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An evaluation of Thiomicrospira, Hydrogenovibrio and Thioalkalimicrobium: reclassification of 4 species of Thiomicrospira to each Thiomicrorhabdus gen. nov. and Hydrogenovibrio, and reclassification of all 4 species of Thioalkalimicrobium to Thiomicrospira.

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Abstract:	Thiomicrospira spp. are small sulfur-oxidising chemolithoautotrophic members of the Gammaproteobacteria. Whilst the type species Tms. pelophila and closely related Tms. thyasirae exhibit canonical spiral morphology under sub-optimal growth conditions, most species are vibrios or rods. The 16S rRNA gene diversity is vast, with identities as low as 91.6 % to Tms. pelophila versus Tms. frisia, for example. Thiomicrospira was examined with closely related genera Hydrogenovibrio and Thioalkalimicrobium and, to rationalise organisms on the basis of the 16S rRNA gene phylogeny, physiology and morphology, we reclassify Tms. kuenenii, Tms. crunogena, Tms. thermophila and Tms. halophila to Hydrogenovibrio kuenenii comb. nov., H. crunogenus corrig. comb. nov., H. thermophilus corrig. comb. nov., and H. halophilus corrig. comb. nov. We reclassify Tms. frisia, Tms. arctica, Tms. psychrophila and Tms. chilensis to Thiomicrorhabdus gen. nov., as Tmr. frisia comb. nov., Tmr. arctica comb. nov., Tmr. psychrophila comb. nov. and Tmr. chilensis comb. nov. - the type species of Thiomicrorhabdus is Tmr. frisia. We demonstrate Thioalkalimicrobium spp. fall in the genus Thiomicrospira sensu stricto, thus reclassifying them to Tms. aerophila corrig. comb. nov., Tms. microaerophila corrig. comb. nov., Tms. cyclica corrig. comb. nov.and Tms. sibirica corrig. comb. nov. We provide emended descriptions of the genera Thiomicrospira and Hydrogenovibrio and of Tms. thyasirae.

An evaluation of *Thiomicrospira*, *Hydrogenovibrio* and *Thioalkalimicrobium*: reclassification of 4 species of *Thiomicrospira* to each *Thiomicrorhabdus* gen. nov. and *Hydrogenovibrio*, and reclassification of all 4 species of *Thioalkalimicrobium* to *Thiomicrospira.*

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RUNNING TITLE: Reclassification of *Thiomicrospira* spp. and *Thioalkalimicrobium* spp.

ABBREVIATIONS: We have used 3-letter abbreviations for genera with similar names, namely *Thiomicrospira* (*Tms*.), *Thiomicrorhabdus* (*Tmr*.) and *Thioalkalimicrobium* (*Tam.*) and retain a single letter for *Thiobacillus* (*T.*). Similarly, we refer to *Halothiobacillus* with 3-letters (*Htb.*) as we used the single letter for *Hydrogenovibrio* (*H.*).

Abstract

Thiomicrospira spp. are small sulfur-oxidising chemolithoautotrophic members of the *Gammaproteobacteria*. Whilst the type species *Tms. pelophila* and closely related *Tms. thyasirae* exhibit canonical spiral morphology under sub-optimal growth conditions, most species are vibrios or rods. The 16S rRNA gene diversity is vast, with identities as low as 91.6 % to *Tms. pelophila versus Tms. frisia*, for example. *Thiomicrospira* was examined with closely related genera *Hydrogenovibrio* and *Thioalkalimicrobium* and, to rationalise organisms on the basis of the 16S rRNA gene phylogeny, physiology and morphology, we reclassify *Tms. kuenenii*, *Tms. crunogena*, *Tms. thermophila* and *Tms. halophila* to *Hydrogenovibrio kuenenii* comb. nov., *H. crunogenus* corrig. comb. nov., *H. thermophilus* corrig. comb. nov., and *H. halophilus* corrig. comb. nov. We reclassify *Tms. frisia*, *Tms. arctica*, *Tms. psychrophila* and *Tms. chilensis* to *Thiomicrorhabdus* gen. nov., as *Tmr. frisia* comb. nov., *Tmr. arctica* comb. nov., *Tmr. psychrophila* comb. nov. and *Tmr. chilensis* comb. nov. – the type species of *Thiomicrorhabdus* is *Tmr. frisia*. We demonstrate *Thioalkalimicrobium* spp. fall in the genus *Thiomicrospira sensu stricto*, thus reclassifying them to *Tms. aerophila* corrig. comb. nov., *Tms. microaerophila* corrig. comb. nov., *Tms. cyclica* corrig. comb. nov.and *Tms. sibirica* corrig. comb. nov. We provide emended descriptions of the genera *Thiomicrospira* and *Hydrogenovibrio* and of *Tms. thyasirae*.

The genus *Thiomicrospira* (Kuenen & Veldkamp, 1972; Approved Lists, 1980) falls within the family *Piscirickettsiaceae* in the order *Thiorichales* of the class *Gammaproteobacteria.* It was circumscribed originally by Kuenen & Veldkamp (1972) on the basis of one isolate (*Tms. pelophila* – a sulfur-oxidising obligate chemolithoautotroph), which had unique properties *versus Thiobacillus* spp., *viz*. thinner, spiral or comma-shaped cells and a very high tolerance of sulfide *versus T. thioparus*, and could be isolated by passing samples through a 0.22 μm filter (*Tms. pelophila* 0.2 – 0.3 μm diameter). There are currently 10 species with validly published names (Figure 1a), showing considerable metabolic and morphological diversity. Closely affiliated to this genus on the basis of 16S rRNA gene sequences are the monospecific genus *Hydrogenovibrio* (Nishihara *et al.*, 1991), comprising one hydrogen-oxidising chemolithoautotroph (which also uses sulfur species) and the genus *Thioalkalimicrobium* (Sorokin *et al*., 2001, also referred to for a time as "*Thialkalimicrobium*"), comprising several obligately alkaliphilic sulfur-oxidising chemolithoautotrophs. On the basis of the 16S rRNA (*rrs*) gene (as shown in Figure 1a), the genus *Thiomicrospira* currently falls into 3 clades – for the purposes of this study, we refer to them as "Clade A" (*Tms. pelophila* [type species], *Tms. thyasirae, Tam. aerophilum* [type species], *Tam. microaerophilum, Tam. cyclicum* and *Tam. sibiricum*), "Clade B" (*Tms. frisia, Tms. chilensis, Tms. arctica, Tms. psychrophila*) and "Clade C" (*Tms. thermophila, Tms. crunogena, Tms. kuenenii* and *H. marinus* [type species]). As can be seen from Figure 1a, there is considerable phylogenetic distance between Clade A and Clade B or Clade C (*e.g.* 16S rRNA gene identity *Tms. pelophila* to *Tms. arctica*, 92.9%, and to *Tms. crunogena*, 92.1%), and members of the genus *Thioalkalimicrobium* are more closely related to the type species of *Thiomicrospira* than members of Clade B or Clade C are (gene identities of *Tms. pelophila* to *Thioalkalimicrobium* spp. range from 95.9 to 97.3 %). As such, here we evaluate the taxonomy and systematics of *Thiomicrospira*, *Hydrogenovibrio* and *Thioalkalimicrobium*

using a polyphasic approach to rationalise species and to circumscribe a novel genus, *Thiomicrorhabdus* gen. nov. We also expand *Hydrogenovibrio* to circumscribe other species, currently regarded as *Thiomicrospira* spp. Furthermore, we also confirm that *Thioalkalimicrobium* is not distinct from *Thiomicrospira* and thus reclassify all 4 species into *Thiomicrospira*. Accordingly, we provide emended descriptions of *Thiomicrospira* and *Hydrogenovibrio* and of *Tms. thyasirae*.

Two principle phylogenetic analyses were performed – Figure 1a shows a maximum likelihood tree (Tamura-Nei model) of 16S rRNA genes from species of the 3 genera considered here, restricted to type strains of species with validly published names, using that from *Galenea microaerophila* P2D^{T}, also from the *Piscirickettsiaceae*, as the out-group. Figure 1b shows additionally *Thiomicrospira* and *Hydrogenovibrio* strains for which genome sequences are publically available in the Integrated Microbial Genomes (IMG) database, based on the first complete 16S rRNA gene identified in their genome (a full list of Genome IDs is given in Table 2). Figure 2 shows unrooted maximum likelihood trees of amino acid sequences derived from 53-gene concatamers comprised of ribosomal protein genes, concatenated using the ribosomal multilocus sequence typing (rMLST) platform (Jolley *et al.* 2012). Genes used were *rpsA through rpsU; rplA* through *rplF*; *rplL* though *rplX*, and *rpmA* through *rpmJ* genes, with full details and rationale given by Jolley *et al.*, 2012. This latter tree comprises sequences only from organisms for which a whole-genome sequence is present in a public database (curated in Table 2, with their origins of isolation where known), thus does not represent all species in Figure 1a. The concatamers in Figure 2 and the genes in Figure 1 were aligned using the MUSCLE algorithm (Edgar, 2004) in MEGA 7.0.2 (Kumar *et al.*, 2016) and trees were built using the maximum-likelihood algorithm using either the Tamura-Nei model (DNA, Tamura & Nei, 1993) or the Jones-Taylor-Thornton model (amino acids, Jones *et al*., 1992). Bootstrap values at nodes represent 5,000 resamplings of each tree and

are given where ≥ 70 %. The 3 clades mentioned as evident in Figure 1 are reflected in the overall topology of the 53-gene concatamer trees shown in Figure 2, based on derived amino acyl sequences (since G+C fractions vary greatly across the organisms here, use of amino acyl sequences effectively allow for that variable), showing that the 16S rRNA gene phylogeny is probably a reliable reflection of the speciation and of relationships.

