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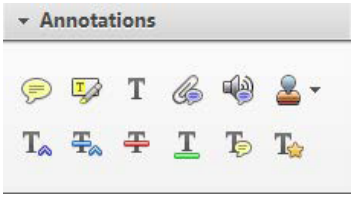

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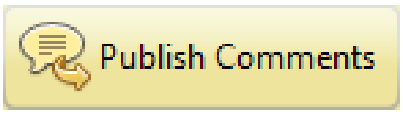

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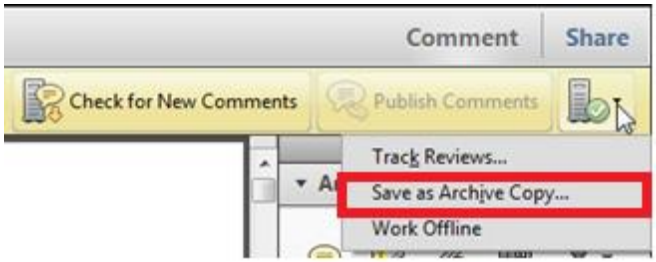

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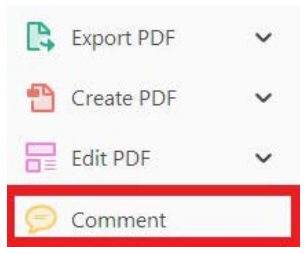

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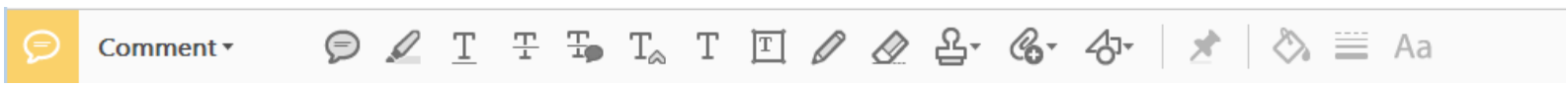

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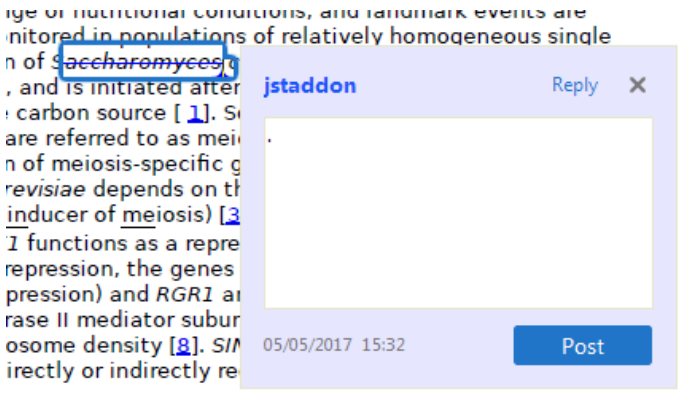

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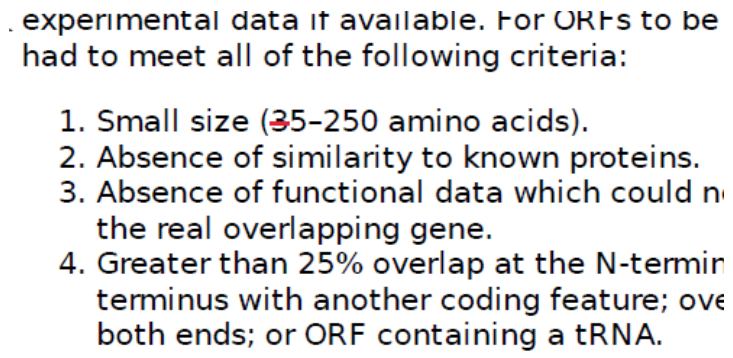
experimental data if available. For ORFs to be had to meet all of the following criteria:

1. Small size (~~3~~5–250 amino acids).
2. Absence of similarity to known proteins.
3. Absence of functional data which could n the real overlapping gene.
4. Greater than 25% overlap at the N-termir terminus with another coding feature; ove both ends; or ORF containing a tRNA.

3. Commenting Tool – for highlighting a section to be changed to bold or italic or for general comments.

Use these 2 tools to highlight the text where a comment is then made.

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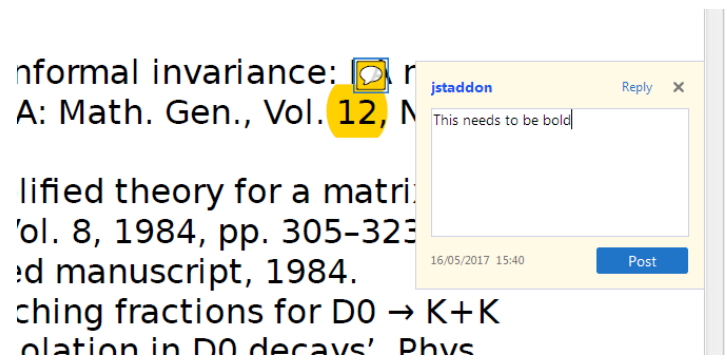

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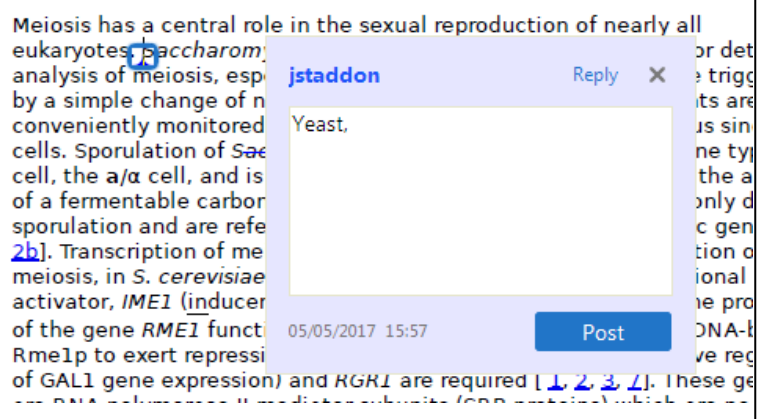

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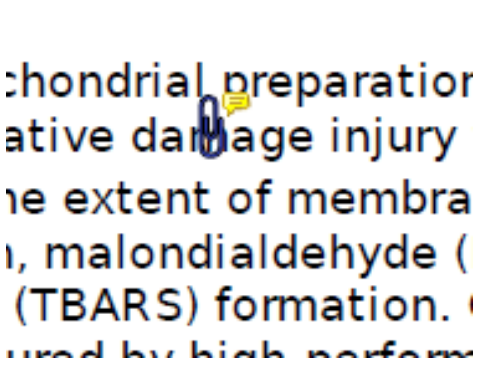

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- Click on  .
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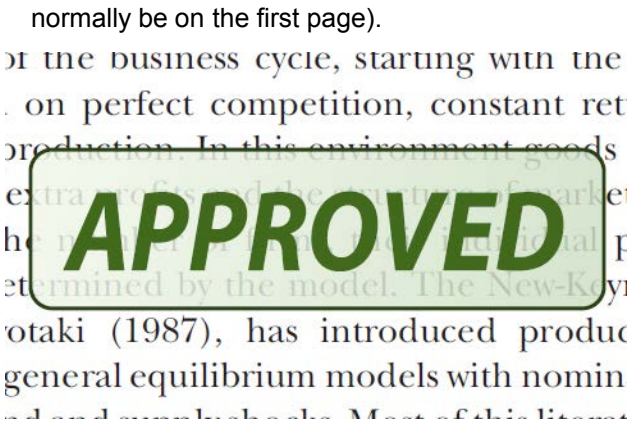
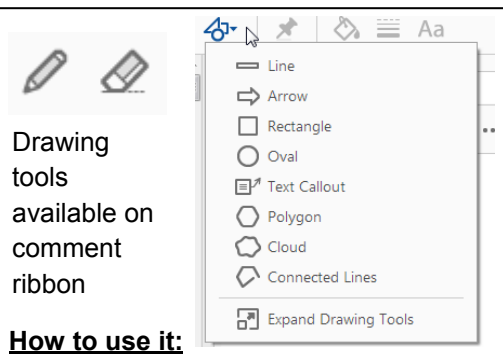
- Fill in any details and then click on the proof where you'd like the stamp to appear. (Where a proof is to be approved as it is, this would normally be on the first page).





Drawing tools available on comment ribbon

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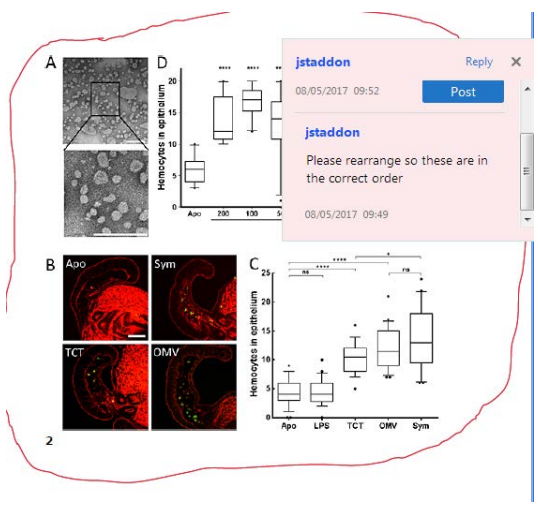

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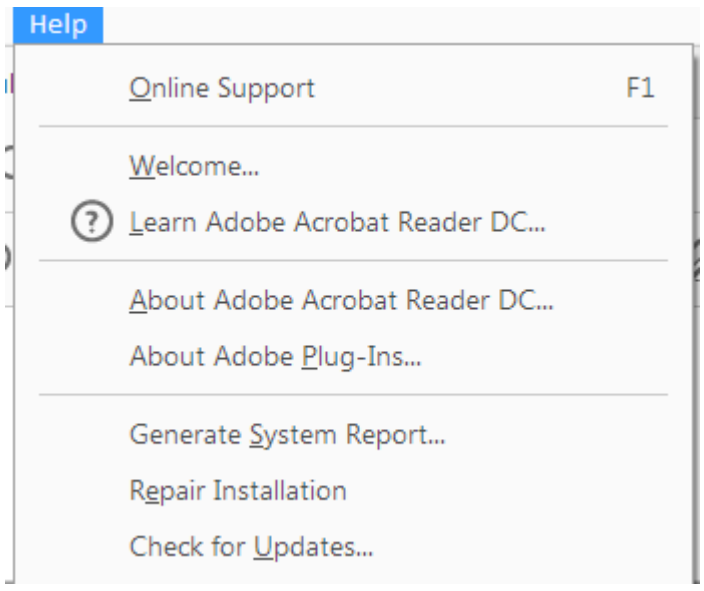

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Environmental Microbiology (2018) 00(00), 00–00 doi:10.1111/1462-2920.14090

Genomes of ubiquitous marine and hypersaline *Hydrogenovibrio*, *Thiomicrorhabdus* and *Thiomicrospira* spp. encode a diversity of mechanisms to sustain chemolithoautotrophy in heterogeneous environments

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Summary

Chemolithoautotrophic bacteria from the genera *Hydrogenovibrio, Thiomicrorhabdus* and *Thiomicrospira* are common, sometimes dominant, isolates from sulfidic habitats including hydrothermal vents, soda and salt lakes and marine sediments. Their genome sequences confirm their membership in a deeply branching clade of the *Gammaproteobacteria*. Several adaptations to heterogeneous habitats are apparent. Their genomes include large numbers of genes for sensing and responding to their environment (EAL- and GGDEF-domain proteins and methyl-accepting chemotaxis proteins) despite their small sizes (2.1–3.1 Mbp). An array of sulfur-oxidizing complexes are encoded, likely to facilitate these organisms' use of multiple forms of reduced sulfur as electron donors. Hydrogenase genes are present in some taxa, including group 1d and 2b hydrogenases in *Hydrogenovibrio marinus* and *H. thermophilus* MA2-6, acquired via horizontal gene transfer. In addition to high-affinity *cbb*$_3$ cytochrome *c* oxidase, some also encode cytochrome *bd*-type quinol oxidase or

ba_3-type cytochrome c oxidase, which could facilitate growth under different oxygen tensions, or maintain redox balance. Carboxysome operons are present in most, with genes downstream encoding transporters from four evolutionarily distinct families, which may act with the carboxysomes to form CO_2 concentrating mechanisms. These adaptations to habitat variability likely contribute to the cosmopolitan distribution of these organisms.

