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Recovery of circular and high quality cable bacteria genomes from complex microbial communities

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Introduction

Cable bacteria are centimeter-long filamentous bacteria that can conduct long-distance electron transfer through coupling of sulfide oxidation and oxygen reduction. To elucidate the unique physiology of cable bacteria, reliable genome reference sequences are required. Although publically available cable bacteria genomes are often fragmented and incomplete due to using short reads for genome-centric metagenomics, long-read DNA sequencing technologies, such as Nanopore sequencing, are yet to be applied for the recovery of cable bacteria genomes.

Conclusions

- 4 high quality genome drafts of cable bacteria were recovered (2 *Electronema*, 2 *Electrothrix*)
- Both *Electronema* genomes were circular, while one 1 *Electrothrix* genome was single-contig
- The single-contig *Electrothrix* and 1 circular *Electronema* genomes were of novel species

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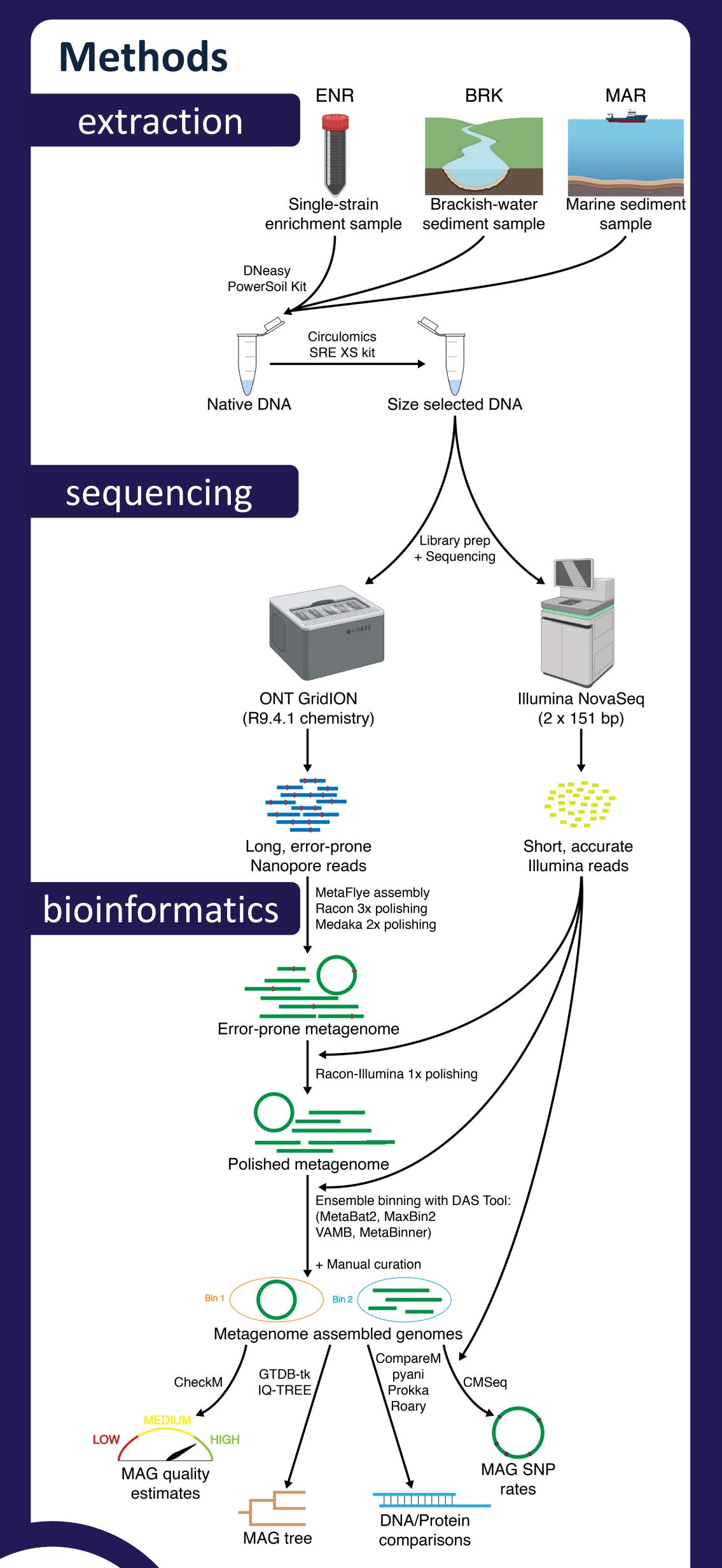
The novel species circular *Electronema* genome featured the most unique gene content and \bullet challenges the current definition of genus Candidatus Electronema

