

Supplementary Material

Phylogenomic Analysis of the Gammaproteobacterial Methanotrophs (Order *Methylococcales*) Calls for the Reclassification of Members at the Genus and Species Levels

Fabini D. Orata*, Jan P. Meier-Kolthoff, Dominic Sauvageau[†], and Lisa Y. Stein[†]

*Correspondence: Fabini D. Orata, orata@ualberta.ca

[†]These authors contributed equally to this work.

1. Supplementary Tables

Supplementary Table S1. 16S rRNA and PmoA sequences used in this study.

Species and strain	Accession numbers	
	16S rRNA	PmoA
<i>Clonothrix fusca</i> AW-b*	DQ984190.1	ABL64049.1
<i>Crenothrix polyspora</i> clone 4*	DQ295895.1	—
<i>Methylobacter luteus</i> ACM 3304 ^T	AF304195.1	—
<i>Methylobacter luteus</i> IMV-B-3098*	—	ATYJ01
<i>Methylobacter marinus</i> A45 ^T	AF304197.1	ARVS01
<i>Methylobacter psychrophilus</i> Z-0021 ^T	AF152597.1	AAX48776.1
<i>Methylobacter tundripaludum</i> SV96 ^T	AJ414655.1	AEGW02
<i>Methylobacter whittenburyi</i> ACM 3310 ^T	X72773.1	NA
<i>Methylocaldum gracile</i> VKM-14L ^T	U89298.1	AAC04380.1
<i>Methylocaldum marinum</i> S8 ^T	AB894129.1	BAO51827.1
<i>Methylocaldum szegediense</i> OR2 ^T	U89300.1	AAC04381.1
<i>Methylocaldum tepidum</i> LK6 ^T	U89297.1	AAC04382.1
<i>Methylococcus capsulatus</i> Texas ^T	AJ563935.1	AUKJ01
<i>Methylococcus mobilis</i> LMD 77.28 ^T	HQ676599.1	—
<i>Methylococcus thermophilus</i> IMV-2Yu ^T	X73819.1	—
<i>“Methylocucumis oryzae”</i> Sn10-6 ^T	KP793700.1	LAJX01
<i>Methylogaea oryzae</i> JCM 16910 ^T	EU672873.1	BBDL01
<i>Methyloglobulus morosus</i> KoM1 ^T	JN386974.1	AYLO01
<i>Methylohalobius crimeensis</i> 10Ki ^T	AJ581837.1	BAM71040.1
<i>Methylomagnum ishizawai</i> RS11D-Pr ^T	AB669155.1	BAK78887.1
<i>Methylomarinovum caldicuralii</i> IT-9 ^T	AB301718.2	BAF62078.2
<i>Methylomarinum vadi</i> IT-4 ^T	AB301717.2	JPON01
<i>Methylomicrobium agile</i> ATCC 35068 ^T	X72767.1	JPOJ01
<i>Methylomicrobium album</i> BG8 ^T	X72777.1	CM001475.1
<i>Methylomicrobium alcaliphilum</i> 20Z ^T	EF495157.1	FO082060.1
<i>“Methylomicrobium buryatense”</i> 5B ^T	AF307138.1	AAL09399.1
<i>Methylomicrobium japanense</i> NI ^T	D89279.1	BAE86885.1
<i>Methylomicrobium kenyense</i> AMO1 ^T	AJ132384.1	NA
<i>Methylomicrobium pelagicum</i> AA-23 ^T	X72775.1	—

Species and strain	Accession numbers	
	16S rRNA	PmoA
<i>Methylomonas aurantiaca</i> JB103 ^T	X72776.1	–
“ <i>Methylomonas denitrificans</i> ” FJG1 ^T	CP014476.1	CP014476.1
<i>Methylomonas fodinarum</i> LD2 ^T	X72778.1	–
<i>Methylomonas koyamae</i> JCM 16701 ^T	AB538964.1	BBCK01
<i>Methylomonas lenta</i> R-45377 ^T	FR798962.1	CCW45942.1
<i>Methylomonas methanica</i> NCIMB 11130 ^T	AF304196.1	LUUF01
<i>Methylomonas paludis</i> MG30 ^T	HE801216.1	CCH22593.1
“ <i>Methylomonas rubra</i> ” 15sh ^T	AF304194.1	–
<i>Methylomonas scandinavica</i> SR5 ^T	AJ131369.1	–
<i>Methyloparacoccus murrellii</i> R-49797 ^T	HF558990.1	CCW45946.1
<i>Methyloprofundus sedimenti</i> WF1 ^T	KF484906.1	LPUF01
<i>Methylosarcina fibrata</i> AML-C10 ^T	AF177296.1	ARCU01
<i>Methylosarcina lacus</i> LW14 ^T	AY007296.1	AZUN01
<i>Methylosarcina quisquiliarum</i> AML-D4 ^T	AF177297.1	AAF04266.1
<i>Methylosoma difficile</i> LC 2 ^T	DQ119050.1	ABD13901.1
<i>Methylosphaera hansonii</i> AM6 ^T	U67929.1	–
<i>Methyloterricola oryzae</i> 73a ^T	JYNS01	JYNS01
“ <i>Methylothermus subterraneus</i> ” HTM55 ^T	AB536747.2	BAI52935.1
“ <i>Methylothermus thermalis</i> ” MYHT ^T	AY829009.1	AAX37294.1
<i>Methylovulum miyakonense</i> HT12 ^T	AB501287.1	AQZU01
<i>Methylovulum psychrotolerans</i> Sph1 ^T	KT381578.1	PGFZ01
<i>Methylosinus sporium</i> ACM 3306 ^T	Y18946.1	PWB93641.1
<i>Methylosinus trichosporium</i> OB3b ^T	Y18947.1	ATQ66669.1

