Proposal of *Giesbergeria voronezhensis* gen. nov., sp. nov. and *G. kuznetsovii* sp. nov. and reclassification of [*Aquaspirillum*] *anulus*, [*A.*] *sinuosum* and [*A.*] *giesbergeri* as *Giesbergeria anulus* comb. nov., *G. sinuosa* comb. nov. and *G. giesbergeri* comb. nov., and [*Aquaspirillum*] *metamorphum* and [*A.*] *psychrophilum* as *Simplicispira metamorpha* gen. nov., comb. nov. and *S. psychrophila* comb. nov.

Margarita Grabovich,[1]† Ekaterina Gavrish,[2]†‡ Jan Kuever,[3]§ Anatoly M. Lysenko,[4] Daria Podkopaeva[1] and Galina Dubinina[4]

Correspondence
Margarita Grabovich
margarita_grabov@mail.ru

[1]Department of Biochemistry and Plant Physiology, Faculty of Biology, Voronezh State University, Voronezh, Russia

[2]All Russian Collection of Microorganisms (VKM), G. K. Skryabin Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, Pushchino, Moscow Region, 142290, Russia

[3]Max-Planck-Institute for Marine Microbiology, Celsiusstraße 1, D-28359 Bremen, Germany

[4]S. N. Winogradsky Institute of Microbiology of the Russian Academy of Sciences, Pr. 60-letiya Octyabrya 7/2, Moscow, 117811, Russia

Five Gram-negative, motile, spiral-shaped strains were isolated from a sulfide spring (D-412^T), active sludge of wastewater (D-419^T, D-420, D-424) and industrial wastewater (D-416). Comparative 16S rRNA gene sequence analysis showed that the isolates belong to the family *Comamonadaceae*, within the class *Betaproteobacteria*, but fall into a distinct cluster. On the basis of phenotypic, chemotaxonomic and phylogenetic data, a new genus, *Giesbergeria* gen. nov., is proposed, including five species. The type species of the genus is *Giesbergeria voronezhensis* sp. nov. (type strain D-419^T = DSM 12825^T = CIP 107340^T = VKM B-2350^T) and other novel members of the genus are *Giesbergeria kuznetsovii* sp. nov. (type strain D-412^T = DSM 12827^T = VKM B-2352^T), *Giesbergeria giesbergeri* comb. nov. (basonym *Aquaspirillum giesbergeri*), *Giesbergeria sinuosa* comb. nov. (basonym *Aquaspirillum sinuosum*) and *Giesbergeria anulus* comb. nov. (basonym *Aquaspirillum anulus*). Using the same criteria, isolate D-416 (= DSM 12826) was identified as a strain of [*Aquaspirillum*] *metamorphum*. Strain D-416, the type strain of [*A.*] *metamorphum* and the type strain of [*Aquaspirillum*] *psychrophilum* form a distinct cluster within the family *Comamonadaceae* (97–97·2% 16S rRNA gene sequence similarity) and share phenotypic and chemotaxonomic properties. Therefore, it is proposed that these strains are reclassified as members of a new genus, *Simplicispira* gen. nov., as *Simplicispira metamorpha* comb. nov. (the type species) and *Simplicispira psychrophila* comb. nov., respectively.

Published online ahead of print on 28 October 2005 as DOI 10.1099/ijs.0.64027-0.

†These authors contributed equally to the work.

‡Present address: Department of Biology, Northeastern University, 360 Huntington Avenue, Boston, MA 02115, USA.

§Present address: Bremen Institute for Materials Testing, Foundation Institute for Materials Science, Paul-Feller-Straße 1, D-28199 Bremen, Germany.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains D-419^T, D-420, D-412^T and D-416 are AY780905–AY780907 and AY780904, respectively.

Tables detailing the strains used in this study, the fatty acid profiles of strains and the DNA–DNA relatedness between strains and images showing the morphology of *Giesbergeria voronezhensis* sp. nov. and *Giesbergeria kuznetsovii* sp. nov. are available as supplementary material in IJSEM Online.