Results



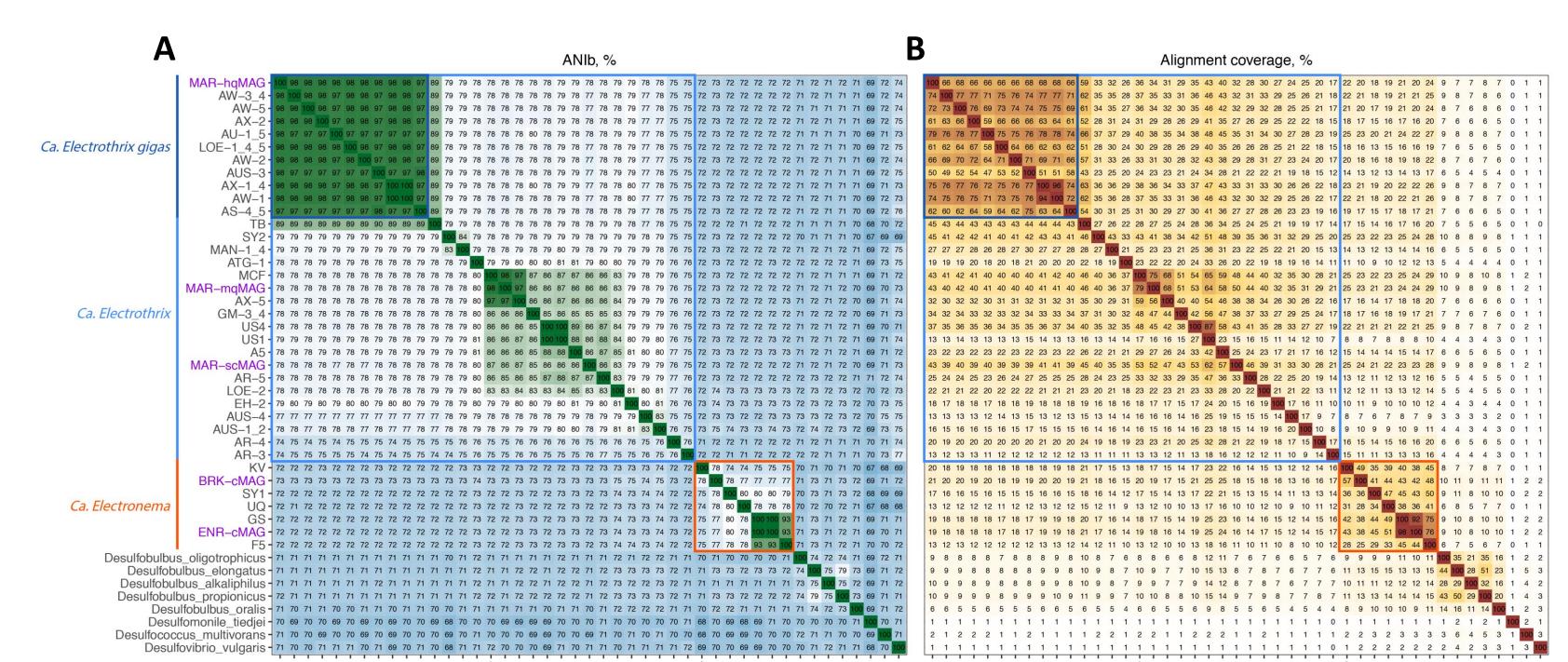
Aim

Recovery and characterization of circular or high quality cable bacteria genome drafts using longread Nanopore sequencing.