Sequence versions are indicated in the NCBI accession numbers. The *PmoA* sequences of *M. whittenburyi* ACM 3310^T and *M. kenyense* AMO1^T were obtained from the MicroScope database and therefore have no NCBI accession numbers. Sequences that were not available from type strains were taken from different representative strains (indicated by asterisks). NA, not applicable; –, no sequence available.

Supplementary Table S2. Whole-genome sequences used in this study.

Genome	Accession number
<i>Crenothrix polyspora</i> RSM_CP1	FUKI01
<i>Crenothrix polyspora</i> RSM_CP2	FUKJ01
<i>Crenothrix</i> sp. D3	MBQZ01
<i>Methylobacter luteus</i> IMV-B-3098	ATYJ01
<i>Methylobacter marinus</i> A45 ^T	ARVS01
<i>Methylobacter</i> sp. BBA5.1	JQKS01
<i>Methylobacter</i> sp. BC.3.60	PESA01
<i>Methylobacter</i> sp. BC.3.94	PERX01
<i>Methylobacter</i> sp. DS2.3.46	PERP01
<i>Methylobacter</i> sp. DS3.3.25	PERK01
<i>Methylobacter</i> sp. UBA2091	DCZF01
<i>Methylobacter</i> sp. UBA6696	DKJI01
<i>Methylobacter</i> sp. UBA6701	DKJD01
<i>Methylobacter</i> sp. UBA6712	DKIS01
<i>Methylobacter</i> sp. WM.3.3	PERF01
<i>Methylobacter tundripaludum</i> 21/22	JMLA01
<i>Methylobacter tundripaludum</i> 31/32	JPOH01
<i>Methylobacter tundripaludum</i> OWC-DMM	PTIZ01
<i>Methylobacter tundripaludum</i> OWC-G53F	PTIY01
<i>Methylobacter tundripaludum</i> SV96 ^T	AEGW02
<i>Methylobacter tundripaludum</i> UBA6706	DKIY01
<i>Methylobacter whittenburyi</i> ACM 3310 ^T	NA
<i>Methylobacter whittenburyi</i> UCM-B-3033	JQNS01
<i>Methylocaldum</i> sp. 14B	MSCV01
<i>Methylocaldum</i> sp. SAD2	MUGL01
<i>Methylocaldum szegediense</i> O-12	ATXX01
<i>Methylococcaceae</i> bacterium NSM2-1	NQJI01
<i>Methylococcaceae</i> bacterium NSO1	NQJJ01
<i>Methylococcaceae</i> bacterium NSP1-1	NQJG01
<i>Methylococcaceae</i> bacterium NSP1-2	NQJH01
<i>Methylococcaceae</i> bacterium TMED282	NHMB01
<i>Methylococcaceae</i> bacterium TMED69	NHDW01
<i>Methylococcaceae</i> bacterium UBA1114	DCBF01
<i>Methylococcaceae</i> bacterium UBA1147	DBZZ01
<i>Methylococcaceae</i> bacterium UBA2778	DEII01
<i>Methylococcaceae</i> bacterium UBA2780	DEIG01
<i>Methylococcaceae</i> bacterium UBA3127	DEZR01
<i>Methylococcaceae</i> bacterium UBA4132	DFWO01
<i>Methylococcaceae</i> bacterium UBA5071	DIAT01
<i>Methylococcaceae</i> bacterium UBA6146	DIUY01
<i>Methylococcaceae</i> bacterium UBA659	DBNZ01
<i>Methylococcaceae</i> bacterium UBA662	DBNW01
<i>Methylococcaceae</i> bacterium UBA7658	DLHW01
<i>Methylococcaceae</i> bacterium UBA975	DBBV01
<i>Methylococcales</i> bacterium OPU3 GD OMZ	MPSY01