Introduction

Members of the genera *Hydrogenovibrio, Thiomicrorhabdus* and *Thiomicrospira* are common in sulfidic habitats worldwide, present and often numerous at hydrothermal vents, terrestrial hot springs, soda and salt lakes and coastal sediments (Table 1; Kuenen and Veldkamp, 1972; Jannasch, 1985; Brinkhoff and Muyzer, 1997; Kato *et al*., 2009; Brazelton and Baross, 2010; Sorokin *et al*., 2011). These organisms, formerly classified in the genera *Hydrogenovibrio, Thiomicrospira* and *Thioalkalimicrobium*, have been taxonomically reassigned based on a polyphasic analysis, including the 16S rRNA gene and 53 ribosomal protein gene sequences (Table 1; Boden *et al*., 2017b). These obligate aerobes all grow chemolithoautotrophically on reduced sulfur compounds; some are also able to grow on molecular hydrogen (Table 1). All fix CO_2 using the transaldolase variant of the Calvin–Benson–Bassham cycle (Kuenen and Veldkamp, 1972; Nishihara *et al*., 1991; Muyzer *et al*., 1995; Ahmad *et al*., 1999; Brinkhoff *et al*., 1999b,c; Rainey *et al*., 2001; Sorokin *et al*., 2002; Knittel *et al*., 2005; Sorokin *et al*., 2006; 2007; Hansen and Perner, 2015; Zhang *et al*., 2016).

Hydrogenovibrio crunogenus XCL-2 was the first member of these genera to have its genome sequenced, and its genome has some puzzling features (Scott *et al*., 2006). Low numbers of membrane transporters reflect its obligately autotrophic lifestyle, although there are a surprisingly high number of genes encoding methyl-accepting chemotaxis proteins, and it was proposed that enhanced capability of sensing, and motility toward, microhabitats in which nutrients were available could compensate for this species' ability to use rather few nutrients for growth (Scott *et al*., 2006). The capability of using reduced sulfur compounds as electron donors is reflected by the presence of genes encoding the 'Sox' complex (*soxABCDXYZ*) associated with the Kelly–Friedrich pathway and sulfide:quinone oxidoreductase (EC 1.8.5.4) (Scott *et al*., 2006). Likewise, genes encoding a [NiFe] hydrogenase suggest the potential for growth on molecular hydrogen, although the presence of this gas does not facilitate growth by this strain under the provided conditions (Hansen and Perner, 2016).

The *H. crunogenus* XCL-2 genome suggests a departure from previous models of dissolved inorganic carbon (DIC) uptake in autotrophic microorganisms. *H. crunogenus* XCL-2 has a CO_2-concentrating mechanism (CCM) that facilitates growth under low DIC conditions (Dobrinski *et al*., 2005). Like the many species of the '*Cyanobacteria*' in which CCMs have been well characterized, *H. crunogenus* XCL-2 can generate an elevated concentration of intracellular DIC (Dobrinski *et al*., 2005; Price, 2011). This large pool of intracellular DIC drives carbon fixation by carboxysomes, intracellular microcompartments containing RubisCO (ribulose 1,5-bisphosphate carboxylase/oxygenase; EC 4.1.1.39) and carbonic anhydrase (EC 4.2.1.1; Cannon *et al*., 2010; Menning *et al*., 2016). The ability to generate intracellular DIC concentrations $>$ 100-fold higher than extracellular suggests active DIC uptake (Dobrinski *et al*., 2005), but genes orthologous to any of the DIC transporters characterized in the '*Cyanobacteria*' are absent in the *H. crunogenus* XCL-2 genome (Menning *et al*., 2016). In *H. crunogenus*, DIC uptake is facilitated by a novel two-component transporter. One subunit of this transporter is a member of a 'domain of unknown function' protein family (PFAM10070; Bateman *et al*., 2002), while the other subunit, which is predicted to be a transmembrane protein, belongs to a protein family that includes proton transporters (PFAM00361; Mangiapia *et al*., 2017).

Hydrogenovibrio crunogenus XCL-2 genome data also predict a peculiar citric acid cycle, in which 2-oxoglutarate dehydrogenase (EC 1.2.4.2/2.3.1.61/1.8.1.4) and malate dehydrogenase (EC 1.1.3.7) are absent (Scott *et al*., 2006). Malate:quinone oxidoreductase (EC 1.1.5.4) is encoded in the genome (Scott *et al*., 2006), is active in membrane fractions (Quasem *et al*., 2017), and could act as a replacement for malate dehydrogenase. The 2-oxoglutarate:ferredoxin oxidoreductase (EC 1.2.7.3) activity is absent, suggesting that this obligate autotroph may have a wishbone-shaped 'citric acid pathway', with an absence of interconversion of 2-oxoglutarate and succinyl-coA (Smith *et al*., 1967; Wood *et al*., 2004). However, genes encoding 2-oxoglutarate decarboxylase and succinic semialdehyde dehydrogenase are present, which could potentially close the citric acid cycle by converting 2-oxoglutarate to succinyl-CoA (Quasem *et al*., 2017).

At the time of its sequencing, placing these and other features of the *H. crunogenus* XCL-2 genome within an evolutionary context was complicated by the absence of genome sequences of close relatives among the basal *Gammaproteobacteria* (Williams *et al*., 2010). In order to determine whether the peculiar aspects of *H. crunogenus* XCL-2 described above are unique to the species, or are typical for the lineage, the genomes of eleven additional members of *Hydrogenovibrio, Thiomicrorhabdus* and *Thiomicrospira* were sequenced, representing the geographic, habitat and phylogenetic breadth of these genera, and

Table 1. Members of the genera *Hydrogenovibrio, Thiomicrorhabdus* and *Thiomicrospira* with sequenced genomes.

Taxon	Basonym[a]	Habitat	Genome published	Electron donors[b,c]	Organic carbon oxidation or assimilation[c,d]	Opti-mum pH[c]	Opti-mum °C[c]	Opti-mum Na^+ (M)[c]
H. crunogenus XCL-2	*Thiomicrospira crunogena* XCL-2	East Pacific Rise deep-sea hydrothermal vent (Ahmad *et al.*, 1999)	(Scott *et al.*, 2006)	$S_2O_3^{-2}$, H_2S, S^0	–			
Hydrogenovibrio sp. XS5	*Thiomicrospira* sp. XS5	Red Sea deep brine-water interface (Zhang *et al.*, 2016)	(Zhang *et al.*, 2016)	$S_2O_3^{-2}$				
H. thermophilus JR2 DSM 25194	*Thiomicrospira* sp. JR2	Northeast Pacific deep-sea hydrothermal vent	This work	$S_2O_3^{-2}$				
H. thermophilus MA2–6	*Thiomicrospira* sp. MA2–6	Mid-Atlantic deep-sea hydrothermal vent (Muyzer *et al.*, 1995)	This work	$S_2O_3^{-2}$				
H. halophilus	*Thiomicrospira halophila*	Hypersaline lake (Russia) (Sorokin *et al.*, 2006)	This work	$S_2O_3^{-2}$, H_2S, S^0		7.5–7.8		1.5
Hydrogenovibrio sp. WB1	*Thiomicrospira* sp. WB1	Red Sea deep brine-water interface (Zhang *et al.*, 2016)	(Zhang *et al.*, 2016)	$S_2O_3^{-2}$				
H. kuenenii	*Thiomicrospira kuenenii*	Wadden Sea mud flat (Brinkhoff *et al.*, 1999a)	This work	$S_2O_3^{-2}$, H_2S, S^0, $S_4O_6^{-2}$	NG	6.0		0.42
H. marinus DSM 11271	N/A	Surface seawater near Japan (Nishihara *et al.*, 1991)	This work and (Jo *et al.*, 2014)	H_2, $S_2O_3^{-2}$, S^0, $S_4O_6^{-2}$	NG	6.5	37	0.5
Hydrogenovibrio sp. Milos-T1	*Thiomicrospira* sp. Milos-T1	Aegean shallow hydrothermal vent (Brinkhoff *et al.*, 1999b)	This work	$S_2O_3^{-2}$				
Tmr. frisia Kp2 DSM 25197	*Thiomicrospira* sp. Kp2	Northeast Pacific deep-sea hydrothermal vent	This work	$S_2O_3^{-2}$				
Thiomicrorhabdus sp. Milos-T2	*Thiomicrospira* sp. Milos-T2	Aegean shallow hydrothermal vent (Brinkhoff *et al.*, 1999b)	This work	$S_2O_3^{-2}$				
Tmr. arctica	*Thiomicrospira arctica*	Arctic Ocean coastal sediments near Svalbard (Knittel *et al.*, 2005)	This work	$S_2O_3^{-2}$, S^0, $S_4O_6^{-2}$	NG	7.3	11–13	0.25
Tmr. chilensis	*Thiomicrospira chilensis*	Chile continental shelf sediment (Brinkhoff *et al.*, 1999c)	This work	$S_2O_3^{-2}$, H_2S, S^0, $S_4O_6^{-2}$	NG	7.0	32–37	
Tms. aerophila	*Thioalkalimicrobium aerophilum* AL3	Hypersaline lake (Russia) (Rainey *et al.*, 2001)		$S_2O_3^{-2}$, H_2S, S^0, PS, $S_4O_6^{-2}$	A, NG	10		
Tms. cyclica	*Thioalkalimicrobium cyclicum*	Hypersaline lake (US) (Sorokin *et al.*, 2002)	(Kappler *et al.*, 2016)	$S_2O_3^{-2}$, H_2S, PS, $S_4O_6^{-2}$		9.5		0.3
Thiomicrospira sp. ALE5	*Thioalkalimicrobium* sp. ALE5	Hypersaline lake (Egypt) (Sorokin *et al.*, 2011)		$S_2O_3^{-2}$, H_2S, PS		9.5–10		
Tms. microaerophila				$S_2O_3^{-2}$, H_2S	A, NG	8–9	25–28	0.4–0.5

Table 1. *cont.*

Taxon	Basonym[a]	Habitat	Genome published	Electron donors[b,c]	Organic carbon oxidation or assimilation[c,d]	Opti-mum pH[c]	Opti-mum °C[c]	Opti-mum Na^+ (M)[c]
	Thioalkalimicrobium microaerophilum	Hypersaline lake (US) (Sorokin *et al.*, 2007)						
Tms. pelophila	N/A	Wadden Sea mud flat (Kuenen and Veldkamp, 1972)	This work	$S_2O_3^{-2}$, H_2S, S^0, $S_4O_6^{-2}$	A, NG	6.5–7.5	28–30	

a. Boden *et al.*, 2017b.
b. PS, polysulfide.
c. Data from (Kuenen and Veldkamp, 1972; Jannasch *et al.*, 1985; Nishihara *et al.*, 1991; Muyzer *et al.*, 1995; Brinkhoff *et al.*, 1999a,b,c; Rainey *et al.*, 2001; Sorokin *et al.*, 2002; Knittel *et al.*, 2005; Sorokin *et al.*, 2007; 2008; 2011; Zhang *et al.*, 2016; Ang *et al.*, 2017; Quasem *et al.*, 2017).
d.-, no assimilation of organic compounds, and no growth in the absence of reduced sulfur compounds; NG, no growth in the absence of inorganic electron donors; A, organic carbon assimilation.

genome comparisons among these organisms were expanded to include members of this lineage sequenced by other projects (Table 1).

Results and discussion

Ribosomal protein supertree

As previously described (Boden *et al.*, 2017b), the supertree constructed from amino acid sequences derived from ribosomal protein genes places the genera *Thiomicrospira, Thiomicrorhabdus* and *Hydrogenovibrio* together in a well-supported clade (Fig. 1). Since *Thiomicrospira* sp. Milos T1, XS-5 and WB-1 fall within genus *Hydrogenovibrio*, and *Thiomicrospira* sp. Milos-T2 falls within the genus *Thiomicrorhabdus*, they will be referred to here with those genus designations (Table 1). As also previously shown (Boden *et al.*, 2017b), on the basis of 16S rRNA gene sequence identities (100% vs. type strain I78) and genome-wide average nucleotide identities (96.5% between JR2 and MA2–6), *Thiomicrospira* sp. JR2 and MA2–6 are strains of *H. thermophilus* (values calculated relative to type strain I78), and will be referred to here as such (e.g., *H. thermophilus* JR2). *Thiomicrorhabdus* sp. KP2 is a strain of *Tmr. frisia* (99.5% 16S sequence identity with type strain JB-A2).

