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Reclassification of Thiobacillus aquaesulis (Wood & Kelly, 1995) as Annwoodia aquaesulis gen. nov., comb. nov. Transfer of Thiobacillus (Beijerinck, 1904) from the Hydrogenophilales to the Nitrosomonadales, proposal of Hydrogenophilalia class. nov. within the 'Proteobacteria', and 4 new families within the orders Nitrosomonadales and Rhodocyclales.

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Reclassification of *Thiobacillus aquaesulis* (Wood & Kelly, 1995) as *Annwoodia aquaesulis* gen. nov., comb. nov. Transfer of *Thiobacillus* (Beijerinck, 1904) from the *Hydrogenophilales* to the *Nitrosomonadales*, proposal of *Hydrogenophilalia* class. nov. within the '*Proteobacteria*', and 4 new families within the orders *Nitrosomonadales* and *Rhodocyclales*.

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Abstract

The genus *Thiobacillus* comprises 4 species with validly published names, of which *T. aquaesulis* DSM 4255^T (= ATCC 43788^T) is the only species that can grow heterotrophically or mixotrophically – the rest being obligate autotrophs - and has a significant metabolic difference in not producing tetrathionate during the oxidation of thiosulfate during autotrophic growth. On the basis of this and differential chemotaxonomic properties and a 16S rRNA gene identity of 93.4% to the type species *Thiobacillus thioparus* DSM 505^T, we propose that it is moved to a novel genus *Annwoodia* gen. nov., for which the type species is *Annwoodia aquaesulis* gen. nov., comb. nov. We confirm the position of the genus *Thiobacillus* in the *Betaproteobacteria* falls within the *Nitrosomonadales* rather than the *Hydrogenophilales* as previously proposed. Within the *Nitrosomonadales* we propose the circumscription of genera to form the *Thiobacilliaceae* fam. nov. and the *Sterolibacteriaceae* fam. nov. We propose the merging of the family *Methylophilaceae* into the *Nitrosomonadales,* and that the *Sulfuricellaceae* be merged into the *Gallionellaceae,* leaving the orders *Methylophilales* and *Sulfuricellales* defunct. In the *Rhodocyclales* we propose the *Azonexaceae* fam. nov. and the *Zoogloeaceae* fam. nov. We also reject the *Hydrogenophilales* from the *Betaproteobacteria* on the basis of a very low16S rRNA gene identity with the class-proper as well as physiological properties, forming the *Hydrogenophilalia* class. nov. in the '*Proteobacteria*'. We provide emended descriptions of *Thiobacillus*, *Hydrogenophilales*, *Hydrogenophilaceae*, *Nitrosomonadales*, *Gallionellaceae*, *Rhodocyclaceae* and the *Betaproteobacteria.*

The genus *Thiobacillus* (Beijerinck, 1904) in the *Betaproteobacteria* is currently considered as a member of the family *Hydrogenophilaceae* in the order *Hydrogenophilales* (Garrity, 2005*a*). The genus has historically comprised over 30 species though the majority have been transferred to other genera or lost (Boden *et al*. 2012). The genus is regarded as containing 4 species with validly published names – *T. thioparus* (type species, Beijerinck, 1904 – *N.B.* the type strain is Starkey's Culture C^T (= Starkey^T = DSM 505^T) isolated from agricultural soil – Starkey, 1935), *Thiobacillus thiophilus* (Kellermann and Griebler, 2009, isolated from aquifer sediment), *Thiobacillus denitrificans* (*ex.* Beijerinck (1904), Kelly and Harrison 1989, type strain is a sewage isolate from Hutchinson *et al*., 1967) and *Thiobacillus aquaesulis* (Wood & Kelly, 1995 (Validation List 52, 1995), isolated from a thermal sulfur spring, Wood & Kelly, 1988).

All but one of the *Thiobacillus* spp. with validly published names are obligate chemolithoautotrophs (assimilating carbon *via* the transaldolase variant of the Calvin-Benson-Bassham cycle (Beller *et al*., 2006, Hutt *et al.* submitted), utilising reduced sulfur species and elementary sulfur as energy sources, but *T. aquaesulis* can grow heterotrophically on complex media (Wood & Kelly, 1988). It is known that *Thiobacillus* spp. have incomplete versions of Krebs' cycle and instead use some of the enzymes of the cycle in the form of Smith's biosynthetic horseshoe (Smith *et al*., 1967), through which intermediates and amino acids *etc* are synthesised, but heterotrophic growth is not possible. The absence of fumarase and 2-oxoglutarate dehydrogenase has been observed in the genomes of *T. denitrificans* (Beller *et al.* 2006) and *T. thioparus* (Hutt *et al., submitted*) – reviewed by Wood *et al.* (2004), along with other enzymes of Krebs' cycle in the latter. Since *T. aquaesulis* grows on complex media (but not in minimal media supplemented with C_1 compounds, sugars or amino acids individually, Wood & Kelly, 1988), it would be anticipated to have a full version of Krebs' cycle or has some manner of bypass that permits growth in the absence of these core enzymes. It is worth noting that the same lesions of Krebs' cycle are found in all obligate autotrophs examined thus far (Wood *et al*., 2004; Boden *et al*., 2016) and whilst larger lesions are found in some species (*e.g.* Boden *et al*., 2016), 2-oxoglutarate dehydrogenase and usually fumarase and/or succinate dehydrogenase and/or succinyl-coenzyme A synthetase are commonly absent. As such, a facultative autotroph so closely related to obligate autotrophs must have significant core metabolic differences.

Thiosulfate oxidation in organisms that use the Kelly-Trudinger pathway (Boden *et al*., 2016, aka the "S_4I" or "tetrathionate intermediary" pathway) always produce tetrathionate as a detectable intermediate in the medium in the early stages of growth, often alongside trithionate and/or pentathionate, but this varies considerably (observed in dozens of studies from the 1930s onwards, 1930s to 1960s studies are reviewed in Roy & Trudinger, 1970, later studies in Boden *et al*., 2012 and Boden *et al*. 2016). *Thiobacillus* spp. use the Kelly-Trudinger pathway and indeed produce tetrathionate, which is accompanied by an increase in culture pH in the first 24h of growth whilst 100% of thiosulfate is oxidised to tetrathionate, after which tetrathionate is oxidised and the pH falls, usually to around pH 4.5 to pH 5.5, with concomitant production of sulfate. In batch cultures or oxygen-limited chemostats, elementary sulfur is often also produced as an intermediate and is observable within cells of *Thiobacillus* spp. as granules in the periplasmic space (Katayama-Fujimura *et al*., 1984). Whilst *T. aquaesulis* still produces elementary sulfur during growth on thiosulfate, it does not produce detectable levels of any polythionates in the medium, nor does the pH increase during the early stages of growth. The final pH of cultures is usually higher – pH 6.0 or so – even when grown on media with the same starting pH as *T. thioparus*. These differences indicate a potential variant of the Kelly-Trudinger pathway for thiosulfate metabolism that does not proceed *via* tetrathionate (or other polythionates) yet still *permits* growth on tetrathionate and trithionate as sole energy sources. Whilst this tetrathionate-free metabolism could superficially indicate that the Kelly-Friedrich pathway (“Sox”) pathway is in use, the production of elementary sulfur in this way would probably be indicative of this not being the case. There is an implication of a different evolution of the sulfur-oxidation pathway in *T. aquaesulis*, distinct from *Thiobacillus* spp.

Owing to these core physiological differences and alongside phylogenetic and chemotaxonomic data, we present here a case for the reclassification of *T. aquaesulis* to a novel genus. During phylogenetic analyses on the basis of the 16S rRNA (*rrs*) gene, it became apparent that *Thiobacillus* spp. cluster at a great distance from other members of the *Hydrogenophilales* (mean pairwise distance 86.4%), therefore we also consider the position of these taxa within the *Betaproteobacteria* and propose multiple revisions to this class with the creation of several new families and revisions to orders. We also propose the removal of the *Hydrogenophilales* from the *Betaproteobacteria* to form the *Hydrogenophilalia* class. nov.

All chemicals were obtained from Sigma-Aldrich (Poole, UK) and were of “analytical” grade or higher, unless otherwise stated. Glass-distilled deionised water (*i.e.* deionised water that was then glass-distilled) and ‘Class A’ volumetric glassware were used to prepare all media and for all analyses. A calibrated 4-decimal place balance was used throughout. *Thiobacillus aquaesulis* DSM 4255^T and *T. thioparus* DSM 505^T were obtained from the Leibniz-Institut DSMZ Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH. *T. denitrificans* NCIMB 9548^T was a kind gift of Dr Peter Green (NCIMB Ltd, Aberdeen, UK). All strains were maintained in E-basal salts (EBS, Wood & Kelly 1985) (g/1,000.0mL) containing NH_4Cl (0.40), $MgSO_4{\cdot}7H_2O$ (0.80), KH_2PO_4 (4.00), K_2HPO_4 (4.00) and trace metals solution (Kelly & Wood 1998, 10.0mL). EBS was supplemented with 20mM sodium thiosulfate for maintenance and solidified with 15.00g/L Oxoid No 1 agar that had been washed with glass-distilled water and dried before use. Growth on carbon disulfide (contaminating carbonyl sulfide removed by washing according to Skidmore, 1979), dimethylsulfide and dimethyldisulfide was tested per Boden *et al*. 2010, using EBS as the basal salts. All chemotaxonomic tests and microscopy were performed according to Boden *et al*. (2010), using high molecular weight genomic DNA standards from Sigma-Aldrich to calibrate for G+C content determination (*Escherichia coli* Strain B, 50.0 mol%, Cat. No. D4889; and *Micrococcus luteus* ATCC 4698^T 71.9 mol%, Cat. No. D8259, G+C contents are as certified by manufacturer), along with genomic DNA from *Thermithiobacillus tepidarius* (66.8 mol%, Boden *et al*., 2016). G+C contents for *T. thioparus* DSM 505^T and *T. aquaesulis* DSM 4255^T were checked using this method (acid denaturation, Fredericq *et al.* 1969) and gave good agreement with the originally published data (Table I). Whilst this method is not widely used, we have found agreement with data obtained from it to within 1.0 mol% with data from genome sequences providing it is properly calibrated on every use (*cf.* Boden *et al*. 2010; Boden *et al*. 2011).

Phylogenetic analyses of the *Betaproteobacteria* were performed using MEGA 7.0.20 (Kumar *et al*. 2016) on the basis of 16S rRNA gene sequences from taxa with validly published names – almost 500 sequences. All members of the *Betaproteobacteria* based on the www.bacterio.net website and the 2005 release of Bergey's manual (Garrity *et al*., 2005*b*) were included in the analyses *pace Gallionella* as no *bone fide* cultures (thus sequences) exist – as such, the *Gallionellaceae* were represented only by the genus *Ferriphaselus* for the sake of the analysis. Genera *incertae sedis* were included in the analysis as long as they were considered *Betaproteobacteria*. Alignments were performed using the MUSCLE algorithm (Edgar *et al*., 2004) without using the pre-sets for large datasets that reduce accuracy. Phylogenetic trees were constructed using the maximum likelihood method with the Tamura-Nei model (Tamura & Nei, 1993) and pairwise deletion of gaps. 5,000 bootstrap replicates were performed and values at nodes are shown if greater than 70%. The outgroup was the genus *Allochromatium* from the *Gammaproteobacteria*.

For phylum-level phylogenetic analyses to determine the position of classes therein, 16S rRNA gene sequences from the *Betaproteobacteria* were aligned in the same way with representatives from all other classes of the '*Proteobacteria*' (*Alphaproteobacteria*, *Gammaproteobacteria*, *Deltaproteobacteria*, *Epsilonproteobacteria*, *Acidithiobacillia*, *Oligoflexia* and '*Zetaproteobacteria*'), using the same approach as Nakai *et al.* (2014) when proposing the class *Oligoflexia* – representatives were used for each other class rather than using the entire of each class which would give a dataset so divergent (> 4,000 sequences) that it would be troublesome to analyse in a precise manner. The outgroup for these analyses (*pace* maximum parsimony) was the phylum '*Aquificeae*', represented by the genera *Aquifex* and *Hydrogenothermus*. Trees were constructed using maximum likelihood, neighbour joining, minimum evolution (all Tamura-Nei model) and maximum parsimony (unrooted, tree bisection-reduction search method) methods, all with 5,000 bootstrap replications and partial or pairwise deletion of gaps at 95 % cut-off. Supplementary Table S1 gives the full details of all sequences used in these trees. The maximum likelihood, neighbour joining and minimum evolution trees were also reconstructed using a dataset that contained the full *Betaproteobacteria* (> 500 sequences) rather than just representative taxa, which gave identical results *viz*. class-level relationships. Whilst complete, even in collapsed form these tree were too physically large to be practical for publication given the page size of the journal, and the representative taxa trees give identical class-level relationships with near-identical bootstrap values in any case. We have retained these complete *Betaproteobacteria* trees as Supplementary Figures S1 – S3, for reference.

Ribosomal multilocus sequence typing (rMLST, Jolley *et al*. 2012) was also used with regard to phylum-level analyses. Representatives from the '*Proteobacteria*' and the '*Aquificeae*' for which genome sequences are available in the PupMLST database (www.pubmlst.org), with an emphasis on using the type species of the type genera of families across the classes of the '*Proteobacteria*', with type strains used where available. Where type species were not available, other members of the same genus or family were used. Genes encoding 53 ribosomal proteins (*rps*, *rpl* and *rpm* genes) were extracted from each genome using the rMLST platform, using only complete genes, trimmed to the same length and concatenated. Concatamers were downloaded into MEGA, translated into derived amino acyl sequences using the bacterial genetic code and aligned using MUSCLE. Phylogenetic trees were reconstructed from amino acid alignments per the 16S rRNA gene trees but using the Jones-Taylor-Thornton algorithm (Jones *et al*. 1992) and, again, using the phylum '*Aquificeae*' as the outgroup.

Pairwise sequence identities for the 16S rRNA gene were inferred from pairwise distances determined in MEGA for MUSCLE alignments. For comparing 16S rRNA gene sequence identities at genus level, the recommended genus threshold of Yarza *et al.* (2014, hereafter 'Yarza threshold') was used as a guide (94.5 %). For higher taxa, we have used the median values (hereafter 'Yarza median') for family (92.25 %), order (89.20 %), class (86.35 %) and phylum (83.68 %) since the Yarza thresholds do not really apply to the class *Betaproteobacteria*, given the intraclass mean pairwise identity is 88.80%, these values would place most of the class in one single family in one single order and thus not follow the generally accepted taxonomy and systematics of this class. The Yarza medians gave better agreement as the order cut-off would then be 89.20%, greater than the mean intraclass sequence identity, thus able to divide the class fairly accurately into the extant orders. We have used these values as a *guide* for circumscribing higher taxa in this study, but not as an exact 'rule'. We have not considered the *Burkholderiales* or *Neisseriales* in our analyses other than including them in maximum likelihood trees for the sake of completeness.