Genome	Accession number
<i>Methylococcus capsulatus</i> Bath	AE017282.2
<i>Methylococcus capsulatus</i> Texas ^T	AUKJ01
<i>Methylococcus</i> sp. UBA6136	DIVI01
“ <i>Methylococcus oryzae</i> ” Sn10-6 ^T	LAJX01
<i>Methylogaea oryzae</i> JCM 16910 ^T	BBDL01
<i>Methyloglobulus morosus</i> KoM1 ^T	AYLO01
<i>Methylohalobius crimeensis</i> 10Ki ^T	ATXB01
<i>Methylomagnum ishizawai</i> 175	FXAM01
<i>Methylomarinum vadi</i> IT-4 ^T	JPON01
<i>Methylomicrobium agile</i> ATCC 35068 ^T	JPOJ01
<i>Methylomicrobium album</i> BG8 ^T	CM001475.1
<i>Methylomicrobium alcaliphilum</i> 20Z ^T	FO082060.1
“ <i>Methylomicrobium buryatense</i> ” 5G	AOTL01
<i>Methylomicrobium kenyense</i> AMO1 ^T	NA
“ <i>Methylomonas denitrificans</i> ” FJG1 ^T	CP014476.1
<i>Methylomonas koyamae</i> JCM 16701 ^T	BBCK01
<i>Methylomonas koyamae</i> LM6	CP023669.1
<i>Methylomonas koyamae</i> R-45378	LUUJ01
<i>Methylomonas koyamae</i> R-45383	LUUK01
<i>Methylomonas koyamae</i> R-49807	LUUL01
<i>Methylomonas lenta</i> R-45370	LUUI01
<i>Methylomonas methanica</i> MC09	CP002738.1
<i>Methylomonas methanica</i> NCIMB 11130 ^T	LUUF01
<i>Methylomonas methanica</i> R-45363	LUUG01
<i>Methylomonas methanica</i> R-45371	LUUH01
<i>Methylomonas</i> sp. 11b	AZ XK01
<i>Methylomonas</i> sp. BC.006	PESC01
<i>Methylomonas</i> sp. DH-1	CP014360.1
<i>Methylomonas</i> sp. DS1.022	PERS01
<i>Methylomonas</i> sp. DS1.3.54	PERR01
<i>Methylomonas</i> sp. DS2.3.37	PERQ01
<i>Methylomonas</i> sp. FW.001	PERW01
<i>Methylomonas</i> sp. FW.007	PERU01
<i>Methylomonas</i> sp. FW.017	PERV01
<i>Methylomonas</i> sp. Kb3	PIZT01
<i>Methylomonas</i> sp. LW13	JNLB01
<i>Methylomonas</i> sp. LWB	MKMC01
<i>Methylomonas</i> sp. MK1	AQOV01
<i>Methyloprofundus sedimenti</i> WF1 ^T	LPUF01
<i>Methylosarcina fibrata</i> AML-C10 ^T	ARCU01
<i>Methylosarcina lacus</i> LW14 ^T	AZUN01
<i>Methyloterricola oryzae</i> 73a ^T	JYNS01
<i>Methylothermaceae</i> bacterium B42	LSNW01
<i>Methylovulum miyakonense</i> HT12 ^T	AQZU01
<i>Methylovulum psychrotolerans</i> HV10-M2	CP022129.1
<i>Methylovulum psychrotolerans</i> Sph1 ^T	PGFZ01
<i>Methylosinus sporium</i> ACM 3306 ^T	PUIV01

Genome	Accession number
<i>Methylosinus trichosporium</i> OB3b ^T	CP023737.1

Sequence versions are indicated in the NCBI accession numbers. The genome sequences of M. whittenburyi ACM 3310^T and M. kenyense AMO1^T were obtained from the MicroScope database and therefore have no NCBI accession numbers. NA, not applicable.

Values are shown for AAI (green–purple, lower left) and POCP (yellow–blue, upper right). Boxed regions indicate inferred genus clusters with at least two members based on AAI comparisons, as well as monophyly in the genome-based phylogeny. POCP was not used to infer genera. Asterisks after reclassified names indicate later heterotypic synonyms of their senior counterparts in the same species clusters as shown in Figure 2.

Supplementary Table S5. Reclassification of the *Methylococcales* genomes.