Functional gene comparisons were made using 'housekeeping' genes encoding DNA gyrase (EC 5.99.1.3) subunit B (*gyrB*); the DNA repair protein (previously "recombinase A") RecA (*recA*), and the F_1-sector of the two-sector proton translocating ATPase (EC 3.6.3.14) beta subunit (*atpD*). We have also used the ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCO, EC 4.1.1.39) genes encoding subunits of two variants of the enzyme – namely the large subunit of form IA RuBisCO (*cbbL*) and form II RuBisCO (*cbbM*) – the holoenzyme of form IA contains 8 CbbL and 8 CbbS subunits; that of form II is formed of dimers of CbbM subunits, which are evolutionarily related to CbbL from form IA. Form IA can be subdivided into form IAc (carboxysome-associated) and form IAq (cytoplasmic, Badger & Bek, 2008; Tabita *et al*. 2008, Tourova *et al*., 2006).. It is worth noting that not all RuBisCO forms are represented in all of the organisms studied herein, indeed some have all types, some have none.

It can be seen from Figure 1b that genome sequenced strains JR2, XS5 and MA2-6 (=DSM 13155) are probably all strains of *Tms. thermophila* (16S rRNA gene identities to *Tms. thermophila* I78^T: 100.0%, 99.0 % and 100.0 %, respectively); XCL-2 is a strain of *Tms. crunogena* (16S rRNA gene identity to *Tms. crunogena* ATCC 35932^T: 99.8 %), and Kp2 is a strain of *Tms. frisia* (16S rRNA gene identity to *Tms. frisia* JB-A2^T: 99.5 %). Using *in silico* DNA-DNA hybridisation (dDDH) for strains with genome sequences available, we can confirm that JR2 and MA2-6 are strains of the same species (70.60 % hybridisation). The

other genome sequence strains could represent novel taxa and are thus considered at the end of this study.

Phylogenetic trees showing derived amino acid sequences from *gyrB*, *recA* and *atpD* are given in Figure 3 and the RuBisCO trees (CbbL and CbbM) are given as Supplementary Figures S1 and S2, respectively. It can be seen that GyrB and RecA trees give good agreement with both the 16S rRNA gene and the 53-gene concatemer trees in terms of the overall topology, and all place *Thioalkalimicrobium* spp. and *Tms. pelophila* together, in a well-supported clade. The AtpD tree shows a different overall topology for Clades B and C but gives Clade A as a single group, per RecA and GyrB. These data together with the 16S rRNA gene identities being greater than the Yarza *et al*. (2014) proposed cut-offs for higher taxonomic ranks indicate that *Thioalkalimicrobium* spp. and *Thiomicrospira sensu stricto* (*viz*. *Tms. pelophila* and *Tms. thyasirae*) fall within the same genus (16S identities of *Thioalkalimicrobium* spp. to *Tms. pelophila* are 95.9 – 97.3 %, as given in Table 1). Since *Thiomicrospira* (1972) takes priority over *Thioalkalimicrobium* (2001), we propose that on this basis, that this genus be named *Thiomicrospira* in accordance with the *Code* and that the four *Thioalkalimicrobium* spp. be circumscribed as *Thiomicrospira* spp., with *Tms. pelophila* remaining as the type species and corrigendum of specific epithets of *Thioalkalimicrobium* spp. to change gender from neuter to feminine to match the gender of *Thiomicrospira*. This is reinforced by phenotypic properties in common, *viz*. the presence of carboxysomes (*c*.70 % of species); growth rates on thiosulfate of 0.22 – 0.33 h^{-1} (75 % of species); rod to vibrioid morphology, which curves or spirals with stress, age or growth rate; sodium chloride maxima of *c*.1,200 mM, and G+C fractions of 45.6 – 49.6 mol%. The latter falls well within the range of about 10 mol% difference, within which most genera fall and within the range of 5 mol% difference within which many species fall (Fournier *et al*., 2005).

The question then stands regarding if the four *Thioalkalimicrobium* spp. are indeed members of one species on the basis of their highly similar 16S rRNA genes (identity > 97 %) and G+C fractions; however, average nucleotide identities (ANI) of the genome sequences of *Tms. pelophila versus Thioalkalimicrobium* spp. are 73.13 – 74.02 %, far lower than the proposed cut-off for ANI of 95 % for members of the same species (Figueras *et al*., 2014), which indicates that they thus belong to separate species. From *in silico* DNA-DNA hybridisation (dDDH) using the genome-to-genome distance calculator of the DSMZ (GGDC v. 2.1, BLAST+ alignment method and taking data from Formula 2, as recommended in Meier-Kolthoff *et al*., 2013), this reinforces the ANI data, with dDDH values for *Thioalkalimicrobium* spp. to one another falling at or below 21.60 % hybridisation – this being far below the cut-off of 70.00 % above which members of the same species will typically fall. Full dDDH data are included in Supplementary Table S1.

It is worth noting that the *in vitro* determinations of G+C fractions for *Thioalkalimicrobium* spp. as reported in previous studies (Table 1) are very close (48.9 – 49.6 mol%), but the *in silico* values obtained by us from genome sequence data are 45.55 – 46.98 mol%, much more similar to *Tms. pelophila* (45.7 mol% *in vitro*, 44.46 mol% *in silico*), further evidencing their similarity as one genus. All members of this genus studied contain form IAc RuBisCO genes and *Tms. pelophila* also contains form II RuBisCO, but no members have form IAq RuBisCO, which implies that they all indeed use carboxysomes.

It can be seen from both the 16S rRNA gene tree (Figure 1a), a gene identity of 100 % and G+C fractions that are near identical (45.6 mol % and 45.7 mol%) that *Tms. thyasirae* is very similar to *Tms. pelophila* and the question has been raised previously regarding its validity as a separate species (Brinkhoff *et al.*, 2005). It is worth noting that in Brinkhoff and colleagues' study, they could not reproduce the heterotrophic growth of *Tms. thyasirae* (*sensu* DSM 5322^{T}) originally reported by Wood & Kelly (1993, previously *'Thiobacillus thyasiris*' [*sic.*],

Wood & Kelly, 1989), nor could they find evidence of ubiquinone-10 as the dominant quinone as reported originally, and found ubiquinone-8 only. Where *Tms. pelophila* was isolated from tidal mud off the Frysian Islands, Netherlands (Kuenen & Veldkamp, 1972), *Tms. thyasirae* was isolated from the gill-tissue of *Thyasira flexuosa* Montagu (a salt-water clam), in turn obtained from marine sediments off of Jennycliff, in the Plymouth Sound, UK. *Tms. thyasirae* was found to comprise short rods that elongated into spirals with age or stress (Wood & Kelly, 1993), similar to *Tms. pelophila*, but could grow heterotrophically (Wood & Kelly, 1989) and contained carboxysomes during mixotrophic growth on thiosulfate and acetate in continuous culture (Lanaras *et al.*, 1991), in which cells were rod-shaped. However, the clearly very closely related *Tms. pelophila* does not ordinarily contain carboxysomes (Kuenen & Robertson, 1989), though its genome sequence contains a carboxysome operon with the canonically carboxysome-associated form IAc RuBisCO, and other *Piscirickettsiaceae* isolates such as *Tms. crunogena* XCL-2 do (Menning, 2012). We have obtained personal communications of the full history of *Tms. thyasirae* strains, from the original authors, which are included in the Supplementary Information and from these, we conclude that *Tms. thyasirae* DSM 5322^{T} and *Tms. thyasirae* TG-2^{T} were identical but that a small, similarly shaped heterotrophic consort became present as a low-level undetected contaminant – perhaps from the *Alphaproteobacteria* given its production of ubiquinone-10 (verified as the dominant respiratory quinone by spectrophotometric assay of spots eluted from repeated chromatograms, A. P. Wood, *personal communication*). The presence of carboxysomes in *Tms. thyasirae* we deem valid, along with the pleomorphy but take the view of Brinkhoff *et al*. (2005), that this species produces ubiquinone-8. It is worth noting that Wood & Kelly originally reported heterotrophic growth of this strain – not observed in other members of *Thiomicrospira sensu* Clade A – but this was not found in studies of DSM 5322^{T} by Brinkhoff *et al*. (2005). Wood (*personal communication*) has reported to us that this strain

only grew heterotrophically on a small range of carbon sources (cellobiose, acetate or yeast extract) after significant periods of incubation of thiosulfate-grown inoculum in basal salts supplemented with *e.g.* cellobiose, much as had previously been demonstrated by the same team with *Paracoccus versutus* (‘*Thiobacillus* A2’ or ‘*Thiobacillus versutus*’) both growing on methanol and fixing carbon-14 from [^{14}C]-methanol only after 2-3 weeks incubation using a thiosulfate-grown inoculum (Wood & Kelly, 1982; Wood & Kelly, 1984). Whilst *Tms. pelophila* is motile (described (Kuenen & Veldkamp, 1972) as apparently monotrichous, but platinum-shadowed electron micrographs in Kuenen & Veldkamp, 1972, and Brinkhoff *et al*. 2005 appear to show amphitrichous cells, with flagellar hooks clearly visible at both ends of the cell, particularly clear in the latter reference), *Tms. thyasirae* is not motile and lacks flagella. The latter has a much slower maximum specific growth rate on thiosulfate (0.07 h^{-1} *versus* 0.45 h^{-1}), and both species have similar pH optima, though *Tms. pelophila* can tolerate acidity to pH 5.0 *versus* pH 7.0 in *Tms. thyasirae*, though the latter tolerates NaCl to 3,000 mM *versus* 1,240 mM (17.5 versus 7.0 % (*w/v*)) in *Tms. pelophila*. This property makes *Tms. thyasirae* the second most halotolerant of *Thiomicrospira sensu lato* after *Tms. halophila* (*H. halophilus* corrig. comb. nov.), isolated from a hypersaline lake in Siberia, Russia. Both species have identical substrate profiles and produce elementary sulfur during the oxidation of thiosulfate at neutrality (Brinkhoff *et al.*, 2005), whilst *Tms. pelophila* has an obligate requirement for vitamin B_{12}, whereas *Tms. thyasirae* does not. These data make it difficult to rule out a significant metabolic difference between the two species and thus DNA-DNA hybridisation and/or genomic studies are needed to ascertain their relationship. At this stage, we have emended the description of *Tms. thyasirae*, *viz*. quinone, carboxysome and fatty acid production (with data from Brinkhoff *et al*., 2005; Lanaras *et al*., 1991 and Fullarton *et al*., 1995).