The order *Thiotrichales*, within which the genera *Thiomicrospira*, *Thiomicrorhabdus* and *Hydrogenovibrio* are circumscribed, is represented on the supertree by many well-supported clades, some of which are intermixed with clades of the *Chromatiales* (Fig. 1). This is also the case with many of the orders of the *Gammaproteobacteria* on this and other supertrees (Williams *et al.*, 2010; Ramulu *et al.*, 2014), suggesting that the taxonomy of the *Gammaproteobacteria* should be revised.

Genome structures and general features

Genome sizes among permanent draft and finished genome sequences of the genera *Thiomicrospira, Thiomicrorhabdus* and *Hydrogenovibrio* (2.1–3.1 Mb; Table 2) fall within the range of sizes of genomes of other free-living autotrophs from the phylum '*Proteobacteria*' (1.7–10.1 Mb; Supporting Information Table S1). Their genomes are larger than those of the vertically transmitted chemolithoautotrophic symbionts of vesicomyid clams (1.0–1.2 Mb), which reflects genome reduction in the symbionts (Kuwahara *et al.*, 2007; Newton *et al.*, 2007; 2008). Their genomes are smaller than those from organisms with additional capabilities such as photosynthesis, denitrification or heterotrophic growth (Supporting Information Table S1). The rRNA operon copy numbers (3–4) are at the high range of what is observed in other autotrophic members of the *Gammaproteobacteria* (Supporting Information Table S1), which may help them to respond more quickly to

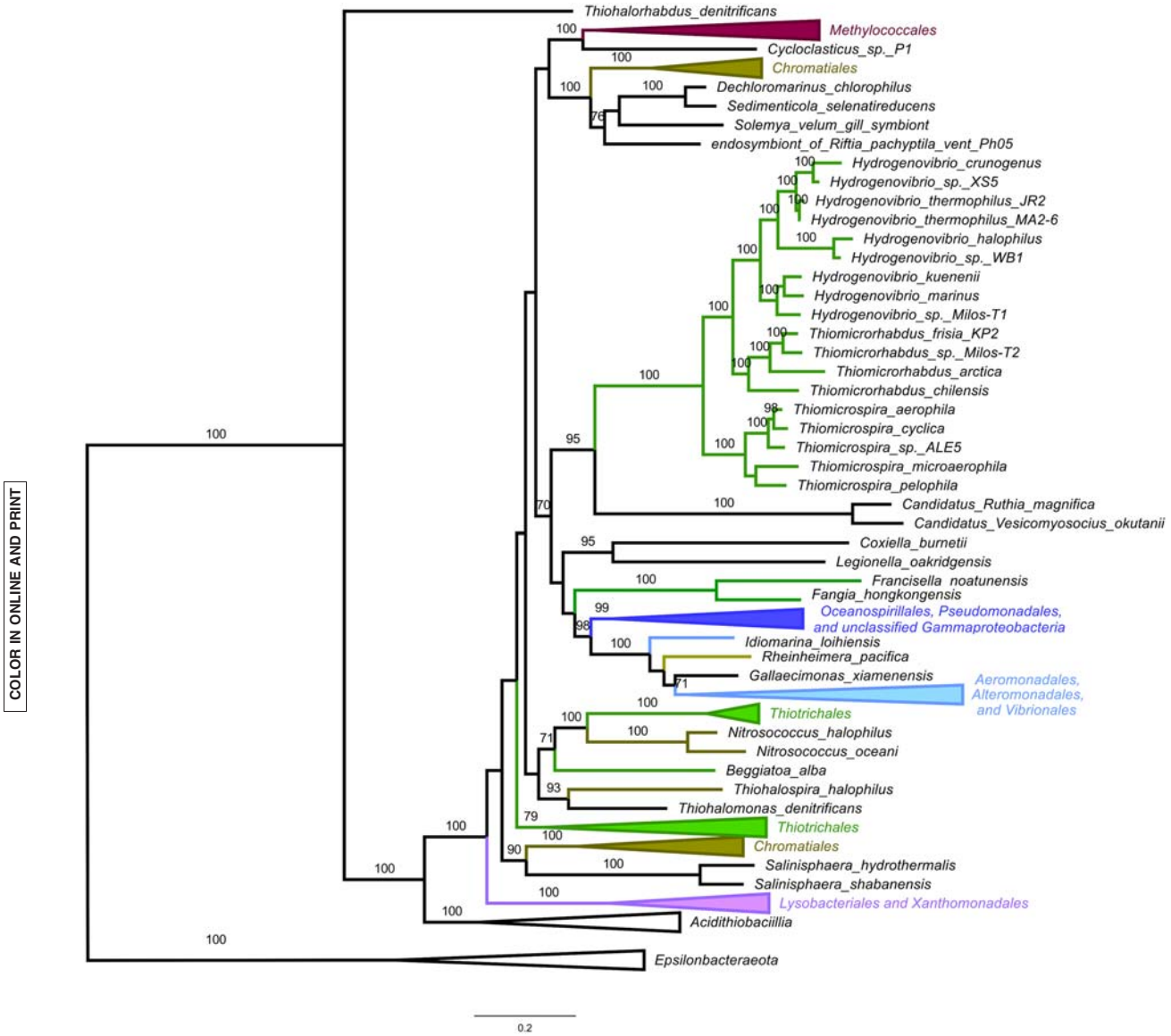

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Fig. 1. Maximum likelihood analysis of concatenated alignments of amino acid sequences derived from gene sequences encoding ribosomal proteins. Members of the order *Chromatiales* are indicated by olive-coloured clades and branches, while members of the *Thiotrichales* have green clades and branches. Bootstrap values > 70% from 200 resamplings of the alignment are shown, and the tree is unrooted. The scale bar represents the number of substitutions per site.

changes in their environment (Klappenbach *et al.*, 2000) and to have higher maximum specific growth rates (Roller *et al.*, 2016). Indeed, members of these genera are among the first to appear in media inoculated from environmental samples (K. Scott and R. Boden, unpubl. data).

Many members of *Hydrogenovibrio* and *Thiomicrorhabdus* have large tandem repeats preceding a gene encoding protein kinase (e.g., *Tcr_0106*; Fig. 2). *Hydrogenovibrio halophilus* lacks an ortholog to this protein kinase, as well as the repeated regions. For those organisms whose tandem repeat regions have been completely sequenced, repeat lengths range from 279 to 393 nucleotides in length, with 5 to > 27 copies in tandem (upper range is at the end of a contig; Fig. 2). Some members of these two genera whose draft genome sequences currently lack large tandem repeats are likely to also have them; in these organisms, orthologs to the protein kinases described above are present at the 5′ end of their contigs and the absence of tandem repeats may result from the difficulties in sequencing and assembly of these regions (Treangen and Salzberg, 2012). In other organisms, repeated regions such as these are hotspots for genome rearrangement that can serve to generate diversity of gene products (if falling within a gene) or diversity in gene

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Table 2. Genome sequencing results.

Taxon	# Contigs	# Scaffolds	Average coverage (fold)	Estimated genome size (Mb)	Gene count	GC content (%)	# rRNA operons	tRNA count
H. thermophilus JR2	7	1	1031[a]	2.6	2444	50	3	44
H. thermophilus MA2–6	1	1	224.8	2.7	2546	50	3	45
H. halophilus	3	1	3302	2.4	2238	55	3	45
H. kuenenii	2	2	96.1	2.5	2289	42	3	43
H. marinus DSM 11271	3	3	284.5	2.6	2554	44	4	45
Hydrogenovibrio sp. Milos-T1	1	1	181.3	2.3	2253	44	3	44
Tmr. frisia Kp2	13	3	1455	2.7	2526	40	3	45
Thiomicrorhabdus sp. Milos-T2	8	2	1000.3	2.6	2382	38	3	46
Tmr. arctica	8	6	631.6	2.6	2337	42	3	45
Tmr. chilensis	5	2	679.5	2.4	2285	48	2	43
Tms. pelophila	1	1	178.4	2.1	2040	44	2	41

a. Illumina coverage; 454 coverage was 35.9-fold.

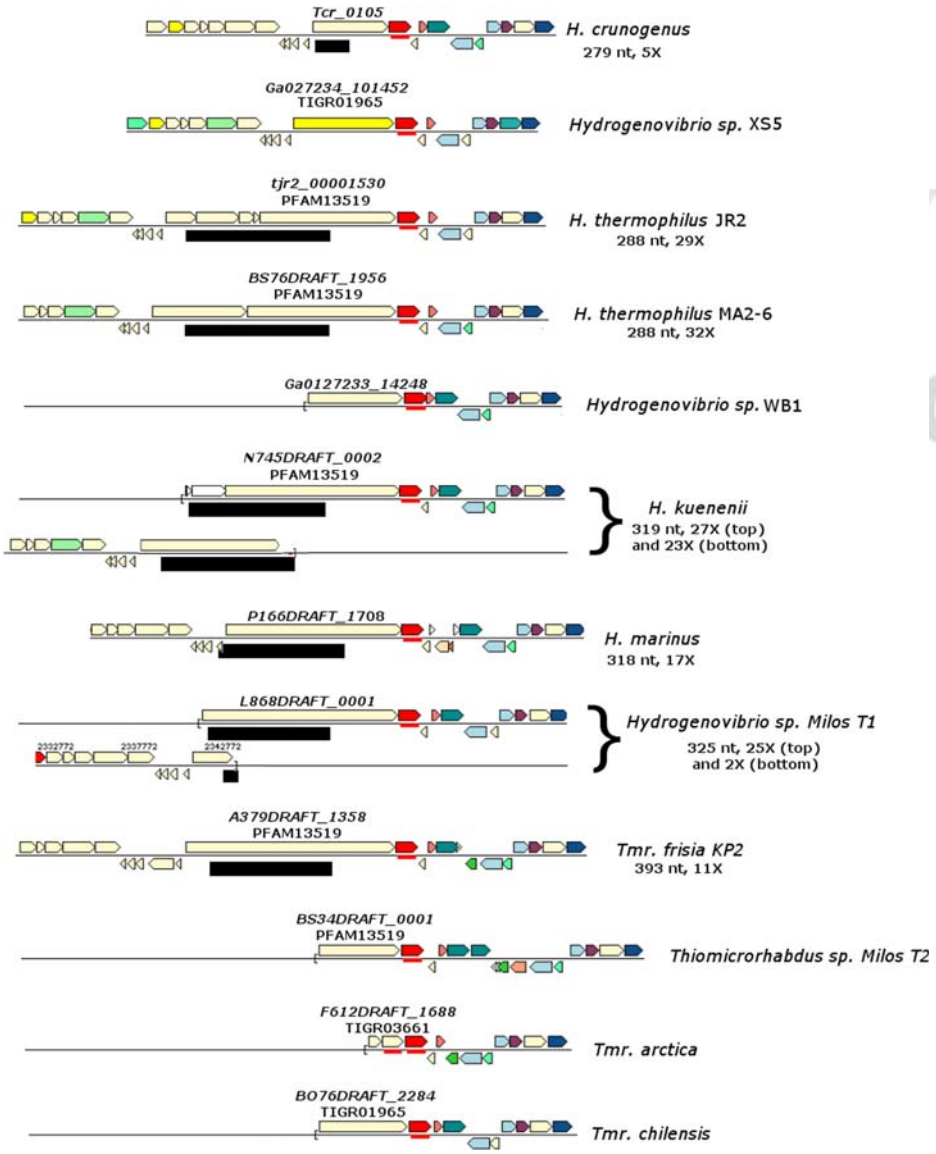

Fig. 2. Genome context for large tandem repeat regions. Genes are coloured by COG membership. Red genes encode protein kinase. Regions with large tandem repeats are indicated with black bars, and the size (in nucleotides) and number of tandem repeats is indicated next to the taxon name. Lines without genes indicate the ends of contigs. Locus tags and protein family membership for the large genes 5' to the protein kinase genes are indicated above the genes. PFAM13519— von Willebrand factor type A domain; TIGR01965—VCBS_repeat; TIGR03661—type I secretion C-terminal target domain.

