Confirmation of *Thiomonas delicata* (formerly *Thiobacillus delicatus*) as a distinct species of the genus *Thiomonas* Moreira and Amils 1997 with comments on some species currently assigned to the genus

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The transfer of *Thiobacillus delicatus* to the genus *Thiomonas* as a distinct species, *Thiomonas delicata* (type strain NBRC 14566T), is confirmed by its morphological and physiological properties, DNA–DNA hybridization and the grouping of its 16S rRNA gene sequence with those of other species of the genus. An emended formal description of *Thiomonas delicata* is given. The status of *Thiomonas cuprina* DSM 5495T as a member of the genus is reconsidered.

The genus *Thiomonas* was established by David Moreira and Ricardo Amils to comprise four species of chemolithotrophic autotrophs that formed a phylogenetic cluster within the β-1 subgroup of the Betaproteobacteria (Moreira & Amils, 1997). All four species (*Thiomonas intermedia*, *Thiomonas perometabolis*, *Thiomonas thermosulfata* and *Thiomonas cuprina*) were previously assigned to *Thiobacillus* but were found, by comparison of 5S and 16S rRNA gene sequences and by restriction fragment length analysis, to be rather remote from members of that genus (Moreira & Amils, 1997). Their closest phylogenetic neighbours within *Thiobacillus* were *Thiobacillus thioparus* and *Thiobacillus denitrificans*, which are in the β-2 subgroup of the Betaproteobacteria (Lane et al., 1992). The 16S rRNA gene sequence similarity between *Thiobacillus thioparus* and *Thiomonas intermedia* was only 87%. Additional defining characteristics of the four *Thiomonas* species included the G+C content of their DNA being between 61 and 69 mol%, the presence of ubiquinone Q-8, the inability to denitrify nitrate to dinitrogen and optimum growth as mixotrophs when supplied with both a reduced inorganic sulfur compound and a variety of organic substrates, but the ability also to grow chemolithoautotrophically or as chemoorganotrophs (Katayama-Fujimura et al., 1982, 1984; Moreira & Amils, 1997).

Moreira & Amils (1997) did not include *Thiobacillus delicatus* in the new genus as *Thiomonas delicata*, as insufficient phylogenetic data were then available, but they indicated that its affiliation with this mixotrophic group needed further investigation. *Thiobacillus delicatus* was originally isolated and characterized about 30 years ago (Mizoguchi et al., 1976; Katayama-Fujimura et al., 1982) and the name was formally revived with an emended species description by Katayama-Fujimura et al. (1984). It was retained in the list of species of the genus *Thiobacillus* in the 1989 and 2005 editions of Bergey’s Manual of Systematic Bacteriology (Kelly & Harrison, 1989; Kelly et al., 2005). However, Kelly & Wood (2005) proposed that *Thiobacillus delicatus* should also be transferred to the genus *Thiomonas* as *Thiomonas delicata*, on the basis of its physiological and biochemical properties (Table 1), subject to its phylogenetic relationship being confirmed by 16S rRNA gene sequencing (Kelly et al., 2005; Kelly & Wood, 2005). This has now been done and we report that *Thiobacillus delicatus* should henceforth be known as *Thiomonas delicata* (Kelly & Wood, 2005, 2006).

A nearly complete 16S rRNA gene sequence (1456 nucleotides) of *Thiomonas delicata* strain THI 091T (NBRC 14566T) was determined at the NITE Biological Resource Center (NBRC) and was deposited with GenBank/EMBL/ DDBJ, under accession number AB245481. The sequence shared 93·4% similarity with that of the type species, *Thiomonas intermedia* (Table 1). Comparison with database

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Thiomonas delicata* NBRC 14566T is AB245481.
sequences for other Thiomonas species using the BLAST algorithm (http://www.ncbi.nlm.nih.gov/blast/; Altschul et al., 1997) showed 99.9% similarity of the Thiomonas delicata sequence with that of ‘Thiomonas arsenivorans’ (Battaglia-Brunet et al., 2006) and with those of Thiomonas sp. strain RCASK1 (AJ879998), Thiomonas sp. C19 (AF460989) and Thiomonas sp. CO2 (AF460988), with similarities of 93.4% with Thiomonas intermedia and Thiomonas peromtabelalis, 91.1% with Thiomonas thermosulfata and 88.8% with Thiomonas curipina (Fig. 1).