The 16S rRNA, RecA, GyrB and AtpD trees shown in Figures 1 and 2 also indicate that a large clade of closely related organisms (Clade C) is consistently found on the basis of 3 of these genes but not AtpD, in which it is a polyphyletic group. The 16S rRNA, RecA, GyrB and 53-gene concatamer data support Clade C as a clear line of descent, within which the 16S rRNA gene identities to *Tms. pelophila* are very low – 92.1 – 94.4 % (Table 1) – and fall below Yarza's cut-off for genus but above the cut-off for family (86.5 %) indicating that these form a separate genus in the same family as *Thiomicrospira*. Within Clade C, *Hydrogenovibrio* is already validly published as a genus name (published in 1991) and thus would take priority over any new name in accordance with the *Code* – as such, we propose naming Clade C (*viz. H. marinus, Tms. kuenenii, Tms. halophila, Tms. crunogena, Tms. thermophila*) as *Hydrogenovibrio*, with *H. marinus* taking priority and thus remaining the type species and corrigendum of specific epithets from feminine to masculine to match *Hydrogenovibrio*. This is supported by the broadly similar growth rates on thiosulfate (0.25 – 0.8 h^{-1}), uniformly vibrioid morphologies without pleomorphy, similar electron donor profiles *viz*. inorganic sulfur oxyanions, motility from a monotrichous flagellum and either growth on molecular hydrogen as an electron donor and/or the presence of [NiFe]-hydrogenase genes in the genome sequence. Whilst it could be argued that *Hydrogenovibrio* may not be the most obvious name for this genus in terms of only 'describing' known properties of two species, it takes priority under the *Code* and there is no requirement under the *Code* for any genus name to be fully representative of every (or, indeed, any) species therein, thus *Hydrogenovibrio* cannot be avoided. Again, it is worth noting that the G+C contents of *Hydrogenovibrio* would now range from 44.1 – 56.6 mol% based on *in vitro* determinations reported in the literature, which is rather large for a genus, but *in silico* determinations also range 41.5 – 54.9 mol%. This is chiefly because *Tms. halophila* has a high G+C content presumably as a stress adaptation. It also clusters distantly from the rest of this clade in all trees with a deep branch

and has a 16S rRNA gene identity to *H. marinus* (type species) of 95.6 %, which is above the Yarza genus cut-off, thus we do not consider it to be a member of a separate genus. This genus contains form IAc and/or form IAq and form II RuBisCO genes, implying that carboxysomes are not used by all members of the genus. It is worth noting that whilst *Tms. thermophila* was reported as "[using]…molecular nitrogen as [a] nitrogen source" by Takai *et al.* (2004), the 2 genome sequenced strains that are likely strains of this species (JR2 and MA2-6) do not contain the genes encoding canonical diazotrophy *i.e.* the molybdenum-iron (*nif*) or vanadium (*vnf*) nitrogenases – as such, this property of the genus probably requires further scrutiny to rule out the possibility of growth on dissolved nitrogen compound 'carry over' from the inoculum, or of atmospheric ammonia dissolving in slightly acidic media and providing a source of dissolved nitrogen, as has been previously observed as a source of error when determining diazotrophy in acid-producing chemolithoautotrophic *Bacteria* (*e.g.* Mackintosh, 1971 and 1978).

Clade B is supported fully by 16S rRNA, 53-gene concatamer, GyrB and RecA trees and partially by the AtpD tree. This clade also shows a large distance from *Tms. pelophila*, with 16S rRNA gene identities of 91.6 – 92.9 %, again falling below the Yarza cut-off for genus but above that for family, thus indicating that this clade should be circumscribed as a separate genus in the same family as *Thiomicrospira*, for which we propose the name *Thiomicrorhabdus* gen. nov., with *Tms. frisia* (*Tmr. frisia* gen. nov., comb. nov.) taking priority as the type species of this novel genus – we have selected this genus name to accurately describe members of this clade whilst retaining the feminine gender to avoid corrigendum of specific epithets in an effort to retain at least *some* continuity from the old taxonomy to the new! The circumscription of this clade as a genus is supported by G+C contents in the range of 39.6 to 49.9 mol% (*in vitro* – the *in silico* values are 41.9 to 48.9 mol%), a uniform rod-shaped morphology without pleomorphy, motility by a monotrichous

flagellum, production of elementary sulfur from thiosulfate at neutrality by all species, maximum salt concentrations of 1,240 mM and a dominance of palmitoleic ($C_{16:1}$) and vaccenic ($C_{18:1}$) acids in the fatty acid fraction. Forms IAc and/or IAq and Form II RuBisCO are found in this group, again, implying carboxysomes are not in use by all species.

From the genome sequenced strains considered in Figure 1b and Figure 2, strain Milos-T2 (= DSM 13229, Brinkhoff *et al.,* 1999*c*) could represent a novel species of *Thiomicrorhabdus* (16S rRNA gene identity to *Tmr. frisia* gen. nov. comb. nov. is 97.7 %) and strain Milos-T1 (= DSM 13190, Brinkhoff *et al*., 1999*c*) is a novel species of *Hydrogenovibrio* (16S rRNA gene identity to *H. kuenenii* comb. nov. is 95.9 %). Strain WB1 could be a novel species of *Hydrogenovibrio* (16S rRNA gene identity to *H. halophilus* HL 5^T: 97.4 %). It is of course the case that significant physiological and chemotaxonomic studies and deposit into two international culture collections are required to be able to validly publish names for these strains, thus we cannot state more than this at this time.

Conclusions and recommendations

We propose the reclassification of 8 species of *Thiomicrospira* that have validly published names since they do not phylogenetically fall within the *Thiomicrospira* genus and have different but consistent morphologies and physiologies. As Clade A contains the type species *Tms. pelophila,* it must be retained with the name *Thiomicrospira*, thus *Tam. cyclicum, Tam. aerophilum, Tam. sibiricum* and *Tam. microaerophilum* are circumscribed into this genus. We propose that Clade B, which comprises *Tms. arctica, Tms. psychrophila, Tms. chilensis* and *Tms. frisia,* is circumscribed to form a new genus *Thiomicrorhabdus* gen. nov. on the basis of 16S rRNA gene affiliation, morphology and physiology. We propose the type species be *Thiomicrorhabdus frisia* comb. nov., on the basis of being the oldest validly published species in this new genus. We propose that Clade C, comprising *H. marinus*, Tms. *kuenenii,*

Tms. halophila, *Tms. crunogena* and *Tms. thermophila*, be circumscribed into the genus *Hydrogenovibrio* on the basis of it being an extant validly published name and thus taking priority, with a gender-change of each specific epithet from feminine to masculine in each new combination. The type species will remain *H. marinus*. On the basis of Yarza and colleagues' (2014) recommendation that families are circumscribed on the basis of 86.5 % identity of the 16S rRNA gene, all taxa in this study still fall within the *Piscirickettsiaceae* of the *Thiotrichales* of the *Gammaproteobacteria*.

Description of *Thiomicrorhabdus* gen. nov.

Thiomicrorhabdus (Thi.o.mi.cro.rhab'dus. Gr. n. *theion*, L. transliteration *thium*, sulfur; Gr. adj. *mikrós*, small; Gr. fem. n. *rhabdos* , N.L. transliteration *rhabdus*, rod or wand. N.L. fem. n. *Thiomicrorhabdus*, small sulfur-oxidising rod).

Gram negative. Cells when grown in liquid media are rod-shaped. Typical cell lengths are 0.8 – 2.7 μm and diameters are 0.3 – 0.6 μm, wider than *Thiomicrospira* spp. Does not form endospores or exospores. Uses molecular oxygen as the sole terminal electron acceptor. Has a cbb_3-type cytochrome *c* oxidase (EC 1.9.3.1).

Forms white to yellow, entire colonies on thiosulfate-agar, which are coated in small granules of elementary sulfur. Motile, cells are monotrichous when grown in liquid media.

Obligately chemolithoautotrophic with heterotrophy never observed. Can use thiosulfate, tetrathionate or sulfide as sole electron donors but not molecular hydrogen, thiocyanate, sulfite, iron or manganese. Some species can use elementary sulfur as a sole electron donors. Fix carbon dioxide *via* the transaldolase-variant Calvin-Benson-Bassham cycle. All species use ammonium as a nitrogen source. Does not fix dinitrogen. No nitrogenase or hydrogenase genes observed in genome sequences. Has form IAc and/or form IAq, and form II RuBisCo.

All species produce elementary sulfur when growing on thiosulfate at neutrality, but at varying degrees. Never auxotrophic for vitamin B_{12}. Growth occurs from pH 4.2 to pH 9.0 but range varies with species – pH optima are pH 6.5 to 8.5. Grows from -2 °C to 42 °C with optima of 11.5 °C to 35 °C, varying by species. NaCl is required for growth, with minima of 40 – 100 mM, maxima of 1,240 mM across the genus and optima of 250 – 470 mM. Does not reduce nitrate to nitrite.

G+C fractions of genomic DNA are 39.6 – 49.9 mol%. Dominant respiratory quinone is ubiquinone-8. Dominant fatty acids include palmitoleic ($C_{16:1}$), vaccenic ($C_{18:1}$), palmitic ($C_{16:0}$), stearic ($C_{18:0}$) and myristoleic ($C_{14:1}$) acids. Members of the *Piskirickettsiaceae* in the *Thiotrichales* of the *Gammaproteobacteria.*

Type species: *Thiomicrorhabdus frisia* (Basonym: *Thiomicrospira frisia*) Brinkhoff *et al.* 1999.

Description of *Thiomicrorhabdus frisia* comb. nov.