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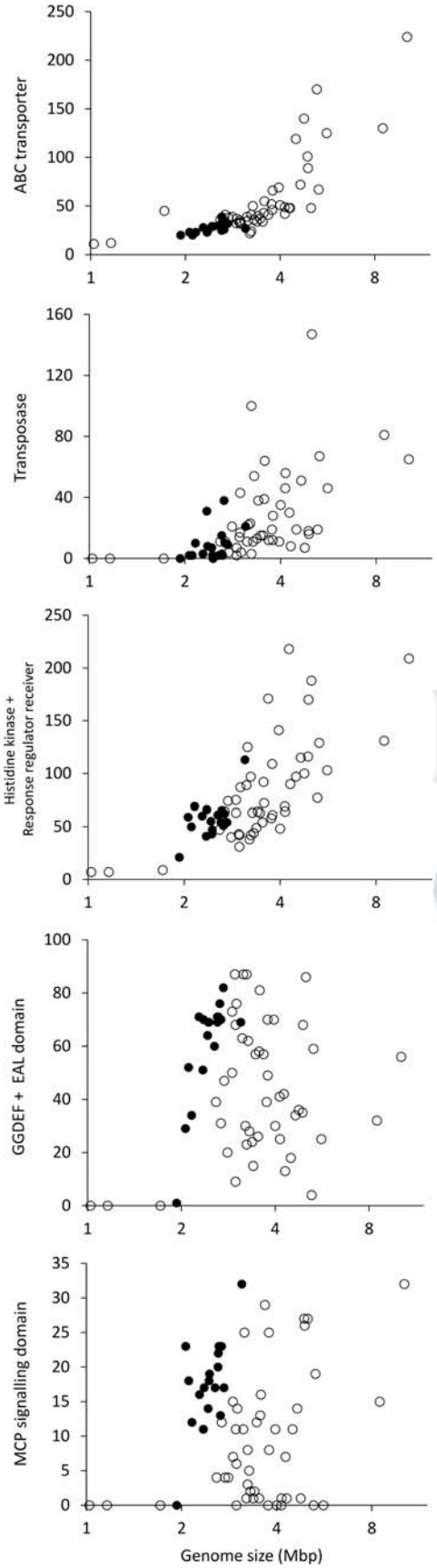

Fig. 3. Number of genes falling within selected Pfams in genomes of members of the genera *Hydrogenovibrio*, *Thiomicrorhabdus* and *Thiomicrospira* (solid black circles) and obligately or facultatively autotrophic members of the *Proteobacteria* (open circles). ABC transporter—members of Pfam00005; Transposase—members of Pfams 00872, 01527, 01609, 01797, 02371, 05598, 09299, 12762 and 12784; Histidine kinase + response regulator receiver—members of Pfams 02518 and 00072; GGDEF + EAL domain—members of Pfams 00990 and 00563; MCP signalling domain—members of Pfam00015.

regulation (if falling upstream of a gene; Zhou *et al.*, 2014). Genes encoding large proteins (up to 4456 aa) have been annotated upstream from the protein kinase genes, and in many cases these large genes include the large tandem repeats (Fig. 2). These genes are variously annotated as Ig-like domain proteins or VCBS repeat containing proteins, and many fall within PFAM13519 (von Willebrand factor type A domain) or TIGR01965 (VCBS repeat). Both of these protein families include extracellular proteins involved in cell adhesion. When the amino acid sequences predicted from these genes are compared among the organisms studied here, they align poorly due to very low sequence similarities (~ 35%). Perhaps the tandem repeat regions serve to introduce variability in adhesion-related proteins, which could provide a mechanism for evading phage and other predators.

Putative prophages are present in the *Tmr. frisia* and *H. marinus* genomes (Supporting Information Fig. S1). These genome regions have G + C fractions that differ from their hosts (in mol%:mol% – *Tmr. frisia* KP2 prophage:host %GC = 44:40; *H. marinus* = 41:44), and they encode many phage-related proteins (Supporting Information Fig. S1). Perhaps the most tantalizing finding in the *H. marinus* prophage genome is alluded to by *P166DRAFT_0452-5*, a retroelement diversity generating mini-operon first discovered in *Bordatella* phage (Doulatov *et al.*, 2004). Diversity-generating retroelements (DGRs) are a family of genetic elements that function to diversify DNA sequences and the proteins they encode (Medhekar and Miller, 2007). Using an error-prone reverse transcriptase (*P166DRAFT_0452*) causes tropism switching that enables the phage to match the diversity generation of the host (Doulatov *et al.*, 2004). Thus, this phage has the putative capability to infect host cells of nearly limitless diversity. Usually the recipient of the diversity generation is genomically nearby, and in this case it may be the 'DUF3751: phage tail collar fibre protein-short tail fibre protein gp12' (*P166DRAFT_0457*).

Certain gene families are particularly well-represented in these genomes, when compared with other autotrophic members of the phylum '*Proteobacteria*' whose genomes have been completely sequenced (Fig. 3; Supporting Information Table S2). Based on membership in Pfams (Bateman *et al.*, 2002), genes encoding ABC transporters are abundant, though less so than in other autotrophic

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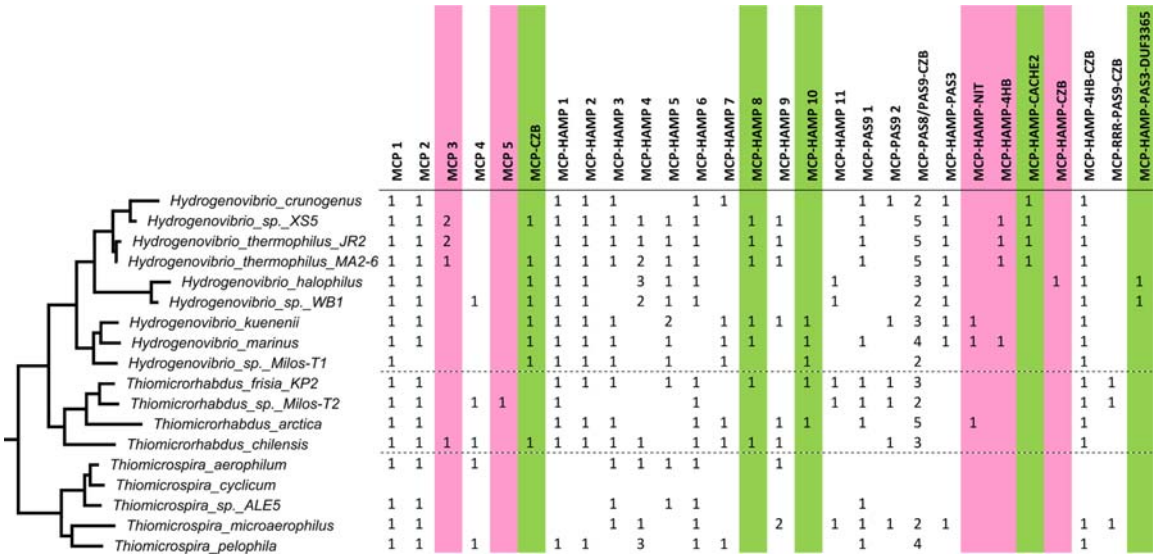

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Fig. 4. Clusters of genes encoding proteins with methyl-accepting chemotaxis protein signalling domains are sorted by taxa. Numbers of genes from each cluster present in each genome are listed for each taxon. Gene clusters that are absent from genus *Thiomicrospira*, but present in the genera *Thiomicrorhabdus* and *Hydrogenovibrio*, are shaded. Pink shading indicates clusters whose genes have no full length matches beyond these taxa, though some have many partial-length matches within them. Green shading indicates clusters with full-length matches in other members of *Gammaproteobacteria*. Gene clusters are named by Pfam domains present in their member's sequences: CACHE2—Pfam08269, Cache domain; CZB—Pfam13682, chemoreceptor zinc-binding domain; DUF3365—Pfam11845, Protein of unknown function; HAMP—Pfam00672, HAMP domain; MCP—Pfam00015, methyl-accepting chemotaxis protein signalling domain; NIT—Pfam08376, nitrate and nitrite sensing; PAS3—Pfam08447, PAS domain; PAS8—Pfam13188, PAS domain; PAS9—Pfam13426, PAS domain; RRR—Pfam00072, response regulator receiver domain; 4HB—Pfam12729, four helix bundle sensory module.

'Proteobacteria' with larger genomes (Fig. 3). Abundance of these transporters is a bit puzzling, given the requirements for chemolithoautotrophic growth consist of rather few nutrients, and also given this group's limited ability to assimilate organic carbon (Table 1). *Hydrogenovibrio* sp. Milos T1 and *Thiomicrorhabdus* sp. Milos T2 have the highest number of transposase genes of the genomes sequenced here (Fig. 3), which is interesting as these two organisms were isolated from the same habitat, but are not particularly closely related (Brinkhoff *et al.*, 1999b). In general, transposase gene frequencies are relatively low (Fig. 3), which may reflect the tendency for smaller genomes to contain fewer transposons (Touchon and Rocha, 2007).

Some gene families whose members facilitate sensing and responding to the environment are abundant in these genomes (Fig. 3). Genes encoding two-component regulatory systems (histidine kinase, response regulators) are slightly elevated in abundance based on genome sizes, but generally fall along an overall trend of increasing numbers with genome size (Galperin, 2005). Genes encoding GGDEF and EAL-domain proteins are particularly abundant in these taxa. In other organisms, these proteins synthesize and degrade the second-messenger cyclic di-GMP, which is involved in regulating motility, chemotaxis, biofilm formation and other interactions with organisms' biotic and abiotic environment (Römling *et al.*, 2013). The abundance of GGDEF and EAL-domain proteins, as well as methyl-accepting chemotaxis proteins, should make these organisms quite responsive to their spatially and temporally variable habitats.

Methyl-accepting chemotaxis proteins

Genes with MCP domains are very abundant in members of *Hydrogenovibrio, Thiomicrorhabdus* and *Thiomicrospira* (11–23 genes per genome; Fig. 3), particularly when compared with other members of family *Piscirickettsiaceae* (0–13 genes per genome, median = 4). The abundance of genes encoding MCP-domain proteins may result from inhabiting spatially and/or temporally variable sediment and hydrothermal vent communities; organisms inhabiting such environments tend to carry more MCP genes (Lacal *et al.*, 2010). The absence of such genes in *Tms. cyclica* is striking, and it also lacks genes encoding motility-related signalling and structures (*che* and *fli* operons), consistent with this species' nonmotile phenotype (Sorokin *et al.*, 2002). Perhaps nonmotility reflects the relative stability of the stratified hypersaline lake from which this species was isolated.

MCP genes among the genera *Hydrogenovibrio, Thiomicrorhabdus* and *Thiomicrospira* fall into 28 clusters (Fig. 4). F4 Their predicted amino termini, which include their sensory domains, likely responsive to a variety of ligands, are less conserved than their carboxy termini, as has been noted

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Table 3. Electron transport-related components encoded in genomes from the genera *Hydrogenovibrio, Thiomicrorhabdus* and *Thiomicrospira*.

Taxon	SDH[a,b]	Sqr types	Sox	[NiFe]	[FeFe]	NDH	bc_1	cbb_3	*bd*	ba_3
H. crunogenus XCL-2		A, F	ABCDXYZ	1b		I	1	1		
Hydrogenovibrio sp. XS5	1	A, F	ABCDXYZ			I, II	1	1	1	
H. thermophilus JR2	1	A, F	ABCDXYZ			I, II	1	1	1	
H. thermophilus MA2–6	1	A, F	ABCDXYZ	1d, 2b		I, II	1	1	1	
H. halophilus	3	A, F	ABCDXYZ		1	I	1	1		
Hydrogenovibrio sp. WB1	3	A, F	ABCDXYZ		1	I	1	1		
H. kuenenii	1	A, F	ABCDXYZ		1	I, II	1	1		
H. marinus DSM 11271	1	A, F	ABCDXYZ	1d, 2b	1	I, II	1	1		
Hydrogenovibrio sp. Milos-T1	1	A, E, F	ABCDXYZ			I, II	1	1	1	
Tmr. frisia Kp2	4	A, F	ABCDXYZ		1	I	1	1		
Thiomicrorhabdus sp. Milos-T2	3	A, F	ABCDXYZ			I	1	1		1
Tmr. arctica		A, F	ABCDXYZ			I	1	1		
Tmr. chilensis	4	A, F	ABCDXYZ		1	I	1	1		
Tms. aerophila	3	B, F	ABCDXYZ			I	1	1		
Tms. cyclica	1	B, F	ABCDXYZ			I	1	1		
Thiomicrospira sp. ALE5	3	B, F	ABCDXYZ			I	1	1		
Tms. microaerophila	4	B, E, F	ABCDXYZ	1b		I	1	1		
Tms. pelophila	2	A, B, F	ABCDXYZ		1	I	1	1		

a. Abbreviations: SDH, flavocytochrome *c* sulfide dehydrogenase; Sqr types, sulfide:quinone oxidoreductases A, B, C, D, E or F; DSR, dissimilatory sulfite reductase system; Sox, Sox/thiosulfate-oxidizing multi-enzyme system, ABXYZ (incomplete) or ABCDXYZ (complete); Sgp, sulfur globule proteins A/B or C; Sor, sulfite dehydrogenase; Soe, sulfite oxidizing enzyme SoeABC; APS red, adenosine phosphosulfate reductase; [NiFe], group 1b, 1d or 2b [NiFe] hydrogenase; [FeFe], [FeFe] hydrogenase; NDH, type I and II NADH:quinone oxidoreductase; bc_1, cytochrome bc_1 complex; cbb_3, cbb_3-type cytochrome *c* oxidzse; *bd*, cytochrome *bd*-type quinol oxidase; ba_3, ba_3-type cytochrome *c* oxidase.
b. Numbers indicate the number of copies of a particular complex that are encoded by the genome.