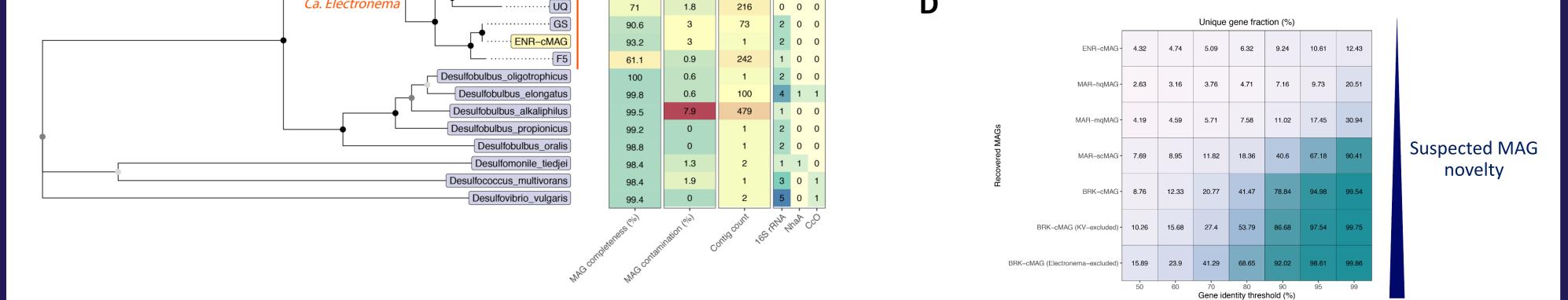
Recovered cable bacteria MAG statistics

	ENR-cMAG	BRK-cMAG	MAR-scMAG	MAR-hqMAG	MAR-mqMAG
MAG Completeness, %	93.2	94.1	92.0	90.2	93.9
MAG Contamination, %	MAG Contamination, % 3.0		2.6	4.0	7.1
Contig count	Contig count 1		1	37	9
SNP rate, %	SNP rate, % 0.002		0.8	1.4	1.0
CDS count	CDS count 2,847		3,409	2,849	3,484
Hypothetical CDS, %	Hypothetical CDS, % 56.4		58.5	58.5 55.5	
MIMAG quality ranking	HQ	HQ	HQ	HQ	MQ
Species taxonomy ranking	Electronema	Novel	Novel	Electrothrix gigas	Electrothrix
Species taxonomy ranking	GS	Electronema	Electrothrix	Electrotinix gigus	aarhusiensis



Whole-genome-based comparisons for cable bacteria MAGs. HQ and MQ cable bacteria MAGs (and outgroups used for tree building) were compared to each other using BLAST-based average nucleotide identity (ANIb, A) and genome alignment fractions (B). Using ANI of 75-77 % as genus boundary, all cable bacteria MAGs from MAR sample were classified as Candidatus Electrothrix, while MAGs from BRK and ENR were attributed to Candidatus Electronema.