Recent studies using molecular genetic techniques have revealed a phylogenetic heterogeneity in representatives of the genus *Aquaspirillum* (Schleifer *et al.*, 1991; Pot *et al.*, 1992; Sakane & Yokota, 1994; Hamana *et al.*, 1994; Ding & Yokota, 2002). A combination of phylogenetic analysis and phenotypic/chemotaxonomic characterization for many *Aquaspirillum* species has resulted in the description of novel genera and/or novel species within the *Alphaproteobacteria* and *Betaproteobacteria* (Schleifer *et al.*, 1991; Wen *et al.*, 1999; Ding & Yokota, 2002; Cleenwerck *et al.*, 2003).

We have isolated several heterotrophic, spiral-shaped strains from a number of freshwater habitats (Grabovich, 1984; Grabovich *et al.*, 1987; Dubinina *et al.*, 1993). Their phenotypic and genotypic properties led them to be classified as representatives of the genus *Aquaspirillum* (Grabovich *et al.*, 1987; Dubinina *et al.*, 1993). Strains D-419^T, 420 and 424 were proposed as a novel species, '*Aquaspirillum voronezhense*', and strain 412^T was proposed as '*Aquaspirillum kuznetsovi*', but these names have not been validly published (Grabovich *et al.*, 1987, Dubinina *et al.*, 1993). In this paper, we present the results of a polyphasic taxonomic study of freshwater spiral-shaped isolates and the revision of some closely related species previously belonging to the genus *Aquaspirillum*; [*Aquaspirillum*] *giesbergeri*, [*Aquaspirillum*] *sinuosum*, [*Aquaspirillum*] *anulus*, [*Aquaspirillum*] *metamorphum* and [*Aquaspirillum*] *psychrophilum*.

All strains used in this study are shown in Supplementary Table S1 (see supplementary data in IJSEM Online). Cultivation of the strains was carried out in a modified PSS medium (Caraway & Krieg, 1974) of the following composition (l^{-1}), 1 g $(NH_4)_2SO_4$, 1 g ammonium molybdate, 0·03 g $CaCl_2.2H_2O$, 1 g sodium succinate and 2 g peptone, at pH 7·0. Vitamins and microelements were added before inoculation (Pfennig & Lippert, 1966). The incubation temperature was 28 °C. The morphology of cells from 18 h cultures was studied with a phase-contrast microscope (NU–2; Zeiss) and in a transmission electron microscope (JEM-100C: JEOL) at an acceleration voltage of 80 kV. Preparations fixed with OsO_4 were contrasted with a 2 % solution of NH_4MoO_4. Physiological and biochemical properties were determined as described previously (Dubinina *et al.*, 1993). The ability to use different carbon and nitrogen sources was tested by removing peptone and succinate from the medium. Carbon and nitrogen sources were added to the medium at a concentration of 1 g l^{-1}. The ability of the bacteria to utilize the test substrate was assayed after three reinoculations on the appropriate medium.

Isoprenoid quinones were extracted and purified as described previously (Collins & Jones, 1981). The fatty acid content was determined from 4–6 mg lyophilized biomass after acid methanolysis. Fatty acid methyl esters were analysed on a specialized chromatograph from the Microbial Identification System (Sherlock; MIDI Inc.) (Stead *et al.*, 1992). DNA was isolated from 5 l batch cultures grown aerobically on modified PSS medium according to the method of Marmur (1961). The DNA G+C content was determined by thermal denaturation as described previously (Owen *et al.*, 1976). DNA of *Escherichia coli* K-12 (=DSM 498) (51·7 mol%) was used as the reference. Levels of DNA–DNA binding were determined by measuring the renaturation rates of denatured DNA at the optimal renaturation temperatures as recommended by De Ley *et al.* (1970).