Properties of DSM 4255^T *versus Thiobacillus* spp. are given in Table I – it can be seen that the identity of the 16S rRNA gene of this strain to *Thiobacillus* spp. is ≤ 93.4 % - below the Yarza threshold of 94.5 % (Yarza *et al.* 2014), indicating that DSM 4255^T does not fall within the same genus as the 3 *Thiobacillus* spp. included in Table I. DSM 4255^T does not form any other close affiliations on the basis of the 16S rRNA gene sequence and thus does not fall into other extant genera. Cells are shorter and thinner than those of *Thiobacillus* spp., it grows heterotrophically, a property not seen in true *Thiobacillus* spp., has a higher optimum growth temperature than all known *Thiobacillus* spp., a higher G+C content, cannot use nitrate as a nitrogen source and does not accumulate tetrathionate or other polythionates in the medium during growth on thiosulfate, implying a different sulfur oxidation pathway. True *Thiobacillus* spp. use the Kelly-Trudinger pathway (Boden *et al*., 2016), in which 100% of thiosulfate is oxidised to tetrathionate by a cytochrome-linked thiosulfate dehydrogenase, prior to oxidation to sulfate – in DSM 4255^T, tetrathionate is either not produced as an intermediate or is converted to sulfate with such rapidity that it does not build up in the medium to any detectable degree (in our hands or in those of Wood & Kelly, 1988). This is a significant metabolic deviation from true *Thiobacillus* spp. and may also comprise a unique evolutionary variation of the Kelly-Trudinger pathway.

It is also worth noting that the dominant fatty acids present in *Thiobacillus* spp. ($C_{15:0}$, $C_{17:0}$, $C_{17:1}$, $C_{16:0}$) differ somewhat from those in DSM 4255^T ($C_{16:0}$, $C_{16:1}$, $C_{15:0}$, $C_{17:1}$), when all grown autotrophically on thiosulfate (Agate & Vishniac, 1973; Fullarton *et al.*, 1995). The phylogenetic position of DSM 4255^T is shown in Figure 1, in which it can be seen that it falls apart from *Thiobacillus* spp. – on this basis we propose that DSM 4255^T form a novel genus – *Annwoodia* gen. nov. – and the new combination *Annwoodia aquaesulis* comb. nov. for the type species.

The genus *Thiobacillus* per *Bergey's Manual of Systematic Bacteriology*, 2nd Edition (Kelly *et al.*, 2005) falls within the *Hydrogenophilales*; however, our more detailed phylogenetic analysis of the entire *Betaproteobacteria* (Figures 1 and 2) – which obviously contains more taxa than in 2003/4 when analyses for the 2005 edition of the *Manual* were presumably performed - shows that these taxa are quite unrelated to this order (identity 87.6% - below the Yarza median for the rank of order), though they can appear superficially similar in trees we constructed containing smaller numbers of sequences, and particularly neighbour-joining trees (*data not shown*). The *Betaproteobacteria* contains the orders *Burkholderiales* (type order), *Neisseriales*, *Methylophilales, Sulfuricellales, Rhodocyclales*, *Hydrogenophilales* and *Nitrosomonadales,* all with validly published names (Garrity *et al*., 2005*b* and Watanabe *et a*l. 2015). *Thiobacillus* spp. and DSM 4255^T form a clade within the *Nitrosomonadales*, which contains in turn 3 families with validly published names – *Nitrosomonadaceae* (type family), *Spirillacae* and *Gallionellaceae*. Figure 1 shows that the orders *Methylophilales* and *Sulfuricellaes* cluster within the *Nitrosomonadales* and on the basis of pairwise distances falling below the Yarza median for family, are probably not separate families but fall within this order. On this basis, we propose that the *Methylophilaceae* and *Sulfuricellaceae* are transferred to the *Nitrosomonadales* and that the former forms a family within that order; however, the *Sulfuricellaecae* have ≥92.5 % pairwise identity with the only sequence available for the *Gallionellaceae* (at or above the Yarza median for the rank of family, *viz.* 92.25%), thus we propose that the genera of the *Sulfuricellaceae* and *Gallionellaceae* should be circumscribed as one family and that the name *Gallionellaceae* (Henrici & Johnson, 1935) takes priority over *Sulfuricellaceae* (Watanabe *et al*., 2015), according to Rule 47a of the *Bacteriological Code*. This leaves the orders *Methylophilales* and *Sulfuricellales* defunct. We propose that the other two clades within the *Nitrosomonadales* are each circumscribed to form families - the *Thiobacillaceae* fam. nov. and the *Sterolibacteriaceae* fam. nov., named on the basis of the earliest legitimate genus in accordance with the *Code*, and circumscribed on the basis of pairwise 16S rRNA gene distances falling above the Yarza median within each group as well as clustering into distinct phylogenetic clades in Figure 1 and 2 – the genera that these circumscribe are given in the descriptions of these novel taxa below. It is worth noting that genera of the *Sterolibacteriaceae* were formerly assigned to the *Rhodocyclales* rather than the *Nitrosomonadales*; however, our analyses demonstrate affiliation with the *Nitrosomonadales* on the basis of maximum likelihood trees of the whole class *Betaproteobacteria*. It can be seen from Figure 1 that a small clade of genera *incertae sedis* falls within the *Nitrosomonadales*: these are *Chitinovorax, Rivicola* and *Leeia*. These genera may warrant a further family being defined (the "*Leeiaceae*") in the *Nitrosomonadales*, or, may fall within the latter, but we do not have sufficient data to recommend either at this time.

In the 2005 revision of *Bergey's Manual* and on www.bacterio.net, the order *Rhodocyclales*, contains one family – the *Rhodocyclaceae* – and no further divisions (Garrity et al., 2005*c*). In addition to the circumscribing of some genera of this family into the *Nitrosomonadales*, on the basis of our analyses we propose circumscribing two novel families in the *Rhodocyclales* (Figure 1) - the *Azonexaceae* fam. nov. and the *Zoogloeaceae* fam. nov., leaving 4 genera in the *Rhodocyclaceae* (type family). *Azovibrio restrictus* S5b2^T remains *incertae sedis* at this point as it falls between these novel taxa somewhat and has sequence identity from the type genera thereof of 92.7 % (*Rhodocyclaceae*) and 91.5 % (*Zoogloeaceae*), even though it apparently clusters separately from the *Rhodocyclaceae*. We therefore do not confirm which family this genus should be assigned to and further work will probably be required since it may represent the type genus of the "*Azovibrionaceae*", or may form part of the one of the above novel families.

The order *Hydrogenophilales* clusters very distantly from the other *Betaproteobacteria* and has a sequence identity from type genus to type genus of the *Burkholderiales* (type order of the class) of exactly 86.35 % - the same value as the Yarza median for the rank of class. It can be seen from Figure 2 that the branching of the two genera of the *Hydrogenophilales* is indeed separate from both the *Betaproteobacteria* and all other classes in the phylum, in terms of forming a distinct branch from the *Betaproteobacteria* from a well-supported node (bootstrap value 99 - 100 % across all 4 tree reconstruction methods, even in those in which the reconstruction of other regions of the phylum were poor and supported only by low bootstrap values). These data indicate that whilst both of these taxa do belong to the '*Proteobacteria*', they are confidently distinct from one another, although pairwise distances much also be considered in addition to tree topology. The pairwise distance from *Hydrogenophilus thermoluteolus* (type species of type genus of type family of type order of the *Hydrogenophilalia* class. nov.) to the equivalent species of the other classes of the '*Proteobacteria*' (*Alphaproteobacteria*, 79.1 %; *Gammaproteobacteria*, 85.70 %; *Deltaproteobacteria*, 79.40 %; *Epsilonproteobacteria*, 66.60 % *Oligoflexia*, 82.80 %; *Acidithiobacillia*, 83.30 % and '*Zetaproteobacteria*', 81.20%) are such that the *Hydrogenophilales* are not affiliated to any class of the phylum on this basis either. It is worth noting that whilst our analysis replicated the broadly accepted structure of the phylum '*Proteobacteria*' in most trees shown, the *Oligoflexia* in our analyses fell within the clade representing the *Deltaproteobacteria* and (in all tree drawing methods employed) formed a branch from a well-supported node (bootstrap value 100 %) in all cases alongside *Pseudobacteriovorax antillogorgiicola* in the *Bdellovibrionales* (pairwise distance 89.1 %, with similar distances to others in this order, which cluster around it in the tree), and thus may warrant further study to ascertain the relative positions of these two classes.

Figure 3 reconstructs the phylogeny of the '*Proteobacteria*' from a 53-gene concatamer of ribosomal protein genes, analysed at amino acid level – in this analysis, the phylogeny is well-constructed with a majority of well-supported nodes and matching the generally accepted topology of the phylum. The *Hydrogenophilalia* class. nov. are, again, branched distinct from the *Betaproteobacteria* from a fully supported node, reinforcing their separation from the latter. Unlike Figure 2, this analysis was sufficient to separate the *Oligoflexia* from the *Deltaproteobacteria*. The same topology with regard to the *Hydrogenophilalia* class. nov. versus the *Betaproteobacteria* is also supported from well-supported nodes (95 -100% of bootstrap replicates) in neighbour-joining and minimum evolution trees (Supplementary Figures S4 and S5).

Table 2 gives properties of each family examined in this study, alongside the *Burkholderiales* and *Neisseriales* for reference. All genera examined contained palmitic acid ($C_{16:0}$) amongst the dominant fatty acids and ubiquinone-8 (UQ-8) as their dominant respiratory quinone, as well as each family having aerobic representitives. Most of the *Rhodocyclales* also contained unsaturated fatty acids $C_{16:1}$ and $C_{18:1}$, but the *Zoogloeaceae* fam. nov. are united by their presence of 3-hydroxycapric acid ($C_{10:0}$ 3-OH) as one of their dominant fatty acids in many genera, distinguishing this family from the rest of the order. The respiratory quinones of this order are effectively unified by their variability as two families contain a second quinone at a significant concentration, *viz*. menaquinone-8 (MK-8) in *Rhodocyclaceae* and rhodoquinone-8 (RQ-8) in *Zoogloeaceae* fam. nov. (the latter also found in two genera of the *Burkholderiales*). The *Nitrosomonadales* often contain stearic acid ($C_{18:0}$) at a high concentration but three families – the *Sterolibacteriaceae* fam. nov., the *Spirillaceae* and the *Gallionellaceae* – also contained significant amounts of hydroxylated fatty acids 2-hydroxypentadecylic acid ($C_{15:0}$ 2-OH) and/or $C_{10:0}$ 3-OH. In contrast, the *Hydrogenophilales* class. nov. are distinguished by their high concentrations of ω-cyclohexyl fatty acids (*viz.*

cyclohexylmargaric ($C_{17:0}$ *cyclo*) and cyclohexylnonadecylic acids ($C_{19:0}$ *cyclo*)) in both known genera, accounting for up to *c*. 30 % of the fatty acid content in *Tepidiphilus* spp. (Poddar *et al*., 2014). These fatty acids are relatively rare in nature, and in the domain *Bacteria* are conserved to a relatively small number of taxa – canonical examples being species of the genera *Alicyclobacillus* (‘*Firmicutes*’), *Sulfobacillus* (*Clostridia*), *Propionibacterium* and *Curtobacterium* (*Actinobacteria*) – but not commonly in the ‘*Proteobacteria*’, except at trace amounts (Suzuki et al., 1981; Kusano *et al*., 1997; da Costa *et al.*, 2011), with the obvious exception of *Pandoraea* spp. in the *Betaproteobacteria* (*Burkholderiales*). Where they do occur, they are usually considered as an adaptation to heat or low pH, thus it is no surprise to find them in an order united by temperature optima of 50-65 °C and which are universally isolated from high-temperature extreme environments. Their pH optima (6.5 – 8.0) do not indicate acidophilia, thus low pH is not likely to be the cause of the evolution of these fatty acids in this group. This, along with their profile as obligate thermophiles (distinct from the *Betaproteobacteria* examined in Table 2) represents a significant physiological difference from the *Betaproteobacteria sensu stricto*, further evidencing their status as a separate class.

Morphologically, there is much variation across these groups, just as in the *Burkholderiales* and *Neisseriales* – the *Nitrosomonadales* contains a great diversity of rods, cocci, spirillae, reniform cells and stalk-forming cells (*sensu* iron oxidisers, rather than *Hyphomicrobium*-esque stalks), but most families are united in their morphology *e.g.* the *Thiobacillaceae* fam. nov. are all rods, the *Spirillaceae* are all spirillae. Flagellation is similarly fairly uniform at family level, with atrichous, monotrichous or amphitrichous cells observed each in separate families. It is worth noting that the *Nitrosomonadales* is listed as ‘atrichous’ but a few *Nitrosomonas* spp. are monotrichous under the right conditions (Garrity *et al*. 2005*e*).

Metabolically, the *Rhodocyclales* contain a wide array of terminal electron acceptors and are united by their widespread anaerobic growth, but we must, as always, exercise caution that workers studying the *Nitrosomonadales* may simply not have routinely tested *e.g.* (per)chlorate as a terminal electron acceptor and thus use of these apparently more exotic electron acceptors may actually be widespread. The *Nitrosomonadales* mostly only grow by respiration of oxygen or nitrate, though several members of the *Sterolibacteriaceae* fam. nov. can also use Fe(II) or Mn(IV) as terminal electron acceptors.

Heterotrophy across the *Nitrosomonadales* and *Rhodocyclales* is relatively restricted in comparison to the *Neisseriales* and *Burkholderiales*, often to less routine carbon sources such as sterols or aromatics – more so in the former order - with sugars and complex media only used by some families. Methylotrophy is present in the *Methylophilaceae* and *Sterolibacteriaceae* fam. nov., as is C_1 autotrophy (dissimilation of C_1 compounds to carbon dioxide which is then assimilated *via* the Calvin-Benson-Bassham cycle, Anthony, 1982) in the *Thiobacillaceae* fam. nov., namely on methylated sulfur compounds. Methylotrophy is also found in some *Neisseriales*, thus is relatively widespread across the class.

Autotrophy in the *Nitrosomonadales* is associated with carboxysome production in the *Nitrosomonadaceae* and *Thiobacillaceae* fam. nov. but none are observed in electron micrographs of autotrophic *Sterolibacteriaceae* fam. nov., or *Gallionellaceae* – it is interesting to note that carboxysomes are not observed elsewhere in the *Betaproteobacteria* but are prevalent in autotrophic *Gammaproteobacteria* and the *Acidithiobacillia*, which both share common ancestry with the *Betaproteobacteria* (Figure 2). Most autotrophs in the *Nitrosomonadales* use reduced sulfur species; however, the *Nitrosomonadaceae* use ammonium or nitrite but not sulfur as an electron donor, and the *Gallionellaceae* use ferrous iron, forming three distinct functional guilds of autotroph that marry with the phylogeny at family level. A similar diversity of autotrophic energy sources is found in the

Burkholderiales. In contrast, autotrophy in the *Rhodocyclales* is not associated with carboxysome production and is either photoautotrophic (*Rhodocyclaceae*) or lithoautotrophic (*Zoogloeaceae* fam. nov.) with only molecular hydrogen as an electron donor. The *Hydrogenophilalia* class. nov. contains both autotrophs and heterotrophs, but only molecular hydrogen has been identified as an electron donor thus far. It is worth noting that, as in the *Acidithiobacillia* and *Thiotrichales*, use of molecular hydrogen for autotrophic growth is relatively promiscuous and has little continuity with phylogeny (Hedrich & Johnson, 2013; Boden *et al., submitted*), and is commonly found across the *Betaproteobacteria* in lineages that do not otherwise contain autotrophs.

