Taxonomic evaluation of the genera *Ruegeria* and *Silicibacter*: a proposal to transfer the genus *Silicibacter* Petursdottir and Kristjansson 1999 to the genus *Ruegeria* Uchino *et al.* 1999

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The taxonomic positions of the genera *Ruegeria* and *Silicibacter* were evaluated by a polyphasic investigation. It was evident from 16S rRNA gene sequence analysis that both genera are closely related as they formed a monophyletic clade with high sequence similarities (96.9–98.2 %). Several properties commonly found in these taxa strongly suggest that they should be classified in the same genus. Further, a comparative study based on DNA–DNA hybridization, phenotypic characterization and chemotaxonomic analysis indicated that the members of this clade, namely *Ruegeria atlantica*, *Silicibacter lacuscaerulensis* and *Silicibacter pomeroyi*, can be readily differentiated from each other. On the basis of the polyphasic data obtained in this study, all species of the genus *Silicibacter* should be transferred to the genus *Ruegeria*, since the latter has nomenclatural priority. It is therefore proposed that *Silicibacter lacuscaerulensis* and *Silicibacter pomeroyi* are transferred to the genus *Ruegeria* as *Ruegeria lacuscaerulensis* comb. nov. and *Ruegeria pomeroyi* comb. nov.

The genus *Ruegeria* was proposed by Uchino *et al.* (1998) to accommodate three species previously assigned as *Agrobacterium atlanticus* (Rüger & Höfle, 1992), *Agrobacterium gelatinovorum* (Rüger & Höfle, 1992) and *Roseobacter algicola* (Lafay *et al.*, 1995). Recently, *Ruegeria gelatinovorans* and *Ruegeria algicola* were transferred to other genera as *Thalassobius gelatinovorus* (Arahal *et al.*, 2005) and *Marinovum algicola* (Martens *et al.*, 2006), respectively. Therefore, at present *Ruegeria atlantica* is the type and only species of the genus *Ruegeria*. The genus *Silicibacter*, with the type species *Silicibacter lacuscaerulensis*, was created by Petursdottir & Kristjansson (1997) to describe mesophilic, moderately halophilic alphaproteobacteria isolated from the Blue Lagoon geothermal lake in Iceland. An additional *Silicibacter* species, *Silicibacter pomeroyi*, was subsequently described by González *et al.* (2003).

However, several subsequent taxonomic studies (González *et al.*, 2003; Macián *et al.*, 2005; Martínez-Cánovas *et al.*, 2004; Yi & Chun, 2004) have demonstrated that the genera *Ruegeria* and *Silicibacter* show a close phylogenetic relationship in which members of both genera have always formed a monophyletic clade. In this study, we present a critical taxonomic evaluation of the members of the two genera and propose the combination of the genus *Silicibacter* with the genus *Ruegeria*, since *Ruegeria* has nomenclatural priority.

R. atlantica KCTC 12017^T, *S. lacuscaerulensis* DSM 11314^T and *S. pomeroyi* DSM 15171^T were obtained from the respective culture collections and maintained on marine agar 2216 (MA; Difco).

Phylogenetic analysis was carried out using the previously published 16S rRNA gene sequences (Fig. 1). The sequences were aligned manually based on bacterial 16S rRNA secondary structure using the jPHYDIT program (Jeon *et al.*, 2005). The regions available for all sequences (positions 52–1437; *Escherichia coli* numbering system), excluding positions likely to show ambiguous alignment (positions 62–88 and 994–1026), were used to generate phylogenetic trees. Evolutionary distance matrices for the neighbour-joining (Saitou & Nei, 1987) tree were generated according to the model of Jukes & Cantor (1969). Maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) trees were created using the PAUP* 4.0b10 program with the

Abbreviation: PHB, poly-β-hydroxybutyrate.

The polar lipid compositions of the type strains of *Ruegeria atlantica*, *Silicibacter lacuscaerulensis* and *Silicibacter pomeroyi* are shown in a supplementary figure in IJSEM Online.