Almost-complete 16S rRNA gene sequences were amplified by PCR with the universal eubacterial primers 27f and 1492r and aligned using CLUSTAL_X software (Thompson *et al.*, 1997). An evolutionary-distance matrix was calculated using the Jukes and Cantor algorithm (Jukes & Cantor, 1969). The phylogenetic tree was constructed using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods. Bootstrap analyses were based on 1000 resamplings. The PAUP 4.0b10 (Swofford, 1998) and TREECON (Van de Peer & De Wachter, 1994) software packages were used for the analysis.

The nucleotide sequences for the 16S rRNA gene (1420–1460 nt) of isolates D-412^T, D-419^T, D-420 and D-416 were obtained. Phylogenetic analysis placed the studied strains within the family *Comamonadaceae*. The phylogenetic tree constructed using the neighbour-joining algorithm is shown in Fig. 1.

Strains D-412^T, D-419^T and D-420 formed a stable phylogenetic cluster, with a 100 % bootstrap value, together with the type strains of [*Aquaspirillum*] *anulus*, [*A.*] *giesbergeri* and [*A.*] *sinuosum*. The 16S rRNA gene sequence of strains D-419^T and D-412^T showed 99·9 % similarity to those of strain D-420 and the type strain of [*A.*] *anulus*, respectively. Sequence similarities between isolates D-412^T, D-419^T and D-420 and other representatives of this cluster were 98·4–99 %. Strain D-416 had 99·9 % sequence similarity to the type strain of [*A.*] *metamorphum*. Both strains were grouped together with [*A.*] *psychrophilum*, forming a coherent cluster with a bootstrap value of 91 % and high 16S rRNA gene sequence similarity (97·2–97·3 %).

The morphological and physiological properties of all studied strains are given in Tables 1, 2 and 3 and the species description. The cell morphology of two isolates can be seen in Supplementary Fig. S1 (see supplementary data in IJSEM Online).

All isolates contain Q-8 as the major isoprenoid quinone of the respiratory chain. Major fatty acids are 16 : 1, 16 : 0 and 18 : 1. The content of minor components of fatty acids varied significantly. All strains showed 10 : 0 3-OH, which was the only hydroxy fatty acid present. A detailed fatty acid profile of the cells is shown in Supplementary Table S2 (see supplementary data in IJSEM Online)

The high level of DNA–DNA hybridization (87–99 %) for strains D-419^T, D-420 and D-424 indicates that the strains represent a single species (see Supplementary Table S3 in IJSEM Online). For strain D-419^T and its close relatives (D-412^T, [*A.*] *anulus*, [*A.*] *giesbergeri* and [*A.*] *sinuosum*), the