for MCPs in general (Wuichet *et al*., 2007). Many of the MCP genes carried by these taxa encode amino-terminal PAS domains that might be involved in energy taxis, in which the redox state of components of the electron transport chain is sensed by redox-sensitive MCP proteins, which results in motility to microenvironments with an optimal concentration of reductant and oxidant. Organisms capable of energy taxis often have large numbers of MCP genes (Alexandre *et al*., 2004), and this is the case for the genera studied here. MCP genes with PAS domains comprise the largest of the 28 clusters (MCP-PAS8/PAS9-CZB; Fig. 4). This cluster includes multiple genes from many of these genomes. Phylogenetic analysis of these genes does not cluster them by species, suggesting duplication prior to divergence of these species (data not shown). Eighteen of the clusters, including this large PAS domain cluster, contain genes from genus *Thiomicrospira* and either *Hydrogenovibrio* or *Thiomicrorhabdus*, and likely reflect the presence of these genes in the shared ancestor of these three genera (Fig. 4). Ten clusters present in the genera *Hydrogenovibrio* and *Thiomicrorhabdus* do not have full-length matches within genus *Thiomicrospira*. Five of these clusters have full-length matches elsewhere in the *Gammaproteobacteria* and appear to have been acquired by the lineage leading to the genera *Hydrogenovibrio* and *Thiomicrorhabdus* subsequent to its divergence from genus *Thiomicrospira*. The other five clusters of genes absent from genus *Thiomicrospira* have many high-similarity partial-length hits that fell within the genera *Hydrogenovibrio, Thiomicrorhabdus* and *Thiomicrospira*. Based on these observations, no genes appear to have been horizontally transferred from distantly related organisms. Perhaps this is because the MCP domain needs to be sufficiently conserved to communicate with the other components of the chemotaxis apparatus (e.g., CheW, CheA; Wadhams and Armitage, 2004).

Electron transport chains

Similar to other chemolithoautotrophs (e.g., Dmytrenko *et al*., 2014; Flood *et al*., 2016), genomes from members of the genera *Hydrogenovibrio, Thiomicrorhabdus* and *Thiomicrospira* encode a variety of complexes to introduce electrons stripped from inorganic compounds into the electron transport chain, and to act as terminal oxidases. Given that several genomes from within this clade of microorganisms have been sequenced, they provide a unique opportunity for illuminating how these complexes were acquired. All 18 of the species examined carry the genetic potential to oxidize reduced sulfur compounds via sulfide:quinone oxidoreductases (EC 1.8.5.4; subdivided into clades sqrA–sqrF and sqrX; Gregersen *et al*., 2011). All carry *sqrF*, while *sqrA, sqrB* and *sqrE* are distributed less evenly among taxa (classified by comparison to sequences in Gregersen *et al*., 2011; Table 3). As a result, several taxa carry multiple sulfide:quinone oxidoreductase genes, and this is also the case for other members of *'Proteobacteria'* (Supporting Information Table S3). In

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Chlorobaculum tepidum, genes encoding multiple forms of this enzyme are differentially induced by sulfide concentrations (Chan *et al.*, 2009); this may also be the case for these organisms. Most of the members of *Hydrogenovibrio, Thiomicrorhabdus* and *Thiomicrospira* also carry one or multiple copies of flavocytochrome *c* sulfide dehydrogenase (SDH; EC 1.8.1.19; Table 3). These genomes encode a particularly large number of copies of this enzyme (up to 4); other members of *'Proteobacteria'* typically carry fewer (1–2; Supporting Information Table S3). Expression of these paralogs may be differentially induced, as has been described above for *sqr* genes. Members of the genera *Hydrogenovibrio, Thiomicrorhabdus* and *Thiomicrospira* carry genes encoding the complete thiosulfate-oxidizing multienzyme system ('Sox complex', encoded by *soxABCDXYZ*) associated with the Kelly–Friedrich pathway, which can oxidize thiosulfate completely to sulfate without the formation of any free intermediates in the cell or locale. These genes are not collocated in a single operon, similar to *H. crunogenus* (Scott *et al.*, 2006), suggesting that they may be differentially regulated. Most of the the *'Proteobacteria'* surveyed here carry *soxABXYZ* instead (Supporting Information Table S3); this 'incomplete' Sox complex may produce polysulfide or elemental sulfur (Frigaard and Dahl, 2009). Given the possibility for differential regulation of *sox* genes in *Hydrogenovibrio, Thiomicrorhabdus* and *Thiomicrospira*, their Sox complexes may not always include SoxCD proteins, or could use alternative cytochromes to fulfill their role. The presence of Sqr and SDH genes (and the potential to synthesize SoxABXYZ systems) is consistent with elemental sulfur production by these cells (Jannasch *et al.*, 1985; Brinkhoff *et al.*, 1999a,c; Takai *et al.*, 2004; Knittel *et al.*, 2005). Genes encoding sulfur globule proteins homologous to those present in *Allochromatium vinosum* are absent, which may indicate that other proteins encase sulfur globules, as has been observed in (Hanson *et al.*, 2016), or that the globules do not have protein coats.

Known pathways for sulfite metabolism are absent from the genomes of members of *Hydrogenovibrio, Thiomicrorhabdus* and *Thiomicrospira*, which distinguishes them from other *'Proteobacteria'* (Supporting Information Table S3) and is consistent with an inability to use sulfite as their sole electron donor (Brinkhoff *et al.*, 1999a,c; Rainey *et al.*, 2001; Sorokin *et al.*, 2002; Takai *et al.*, 2004; Knittel *et al.*, 2005). As has been noted for *H. crunogenus* (Scott *et al.*, 2006), members of *Hydrogenovibrio, Thiomicrorhabdus* and *Thiomicrospira* lack dissimilatory sulfite reductase (DSR); this enzyme is common in photosynthetic sulfur oxidizing bacteria, which use it to oxidize sulfide and elemental sulfur to sulfite (Frigaard and Dahl, 2009). This complex is also encoded in the genomes of many chemolithoautotrophic members of *'Proteobacteria'* (Supporting Information Table S3). Its absence in *Hydrogenovibrio, Thiomicrorhabdus* and *Thiomicrospira* is consistent with previous studies which noted that the DSR complex is absent from organisms carrying genes encoding a complete Sox complex that includes SoxCD (Frigaard and Dahl, 2009; Gregersen *et al.*, 2011). Genes encoding enzymes to oxidize sulfite directly (SorAB, SoeABC) or indirectly (dissimilatory APS reductase) are also absent from their genomes, though these genes are common among other *'Proteobacteria'* (Supporting Information Table S3).

The presence of genes encoding the Sox complex and sulfide:quinone oxidoreductase in all sequenced members of *Hydrogenovibrio, Thiomicrorhabdus* and *Thiomicrospira* suggests that their common ancestor was a sulfur-oxidizing chemolithotroph. All of them also carry genes encoding NDH-1-type NADH:quinone oxidoreductase (NADH dehydrogenase, EC 1.6.5.3), cytochrome bc_1 complex (EC 1.10.2.2) and a cbb_3-type cytochrome c oxidase (EC 1.9.3.1), which facilitates growth under low oxygen conditions (Pitcher and Watmough, 2004) and has been purified and characterized from *Tms. aerophila* (Sorokin *et al.*, 1999). The presence of a high-affinity cbb_3 cytochrome *c* oxidase in all species examined suggests that the common ancestor to these organisms was capable of growth under low oxygen conditions as well.

Other components of electron transport chains are not universally encoded by the genomes of these organisms. Genes encoding hydrogenases are unevenly distributed among *Hydrogenovibrio, Thiomicrorhabdus* and *Thiomicrospira*. Among these are genes encoding four types of hydrogenase (EC 1.12.1._; [FeFe], as well as [NiFe] groups 1b, 1d and 2b) (Hansen and Perner, 2016). [FeFe] hydrogenases are present in some of the taxa sequenced here (Table 3), though absent from other members of the *'Proteobacteria'* that were examined (Supporting Information Table S4). Genome contexts of these [FeFe]-hydrogenase genes do not suggest a function for this hydrogenase, or a direction to the catalytic activity (i.e., either producing or consuming H_2). Group 1b [NiFe]-hydrogenase genes are present in *H. crunogenus* (Scott *et al.*, 2006) and *Tms. microaerophila* ASL8-2. Matches to these genes largely fall within genomes of members of the phylum *Epsilonbacteraeota*, and group 1b hydrogenases are absent from the other *Gammaproteobacteria* queried here (Supporting Information Table S4). In *Tms. microaerophila* ASL8-2, as has been described in *H. crunogenus*, the small-subunit-encoding gene includes an NADH-binding domain; this, plus the absence of a *b*-type subunit-encoding gene, suggests that the redox partner for this enzyme could be cytoplasmic (Scott *et al.*, 2006). Hydrogenases using NADH as the redox partner are often reversible (Lubitz *et al.*, 2014), so the enzyme encoded by these genes may function either to use H_2 as an electron donor, or to produce this gas to maintain redox balance,

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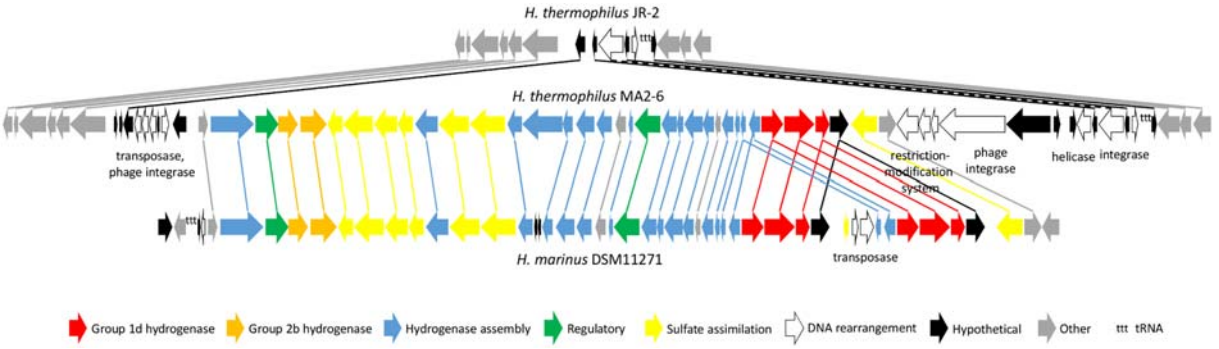

Fig. 5. Genome context and synteny of genes encoding and associated with hydrogenase enzymes in *H. thermophilus* MA2–6 and *H. marinus*. A region of the genome from *H. thermophilus* JR-2, which lacks hydrogenase, is provided for comparison, as it shows the likely site of insertion for this hydrogenase genomic island subsequent to the divergence of these two strains of *H. thermophilus*. Lines link orthologous genes.

which would be consistent with life in a highly variable environment. Group 1b [NiFe]-hydrogenase genes are also present in *H. crunogenus* TH55. Molecular hydrogen use by this organism is suggested by hydrogen consumption from culture headspace, and increased cell counts in the presence of this gas, demonstrating chemolithoautotrophic growth on molecular hydrogen (Hansen and Perner, 2015; 2016). Hydrogen use by *H. crunogenus* XCL-2 was not detected under these or a variety of other cultivation conditions, and there are no mutations in its hydrogenase or hydrogenase-associated genes that explain this lack of activity (Hansen and Perner, 2016). The function of the group 1b [NiFe]-hydrogenase remains mysterious in *H. crunogenus* XCL-2.