Α		В				C	
							MAR-hqMAG - 52 53 51 60 64 62 63 62 69 69 69 68 68 68 68 71 74 78 78 80 80 79 79 80 81 79 79 80 80 79 79 80 81 90 97 98 98 97 98 98 97 98 98 98 98 98 98
	MAR-hqMAG	90.2	4	37	2 1 0		AW-3_4 - 52 53 51 60 64 62 63 62 69 69 69 67 68 68 68 71 74 78 77 80 80 79 79 80 81 79 79 79 79 79 79 79 79 79 80 80 90 97 98 98 97 98 98 97 97 98 98 77 97 98 98 97 97 98 98 97 97 98 98 97 97 98 98 97 97 98 98 97 97 97 97 97 97 97 97 97 97 97 97 97
	L(AW-3_4)	94.6	3.7	354	1 1 0		AX-2 - 52 53 51 60 64 63 63 63 69 69 69 68 68 68 69 71 74 77 77 80 80 79 79 80 80 79 79 79 79 79 79 79 79 80 80 89 97 98 98 97 98 98 97 98 98 97 8 98 97 98 98
	- · · · · · · (AW–5)	93.5	4	444	1 1 0	Ca. Electrothrix gigas	AU-1_5 - 52 53 51 60 64 62 63 62 69 69 69 69 67 68 68 68 71 74 78 77 80 80 79 79 79 79 79 79 79 79 79 79 79 79 79
	AX-2	89.2	4.5	818	1 1 0	55	AW_2 - 52 53 52 60 64 62 63 63 69 69 69 67 68 68 68 71 74 77 77 98 67 97 98 68 79 78 79 79 79 79 79 80 80 90 97 98 98 97 64 67 65 69 70 62
	IL AU−1_5	95.2	3	163	1 1 0		AUS-3 - 52 53 51 61 64 63 63 63 69 69 69 67 68 68 69 71 74 77 77 98 67 97 80 80 79 79 80 80 79 79 80 80 99 98 97 97 57 57 56 57 58 57 51
	LOE-1_4_5	86.9	2.2	784	1 1 0		AV-1-4 52 53 51 60 64 62 63 62 69 69 69 69 68 68 68 71 74 77 78 80 80 79 79 80 81 79 79 79 79 79 79 79 79 79 79 79 79 79
	[<u>LOL</u> 1 <u>_</u> + <u>_</u> 5]	89.5		696	1 1 0		AS-4_5 - 52 53 51 60 64 63 63 63 69 69 69 67 68 68 68 71 74 78 77 79 79 79 79 79 78 79 79 79 79 78 60 90 66 67 62 63 61 66 65 60
		80	2.5				SY2 - 52 53 51 60 63 62 63 62 69 69 69 68 68 69 69 71 74 77 77 98 08 80 80 81 81 80 79 80 80 80 81 81 80 79 80 80 80 81 81 80 79 80 80 80 81 81 80 79 80 80 80 81 81 80 79 80 80 80 80 81 81 80 79 80 80 80 80 80 80 80 80 80 80 80 80 80
	Ca. Electrothrix gigas		3.3	998	1 1 0		MAN-1_4 - 52 54 51 61 64 63 63 62 70 69 69 68 68 69 69 71 74 77 78 79 79 79 80 81 82 80 79 79 80 80 79 42 35 40 42 42 37 40 40 42 39 41 41 39 ATG-1 - 52 54 52 61 64 63 64 63 70 69 69 68 68 69 69 71 75 77 78 79 79 80 81 81 82 81 79 81 81 81 28 32 27 32 33 33 00 31 31 31 32 32 29 AA
Bootstrap support:		94.6	2.5	320	1 1 0		ATG-1 - 52 54 52 61 64 63 64 63 70 69 69 68 68 69 69 71 75 77 78 79 79 80 81 81 82 81 79 81 81 81 28 32 27 32 33 33 03 11 31 31 31 32 32 29 AAI MCF - 52 53 51 60 64 62 63 62 70 70 70 68 68 68 69 72 75 80 80 80 84 86 88 78 88 78 79 79 83 33 85 244 48 53 53 40 48 46 54 47 52 53 50 100
	T	95.2	3.6	407	1 1 0		MAR-mqMAG - 52 53 51 60 64 62 64 63 70 70 70 68 68 69 69 72 75 79 80 80 84 86 88 87 88 87 87 97 70 32 39 54 46 48 53 56 40 50 47 56 47 53 54 52 90
