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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 four new families within the orders Nitrosomonadales and Rhodocyclales

Boden, R

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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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Corresponding Author:	Rich Boden, Ph.D B.Sc (Lond.) PGCert University of Plymouth Plymouth, Devon UNITED KINGDOM
First Author:	Rich Boden, Ph.D B.Sc (Lond.) PGCert
Order of Authors:	Rich Boden, Ph.D B.Sc (Lond.) PGCert  Lee P Hutt, Ph.D B.Sc  Alex W Rae
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Abstract:	The genus <i>Thiobacillus</i> comprises 4 species with validly published names, of which <i>T. aquaesulis</i> DSM 4255T (= ATCC 43788T) 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 <i>Thiobacillus thioparus</i> DSM 505T, we propose that it is moved to a novel genus <i>Annwoodia</i> gen. nov., for which the type species is <i>Annwoodia aquaesulis</i> gen. nov., comb. nov. We confirm the position of the genus <i>Thiobacillus</i> 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 <i>Thiobacillaceae</i> fam. nov. and the <i>Sterolibacteriaceae</i> fam. nov. We propose the merging of the family <i>Methylophilaceae</i> into the Nitrosomonadales, and that the <i>Sulfuricellaceae</i> be merged into the <i>Gallionellaceae</i> , leaving the orders <i>Methylophilales</i> and <i>Sulfuricellales</i> defunct. In the Rhodocyclales we propose the <i>Azonexaceae</i> fam. nov. and the <i>Zoogloaceae</i> fam. nov. We also reject the Hydrogenophilales from the Betaproteobacteria on the basis of a very low 16S 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 <i>Thiobacillus</i> , <i>Hydrogenophilales</i> , <i>Hydrogenophilaceae</i> , <i>Nitrosomonadales</i> , <i>Gallionellaceae</i> , <i>Rhodocyclaceae</i> and the Betaproteobacteria.



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

6    Rich Boden<sup>1,2</sup>, Lee P. Hutt<sup>1,2</sup> and Alex W. Rae<sup>1</sup>

7        1. School of Biological and Marine Sciences, Plymouth University, Plymouth, UK.  
8        2. Sustainable Earth Institute, Plymouth University, Plymouth, UK.

9        \* Corresponding author [rich.boden@plymouth.ac.uk](mailto:rich.boden@plymouth.ac.uk)

10      Portland Square A421, Plymouth University, Plymouth, UK

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12      **Keywords:** *Thiobacillus*, *Annwoodia*, *Betaproteobacteria*, *Nitrosomonadales*,  
13      Chemolithoautotrophy, *Hydrogenophilalia*

14

15    **Abstract**

16    The genus *Thiobacillus* comprises 4 species with validly published names, of which *T.*  
17    *aquaesulis* DSM 4255<sup>T</sup> (= ATCC 43788<sup>T</sup>) is the only species that can grow heterotrophically  
18    or mixotrophically – the rest being obligate autotrophs - and has a significant metabolic  
19    difference in not producing tetrathionate during the oxidation of thiosulfate during  
20    autotrophic growth. On the basis of this and differential chemotaxonomic properties and a  
21    16S rRNA gene identity of 93.4% to the type species *Thiobacillus thioparus* DSM 505<sup>T</sup>, we  
22    propose that it is moved to a novel genus *Annwoodia* gen. nov., for which the type species is  
23    *Annwoodia aquaesulis* gen. nov., comb. nov. We confirm the position of the genus  
24    *Thiobacillus* in the *Betaproteobacteria* falls within the *Nitrosomonadales* rather than the  
25    *Hydrogenophilales* as previously proposed. Within the *Nitrosomonadales* we propose the  
26    circumscription of genera to form the *Thiobacilliaceae* fam. nov. and the *Sterolibacteriaceae*  
27    fam. nov. We propose the merging of the family *Methylophilaceae* into the  
28    *Nitrosomonadales*, and that the *Sulfuricellaceae* be merged into the *Gallionellaceae*, leaving  
29    the orders *Methylophilales* and *Sulfuricellales* defunct. In the *Rhodocyclales* we propose the  
30    *Azonexaceae* fam. nov. and the *Zoogloeaceae* fam. nov. We also reject the  
31    *Hydrogenophilales* from the *Betaproteobacteria* on the basis of a very low 16S rRNA gene  
32    identity with the class-proper as well as physiological properties, forming the  
33    *Hydrogenophilalia* class. nov. in the ‘*Proteobacteria*’. We provide emended descriptions of  
34    *Thiobacillus*, *Hydrogenophilales*, *Hydrogenophilaceae*, *Nitrosomonadales*, *Gallionellaceae*,  
35    *Rhodocyclaceae* and the *Betaproteobacteria*.

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41 The genus *Thiobacillus* (Beijerinck, 1904) in the *Betaproteobacteria* is currently considered  
42 as a member of the family *Hydrogenophilaceae* in the order *Hydrogenophilales* (Garrity,  
43 2005a). The genus has historically comprised over 30 species though the majority have been  
44 transferred to other genera or lost (Boden *et al.* 2012). The genus is regarded as containing 4  
45 species with validly published names – *T. thioparus* (type species, Beijerinck, 1904 – *N.B.*  
46 the type strain is Starkey's Culture C<sup>T</sup> (= Starkey<sup>T</sup> = DSM 505<sup>T</sup>) isolated from agricultural  
47 soil – Starkey, 1935), *Thiobacillus thiophilus* (Kellermann and Griebler, 2009, isolated from  
48 aquifer sediment), *Thiobacillus denitrificans* (*ex.* Beijerinck (1904), Kelly and Harrison 1989,  
49 type strain is a sewage isolate from Hutchinson *et al.*, 1967) and *Thiobacillus aquaesulis*  
50 (Wood & Kelly, 1995 (Validation List 52, 1995), isolated from a thermal sulfur spring, Wood  
51 & Kelly, 1988).

52 All but one of the *Thiobacillus* spp. with validly published names are obligate  
53 chemolithoautotrophs (assimilating carbon *via* the transaldolase variant of the Calvin-  
54 Benson-Bassham cycle (Beller *et al.*, 2006, Hutt *et al.* submitted), utilising reduced sulfur  
55 species and elementary sulfur as energy sources, but *T. aquaesulis* can grow heterotrophically  
56 on complex media (Wood & Kelly, 1988). It is known that *Thiobacillus* spp. have incomplete  
57 versions of Krebs' cycle and instead use some of the enzymes of the cycle in the form of  
58 Smith's biosynthetic horseshoe (Smith *et al.*, 1967), through which intermediates and amino  
59 acids *etc* are synthesised, but heterotrophic growth is not possible. The absence of fumarase  
60 and 2-oxoglutarate dehydrogenase has been observed in the genomes of *T. denitrificans*  
61 (Beller *et al.* 2006) and *T. thioparus* (Hutt *et al.*, *submitted*) – reviewed by Wood *et al.*  
62 (2004), along with other enzymes of Krebs' cycle in the latter. Since *T. aquaesulis* grows on  
63 complex media (but not in minimal media supplemented with C<sub>1</sub> compounds, sugars or  
64 amino acids individually, Wood & Kelly, 1988), it would be anticipated to have a full version

65 of Krebs' cycle or has some manner of bypass that permits growth in the absence of these  
66 core enzymes. It is worth noting that the same lesions of Krebs' cycle are found in all  
67 obligate autotrophs examined thus far (Wood *et al.*, 2004; Boden *et al.*, 2016) and whilst  
68 larger lesions are found in some species (*e.g.* Boden *et al.*, 2016), 2-oxoglutarate  
69 dehydrogenase and usually fumarase and/or succinate dehydrogenase and/or succinyl-  
70 coenzyme A synthetase are commonly absent. As such, a facultative autotroph so closely  
71 related to obligate autotrophs must have significant core metabolic differences.