Each family of the *Rhodocyclales* is united by a small range of G+C contents (3.5, 3.7 or 9.7 mol% ranges, which are actually inside of the *c*.10 mol% range within which most congeneric species fall (Fournier *et al*., 2005), and order range of 9.7 mol%). The *Nitrosomonadales* has broader ranges within some families (2.0, 3.5, 4.5, 11.9, 14.4 or 15.0 mol% ranges and order range of 30.0 mol%), with the *Nitrosomonadaceae* and *Methylophilaceae* having the largest ranges. The orders *Neisseriales* and *Burkholderiales* have order ranges of 28.8 and 32.9 mol%, respectively, suggesting that the ranges of the *Nitrosomonadales* and *Rhodocyclales* following our revisions are not excessively diverse. The very low G+C contents of the *Spirillaceae* are somewhat unexpectedly low in terms of the other families examined in the order, but they do fall somewhat distantly from the rest of the order and form one of the deeper branches therein (Figure 3). Similarly, in the *Burkholderiales*, *Thermothrix* spp. have an ‘unusually’ low G+C content for the order (39.7 mol%). The pairwise distance from *Nitrosomonas europaea* (type species of type genus of type family) to *S. winogradskyi* (neotype species of *Spirillum*) is 86.80 %, below the Yarza median for the rank of order (89.20 %), indicating that the *Spirillaceae* may represent a separate order; however, this will obviously require further investigation.

Metabolically, the *Hydrogenophilales* contain a genus of each autotrophs and heterotrophs, the former using molecular hydrogen as an electron donor. They are unified by the same dominant respiratory quinone (ubiquinone-8) and a relatively small range in G+C contents – 58.6 – 65.0 mol%.

On the basis of phylogenetic, metabolic and fatty acid diversity distinct from the *Betaproteobacteria* examined, we therefore propose that the order *Hydrogenophilales* be circumscribed to form a novel class, the *Hydrogenophilia* class. nov. – and that the order (*Hydrogenophilales*) and the only family therein (*Hydrogenophilaceae*) be revised to exclude the genus *Thiobacillus,* which is within the *Betaproteobacteria sensu stricto*.

Emended description of the *Betaproteobacteria* (Garrity *et al*., 2006, Garrity et al., 2005*b*)

Betaproteobacteria (Be.ta.pro.te.o.bac.te'ri.a. Gr. n. *beta* (*βήτα*), name of the second letter of the Greek alphabet; Gr. n. *Proteus* ocean god able to change shape; Gr. n. *bakterion* (*βακτήριων*) a small rod or staff; N.L. fem. pl. n. *Betaproteobacteria*, class of the *Bacteria* having 16S rRNA gene sequences related to those of the order *Spirillales*).

This class is circumscribed on the basis of 16S rRNA gene sequences, ribosomal protein gene concatamer analysis, physiology and metabolism, and contains the orders *Burkholderiales*, *Neisseriales*, *Rhodocyclales* and *Nitrosomonadales*. Ubiquinone-8 is always found as one of the dominant respiratory quinones, but menaquinones and rhodoquinones are also found as major components in some orders. Metabolically and morphologically diverse. G+C contents range from about 35 – 75 mol%. $C_{16:0}$ is typically found amongst dominant fatty acids, along with $C_{16:1}$ and $C_{18:1}$.

Type order: *Burkholderiales* (Garrity *et al*., 2006).

Description of *Hydrogenophilalia* class. nov.

Hydrogenophilalia (Hy.dro.ge.no.phi.la'li.a. N.L. masc. n. *Hydrogenophilus* type genus of the type family of the type order; -alia suffix to denote class; N.L. fem. pl. n. *Hydrogenophilalia*, class of the *Bacteria* having 16S rRNA gene sequences related to those of the order *Hydrogenophilales*).

This class has been circumscribed on the basis of 16S rRNA gene sequences, ribosomal protein gene concatamers and a phylogenetic distance from the *Betaproteobacteria* greater than the Yarza median for class. It shows no affinity to other classes. This class comprises one order – the *Hydrogenophilales* (type order), containing thermophilic, heterotrophic organisms (using fatty acids, amino acids, aromatic acids and complex media) and autotrophic organisms that use molecular hydrogen as an energy source. Carboxysomes are not observed in electron micrographs of autotrophic members. Contain ubiquinone-8 as the dominant respiratory quinone and menaquinones and rhodoquinones are not observed. Contain ω-cyclohexyl saturated fatty acids as major components of the lipid fraction, probably as an adaptation to high temperature.

Type order: *Hydrogenophilales* (Garrity *et al*. 2006)

Emended description of *Hydrogenophilales* (Garrity *et al.* 2006, Garrity *et al.* 2005*a*)

Hydrogenophilales (Hy.dro.ge.no.phi.la'les. N.L. masc. n. *Hydrogenophilus* type genus of the type family; -ales suffix to denote order; N.L. fem. pl. n. *Hydrogenophilales*, the *Hydrogenophilus* order).

This order is re-defined following the circumscribing of members of this family to form the *Thiobacillaceae* in the *Nitrosomonadales*. On the basis of 16S rRNA gene sequences, comprises the *Hydrogenophilaceae*.

Type family: *Hydrogenophilaceae* (Garrity *et al*., 2006)

Emended description of the *Hydrogenophilaceae* (Garrity *et al*. 2006, Garrity *et al.* 2005*d*)

Hydrogenophilaceae (Hy.dro.ge.no.phi.la.ce'ae. N.L. masc. n. *Hydrogenophilus* type genus; -aceae suffix to denote family; N.L. fem. pl. n. *Hydrogenophilaceae*, the *Hydrogenophilus* family).

This order’s description is as given by Garrity *et al.* (2005*d*) with the genera contained therein being *Hydrogenophilus* (type genus) and *Tepidiphilus*. Family contains obligately thermophilic autotrophs using molecular hydrogen and heterotrophs, which are obligately respiratory, using oxygen or nitrate. Contain ω-cyclohexyl fatty acids as major components. Heterotrophic members principally metabolise fatty acids, amino acids, aromatics or complex media. G+C fractions in the family are typically in the region of 60 mol%. Oxidase and catalase positive.

Type genus: *Hydrogenophilus* (Hayashi *et al.* 1999)

Emended description of *Nitrosomonadales* (Garrity *et al*., 2006, Garrity *et al.* 2005*e*)

Nitrosomonadales (Ni.tro.so.mo.na.da'les. N.L. masc. n. *Nitrosomonas* type genus of the type family; -ales suffix to denote order; N.L. fem. pl. n. *Nitrosomonadales*, the *Nitrosomonas* order).

This order is re-defined following the addition of members on the basis of 16S rRNA gene sequence analysis. Contains the families *Nitrosomonadaceae, Gallionellaceae,* *Methylophilaceae*, *Spirillaceae*, *Thiobacillaceae* and *Sterolibacteriaceae*. Contains a broad diversity of metabolisms including autotrophs, nitrogen fixing heterotrophs, iron-oxidisers and methylotrophs. All families are mesophilic and use ubiquinone-8 as the dominant respiratory quinone.

Type family: *Nitrosomonadaceae* (Garrity *et al*., 2006)

Emended description of *Gallionellaceae* (Henrici and Johnson, 1935)

Gallionellaceae (Gal.li.o.nel.la.ce'ae. N.L. fem. n. *Gallionella* type genus; -*aceae* suffix to denote family; N.L. fem. pl. n. *Gallionellaceae,* the *Gallionella* family).

This family is re-defined following the circumscribing of members of the *Sulfuricellaceae* to this family on the basis of 16S rRNA gene pairwise identities. Comprises iron-oxidising bacteria and sulfur-oxidising bacteria, now circumscribing members of *Sulfuricellaceae* – contains the genera *Gallionella*, *Ferriphaselus, Sulfuricella*, *Sulfurirhabdus* and *Sulfuriferula*. Oxidase variable and catalase negative. Motile organisms are monotrichous. Cells vary from rod-shaped to reniform with stalks. Metabolism is heterotrophic, autotrophic and/or mixotrophic. Obligately respiratory using oxygen or nitrate as terminal electron acceptors. Possess ubiquinone-8 as the dominant respiratory quinone. G+C contents are generally around 50-70 mol%. Autotrophic genera use molecular hydrogen, reduced sulfur or ferrous iron as electron donors, the latter producing stalks thought to be ferric-iron-based. Many members contain hydroxylated saturated fatty acids as major components.

Type genus: *Gallionella* (Ehrenberg, 1838 *NB:* no *bone fide* specimens in culture)

Description of *Thiobacillaceae* fam. nov.

Thiobacillaceae (Thi.o.ba.cil.la.ce'ae. N.L. fem. n. *Thiobacillus* type genus; -*aceae* suffix to denote family; N.L. fem. pl. n. *Thiobacillaceae,* the *Thiobacillus* family).

This family is circumscribed on the basis of 16S rRNA gene sequences and comprises the genera *Thiobacillus* and *Annwoodia*. Obligate or facultative autotrophs and mixotrophs using reduced sulfur oxyanions, elementary sulfur or methylated sulfur species as sole energy sources for chemolithoautotrophic metabolism. Fix carbon during autotrophic growth *via* the transaldolase variant of the Calvin-Benson-Bassham cycle. Have $C_{16:0}$ and $C_{16:1}$ as the dominant fatty acids. Ubiquinone-8 is the dominant respiratory quinone. Obligately respiratory using oxygen and/or nitrate as the terminal electron acceptor. Oxidase and catalase positive. Carboxysomes are produced by some genera during autotrophic growth. G+C contents are around 60-65 mol%.

Type genus: *Thiobacillus* (Beijerinck 1904; Wood and Kelly, 2000).

Emended description of *Thiobacillus* (Beijerinck, 1904)

Thiobacillus (Thi.o.ba.cil 'lus. Gr. n. *theios,* sulfur, L. transliteration *theios*, sulfur. L. n. *bacillus* a small rod; N.L. masc. n. *Thiobacillus* sulfur rodlet).

Contains autotrophic sulfur-oxidising bacteria obtaining their energy from the oxidation of reduced sulfur oxyanions, namely thiosulfate and tetrathionate plus other sulfur species such as polythionates, elementary sulfur and/or methylated sulfur compounds. G+C contents of genomic DNA range from 61.5 to 66.0 mol%. No heterotrophy or mixotrophy is observed. Growth temperatures are under 30 °C and usually mesophilic and sometimes psychrotolerant. Catalase and oxidase positive when grown aerobically on thiosulfate agar. Produce tetrathionate as a detectable intermediate of thiosulfate oxidation, released into the medium, often with trithionate and pentathionate. Oxidise thiosulfate using the Kelly-Trudinger pathway and fix carbon dioxide with the transaldolase variant of the Calvin-Benson-Bassham cycle. Some species contain carboxysomes detectable using transmission electron microscopy, which are repressed by growth at elevated carbon dioxide partial pressures. Most species form polyphosphate (volutin) granules during aerobic growth on thiosulfate. Fatty acids include $C_{16:0}$, $C_{16:1}$, $C_{15:0}$ and $C_{17:1}$ when grown autotrophically on thiosulfate.

Type species: *Thiobacillus thioparus* (Beijerinck, 1904)

Description of *Annwoodia* gen. nov.

Annwoodia (Ann.wood'i.a. N.L. fem. n. *Annwoodia*, named to honour Dr Ann Patricia Wood (b. 1952) formerly of University of Warwick and King's College London in the United Kingdom; British microbial physiologist and taxonomist who first described the type strain and made significant contributions to the taxonomy of the 'sulfur bacteria' and methylotrophic '*Proteobacteria*', their physiology and ecology).

Catalase positive, oxidase positive. Short motile rods capable of chemolithoautotrophic growth on reduced sulfur species or heterotrophic growth on complex media, but not on sugars, amino acids, formate or methylated amines in defined media. Mixotrophic growth on nutrient broth supplemented with thiosulfate. Growth on thiosulfate does not result in tetrathionate build-up in media, though elementary sulfur is formed in batch cultures or oxygen-limited chemostats. No intermediates of growth are detected in thiosulfate-limited chemostat cultures and sulfur species are oxidised completely to sulfate, with concomitant fall in culture pH – this is contrary to *Thiobacillus* spp. in which pH rises in the first 24h due to tetrathionate formation and then falls. Dominant respiratory quinone is ubiquinone-8 (UQ8).

Type species is *Annwoodia aquaesulis* (Wood & Kelly, 1995) comb. nov.

Description of *Annwoodia aquaesulis* (Wood & Kelly, 1995) comb. nov.

Annwoodia aquaesulis (a.quae.su'lis. N.L. fem. gen. n. of or from the waters of Sulis Minerva – in this case relating to the Temple of Sulis-Minerva (Goddess of Wisdom), now the Roman

Baths at Bath, UK – source of isolation). Basonym: *Thiobacillus aquaesulis* (Wood & Kelly, 1995).

Short rods 0.3 × 0.9 µm containing volutin (polyphosphate) granules. Motile by means of single polar flagella. Gram negative. Does not produce endospores or exospores. Does not accumulate polyhydroxybutyrate. Colonies on thiosulfate agar grown at 43-45 °C are 1-2 mm diameter, circular, convex, smooth and entire; off-white colonies become white then yellow with age as elementary sulfur builds up. Colonies on thiosulfate agar have a faint "burnt matches" smell. Grows on thiosulfate, trithionate, tetrathionate and elementary sulfur but not thiocyanate, molecular hydrogen, dimethylsulfide, dimethyldisulfide or carbon disulfide as sole energy sources. Grows anaerobically with denitrification but cannot use nitrate as a nitrogen source. Uses ammonium as a nitrogen source. Cultures with an initial pH 7.0 – 9.0 drop to pH 6.0 – 7.0. Grows from 30 to 55 °C with an optimum temperature of 45 °C. Thiosulfate-grown cultures at the optimal growth pH and temperature reach a specific growth rate of 0.29 h^{-1} in the exponential phase. The G+C content of genomic DNA is 65.7 mol% (T_m). Dominant fatty acids are $C_{16:0}$ and $C_{16:1}$ when grown autotrophically on thiosulfate.

Type strain is ATCC 43788^T = DSM 4255^T, isolated from the thermal sulfur spring at the Roman Baths, Bath, UK.

Description *of Sterolibacteriaceae* fam. nov.

Sterolibacteriaceae (Ste.rol.i.bac.te.ri.a.ce'ae. N.L. neuter. n. *Sterolibacterium* type genus; -aceae suffix to denote family; N.L. fem. pl. n. *Sterolibacteriaceae,* the *Sterolibacterium* family).