• 90–100 %	L AS-4_5	92.5	4.1	808	1 1 0	Ca. Electrothrix	AX-5 - 52 53 52 60 64 62 63 63 70 69 69 68 68 69 72 75 79 79 79 79 78 84 86 87 87 88 87 86 56 55 31 36 41 35 41 43 43 37 41 40 43 41 42 43 39 GM-3 4 52 53 51 60 64 62 63 62 69 69 69 68 68 68 69 71 75 78 78 78 78 78 78 82 84 86 85 86 85 44 49 48 30 34 42 37 41 43 43 36 41 39 43 40 43 43 40 43 43 40 43 43 40 43 41 42 43 40 43 40 43 41 42 43 40 43 40 43 41 42 43 40 40 40 40 40 40 40 40 40 40 40 40 40
• 70–90 %		74.4	6.1	539	1 1 0		US4 - 52 53 51 60 64 62 63 62 69 69 69 68 68 68 69 72 75 79 80 79 84 88 87 89 99 43 42 54 53 32 36 50 44 46 50 51 39 46 43 52 44 49 50 47
• 0–70 %	SY2	98.8	1.2	240	0 0 0		US1 - 52 54 52 61 65 64 64 63 69 70 70 69 69 69 70 72 76 79 79 80 84 88 88 89 35 25 25 27 27 22 24 25 27 27 28 28 26 27 27 27 27 28 28 28 26 27 27 27 28 28 28 26 27 27 27 28 28 28 26 27 27 27 28 28 28 28 28 28 28 28 28 28 28 28 28
	• • • • • • • • • MAN-1_4	77.4	3.6	1177	1 2 0		MAR-scMAG - 51 53 51 60 64 62 63 62 69 69 69 68 68 69 69 72 75 79 79 80 83 87 40 26 55 45 45 60 56 33 39 55 45 48 53 54 40 49 46 56 46 52 54 52
MAG origin:	ATG-1	65.7	3.6	1441	2 1 0		AR-5 52 53 51 60 64 62 63 63 69 69 69 68 68 68 72 75 78 78 78 78 82 41 29 25 37 36 39 38 40 30 31 37 32 36 37 37 33 36 36 37 35 37 37 33 OF-2 52 54 51 61 65 63 65 63 70 70 70 70 69 69 70 70 72 75 79 79 78 30 33 26 23 31 29 32 32 33 26 33 34 28 33 34 34 30 34 33 34 33 34 34 31 0000000000
Previous studies		91.3					EH_2 - 52 54 52 61 65 63 65 64 70 69 69 68 69 69 69 71 74 78 78 28 26 28 23 19 27 26 28 29 29 24 29 30 26 31 32 32 29 31 31 31 30 31 31 28
Current study			2.4	143	2 3 0		AUS-4 - 52 53 52 60 64 62 64 62 69 69 69 69 69 69 68 68 69 71 73 81 24 26 26 27 22 21 27 25 28 27 28 25 27 28 23 27 27 27 27 27 27 27 27 27 27 27 27 27
	MAR-mqMAG	93.8	7.1	9	2 3 0		AR-4 - 52 54 52 60 64 62 63 62 71 70 70 68 69 69 69 74 22 23 25 27 32 37 27 23 37 34 34 37 37 27 29 36 31 35 36 37 31 35 34 36 33 36 36 33 50 50 50 50 50 50 50 50 50 50 50 50 50
	AX-5	83.2	3.3	1174	1 3 0		AR-3 - 51 53 51 60 64 62 63 63 71 71 71 70 69 70 70 27 19 19 21 23 26 30 23 19 28 27 27 30 29 23 25 29 26 29 30 30 26 29 28 29 28 30 29 28 29 28 29 28 29 28 30 29 28 29 28 29 28 30 29 28 29 28 29 28 30 29 28 29 28 29 28 29 28 30 29 28 29 28 29 28 30 29 28
	GM-3_4	87.6	3.3	1190	2 3 3		BRK-cMAG - 52 53 52 61 64 63 63 62 76 76 76 76 76 76 76 76 76 76 76 76 76
		87.7	1.8	307	1 2 1	Ca. Electronema	SY1 - 52 53 52 61 64 63 64 62 80 80 80 79 63 64 29 33 25 23 25 30 31 48 36 26 45 37 36 47 46 27 35 50 43 45 49 50 38 44 42 51 43 48 49 47
	US1	51.7	0.9	605	1 0 2		UQ = 51 52 51 60 63 62 63 61 80 80 79 58 55 57 26 29 23 20 22 72 74 132 26 40 31 31 41 40 24 31 41 40 40 43 44 35 40 37 45 39 43 43 42 50 43 43 42 50 43 43 42 50 43 43 44 42 50 44 42 50 44 44 50 44 40 40 44 40 40 44 40 40 44 40 40 40