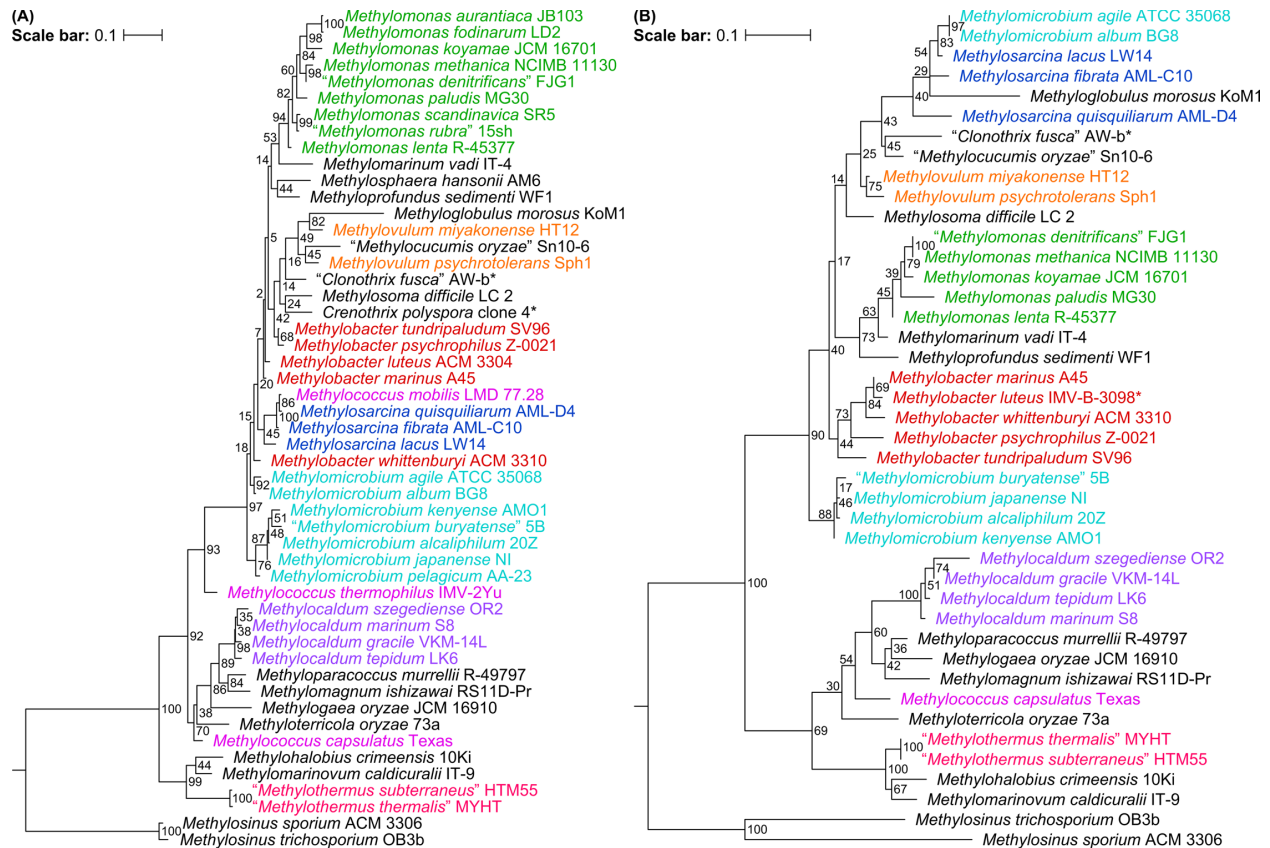
Previous name	Name after reclassification	Genus cluster	Species cluster	G+C of DNA (mol%)	Genome size (bp)
<i>Crenothrix polyspora</i> RSM_CP1	<i>Crenothrix polyspora</i> RSM_CP1	6	20	44.24	3,921,762
<i>Crenothrix polyspora</i> RSM_CP2	<i>Crenothrix</i> sp. RSM_CP2	6	21	44.73	3,527,685
<i>Crenothrix</i> sp. D3	<i>Methylococcaceae</i> bacterium D3	4	17	42.18	3,633,770
<i>Methylobacter luteus</i> IMV-B-3098	<i>Methylobacter luteus</i> IMV-B-3098	1	10	51.13	5,027,778
<i>Methylobacter marinus</i> A45 ^T	<i>Methylobacter marinus</i> A45 ^T	1	9	52.67	4,987,856
<i>Methylobacter</i> sp. BBA5.1	<i>Methylobacter marinus</i> BBA5.1	1	9	52.24	5,071,908
<i>Methylobacter</i> sp. BC.3.60	<i>Methylococcaceae</i> bacterium BC.3.60	7	22	45.33	1,321,518
<i>Methylobacter</i> sp. BC.3.94	<i>Methylovulum miyakonense</i> BC.3.94	2	11	50.52	4,467,756
<i>Methylobacter</i> sp. DS2.3.46	<i>Methylococcaceae</i> bacterium DS2.3.46	15	32	48.79	3,075,382
<i>Methylobacter</i> sp. DS3.3.25	<i>Methylococcaceae</i> bacterium DS3.3.25	15	32	49.80	2,753,434
<i>Methylobacter</i> sp. UBA2091	<i>Methylobacter</i> sp. UBA2091	1	1	49.28	3,970,437
<i>Methylobacter</i> sp. UBA6696	<i>Methylobacter</i> sp. UBA6696	1	1	49.27	3,961,971
<i>Methylobacter</i> sp. UBA6701	<i>Methylobacter</i> sp. UBA6701	1	2	49.26	3,793,708
<i>Methylobacter</i> sp. UBA6712	<i>Methylobacter</i> sp. UBA6712	1	2	48.86	4,212,994
<i>Methylobacter</i> sp. WM.3.3	<i>Methylococcaceae</i> bacterium WM.3.3	15	32	49.39	2,673,132
<i>Methylobacter tundripaludum</i> 21/22	<i>Methylobacter</i> sp. 21/22	1	3	49.46	4,665,210