This family is circumscribed on the basis of 16S rRNA gene sequences and comprises the genera *Sterolibacterium* (type genus), *Methyloversatilis*, *Sulfurisoma*, *Sulfuritalea*, *Denitratisoma* and *Georgfuchsia*. Varied metabolism from methylotrophs, facultative autotrophs, denitrifiers and obligate anaerobes using ferric iron or manganic manganese as terminal electron acceptors. Autotrophic members use reduced sulfur or ferrous iron as electron donors. Oxidase positive but catalase variable. Usually contain hydroxylated saturated fatty acids as major components. Ubiquinone-8 is the dominant respiratory quinone.

Type genus: *Sterolibacterium* (Tarlera and Denner 2003).

Emended description of *Rhodocyclaceae* (Garrity *et al*. 2006 – Garrity *et al*., 2005*c*)

Rhodocyclaceae (Rho.do.cy.cla.ce'ae. N.L. masc. n. *Rhodocyclus* type genus; *-aceae* suffix to denote family; N.L. fem. pl. n. *Rhodocyclaceae,* the *Rhodocyclus* family).

This family is re-defined following the circumscribing of members of this family to form new families. On the basis of 16S rRNA gene sequences, comprises the genera *Rhodocyclus* (type genus), *Propionibacter*, *Propionivibrio*, and *Azospira*. Family contains nitrogen-fixing aerobes and anaerobes as well as photoheterotrophic anaerobes which can ferment organics. Ubiquinone-8 and menaquinone-8 are the dominant respiratory quinones. Many genera are anaerobic. Autotrophic members can only use molecular hydrogen as an electron donor. Heterotrophic growth is quite restricted to fatty acids *etc* in most genera. Oxidase and catalase negative. Large diversity of cell morphologies including curved rods, rings and spirillae. Hydroxylated and cyclic fatty acids are not observed as major components.

Type genus: *Rhodocyclus* (Pfennig, 1978)

Description of *Zoogloeaceae* fam. nov.

Zoogloeaceae (Zo.o.gloe.a.ce'ae. N.L. fem. n. *Zoogloea* type genus; *-aceae* suffix to denote family; N.L. fem. pl. n. *Zoogloeaceae,* the *Zoogloea* family).

This family is circumscribed on the basis of 16S rRNA gene sequences and physiology, and comprises the genera *Zoogloea* (type genus), *Thauera*, *Azoarcus* and *Uliginosibacterium*.

Family comprises strict aerobes that fix nitrogen, zoogloeae-forming denitrifiers and more generalist heterotrophs. Ubiquinone-8 and rhodoquinone-8 are the dominant respiratory quinones. May contain hydroxylated saturated fatty acids as major components. Oxygen, nitrate, nitrite, nitrous oxide and selenate are used as terminal electron acceptors. Molecular hydrogen can be used as an electron donor by autotrophic members. Oxidase and catalase positive. Cells are usually rod-shaped and monotrichous or ‘degenerately peritrichous’. ω-hydroxylcapric acid ($C_{10:0}$ 3-OH) is observed as major component in most known genera.

Type genus: *Zoogloea* (Itzigsohn 1868)

Description of *Azonexaceae* fam. nov.

Azonexaceae (A.zo.nex.a.ce'ae. N.L. masc. n. *Azonexus* type genus; *-aceae* suffix to denote family; N.L. fem. pl. n. *Azonexaceae,* the *Azonexus* family).

This family is circumscribed on the basis of 16S rRNA gene sequences and comprises the genera *Azonexus* (type genus), *Dechloromonas*, *Ferribacterium* and *Quatrionicoccus*. Family comprises facultative anaerobes respiring (per)chlorate; obligate aerobes fixing nitrogen; strict anaerobes respiring ferric iron and more generalist heterotrophs. Ubiquinone-8 is the dominant respiratory quinone. Catalase positive but oxidase variable across genera. Hydroxylated and cyclic fatty acids are not observed as major components.

Type genus: *Azonexus* (Reinhold-Hurek and Hurek, 2000).

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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Table 1. Comparative properties of DSM 4255^T *versus Thiobacillus* spp. Data are obtained from Wood & Kelly (1988), Kelly & Harrison (1989), Kellermann & Griebler (2009), Beller *et al*. (2006), Hutt *et al*. (*submitted*) and this study. * experimental (T_m confirmed with acid denaturation), ** genome sequence data.

Table 2. Comparative properties of the *Hydrogenophilales versus* the other families of the *Betaproteobacteria* considered in this study, with families and orders as defined in our formal descriptions thereof. The orders *Burkholderiales* and *Neisseriales* are included for comparison. Data given are typical of each taxon and are taken from Garrity *et al*. (2005*a-g*) and are updated to include taxa published since 2005. + = positive or present. – negative or absent. *w* = weakly positive. ± = variable across this taxon. *N.D.* = no data available. UQ-8, ubiquinone-8; MK-8, menaquinone-8; RQ-8, rhodoquinone-8. Features in parentheses are limited to a small number of genera in that taxon.

Footnotes:

* a small number of species are monotrichous;

† One species in this family is mixotrophic or heterotrophic;

‡ Found only as a major component in *Pandoraea* spp.

§ *Thermothrix* (*Burkholderiales*) is the notable exception, growing optimally at 73 °C (Odintsova *et al.*, 1996)

Figure 1. Phylogenetic tree on the basis of the 16S rRNA (*rrs*) gene, showing the position of DSM 4255^T (bold) and *Thiobacillus* spp. within the *Betaproteobacteria sensu* Garrity *et al*. (2005*b*). This includes the type strains of every species with a validly published name, but only the *Rhodocyclales* and *Nitrosomonadales* are shown, for clarity – the entire tree is given in Figure 4. MUSCLE was used to align nucleotide sequences and the tree reconstructed using the maximum likelihood method based on the Tamura-Nei model within MEGA 7.0.20. After 5,000 bootstrap replicate constructions, the tree with the highest log likelihood (-90516) is shown, with values next to nodes indicate the percentage of constructions in which the topology was preserved (values <70 % are omitted for clarity). All positions with < 95 % site coverage were omitted from the final analysis, which used 1,303 nt. Branch lengths are to scale and indicate the number of substitutions per site, with the scale bar indicating 2 substitutions per site. The outgroup is the genus *Allochromatium* from the *Gammaproteobacteria*. Names of orders and families in the tree itself are the extant names/circumscriptions, with the novel groupings indicated by bars to the right, showing orders (green) and families (black).

Figure 2. Phylogenetic trees on the basis of the 16S rRNA (*rrs*) gene, showing the position of the two genera of the proposed *Hydrogenophilalia* class. nov. (diagonal shading) *versus* the classes of the '*Proteobacteria*'. The full list of 94 sequences used is given in Supplementary Table S1. Trees were reconstructed from MUSCLE alignments of the gene using the maximum likelihood, neighbour-joining and minimum evolution methods (all using the Tamura-Nei model) or the maximum parsimony method (tree bisection-reconnection method) within MEGA 7.0.20. After 5,000 bootstrap replicate constructions, the trees with the highest confidence are shown, with values at nodes indicating the percentage of constructions in which the topology was preserved (values <70 % are omitted for clarity). All positions with <95 % site coverage were omitted from the final analyses, which used 1,705 nt. Branch

lengths are to scale and indicate the number of substitutions per site, with scale bars representing 5 substitutions per site for all except maximum parsimony, in which the scale bar represents the number of changes across the whole sequence. The outgroup for the maxmimum likelihood, minimum evolution and neighbour-joining trees is the phylum '*Aquificae*'; the maximum parsimony tree is unrooted. Versions using the complete *Betaproteobacteria* are given in Supplementary Figures S1 – S3 (maximum likelihood, neighbour joining and minimum evolution, respectively) and show the same class-level topology. A complete *Betaproteobacteria* maximum parsimony tree was not attempted owing to the computational time required and the poor reconstruction of the phyla in the version in this figure.

Figure 3. Maximum likelihood tree reconstructed from amino acid alignments using sequences derived from 53-gene concatamers of ribosomal protein genes of the *rps*, *rpl* and *rpm* operons, confirming the position of the proposed *Hydrogenophilalia* class. nov. as distinct from the *Betaproteobacteria* and all other classes of the '*Proteobacteria*'. Gene sequences were extracted and concatenated from genome sequences held in the ribosomal multilocus sequence typing (rMLST) public database using the rMLST platform, ignoring any incomplete genes or genes at the ends of contigs. Concatamers were translated using the bacterial genetic code and amino acyl sequences aligned using MUSCLE. Tree was reconstructed using the maximum likelihood method and the Jones-Taylor-Thornton model within MEGA 7.0.20. After 5,000 bootstrap replicate constructions, the tree with the highest log likelihood (-417827) is shown, with values next to nodes indicate the percentage of constructions in which the topology was preserved (values <70 % are omitted for clarity). All positions with < 95 % site coverage were omitted from the final analysis, which used 4,289 amino acyl residues (from 12,867 nt). Branch lengths are to scale and indicate the number of substitutions per site, with the scale bar indicating 10 substitutions per site. The outgroup is

the phylum ‘*Aquificae*’. Supplementary Table S2 gives full details of sequences employed in this analysis.

Figure 4. Phylogenetic tree on the basis of the 16S rRNA (*rrs*) gene, showing the position and names of new higher taxa proposed in this study, within the *‘Proteobacteria*’ – this is a redrawing of Figure 1, showing the entire *Betaproteobacteria* and *Hydrogenophilalia* class. nov., with all taxa collapsed for clarity at class, order or family, with novel taxa proposed in this study indicated such that their relationships to one another are clear. All properties of the tree and the alignment are identical to Figure 1. Comparison between Figure 1 and Figure 2 will allow understanding how original and revised systematics of the *Betaproteobacteria* relate to one another.

Characteristic	**DSM 4255^{T}**	***Thiobacillus thioparus* DSM 505^{T}**	***Thiobacillus denitrificans* DSM 12475^{T}**	***Thiobacillus thiophilus* DSM 19892^{T}**
16S rRNA gene identity (%) to:				
T. thioparus DSM 505^{T}	93.1	100	97.6	97.6
T. denitrificans DSM 12475^{T}	93.4	97.6	100	97.6
T. thiophilus DSM 19892^{T}	93.1	97.6	97.6	100
DSM 4255^{T}	100	93.1	93.4	93.1
Cell length (µm)	0.9	1.7	1.0-3.0	1.8-2.5
Cell width (µm)	0.3	0.5	0.5	0.5-0.8
Source of type strain	Thermal sulfur spring	Agricultural soil	Sewage	Aquifer
Colony colour on thiosulfate-agar, under air	White, yellow with age	White, pink or brown with age	Clear, white with age	White, yellow with age
Metabolic mode	Facultative heterotroph	Obligate autotroph	Obligate autotroph	Obligate autotroph
Use of nitrate as:				
Nitrogen source	-	+	±	*N.D.*
Electron acceptor	+	-	+	+
Temperature range	30-55	*N.D.*	*N.D.*	-2-30
Temperature optimum	45	25-30	28-32	25-30
pH range	7.0 – 9.0	5.0-9.0	*N.D.*	6.3-8.7
pH optimum	7.6	6.0-8.0	6.8-7.4	7.5-8.3
Tetrathionate accumulation in thiosulfate batch culture	-	+	+	+
Energy sources for autotrophic growth (all grow on thiosulfate and tetrathionate)				
Trithionate	+	+	*N.D.*	-
Elementary sulfur	+	-	+	-
Thiocyanate	-	+	+	-
Sulfide	+	+	+	-
Dimethylsulfide	-	+	-	*N.D.*
Dimethyldisulfide	-	+	-	*N.D.*
Carbon disulfide	-	*w*	-	*N.D.*
G+C content (mol%)	65.7*	62.3**	66.0**	61.5*

Class	***Hydrogenophilalia***	***Betaproteobacteria***										
Order	***Hydrogenophilales***	***Rhodocyclales***			***Nitrosomonadales***						***Burkholderiales***	***Neisseriales***
Family	***Hydrogenophilaceae***	***Rhodocylaceae***	***Azonexaceae***	***Zoogloeaceae***	***Nitrosomonadaceae***	***Methylophilaceae***	***Thiobacillaceae***	***Sterolibacteriaceae***	***Spirillaceae***	***Gallionellaceae***		
Flagellation or other motility	Monotrichous	Monotrichous or atrichous	Monotrichous or atrichous	Monitrichous or 'degenerately peritrichous'	Atrichous*	Atrichous	Monotrichous	*N.D.*	Amphitrichous	Monotrichous	Atrichous, monotrichous, lophotrichous or amphitrichous.	Atrichous, monotrichous, lophotrichous or amphitrichous. Gliding motility in some genera.
Morphology	Rods	Curved rods, rings, spirillae,	Curved rods or cocci	Rods	Rods or cocci	Rods	Rods	Rods	Spirillae	Reniform or curved rods with stalks, or rods	Rods, curved rods, spirillae, multicellular 'tablets'	Cocci, rods, spirillae, multicellular 'ribbons' or filaments
Metabolic mode	Heterotrophic or autotrophic	Photoheterotrophic or photoautotrophic	Heterotrophic	Heterotrophic or autotrophic	Autotrophs or mixotrophs†	Facultative methylotrophs	Autotrophs or restricted heterotrophs†	Autotrophs, heterotrophs, facultative methylotrophs	Heterotrophs	Autotrophs, heterotrophs or mixotrophs†	Heterotrophs and some facultative autotrophs and photoheterotrophs	Heterotrophs, facultative methylotrophs and chemolitho-heterotrophs
Oxidase	+	-	±	+	+	±	+	+	+	±	+	+
Catalase	+	-	+	+	+	+	+	±	*w*	-	±	±
Energy sources (if autotrophic)	Molecular hydrogen	Molecular hydrogen	*N/A*	Molecular hydrogen	Ammonium, nitrite, molecular hydrogen	*N/A*	Reduced sulfur species	Reduced sulfur, molecular hydrogen	*N/A*	Fe(II), molecular hydrogen, reduced sulfur.	Molecular hydrogen, reduced sulfur,	*N/A*
Carbon sources (if heterotrophic or mixotrophic)	Fatty acids, amino acids, aromatics, complex media	Fatty acids	Fatty acids, alcohols, amino acids, sugars	Krebs' cycle intermediates, aromatics, alcohols, sugars	Fatty acids†	C_1 compounds, sugars, complex media	Complex media†	Sterols, fatty acids, C_1 compounds, Krebs' cycle intermediates, aromatics	Krebs' cycle intermediates	Sugars, Krebs' cycle intermediates, carboxylates, alcohols	Complex media, sugars, amino acids, Krebs' cycle intermediates *etc.* Aromatics,	Complex media, sugars, amino acids, Krebs' cycle intermediates *etc.* Methanol in methylotrops.
Electron acceptors	Oxygen, nitrate	Oxygen, fermentation	Oxygen, (per)chlorate, nitrate, Fe(III)	Oxygen, nitrate, nitrite, nitrous oxide, selenate	Oxygen, nitrate	Oxygen	Oxygen, nitrate	Oxygen, nitrate, Fe(III), Mn(IV)	Oxygen,	Oxygen, nitrate	Oxygen, fermentation,	Oxygen, fermentation, nitrate, elementary sulfur, Fe(III)
Carboxysomes	-	-	-	-	+	-	+	-	-	-	-	-
Dominant fatty acids	$C_{16:0}$, $C_{19:0}$ *cyclo* $C_{17:0}$ *cyclo*	$C_{16:0}$, $C_{16:1}$, $C_{18:1}$	$C_{16:0}$, $C_{16:1}$, $C_{18:1,}$	$C_{10:0}$ 3-OH $C_{16:0}$, $C_{16:1}$, $C_{18:1,}$ $C_{17:0,}$	$C_{16:0}$, $C_{16:1}$	$C_{16:0}$, $C_{16:1}$	$C_{16:0}$, $C_{16:1}$, $C_{15:0}$, $C_{17:1}$, $C_{18:0}$, $C_{18:1}$	$C_{16:1}$, $C_{16:0}$, $C_{15:0}$ 2-OH, $C_{10:0}$ 3-OH	$C_{16:0}$, $C_{16:1}$, $C_{18:1,}$	$C_{16:1}$, $C_{16:0}$, $C_{18:0}$, $C_{18:1}$ $C_{10:0}$ 3-OH $C_{15:0}$ 2-OH,	$C_{12:0,}$ $C_{14:0,}$ $C_{16:0,}$ $C_{16:1,}$ $C_{18:1,}$ $C_{18:0,}$ $C_{12:0}$ 2-OH $C_{14:0}$ 3-OH $C_{16:0}$ 2-OH $C_{17:0}$ *cyclo*§	$C_{16:1}$, $C_{16:0}$, $C_{18:0}$, $C_{18:1,}$ $C_{12:0,}$ $C_{12:0}$ 3-OH
Respiratory quinones	UQ-8	UQ-8, MK-8	UQ-8	UQ-8, RQ-8	UQ-8	UQ-8	UQ-8	UQ-8	*N.D.*	*N.D.*	UQ-8 (RQ-8)	UQ-8
G+C content (mol%)	58.6 – 65.0	61.6-65.3	63.5-67.0	59.3-69.0	45.0-56.9	42.6-57.0	61.5-66.0	63.5-67.0	36.0-38.0	51.0-66.0	39.7-72.6	40.0-68.8
Temperature profile	Thermophiles	Mesophiles**										