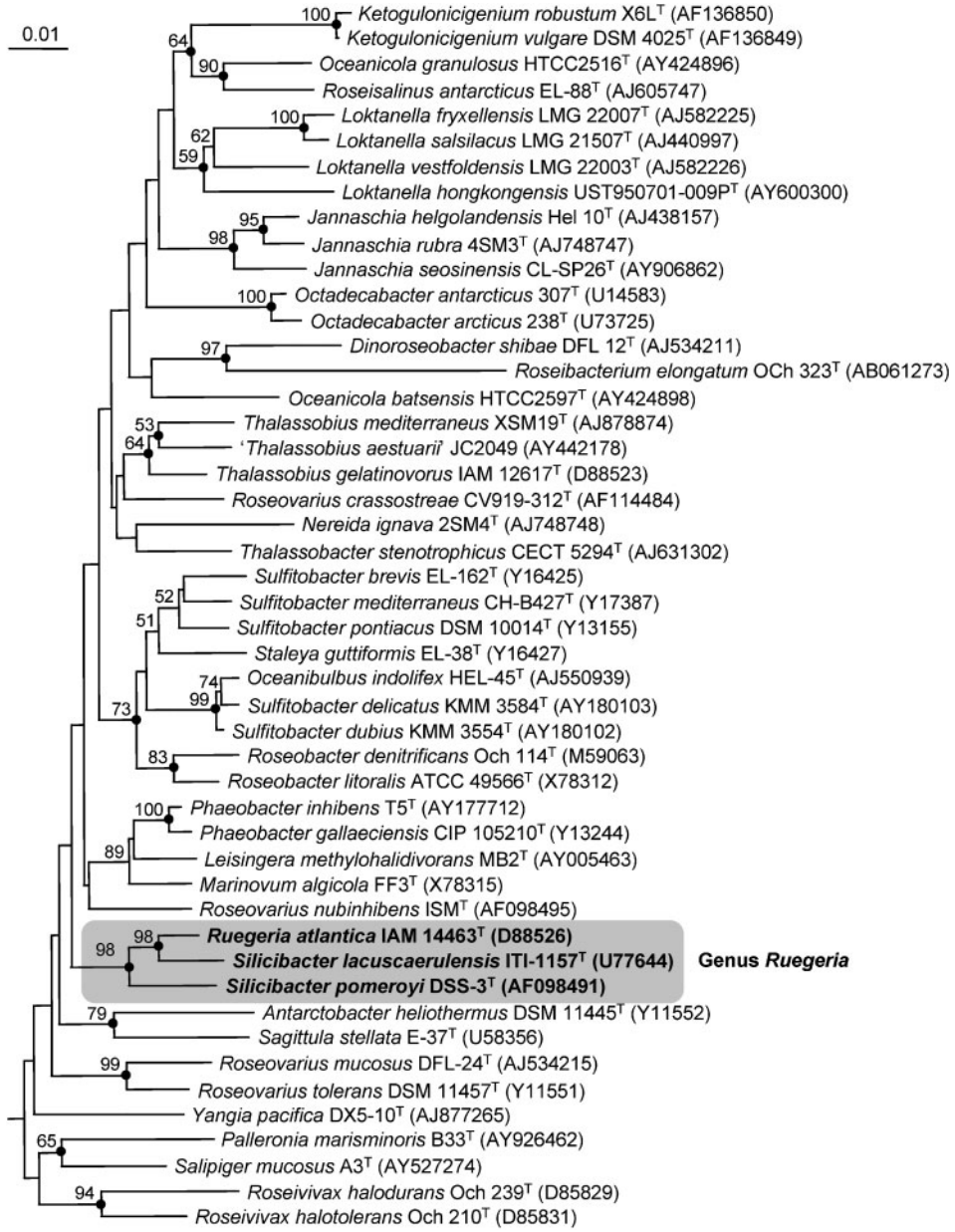

Fig. 1. Phylogenetic positions of the members of the genera *Ruegeria* and *Silicibacter* based on 16S rRNA gene sequences. The tree was created using the neighbour-joining method; numbers above the lines are bootstrap values (>50 %) from 1000 resampled datasets. Solid circles indicate that the corresponding nodes (groupings) were also recovered in maximum-likelihood and maximum-parsimony trees. *Rickettsia prowazekii* ATCC VR-142^T (M21789), *Rhodobacter capsulatus* ATCC 11166^T (D16428), *Rhodobaca bogoriensis* LBB1^T (AF248638) and *Roseinatronobacter thiooxidans* ALG 1^T (AF249749) were used as outgroups (not shown). Bar, 0.01 nucleotide substitutions per position.