72 Thiosulfate oxidation in organisms that use the Kelly-Trudinger pathway (Boden *et al.*, 2016,  
73 aka the "S<sub>4</sub>I" or "tetrathionate intermediary" pathway) always produce tetrathionate as a  
74 detectable intermediate in the medium in the early stages of growth, often alongside  
75 trithionate and/or pentathionate, but this varies considerably (observed in dozens of studies  
76 from the 1930s onwards, 1930s to 1960s studies are reviewed in Roy & Trudinger, 1970,  
77 later studies in Boden *et al.*, 2012 and Boden *et al.* 2016). *Thiobacillus* spp. use the Kelly-  
78 Trudinger pathway and indeed produce tetrathionate, which is accompanied by an increase in  
79 culture pH in the first 24h of growth whilst 100% of thiosulfate is oxidised to tetrathionate,  
80 after which tetrathionate is oxidised and the pH falls, usually to around pH 4.5 to pH 5.5,  
81 with concomitant production of sulfate. In batch cultures or oxygen-limited chemostats,  
82 elementary sulfur is often also produced as an intermediate and is observable within cells of  
83 *Thiobacillus* spp. as granules in the periplasmic space (Katayama-Fujimura *et al.*, 1984).

84 Whilst *T. aquaesulic* still produces elementary sulfur during growth on thiosulfate, it does not  
85 produce detectable levels of any polythionates in the medium, nor does the pH increase  
86 during the early stages of growth. The final pH of cultures is usually higher – pH 6.0 or so –  
87 even when grown on media with the same starting pH as *T. thioparus*. These differences  
88 indicate a potential variant of the Kelly-Trudinger pathway for thiosulfate metabolism that  
89 does not proceed *via* tetrathionate (or other polythionates) yet still *permits* growth on

90 tetrathionate and trithionate as sole energy sources. Whilst this tetrathionate-free metabolism  
91 could superficially indicate that the Kelly-Friedrich pathway (“Sox”) pathway is in use, the  
92 production of elementary sulfur in this way would probably be indicative of this not being the  
93 case. There is an implication of a different evolution of the sulfur-oxidation pathway in *T.*  
94 *aquaesulis*, distinct from *Thiobacillus* spp.

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

104 All chemicals were obtained from Sigma-Aldrich (Poole, UK) and were of “analytical” grade  
105 or higher, unless otherwise stated. Glass-distilled deionised water (*i.e.* deionised water that  
106 was then glass-distilled) and ‘Class A’ volumetric glassware were used to prepare all media  
107 and for all analyses. A calibrated 4-decimal place balance was used throughout. *Thiobacillus*  
108 *aquaesulis* DSM 4255<sup>T</sup> and *T. thioparus* DSM 505<sup>T</sup> were obtained from the Leibniz-Institut  
109 DSMZ Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH. *T. denitrificans*  
110 NCIMB 9548<sup>T</sup> was a kind gift of Dr Peter Green (NCIMB Ltd, Aberdeen, UK). All strains  
111 were maintained in E-basal salts (EBS, Wood & Kelly 1985) (g/1,000.0mL) containing  
112 NH<sub>4</sub>Cl (0.40), MgSO<sub>4</sub>·7H<sub>2</sub>O (0.80), KH<sub>2</sub>PO<sub>4</sub> (4.00), K<sub>2</sub>HPO<sub>4</sub> (4.00) and trace metals solution  
113 (Kelly & Wood 1998, 10.0mL). EBS was supplemented with 20mM sodium thiosulfate for  
114 maintenance and solidified with 15.00g/L Oxoid No 1 agar that had been washed with glass-

115 distilled water and dried before use. Growth on carbon disulfide (contaminating carbonyl  
116 sulfide removed by washing according to Skidmore, 1979), dimethylsulfide and  
117 dimethyldisulfide was tested per Boden *et al.* 2010, using EBS as the basal salts. All  
118 chemotaxonomic tests and microscopy were performed according to Boden *et al.* (2010),  
119 using high molecular weight genomic DNA standards from Sigma-Aldrich to calibrate for  
120 G+C content determination (*Escherichia coli* Strain B, 50.0 mol%, Cat. No. D4889; and  
121 *Micrococcus luteus* ATCC 4698<sup>T</sup> 71.9 mol%, Cat. No. D8259, G+C contents are as certified  
122 by manufacturer), along with genomic DNA from *Thermithiobacillus tepidarius* (66.8 mol%,  
123 Boden *et al.*, 2016). G+C contents for *T. thioparus* DSM 505<sup>T</sup> and *T. aquaesulus* DSM 4255<sup>T</sup>  
124 were checked using this method (acid denaturation, Fredericq *et al.* 1969) and gave good  
125 agreement with the originally published data (Table I). Whilst this method is not widely used,  
126 we have found agreement with data obtained from it to within 1.0 mol% with data from  
127 genome sequences providing it is properly calibrated on every use (*cf.* Boden *et al.* 2010;  
128 Boden *et al.* 2011).

129 Phylogenetic analyses of the *Betaproteobacteria* were performed using MEGA 7.0.20  
130 (Kumar *et al.* 2016) on the basis of 16S rRNA gene sequences from taxa with validly  
131 published names – almost 500 sequences. All members of the *Betaproteobacteria* based on  
132 the [www.bacterio.net](http://www.bacterio.net) website and the 2005 release of Bergey's manual (Garrity *et al.*, 2005b)  
133 were included in the analyses *pace* *Gallionella* as no *bona fide* cultures (thus sequences) exist  
134 – as such, the *Gallionellaceae* were represented only by the genus *Ferriphaselus* for the sake  
135 of the analysis. Genera *incertae sedis* were included in the analysis as long as they were  
136 considered *Betaproteobacteria*. Alignments were performed using the MUSCLE algorithm  
137 (Edgar *et al.*, 2004) without using the pre-sets for large datasets that reduce accuracy.  
138 Phylogenetic trees were constructed using the maximum likelihood method with the Tamura-  
139 Nei model (Tamura & Nei, 1993) and pairwise deletion of gaps. 5,000 bootstrap replicates

140 were performed and values at nodes are shown if greater than 70%. The outgroup was the  
141 genus *Allochromatium* from the *Gammaproteobacteria*.