The 16S rRNA gene sequence of Thiomonas delicata differs from that of ‘Thiomonas arsenivorans’ and several other database sequences of Thiomonas spp. by only one to three nucleotides, but Thiomonas delicata and ‘Thiomonas arsenivorans’ are distinguished from each other by a number of physiological and biochemical characteristics (Table 1). In particular, Thiomonas delicata has the following properties that are not shared with ‘Thiomonas arsenivorans’: it is non-motile, has more restricted ranges of temperature and pH for growth and optimum growth, can grow as an anaerobe using nitrate reduction to nitrite, but does not show heterotrophic growth on single organic compounds (glucose, fructose, ethanol, propanol, glycerol, oxalate, gluconate, pyruvate, lactate, malate, succinate, 2-oxoglutarate, citrate, alanine, serine, aspartate, glutamate, proline and histidine). Whereas ‘Thiomonas arsenivorans’ grows autotrophically on arsenite and chemo-organotrophically on a wide range of organic acids and sugars (Battaglia-Brunet et al., 2006), best growth of Thiomonas

Table 1. Comparison of Thiomonas delicata NBRC 14566T with the type strains of other species of Thiomonas

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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</thead>
<tbody>
<tr>
<td>DNA G+C content (mol%)</td>
<td>66–67</td>
<td>65</td>
<td>65–67</td>
<td>65–66</td>
<td>61</td>
<td>66–69</td>
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<tr>
<td>16S rRNA gene sequence similarity with Thiomonas intermedia (%)</td>
<td>93</td>
<td>93</td>
<td>97</td>
<td>94</td>
<td>1</td>
<td>87–3</td>
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<td>DNA–DNA hybridization with Thiomonas intermedia DNA (%)</td>
<td>25–28</td>
<td>49</td>
<td>(100)</td>
<td>31–35</td>
<td>NA</td>
<td>NA</td>
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<tr>
<td>Cell width (μm)</td>
<td>0–4–0–6</td>
<td>0–3–0–5</td>
<td>0–6–0–8</td>
<td>0–4–0–5</td>
<td>0–9</td>
<td>0–3–0–5</td>
</tr>
<tr>
<td>Cell length (μm)</td>
<td>1–0–1–6</td>
<td>1–0–2–0</td>
<td>1–0–1–4</td>
<td>1–1–1–7</td>
<td>1–3–2–3</td>
<td>1–0–4–0</td>
</tr>
<tr>
<td>Motility</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flagellation</td>
<td>–</td>
<td>–</td>
<td>Polar</td>
<td>Polar</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ubiquinone</td>
<td>Q-8</td>
<td>NA</td>
<td>Q-8</td>
<td>Q-8</td>
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<tr>
<td>Major cellular fatty acids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<tr>
<td>3-Hydroxydecanoic acid</td>
<td>NA</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>3-Hydroxy-8-methylnonanoic acid</td>
<td>NA</td>
<td>+</td>
<td>NA</td>
<td>NA</td>
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<td>NA</td>
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<tr>
<td>3-Hydroxydecanoic acid</td>
<td>–</td>
<td>NA</td>
<td>+</td>
<td>+</td>
<td>NA</td>
<td>NA</td>
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<tr>
<td>Growth on oxidation</td>
<td>Thiosulfate</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Tetrathionate</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Fe(II)</td>
<td>NA</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Arsenite</td>
<td>NA</td>
<td>+</td>
<td>–</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
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<tr>
<td>Transitory formation of polythionates during thiosulfate oxidation</td>
<td>+</td>
<td>NA</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Chemo-organotrophic growth with single organic compounds</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
<td>+/-</td>
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<tr>
<td>Nitrate reduction to nitrite</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Optimum pH</td>
<td>5–5–6</td>
<td>0–4–0–7</td>
<td>5–5–6</td>
<td>5–5–6</td>
<td>5–2–5</td>
<td>6–0–4–0</td>
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<tr>
<td>pH limits for growth</td>
<td>5–0–7</td>
<td>5–0–7</td>
<td>5–0–7</td>
<td>5–0–7</td>
<td>4–3–7</td>
<td>8–0–6–5</td>
</tr>
<tr>
<td>Temperature limits for growth (°C)</td>
<td>15–42</td>
<td>4 to &lt;45</td>
<td>15–37</td>
<td>15–42</td>
<td>34–65</td>
<td>15–63</td>
</tr>
</tbody>
</table>