Genes encoding group 1d (oxygen-tolerant) and 2b (sensory) hydrogenases are present in both *H. marinus* and *H. thermophilus* MA2–6, and both are capable of growth with hydrogen gas as the electron donor (Hansen and Perner, 2016). Genes encoding group 1d hydrogenase are common among the members of *Gammaproteobacteria* (Supporting Information Table S4). For both *H. marinus* and *H. thermophilus* MA2–6, genes nearby encode enzymes for assimilatory sulfate reduction (subunits 1 and 2 of sulfate adenylyltransferase, thioredoxin-dependent adenylylsulfate APS reductase; Bick *et al*., 2000); perhaps this collocation assists with the synthesis of iron sulfur clusters for hydrogenase enzymes. Together, these genes liberate *H. marinus* and *H. thermophilus* MA2–6 from any requirement for reduced sulfur compounds either as electron donors or biosynthetic substrates; indeed, both strains grow on hydrogen gas in the absence of reduced sulfur sources (Nishihara *et al*., 1991, K. Scott, unpublished). This ability could be an advantage as some vent habitats are hydrogen-rich and sulfide poor (Kelley *et al*., 2005).

The chromosome regions encoding the 1d and 2b hydrogenases in *H. marinus* and *H. thermophilus* MA2–6 are strikingly similar; the order of the genes encoding these hydrogenases and the many proteins needed to assemble them are nearly identical (Fig. 5), despite a lack of conservation of gene order in other genomes encoding these hydrogenases. The predicted amino acid sequences of the large and small subunits of these hydrogenases share high sequence identities between these two species (e.g., for 1d, 92% and 96% identical respectively). Given the absence of group 2b and 1d hydrogenases in the other members of genus *Hydrogenovibrio*, including species closely related to *H. marinus* and *thermophilus* MA2–6, it seems likely that these two taxa acquired these genes independently via horizontal gene transfer. Given the similarity in gene order and sequence, they both likely acquired this hydrogenase gene cluster from similar organisms. Phylogenetic analysis of the group 2b and 1d hydrogenase gene sequences from genus *Hydrogenovibrio* places them together on a long branch (data not shown), precluding inference about the taxonomic affiliation of the donor organisms. For *H. thermophilus* MA2–6, there are many signs that acquisition of these genes was a relatively recent phage-mediated acquisition, as the hydrogenase gene cluster is surrounded by phage components (Fig. 5). *H. thermophilus* JR2, whose genome is otherwise syntenous with *H. thermophilus* MA2–6, lacks both these phage components and the gene cluster, suggesting hydrogenase acquisition by *H. thermophilus* MA2–6 subsequent to the *H. thermophilus* MA2–6/JR2 divergence. Hydrogenase acquisition by horizontal gene transfer may be widespread (Greening *et al*., 2016). *Tmr. hydrogeniphilia*, a recently isolated member of the *genus Thiomicrorhabdus*, grows chemolithoautotrophically on molecular hydrogen. *Tmr. hydrogeniphila* is very closely related to *Tmr. frisia*, and is the only member thus far of its genus to have this capability demonstrated (Watsuji *et al*., 2016; Boden *et al*., 2017a). The ability to grow with hydrogen as the electron donor is also observed in some species of

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Acidithiobacillus; the distribution of this trait does not suggest vertical transmission from the ancestral *Acidithiobacillus* (Hedrich and Johnson, 2013).

The recent isolation of an organism falling within genus *Hydrogenovibrio* that can grow with ferrous iron as its electron donor (Barco *et al*., 2017) raises the possibility that the organisms sequenced here might also have this capability. However, their genomes do not include homologs of the outer membrane proteins canonically associated with iron oxidization or reduction [Cyc2 (and associated alternative complex III), MtoA, OmpB or OmcB; Liu *et al*., 2012; 2014; Barco *et al*., 2015; Kato *et al*., 2015]. Despite this absence of genetic evidence, it would be worthwhile to test these organisms for the ability to use ferrous iron as an electron donor, since mechanisms for iron oxidation have yet to be described for many organisms with this capability (He *et al*., 2017).

In addition to this diversity in mechanisms for input of reductant into the electron transport chains of these organisms, there is diversity in electron transport complexes as well (Table 3). All of these organisms carry genes encoding complex I (NADH:ubiquinone reductase, EC 1.6.5.3). Some members of *Hydrogenovibrio* carry genes encoding alternative (type II) NADH:ubiquinone reductase (EC 1.6.5.9), which is common among *'Proteobacteria'* (Marreiros *et al*., 2016), though not among the autotrophic organisms surveyed here (Supporting Information Table S4). Type II NADH:ubiquinone reductase oxidizes NADH without directly contributing to proton potential, which may function to maintain cellular NADH/NAD^+ ratios (Kerscher *et al*., 2008). In addition to the cbb_3 cytochrome c oxidase complex noted above, some members of *Hydrogenovibrio* also carry genes encoding cytochrome *bd*-type quinol oxidase (EC 1.10.3.10), while *Thiomicrorhabdus* sp. Milos T2 has ba_3-type cytochrome *c* oxidase (EC 1.10.3.11; Table 3). Biochemically characterized cytochrome *bd*-type quinol oxidases from other organisms, as well as cbb_3 cytochrome *c* oxidase, have high affinities for oxygen and facilitate growth under low-oxygen conditions (Pitcher and Watmough, 2004; Borisov *et al*., 2011), which would be helpful in many of the habitats where these genera are found. Indeed, both of these complexes are quite common among autotrophic *'Proteobacteria'*, which should facilitate growth in low-oxygen habitats where reduced inorganic electron donors are present (Supporting Information Table S4). Alternatively, the cytochrome *bd*-type oxidase and ba_3 complexes may provide mechanisms for redox balance. The ba_3 cytochrome *c* oxidase from '*Aquifex aeolicus*' uses both quinol and ferrocytochrome *c* as electron donors. Quinol oxidation by this complex may function to maintain redox balance when the quinone pool becomes excessively reduced (Gao *et al*., 2012). It has also been suggested that the *bd* complex may function to minimize oxidative damage when oxygen tensions rise (Poole and Hill, 1997; Ramel *et al*., 2015).

Much of the diversity in electron transport chain components is present in the genera *Hydrogenovibrio* and *Thiomicrorhabdus*, but not in genus *Thiomicrospira* (Table 3). The sequenced members of genus *Thiomicrospira* were all isolated from sediment habitats and hypersaline and soda lakes, while many of the sequenced members of the genera *Hydrogenovibrio* and *Thiomicrorhabdus* were isolated from hydrothermal vent environments (Table 1). The additional electron transport chain components found in these organisms could function to maintain electron transport activity when oxygen tensions or sulfide concentrations vary; perhaps they lend these organisms a degree of 'redox versatility' that provides an advantage in the extreme spatial and temporal heterogeneity of vent environments.

Carboxylases, carboxysomes and associated transporters

All of these organisms carry genes encoding the Calvin–Benson–Bassham (CBB) cycle; none carry genes encoding ATP-dependent citrate lyase (EC 2.3.3.8), or citryl-coA synthetase (EC 6.2.1.18)/citryl-coA ligase (EC 4.1.3.34), which are present in organisms using the reductive citric acid (Arnon-Buchanan) cycle for carbon fixation (Aoshima *et al*., 2004a,b; Hügler *et al*., 2007). Absence of a gene encoding sedoheptulose-bisphosphatase (EC 3.1.3.37) suggests that these organisms either have a bifunctional bisphosphatase, capable of acting both on fructose 1,6-bisphosphate as well as on sedoheptulose 1,7-bisphosphate (Yoo and Bowien, 1995), or that they use the transaldolase variant of the CBB cycle (Strøm *et al*., 1974; Boden *et al*., 2017b). Other organisms lacking these enzymes have been found to carry pyrophosphate-dependent phosphofructokinase (EC 2.7.1.90), which is reversible and can act on sedoheptulose 1,7-bisphosphate (Reshetnikov *et al*., 2008). This does not appear to be the case for the organisms studied here. Genes encoding pyrophosphate-dependent phosphofructokinase are often adjacent on the chromosome to genes encoding proton translocating pyrophosphate synthase (Kleiner *et al*., 2012); these genes are absent from the organisms studied here. It was originally suggested that enzyme from *H. crunogenus* might use pyrophosphate (Scott *et al*., 2006); however, amino acid sequences predicted from phosphofructokinase genes from this organism, as well as other members of *Hydrogenovibrio, Thiomicrorhabdus* and *Thiomicrospira* are consistent with ATP as a substrate; they have a glycine residue at position 104, and a lysine at 124 (positions relative to ATP-dependent enzyme from *Escherichia coli*), as do biochemically characterized ATP-dependent enzymes (Bapteste *et al*., 2003).

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Table 4. Presence of genes encoding RubisCO, and enzymes for acetate assimilation and metabolism in the genera *Hydrogenovibrio, Thiomicrorhabdus* and *Thiomicrospira*.

Taxon	Form IAc[a,b]	Form IAq	Form II	AK[c]	PTA	OFOR
H. crunogenus XCL-2	1	1	1			1
Hydrogenovibrio sp. XS-5	1	1	1	1	1	1
H. thermophilus JR2	1	1	1	1	1	1
H. thermophilus MA2–6	1	1	1	1	1	1
H. halophilus	1	1	1	1	1	1
Hydrogenovibrio sp. WB-1	1	1		1	1	1
H. kuenenii	1	1	1	1	1	
H. marinus	1	1	1	1	1	
Hydrogenovibrio sp. Milos-T1	1		1	1	1	
Tmr. frisia KP2	1	1	1			
Thiomicrorhabdus sp. Milos-T2		1	1			
Tmr. arctica		1	1			
Tmr. chilensis	1	1	1			
Tms. aerophila	1					
Tms. cyclica	1					
Thiomicrospira sp. ALE5	1					
Tms. microaerophila	1					
Tms. pelophila	1		1			

a. Form IAc = form IA carboxysomal RubisCO; Form IAq = form IA noncarboxysomal RubisCO; form II = form II RubisCO.
b. Numbers indicate the number of copies of a particular enzyme that are encoded by the genome.
c. AK, acetate kinase; PTA, phosphotransacetylase; OFOR, oxoacid:ferredoxin oxidoreductase.

Many of these organisms' genomes encode multiple RubisCO enzymes (form IAc, carboxysomal form IA RubisCO; form IAq, noncarboxysomal form IA RubisCO; form II; Table 4; Supporting Information Fig. S2; Boden *et al*., 2017b). Two, and often three, genes encoding RubisCO are present in genomes from members of *Hydrogenovibrio* and *Thiomicrorhabdus* (Table 4); it is more common among *'Proteobacteria'* for genomes to include one or two of these genes (Supporting Information Table S5). RubisCO enzymes have very large differences in K_{CO2} values and specificity for CO_2 versus O_2; noncarboxysomal form I enzymes typically have higher affinities for CO_2, as well as being more specific for CO_2 (Badger and Bek, 2008), while carboxysomal form I enzymes are packed into carboxysomes as part of CCMs (Menon *et al*., 2008). Encoding more versions of this enzyme may facilitate autotrophic growth in the presence of a broader range of CO_2 concentrations. For *H. marinus* and *H. crunogenus*, it has been demonstrated that these RubisCO genes are differentially transcribed in response to the concentration of CO_2 in the growth medium (Yoshizawa *et al*., 2004; Dobrinski *et al*., 2012), and having multiple enzymes is believed to facilitate stable rates of carbon fixation at a broad range of CO_2 and O_2 concentrations (Tabita, 1999; Badger and Bek, 2008).

Most genomes encode carboxysomal form IAc enzyme, consistent with the presence of carboxysomes and CO_2 concentrating mechanisms similar to what has been described in members of the '*Cyanobacteria'* as well as *Hydrogenovibrio crunogenus* (Dobrinski *et al*., 2005; Price *et al*., 2008; Boden *et al*., 2017b). Carboxysome presence has been previously verified via electron micrographs of *H. crunogenus* (Dobrinski *et al*., 2012; Menning *et al*., 2016), *H. marinus* (Yoshizawa *et al*., 2004), *Tms. aerophila* (Rainey *et al*., 2001) and *Tms. cyclica* (Sorokin *et al*., 2002). However, carboxysomes and CCMs are not inevitable among these genera; *Thiomicrorhabdus* sp. Milos-T2 and *Tmr. arctica* lack carboxysomal RubisCO genes, as well as genes encoding shell proteins and other components of carboxysomes. It is possible that these genes are encoded on portions of the genomes that remain to be sequenced. Alternatively, these taxa may have ~~alternative~~ mechanisms ~~beyond CCMs~~ for coping with periods of low CO_2 availability.