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

163 Ribosomal multilocus sequence typing (rMLST, Jolley *et al.* 2012) was also used with regard  
164 to phylum-level analyses. Representatives from the ‘*Proteobacteria*’ and the ‘*Aquificeae*’ for

165 which genome sequences are available in the PupMLST database ([www.pubmlst.org](http://www.pubmlst.org)), with  
166 an emphasis on using the type species of the type genera of families across the classes of the  
167 ‘*Proteobacteria*’, with type strains used where available. Where type species were not  
168 available, other members of the same genus or family were used. Genes encoding 53  
169 ribosomal proteins (*rps*, *rpl* and *rpm* genes) were extracted from each genome using the  
170 rMLST platform, using only complete genes, trimmed to the same length and concatenated.  
171 Concatamers were downloaded into MEGA, translated into derived amino acyl sequences  
172 using the bacterial genetic code and aligned using MUSCLE. Phylogenetic trees were  
173 reconstructed from amino acid alignments per the 16S rRNA gene trees but using the Jones-  
174 Taylor-Thornton algorithm (Jones *et al.* 1992) and, again, using the phylum ‘*Aquificeae*’ as  
175 the outgroup.

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

Properties of DSM 4255<sup>T</sup> *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<sup>T</sup> does not fall within the same genus as the 3 *Thiobacillus* spp. included in Table I. DSM 4255<sup>T</sup> 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<sup>T</sup>, 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<sub>15:0</sub>, C<sub>17:0</sub>, C<sub>17:1</sub>, C<sub>16:0</sub>) differ somewhat from those in DSM 4255<sup>T</sup> (C<sub>16:0</sub>, C<sub>16:1</sub>, C<sub>15:0</sub>, C<sub>17:1</sub>), when all grown autotrophically on thiosulfate (Agate & Vishniac, 1973; Fullarton *et al.*, 1995). The phylogenetic position of DSM 4255<sup>T</sup> 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<sup>T</sup> form a novel genus – *Annwoodia* gen. nov. – and the new combination *Annwoodia aquaesulis* comb. nov. for the type species.

214 The genus *Thiobacillus* per *Bergey's Manual of Systematic Bacteriology*, 2<sup>nd</sup> Edition (Kelly  
215 *et al.*, 2005) falls within the *Hydrogenophilales*; however, our more detailed phylogenetic  
216 analysis of the entire *Betaproteobacteria* (Figures 1 and 2) – which obviously contains more  
217 taxa than in 2003/4 when analyses for the 2005 edition of the *Manual* were presumably  
218 performed - shows that these taxa are quite unrelated to this order (identity 87.6% - below the  
219 Yarza median for the rank of order), though they can appear superficially similar in trees we  
220 constructed containing smaller numbers of sequences, and particularly neighbour-joining  
221 trees (*data not shown*). The *Betaproteobacteria* contains the orders *Burkholderiales* (type  
222 order), *Neisseriales*, *Methylophilales*, *Sulfuricellales*, *Rhodocyclales*, *Hydrogenophilales* and  
223 *Nitrosomonadales*, all with validly published names (Garrity *et al.*, 2005b and Watanabe *et*  
224 *al.* 2015). *Thiobacillus* spp. and DSM 4255<sup>T</sup> form a clade within the *Nitrosomonadales*,  
225 which contains in turn 3 families with validly published names – *Nitrosomonadaceae* (type  
226 family), *Spirillaceae* and *Gallionellaceae*. Figure 1 shows that the orders *Methylophilales* and  
227 *Sulfuricellales* cluster within the *Nitrosomonadales* and on the basis of pairwise distances  
228 falling below the Yarza median for family, are probably not separate families but fall within  
229 this order. On this basis, we propose that the *Methylophilaceae* and *Sulfuricellaceae* are  
230 transferred to the *Nitrosomonadales* and that the former forms a family within that order;  
231 however, the *Sulfuricellaecae* have ≥92.5 % pairwise identity with the only sequence  
232 available for the *Gallionellaceae* (at or above the Yarza median for the rank of family, *viz.*  
233 92.25%), thus we propose that the genera of the *Sulfuricellaceae* and *Gallionellaceae* should  
234 be circumscribed as one family and that the name *Gallionellaceae* (Henrici & Johnson, 1935)  
235 takes priority over *Sulfuricellaceae* (Watanabe *et al.*, 2015), according to Rule 47a of the  
236 *Bacteriological Code*. This leaves the orders *Methylophilales* and *Sulfuricellales* defunct. We  
237 propose that the other two clades within the *Nitrosomonadales* are each circumscribed to  
238 form families - the *Thiobacillaceae* fam. nov. and the *Sterolibacteriaceae* fam. nov., named

239 on the basis of the earliest legitimate genus in accordance with the *Code*, and circumscribed  
240 on the basis of pairwise 16S rRNA gene distances falling above the Yarza median within  
241 each group as well as clustering into distinct phylogenetic clades in Figure 1 and 2 – the  
242 genera that these circumscribe are given in the descriptions of these novel taxa below. It is  
243 worth noting that genera of the *Sterolibacteriaceae* were formerly assigned to the  
244 *Rhodocyclales* rather than the *Nitrosomonadales*; however, our analyses demonstrate  
245 affiliation with the *Nitrosomonadales* on the basis of maximum likelihood trees of the whole  
246 class *Betaproteobacteria*. It can be seen from Figure 1 that a small clade of genera *incertae*  
247 *sedis* falls within the *Nitrosomonadales*: these are *Chitinovorax*, *Rivicola* and *Leeia*. These  
248 genera may warrant a further family being defined (the “*Leeiaceae*”) in the  
249 *Nitrosomonadales*, or, may fall within the latter, but we do not have sufficient data to  
250 recommend either at this time.

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

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

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

297 Table 2 gives properties of each family examined in this study, alongside the *Burkholderiales*  
298 and *Neisseriales* for reference. All genera examined contained palmitic acid ( $C_{16:0}$ ) amongst  
299 the dominant fatty acids and ubiquinone-8 (UQ-8) as their dominant respiratory quinone, as  
300 well as each family having aerobic representitives. Most of the *Rhodocyclales* also contained  
301 unsaturated fatty acids  $C_{16:1}$  and  $C_{18:1}$ , but the *Zoogloaceae* fam. nov. are united by their  
302 presence of 3-hydroxycapric acid ( $C_{10:0}$  3-OH) as one of their dominant fatty acids in many  
303 genera, distinguishing this family from the rest of the order. The respiratory quinones of this  
304 order are effectively unified by their variability as two families contain a second quinone at a  
305 significant concentration, *viz.* menaquinone-8 (MK-8) in *Rhodocyclaceae* and rhodoquinone-  
306 8 (RQ-8) in *Zoogloaceae* fam. nov. (the latter also found in two genera of the  
307 *Burkholderiales*). The *Nitrosomonadales* often contain stearic acid ( $C_{18:0}$ ) at a high  
308 concentration but three families – the *Sterolibacteriaceae* fam. nov., the *Spirillaceae* and the  
309 *Gallionellaceae* – also contained significant amounts of hydroxylated fatty acids 2-  
310 hydroxypentadecylic acid ( $C_{15:0}$  2-OH) and/or  $C_{10:0}$  3-OH. In contrast, the *Hydrogenophilales*  
311 class. nov. are distinguished by their high concentrations of  $\omega$ -cyclohexyl fatty acids (*viz.*