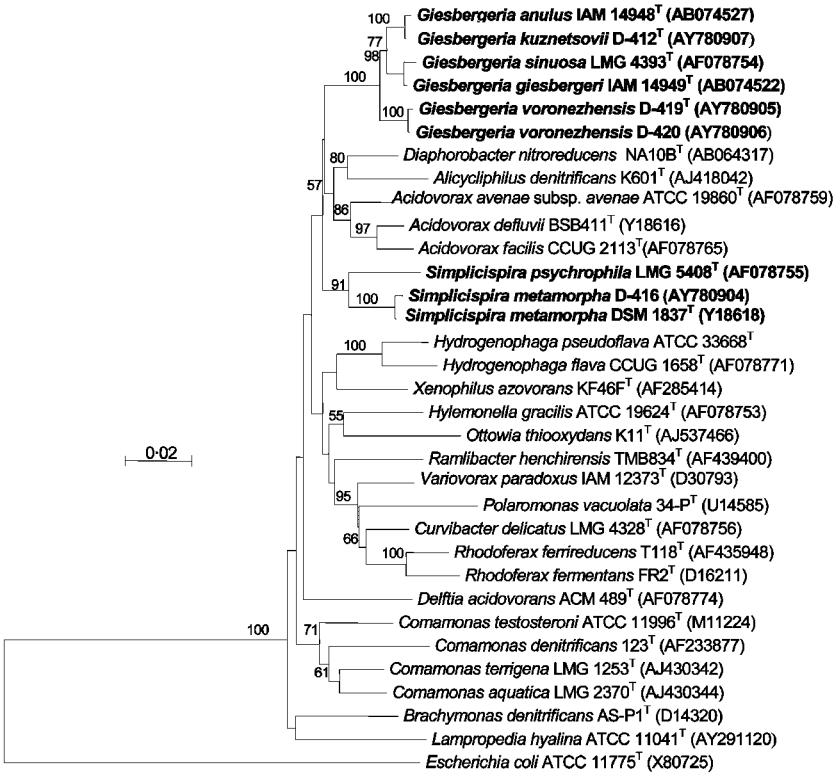

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequence analysis showing the position of investigated strains and members of the family *Comamonadaceae*. The tree was constructed by the neighbour-joining method. Bootstrap values from 1000 resamplings are shown at the branch points. Bar, 2 nucleotide substitutions per 100 nucleotides.

DNA–DNA hybridization value was less than 39 %. Strain D-412^T showed slightly higher values of DNA–DNA hybridization with the type strains of [*A.*] *anulus*, [*A.*] *giesbergeri* and [*A.*] *sinuosum* (36, 52 and 50 %, respectively). Although the 16S rRNA gene sequence of strain D-412^T is almost identical to that of the type strain of [*A.*] *annulus*, its physiological properties are different (Table 1). The ability to use additional organic compounds requires genes or complete pathways which might not be present in the type strain of [*A.*] *annulus*. This is also reflected in the low level of DNA–DNA hybridization between these two strains. The level of DNA–DNA hybridization between the three type strains of the *Aquaspirillum* species was 17–45 %. The high DNA–DNA hybridization value between strain D-416 and the type strain of [*A.*] *metamorphum* DSM 1837^T (95 %) indicated that the strains represent a single species.

Polyphasic analysis demonstrates that isolate D-412^T and strains D-419^T, D-420 and D-424 are representatives of two separate species and are closely related to [*A.*] *anulus*, [*A.*] *giesbergeri* and [*A.*] *sinuosum*, which are misclassified species of the genus *Aquaspirillum* within the family *Comamonadaceae* (Wen *et al.*, 1999; Ding & Yokota, 2002). Therefore, we propose to combine these species into a novel genus within the family *Comamonadaceae*, *Giesbergeria* gen. nov., with *Giesbergeria voronezhensis* sp. nov. as the type species. The type strain of *G. voronezhensis* is strain D-419^T, with strains D-420 and D-424 as reference strains. Four other species of the genus are proposed, *Giesbergeria kuznetsovii* sp. nov. (type strain D-412^T), *Giesbergeria anulus* comb. nov., *Giesbergeria giesbergeri* comb. nov. and *Giesbergeria sinuosa* comb. nov. The species of the novel genus can be differentiated from each other by phenotypic features (Table 1). Representatives of the novel genus differ from the recognized genera of the family *Comamonadaceae* in cell morphology and cell size, the spectrum of substrates used, fatty acid content and in an inability to perform denitrification (Tables 1 and 3).

The phylogenetic positions of [*A.*] *metamorphum* and [*A.*] *psychrophilum* indicate that both species are misclassified as members of the genus *Aquaspirillum*. The two species form a separate cluster (Fig. 1) from the genus *Aquaspirillum* and share different phenotypic and chemotaxonomic properties (Tables 2 and 3). Therefore, we propose to reclassify the two species into a novel genus, *Simplicispira* gen. nov., as *Simplicispira metamorpha* comb. nov. (the type species) and *Simplicispira psychrophila* comb. nov.

Description of *Giesbergeria* gen. nov.