Figure 1

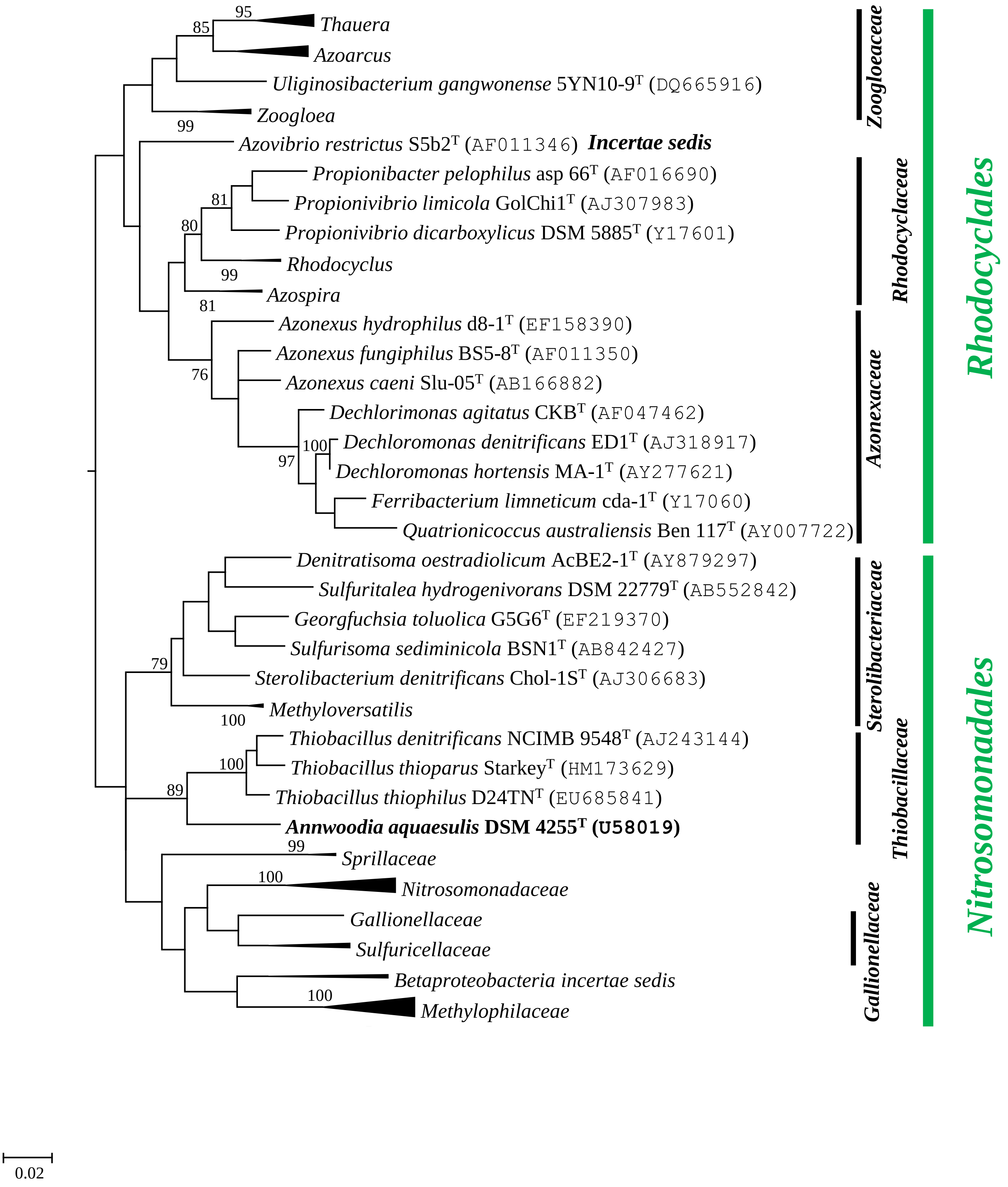
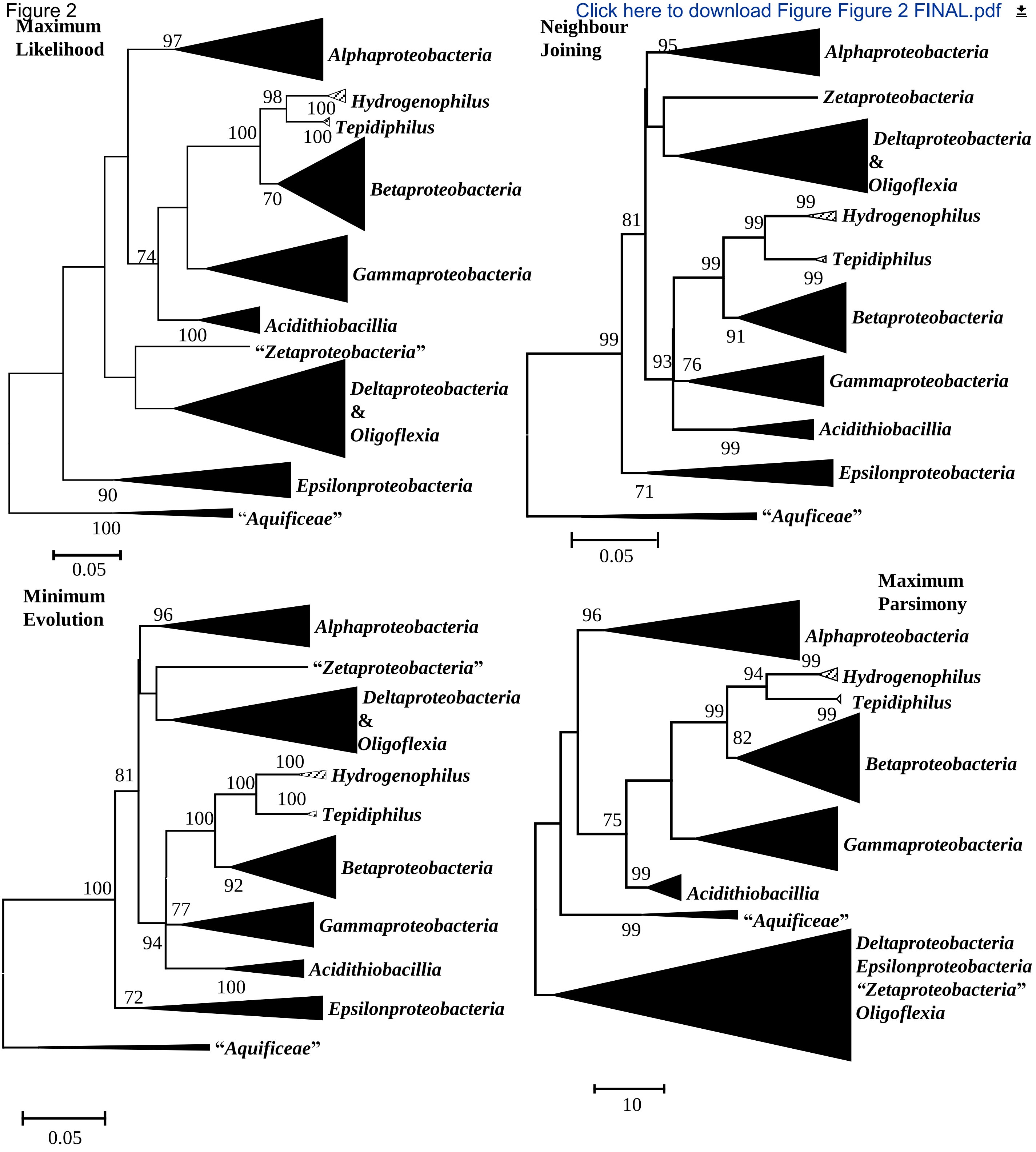
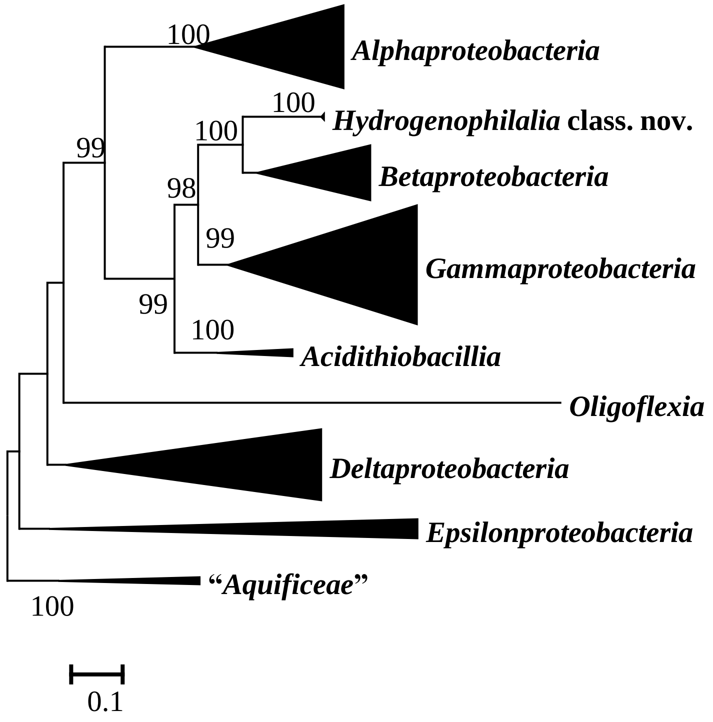
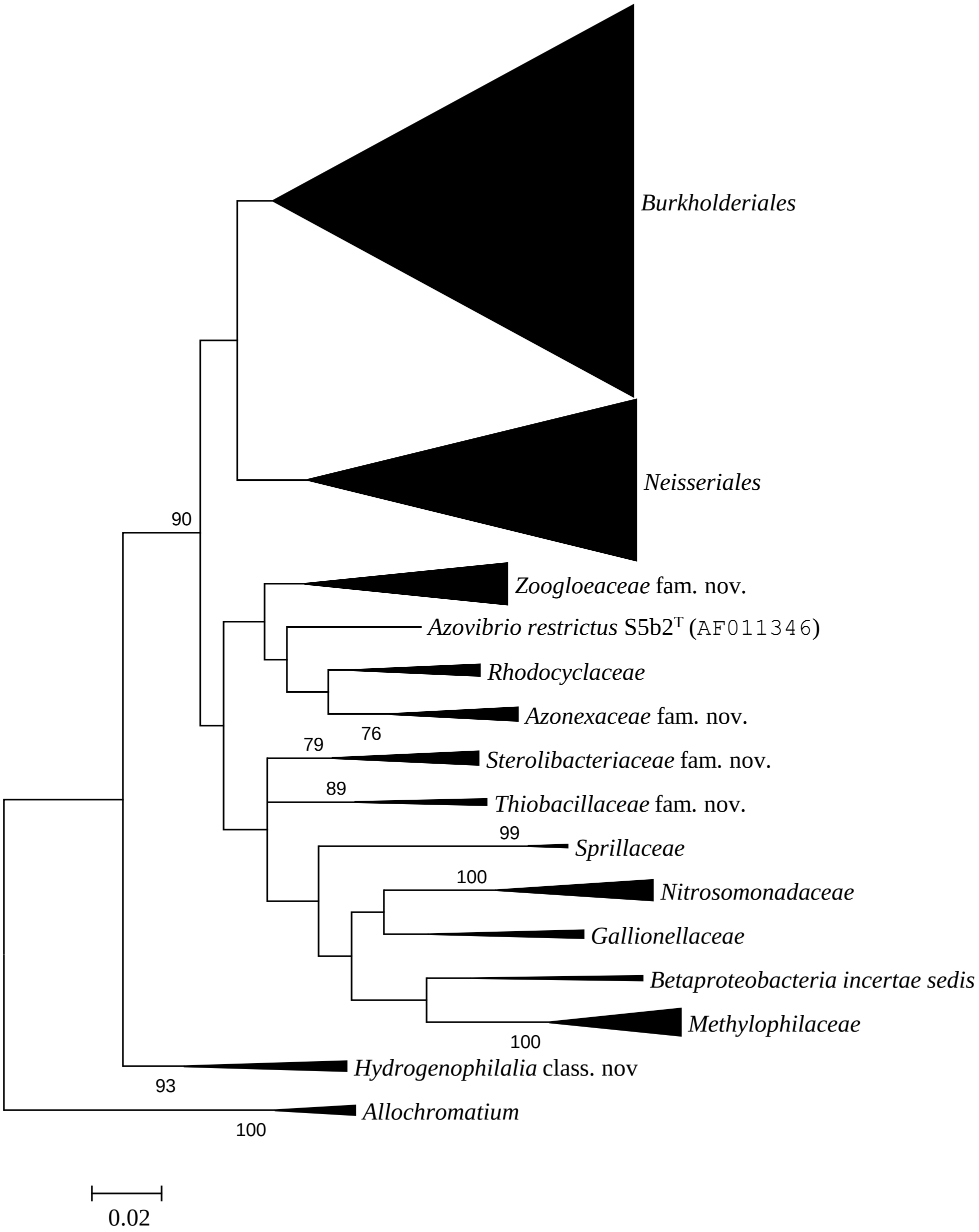