312 cyclohexylmargaric ( $C_{17:0}$  *cyclo*) and cyclohexylnonadecylic acids ( $C_{19:0}$  *cyclo*)) in both  
313 known genera, accounting for up to *c.* 30 % of the fatty acid content in *Tepidiphilus* spp.  
314 (Poddar *et al.*, 2014). These fatty acids are relatively rare in nature, and in the domain  
315 *Bacteria* are conserved to a relatively small number of taxa – canonical examples being  
316 species of the genera *Alicyclobacillus* ('*Firmicutes*'), *Sulfovibacillus* (*Clostridia*),  
317 *Propionibacterium* and *Curtobacterium* (*Actinobacteria*) – but not commonly in the  
318 '*Proteobacteria*', except at trace amounts (Suzuki *et al.*, 1981; Kusano *et al.*, 1997; da Costa  
319 *et al.*, 2011), with the obvious exception of *Pandoraea* spp. in the *Betaproteobacteria*  
320 (*Burkholderiales*). Where they do occur, they are usually considered as an adaptation to heat  
321 or low pH, thus it is no surprise to find them in an order united by temperature optima of 50–  
322 65 °C and which are universally isolated from high-temperature extreme environments. Their  
323 pH optima (6.5 – 8.0) do not indicate acidophilia, thus low pH is not likely to be the cause of  
324 the evolution of these fatty acids in this group. This, along with their profile as obligate  
325 thermophiles (distinct from the *Betaproteobacteria* examined in Table 2) represents a  
326 significant physiological difference from the *Betaproteobacteria sensu stricto*, further  
327 evidencing their status as a separate class.

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

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

343 Heterotrophy across the *Nitrosomonadales* and *Rhodocyclales* is relatively restricted in  
344 comparison to the *Neisseriales* and *Burkholderiales*, often to less routine carbon sources such  
345 as sterols or aromatics – more so in the former order - with sugars and complex media only  
346 used by some families. Methylotrophy is present in the *Methylophilaceae* and  
347 *Sterolibacteriaceae* fam. nov., as is C<sub>1</sub> autotrophy (dissimilation of C<sub>1</sub> compounds to carbon  
348 dioxide which is then assimilated *via* the Calvin-Benson-Bassham cycle, Anthony, 1982) in  
349 the *Thiobacillaceae* fam. nov., namely on methylated sulfur compounds. Methylotrophy is  
350 also found in some *Neisseriales*, thus is relatively widespread across the class.

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

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

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

386 Metabolically, the *Hydrogenophilales* contain a genus of each autotrophs and heterotrophs,  
387 the former using molecular hydrogen as an electron donor. They are unified by the same  
388 dominant respiratory quinone (ubiquinone-8) and a relatively small range in G+C contents –  
389 58.6 – 65.0 mol%.

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

395

396 **Emended description of the *Betaproteobacteria* (Garrity et al., 2006, Garrity et al.,  
397 2005b)**

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

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

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

410 **Description of *Hydrogenophilalia* class. nov.**

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

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

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

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

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

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

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

434 **Emended description of the *Hydrogenophilaceae* (Garrity *et al.* 2006, Garrity *et al.*  
435 2005d)**

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

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

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

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

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

451 This order is re-defined following the addition of members on the basis of 16S rRNA gene  
452 sequence analysis. Contains the families *Nitrosomonadaceae*, *Gallionellaceae*,

453 *Methylophilaceae*, *Spirillaceae*, *Thiobacillaceae* and *Sterolibacteriaceae*. Contains a broad  
454 diversity of metabolisms including autotrophs, nitrogen fixing heterotrophs, iron-oxidisers  
455 and methylotrophs. All families are mesophilic and use ubiquinone-8 as the dominant  
456 respiratory quinone.

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

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

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

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

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

473 **Description of *Thiobacillaceae* fam. nov.**

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

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

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

486 **Emended description of *Thiobacillus* (Beijerinck, 1904)**

487 *Thiobacillus* (Thi.o.ba.cil 'lus. Gr. n. *theios*, sulfur, L. transliteration *theios*, sulfur. L. n.  
488 *bacillus* a small rod; N.L. masc. n. *Thiobacillus* sulfur rodlet).  
489 Contains autotrophic sulfur-oxidising bacteria obtaining their energy from the oxidation of  
490 reduced sulfur oxyanions, namely thiosulfate and tetrathionate plus other sulfur species such  
491 as polythionates, elementary sulfur and/or methylated sulfur compounds. G+C contents of  
492 genomic DNA range from 61.5 to 66.0 mol%. No heterotrophy or mixotrophy is observed.  
493 Growth temperatures are under 30 °C and usually mesophilic and sometimes psychrotolerant.  
494 Catalase and oxidase positive when grown aerobically on thiosulfate agar. Produce  
495 tetrathionate as a detectable intermediate of thiosulfate oxidation, released into the medium,  
496 often with trithionate and pentathionate. Oxidise thiosulfate using the Kelly-Trudinger  
497 pathway and fix carbon dioxide with the transaldolase variant of the Calvin-Benson-Bassham  
498 cycle. Some species contain carboxysomes detectable using transmission electron  
499 microscopy, which are repressed by growth at elevated carbon dioxide partial pressures. Most

500 species form polyphosphate (volutin) granules during aerobic growth on thiosulfate. Fatty  
501 acids include C<sub>16:0</sub>, C<sub>16:1</sub>, C<sub>15:0</sub> and C<sub>17:1</sub> when grown autotrophically on thiosulfate.

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

503

504 **Description of *Annwoodia* gen. nov.**

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

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

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

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

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

524 Baths at Bath, UK – source of isolation). Basonym: *Thiobacillus aquaesulus* (Wood & Kelly,  
525 1995).

526 Short rods  $0.3 \times 0.9 \mu\text{m}$  containing volutin (polyphosphate) granules. Motile by means of  
527 single polar flagella. Gram negative. Does not produce endospores or exospores. Does not  
528 accumulate polyhydroxybutyrate. Colonies on thiosulfate agar grown at 43–45 °C are 1–2 mm  
529 diameter, circular, convex, smooth and entire; off-white colonies become white then yellow  
530 with age as elementary sulfur builds up. Colonies on thiosulfate agar have a faint “burnt  
531 matches” smell. Grows on thiosulfate, trithionate, tetrathionate and elementary sulfur but not  
532 thiocyanate, molecular hydrogen, dimethylsulfide, dimethyldisulfide or carbon disulfide as  
533 sole energy sources. Grows anaerobically with denitrification but cannot use nitrate as a  
534 nitrogen source. Uses ammonium as a nitrogen source. Cultures with an initial pH 7.0–9.0  
535 drop to pH 6.0–7.0. Grows from 30 to 55 °C with an optimum temperature of 45 °C.  
536 Thiosulfate-grown cultures at the optimal growth pH and temperature reach a specific growth  
537 rate of  $0.29 \text{ h}^{-1}$  in the exponential phase. The G+C content of genomic DNA is 65.7 mol%  
538 ( $T_m$ ). Dominant fatty acids are C<sub>16:0</sub> and C<sub>16:1</sub> when grown autotrophically on thiosulfate.  
539 Type strain is ATCC 43788<sup>T</sup> = DSM 4255<sup>T</sup>, isolated from the thermal sulfur spring at the  
540 Roman Baths, Bath, UK.