		63.4	1.2	472	1 1 0		ENR-cMAG - 52 53 52 60 64 63 64 63 94 93 57 64 60 62 28 32 24 22 24 28 29 46 33 25 42 35 34 45 44 26 35 47 41 43 47 48 36 42 40 49 41 46 47 45
		92	2.5	1	1 3 2	Desulfobulbu	LS_Oligotrophicus - 52 54 51 63 73 70 74 30 35 36 33 37 36 36 18 23 15 15 15 19 20 32 26 20 31 25 24 32 31 18 24 33 29 31 33 34 26 31 28 35 30 33 34 32
	AR-5	74	2.8	1341	1 3 2	Desulfob	Dulbus_elongatus - 52 54 51 64 78 72 62 28 33 34 31 35 35 34 17 23 15 14 15 18 19 31 24 18 31 24 23 31 30 17 22 32 27 29 32 33 24 29 27 33 28 32 32 31
			2.0				Jlbus_alkaliphilus 52 54 51 63 72 53 53 53 53 33 30 34 34 18 22 15 16 18 20 32 24 33 31 17 22 33 27 29 32 23 24 29 27 34 28 32 33 20 25 24 18 30 25 24 33 31 17 22 33 27 29 32 23 24 29 27 34 28 32 33 31 17 22 33 21 21 33 31 37 23 23 24 29 27 34 28 32 34 3
	LOE-2	73.2	3	1070	1 2 0	Desu	sulfobulbus_oralis - 52 53 51 42 38 42 45 30 34 35 32 36 35 35 17 22 15 14 15 18 19 30 24 19 30 23 22 29 28 17 22 30 28 28 31 32 24 28 27 32 27 31 31 30
	EH-2	58.1	1.8	1297	1 2 0		ulfomonile_tiedjei <mark>- 51 53</mark> 14 17 15 17 16 11 14 14 12 14 13 13 7 8 6 5 6 8 8 12 9 6 11 10 9 13 12 7 9 13 10 11 12 13 9 11 10 13 11 12 12 12 ccus_multivorans - <mark>53</mark> 20 21 27 26 26 27 16 20 20 18 21 21 20 11 13 9 9 9 12 12 19 14 11 18 15 15 20 18 10 14 20 16 17 19 20 14 17 16 20 17 19 19 19
	AUS-4	51.9	1.9	1393	1 1 0		Ilfovibrio_vulgaris - 22 18 22 24 20 23 24 15 18 18 16 18 18 9 11 8 7 8 10 10 15 12 9 15 12 12 16 15 9 12 16 14 15 16 16 12 15 13 17 14 16 16 16
	Ca. Electrothrix AUS-1_2	63.1	3.1	1349	1 1 0		
	AR-4	77.6	2.7	1246	1 0 1		The second
	AR-3	65.8	2.5	1561	1 1 3		NAL NAL AND
	•	86.9	3	89	0 1 0		
	BRK-cMAG	94	3.3	1	2 1 0		Contraction of the second seco
	SY1	92.9	1.2	125	0 1 0		\checkmark
	Ca. Electronema	71	1.8	216	0 0 0	Π	
		/1	1.0	210	0 0 0		\mathbf{V}



Gene-based comparisons of cable bacteria MAGs. A) Maximum-likelihood genome tree, built using 120 universal bacterial marker genes and 100 bootstraps for HQ, MQ cable bacteria MAGs, with multiple outgroups included. B) Selected features of cable bacteria MAGs: quality metrics and relevant gene counts. C) Average amino acid identity (AAI) and percentage of conserved proteins (POCP) between cable bacteria MAGs and outgroups. POCP of 50 % was used as genus boundary to further confirm the classification of BRKcMAG as a member of *Candidatus Electronema*. **D)** Unique gene fractions for MAGs recovered in this study, compared to other cable bacteria MAGs (HQ and MQ), at different identity thresholds for gene clustering.

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