95
85
Thauera
Azoarcus
Uliginosibacterium gangwonense 5YN10-9^T (DQ665916)
Zoogloea
99
Azovibrio restrictus S5b2^T (AF011346) ***Incertae sedis***
Propionibacter pelophilus asp 66^T (AF016690)
81
Propionivibrio limicola GolChi1^T (AJ307983)
80
Propionivibrio dicarboxylicus DSM 5885^T (Y17601)
Rhodocyclus
99
Azospira
81
Azonexus hydrophilus d8-1^T (EF158390)
Azonexus fungiphilus BS5-8^T (AF011350)
76
Azonexus caeni Slu-05^T (AB166882)
Dechlorimonas agitatus CKB^T (AF047462)
100
Dechloromonas denitrificans ED1^T (AJ318917)
97
Dechloromonas hortensis MA-1^T (AY277621)
Ferribacterium limneticum cda-1^T (Y17060)
Quatrionicoccus australiensis Ben 117^T (AY007722)
Denitratisoma oestradiolicum AcBE2-1^T (AY879297)
Sulfuritalea hydrogenivorans DSM 22779^T (AB552842)
Georgfuchsia toluolica G5G6^T (EF219370)
Sulfurisoma sediminicola BSN1^T (AB842427)
79
Sterolibacterium denitrificans Chol-1S^T (AJ306683)
Methyloversatilis
100
Thiobacillus denitrificans NCIMB 9548^T (AJ243144)
100
Thiobacillus thioparus Starkey^T (HM173629)
89
Thiobacillus thiophilus D24TN^T (EU685841)
***Annwoodia aquaesulis* DSM 4255^T (U58019)**
99
Sprillaceae
100
Nitrosomonadaceae
Gallionellaceae
Sulfuricellaceae
Betaproteobacteria incertae sedis
100
Methylophilaceae
0.02
Zoogloeaceae
Rhodocyclaceae
Azonexaceae
Sterolibacteriaceae
Thiobacillaceae
Gallionellaceae
Rhodocyclales
Nitrosomonadales

Figure 2


Maximum Likelihood
97
Alphaproteobacteria
98
100
Hydrogenophilus
100
100
Tepidiphilus
70
Betaproteobacteria
74
Gammaproteobacteria
Acidithiobacillia
100
"Zetaproteobacteria"
Deltaproteobacteria & Oligoflexia
90
Epsilonproteobacteria
100
"Aquificeae"
0.05
Neighbour Joining
95
Alphaproteobacteria
Zetaproteobacteria
Deltaproteobacteria & Oligoflexia
99
99
Hydrogenophilus
Tepidiphilus
81
99
99
Betaproteobacteria
91
99
93
76
Gammaproteobacteria
Acidithiobacillia
99
Epsilonproteobacteria
71
"Aquficeae"
0.05
Minimum Evolution
96
Alphaproteobacteria
"Zetaproteobacteria"
Deltaproteobacteria & Oligoflexia
100
Hydrogenophilus
81
100
100
Tepidiphilus
100
Betaproteobacteria
92
100
77
Gammaproteobacteria
94
Acidithiobacillia
100
72
Epsilonproteobacteria
"Aquificeae"
0.05
Maximum Parsimony
96
Alphaproteobacteria
99
94
Hydrogenophilus
Tepidiphilus
99
99
82
Betaproteobacteria
75
Gammaproteobacteria
99
Acidithiobacillia
"Aquificeae"
99
Deltaproteobacteria Epsilonproteobacteria "Zetaproteobacteria" Oligoflexia
10


100
Alphaproteobacteria
100
Hydrogenophilalia class. nov.
100
Betaproteobacteria
99
98
99
Gammaproteobacteria
99
100
Acidithiobacillia
Oligoflexia
Deltaproteobacteria
Epsilonproteobacteria
"*Aquificeae*"
100
0.1


Burkholderiales
Neisseriales
90
Zoogloeaceae fam. nov.
Azovibrio restrictus S5b2T (AF011346)
Rhodocyclaceae
Azonexaceae fam. nov.
76
79
Sterolibacteriaceae fam. nov.
89
Thiobacillaceae fam. nov.
99
Sprillaceae
100
Nitrosomonadaceae
Gallionellaceae
Betaproteobacteria incertae sedis
Methylophilaceae
100
Hydrogenophilalia class. nov
93
Allochromatium
100
0.02

Supplementary Information

Reclassification of *Thiobacillus aquaesulis* (Wood & Kelly, 1995) as *Annwoodia aquaesulis* gen. nov., comb. nov. Transfer of *Thiobacillus* (Beijerinck, 1904) from the *Hydrogenophilales* to the *Nitrosomonadales*, proposal of *Hydrogenophilalia* class. nov. within the '*Proteobacteria*', and 4 new families within the orders *Nitrosomonadales* and *Rhodocyclales*.

Rich Boden[1,2], Lee P. Hutt[1,2] and Alex W. Rae[1]

Supplementary Table S1. GenBank™ accession numbers and strains used in construction of Figure 2 with their classifications at class, order and family levels.

Supplementary Table S2. rMLST genome ID numbers and strains used in construction of Figure 3 with their classifications at class, order and family levels.

Supplementary Figure S1. Maximum likelihood tree of 16S rRNA genes constructed in an identical manner to that given in Figure 2 but using the whole of the *Betaproteobacteria*.

Supplementary Figure S2. Neighbour-joining tree of 16S rRNA genes constructed in an identical manner to that given in Figure 2 but using the whole of the *Betaproteobacteria*.

Supplementary Figure S3. Minimum-evolution tree of 16S rRNA genes constructed in an identical manner to that given in Figure 2 but using the whole of the *Betaproteobacteria*.

Supplementary Figure S4. Minimum-evolution tree of rMLST 53-gene concatamers analysed at amino acid level in the same way as the maximum likelihood tree given in Figure 3.

Supplementary Figure S5. Neighbour-joining tree of rMLST 53-gene concatamers analysed at amino acid level in the same way as the maximum likelihood tree given in Figure 3.

Class	**Order**	**Family**	**Species (and subspecies/biovar *etc* where applicable)**	**Strain Code**	**Accession No. (GenBank™)**
Phylum: “***Proteobacteria***”					
Alphaproteobacteria	*Rhizobiales*	*Bradyrhizobiaceae*	*Rhodopseudomonas palustris*	ATH 2.1.6^{T}	D25312
		Methylobacteriaceae	*Methylobacterium organophilum*	ATCC 27886^{T}	AB175638
		Beijerinckiaceae	*Beijerinckia indica* subsp. *indica*	ATCC 9039^{T}	CP001016
		Hyphomicrobiaceae	*Hyphomicrobium vulgare*	ATCC 33404^{T}	AB543807
		“*Aurantimonadaceae*”	*Fulvimarina pelagi*	HTCC2506^{T}	AY178860
		Rhizobiaceae	*Rhizobium leguminosum* bv. *viciae*	3Hoq18^{T}	U29386
		Rhodobiaceae	*Rhodobium radiobacter*	ATCC 19358^{T}	AB247615
	Sphingomonadales	*Sphingomonadaceae*	*Sphingomonas paucimobilis*	ATCC 29837^{T}	U37337
		Erythrobacteriaceae	*Erythrobacter longus*	DSM 6997^{T}	AF46535
	Rhodospirillales	*Rhodospirillaceae*	*Rhodospirillum rubrum*	ATCC 11170^{T}	CP000230
			Azospirillum lipoferum	NCIMB 11861^{T}	Z29619
			Defulvicoccus vanus	Ben 114^{T}	AF179678
		Acetobacteriaceae	*Acetobacter aceti*	ATCC 15973^{T}	X74066
	Rickettsiales	*Rickettsiaceae*	*Rickettsia prowazekii*	Brenl^{T}	M21789
Betaproteobacteria	*Burkholderiales*	*Burkholderiaceae*	*Burkholderia cepacia*	ATCC 25416^{T}	U96927
		Oxalobacteriaceae	*Oxalobacter formigenes*	OXB^{T}	U49757
		Comamonadaceae	*Comamonas terrigena*	IMI 359870^{T}	AF078772
		Alcaliginaceae	*Alcaligenes faecalis* subsp. *faecalis*	ATCC 8750^{T}	D88008
		Sutterellaceae	*Sutterella wadsworthensis*	ATCC 51579^{T}	HM037997
	Neisseriales	*Neisseriaceae*	*Neisseria gonorrhoeae*	NCTC 83785^{T}	X07714
		Chromobacteriaceae	*Chromobacterium violaceum*	ATCC 12472^{T}	AE016825
	Nitrosomonadales	*Nitrosomonadaceae*	*Nitrosomonas europaea*	ATCC 25979^{T}	AB070982
		Thiobacillaceae	*Thiobacillus thioparus*	Starkey^{T}	HM173629
			Annwoodia aquaesulis	ATCC 43788^{T}	U58019

		Gallionellaceae	*Ferriphaselus amnicola*	OYT1^T	AB720115
			Sulfuricella denitrificans	NBRC 105220^T	AB506456
		Methylophilaceae	*Methylophilus methylotrophus*	NCIMB 10515^T	AB193724
		Sterolibacteriaceae	*Sterolibacterium denitrificans*	Chol-1S^T	AJ306683
		Spirllaceae	*Spirillum winogradskyi*	D-427^T	AY845251
			Spirillum volutans	ATCC 19554^T	GU585672
	Rhodocyclales	*Rhodocyclaceae*	*Rhodocyclus purpureus*	6770^T	M34132
		Zoogloeaceae	*Zoogloea ramigera*	ATCC 19544^T	X74913
		Azonexaceae	*Azonexus fungiphilus*	BS5-8^T	AF011350
Hydrogenophilalia	*Hydrogenphilales*	*Hydrogenophilaceae*	*Hydrogenophilus thermoluteolus*	TH-1^T	AB009828
			Hydrogenophilus hirschii	yel5a^T	AJ131694
			Hydrogenophilus islandicus	16C^T	EU625664
			Tepidiphilus succinimandens	BON4^T	AY219713
			Tepidiphilus margaritifer	N2-214^T	AJ504663
Gammaproteobacteria	*Chromatiales*	*Chromatiaceae*	*Chromatium okenii*	DSM 169^T	AJ223234
			Thiorhodovibrio winogradskyi	DSM 6702^T	AB016986
	Methylococcales	*Methylothermaceae*	*Methylomarinovum caldicuralii*	IT-9^T	AB301718
		Methylococcaceae	*Methylococcus capsulatus*	Texas^T	AJ563935
	Thiotrichales	*Thiothrichaceae*	*Thiothrix nivea*	DSM 5205^T	L40993
			Thiothrix disciformis	B3-1^T	AB042532
		Piscirickettsiaceae	*Methylophaga marina*	DSM 5989^T	X95459
			Methylophaga thiooxydans	DMS010^T	DQ660915
	Pseudomonadales	*Moraxellaceae*	*Moraxella lacunata*	ATCC 17967^T	D64049
		Pseudomonadaceae	*Pseudomonas aeruginosa*	DSM 50071^T	HE978271
			Pseudomonas chlororaphis subsp. *chlororaphis*	DSM 50083^T	Z76673
	Alteromonadales	*Shewanellaceae*	*Shewanella putrefaciens*	LMG 26268^T	X81623
		Alteromonadaceae	*Altermonas macleodii*	DSM 6062^T	Y18228
	“*Enterobacteriales*”	*Enterobacteriaceae*	*Escherichia coli*	ATCC 11775^T	X80725

	“*Vibrionales*”	*Vibrionaceae*	*Vibrio cholera*	CECT 514^{T}	X76337
Acidithiobacillia	*Acidithiobacillales*	*Acidithiobacillaceae*	*Acidithiobacillus caldus*	DSM 8584^{T}	Z29975
			Acidithiobacillus thiooxidans	ATCC 19377^{T}	Y11596
			Acidithiobacillus albertensis	DSM 14366^{T}	AJ459804
			Acidithiobacillus ferrooxidans	ATCC 23270^{T}	AF465604
		Thermithiobacillaceae	*Thermithiobacillus tepidarius*	DSM 3134^{T}	AJ45801
			Thermithiobacillus plumbiphilus	wk12^{T}	LC088006
“*Zetaproteobacteria*”	“*Mariprofundales*”	“*Mariprofundaceae*”	*Mariprofundus ferrooxydans*	PV-1^{T}	EF493243
Oligoflexia	*Oligoflexales*	*Oligoflexaceae*	*Oligoflexus tunisiensis*	Shr3^{T}	AB540021
Deltaprotebacteria	*Bdellovibrionales*	*Bdellovibrionaceae*	*Bdellovibrio bacteriovorus*	DSM 50701^{T}	AJ292759
		Bacteriovoracaceae	*Bacteriovorax stolpi*	DSM 12778^{T}	AJ288899
		Halobacteriovoracaceae	*Halobacteriovorax marinus*	ATCC BAA-682^{T}	AF084854
		Pseudobacteriovoracaceae	*Pseudobacteriovorax antillogorgicola*	RKEM611^{T}	KJ685394
	Desulfovibrionales	*Desulfonatronaceae*	*Desulfonatronum lacustre*	DSM 10312^{T}	AF418171
		Desulfovibrionaceae	*Desulfovibrio desulfuricans*	ATCC 27774^{T}	M32113
		Desulfomicrobiaceae	*Desulfomicrobium baculatum*	DSM 4028^{T}	CP001629
	Desulfobacterales	*Nitrospinaceae*	*Nitrospina gracilis*	3/211^{T}	FR865038
	Syntrophobacterales	*Syntrophobacteraceae*	*Syntrophobacter wolnii*	DSM 2805M^{T}	X70905
		Syntrophaceae	*Syntrophus buswelii*	DSM 2612M^{T}	X85131
	Desulfarculales	*Desulfarculaceae*	*Desulfarculus baarsii*	DSM 2075^{T}	CP002085
	Desulfobacterales	*Desulfobacteraceae*	*Desulfobacter postgatei*	DSM 2034^{T}	AF418180
	Myxococcales	*Cystobacteraceae*	*Cystobacter fuscus*	DSM 2262^{T}	DQ768109
		Myxococcaceae	*Myxococcus fulvus*	ATCC 25199^{T}	DQ768117
		“*Haliangiaceae*”	*Haliangium ochraceum*	DSM 14365^{T}	CP001804
		Kofleriaceae	*Kofleria flava*	PI vt1^{T}	AJ233944
		Nannocystaceae	*Nannocystis exedens*	DSM 71^{T}	AB084253
		Sandaracinaceae	*Sandaracinus amyloliticus*	NOSO-4^{T}	HQ540311
		Phaselicystidaceae	*Phaselicystis flava*	SBKo001^{T}	EU545827
		Polyangiaceae	*Polyangium spumosum*	DSM 14734^{T}	GU207881