<i>Methylobacter tundripaludum</i> 31/32	<i>Methylobacter</i> sp. 31/32	1	3	49.24	5,047,546
<i>Methylobacter tundripaludum</i> OWC-DMM	<i>Methylobacter</i> sp. OWC-DMM	1	1	49.47	4,607,785
<i>Methylobacter tundripaludum</i> OWC-G53F	<i>Methylobacter</i> sp. OWC-G53F	1	6	50.46	4,180,651
<i>Methylobacter tundripaludum</i> SV96 ^T	<i>Methylobacter tundripaludum</i> SV96 ^T	1	5	49.51	4,846,919
<i>Methylobacter tundripaludum</i> UBA6706	<i>Methylobacter</i> sp. UBA6706	1	4	49.44	3,565,077
<i>Methylobacter whittenburyi</i> ACM 3310 ^T	<i>Methylobacter whittenburyi</i> ACM 3310 ^T *	1	9	51.98	5,440,158
<i>Methylobacter whittenburyi</i> UCM-B-3033	<i>Methylobacter marinus</i> UCM-B-3033	1	9	51.98	5,440,158
<i>Methylocaldum</i> sp. 14B	<i>Methylocaldum</i> sp. 14B	27	59	58.24	5,621,438
<i>Methylocaldum</i> sp. SAD2	<i>Methylocaldum</i> sp. SAD2	27	59	58.18	5,907,411
<i>Methylocaldum szegediense</i> O-12	<i>Methylocaldum szegediense</i> O-12	27	60	57.15	5,011,695
<i>Methylococcaceae</i> bacterium NSM2-1	<i>Methylobacter</i> sp. NSM2-1	1	8	44.82	1,901,400
<i>Methylococcaceae</i> bacterium NSO1	<i>Methylobacter</i> sp. NSO1	1	7	43.92	1,404,415
<i>Methylococcaceae</i> bacterium NSP1-1	<i>Methylobacter</i> sp. NSP1-1	1	7	44.25	1,667,819
<i>Methylococcaceae</i> bacterium NSP1-2	<i>Methylococcaceae</i> bacterium NSP1-2	4	18	42.81	2,718,667
<i>Methylococcaceae</i> bacterium TMED282	<i>Methylococcaceae</i> bacterium TMED282	10	26	43.73	1,499,574
<i>Methylococcaceae</i> bacterium TMED69	<i>Methylococcaceae</i> bacterium TMED69	11	27	38.55	1,973,107
<i>Methylococcaceae</i> bacterium UBA1114	<i>Methylococcaceae</i> bacterium UBA1114	8	23	42.83	2,173,118