*G+C values were determined by using the following methods: 1thermal denaturation (Tm); 2chemical analysis after hydrolysis with perchloric acid; 3HPLC after enzymic digestion; 4UV ratios (Ulitzur, 1972).
†Thiomonas delicata, Thiomonas intermedia and Thiomonas peromtabelalis contain hexadecanoic acid, hexadecenoic acid plus C17 cyclopropane acid, and octadecenoic acid plus C19 cyclopropane acid. NA for the other species.
‡Growth with succinate or glutamate.
§Growth with pyruvate.
||No growth at 10 or 45 °C; 4weak growth at 4 °C, but no growth at 45 °C; ‘No growth at 15 or 50 °C.”
increased the biomass production to 90 mg cell protein

The name *Thiomonas delicata* (Kelly & Wood, 2006) will have taxonomic precedence when validating the names of existing and future isolates of *Thiomonas* that are indistinguishable from it on the basis of their 16S rRNA gene sequences. Such isolates will require assessment of their physiological characteristics and comparative DNA–DNA hybridization with *Thiomonas delicata*. In this respect, there are numerous examples of species, in some cases from the same habitats, which share virtually identical 16S rRNA gene sequences (99–100 %) but show only 0–35 % interspecies DNA–DNA hybridization (Ash et al., 1991; Fox et al., 1992; Martinez-Murcia et al., 1992; Jaspers & Overmann, 2004).

*Thiomonas* species can also exhibit extremely divergent 16S rRNA gene sequences, as is seen with *Thiomonas cuprina*, which shows only 85.9–88.8 % sequence similarity with the other four *Thiomonas* species with validly published names. All five species have a rather distant relationship with strains of the β-1 betaproteobacterium *Burkholderia cepacia* (87–90 % similarity) but, apart from *Thiomonas cuprina*, they show 91.1–99.7 % similarity with each other. On the basis of physiological properties and molecular analyses, Moreira & Amils (1997) argued that *Thiomonas cuprina* should be included in the genus *Thiomonas*. However, the current wealth of betaproteobacterial 16S RNA gene sequences indicates that *Thiomonas cuprina* is not securely placed as a recognized member of the *Thiomonas* clade on phylogenetic trees, being at least as closely related to *B. cepacia* and some other genera as to the *Thiomonas* cluster (Fig. 1; Y. Uchino, additional data not shown). None of the recognized species (or *Thiomonas arsenivorans*), except *Thiomonas cuprina*, has been reported to be capable of autotrophic growth on sulfide minerals. Moreira & Amils (1997) erroneously stated that *Thiomonas cuprina* also could not grow on sulfide minerals, but the type strain grew autotrophically on arsenopyrite, chalcopryite, galena, cadmium sulfide and synthetic FeS (Huber & Stetter, 1990), although it did not oxidize Fe(II). In contrast to the other species of *Thiomonas* described to date, it could not grow on thiosulfate or tetrathionate as energy substrates, being able to use only more reduced sulfur substrates such as elemental sulfur and sulfides. With the exception of *Thiobacillus plumbophilus* (Drobner et al., 1992), this inability to use thiosulfate is unique among all the species currently and previously known as *Thiobacillus* (Kelly & Harrison, 1989; Kelly et al., 2005) and other chemolitho-

delicata is obtained on mixotrophic media with thiosulfate supplemented with Krebs cycle intermediates or amino acids. Optimal growth as a mixotroph is a property reportedly shared with *Thiomonas intermedia*, *Thiomonas perometabolis*, *Thiomonas thermosulfata* and *Thiomonas cuprina* (Moreira & Amils, 1997; Kelly & Wood, 2005). Whereas *Thiomonas arsenivorans* can grow well on yeast extract, *Thiomonas delicata* gave virtually no growth with 0.1 % (w/v) yeast extract alone (about 5 mg cell protein l\(^{-1}\)), but supplementing with 0.5 % (w/v) thiosulfate increased the biomass production to 90 mg cell protein l\(^{-1}\), compared with about 40 mg cell protein l\(^{-1}\) with thiosulfate alone (Mizoguchi et al., 1976). With 0.5 % (w/v) yeast extract, the biomass production was stimulated to about sixfold over that with thiosulfate alone (Katayama-Fujimura et al., 1984). While these properties suggest that *Thiomonas delicata* and *Thiomonas arsenivorans* are separate species, determination of the ability of *Thiomonas delicata* to oxidize Fe(II) and As(III) and DNA–DNA hybridization between them will help to establish whether *Thiomonas arsenivorans* is a distinct species or a subspecies of *Thiomonas delicata*. In this respect, it is noteworthy that the 16S rRNA gene sequences of *Thiomonas intermedia* and *Thiomonas perometabolis* also differ by only four nucleotides (99.7 % similarity), but were shown by DNA–DNA hybridization, 23S rRNA gene and restriction fragment length polymorphism to be distinct species (Katayama-Fujimura et al., 1982; Kelly & Harrison, 1989; Moreira & Amils, 1997).

![Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences showing the relationship of Thiomonas delicata NBRC 14566T to the other four recognized species of Thiomonas and ‘Thiomonas arsenivorans’. The analyses were performed using the neighbour-joining CLUSTAL x (version 1.82) method (Jeanmougin et al., 1998) and evolutionary distances calculated by using the Kimura two-parameter procedure (Kimura, 1980). Escherichia coli (Gammaproteobacteria) was included as an outgroup. Bar, 2 % nucleotide divergence. Numbers at nodes are bootstrap values from 1000 subsamples.](http://ijs.sgmjournals.org)
Paracoccus, Pseudaminobacter and Thiomicrospira. However, Thiomonas cuprina and Thio- bacillus plumbophilus are not closely related phylogenetically, as they show insignificant DNA–DNA hybridization (Drobner et al., 1992) and share only about 85% 16S rRNA gene sequence similarity (estimated by BLAST2 sequence comparison). Also, contrary to the description of Thiomonas cuprina provided by Moreira & Amils (1997), Huber & Stetter (1990) did not report that this species grew best under mixotrophic conditions. Unlike other Thiomonas species, the type strain of Thiomonas cuprina is moderately acidophilic with some strains being extreme acidophiles (Huber & Stetter, 1990). As well as sharing only 86–89% 16S rRNA gene sequence similarity with the other Thiomonas species, Thiomonas cuprina shows only 85–89% sequence similarity with other phylogenetically related Betaproteobacteria, including the type strains of B. cepacia, Leptothrix discolores and Comamonas testosteroni. Thiomonas cuprina also shares 87.5% similarity of its 23S rRNA gene sequence with that of B. cepacia (Moreira & Amils, 1996). Currently, Thiomonas cuprina appears to have no closer phylogenetic neighbours than these genera (Moreira & Amils, 1997; D. P. Kelly, unpublished sequence analyses). It clearly differs from B. cepacia at the genus level (Selenska-Pobell et al., 1998). There is thus a case for considering the promotion of Thiomonas cuprina as a novel genus of moderately acidophilic, metal sulfide-oxidizing, facultatively chemolithoautotrophic Betaproteobacteria.

The description of Thiomonas delicata is essentially that provisionally proposed by Kelly & Wood (2005), based on that of Katayama-Fujimura et al. (1984), as emended below. The type strain is available from the NBRC (Japan), the Korean Collection of Type Cultures (KCTC) and the DSMZ.

Emended description of Thiomonas delicata (Katayama-Fujimura et al. 1984) Kelly and Wood 2006

Thiomonas delicata (del.i.cat’a. L. fem. adj. delicata delicate).


Rods, usually single, rarely in pairs, 0.4–0.6 μm wide and 1.0–1.6 μm long. Non-motile. Colonies grown on yeast extract-thiosulfate agar (1 mm in diameter) are smooth and circular and change from transparent to whitish-yellow with sulfur. Facultative chemolithotroph and mixotroph. Grows autotrophically with sulfur, thiosulfate and tetrathionate, but not with thiocyanate; accumulates tetrathionate and trithionate transiently during growth on thiosulfate. Incapable of chemo-organotrophic growth on single carbon compounds. Grows mixotrophically with thiosulfate as an energy source in mineral media supplemented with tricarboxylic acid cycle intermediates, amino acids or yeast extract. Optimum growth requires both organic substances and thiosulfate or sulfur. Facultative anaerobe; reduces nitrate and produces nitrite in mixotrophic and autotrophic media with thiosulfate or tetrathionate, but does not denitrify nitrate to nitrogen gas. Ammonium salts, nitrate, urea, glutamate or aspartate can be used as a nitrogen source. Optimum temperature, 30–35°C; growth range, 15–42°C (no growth at 10 or 45°C). Optimum pH, 5.5–6.5; growth range, pH 5.0–7.0. Isolated from mine water. Distribution unknown. The G+C content of the DNA is 66–67 mol% (Tm, chemical analysis).

The type strain is THI 091T (= NBRC 14566T (formerly IFO 14566T) = KCTC 2851T = DSM 17897T). The culture deposited as IAM 12624T is no longer available from that collection.

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References