Genomes of most members of *Thiomicrospira* encode only carboxysomal RubisCO (form IAc) and not form II (Table 4), confirming results from PCR-based assays for these enzymes (Tourova *et al*., 2006). Most of the members of *Thiomicrospira* studied here were isolated from alkaline hypersaline and soda lakes (Sorokin *et al*., 2002; 2007; 2011). These habitats typically have high concentrations of bicarbonate and carbonate, but their high pH (9–10) likely renders CO_2 scarce (Sorokin *et al*., 2011). The hydrothermal vent or sediment habitats from which all of the other organisms in this study were isolated can have quite high CO_2 concentrations (e.g., Goffredi *et al*., 1997), which would make it an advantage for these organisms to be able to repress carboxysome synthesis. In hypersaline and soda lakes, noncarboxysomal form I RubisCO, or form II RubisCO, which typically has a low affinity for CO_2,

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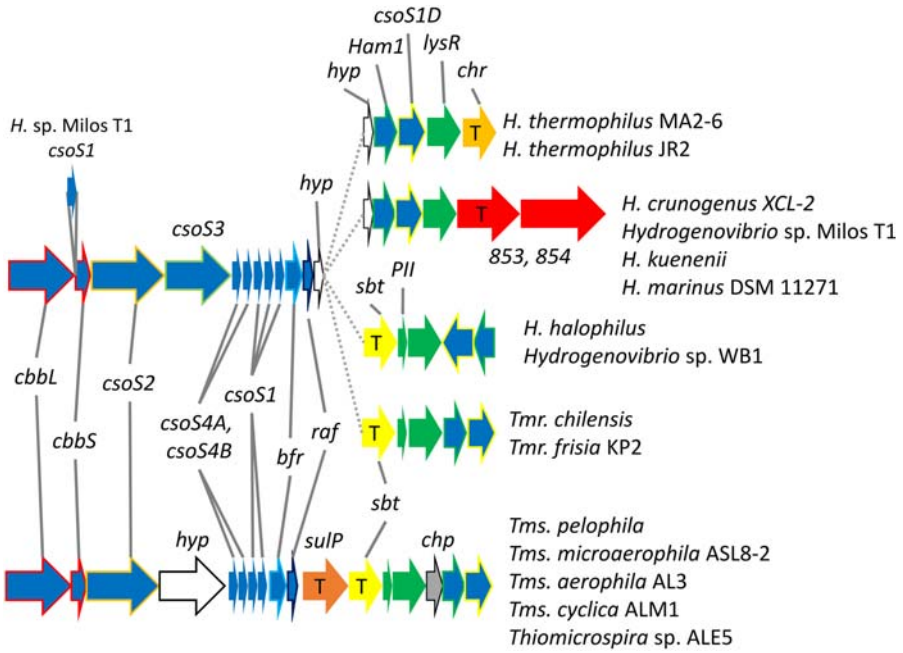

Fig. 6. Carboxysome operon synteny among members of the genera *Hydrogenovibrio, Thiomicrorhabdus*, and *Thiomicrospira*. 'T' marks genes that encode potential transporters. Gene abbreviations are: *bfr*—bacterioferritin; *cbbL*—RubisCO large subunit; *cbbS*—RibisCO small subunit; *chp*—conserved hypothetical protein; *chr*—chromate ion transporter family; *csoS1, csoS2, csos4A, csoS4b*—carboxysome shell proteins; *csoS3*—carboxysome carbonic anhydrase; *Ham1*—Ham1-domain protein; *hyp*—hypothetical protein; *lysR*—LysR-type transcriptional regulator; *PII*—PII signal transduction protein; *raf*—RubisCO assembly factor; *sbt*—sodium-dependent bicarbonate transporter family; *sulP*—sulfate transporter family; *853, 854*—homologs to *Tcr_0853, 0854*.

would function poorly and may explain the absence of these forms of the enzyme among the members of *Thiomicrospira* isolated from these habitats. Consistent with this, *Thiomicrospira pelophila*, the sole sequenced member of its genus to encode form II RubisCO, was isolated from coastal sediment, which is likely to have higher CO_2 concentrations, and grows optimally near neutral pH (Kuenen and Veldkamp, 1972).

Carboxysome operons among these organisms differ from those typically present in other chemolithoautotrophic bacteria (Axen *et al*., 2014) in that they lack *cbbO* and *cbbQ*, encoding RubisCO accessory proteins, as well as homologs to *parA,* which may play a role in carboxysome positioning in other organisms. Among the organisms studied here, carboxysome operon structure is generally well conserved (Fig. 6). An intriguing deviation is that *Tms. pelophila* and other members of *Thiomicrospira* lack *csoS3*, encoding a carboxysomal carbonic anhydrase (Cannon *et al*., 2010). In its place are genes that are unique to these taxa. *Tms. pelophila* gene *N746DRAFT_0321* and *Tms. microaerophila* ASL8-2 gene *NA59DRAFT_00206* have no homologs in IMG or NCBI non-redundant protein databases. *Thicy_1562, Thiae_1973* and *MZ34DRAFT_1177*, which are the corresponding genes in *Tms. cyclica* ALM1, *Tms. aerophila* AL3 and *Thiomicrospira* sp. ALE5, are homologous to each other. Given that carbonic anhydrase activity is key to the functioning of carboxysomes in other organisms (Cannon *et al*., 2010), its potential absence in these taxa is puzzling. One possibility is that the carboxysomal carbonic anhydrase for these organisms is encoded elsewhere on their chromosomes. Indeed, some of them do carry alpha carbonic anhydrase genes (*Thiae_1542, MZ34DRAFT_1567, N746DRAFT_1499, NA59DRAFT_02538*). Alternatively, the novel genes present in the carboxysome operons of these organisms may encode novel carbonic anhydrase enzymes. A third possibility is that these carboxysomes function in the absence of carbonic anhydrase activity, which would be particularly surprising, given the presence of this enzyme in all other carboxysomes characterized to date (Cannon *et al*., 2010).

Potential transporters are encoded 3′ to the carboxysome operons and may play a role in dissolved inorganic carbon uptake. These genes are quite diverse, encoding proteins that are members of four evolutionarily distinct transporter families (Saier *et al*., 2014): major facilitator superfamily SulP/BicA (MFS), HCO_3^-:Na^+ symporter (SbtA), chromate ion transporter (CHR). The fourth consists of a two-component DIC transporter in which one subunit is a homolog of the NADH dehydrogenase chain L (NDL). Members of three of these families have been implicated in DIC uptake: two in the '*Cyanobacteria*' [BicA (Price *et al*., 2004), SbtA (Shibata *et al*., 2002)], and one in *H. crunogenus* [encoded by *Tcr_0853* and *Tcr_0854;* Mangiapia *et al*. (2017)]. Some species carry representatives of several of these transporter families either downstream from the carboxysome operon or elsewhere on the chromosome (Fig. 6). Since the carboxysome operon falls at the end of a genome scaffold for *Hydrogenovibrio* sp. XS5, it was not possible to determine which, if any, potential transporter genes might be present 3′ to this locus.

If indeed genes from all four of these transporter families encode DIC transporters, it is interesting that such a

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diversity of transporters exists among these organisms. It can be anticipated that transporters from different families will differ from each other with respect to maximal rates and affinities, and in symported/antiported compounds and stoichiometries, providing advantages under certain growth conditions. They are particularly abundant and diverse among members of genus *Thiomicrospira*. Perhaps the added transporters provide a selective advantage in the extremely low CO_2 alkaline habitats from which most of the members of *Thiomicrospira* were isolated (Sorokin *et al*., 2011), or compensate for a lack of carboxysomal carbonic anhydrase in these species.

Central carbon metabolism

All of the target genomes carry genes encoding the same version of Embden–Meyerhof–Parnas glycolysis/gluconeogenesis and the citric acid cycle described for *H. crunogenus*, lacking all NADH-specific dehydrogenases (isocitrate EC 1.1.1.41, 2-oxoglutarate EC 1.2.4.2/2.3.1.61/1.8.1.4 and malate EC 1.1.3.7) except pyruvate dehydrogenase (EC 1.2.4.1/2.3.1.12/1.8.1.4; Quasem *et al*., 2017). Instead, they carry genes less common among members of the *'Proteobacteria'*:monomeric $NADP^+$ isocitrate dehydrogenase (EC 1.1.1.42; (Yasutake *et al*., 2002), and malate:quinone oxidoreductase (E.C. 1.1.5.4; Quasem *et al*., 2017). It is possible that they have incomplete citric acid cycles (Smith's horseshoe), as is often the case for obligate autotrophs (e.g., Boden *et al*., 2016; Hutt *et al*., 2017) that function primarily to provide biosynthetic intermediates (Smith *et al*., 1967; Wood *et al*., 2004). Alternatively, all of the genomes studied here encode homologs of the genes of the 2-oxoglutarate decarboxylase/succinic semialdehyde dehydrogenase bypass present in some members of the '*Cyanobacteria*' (Zhang and Bryant, 2011), so it is possible that they may be capable of catalysing a complete oxidative citric acid cycle.

Some of these organisms are capable of assimilating exogenous organic carbon (Table 1), and their genomes provide possible mechanisms for doing so. *H. thermophilus* I78 and *H. crunogenus* TH-55 and L-12 (Takai *et al*., 2004) can assimilate acetate and other organic compounds when inorganic electron donors are provided, as can *Tms. pelophila* and *Tms. aerophila* (Kuenen and Veldkamp, 1972; 1973; Rainey *et al*., 2001; Ang *et al*., 2017). All organisms sequenced here have genes encoding acetyl-CoA synthetase (EC 6.2.1.1), which could facilitate assimilation of acetate; some members of *Hydrogenovibrio* also have genes encoding acetate kinase (EC 2.7.2.1 or 2.7.2.12) and phosphotransacetylase (EC 2.3.1.8; Table 4). These organisms lack the genes necessary for the glyoxylate cycle, which, in the absence of a shunt between 2-oxoglutarate and succinyl-CoA would restrict the distribution of the acetate carbons to fatty acid biosynthesis and amino acids derived from 2-oxoglutarate (Wood *et al*., 2004). However, these organisms also all carry oxoacid:-ferredoxin oxidoreductase genes similar to those from *H. crunogenus*. *H. crunogenus* cell extracts have pyruvate:ferredoxin oxidoreductase activity (EC 1.2.7.10; Quasem *et al*., 2017), suggesting that its oxidoreductase genes encode pyruvate: ferredoxin oxidoreductase. Given the amino acid sequence similarities of orthologs from other members of *Hydrogenovibrio* (65%–77%, alpha subunits; 74%–78%, beta subunits), their oxidoreductases may also act on pyruvate. If the acetyl-CoA were converted to pyruvate by this enzyme, it would circumvent the need for a glyoxylate cycle.

Extremophile lifestyles

Two taxa sequenced here are extremophiles (Table 1), capable of growth at high salinity (*H. halophilus*; up to 3.5 M NaCl, optimum 1.5 M; Sorokin *et al*., 2006) or low temperature [*Tmr. arctica;* down to −2°C, optimum 11°C–13°C; (Knittel *et al*., 2005)]. Many halophiles and psychrophiles adapt to these conditions by accumulating compatible solutes such as ectoine, glycine betaine, proline or trehalose (Sleator and Hill, 2002; De Maayer *et al*., 2014). Genome evidence suggests that the ability to synthesize or transport of these molecules is a trait shared by all of the organisms studied here, and not specific to the two extremophiles. All sequenced members of *Hydrogenovibrio, Thiomicrorhabdus* and *Thiomicrospira* have genes encoding the enzymes necessary to synthesize ectoine (diaminobutyrate-2-oxoglutarate transaminase, EC2.6.1.76; diaminobutanoate acetyltransferase, EC 2.3.1.178 and ectoine synthase, 4.2.1.108). This observation is consistent with ectoine synthesis by *Thiomicrospira aerophila* when cultivated at elevated concentrations of Na^+ (Banciu *et al*., 2005). None carry genes encoding the enzymes necessary to synthesize glycine betaine or trehalose; instead, the genomes of *Tmr. arctica* and *Tmr.* Milos T2 carry genes encoding ABC transporters predicted to transport proline or glycine betaine (*F612DRAFT_1896–F612DRAFT_1898, BS34DRAFT_2245–BS34DRAFT_2247*), and all genomes studied here encode members of the BCCT betaine/carnitine/choline transporter family (Pfam02028); some members of this family transport glycine betaine (Ziegler *et al*., 2010). Neither *Tmr. arctica* nor *H. halophilus* appear to have acquired or amplified compatible solute-related genes to facilitate their psychrophilic or halophilic lifestyles.