Giesbergeria (Gies.ber.ger′i.a. N.L. fem. n. *Giesbergeria* named after the researcher G. Giesberger, who made a great contribution to the study of physiology of heterotrophic spirilla).

Gram-negative spiral cells. Cells are motile and have bipolar tufts of flagella. Cells accumulate poly-β-hydroxybutyric acid, some accumulate globules of elemental sulfur. Catalase- and oxidase-positive. Aerobes. Neutrophilic.

Table 1. Differential phenotypic characteristics of strains D-412^T, D-419^T, D-420 and D-424 and type strains of phylogenetically related species

Strains/species: 1, *Giesbergeria kuznetsovii* sp. nov. D-412^T; 2, *Giesbergeria voronezhensis* sp. nov. D-419^T and strains D-420 and D-424; 3, [*A.*] *giesbergeri*; 4, [*A.*] *sinuosum*; 5, [*A.*] *anulus*. +, Positive; −, negative; D, variable; NA, no data. None of the strains utilize glucose, fructose, maltose, arabinose, xylose or galactose. Data shown are from this study, Grabovich *et al.* (1987) and Krieg (1984).

Characteristic	1	2	3	4	5
Cell diameter (μm)	1·2–1·5	1·3–2·1	0·7–1·4	0·6–0·9	0·8–1·4
Wavelength of helix (μm)	4·8–8·0	7·9–14·7	4·5–8·4	8·6–10·5	5·0–13·0
Helix diameter (μm)	1·9–4·0	2·9–6·8	1·2–5·0	1·4–3·5	1·7–4·5
Length of helix (μm)	8·0–60·0	9·2–26·6	4·0–40·0	5·0–42·0	5·0–13·0
Assimilation of:					
Acetate	+	+	D	+	+
Aconitate	+	D	−	−	−
Alanine	+	−	−	−	−
Arginine	+	−	−	−	−
Asparagine	+	D	−	−	−
Aspartate	+	+	−	−	−
Butanol	+	−	−	−	−
Caproate	+	NA	−	−	−
Citrate	−	D	−	−	−
Cysteine	+	−	−	−	−
Ethanol	+	−	−	−	−
Fumarate	+	+	D	+	+
Glutamate	+	NA	−	−	+
Glutamine	+	−	−	−	−
Glycerol	+	−	−	−	−
Glycine	+	D	−	−	−
Histidine	+	D	−	−	−
β-Hydroxybutyrate	−	NA	−	−	−
Isocitrate	+	D	−	−	−
Lactate	+	+	D	+	+
Leucine	+	D	−	−	−
Lysine	+	−	−	−	−
Malate	+	+	D	+	D
Methionine	+	D	−	−	−
Ornithine	+	−	−	−	−
Oxaloacetate	+	+	−	+	−
2-Oxaloglutarate	+	+	−	−	−
Phenylalanine	+	−	−	−	−
Proline	+	D	−	−	−
Propanol	+	−	−	−	−
Propionate	+	NA	−	−	−
Pyruvate	+	+	D	+	D
Serine	+	−	−	−	−
Succinate	+	+	D	+	+
Tryptophan	+	D	−	−	−
Tyrosine	+	D	−	−	−
Valine	+	−	−	−	−
Temperature range for growth (°C)	7–36	7–36	8–36	9–37	3–36
DNA G+C content (mol%)	56·5	57·8–60	57–58	57–59	58–59

Table 2. Differential characteristics of strain D-416 and type strains of phylogenetically related species

Strains/species: 1, D-416; 2, [*A.*] *metamorphum*; 3, [*A.*] *psychrophilum*. +, Positive; −, negative; D, variable; NA, no data. All strains/species were unable to utilize some sugars (glucose, fructose, maltose, arabinose, xylose and galactose), alcohols (ethanol, propanol, butanol and glycerol) and amino acids (tryptophan, methionine, serine, lysine, phenylalanine, tyrosine, cysteine, cystine, glycine, ornithine, arginine, valine and leucine). Data are from this study and Krieg (1984).