heuristic search algorithm (Swofford, 2002). The confidence levels of the branching points were determined by 1000 bootstrap replicates for the neighbour-joining tree (Felsenstein, 1985). The members of the genera *Ruegeria* and *Silicibacter* formed a monophyletic clade with 98 % bootstrap support and were readily differentiated from other genera in the suprageneric *Roseobacter*-clade (Fig. 1). The pairwise sequence similarity values within this clade ranged from 96.9 to 98.2 % (Table 1), which are typical for species that are members of the same genus.

Genomic relatedness was examined to evaluate the species status of the members of the *Ruegeria–Silicibacter* clade. DNA–DNA hybridization was carried out using the method of Huß *et al.* (1983) following the modification of Yi & Chun (2006). The DNA–DNA relatedness values among the test

Table 1. 16S rRNA gene sequence similarities (%, lower left) and DNA–DNA hybridization values (upper right) of the type strains of *R. atlantica*, *S. lacuscaerulensis* and *S. pomeroyi*

Species	1	2	3
1. *R. atlantica* KCTC 12017^T	–	44	41
2. *S. lacuscaerulensis* DSM 11314^T	98.2	–	33
3. *S. pomeroyi* DSM 15171^T	97.3	96.9	–

strains were all below 70 % (Table 1), which confirms that these taxa merit their separate species status (Wayne *et al.*, 1987).

The temperature range for growth (between 5 °C and 55 °C, with intervals of 5 °C) was determined on MA. A requirement for sea salts or NaCl was determined using modified ZoBell medium [ZoBell, 1941; 5 g Bacto peptone (Difco); 1 g yeast extract (Difco); 0.1 g ferric citrate; 1 l distilled water] containing 3.24 g $MgSO_4\,l^{-1}$. Growth under anaerobic conditions was determined in an anaerobic chamber (10 % CO_2, 10 % H_2, 80 % N_2; Sheldon Manufacturing). Standard physiological and biochemical tests were performed as described previously (Smibert & Krieg, 1994). *R. atlantica* was grown on MA at 25 °C, *S. lacuscaerulensis* at 35 °C and *S. pomeroyi* at 30 °C, respectively. Hydrolysis of high molecular mass compounds was tested using MA as the basal medium. Poly-β-hydroxybutyrate (PHB) accumulation was investigated by using Nile blue A staining. Other enzymic activities were determined using API 20NE and API ZYM kits (bioMérieux). Strips were inoculated with a heavy bacterial suspension in half-strength artificial seawater (Sigma). The biochemical and physiological properties are given in the species descriptions and Table 2.

The potential for aerobic anoxygenic photosynthesis was determined at the genetic level by PCR amplification of the *pufLM* genes of the bacterial photosynthesis reaction centre. The primer pair pufLF (5′-CTKTTCGACTTCTGGGTSGG-3′) and pufMR (5′-CCCATGGTCCAGCGCCAGAA-3′) were used for amplification according to Allgaier *et al.* (2003). *Thalassobacter stenotrophicus* LMG 22015, *Roseobacter denitrificans* NBRC 15277^T and *Staleya guttiformis* LMG 19755^T were used as positive controls according to Yi & Chun (2004). *Marinovum algicola* NBRC 16653^T, *Octadecabacter antarcticus* CIP 106731^T and *Thalassobius gelatinovorus* NBRC 15761^T were used as negative controls. As *pufLM* genes were not amplified from species of the genera *Ruegeria* or *Silicibacter*, this implies the absence of aerobic anoxygenic photosynthesis in these strains.