Thiomicrorhabdus frisia (fri'sia. L. fem. adj. *frisia*, of or pertaining to Frisia, coastal region of northwestern Germany and northeastern Netherlands, from where the organism was obtained).

Properties are as given by Brinkhoff *et al*. (1999*a*). Basonym *Thiomicrospira frisia*.

Type species of the genus *Thiomicrorhabdus.*

Type strain = JB-A2^T = ATCC 700878^T = DSM 12351^T.

Description of *Thiomicrorhabdus chilensis* comb. nov.

Thiomicrorhabdus chilensis (chi.len'sis. N.L. fem. adj. *chilensis*, of or pertaining to Chile, country in South America from where the organism was obtained).

Properties are as given by Brinkhoff *et al.* (1999b). Basonym *Thiomicrospira chilensis*.

Type strain is Ch-1^T = ATCC 700858^T = DSM 12352^T.

Description of *Thiomicrorhabdus arctica* comb. nov.

Thiomicrorhabdus arctica (arc'ti.ca. L. fem. adj. *arctica*, northern, arctic, and by extension, the Arctic, referring to the site of isolation.

Properties are as given by Knittel *et al.* (2005). Basonym *Thiomicrospira arctica*.

Type strain is SVAL-E^T = ATCC 700955^T = DSM 13458^T.

Description of *Thiomicrorhabdus psychrophila* comb. nov.

Thiomicrorhabdus psychrophila (psy.chro'phi.la. Gr. adj. *psychros*, cold; N.L. adj. *philus* from Gr. adj. *philos*, friend, someone dearly loved; N.L. fem. adj. *psychrophila*, cold-loving)

Properties are as given by Knittel *et al*. (2005). Basonym *Thiomicrospira psychrophila*.

Type strain is SVAL-D^T = ATCC 700954^T = DSM 13453^T.

Emended description of *Hydrogenovibrio* (Nishihara *et al.* 1991)

Hydrogenovibrio (Hy.dro.ge.no.vi'bri.o. Gr. n. *hydôr*, water; Gr. v. *gennaô*, to beget, to bring forth, to produce; N.L. n. *hydrogenum*, hydrogen, *i.e.* that which produces water; L. v. *vibro* to set in tremulous motion, to move to and fro or to vibrate; N.L. masc. n. *vibrio* that which vibrates, and name of a genus of the *Bacteria* with a curved rod shape (*Vibrio*); N.L. masc. n. *Hydrogenovibrio*, the hydrogen vibrio).

Gram negative. Cells when grown in liquid media are usually vibioid but curved rods are sometimes also found. Typical cell lengths are 0.8 – 3.0 μm and diameters are 0.3 – 0.5 μm, wider than *Thiomicrospira* spp. Does not form endospores or exospores. Uses molecular

oxygen as the sole terminal electron acceptor. Most species grow optimally at oxygen partial pressures below atmospheric levels. Has a *cbb*$_3$-type cytochrome *c* oxidase (EC 1.9.3.1).

Forms cream, white or yellow, entire colonies on thiosulfate-agar, which are coated in small granules of elementary sulfur. Motile, cells are monotrichous when grown in liquid media.

Obligately chemolithoautotrophic with heterotrophy occasionally observed in some species, but mixotrophic growth is not usually observed in this genus. Can use thiosulfate or sulfide as sole electron donors but not thiocyanate, sulfite, iron, manganese. Some species can use tetrathionate or elementary sulfur as sole electron donors. Molecular hydrogen use is found in some species and [NiFe]-hydrogenase (EC 1.12.1.2) genes are encoded for in the genomes of others where hydrogen use has not been observed *in vivo*. Where hydrogenase enzyme activity has been detected, they are membrane-bound and do not reduce NAD(P)$^+$ *in vivo*. Fix carbon dioxide *via* the transaldolase-variant Calvin-Benson-Bassham cycle. All species use ammonium as a nitrogen source – some can also use urea and possess urease. One strain has been reported to be diazotrophic but all others known do not fix dinitrogen or possess *nif* or *vnf* nitrogenase genes. Do not use nitrate or nitrite as nitrogen sources – nitrite is toxic to most species. Has form IAc and/or form IAq, and form II RuBisCO. All strains examined by electron microscopy show carboxysomes when grown autotrophically.

Some species produce elementary sulfur when growing on thiosulfate at neutrality. Never auxotrophic for vitamin B_{12}. Growth occurs from pH 4.0 to pH 8.5 but range varies with species – pH optima are pH 6.0 to 8.0. Grows from 4 °C to 55 °C with optima of 28 °C to 40 °C, varying by species. NaCl is required for growth, with minima of 45 – 500 mM, maxima of 640 – 3,500 mM and optima of 205 – 1,500 mM. Does not reduce nitrate to nitrite.

G+C fractions of genomic DNA are 44.1 – 56.6 mol%. Dominant respiratory quinone is ubiquinone-8. Dominant fatty acids include palmitoleic ($C_{16:1}$), palmitic ($C_{16:0}$), stearic ($C_{18:0}$) acids. Members of the *Piskirickettsiaceae* in the *Thiotrichales* of the *Gammaproteobacteria.*

Type species is *Hydrogenovibrio marinus* (Nishihara *et al.* 1991), isolated from seawater off the coast of Japan.

Description of *Hydrogenovibrio kuenenii* comb. nov.

Hydrogenovibrio kuenenii (kue.nen'.i.i. N.L. gen. n. *kuenenii*, of or pertaining to Kuenen; named for Professor J. Gijs Kuenen, Dutch microbiologist of the Delft School, and proposer of the genus *Thiomicrospira*).

Properties are as given by Brinkhoff *et al.* (1999*a*), with the addition that carboxysomes are observed. Basonym *Thiomicrospira kuenenii*.

Type strain is JB-A1^T = ATCC 700877^T = DSM 12350^T.

Description of *Hydrogenovibrio halophilus* comb. nov.

Hydrogenovibrio halophilus (ha.lo'phi.lus. Gr. n. hals or halos, salt; N.L. adj. *philus* from Gr. adj. *philos*, friend, someone dearly loved; N.L. masc. adj. *halophilus*, salt-loving)

Properties are as given by Sorokin *et al.* (2006) with the addition that carboxysomes are observed. Basonym *Thiomicrospira halophila.*

Type strain is HL 5^T = DSM 15072^T = UNIQEM U 221^T.

Description of *Hydrogenovibrio crunogenus* comb. nov.

Hydrogenovibrio crunogenus (cru.no'ge.nus. Gr. n. *krounos*, spring, Latin transliteration, *crunos*; L. suff. *–genus*, *-gena*, *-genum*, born from (from L. v. *gigno* (in turn from Gr. v. *gennaô*), to beget, to bring forth, to produce); N.L. masc. adj. *crunogenus*, born from a spring)

Properties are as given by Jannasch *et al.* (1985) with the addition that carboxysomes are observed. Basonym *Thiomicrospira crunogena*.

Type strain is TH-55^T = ATCC 35932^T = DSM 12353^T = LMD 84.00^T.

Description of *Hydrogenovibrio thermophilus* comb. nov.

Hydrogenovibrio thermophilus (ther.mo'phi.lus. Gr. n. *thermê*, heat; N.L. adj. *philus* from Gr. adj. *philos*, friend, someone dearly loved; N.L. masc. adj. *thermophilus*, heat-loving)

Properties are as given by Takai *et al.* (2004) noting that the type strain was reported as diazotrophic but from genome sequences of 2 other strains, no *nif* or *vnf* nitrogenase genes are present. Carboxysomes are observed in strains MA2-6 and JR2 but the type strain has not been examined to date. Basonym *Thiomicrospira thermophila.*

Type strain is I78^T = DSM 16397^T = JCM 12397^T.

Emended description of *Thiomicrospira* (Kuenen & Veldkamp, 1972)

Thiomicrospira (Thi.o.mic.ro.spi'ra.Gr. n. *theion*, L. transliteration *thium*, sulfur; Gr. adj. *mikrós*, small; L. fem. n. *spira*, N.L. fem. n. *Thiomicrospira*, small sulfur-oxidising spiral).

Cells are pleomorphic when grown in liquid media, ranging from very thin, curved rods (some so curved that they form an open circle) to vibrios and to spirals depending on stress (pH, oxygen tension *etc*), age and growth rate. Typical cell lengths are 0.8 – 5.0 μm and diameters are 0.2 – 2.0 μm. Strictly aerobic with some microaerophilic species. Cells of most species typically pass through at 0.2 μm filter. Forms white, pink or reddish, entire or spreading colonies on thiosulfate-agar, which are sometimes coated in small granules of white or yellow elementary sulfur. Cells are atrichous, monotrichous, lophotrichous or amphitrichous when grown in liquid media, varying by species. Has a cbb_3-type cytochrome *c* oxidase (EC 1.9.3.1).

Obligately chemolithoautotrophic with heterotrophy not observed, but may take in supplementary carbon sources such as acetate or succinate during mixotrophic growth. Can use thiosulfate, elementary sulfur, tetrathionate, trithionate or sulfide as sole electron donors but not thiocyanate, sulfite, iron, manganese or molecular hydrogen. Some species produce elementary sulfur when growing on thiosulfate at neutrality. Does not fix dinitrogen. Some species can use thiocyanate as a nitrogen source. Some species are auxotrophic for vitamin B_{12}. Growth occurs from pH 5.9-8.0 to pH 8.4-10.0 but range varies with species – pH optima are pH 7.0 to 10.0, varying by species. Grows from 3.5 °C to 42 °C with optima of 25 °C to 40 °C, varying by species. NaCl is required for growth, with minima of 40 – 250 mM, maxima of 1,200 – 3,000 mM and optima of around 430-600 mM. May produce carboxysomes (polyhedral bodies) during autotrophic growth at atmospheric carbon dioxide partial pressures. Has form IAc of RuBisCo and not form IAq, but some species also have form II.