541 **Description of *Sterolibacteriaceae* fam. nov.**

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

545 This family is circumscribed on the basis of 16S rRNA gene sequences and comprises the  
546 genera *Sterolibacterium* (type genus), *Methyloversatilis*, *Sulfurisoma*, *Sulfuritalea*,  
547 *Denitratisoma* and *Georgfuchsia*. Varied metabolism from methylotrophs, facultative

548 autotrophs, denitrifiers and obligate anaerobes using ferric iron or manganic manganese as  
549 terminal electron acceptors. Autotrophic members use reduced sulfur or ferrous iron as  
550 electron donors. Oxidase positive but catalase variable. Usually contain hydroxylated  
551 saturated fatty acids as major components. Ubiquinone-8 is the dominant respiratory quinone.  
552 Type genus: *Sterolibacterium* (Tarlera and Denner 2003).

553 **Emended description of *Rhodocyclaceae* (Garrity *et al.* 2006 – Garrity *et al.*, 2005c)**  
554 *Rhodocyclaceae* (Rho.do.cy.cla.ce'ae. N.L. masc. n. *Rhodocyclus* type genus; -aceae suffix to  
555 denote family; N.L. fem. pl. n. *Rhodocyclaceae*, the *Rhodocyclus* family).

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

565 Type genus: *Rhodocyclus* (Pfennig, 1978)

566 **Description of *Zoogloeaceae* fam. nov.**

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

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

571 Family comprises strict aerobes that fix nitrogen, zoogloae-forming denitrifiers and more  
572 generalist heterotrophs. Ubiquinone-8 and rhodoquinone-8 are the dominant respiratory  
573 quinones. May contain hydroxylated saturated fatty acids as major components. Oxygen,  
574 nitrate, nitrite, nitrous oxide and selenate are used as terminal electron acceptors. Molecular  
575 hydrogen can be used as an electron donor by autotrophic members. Oxidase and catalase  
576 positive. Cells are usually rod-shaped and monotrichous or ‘degenerately peritrichous’.  $\omega$ -  
577 hydroxylcapric acid ( $C_{10:0}$  3-OH) is observed as major component in most known genera.  
578 Type genus: *Zoogloea* (Itzigsohn 1868)

579 **Description of *Azonexaceae* fam. nov.**

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

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

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

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604 **Conflicts of interest**

605 The authors declare that they have no conflicts of interest.

606

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766

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

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

779 Footnotes:

780 \* a small number of species are monotrichous;

781 † One species in this family is mixotrophic or heterotrophic;

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

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

785

786 **Figure 1.** Phylogenetic tree on the basis of the 16S rRNA (*rrs*) gene, showing the position of  
787 DSM 4255<sup>T</sup> (bold) and *Thiobacillus* spp. within the *Betaproteobacteria sensu Garrity et al.*  
788 (2005b). This includes the type strains of every species with a validly published name, but  
789 only the *Rhodocyclales* and *Nitrosomonadales* are shown, for clarity – the entire tree is given  
790 in Figure 4. MUSCLE was used to align nucleotide sequences and the tree reconstructed  
791 using the maximum likelihood method based on the Tamura-Nei model within MEGA 7.0.20.  
792 After 5,000 bootstrap replicate constructions, the tree with the highest log likelihood (-90516)  
793 is shown, with values next to nodes indicate the percentage of constructions in which the  
794 topology was preserved (values <70 % are omitted for clarity). All positions with < 95 % site  
795 coverage were omitted from the final analysis, which used 1,303 nt. Branch lengths are to  
796 scale and indicate the number of substitutions per site, with the scale bar indicating 2  
797 substitutions per site. The outgroup is the genus *Allochromatium* from the  
798 *Gammaproteobacteria*. Names of orders and families in the tree itself are the extant  
799 names/circumscriptions, with the novel groupings indicated by bars to the right, showing  
800 orders (green) and families (black).

801 **Figure 2.** Phylogenetic trees on the basis of the 16S rRNA (*rrs*) gene, showing the position of  
802 the two genera of the proposed *Hydrogenophilalia* class. nov. (diagonal shading) versus the  
803 classes of the ‘*Proteobacteria*’. The full list of 94 sequences used is given in Supplementary  
804 Table S1. Trees were reconstructed from MUSCLE alignments of the gene using the  
805 maximum likelihood, neighbour-joining and minimum evolution methods (all using the  
806 Tamura-Nei model) or the maximum parsimony method (tree bisection-reconnection method)  
807 within MEGA 7.0.20. After 5,000 bootstrap replicate constructions, the trees with the highest  
808 confidence are shown, with values at nodes indicating the percentage of constructions in  
809 which the topology was preserved (values <70 % are omitted for clarity). All positions with  
810 <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  
maximum 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

