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## Supplementary Materials

# Metagenomics Revealed a New Genus ‘Candidatus Thiocaldithrix dubininis’ gen. nov., sp. nov. and a New Species ‘Candidatus Thiothrix putei’ sp. nov. in the Family Thiotrichaceae, Some Members of Which Have Traits of Both Na<sup>+</sup>- and H<sup>+</sup>-Motive Energetics †

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† The study is dedicated to the memory of the outstanding bioenergeticist Prof. V.P. Skulachev.

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**Table S1.** Characteristics of MAGs according to the minimal standards for description of MAGs (Bowers et. al.

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<i>Thiotrichaceae</i> sp. GKL-01	
<b>General genome metadata</b>	
analysis project type	metagenome-assembled genome (MAG)
taxa id	multi marker approach
assembly software	Flye v. 2.9
annotation	NCBI Prokaryotic Genome Annotation Pipeline (PGAP)
<b>Genome quality</b>	
assembly quality	Finished: single 3,251,546 bp long contiguous sequence
completeness score	High Quality Draft: 100%
contamination score	High Quality Draft: 0.6%
completeness software	CheckM2 v. 1.0.1
number of contigs	1
16S recovered	yes
16S recovery software	NCBI Prokaryotic Genome Annotation Pipeline (PGAP)
<b>MAG metadata</b>	
bin parameters	kmer+coverage
binning software	MetaBAT v.2.15

<i>Thiothrix</i> sp. GKL-02	
<b>General genome metadata</b>	
analysis project type	metagenome-assembled genome (MAG)
taxa id	multi marker approach
assembly software	Flye v. 2.9
annotation	NCBI Prokaryotic Genome Annotation Pipeline (PGAP)
<b>Genome quality</b>	
assembly quality	Finished: single 4,277,058 bp long contiguous sequence
completeness score	High Quality Draft: 100%
contamination score	High Quality Draft: 0.71%
completeness software	CheckM2 v. 1.0.1
number of contigs	1
16S recovered	yes
16S recovery software	NCBI Prokaryotic Genome Annotation Pipeline (PGAP)
<b>MAG metadata</b>	
bin parameters	kmer+coverage
binning software	MetaBAT v.2.15

dDDH	CT3	KT	BL	AS	Ku-5	G1	STA	A1	JP2	Q	RT	SSD2	A52	207	GKL-02	GKL-01
CT3	100	33,5	27,9	27,6	40,1	36	22,6	35,1	23,7	32,6	30,5	22,3	38,9	21,5	29,7	13,8
KT	33,5	100	27,8	28,8	48,7	36,4	21,7	33,8	23,6	33,5	27,7	22,9	33,8	21,5	29,3	18,9
BL	27,9	27,8	100	56,4	27,7	28	26,2	27,8	23,9	28,8	24,9	22,4	27,7	26,4	28,2	20
AS	27,6	28,8	56,4	100	28,5	28,6	26,7	28,2	24,7	28,6	25,3	22,6	27,4	26,6	28,9	21,8
Ku-5	40,1	48,7	27,7	28,5	100	38,5	27,3	36,7	23,6	34,7	27,9	22,4	35,9	27,3	40,4	21,8
G1	36	36,4	28	28,6	38,5	100	27,3	37,3	23,8	32,9	30,2	22,7	35	28,4	57,3	19,7
STA	22,6	21,7	26,2	26,7	27,3	27,3	100	28,6	23,8	27,5	26	22,3	26,7	21,8	17,3	17,7
A1	35,1	33,8	27,8	28,2	36,7	37,3	28,6	100	23,4	34,2	30,5	23	46,9	28,9	36	20
JP2	23,7	23,6	23,9	24,7	23,6	23,8	23,8	23,4	100	23,7	25,6	27,3	24,6	28,4	26	19,7
Q	32,6	33,5	28,8	28,6	34,7	32,9	27,5	34,2	23,7	100	27,9	22,3	31,9	26,6	33,8	21,6
RT	30,5	27,7	24,9	25,3	27,9	30,2	26	30,5	25,6	27,9	100	24,9	21,3	27,4	28,5	24,3
SSD2	22,3	22,9	22,4	22,6	22,4	22,7	22,3	23	27,3	22,3	24,9	100	32,3	25,6	22,7	21,4
A52	38,9	33,8	27,7	27,4	35,9	35	26,7	46,9	24,6	31,9	21,3	32,3	100	27,6	36,3	22,1
207	21,5	21,5	26,4	26,6	27,3	28,4	21,8	28,9	28,4	26,6	25,6	25,6	27,6	100	21,5	16,6
GKL-02	29,7	29,3	28,2	28,9	40,4	57,3	17,3	36	26	33,8	27,4	22,7	36,3	21,5	100	20,8
GKL-01	13,8	18,9	20	21,8	21,8	19,7	17,7	20	19,7	21,6	21,4	21,4	22,1	16,6	20,8	100