G+C fractions of genomic DNA are around 45.6 – 49.6 mol%. Dominant respiratory quinone is ubiquinone-8. Members of the *Piskirickettsiaceae* in the *Thiotrichales* of the *Gammaproteobacteria.*

Type species is *Thiomicrospira pelophila* (Kuenen & Veldkamp, 1972), isolated from marine mud of the Wadden Sea, off the coast of the Frysian Islands, Netherlands.

Description of *Thiomicrospira aerophila* comb. nov.

Thiomicrospira aerophila (a.e.ro'phi.la. Gr. n. *aer*, air; N.L. adj. *phila* from Gr. adj. *philos*, friend, someone dearly loved; N.L. fem. adj. *aerophila*, air-loving)

Properties are as given by Sorokin *et al.* (2001). Basonym *Thioalkalimicrobium aerophilum*.

Type strain is AL 3^T = CBS 100465^T = DSM 13739^T.

Description of *Thiomicrospira cyclica* comb. nov.

Thiomicrospira cyclica (cy'cli.ca. L. n. *cyclus*, circle; L. fem. suffix. *–ica*, of or pertaining to; N.L. fem. adj. *cyclica* circle-like).

Properties are as given by Sorokin *et al.* (2002). Basonym *Thioalkalimicrobium cyclicum*.

Type strain is ALM 1^T = DSM 14477^T = JCM 11371^T.

Description of *Thiomicrospira microaerophila* comb. nov.

Thiomicrospira microaerophila (mi.cro.a.e.ro'phi.la. Gr. adj. *mikros*, small; Gr. n. *aer*, air; N.L. adj. *phila* from Gr. adj. *philos*, friend, someone dearly loved; N.L. fem. adj. *microaerophila*, loving low-air concentrations, referring to low-oxygen preference).

Properties are as given by Sorokin *et al.* (2007). Basonym *Thioalkalimicrobium microaerophilum*.

Type strain is ASL8-2^T = DSM 17327^T = UNIQEM $U242^T$.

Description of *Thiomicrospira sibirica* comb. nov.

Thiomicrospira sibirica (si.bi'ri.ca. N.L. fem. adj. *sibirica*, pertaining to Siberia (region of northwestern Asia, the name coming from Sibir, ancient Tartar fortress at the Tobol-Irtysh confluence)).

Properties are as given by Sorokin *et al.* (2001). Basonym *Thioalkalimicrobium sibiricum*.

Type strain is AL 7^T = DSM 13740^T = NCCB 100000^T.

Emended description of *Thiomicrospira thyasirae* (Wood & Kelly, 1995)

Thiomicrospira thyasirae (thy.a.si'rae. N.L. gen. n. *thyasirae*, pertaining to *Thyasira* (a genus of the bivalve mollusc family of the *Thyasiridae*), referring to *Thyasira flexuosa* Montagu, the source of isolation).

Properties are as given by Wood & Kelly (1993) with the exceptions that the species produces ubiquinone-8 as the dominant respiratory quinone, and does not produce ubiquinone-10 in detectable amounts. Produces vaccenic ($C_{18:1}$), stearic ($C_{18:0}$), palmitoleic ($C_{16:1}$), palmitic ($C_{16:0}$) and myristic ($C_{14:0}$) acids as the dominant fatty acids when grown mixotrophically on thiosulfate with acetate. Grows heterotrophically only after long incubations of thiosulfate-grown cells in media with multicarbon compounds as the sole carbon source.

Type strain is TG-2^T = ATCC 51452^T = DSM 5322^T.

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Conflicts of interest

The authors declare that they have no competing interests.

Ethical Statement

No human or animal experiments were conducted in this study.

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Figure 1. Maximum likelihood trees based on the 16S rRNA (*rrs*) gene from *Thiomicrospira*, *Thioalkalimicrobium* and *Hydrogenovibrio* spp. Genes were aligned using MUSCLE in MEGA 7.0.20 and trees build using the Tamura-Nei model with the nearest-neighbour interchange (NNI) heuristic method and partial deletion of gaps. Topologies with the superior log-likelihoods are shown, with numbers at nodes representing the percentage of 5,000 bootstrap replicates for which that topology was preserved (values < 70 % are omitted). *Galenea microaerophila* P2D^{T} was used as the outgroup. GenBank or IMG gene accession numbers are giving in parentheses. Scale bars represent the number of substitutions per site. 1,360 bases were used in each analysis. Type species of genera are emboldened. **Figure 1a** shows the 16S rRNA gene phylogeny of species with validly published names and indicates the 3 clades used in this study. **Figure 1b** adds the 16S rRNA genes from strains for which genome sequences exist in public databases.

Figure 2. Unrooted maximum likelihood tree of amino acid sequences derived from 53 ribosomal protein genes extracted from whole genome sequences publically available in the IMG database and concatenated at DNA level using the rMLST platform, then translated and aligned using MUSCLE in MEGA 7.0.20. Tree was built using the Jones-Taylor-Thornton model with the NNI heuristic method and partial deletion of gaps. Topology with the superior log-likelihood is shown, with numbers at nodes represenging the percentage of 5,000 bootstrap replicates for which that topology was preserved (values < 70 % are omitted). As 53 genes were used for each taxon, gene accession numbers are omitted but Genome ID numbers for each organism are given in Table 2. Scale bar represents the number of substitutions per site. 6,433 amino acids were used in the final analysis (derived from 19,299 bases). Type species of genera are emboldened.

Figure 3. Maximum likelihood tress of amino acid sequences derived from *gyrB, recA* and *atpD* genes extracted from the IMG database and translated and aligned using MUSCLE in

642 MEGA 7.0.20. Tree was built using the Jones-Taylor-Thornton model with the NNI heuristic
643 method and partial deletion of gaps. Topology with the superior log-likelihood is shown, with
644 numbers at nodes represenging the percentage of 5,000 bootstrap replicates for which that
645 topology was preserved (values < 70 % are omitted). Gene ID numbers for IMG are given in
646 parentheses. A total of 802 (GyrB), 341 (RecA) or 549 (AtpD) amino acids were used in each
647 analysis. Scale bars represent the total number of substitutions per site.

648

649 **Table 1.** Comparative properties of all *Thiomicrospira*, *Thioalkalimicrobium* and
650 *Hydrogenovibrio* species with validly published names. Data are either novel and derived
651 from genome mining or are taken from Kuenen & Veldkamp (1972), Knittel *et al*. (2005),
652 Brinkhoff *et al.* (1999a-b), Brinkhoff *et al*. (2005), Jannasch *et al.* (1985), Sorokin *et al.*
653 (2006), Takai *et al.* (2004), Wood & Kelly (1993), Wood & Kelly (1989), Fullarton *et al*.
654 (1995) and Distel & Wood (1992). Carboxysome presence in Clade C type strains
655 demonstrated by Scott *et al.* (*unpublished data*). Values are positive (+), negative (-) or not
656 determined (*N.D.*). * demonstrated in strains JR2 and MA2-6 but type strain remains not
657 determined.

658 **Table 2.** Origins and genome properties of strains of *Thiomicrospira*, *Thioalkalimicrobium*
659 and *Hydrogenovibrio* for which genome sequences have been deposited publically in the
660 Integrated Microbial Genomes (IMG) database.