Previous name	Name after reclassification	Genus cluster	Species cluster	G+C of DNA (mol%)	Genome size (bp)
<i>Methylococcaceae</i> bacterium UBA1147	<i>Methylococcaceae</i> bacterium UBA1147	21	53	53.15	2,103,507
<i>Methylococcaceae</i> bacterium UBA2778	<i>Methylococcaceae</i> bacterium UBA2778	22	54	55.65	3,927,269
<i>Methylococcaceae</i> bacterium UBA2780	<i>Methylococcaceae</i> bacterium UBA2780	9	25	44.90	2,373,821
<i>Methylococcaceae</i> bacterium UBA3127	<i>Methylococcaceae</i> bacterium UBA3127	4	16	42.18	3,099,008
<i>Methylococcaceae</i> bacterium UBA4132	<i>Methylococcaceae</i> bacterium UBA4132	4	16	42.15	3,069,361
<i>Methylococcaceae</i> bacterium UBA5071	<i>Methylococcaceae</i> bacterium UBA5071	4	16	42.15	3,132,147
<i>Methylococcaceae</i> bacterium UBA6146	<i>Methylovulum</i> sp. UBA6146	2	13	48.50	3,187,382
<i>Methylococcaceae</i> bacterium UBA659	<i>Methylococcaceae</i> bacterium UBA659	3	14	51.40	2,140,836
<i>Methylococcaceae</i> bacterium UBA662	<i>Methylococcaceae</i> bacterium UBA662	3	15	52.99	2,387,527
<i>Methylococcaceae</i> bacterium UBA7658	<i>Methyloglobulus</i> sp. UBA7658	14	30	46.81	3,709,136
<i>Methylococcaceae</i> bacterium UBA975	<i>Methylococcaceae</i> bacterium UBA975	8	24	44.76	2,328,141
<i>Methylococcales</i> bacterium OPU3_GD_OMZ	<i>Methylococcaceae</i> bacterium OPU3_GD_OMZ	12	28	42.32	2,094,096
<i>Methylococcus capsulatus</i> Bath	<i>Methylococcus capsulatus</i> Bath	23	55	63.58	3,304,560
<i>Methylococcus capsulatus</i> Texas ^T	<i>Methylococcus capsulatus</i> Texas ^T	23	55	63.56	3,263,814
<i>Methylococcus</i> sp. UBA6136	<i>Methylococcaceae</i> bacterium UBA6136	28	61	57.14	2,440,517
“ <i>Methylocucumis oryzae</i> ” Sn10-6 ^T	“ <i>Methylocucumis oryzae</i> ” Sn10-6 ^T	5	19	43.87	4,583,004
<i>Methylogaea oryzae</i> JCM 16910 ^T	<i>Methylogaea oryzae</i> JCM 16910 ^T	26	58	62.55	3,696,254
<i>Methyloglobulus morosus</i> KoM1 ^T	<i>Methyloglobulus morosus</i> KoM1 ^T	14	31	47.32	4,136,844
<i>Methylohalobius crimeensis</i> 10Ki ^T	<i>Methylohalobius crimeensis</i> 10Ki ^T	29	62	58.28	3,509,607
<i>Methylomagnum ishizawai</i> 175	<i>Methylomagnum ishizawai</i> 175	25	57	62.96	5,480,099
<i>Methylomarinum vadi</i> IT-4 ^T	<i>Methylomarinum vadi</i> IT-4 ^T	17	46	51.29	4,335,162
<i>Methylomicrobium agile</i> ATCC 35068 ^T	<i>Methylomicrobium agile</i> ATCC 35068 ^T	19	50	56.17	4,526,071
<i>Methylomicrobium album</i> BG8 ^T	<i>Methylomicrobium album</i> BG8 ^{T*}	19	50	56.25	4,493,344
<i>Methylomicrobium alcaliphilum</i> 20Z ^T	<i>Methylothemobacterium alcaliphilum</i> 20Z ^T	18	47	48.74	4,796,711
“ <i>Methylomicrobium buryatense</i> ” 5G	“ <i>Methylothemobacterium buryatense</i> ” 5G	18	48	48.71	5,064,796
<i>Methylomicrobium kenyense</i> AMO1 ^T	<i>Methylothemobacterium kenyense</i> AMO1 ^T	18	49	50.16	4,474,416
“ <i>Methylomonas denitrificans</i> ” FJG1 ^T	“ <i>Methylomonas denitrificans</i> ” FJG1 ^{T*}	16	38	51.66	5,172,098
<i>Methylomonas koyamae</i> JCM 16701 ^T	<i>Methylomonas koyamae</i> JCM 16701 ^T	16	39	56.05	4,954,151
<i>Methylomonas koyamae</i> LM6	<i>Methylomonas koyamae</i> LM6	16	39	56.23	5,080,660
<i>Methylomonas koyamae</i> R-45378	<i>Methylomonas</i> sp. R-45378	16	40	56.06	5,115,236
<i>Methylomonas koyamae</i> R-45383	<i>Methylomonas</i> sp. R-45383	16	41	55.85	5,414,320
<i>Methylomonas koyamae</i> R-49807	<i>Methylomonas koyamae</i> R-49807	16	39	55.86	5,179,245
<i>Methylomonas lenta</i> R-45370	<i>Methylomonas lenta</i> R-45370	16	43	46.63	4,703,858
<i>Methylomonas methanica</i> MC09	<i>Methylomonas</i> sp. MC09	16	44	51.31	5,051,681
<i>Methylomonas methanica</i> NCIMB 11130 ^T	<i>Methylomonas methanica</i> NCIMB 11130 ^T	16	38	51.67	5,023,270