**Figure S1.** Heatmap of pairwise dDDH values for the assembled genomes of *Thiothrix*. *T. winogradskyi* CT3<sup>T</sup> (GCA\_021650935.1); '*Ca. Thiothrix sulfatifontis*' KT (GCA\_022828425.1); *T. lacustris* BL<sup>T</sup> (GCF\_000621325.1); *T. litoralis* AS<sup>T</sup> (GCF\_017901135.1); *T. subterranea* Ku-5<sup>T</sup> (GCF\_016772315.1); *T. caldifontis* G1<sup>T</sup> (GCF\_900107695.1); *T. unzii* A1<sup>T</sup> (GCA\_017901175.1); *T. nivea* JP2<sup>T</sup> (GCF\_000260135.1); *T. fructosivorans* Q<sup>T</sup> (GCA\_017349355.1); *Ca. Thiothrix moscovensis* RT (GCA\_016292235.1); *Ca. Thiothrix singaporensis* SSD2 (GCA\_013693955.1); *Ca. Thiothrix anitrata* A52 (GCF\_017901155.1); *Thiothrix* sp. 207 (GCA\_018813855.1); *Thiothrix* sp. STA\_22 (GCA\_028714775.1); *Thiothrixaceae* sp. GKL-01 (GCA\_029972135.1); *Thiothrix* sp. GKL-02 (GCA\_029972225.1).

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### Text S1. Accession numbers of the subunit c gene of F<sub>0</sub>F<sub>1</sub>-ATPases