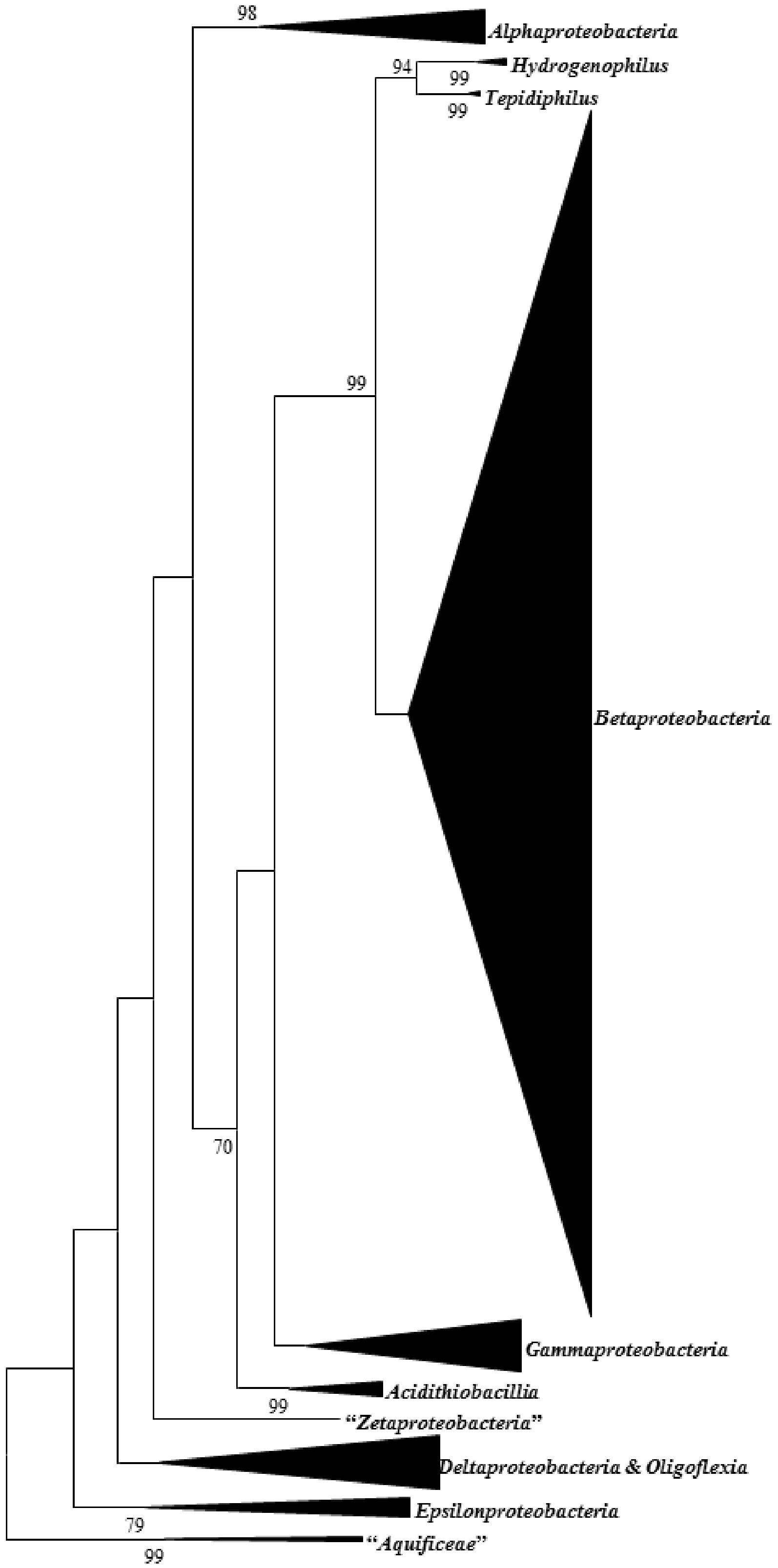
	Desulfuromonadales	*Geobacteraceae*	*Geobacter metallireducens*	GS-15^T	L07834
Epsilonproteobacteria	*Campylobacterales*	“*Hydrogenimonaceae*”	*Hydrogenimonas thermophile*	EP1-55-1%^{T}	AB105048
		Campylobacteraceae	*Campylobacter fetus* subsp. *fetus*	ATCC 27374^T	DQ174127
			Arcobacter nitrofigilis	DSM 16994^T	CP001999
		Helicobacteraceae	*Helicobacter pylori*	ATCC 43504^T	U01330
			Wolinella succinogenes	ATCC 29543^T	M88159
			Sulfurimonas autotrophica	DSM 16294^T	CP002205
	Nautiliales	*Nautiliaceae*	*Nautilia lithotrophica*	525^T	AJ04370
			Caminibacter hydrogeniphilus	AM1116^T	AJ309655
Phylum: **“*Aquificeae*”**					
Aquificeae	*Aquificales*	*Aquificaceae*	*Aquifex pyrophilus*	DSM 6858^T	M83548
		Hydrogenothermaceae	*Hydrogenothermus marinus*	DSM 12046^T	AJ292525

Class	**Order**	**Family**	**Species (and subspecies/biovar *etc* where applicable)**	**Strain Code**	**Genome ID (rMLST)**
Phylum: “***Proteobacteria***”					
Alphaproteobacteria	“*Parvularculales*”	“*Parvularculaceae*”	*Parvularcula bermudensis*	HTCC2503^{T}	1390
	Caulobacterales	*Caulobacteraceae*	*Caulobacter vibrioides*	T5M6	169492
		Hyphomonadaceae	*Hyphomonas polymorpha*	PS728^{T}	173126
	Kiloniellales	*Kiloniellaceae*	*Kiloniella laminariae*	LD81^{T}	60463
	Magnetococcales	*Magnetococcaceae*	*Magnetococcus marinus*	MC-1^{T}	1103
	Rhizobiales	*Bradyrhizobiaceae*	*Bradyrhizobium japonicum*	ATCC 10324^{T}	310
		“*Aurantimonadaceae*”	*Aurantimonas coralicida*	WP1^{T}	166879
		Bartonellaceae	*Bartonella bacilliformis*	KC583^{T}	305
		Methylobacteriaceae	*Methylobacterium extorquens*	AM1	318
		Brucellaceae	*Brucella melitensis*	16M^{T}	568
		Beijerinckiaceae	*Beijerinckia indica* subsp. *indica*	ATCC 9039^{T}	310
		Cohaesibacteriaceae	*Cohaesibacter gelatinilyticus*	CL-GR15^{T}	204853
		Hyphomicrobiaceae	*Hyphomicrobium sulfonivorans*	S1^{T}	173186
		Methylocystaceae	*Methylocystis parvus*	OBBP^{T}	59626
		Phyllobacteriaceae	*Phyllobacterium myrisinacearum*	YR531	59766
		Rhizobiaceae	*Rhizobium leguminosum* bv. *trifoli*	WSM1325	68001
		Xanthobacteraceae	*Xanthobacter autotrophicus*	Py2	250
	Sphingomonadales	*Sphingomonadaceae*	*Sphingomonas paucimobilis*	ATCC 29837^{T}	183314
		Erythrobacteriaceae	*Erythrobacter longus*	DSM 6997	171386
	Rhodobacterales	*Rhodobacteraceae*	*Rhodobacter capsulatus*	DSM 938	209652
	Rhodospirillales	*Acetobacteriaceae*	*Acetobacter aceti* subsp. *aceti*	ATCC 15973^{T}	69159
		Rhodospirillaceae	*Rhodospirillum rubrum*	ATCC 11170^{T}	885
	Sneathiellales	*Sneathiellaceae*	Sneathiella glossod	JCM 23214	183245
Betaproteobacteria	*Burkholderiales*	*Burkholderiaceae*	*Burkholderia cepacia*	FL-2-3-10-S1-D0	167561

		Oxalobacteriaceae	*Oxalobacter formigenes*	OXCC13	1461
		Comamonadaceae	*Comamonas thiooxydans*	DSM 17888^{T}	146318
		Alcaliginaceae	*Alcaligenes faecalis* subsp. *phenolicus*	DSM 16503^{T}	69234
		Sutterellaceae	*Sutterella wadsworthensis*	2_1_59BFAA	60124
	Neisseriales	*Neisseriaceae*	*Neisseria gonorrhoeae*	FA1090	147
		Chromobacteriaceae	*Chromobacterium violaceum*	ATCC 12472^{T}	893
	Nitrosomonadales	*Nitrosomonadaceae*	*Nitrosomonas europaea*	ATCC 19718	1457
		Thiobacillaceae	*Thiobacillus thioparus*	Starkey^{T}	60145
			Thiobacillus denitrificans	ATCC 23644^{T}	905
		Gallionellaceae	*Ferriphaselus amnicola*	OYT1^{T}	147960
			Sulfuricella denitrificans	NBRC 105220^{T}	58775
		Methylophilaceae	*Methylophilus methylotrophus*	NCIMB 10515^{T}	58935
		Sterolibacteriaceae	*Sterolibacterium denitrificans*	Chol-1S^{T}	186758
	Rhodocyclales	*Azonexaceae*	*Azonexus hydrophilus*	DSM 23864^{T}	68312
Hydrogenophilalia	*Hydrogenphilales*	*Hydrogenophilaceae*	*Tepidiphilus succinatimandens*	BON4^{T}	155900
			Tepidiphilus margaritifer	N2-214^{T}	68982
Gammaproteobacteria	*Chromatiales*	*Ectothiorhodospiraceae*	*Ectothiorhodospira mobilis*	DSM 4180	205292
		Halothiobacillaceae	*Halothiobacillus neapolitanus*	ParkerX^{T}	110147
		Woeseiaceae	*Woeseiea oceani*	XK5^{T}	215965
	Legionellales	*Coxiellaceae*	*Coxiella burnetii*	CbuG_Q212	621
		Legionellaceae	*Legionella pneumophila* subsp. *pneumophila*	Philadelphia1^{T}	67744
	Lysobacterales	*Lysobacteraceae*	*Lysobacter enzymogenes*	ATCC 29487	207164
		Rhodanobacteraceae	*Rhodanobacter lindaniclasticus*	PRJNA 89551	90277
	Acidiferrobacterales	*Acidiferrobacteraceae*	*Acidiferrobacter thiooxydans*	ZJ	201216
	Aeromonadales	*Aeromonadaceae*	*Aeromonas hydrophila* subsp. *hydrophila*	ATCC 7966^{T}	230
		Succinivibrionaceae	*Succinivibrio dextrinosolvens*	H5	194891
	Methylococcales	*Methylococcaceae*	*Methylococcus capsulatus*	Texas^{T}	69142
	Nevskiales	"*Salinisphaeraceae*"	*Salinisphaera shabanensis*	EIL3A^{T}	60624
		Algiphilaceae	*Algiphilus aromaticivorans*	DG1254^{T}	166578

		Nevskiaceae	*Nevskia ramose*	DSM 11499^{T}	60532
	Oceanospirillales	"*Saccharospirillaceae*"	*Saccharospirillum impatiens*	EL-105^{T}	68938
		Alcanivoraceae	*Alcanivorax borkumensis*	SK2^{T}	1478
		Hahellaceae	*Hahella chejuensis*	KCTC 2396	936
		Halomonadaceae	*Halomonas elgonaga*	DSM 2581	55558
		Oceanospirillaceae	*Oceanospirillum beijerinckii* subsp. *beijerickii*	DSM 7166^{T}	68800
	Thiotrichales	*Thiothrichaceae*	*Thiothrix nivea*	DSM 5205^{T}	60666
	Pseudomonadales	*Pseudomonadaceae*	*Pseudomonas aeruginosa*	DSM 50071^{T}	152515
	Alteromonadales	*Colwelliaceae*	*Colwellia psychrerythraea*	34H	1495
		Alteromonadaceae	*Altermonas macleodii*	DSM 6062^{T}	1532
		Ferriomonadaceae	*Ferrimonas balaerica*	DSM 9769^{T}	952
		Idiomarinaceae	*Idiomarina abyssalis*	KMM227	148224
		Moritellaceae	*Moritella marina*	ATCC 15381^{T}	58902
		Pseudoalteromonadaceae	*Pseudoalteromonas haloplanktis*	TAC125	952
		Psychromonadaceae	*Psychromonas ingrahamii*	37	946
		Shewanellaceae	*Shewanella putrefaciens*	CN-32	373
	Cardiobacteriales	*Cardiobacteriaceae*	*Cardiobacterium hominis*	ATCC 15826^{T}	1537
	Cellvibrionales	*Cellvibrionaceae*	*Cellvibrio japonicas*	UEDA107	1491
		Halieaceae	*Haliea salexigens*	DSM 19537	68605
		Microbulbiferaceae	*Microbulbifer variabilis*	Ni-2088^{T}	60509
		Porticoccaceae	"*Porticoccus hydrocarbonoclasticus*"	MCTC13d	177734
		Spongiibacteraceae	*Spongiibacter marinus*	DSM 19753	68725
	"*Enterobacteriales*"	*Enterobacteriaceae*	*Escherichia coli*	K12 MG1655	21
	"*Vibrionales*"	*Vibrionaceae*	*Vibrio cholera*	M66-2	212
Acidithiobacillia	*Acidithiobacillales*	*Acidithiobacillaceae*	*Acidithiobacillus thiooxidans*	ATCC 19377^{T}	58968
		Thermithiobacillaceae	*Thermithiobacillus tepidarius*	DSM 3134^{T}	68989
Oligoflexia	*Oligoflexales*	*Oligoflexaceae*	*Oligoflexus tunisiensis*	Shr3^{T}	208798
Deltaprotebacteria	*Bdellovibrionales*	*Bdellovibrionaceae*	*Bdellovibrio bacteriovorus*	HD100	909