Characteristic	1	2	3
Cell diameter (μm)	0·9–1·3	0·7–1·3	0·7–0·9
Wavelength of helix (μm)	−*	7·5–12·0	5·5–6·5
Helix diameter (μm)	−*	2·2–3·5	1·0–1·4
Length of helix (μm)	3·5–8·5	3·5–11·0	1·5–14·0
Poly-β-hydroxybutyrate formation	+	+	−
Assimilation of:			
Acetate	+	+	−
Aconitate	−	−	NA
Alanine	−	+	NA
Aspartate	−	+	NA
Butyrate	NA	+	−
Caproate	NA	−	NA
Citrate	−	−	−
Fumarate	+	+	−
Glutamate	−	+	NA
Glutamine	−	+	NA
β-Hydroxybutyrate	NA	−	NA
Isocitrate	−	−	NA
Lactate	+	+	−
Malate	+	−	−
Malonate	NA	+	−
Oxalaceate	+	+	NA
2-Oxoglutarate	−	+	NA
Proline	+	−	NA
Propionate	NA	D	−
Pyruvate	+	+	−
Succinate	+	+	−
Urease	+	−	NA
Anaerobic growth with nitrate	−	−	+
Temperature range for growth (°C)	3–38	3–38	2–25
DNA G+C content (mol%)	63	63	65

*Under standard conditions, cells have less than one helical turn.

Mesophilic. Do not grow with 3 % NaCl. Do not reduce nitrate to nitrite. Chemoorganoheterotrophs. Major fatty acids are 16 : 1 and 16 : 0. The latter acid may vary between representatives of the genus. The major respiratory ubiquinone is Q-8. The DNA G+C content is 56·5–60 mol%. The type species is *Giesbergeria voronezhensis*.

Description of *Giesbergeria voronezhensis* sp. nov.

Giesbergeria voronezhensis (vo.ro.nezh.en′sis. N.L. fem. adj. *voronezhensis* pertaining to Voronezh, the place from where the first strains were isolated).

Spiral cells, 1·3–2·1 μm in diameter, one to three helices, helix diameter 2·9–6·8 μm. Accumulates polyphosphate granules inside the cells and forms globules of elemental sulfur in the presence of sulfide. The pH for growth ranges from 6·0 to 9·0. The optimum growth temperature is 30 °C. Utilizes a wide range of organic acids for growth, including acetate, succinate, malate, fumarate, benzoate, isocitrate, formate, 2-oxoglutarate, oxaloacetate, pyruvate, salicylate, lactate and glyoxylate. Capable of growing on some amino acids as carbon sources. Does not utilize sugars or alcohols. Uses ammonium salts, casein hydrolysate, yeast extract, peptone, aspartate, glutamate and cysteine as nitrogen sources. Does not hydrolyse casein or starch. Does not use nitrates, sulfates, thiosulfate or fumarate as electron acceptors. Possesses urease activity. Forms hydrogen sulfide from cysteine. Does not form indole. Forms coloured products on a medium with benzoate. Predominant cellular fatty acids are 16 : 0, 16 : 1 and 14 : 0. The DNA G+C content is 58·5–60 mol%.

The type strain, D-419^T (=DSM 12825^T=CIP 107340^T=VKM B-2350^T), was isolated from active sludge from a wastewater aeration tank, Russia.

Description of *Giesbergeria kuznetsovii* sp. nov.

Giesbergeria kuznetsovii (kuz.net.so′vi.i. N.L. gen. n. *kuznetsovii* named after Sergey Kuznetsov, a Russian microbiologist who has made a great contribution to the study of microbial ecology).