The gene accession numbers retrieved from UniProtKB and NCBI were as follows: *Thiothrix lacustris* BL - A0A1Y1QH02\_9GAMM and A0A1Y1QMK8\_9GAMM, *Thiothrix litoralis* AS - WP\_210224368.1 and QTR46012.1, *Thiothrix winogradskyi* CT3 - WP\_236501573.1 and UJS24521.1, *Thiothrix unzii* A1 - WP\_210218492.1 and WP\_210219344.1, *Thiothrix fructosivorans* Q - A0A8B0SKJ4\_9GAMM and QTX11484.1, *Leucothrix arctica* IMCC 9719 - WP\_109826210.1 and WP\_109824171.1, *Leucothrix mucor* DSM 2834 - WP\_022954344.1 and A0A7V2WW86\_LEUMU, *Leucothrix pacifica* XH 122 - A0A317COL7\_9GAMM and A0A317CP54\_9GAMM, *Cocleimonas flava* KMM 3898 - WP\_131905866.1 and A0A4V2P7R0\_9GAMM, *Thiolinea eikelboomii*-AP3 - A0A1T4XYD5\_9GAMM, uncultured *Thiotrichaceae* bacterium - CAA6810787.1, *Ca. Thiothrix anitrata* A52 - QTR51456.1, '*Ca. Thiothrix sulfatifontis*' KT - UOG92218.1, *Thiothrix subterranea* Ku-5 - WP\_202716014.1, '*Ca. Thiotrichaceae* bacterium' GKL-01 - WGZ91550.1, *Thiothrix caldifontis* G1 - WP\_093066078.1, *Thiothrix nivea* JP2 - WP\_002709454.1, *Ca. Thiothrix singaporensis* SSD2 - QLQ34252.1, *Beggiatoa leptomitiformis* D-402 - WP\_201800170.1, *Beggiatoa alba* B18LD - I3CK18\_9GAMM, *Thioflexithrix pseupsensis* KCTC 62399 - WP\_086489165.1, *Thermotoga maritima* DSM 3109 - Q9X1V0|ATPL\_THEMEA, *Ilyobacter tartaricus* DSM 2382 - Q8KRV3|ATPL\_ILYTA, *Propionigenium modestum* DSM 2376 - CAA37840.1. ATPase genes of the following species were retrieved from the corresponding genomes in the RAST database using BLAST: *Thiothrix* sp. GKL-02 ('*Ca. Thiothrix putei*' GKL-02) - GCA\_029972225.1, *Thiothrix* sp. STA\_22 - GCA\_028714775.1, *Thiofilum flexile* DSM 14609 - GCF\_000380185.1, *Thiolinea disciformis* B31 - GCF\_000371925.1, *Ca. Thiothrix moscovensis* RT - GCA\_016292235.1, *Thiothrix* sp. SSD2 (*Ca. Thiothrix singaporensis* SSD2) - GCA\_013693955.1, *Thiothrix* sp. 207 - GCA\_018813855.1.

### Text S2. Accession numbers of the membrane PPase gene *hppA*

*Cocleimonas flava* KMM 3898 - WP\_131904771.1, *Ca. Thiothrix moscovensis* RT - MBJ6611069.1, *Methylophaga thiooxydans* DSM 22068 - A0A0A0BCX4\_9GAMM, *Methylophaga frappieri* DSM 25690 - WP\_014705011.1, *Methylophaga marina* DSM 5689 - WP\_286304297.1, *Methylophaga sulfidovorans* DSM 11578 - A0A1I3ZW22\_9GAMM, *Methylophaga aminisulfidovorans* SK1 MP - F5T014\_9GAMM, *Methylophaga thalassica* DSM 5690 - WP\_284722875.1, *Beggiatoa leptomitiformis* D-402 - A0A2N9YBH2\_9GAMM, *Beggiatoa alba* B18LD - WP\_002682680.1, *Thioflexithrix pseupsensis* (*Thioflexithrix pseupsensis* KCTC 62399) - A0A251XAU4\_9GAMM, *Thiothrix subterranea* Ku-5 - WP\_202715708.1, *Thiothrix nivea* JP2 DSM 5205 - WP\_002709561.1, *Thiothrix caldifontis* G1 - WP\_093064126.1, *Ca. Thiothrix singaporensis* SSD2 - QLQ33052.1, *Thiothrix fructosivorans* Q - WP\_207251832.1, *Thiothrix litoralis* AS - WP\_210221415.1, *Thiothrix unzii* A1 - WP\_210218856.1, *Ca. Thiothrix anitrata* A52 - WP\_228292382.1, *Thiothrix winogradskyi* CT3 - WP\_236500955.1, '*Ca. Thiothrix sulfatifontis*' KT - UOG93112.1, *Leucothrix mucor* DSM 2157 - A0A7V2T1F4\_LEUMU, *Azospirillum lipoferum* DSM 1691 (strain 4B) - A0A5A9GUE1\_AZOLI, *Azospirillum thermophilum* CFH 70021 - A0A2S2CQR0\_9PROT. PPase genes (*hppA*) of the following species were retrieved from the corresponding genomes in the RAST database using BLAST: *Thiothrix* sp. GKL-02 ('*Ca. Thiothrix putei*' GKL-02) - GCA\_029972225.1, *Thiothrix* sp. 207 - GCA\_018813855.1.