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

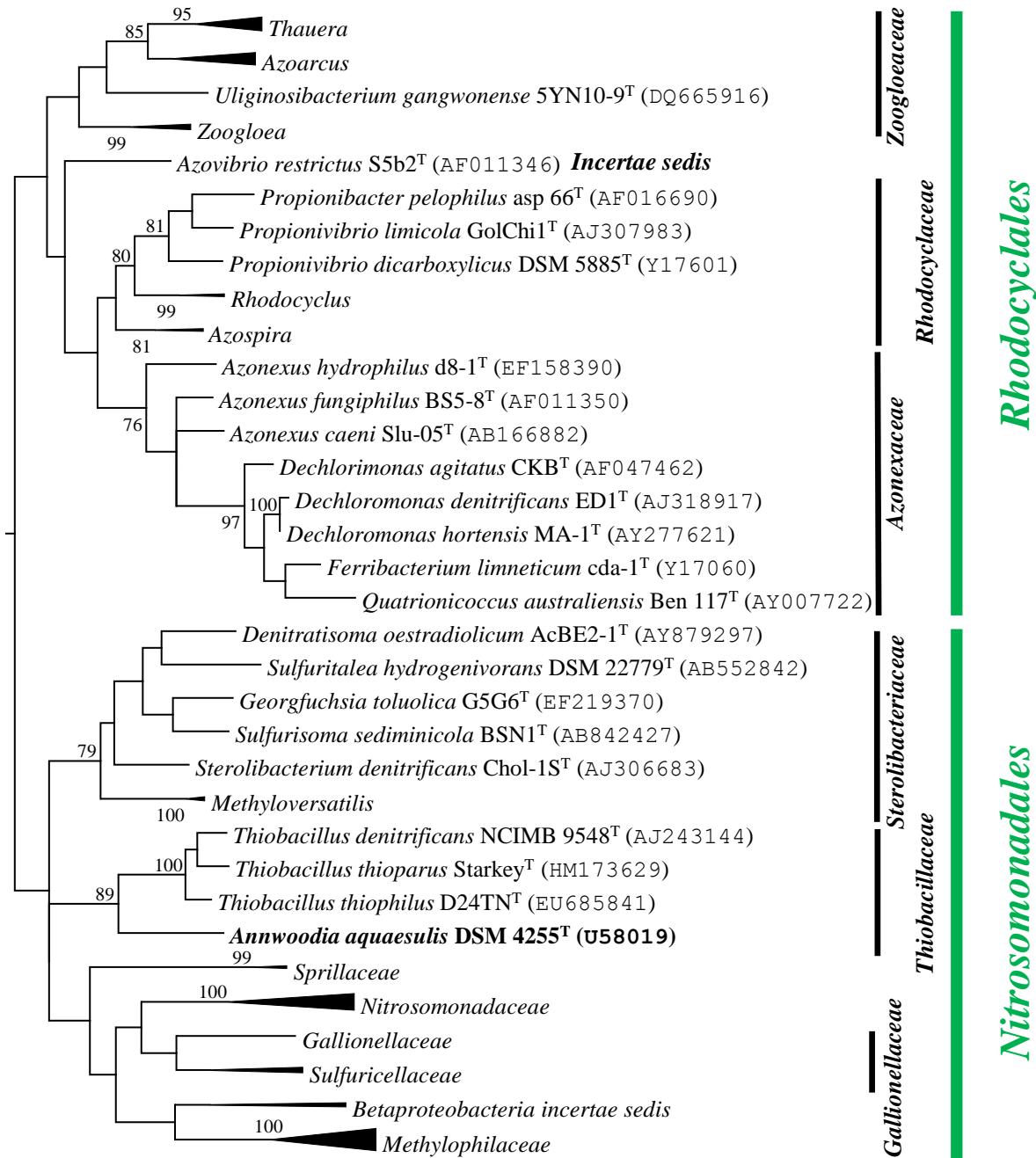
838 **Figure 4.** Phylogenetic tree on the basis of the 16S rRNA (*rrs*) gene, showing the position  
839 and names of new higher taxa proposed in this study, within the ‘*Proteobacteria*’ – this is a  
840 redrawing of Figure 1, showing the entire *Betaproteobacteria* and *Hydrogenophilalia* class.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

846

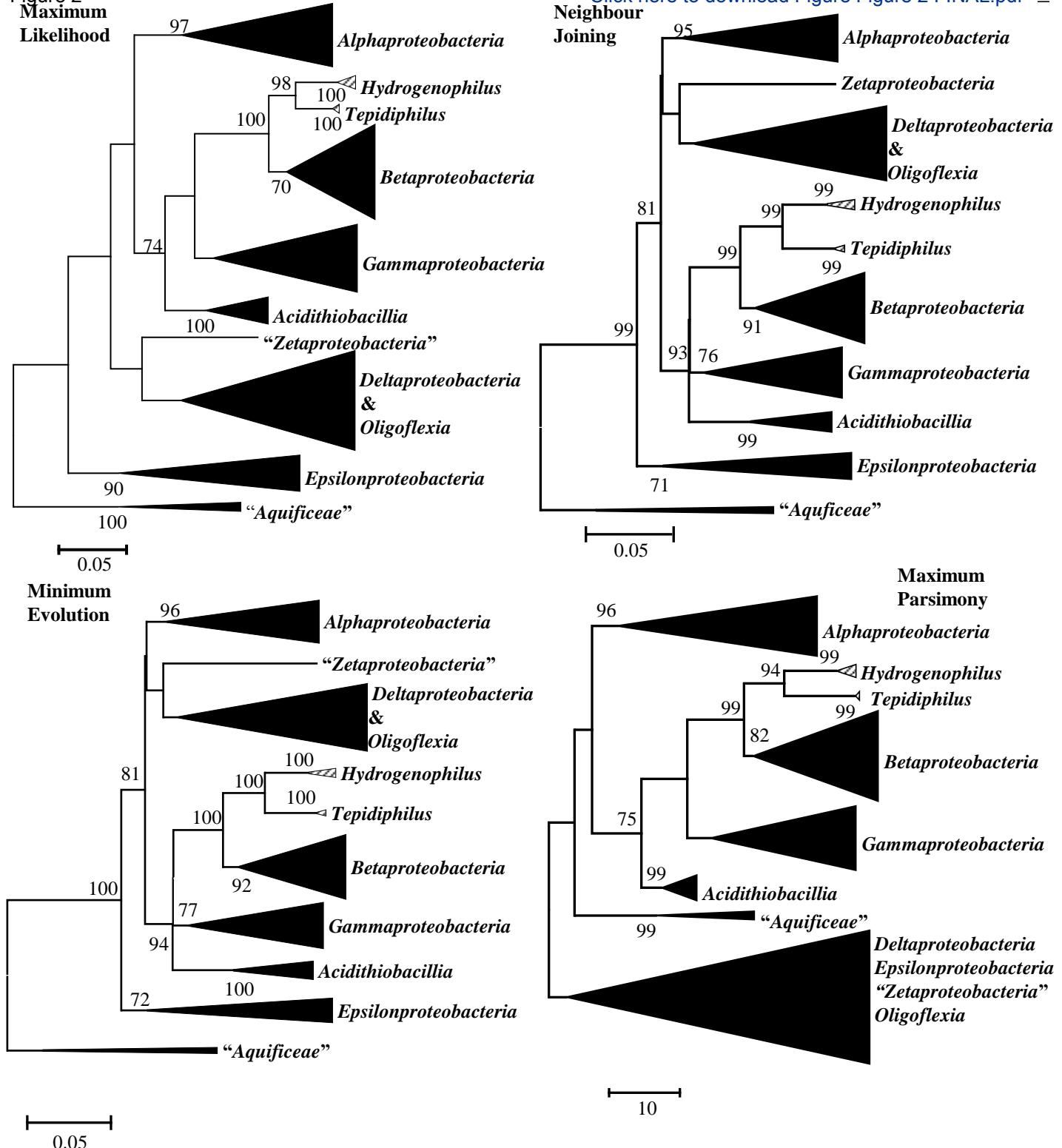
Characteristic	DSM 4255 <sup>T</sup>	<i>Thiobacillus thioparus</i> DSM 505 <sup>T</sup>	<i>Thiobacillus denitrificans</i> DSM 12475 <sup>T</sup>	<i>Thiobacillus thiophilus</i> DSM 19892 <sup>T</sup>
<b>16S rRNA gene identity (%) to:</b>				
<i>T. thioparus</i> DSM 505 <sup>T</sup>	93.1	100	97.6	97.6
<i>T. denitrificans</i> DSM 12475 <sup>T</sup>	93.4	97.6	100	97.6
<i>T. thiophilus</i> DSM 19892 <sup>T</sup>	93.1	97.6	97.6	100
DSM 4255 <sup>T</sup>	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
<b>Use of nitrate as:</b>				
Nitrogen source	-	+	±	<i>N.D.</i>
Electron acceptor	+	-	+	+
Temperature range	30-55	<i>N.D.</i>	<i>N.D.</i>	-2-30
Temperature optimum	45	25-30	28-32	25-30
pH range	7.0 – 9.0	5.0-9.0	<i>N.D.</i>	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	-	+	+	+
<b>Energy sources for autotrophic growth (all grow on thiosulfate and tetrathionate)</b>				
Trithionate	+	+	<i>N.D.</i>	-
Elementary sulfur	+	-	+	-
Thiocyanate	-	+	+	-
Sulfide	+	+	+	-
Dimethylsulfide	-	+	-	<i>N.D.</i>
Dimethyldisulfide	-	+	-	<i>N.D.</i>
Carbon disulfide	-	w	-	<i>N.D.</i>
G+C content (mol%)	65.7*	62.3**	66.0**	61.5*