Spiral cells, 1·2–1·5 μm in diameter, one to four helix loops, helix diameter 1·9–4·0 μm. Accumulates polyphosphate granules inside cells and forms globules of elemental sulfur in the presence of sulfide. The pH for growth ranges from 6·0 to 8·5. The optimum growth temperature is 28 °C. Utilizes a wide range of organic acids for growth, including acetate, succinate, malate, fumarate, benzoate, isocitrate, formate, 2-oxoglutarate, oxaloacetate, pyruvate, salicylate, lactate and glyoxylate. Uses the following alcohols: propanol, mannitol, glycerol, ethanol and butanol. Utilizes all tested amino acids. Does not utilize sugars. Uses ammonium salts, casein hydrolysate, yeast extract, peptone, aspartate, glutamate and cysteine as nitrogen sources. Does not hydrolyse casein or starch. Does not use nitrates, sulfates, thiosulfate or fumarate as electron acceptors. Possesses urease activity. Forms hydrogen sulfide from cysteine. Does not form indole. The predominant cellular fatty acids are 16 : 1 and 16 : 0. The DNA G+C content is 56·5 mol%.

The type strain, D-412^T (=DSM 12827^T=VKM B-2352^T), was isolated from a sulfide spring, Russia.

Table 3. Differential characteristics of the genera *Simplicispira* gen. nov. and *Giesbergeria* gen. nov. and related genera within the family *Comamonadaceae*

This table was adapted from Krieg (1984), Wen *et al.* (1999), Khan & Hiraishi (2002), Mechichi *et al.* (2003) and Spring *et al.* (2004). +, Positive; −, negative; D, variable; NA, no data.

Characteristic	*Simplicispira*	*Giesbergeria*	*Acidovorax*	*Diaphorobacter*	*Alicycliphilus*	*Hylemonella*	*Comamonas*
Cell shape	Spirilla	Spirilla curved-rods	Rods	Rods	Rods	Spirilla	Rods or spirilla
Flagellation	Bipolar tufts	Bipolar tufts	Polar monotrichous or absent	Polar monotrichous	+	Bipolar tufts	Polar, bipolar tufts
Autotrophic growth with H_2	−	−	D	NA	−	−	−
Reduction of nitrate	−	−	+	+	+	−	D
Denitrification	+*	−	D	+	+	−	D
Assimilation of:							
D-Fructose	−	−	+	−	+	−	−
D-Glucose	−	−	D	−	+	+	−
Glycerol	−	−†	+	NA	+	−	D
β-Alanine	−	−†	D	+	+	−	−
Major fatty acids‡	16:0, 16:1	16:0, 16:1	16:0, 16:1, 18:1	16:0, 16:1, 18:1	16:0, 16:1, 18:1	16:0, 16:1	16:0, 16:1, 18:1
3-Hydroxy fatty acids‡	10:0, (8:0*)	10:0	10:0, (8:0§)	10:0	−	10:0, 12:0	10:0
DNA G+C content (mol%)	63–65	56·5–60	62–66	64–65	66	65	59·7–68·7
Source	Freshwater, wastewater, Antarctic mosses	Sulfide spring, freshwater, wastewater, pond water	Soil, freshwater, clinic isolates	Activated sludge	Wastewater	Pond water, stream water	Soil, freshwater, wastewater, clinic isolates

*Positive for *S. psychrophila*.

†Positive for *G. kuznetsovii*.

‡Data from this study and Sakane & Yokota (1994).

§Present in phytopathogenic species.

Description of *Giesbergeria sinuosa* comb. nov.

Giesbergeria sinuosa (sin.u.o′sa. L. fem. adj. *sinuosa* sinuous, full of curves).

Basonym: *Aquaspirillum sinuosum* (Williams and Rittenberg 1957) Hylemon *et al.* 1973 (Approved Lists 1980).

The description is identical to the description given for *Aquaspirillum sinuosum* by Hylemon *et al.* (1973). In addition, the predominant cellular fatty acids are 16 : 1, 16 : 0, 17 : 1 and 18 : 1. The type strain is ATCC 9786^T (=DSM 11556^T).