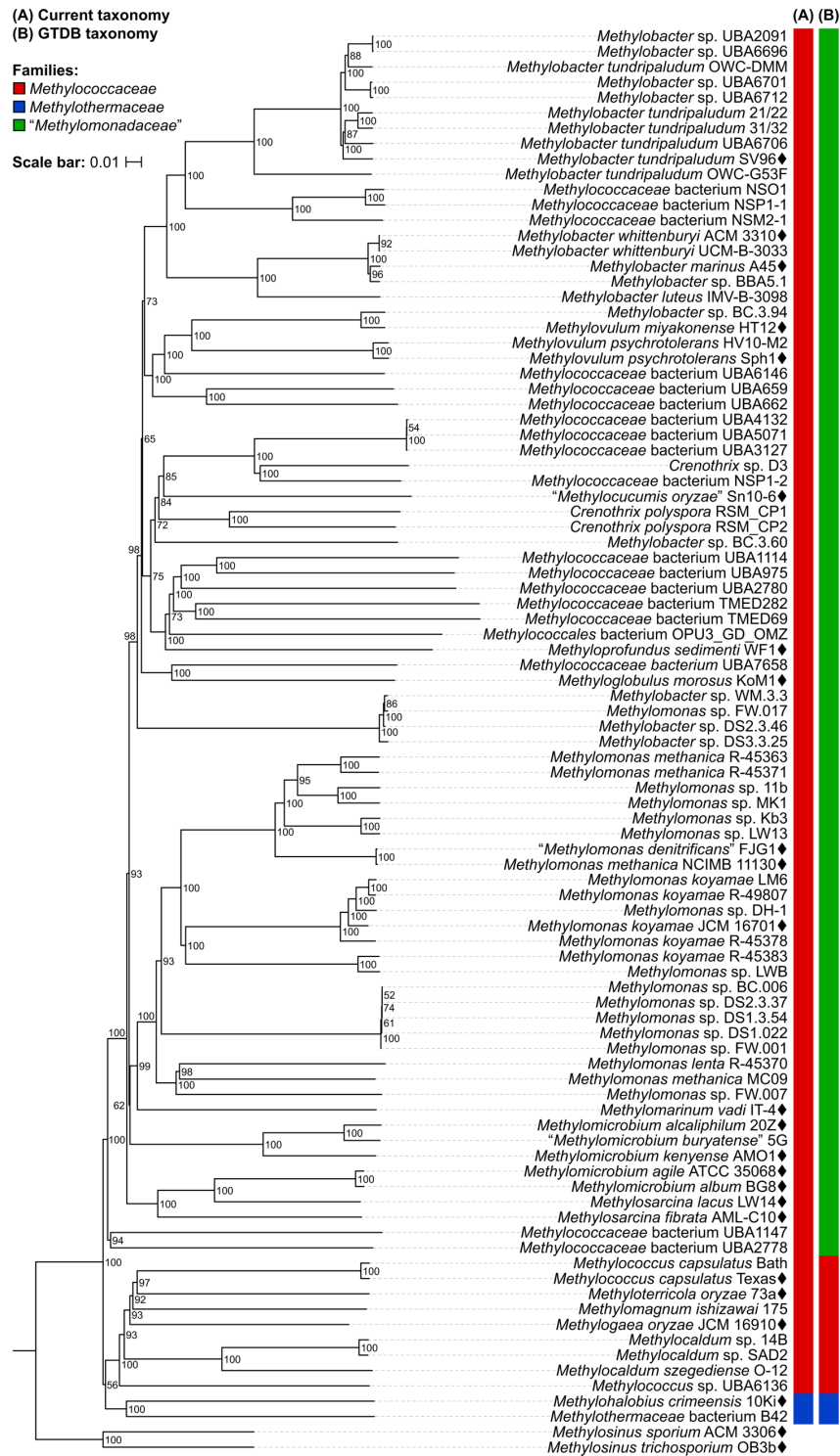
Previous name	Name after reclassification	Genus cluster	Species cluster	G+C of DNA (mol%)	Genome size (bp)
<i>Methylomonas methanica</i> R-45363	<i>Methylomonas</i> sp. R-45363	16	33	51.29	5,407,230
<i>Methylomonas methanica</i> R-45371	<i>Methylomonas</i> sp. R-45371	16	34	51.27	5,482,877
<i>Methylomonas</i> sp. 11b	<i>Methylomonas</i> sp. 11b	16	35	51.40	5,412,616
<i>Methylomonas</i> sp. BC.006	<i>Methylomonas</i> sp. BC.006	16	42	56.11	2,569,055
<i>Methylomonas</i> sp. DH-1	<i>Methylomonas koyamae</i> DH-1	16	39	56.21	5,127,407
<i>Methylomonas</i> sp. DS1.022	<i>Methylomonas</i> sp. DS1.022	16	42	56.48	2,850,821
<i>Methylomonas</i> sp. DS1.3.54	<i>Methylomonas</i> sp. DS1.3.54	16	42	56.50	2,478,482
<i>Methylomonas</i> sp. DS2.3.37	<i>Methylomonas</i> sp. DS2.3.37	16	42	56.14	2,570,809
<i>Methylomonas</i> sp. FW.001	<i>Methylomonas</i> sp. FW.001	16	42	55.96	2,324,165
<i>Methylomonas</i> sp. FW.007	<i>Methylomonas</i> sp. FW.007	16	45	48.36	4,032,484
<i>Methylomonas</i> sp. FW.017	<i>Methylococcaceae</i> bacterium FW.017	15	32	49.21	2,488,664
<i>Methylomonas</i> sp. Kb3	<i>Methylomonas</i> sp. Kb3	16	37	51.81	5,121,132
<i>Methylomonas</i> sp. LW13	<i>Methylomonas</i> sp. LW13	16	37	51.80	5,200,804
<i>Methylomonas</i> sp. LWB	<i>Methylomonas</i> sp. LWB	16	41	55.93	5,365,240
<i>Methylomonas</i> sp. MK1	<i>Methylomonas</i> sp. MK1	16	36	51.47	5,233,591
<i>Methyloprofundus sedimenti</i> WF1 ^T	<i>Methyloprofundus sedimenti</i> WF1 ^T	13	29	41.02	4,290,526
<i>Methylosarcina fibrata</i> AML-C10 ^T	<i>Methylosarcina fibrata</i> AML-C10 ^T	20	52	54.07	4,978,052
<i>Methylosarcina lacus</i> LW14 ^T	<i>Methylomicrobium lacus</i> LW14 ^T	19	51	54.67	4,384,919
<i>Methyloterricola oryzae</i> 73a ^T	<i>Methyloterricola oryzae</i> 73a ^T	24	56	61.10	4,902,365
<i>Methylothermaceae</i> bacterium B42	<i>Methylothermaceae</i> bacterium B42	30	63	51.47	3,040,036
<i>Methylovulum miyakonense</i> HT12 ^T	<i>Methylovulum miyakonense</i> HT12 ^T	2	11	50.66	4,686,311
<i>Methylovulum psychrotolerans</i> HV10-M2	<i>Methylovulum psychrotolerans</i> HV10-M2	2	12	50.88	4,923,391
<i>Methylovulum psychrotolerans</i> Sph1 ^T	<i>Methylovulum psychrotolerans</i> Sph1 ^T	2	12	50.85	5,162,352