Class	<i>Hydrogenophilalia</i>	Betaproteobacteria										Burkholderiales	Neisseriales
Order	<i>Hydrogenophilales</i>	<i>Rhodocyclales</i>			<i>Nitrosomonadales</i>								
Family	<i>Hydrogenophilaceae</i>	<i>Rhodocylaceae</i>	<i>Azonexaceae</i>	<i>Zoogloaceae</i>	<i>Nitrosomonadaceae</i>	<i>Methylophilaceae</i>	<i>Thiobacillaceae</i>	<i>Sterolibacteriaceae</i>	<i>Spirillaceae</i>	<i>Gallionellaceae</i>			
Flagellation or other motility	Monotrichous	Monotrichous or atrichous	Monotrichous or atrichous	Monotrichous 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, cocci	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 chemolithoheterotrophs	
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 <sub>1</sub> compounds, sugars, complex media	Complex media†	Sterols, fatty acids, C <sub>1</sub> 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 <sub>16:0</sub> , C <sub>19:0 cyclo</sub> , C <sub>17:0 cyclo</sub>	C <sub>16:0</sub> , C <sub>16:1</sub> , C <sub>18:1</sub>	C <sub>16:0</sub> , C <sub>16:1</sub> , C <sub>18:1</sub>	C <sub>10:0</sub> 3-OH, C <sub>16:0</sub> , C <sub>16:1</sub> , C <sub>18:1</sub> , C <sub>17:0</sub>	C <sub>16:0</sub> , C <sub>16:1</sub>	C <sub>16:0</sub> , C <sub>16:1</sub> , C <sub>15:0</sub> , C <sub>17:1</sub> , C <sub>18:0</sub> , C <sub>18:1</sub>	C <sub>16:1</sub> , C <sub>16:0</sub> , C <sub>15:0</sub> 2-OH, C <sub>10:0</sub> 3-OH	C <sub>16:0</sub> , C <sub>16:1</sub> , C <sub>18:0</sub> , C <sub>18:1</sub>	C <sub>16:1</sub> , C <sub>16:0</sub> , C <sub>18:0</sub> , C <sub>18:1</sub> , C <sub>10:0</sub> 3-OH, C <sub>15:0</sub> 2-OH	C <sub>16:1</sub> , C <sub>16:0</sub> , C <sub>18:0</sub> , C <sub>18:1</sub> , C <sub>12:0</sub> , C <sub>12:0</sub> 3-OH, C <sub>12:0</sub> 2-OH, C <sub>14:0</sub> 3-OH, C <sub>16:0</sub> 2-OH, C <sub>17:0 cyclo</sub> §	C <sub>12:0</sub> , C <sub>14:0</sub> , C <sub>16:0</sub> , C <sub>18:0</sub> , C <sub>18:1</sub> , C <sub>12:0</sub> , C <sub>12:0</sub> 3-OH	C <sub>16:1</sub> , C <sub>16:0</sub> , C <sub>18:0</sub> , C <sub>18:1</sub> , C <sub>12:0</sub> , C <sub>12:0</sub> 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**											