Description of *Giesbergeria giesbergeri* comb. nov.

Giesbergeria giesbergeri (gies.ber′ge.ri. N.L. gen. n. *giesbergeri* of G. Giesberger, a researcher who made a great contribution to the study of heterotrophic spirilla).

Basonym: *Aquaspirillum giesbergeri* (Williams and Rittenberg 1957) Hylemon *et al.* 1973 (Approved Lists 1980).

The description is identical to that given for *Aquaspirillum giesbergeri* by Hylemon *et al.* (1973). In addition, the predominant cellular fatty acids are 16 : 1, anteiso-15 : 0, 16 : 0 and 18 : 1. The type strain is ATCC 11334^T (=DSM 9157^T=NCIB 9073^T).

Description of *Giesbergeria anulus* comb. nov.

Giesbergeria anulus (an′u.lus. L. masc. n. *anulus* a ring).

Basonym: *Aquaspirillum anulus* (Williams and Rittenberg 1957) Hylemon *et al.* 1973 (Approved Lists 1980).

The description is identical to that given for *Aquaspirillum anulus* by Hylemon *et al.* (1973). In addition, the predominant cellular fatty acids are 16 : 0, 16 : 1 and 18 : 1. The type strain is ATCC 35958^T (=NCIB 9012^T).

Description of *Simplicispira* gen. nov.

Simplicispira (Sim.pli.ci.spi′ra. L. adj. *simplex -icis* simple; L. fem. n. *spira* a spiral; N.L. fem. n. *Simplicispira* a simple spiral).

Cells are polymorphic, weakly curved rods, spiral and motile by the use of bipolar tufts of flagella. Gram-negative. Cells accumulate poly-β-hydroxybutyric acid. Catalase- and oxidase-positive. Aerobes and facultative anaerobes; capable of denitrification. Neutrophilic. Mesophilic. Do not grow with 3 % NaCl. Chemoorganoheterotrophs. The major fatty acids are 16 : 0 and 16 : 1. The major respiratory ubiquinone is Q-8. The DNA G+C content is 63–65 mol%. The type species is *Simplicispira metamorpha*.

Description of *Simplicispira metamorpha* comb. nov.

Simplicispira metamorpha (me.ta.mor′pha. N.L. fem. adj. *metamorpha* changing).

Basonym: *Aquaspirillum metamorphum* (Terasaki 1961) Hylemon *et al.* 1973 (Approved Lists 1980).

The description is identical to that given for *Aquaspirillum metamorphum* by Hylemon *et al.* (1973). In addition, the predominant cellular fatty acids of the type strain are 16 : 0, 16 : 1 and 18 : 1. The reference strain D-416 (=DSM 12826) possesses urease activity and the predominant fatty acids are 16 : 0 and iso-16 : 1, but 15 : 0 and 17 : 0 have also been found. The type strain is ATCC 15280^T (=DSM 1837^T=NBRC 13960^T).

Description of *Simplicispira psychrophila* comb. nov.

Simplicispira psychrophila (psy.chro.phi′la. Gr. adj. *psychros* cold; L. adj. *philos* loving; N.L. fem. adj. *psychrophila* cold-loving).

Basonym: *Aquaspirillum psychrophilum* (Terasaki 1973) Terasaki 1979 (Approved Lists 1980).

The description is identical to that given for *Aquaspirillum psychrophilum* by Hylemon *et al.* (1973). The type strain is DSM 11588^T (=ATCC 33335^T=NBRC 13611^T=LMG 5408^T).

Acknowledgements

This work was supported by grants from the Russian Foundation for Fundamental Research (04-04-48602; 05-04-48229) and a grant from the Program of Fundamental Research of Russian Academy of Sciences 'Molecular and Cell Biology'. We are grateful to Dr G. A. Osipov for performing fatty acid analysis of strains. We thank Dr Jean Euzéby for his help with the Latin nomenclature.

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