Asterisks after reclassified names indicate later heterotypic synonyms of their senior counterparts in the same species clusters.

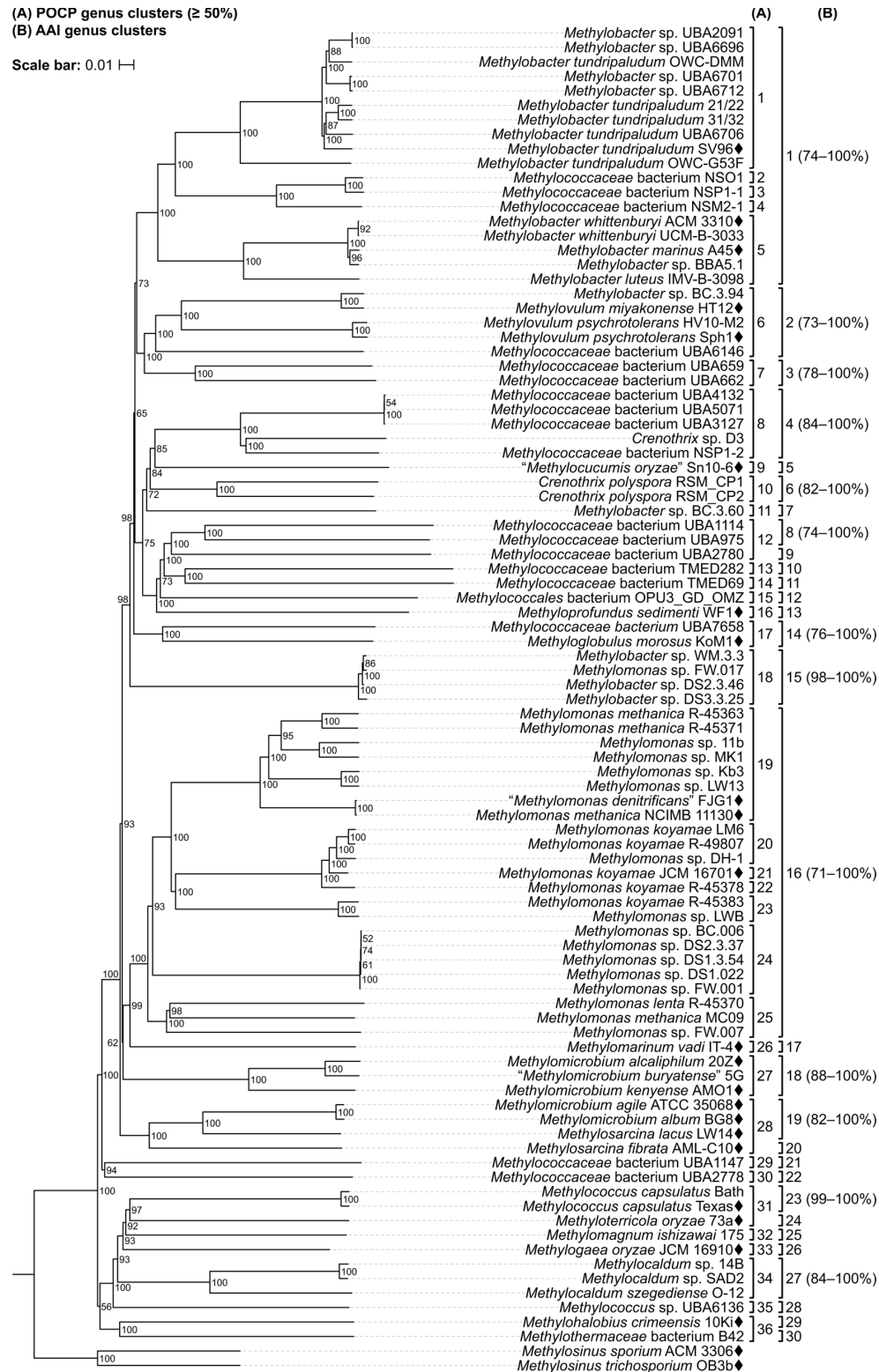
2. Supplementary Figures



Supplementary Figure S1. The phylogenetic relationship of *Methylococcales* type and representative strains based on (A) 16S rRNA and (B) PmoA sequences. The phylogenetic trees are the same trees as in Figure 1 but expanded to show all nodes and bootstrap support.



Supplementary Figure S2. The phylogenetic relationship of the *Methylococcales* genomes and their family designations. The phylogenetic tree is the same tree as in Figure 2. (A) The genus *Crenothrix* is listed under family *Methylococcaceae* in the *Bergey's Manual of Systematics of Archaea and Bacteria* (Bowman, 2016) and the Genome Taxonomy Database (GTDB) (Parks et al., 2018) but is listed under *Crenotrichaceae* in the List of Prokaryotic Names with Standing in Nomenclature (Parte, 2014). (B) GTDB taxonomy proposed family-level reclassification.



Supplementary Figure S3. Inferred genus clusters based on (A) POCP and (B) AAI. The phylogenetic tree is the same tree as in Figure 2. (A) Inferred genus clusters based on monophyly and the 50% POCP threshold for genus delineation (Qin et al., 2014). (B) AAI range values are indicated for every inferred genus cluster based on monophyly and AAI.

3. Supplementary References

Bowman, J.P. (2016). “*Methylococcaceae*,” in *Bergey’s Manual of Systematics of Archaea and Bacteria*, eds. W.B. Whitman, F. Rainey, P. Kämpfer, M. Trujillo, J. Chun, P. De Vos, B. Hedlund & S. Dedysh. (Hoboken, NJ: John Wiley & Sons, Inc.).

Parks, D.H., Chuvochina, M., Waite, D.W., Rinke, C., Skarszewski, A., Chaumeil, P.A., et al. (2018). A standardized bacterial taxonomy based on genome phylogeny substantially revises the tree of life. *Nat. Biotechnol.* 36, 996-1004. doi: 10.1038/nbt.4229.

Parte, A.C. (2014). LPSN—list of prokaryotic names with standing in nomenclature. *Nucleic Acids Res.* 42, D613-D616. doi: 10.1093/nar/gkt1111.

Qin, Q.L., Xie, B.B., Zhang, X.Y., Chen, X.L., Zhou, B.C., Zhou, J., et al. (2014). A proposed genus boundary for the prokaryotes based on genomic insights. *J. Bacteriol.* 196, 2210-2215. doi: 10.1128/JB.01688-14.