	"Clade A" ***Thiomicrospira***						**"Clade B"** ***Thiomicrorhabdus* gen. nov.**				**"Clade C"** ***Hydrogenovibrio***				
	Tms. pelophila	***Tms. thyasirae***	***Tam. aerophilum***	***Tam. cyclicum***	***Tam. microaerophilum***	***Tam. sibiricum***	***Tms. frisia***	***Tms. chilensis***	***Tms. arctica***	***Tms. psychrophila***	***Tms. halophila***	***Tms. thermophila***	***Tms. crunogena***	***Tms. kuenenii***	***Hydrogenovibrio marinus***
16S rRNA gene sequence identity to:															
Tms. pelophila DSM 1534^T	100	100	97.0	97.0	95.9	97.3	91.6	92.0	92.9	92.9	94.4	92.5	92.1	92.6	92.5
Tms. frisia JB-A2^T	91.4	91.4	90.7	91.0	90.3	90.9	100	96.0	96.0	96.0	94.3	94.2	95.5	94.6	94.2
H. marinus MH-110^T	92.9	92.9	92.0	91.4	91.9	92.2	94.2	94.8	94.0	94.1	95.7	96.5	96.7	97.6	100
General properties															
Colony colour	White	*N.D.*	Pink	Reddish	*N.D.*	Pink	White/ yellow	*N.D.*	*N.D.*	*N.D.*	*N.D.*	Cream	White	White/ yellow	*N.D.*
Heterotrophic	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Carboxysomes	-	+	+	+	*N.D.*	+	*N.D.*	*N.D.*	*N.D.*	*N.D.*	+	+*	+	+	+
G+C fraction (mol%) *In vitro* and (*in silico*)	45.7 (44.5)	45.6 (*N.D.*)	49.5 (45.9)	49.6 (47.0)	49.3 (45.55)	48.9 (*N.D.*)	39.6 (*N.D.*)	49.9 (48.1)	42.4 (41.9)	42.5 (*N.D.*)	56.6 (54.9)	44.1 (*N.D.*)	44.2 (*N.D.*)	42.4 (41.5)	44.1 (43.9)
Maximum specific growth rate on thiosulfate under optimal conditions (h^{-1})	0.3	0.07	0.33	*N.D.*	*N.D.*	0.22	0.45	0.4	0.14	0.2	0.25	*N.D.*	0.8	0.35	0.6
Cell morphology															
Length (µm)	1.0-2.0	0.8-1.2	0.8-1.5	1.0	2.0-5.0	0.8-1.5	1.0-2.7	0.8-2.0	1.2-1.5	1.3-1.7	1.0-2.0	0.8-1.5	1.5-3.0	1.0-2.5	1.0-2.0
Width (µm)	0.2-0.3	0.3	0.4-0.5	0.3-0.4	1.0-2.0	0.4-0.5	0.3-0.5	0.3-0.5	0.5-0.6	0.5-0.6	0.3-0.5	0.4-0.7	0.4-0.5	0.3-0.4	0.2-0.5
Shape of cells under optimal and (stress) conditions	Vibrio (Spiral)	Vibrio (Spiral)	Rod (Spiral)	Open ring	Vibrio (rod)	Vibrio	Rod	Rod	Rod	Rod	Vibrio	Curved Rod	Vibrio	Vibrio	Vibrio
Motility	+	-	+	+	+	+	±	+	±	+	+	+	+	+	+
Flagella	1-2	0	3	*N.D.*	1	1	*N.D.*	*N.D.*	1	1	1	1	1	*N.D.*	1
Growth conditions															
pH optimum	7.0	7.5	9.8-10.0	9.5	9.0	9.8-10.0	6.5	7.0	7.3-8.0	7.5-8.5	7.5-7.8	6.0	7.5-8.0	6.0	6.5
pH minimum	5.9	7.0	7.5	7.5	8.0	7.5	4.2	5.3	6.5	6.5	6.5	5.0	5.0	4.0	*N.D.*
pH maximum	6.0	8.4	10.6	10.5	10.0	10.6	8.5	8.5	9.0	9.0	8.5	8.0	8.5	7.5	*N.D.*
Temperature optimum (°C)	28-30	35-40	*N.D.*	*N.D.*	25-28	*N.D.*	32-35	32-27	11.5-13.2	14.6-15.4	30	35-40	28-32	29-33.5	37
Temperature minimum (°C)	3.5	3.5	*N.D.*	*N.D.*	*N.D.*	*N.D.*	3.5	3.5	-2.0	-2.0	20	15	4	3.5	*N.D.*
Temperature maximum (°C)	42	42	41	*N.D.*	*N.D.*	41	39	42	20.8	20.8	43	55	38.5	42	*N.D.*
NaCl optimum (mM)	470	430	*N.D.*	*N.D.*	600	*N.D.*	470	470	250	250	1,500	205-342	*N.D.*	470	500
NaCl minimum (mM)	40	250	*N.D.*	*N.D.*	200	*N.D.*	100	100	40	40	500	51	45	100	*N.D.*
NaCl maximum (mM)	1,240	3,000	1,200	1,500	1,200	1,200	1,240	1,240	1,240	1,240	3,500	1,197	*N.D.*	640	*N.D.*
Physiology															
Tetrathionate as an energy source	+	+	+	+	-	-	+	+	+	+	-	*N.D.*	+	+	+
Elementary sulfur as an energy source	*N.D.*	*N.D.*					*N.D.*	+	*N.D.*	*N.D.*	*N.D.*	*N.D.*	*N.D.*	+	+
Auxotrophic for vitamin B_{12}	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Production of elementary sulfur when growing on thiosulfate at neutrality	+	+	-	-	-	-	±	+	+	+	*N.D.*	+	+	-	-
Molecular hydrogen as an energy source	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Diazotrophy	*N.D.*	*N.D.*	*N.D.*	*N.D.*	*N.D.*	*N.D.*	*N.D.*	*N.D.*	*N.D.*	*N.D.*	*N.D.*	+	-	*N.D.*	-
Dominant fatty acids	*N.D.*	$C_{18:1}$ $C_{16:1}$ $C_{16:0}$ $C_{14:0}$	*N.D.*	*N.D.*	*N.D.*	*N.D.*	*N.D.*	$C_{16:1}$ $C_{18:1}$ $C_{16:0}$ $C_{18:0}$	$C_{16:1}$ $C_{18:1}$ $C_{16:0}$ $C_{14:1}$	$C_{16:1}$ $C_{18:0}$ $C_{16:0}$ $C_{12:1}$	*N.D.*	$C_{16:1}$ $C_{16:0}$ $C_{18:0}$ $C_{18:1}$	*N.D.*	*N.D.*	$C_{16:1}$ $C_{16:0}$ $C_{18:0}$
[NiFe]-hydrogenase genes	-	*N.D.*	-	-	-	*N.D.*	*N.D.*	-	-	*N.D.*	-	*N.D.*	+	-	+
RuBisCO Forms															
Form IAc	+	*N.D.*	+	+	+	+	*N.D.*	+	-	*N.D.*	+	*N.D.*	*N.D.*	+	+
Form IAq	-	*N.D.*	-	-	-	-	*N.D.*	+	+	*N.D.*	+	*N.D.*	*N.D.*	+	+
Form II	+	*N.D.*	-	-	-	-	*N.D.*	+	+	*N.D.*	+	*N.D.*	*N.D.*	+	+

Species	**Strain**	**Genome size (MBp)**	**G+C fraction (mol%)**	**Protein coding genes**	**IMG/M Genome ID Number**	**Source**	**Clade affiliation on basis of 16S rRNA gene**
Tms. pelophila	DSM 1534^{T}	2.11	44.4	1,961	2565957139	Marine mud, Frysian Islands, Netherlands.	Clade A *Thiomicrospira*
Tam. cyclicum	DSM 14477^{T}	1.93	47.0	1,734	2505679009	Mono Lake, California, USA.	
Tam. aerophilum	AL3^{T}	2.16	45.9	2,061	2506783063	Soda lake in Hadyn, Russia.	
Tam. microaerophilum	ASL8-2^{T}	3.10	45.6	2,855	2593339162	Soap Lake, Washington State, USA.	
Tms. chilensis	Ch-1^{T}	2.44	48.1	2,191	2537562247	Marine mud, Bay of Conception, Chile.	Clade B *Thiomicrorhabdus* gen. nov.
Tms. arctica	SVAL-E^{T}	2.55	41.9	2,214	2522572127	Marine sediment, Svalbard.	
Thiomicrospira sp.	Milos-T2	2.66	38.2	2,349	2561511141	Shallow sea hydrothermal vent, Greece.	
Thiomicrospira sp.	Kp2	2.73	39.9	2,411	2503538029	Deep sea hydrothermal vent, North East Pacific Ocean.	
H. marinus	MH-110^{T}	2.61	43.9	2,492	2571042915	Seawater, Shonan Coast, Japan.	Clade C *Hydrogenovibrio*
Tms. kuenenii	JB-A1^{T}	2.45	41.5	2,202	2540341246	Marine sediment, Wadden Sea, Germany.	
Tms. halophila	HL 5^{T}	2.36	54.9	2,127	2517572244	Sediment from hypersaline lake, Kulunda Steppe, Russia.	
Thiomicrospira sp.	Milos-T1	2.34	43.9	2,520	2576861815	Shallow sea hydrothermal vent, Greece	
Thiomicrospira sp.	WB1	2.28	53.7	2,103	2690315833	Brine-seawater interface, Kebrit brine pool, Red Sea.	
Thiomicrospira sp.	XCL-2	2.43	43.1	2,200	637000325	Deep sea hydrothermal vent, Galapagos Rift.	
Thiomicrospira sp.	XS5	2.63	50.1	2,447	2675903511	Brine-seawater interface, Kebrit brine pool, Red Sea.	
Thiomicrospira sp.	MA2-6	2.68	50.1	2,520	2571042363	Mid Atlantic Ridge	
Thiomicrospira sp.	JR-2	2.61	50.5	2,387	2506783050	Deep sea hydrothermal vent,	