		Halobacteriovoracaceae	*Halobacteriovorax marinus*	SJ	4117
	Desulfovibrionales	*Desulfonatronaceae*	*Desulfonatronum lacustre*	DSM 10312^{T}	68483
		Desulfovibrionaceae	*Desulfovibrio desulfuricans*	ATCC 27774	790
		Desulfohalobiaceae	*Desulfohalobium retbaense*	ATCC 49708^{T}	786
		Desulfomicrobiaceae	*Desulfomicrobium baculatum*	DSM 4028^{T}	787
	Desulfobacterales	*Nitrospinaceae*	*Nitrospina gracilis*	3/211^{T}	68765
	Syntrophobacterales	*Syntrophobacteraceae*	*Syntrophobacter fumaroxidans*	MPOB^{T}	915
	Desulfarculales	*Desulfarculaceae*	*Desulfarculus baarsii*	DSM 2075^{T}	4128
	Desulfobacterales	*Desulfobacteraceae*	*Desulfobacter postgatei*	DSM 2034^{T}	60370
		Desulfobulbaceae	*Desulfobulbus propionicus*	ATCC 33891^{T}	4130
	Myxococcales	*Cystobacteraceae*	*Cystobacter fuscus*	DSM 2262^{T}	60364
		Myxococcaceae	*Myxococcus fulvus*	HW-1	55631
		“*Haliangiaceae*”	*Haliangium ochraceum*	DSM 14365^{T}	910
		Nannocystaceae	*Nannocystis exedens*	DSM 71^{T}	208322
		Sandaracinaceae	*Sandaracinus amyloliticus*	NOSO-4^{T}	215369
	Desulfuromonadales	*Desulfuromonadaceae*	*Desulfomonas acetoxidans*	DSM 684^{T}	800
		Geobacteraceae	*Geobacter metallireducens*	GS-15^{T}	746
Epsilonproteobacteria	*Campylobacterales*	“*Hydrogenimonaceae*”	*Hydrogenimonas thermophila*	EP1-55-1%^{T}	206187
		Campylobacteraceae	*Campylobacter fetus* subsp. *fetus*	82-40	330
		Helicobacteraceae	*Helicobacter pylori*	52	67647
	Nautiliales	*Nautiliaceae*	*Nautilia profundicola*	AmH^{T}	1084
Phylum: **“*Aquificeae*”**					
Aquificeae	*Aquificales*	*Aquificaceae*	“*Aquifex aeolicus*”	VF5	834
		Desulfurobacteriaceae	*Desulfurobacterium thermolithotrophicus*	DSM 11699^{T}	4134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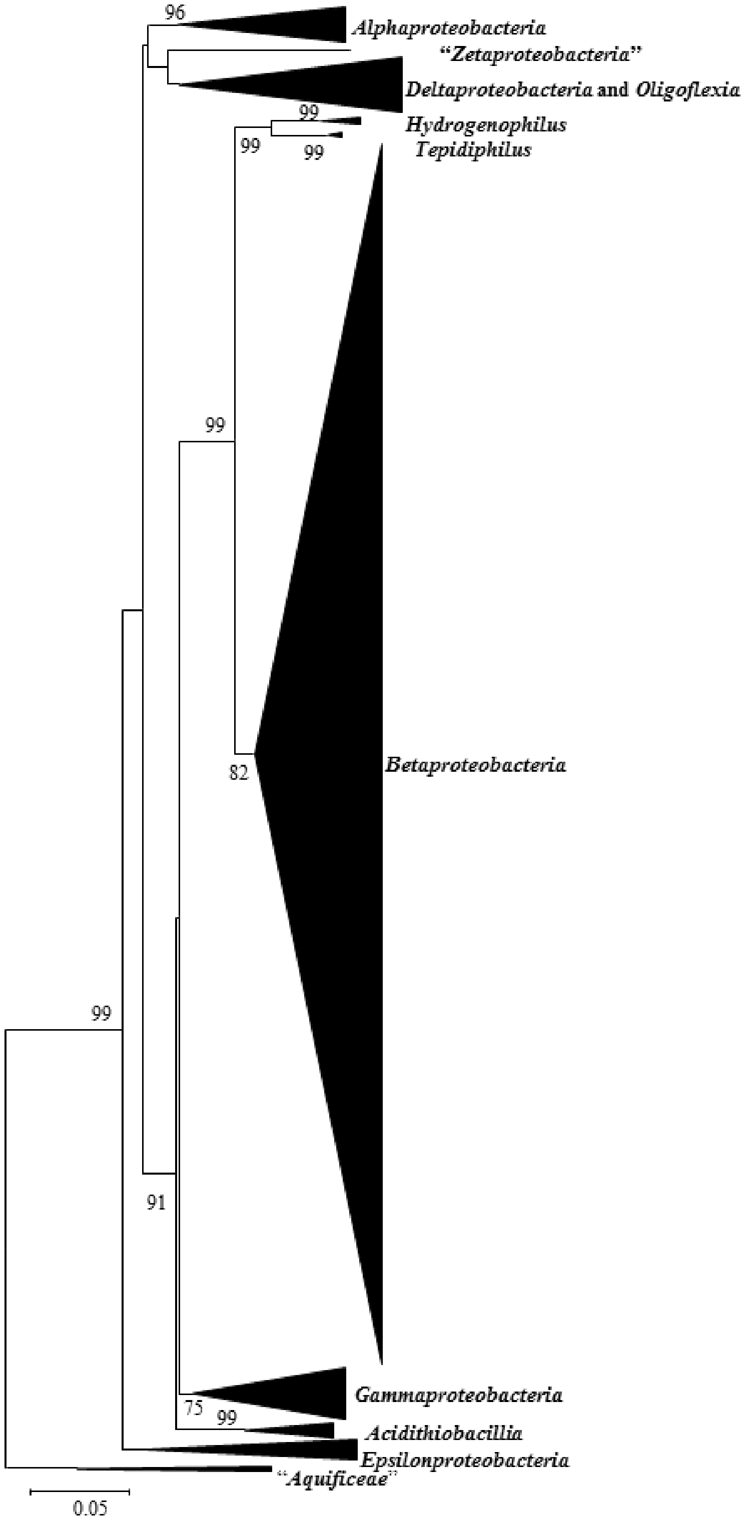
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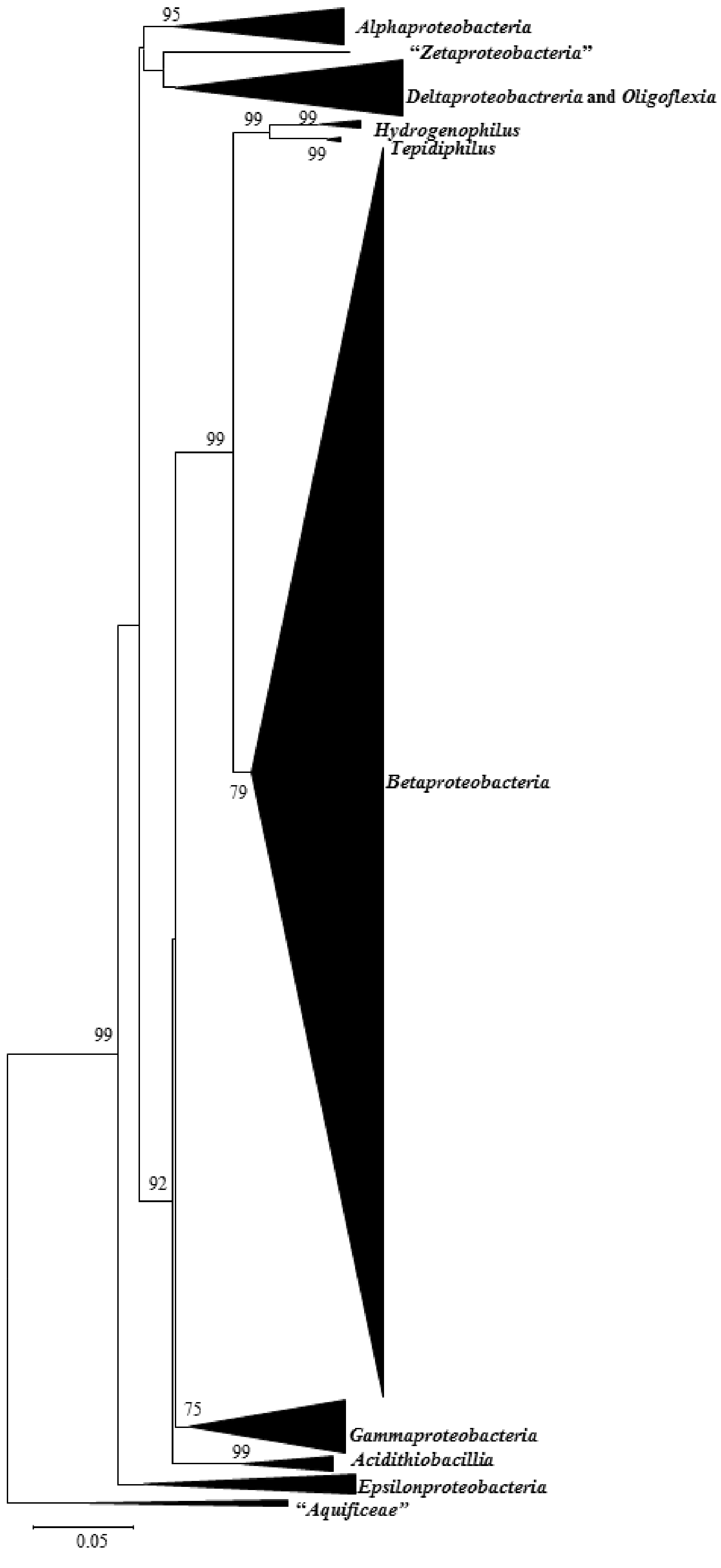
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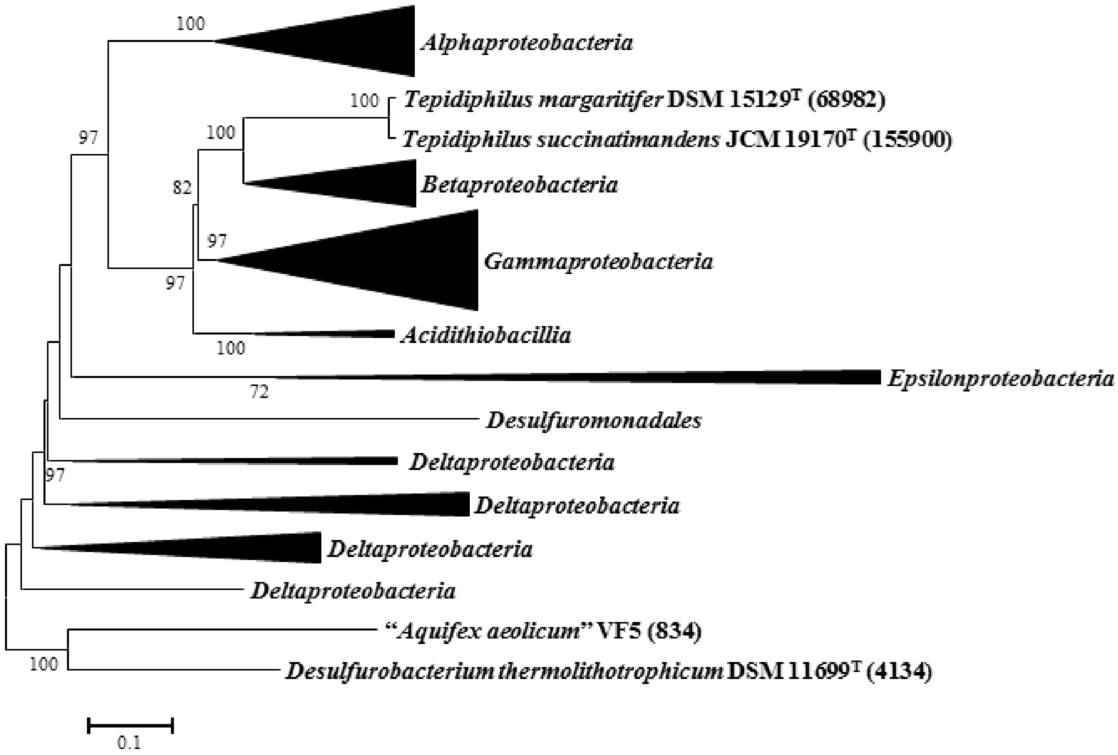
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Tepidiphilus succinatimandens JCM 19170T (155900)
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Betaproteobacteria
82
97
Gammaproteobacteria
97
Acidithiobacillia
100
Epsilonproteobacteria
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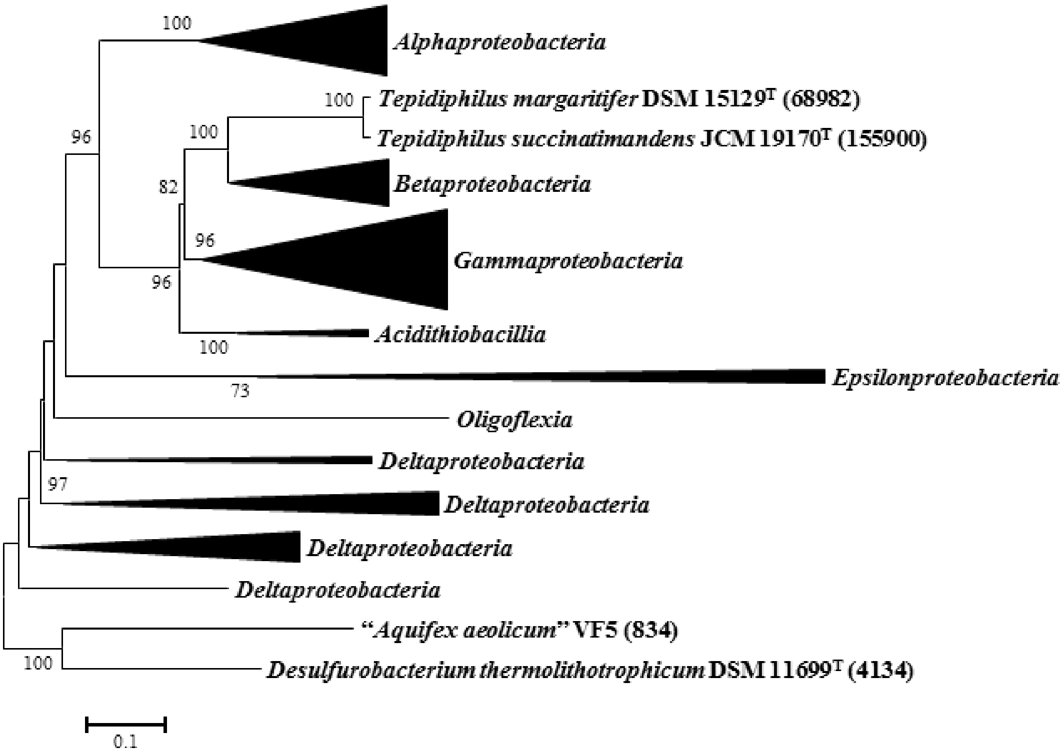
Desulfuromonadales
Deltaproteobacteria
97
Deltaproteobacteria
Deltaproteobacteria
Deltaproteobacteria
"Aquifex aeolicum" VF5 (834)
100
Desulfurobacterium thermolithotrophicum DSM 11699T (4134)
0.1


100
Alphaproteobacteria
100
Tepidiphilus margaritifer DSM 15129T (68982)
100
Tepidiphilus succinatimandens JCM 19170T (155900)
96
82
Betaproteobacteria
96
Gammaproteobacteria
96
Acidithiobacillia
100
Epsilonproteobacteria
73
Oligoflexia
Deltaproteobacteria
97
Deltaproteobacteria
Deltaproteobacteria
Deltaproteobacteria
"Aquifex aeolicum" VF5 (834)
100
Desulfurobacterium thermolithotrophicum DSM 11699T (4134)
0.1