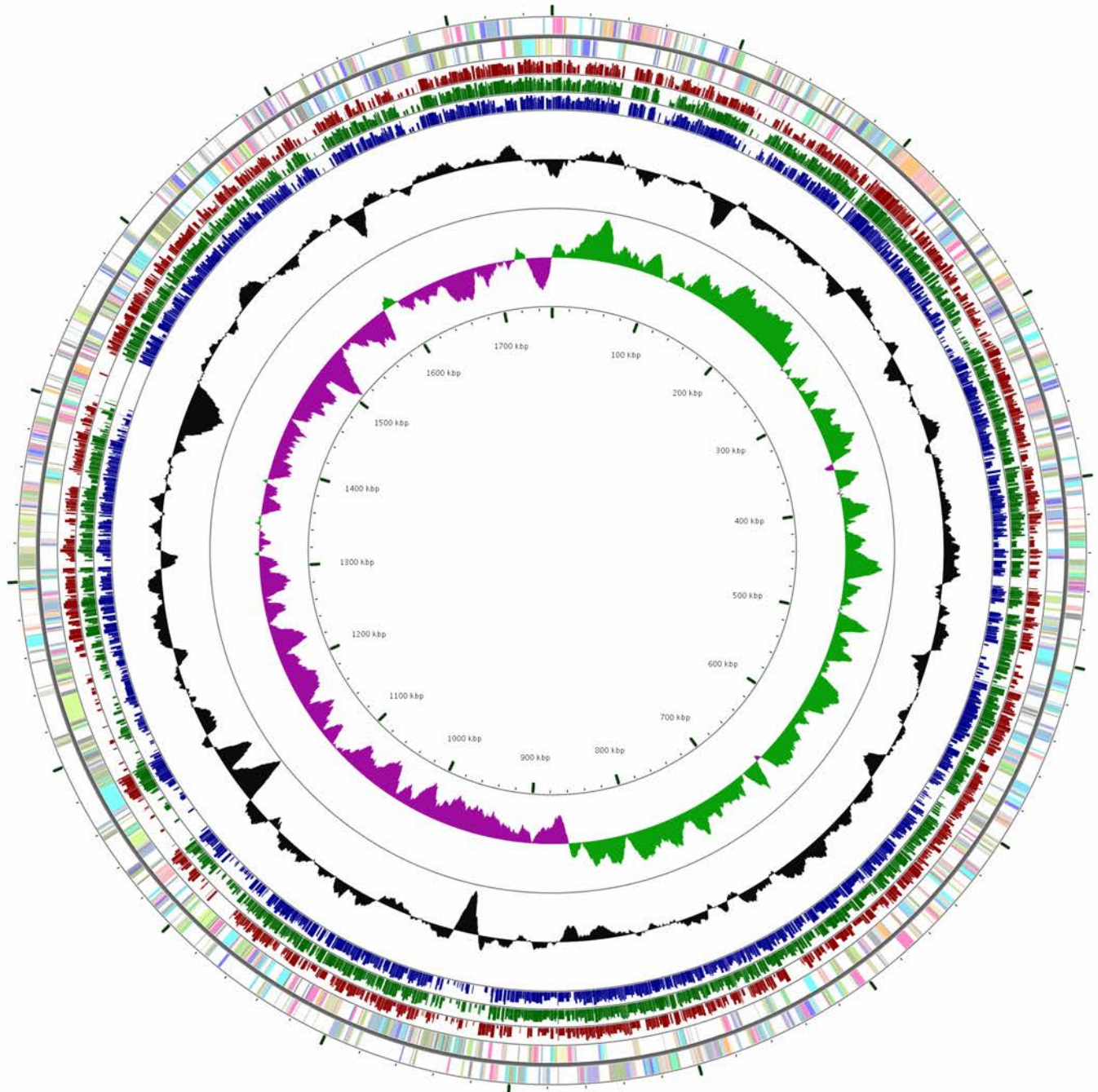
■ A	■ M	■ Y	■ I	■ BLAST results 'Ca. M. tur.' MMS-10A-171
■ B	■ N	■ Z	■ Q	■ BLAST results marine HTCC2181 (OM43)
■ J	■ P	■ C	■ R	■ BLAST results <i>Methylopusillus mobilis</i> JLW8
■ K	■ T	■ G	■ S	■ GC content
■ L	■ U	■ E	■ unknown COG	■ GC skew +
■ D	■ V	■ F	■ tRNA	■ GC skew -
■ O	■ W	■ H	■ rRNA	

Fig. S6: Graphic circular genome map of 'Ca. Methylopusillus turicensis' strain MMS-10A-171. From outside to the center: Genes on forward strand, genes on reverse strand (both coloured in COG categories), BLASTx comparison to 3 closely related taxa ('Ca. Methylopusillus planktonicus' MMS-2-53, *Methylotenera mobilis* JLW8, and *Methylobacillus flagellatus* KT), GC content, GC skew. COG classification was done with the WebMGA server (Wu et al., 2011) and the genome was displayed with the CGView server (Grant et al. 2008).

References:

Wu S, Zhu Z, Fu L, Niu B, Li W (2011). WebMGA: a customizable web server for fast metagenomic sequence analysis. BMC Genomics 12: 444.

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COG categories:

■ A	■ M	■ Y	■ I	■ BLAST results 'Ca. M. pla.' MMS-2-53
■ B	■ N	■ Z	■ Q	■ BLAST results <i>Methylotenera mobilis</i> JLW8
■ J	■ P	■ C	■ R	■ BLAST results <i>Methylobacillus flagellatus</i> KT
■ K	■ T	■ G	■ S	■ GC content
■ L	■ U	■ E	■ unknown COG	■ GC skew +
■ D	■ V	■ F	■ tRNA	■ GC skew -
■ O	■ W	■ H	■ rRNA	