Figure 1

Click here to download Figure Figure 1 16S rRNA gene trees A and B.tif

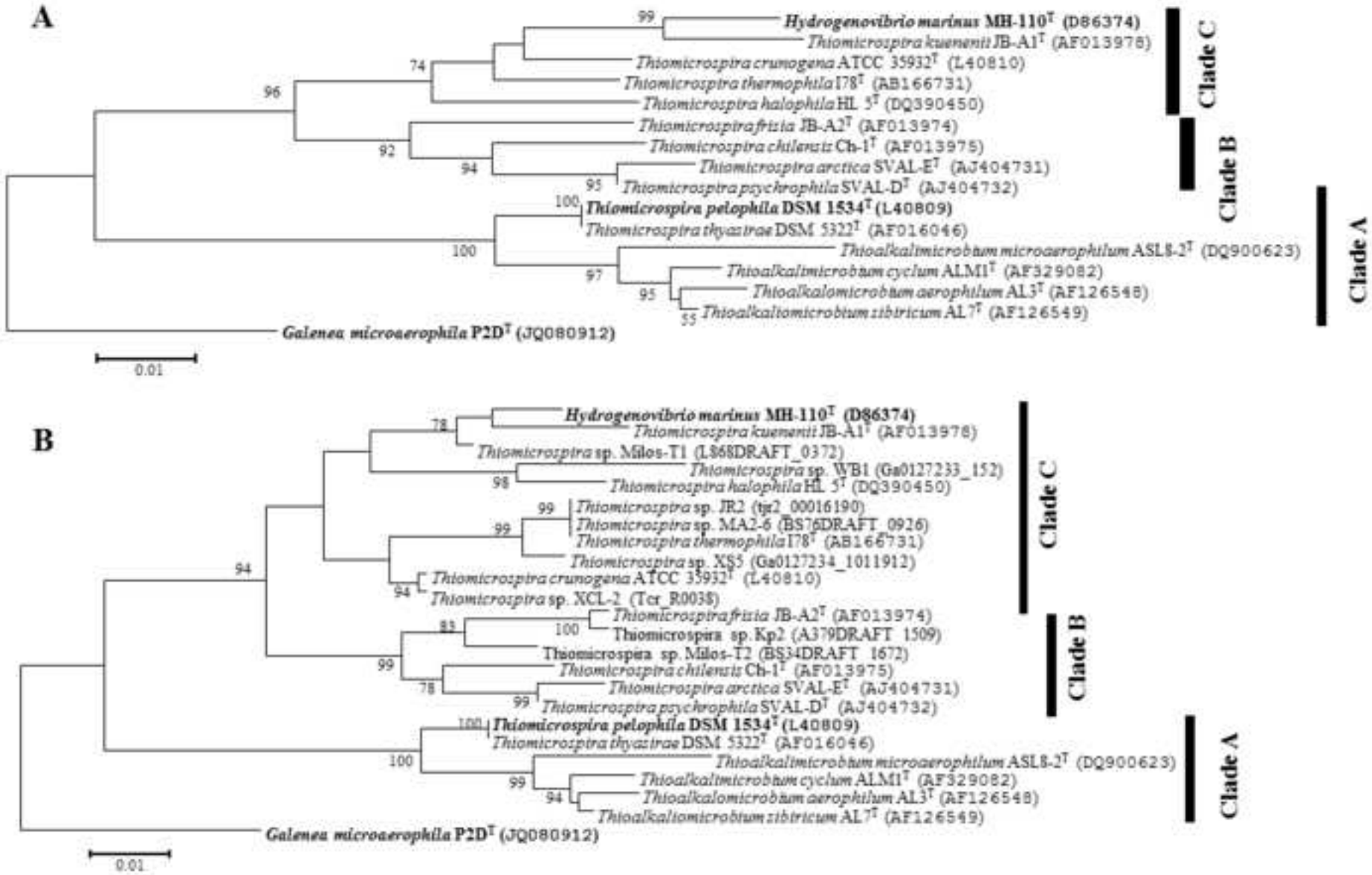


Figure 2

Click here to download Figure Figure 2 AA tree of rMLST.tif

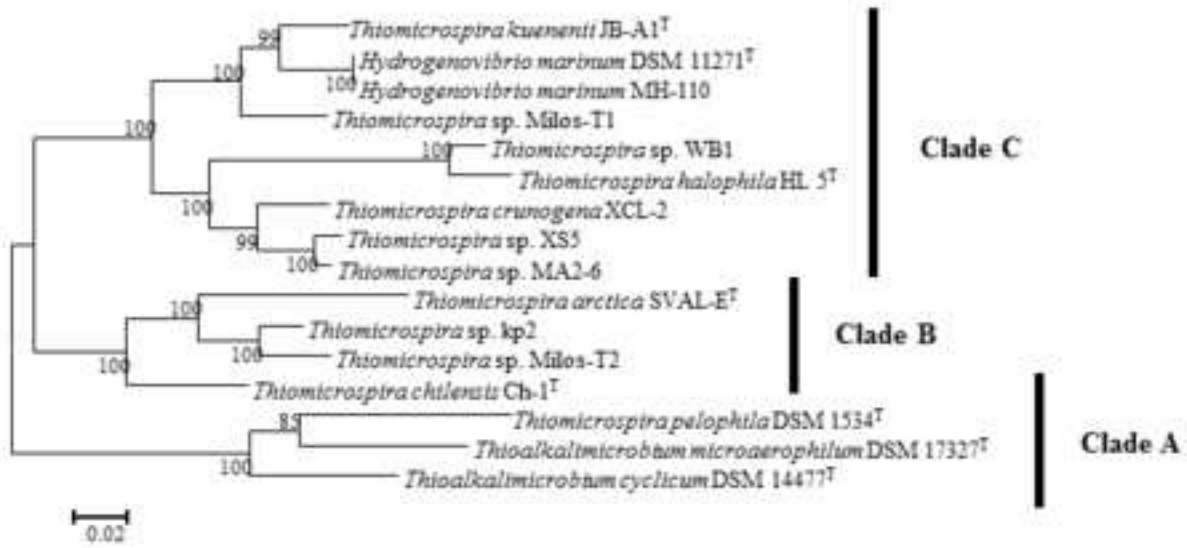
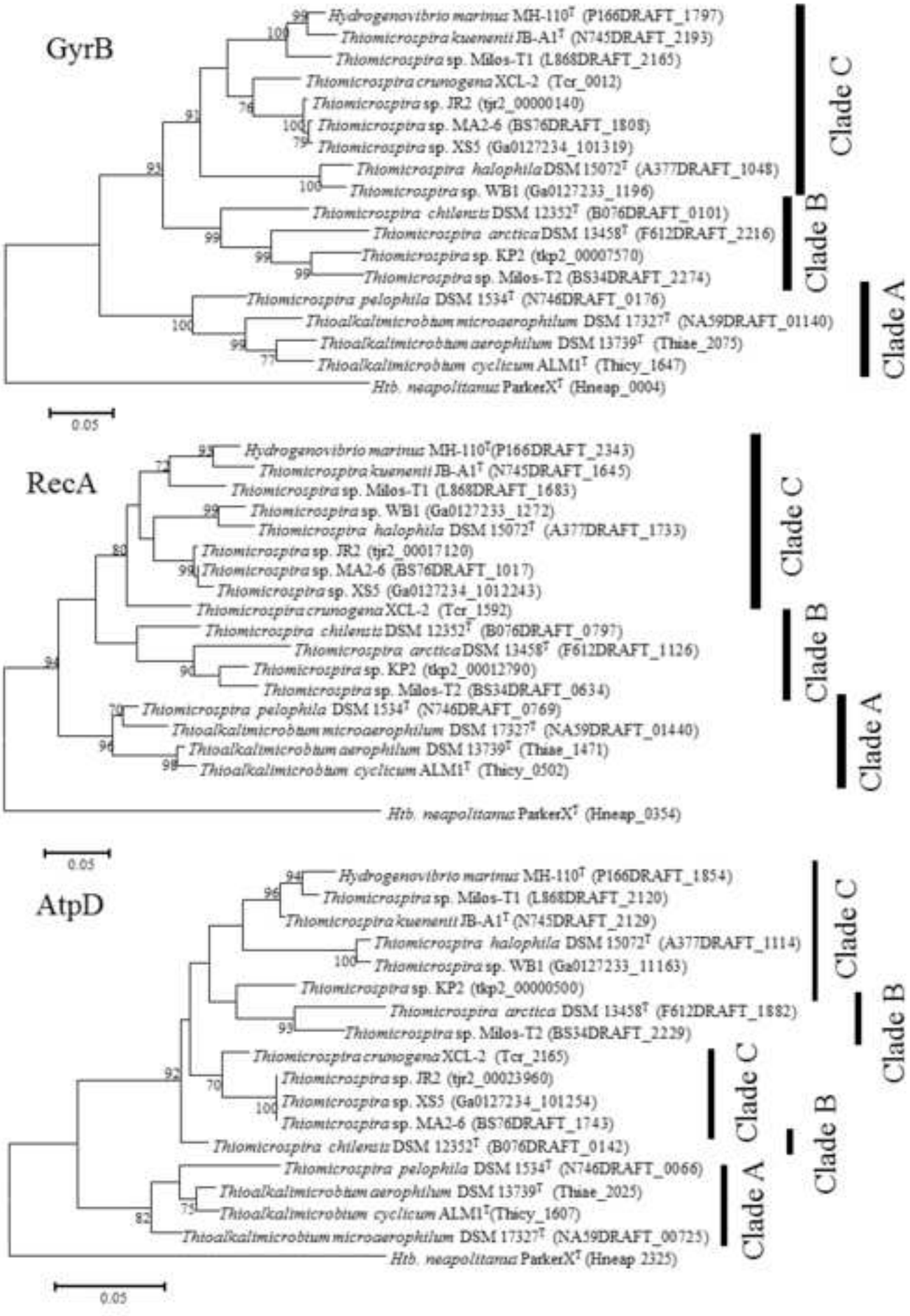


Figure 3

Click here to download Figure Figure 3 GyrB RecA AtpD.tif



An evaluation of *Thiomicrospira*, *Hydrogenovibrio* and *Thioalkalimicrobium*: reclassification of 4 species of *Thiomicrospira* to each *Thiomicrorhabdus* gen. nov. and *Hydrogenovibrio*, and reclassification of all 4 species of *Thioalkalimicrobium* to *Thiomicrospira*.

Rich Boden[1,2*], Kathleen M. Scott[3], John Williams[3], Sydney Russel[3], Kirsten Antonen[3], Alex W. Rae[1] and Lee P. Hutt[1,2]

Supplementary Information:

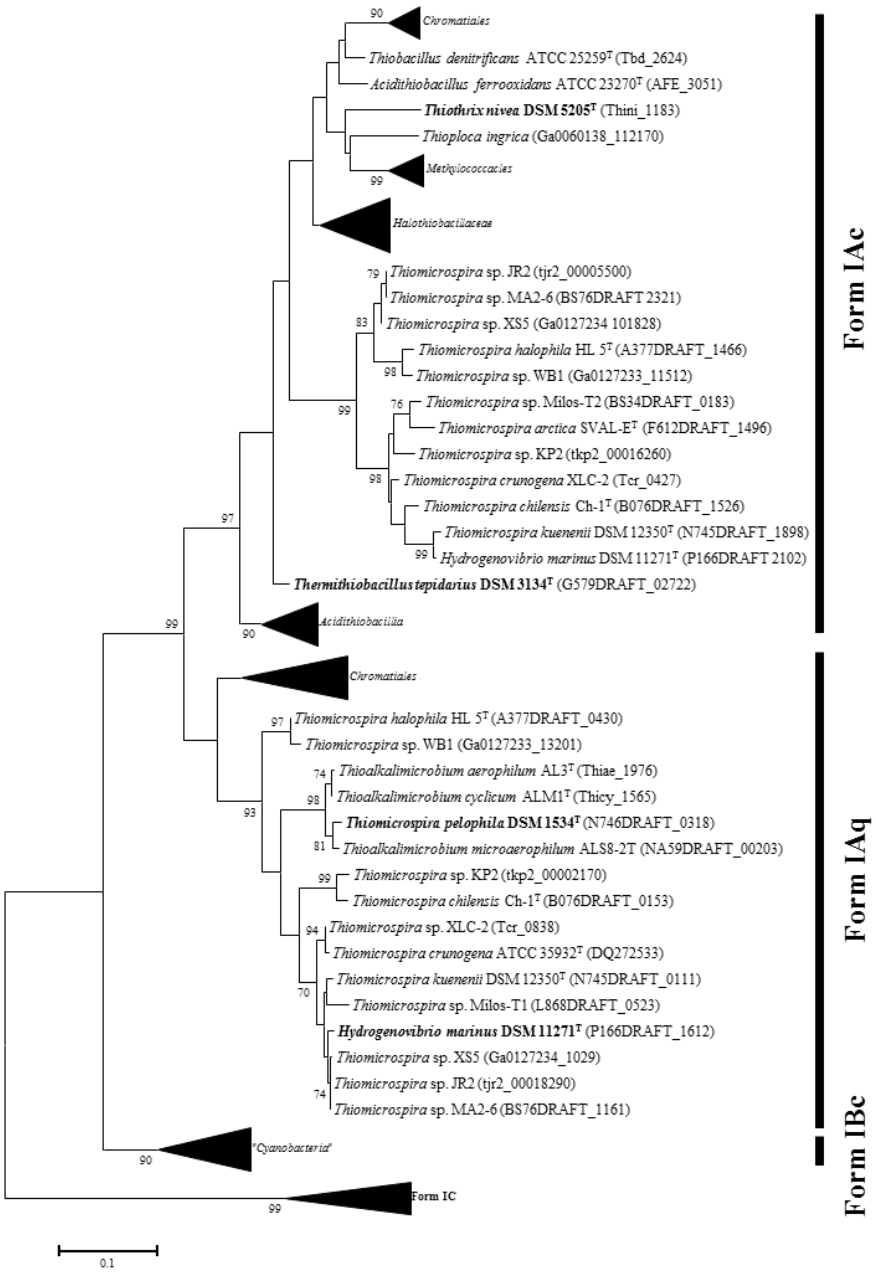
Details of the history of *Thiomicrospira thyasirae* and sequence analysis thereof.