The *Tmr. arctica* genome does have some aspects that may be adaptive to psychrophily. Growth at low temperature requires that proteins are especially flexible (Feller, 2013), making them particularly sensitive to denaturing. To facilitate correct folding of these proteins, particularly at warmer temperatures, some psychrophiles elevate expression of prolyl isomerase and molecular chaperones

(Williams *et al.*, 2011); some also carry added *dnaJ* genes (Riley *et al.*, 2008), presumably as added protection against denatured protein aggregation and facilitate refolding (Han and Christen, 2004). The *Tmr. arctica* genome carries slightly elevated numbers of both sorts of genes relative to other members of its genus. *Tmr. arctica* carries 14 genes belonging to prolyl isomerase PFAMs 00160, 00254, 00639, 13145 and 13616, compared with 12 in *Tmr. chilensis*, 13 in *Tmr. frisia* KP2, and 14 in *Tmr.* sp. Milos T2 and 5 genes encoding DnaJ proteins, compared with 2 in *Tmr.* sp. Milos T2, 4 in *Tmr. chilensis* and 5 in *Tmr. frisia* KP2.

The *Tmr. arctica* genome is unique among all of the organisms sequenced here in carrying a chromosome region encoding the enzymes necessary for extracellular polysaccharide (EPS) synthesis. Other psychrophiles produce EPS when cultivated at the low range of their growth temperatures; EPS also enhances their survival of freezing (Marx *et al.*, 2009). In *Tmr. arctica*, the chromosome region spanning from *F612DRAFT_0075* to *F612DRAFT_0094* includes genes homologous to those involved in EPS production in other organisms (*epsD–I, ExoZ)*, encoding glycosyltransferases, as well as genes encoding molecular machinery necessary to translocate EPS to the extracellular milleau (flippase, beta barrel porin). It is important to note, however, that the production of EPS-like substances has been reported in a nonpsychrophilic sulfur-oxidizing microorganism (Nunoura *et al.*, 2014); further study would be necessary to determine the role of these substances in *Tmr. arctica*.

In other respects, the *Tmr. arctica* genome does not depart from other members of *Thiomicrorhabdus*. When all genes from this organism are translated and amino acid frequencies are tallied, there are no differences in their frequencies, relative to other members of *Thiomicrorhabdus;* the elevated frequencies of lysine or diminished frequencies of proline, acidic or arginine observed in other psychrophiles (Ayala-del-Río *et al.*, 2010) are not apparent in this organism. Additionally, the fatty acid biosynthetic pathways are predicted to be identical in all of these organisms based on genome data, and all encode fatty acid desaturase; any adjustments to maintain membrane fluidity at colder temperatures likely occurs by differential expression of fatty acid biosynthetic genes shared by all members of *Thiomicrorhabdus*, as has been observed for *Shewanella* (Wang *et al.*, 2009).

Conclusions

Members of the genera *Hydrogenovibrio, Thiomicrorhabdus* and *Thiomicrospira* all appear to have multiple adaptations to maintain aerobic chemolithoautotrophic metabolism in their heterogeneous environments. Rather than being able to express multiple physiologies (e.g., denitrification, heterotrophy via diverse carbon sources), they appear to have expanded their versatilities as chemolithoautotrophs by sensing their environment with a large arsenal of methyl-accepting chemotaxis proteins and responding to it via chemotaxis, as well as with an elaborate array of proteins that communicate with the second messenger cyclic di-GMP. They carry multiple complexes for introducing electrons removed from inorganic compounds into their electron transport chains, which eventually arrive at multiple terminal oxidases. Multiple RubisCO enzymes are present to facilitate CO_2 fixation at a variety of CO_2 and O_2 tensions, and multiple transporters are likely to facilitate CO_2 ~~or~~ bicarbonate uptake when these substrates are available at a range of concentrations.

Experimental procedures

Genome sequencing, assembly and annotation

Draft genome sequences were generated at the DOE Joint Genome Institute (JGI) and LANL Genome Science Group. The Pacific Biosciences (PacBio) technology (Eid *et al.*, 2009) was used for *H. thermophilus* MA2–6, *Hydrogenovibrio* sp. Milos-T1, *H. kuenenii, H. marinus* DSM 11271 and *Tms. pelophila*. A Pacbio SMRTbell™ library was constructed and sequenced on the PacBio RS platform. The raw reads were assembled using HGAP (version: 2.1.1; Chin *et al.*, 2013). Illumina technology was used to generate draft sequences for *H. halophilus, Tmr. chilensis, Tmr. arctica, Thiomicrorhabdus* sp. Milos-T2 and *Tmr. frisia* Kp2. An Illumina standard shotgun library and long insert mate pair library was constructed and sequenced using the Illumina HiSeq 2000 platform (Bennett, 2004). All raw Illumina sequence data was passed through DUK, a filtering program developed at JGI, which removes known Illumina sequencing and library preparation artefacts (L. Mingkun, A. Copeland, J. Han, unpubl.). Filtered Illumina reads were assembled using AllpathsLG (Gnerre *et al.*, 2011). The consensus was computationally shredded into 10 kbp overlapping fake reads (shreds). The Illumina draft data was also assembled with Velvet, version 1.1.05 (Zerbino and Birney, 2008), and the consensus sequences were computationally shredded into 1.5 kbp overlapping fake reads (shreds). The Illumina draft data was assembled again with Velvet using the shreds from the first Velvet assembly to guide the next assembly. The consensus from the second VELVET assembly was shredded into 1.5 kbp overlapping fake reads. The fake reads from the Allpaths assembly and both Velvet assemblies and a subset of the Illumina CLIP paired-end reads were assembled using parallel phrap, version 4.24 (High Performance Software, LLC). Possible mis-assemblies were corrected with manual editing in Consed (Ewing and Green, 1998; Ewing *et al.*, 1998; Gordon *et al.*, 1998). Gap closure was accomplished using repeat resolution software (Wei Gu, unpublished), and sequencing of bridging PCR fragments with Sanger and/or PacBio (Cliff Han, unpublished) technologies. The draft genome of *H. thermophilus* JR-2 was generated using a combination of Illumina (Bennett, 2004) and 454 technologies (Margulies *et al.*, 2005). Illumina libraries were constructed as above. A 454 Titanium standard

library and 2 paired end 454 libraries were also generated. The 454 Titanium standard data and the 454 paired end data were assembled together with Newbler, version 2.3-PreRelease-6/30/2009. The Newbler consensus sequences were computation ally shredded into 2 kb overlapping fake reads (shreds). The 454 Newbler consensus shreds, the Illumina VELVET consensus shreds, and the read pairs in the 454 paired end library were integrated using parallel phrap, version SOS-4.24 (High Performance Software, LLC). Illumina data were used to correct potential base errors and increase consensus quality using the software Polisher developed at JGI (Alla Lapidus, unpublished). Possible mis-assemblies were corrected using gapResolution (Cliff Han, unpublished), or Dupfinisher (Han and Chain, 2006). All general aspects of library construction and sequencing performed at the JGI can be found at http://www.jgi.doe.gov.

Genes were identified using Prodigal (Hyatt *et al*., 2010), followed by a round of manual curation using GenePRIMP (Pati *et al*., 2010) for finished genomes and draft genomes in fewer than 20 scaffolds. The predicted CDSs were translated and used to search the National Centre for Biotechnology Information (NCBI) nonredundant database, UniProt, TIGRFam, Pfam, KEGG, COG and InterPro databases. The tRNAScanSE tool (Lowe and Eddy, 1997) was used to find tRNA genes, whereas ribosomal RNA genes were found by searches against models of the ribosomal RNA genes built from SILVA (Pruesse *et al*., 2007). Other non-coding RNAs such as the RNA components of the protein secretion complex and the RNase P were identified by searching the genome for the corresponding Rfam profiles using INFERNAL (Nawrocki *et al*., 2009). Additional gene prediction analysis and manual functional annotation was performed within the integrated microbial genomes (IMG) platform (Markowitz *et al*., 2014) developed by the Joint Genome Institute, Walnut Creek, CA (Markowitz *et al*., 2009). Tandem repeats were identified using Tandem repeat finder (Benson, 1999).

Ribosomal protein supertree

Representative taxa from the *Gammaproteobacteria*, the *'Epsilonbacteraeota'* (Waite *et al*., 2017), and the *Acidithiobacillia* were selected from the Integrated Microbial Genomes database (Markowitz *et al*., 2014). Genes encoding ribosomal proteins were gathered based on membership in Clusters of Orthologous Genes (COG) and Protein FAMilies (PFAM; (Tatusov *et al*., 2001; Bateman *et al*., 2002). Large ribosomal subunits 8, 13, 26 and 29 and small subunit 13 were omitted from this analysis as many strains were missing them. Genes encoding the subunits were aligned independently using the MUSCLE algorithm (Edgar, 2004) as implemented in MEGA 4.0 using default settings (Kumar *et al*., 2008), alignments were concatenated via FABOX (http://users-birc.au.dk/biopv/php/fabox/), and refined with GBLOCKS using stringent criteria (Talavera and Castresana, 2007). Phylogenetic trees were constructed in PhyML 3.0 (Guindon *et al*., 2010) using maximum likelihood (ML) analysis of amino acid sequences. The best fit model of evolution, estimated using smart model selection (SMS) in PhyML 3.0 (Guindon *et al*., 2010) was determined to be the amino acid replacement model of (Le and Gascuel, 2008) with four categories using a discrete Gamma distribution ($G = 0.884$) and a proportion of Invariant sites ($I = 0.143$; '$LG + G + I$'). The consensus tree was visualized using FigTree (Version 1.4.3; Rambaut, 2016).

Comparing genome traits of Hydrogenovibrio*,* Thiomicrorhabdus *and* Thiomicrospira *to other autotrophic members of* 'Proteobacteria'

To identify traits that distinguish members of *Hydrogenovibrio, Thiomicrorhabdus* and *Thiomicrospira*, these genomes were compared with those of other members of *'Proteobacteria'* that use the CBB cycle for carbon fixation. This comparison group was collected based on the presence of form I or form II RubisCO genes in their genomes, and demonstrated ability to grow with CO_2 as the major carbon source, as described in Quasem *et al*. (2017). To compare the distribution of electron transport chain components among these organisms, BLAST queries were undertaken, using genes encoding biochemically characterized enzymes (Brune, 1995; Gregersen *et al*., 2011; Dahl *et al*., 2013; Weissgerber *et al*., 2014). BLAST hits were evaluated based on sequence similarities, phylogenetic analysis, and chromosome collocation (Supporting Information Table S6). To compare the distribution and forms of genes encoding RubisCO, all genes belonging to Pfam00016 (Ribulose bisphosphate carboxylase large chain, catalytic domain) were collected from these genomes, and aligned as described above. A preliminary neighbour-joining tree was used to identify 'RubisCO-like proteins', which are not catalytically active as carboxylases (Tabita *et al*., 2008); these sequences were removed from the alignment. The remaining sequences were re-aligned via MUSCLE, the alignment was refined via GBLOCKS, and phylogenetic trees were constructed as described above ($G = 1.027$, $I = 0.07$; '$LG + G + I$').

Methyl-accepting chemotaxis proteins

All genes encoding methyl-accepting chemotaxis proteins were gathered from target genome sequences in IMG. These genes were identified based on the presence of a methyl-accepting chemotaxis protein signalling domain (PFAM 00015), since this portion of these proteins is conserved (Alexander and Zhulin, 2007). Amino acid sequences predicted from genes were clustered via CD-HIT (http://weizhongli-lab.org/cdhit_suite/cgi-bin/index.cgi; Li *et al*., 2001). Clusters were generated with 30%–90% sequence identity, and a sequence identity of 40% was chosen for clustering, as this value was high enough to produce clusters with sequences that aligned well over their full length. Representative sequences from each cluster were used as query sequences for BLAST searches of IMG to find homologous genes from other organisms beyond the genera *Thiomicrospira, Hydrogenovibrio* and *Thiomicrorhabdus* whose sequences aligned well along their full length.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Genome data of autotrophic microorganisms from the '*Proteobacteria*'.
Table S2. Numbers of genes belonging to selected Pfams.
Table S3. Inorganic sulfur-metabolizing systems encoded in genomes of autotrophic microorganisms from the '*Proteobacteria*'.
Table S4. Electron transport chain components encoded in genomes of autotrophic microorganisms from the '*Proteobacteria*'.
Table S5. Presence of genes encoding RubisCO in genomes of autotrophic microorganisms from the '*Proteobacteria*'.
Table S6. Queries and criteria used to find sulfur metabolizing complexes and electron transport chain components.
Fig. S1. Annotated ORFs of *H. marinus* and *Tmr. frisia* KP2 prophages.
Fig. S2. Maximum likelihood analysis of amino acid sequences predicted from genes encoding form II RubisCO (CbbM) and large subunits from form I (CbbL). The tree is rooted, and bootstrap values are from 1000 resamplings of the alignment. The scale bar represents the number of substitutions per site.