0.02



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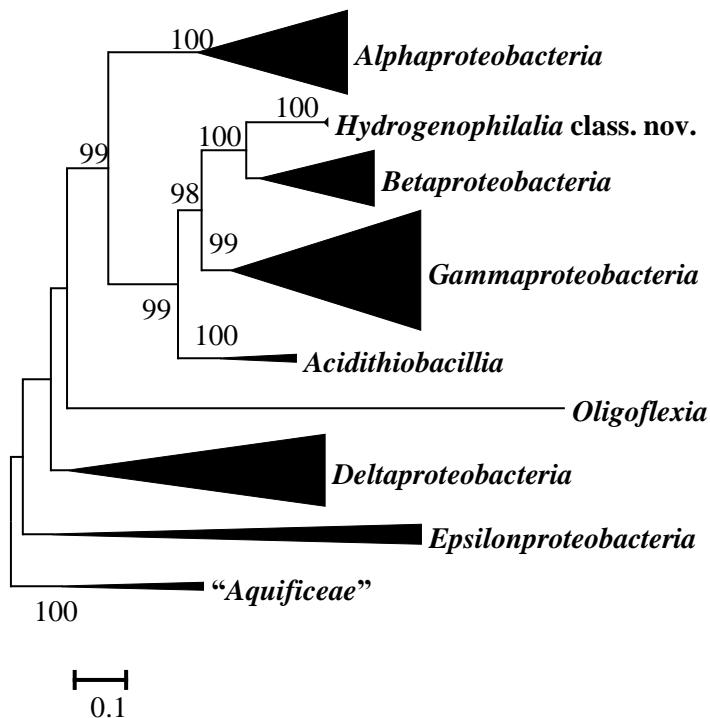
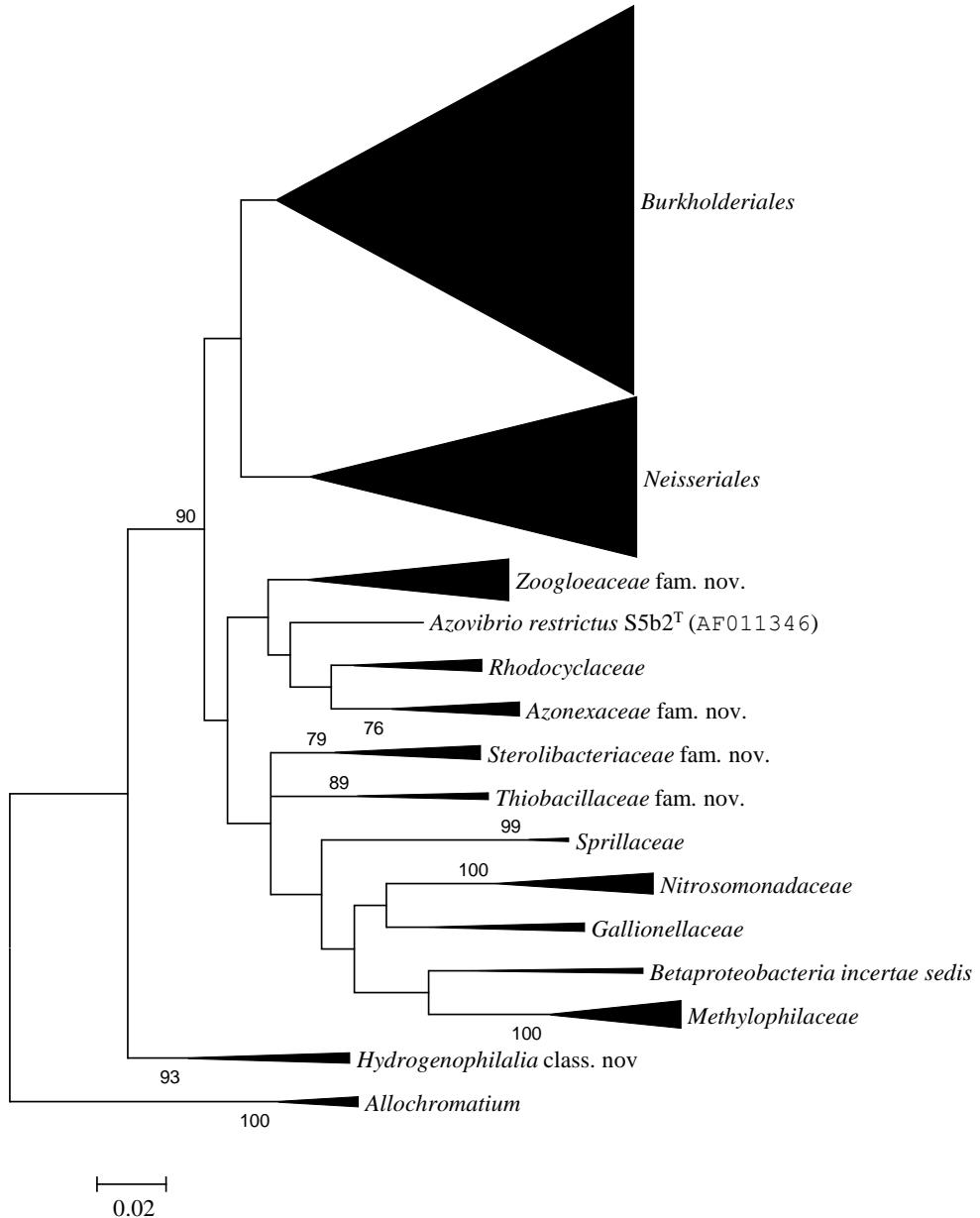


Figure 4

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## 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<sup>1,2</sup>, Lee P. Hutt<sup>1,2</sup> and Alex W. Rae<sup>1</sup>

**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: “ <i>Proteobacteria</i> ”					
<i>Alphaproteobacteria</i>	<i>Rhizobiales</i>	<i>Bradyrhizobiaceae</i>	<i>Rhodopseudomonas palustris</i>	<i>ATH 2.1.6<sup>T</sup></i>	D25312
		<i>Methylobacteriaceae</i>	<i>Methylobacterium organophilum</i>	<i>ATCC 27886<sup>T</sup></i>	AB175638
		<i>Beijerinckiaceae</i>	<i>Beijerinckia indica</i> subsp. <i>indica</i>	<i>ATCC 9039<sup>T</sup></i>	CP001016
		<i>Hypomicrobiaceae</i> “ <i>Aurantimonadaceae</i> ”	<i>Hypomicrobium vulgare</i> <i>Fulvimarina pelagi</i>	<i>ATCC 33404<sup>T</sup></i> <i>HTCC2506<sup>T</sup></i>	AB543807 AY178860
		<i>Rhizobiaceae</i>	<i>Rhizobium leguminosum</i> bv. <i>viciae</i>	<i>3Hoq18<sup>T</sup></i>	U29386
	<i>Sphingomonadales</i>	<i>Rhodobiaceae</i>	<i>Rhodobium radiobacter</i>	<i>ATCC 19358<sup>T</sup></i>	AB247615
		<i>Sphingomonadaceae</i>	<i>Sphingomonas paucimobilis</i>	<i>ATCC 29837<sup>T</sup></i>	U37337
	<i>Rhodospirillales</i>	<i>Erythrobacteriaceae</i>	<i>Erythrobacter longus</i>	<i>DSM 6997<sup>T</sup></i>	AF46535
		<i>Rhodospirillaceae</i>	<i>Rhodospirillum rubrum</i> <i>Azospirillum lipoferum</i> <i>Defulvicoccus vanus</i>	<i>ATCC 11170<sup>T</sup></i> <i>NCIMB 11861<sup>T</sup></i> <i>Ben 114<sup>T</sup></i>	CP000230 Z29619 AF179678
	<i>Rickettsiales</i>	<i>Acetobacteriaceae</i> <i>Rickettsiaceae</i>	<i>Acetobacter aceti</i> <i>Rickettsia prowazekii</i>	<i>ATCC 15973<sup>T</sup></i> <i>Brenl<sup>T</sup></i>	X74066 M21789
<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	<i>Burkholderiaceae</i>	<i>Burkholderia cepacia</i>	<i>ATCC 25416<sup>T</sup></i>	U96927
		<i>Oxalobacteriaceae</i>	<i>Oxalobacter formigenes</i>	<i>OXB<sup>T</sup></i>	U49757
		<i>Comamonadaceae</i>	<i>Comamonas terrigena</i>	<i>IMI 359870<sup>T</sup></i>	AF078772
		<i>Alcaliginaceae</i>	<i>Alcaligenes faecalis</i> subsp. <i>faecalis</i>	<i>ATCC 8750<sup>T</sup></i>	D88008
	<i>Neisseriales</i>	<i>Sutterellaceae</i>	<i>Sutterella wadsworthensis</i>	<i>ATCC 51579<sup>T</sup></i>	HM037997
		<i>Neisseriaceae</i>	<i>Neisseria gonorrhoeae</i>	<i>NCTC 83785<sup>T</sup></i>	X07714
	<i>Nitrosomonadales</i>	<i>Chromobacteriaceae</i>	<i>Chromobacterium violaceum</i>	<i>ATCC 12472<sup>T</sup></i>	AE016825
		<i>Nitrosomonadaceae</i>	<i>Nitrosomonas europaea</i>	<i>ATCC 25979<sup>T</sup></i>	AB070982
		<i>Thiobacillaceae</i>	<i>Thiobacillus thioparus</i> <i>Annwoodia aquaesulnis</i>	<i>Starkey<sup>T</sup></i> <i>ATCC 43788<sup>T</sup></i>	HM173629 U58019

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

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

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

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

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

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

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