Following personal communication from Prof. Donovan P. Kelly and Dr Ann P. Wood (now both retired), it was found in their archives that, when *'Thiobacillus thyasiris*' strain TG-2^T was deposited into Culture Collections in the late 1980s, Dr Hans Hippe of the then DSM wrote back to the authors stating that the strain they had sent was contaminated, which Wood and Kelly had also realised at around the same time, noting that this contamination was present at ≤ 1.0 % on the basis of colony forming unit counts and was not present during physiological characterisation. Dr Wood spent some months re-purifying and verifying a sulfur-oxidising autotroph away from its heterotrophic consort, and sent the former back to Dr Hippe, which was curated as DSM 5322^T. This strain was in turn sent by the DSM to the ATCC, which accepted it without reporting any contamination (A. P. Wood and D. P. Kelly, *personal communication*). An authentic 16S rRNA gene sequence of TG-2^T was obtained by Distel and Wood (1992), curated into the GenBank™ as L01478 and L01479 (partial sequences), which we have concatenated by alignment against that of *Tms. thyasirae* DSM 5322^T (NR_024854), with a small gap present at *c*. 520 bp owing to the join, and then aligned it (MUSCLE) into the data used to generate Figure 1a. This alignment was then used to build a maximum likelihood tree with partial deletion of gaps using the Tamura-Nei model (*data not shown*), which showed extremely close affiliation of TG-2^T with the DSM 5322^T sequence, with the minor ambiguity presumably due to mis-calling of bases from sequencing gels used in the early 1990s (A. P. Wood, *personal communication*).

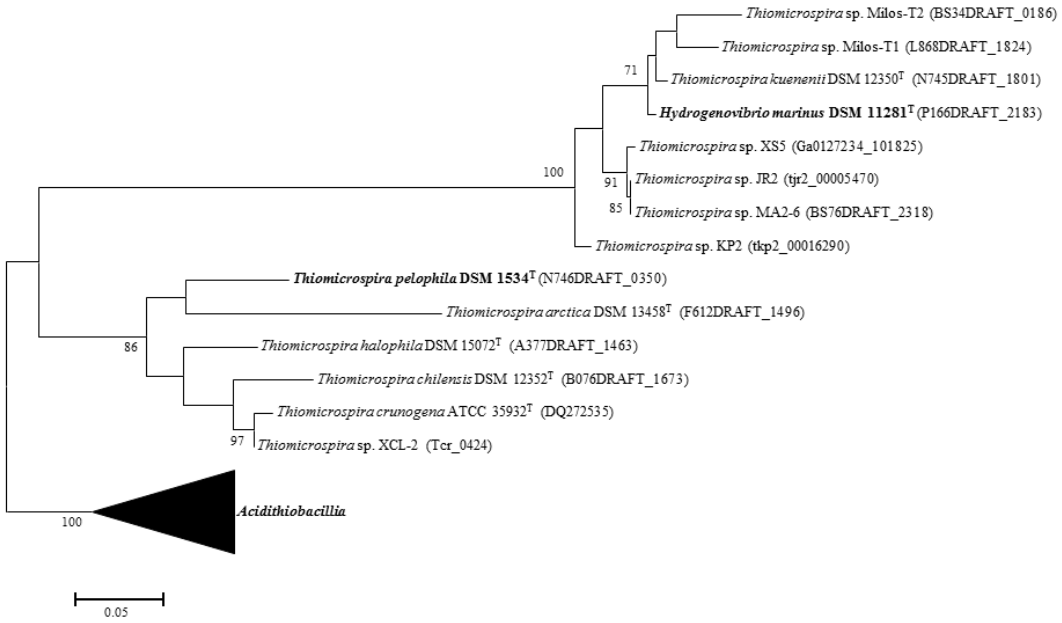
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	100.00															
2	22.40	100.00														
3	20.30	17.20	100.00													
4	19.50	16.60	19.20	100.00												
5	18.80	17.50	19.80	20.80	100.00											
6	19.30	12.50	12.50	19.80	21.60	100.00										
7	18.10	18.00	19.50	19.30	18.60	12.50	100.00									
8	13.20	18.00	21.50	19.10	18.50	12.50	20.90	100.00								
9	18.60	27.50	20.50	20.10	21.30	14.30	20.50	19.10	100.00							
10	19.60	18.60	19.90	19.30	18.40	12.50	20.60	19.90	20.30	100.00						
11	20.00	16.80	20.70	21.70	19.90	12.50	19.90	21.10	22.10	20.50	100.00					
12	19.70	18.00	20.60	19.60	18.80	15.00	21.10	19.70	19.70	19.90	21.50	100.00				
13	20.10	17.80	22.10	19.50	18.60	15.90	20.90	20.50	19.50	19.90	22.00	**70.60**	100.00			
14	19.40	17.70	20.50	19.70	19.60	12.50	20.10	19.90	18.90	18.70	20.90	39.70	39.20	100.00		
15	19.00	30.10	19.90	18.00	18.10	12.50	20.40	19.60	21.60	20.10	20.50	19.50	19.40	18.60	100.00	
16	20.60	18.70	20.30	19.30	19.40	14.90	21.10	19.90	19.80	20.20	21.00	20.80	20.90	20.50	20.30	100.00

Supplementary Table S1. *In silico* DNA-DNA hybridisation ("dDDH") percentage hybridisations obtained for genome sequences using the genome-to-genome distance calculator of the DSMZ (GGDC v. 2.1, BLAST+ alignment method and taking data from Formula 2, as recommended in Meier-Kolthoff *et al*. (2013). Numbers represent genome sequences from organisms listed below. Aside from the 100.00% values obtained from autohybridisation, pairings with values greater than the 70.00 % recommended 'cut off' for strains of the same species are emboldened and underlined.

1. *Thiomicrospira pelophila* DSM 1534^{T}. 2. *Tms. arctica* SVAL-E^{T}. 3. *Hydrogenovibrio marinus* DSM 11271^{T}. 4. *Thioalkalimicrobium aerophilum* AL 3^{T}. 5. *Tam. cyclicum* DSM 14477^{T}. 6. *Tam. microaerophilum* DSM 17327^{T}. 7. *Tms. kuenenii* JB-A1^{T}. 8. *Tms. chilensis* Ch-1^{T}. 9. *Tms. halophila* HL 5^{T}. 10. *Tms. crunogena* XCL-2. 11. *Tms.* sp. MILOS T1. 12. *Tms.* sp. JR2. 13. *Tms*. sp. MA2-6. 14. *Tms*. sp. XS5. 15. *Tms.* sp. WB1. 16. *Tms.* sp. kp2.



Supplementary Figure S1. Maximum likelihood tree of amino acids derived from ribulose-1,5-bisphosphate carboxylase/oxygenase form I large subunit genes (*cbbL*) extracted from the IMG or GenBank™ databases and translated and aligned using MUSCLE in MEGA 7.0.20. Tree was built using the Jones-Taylor-Thornton model with the NNI heuristic method and partial deletion of gaps. Topology with the superior log-likelihood is shown, with numbers at nodes representing the percentage of 5,000 bootstrap replicates for which that topology was preserved (values < 70 % are omitted). Gene ID numbers for IMG or accession numbers for GenBank™ are given in parentheses. A total of 466 amino acids were used, derived from 1,398 bases, in this analysis. Subforms of form I RuBisCO are given based on the properties described by Badger & Bek (2008). Type species of genera are emboldened. Form Ic RuBisCO amino acyl sequences derived from large subunit genes (*cbbL*) from the '*Proteobacteria*' were used as the outgroup.



Supplementary Figure S2. Maximum likelihood tree of amino acids derived from ribulose-1,5-bisphosphate carboxylase/oxygenase form II genes (*cbbM*) extracted from the IMG or GenBank™ databases and translated and aligned using MUSCLE in MEGA 7.0.20. Tree was built using the Jones-Taylor-Thornton model with the NNI heuristic method and partial deletion of gaps. Topology with the superior log-likelihood is shown, with numbers at nodes representing the percentage of 5,000 bootstrap replicates for which that topology was preserved (values < 70 % are omitted). Gene ID numbers for IMG or accession numbers for GenBank™ are given in parentheses. A total of 459 amino acids were used, derived from 1,377 bases, in this analysis. Type species of genera are emboldened. Form II RuBisCO amino acyl sequences derived from *cbbM* genes from the *Acidithiobacillia* were used as the outgroup.

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