Fatty acid methyl esters were prepared from biomass scraped from MA after 3 days of incubation and analysed by GC according to the instructions for the Microbial Identification System (MIDI). The predominant cellular fatty acids in the test strains were $C_{18:1}\omega7c$ and $C_{18:1}$11 methyl $\omega7c$, although *R. atlantica* contained a smaller amount of $C_{18:1}\omega7c$ (Table 3). Phospholipids were extracted, purified and identified as described by Minnikin *et al.* (1984). The polar lipid compositions of the three type strains were very similar, as shown in Supplementary Fig. S1 (available in IJSEM Online). All of the *Ruegeria* and *Silicibacter* strains contained phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol and three or four unidentified phospholipids.

Table 2. Characteristics that differentiate *R. atlantica*, *S. lacuscaerulensis* and *S. pomeroyi*

Strains: 1, *R. atlantica* KCTC 12017^T; 2, *S. lacuscaerulensis* DSM 11314^T; 3, *S. pomeroyi* DSM 15171^T. +, Positive; −, negative; W, weakly positive.

Characteristic	1	2	3
Growth at 45 °C	−	+	−
Motility	−	−*	+*
Reduction of nitrate to nitrogen	+	+	−
Acid phosphatase	−	+	−
β-Galactosidase	+	+	−
α-Glucosidase	W	+	−
Naphthol-AS-BI-phosphohydrolase	−	+	−
Hydrolysis of:			
Aesculin	+	+	−
Gelatin	−	−	+
Tween 80	−	+	+
DNA G+C content (mol%)	55–58	66*	68*

*Data from Rüger & Höfle (1992); Uchino *et al.* (1998); Petursdottir & Kristjansson (1997); González *et al.* (2003).

Even though members of the *Ruegeria*–*Silicibacter* clade have many properties in common, several characteristics can be used to differentiate them (Table 2). The genus name

Table 3. Cellular fatty acid content (%) of type strains

Strains: 1, *R. atlantica* KCTC 12017^T; 2, *S. lacuscaerulensis* DSM 11314^T; 3, *S. pomeroyi* DSM 15171^T. Values less than 1 % are not shown. Data for *S. pomeroyi* are from González *et al.* (2003).

Fatty acid	1	2	3
$C_{10:0}$	3.0	2.7	3.1
$C_{10:0}$ 3-OH	0.5	1.5	0.6
$C_{12:0}$	3.5	2.3	1.1
$C_{12:0}$ 3-OH	5.9	4.1	4.9
$C_{16:0}$	3.4	2.1	7.8
$C_{16:0}$ 2-OH	8.9	0.4	2.8
$C_{18:1}\omega7c$	44.7	72.9	76.3
$C_{18:0}$	2.9	2.1	1.6
$C_{18:1}$11 methyl $\omega7c$	26.9	7.3	1.9
$C_{19:0}$ cy$\omega8c$	0	2.1	0

Silicibacter was effectively published in 1997 (Petursdottir & Kristjansson, 1997) and *Ruegeria* in 1998 (Uchino *et al.*, 1998). However, the name *Ruegeria* has nomenclatural priority over *Silicibacter* as it was validly published in Validation List No. 68 (Uchino *et al.*, 1999) whereas *Silicibacter* appeared in Validation List No. 71 (Petursdottir & Kristjansson, 1999). On the basis of polyphasic evidence presented in this and earlier studies, we propose that *Silicibacter lacuscaerulensis* and *Silicibacter pomeroyi* be transferred to the genus *Ruegeria* as *Ruegeria lacuscaerulensis* comb. nov. and *Ruegeria pomeroyi* comb. nov., respectively.

Emended description of the genus *Ruegeria*

Ruegeria (Rue.ge′ria. N.L. fem. n. *Ruegeria* honouring Rueger, a German microbiologist, for his contribution to the taxonomy of marine species of *Agrobacterium*).

Gram-negative, oxidase- and catalase-positive. Ovoid or rod-shaped cells are motile by a polar flagellum or non-motile. Colonies are convex, opaque, butyrous, circular with entire margins and beige-coloured on MA after 2–3 days. Spores are not formed. Accumulate PHB. Require sea salts for growth. Strict aerobes. Genetic potential for aerobic anoxygenic photosynthesis is not detected. Bacteriochlorophyll *a* is absent. Major isoprenoid quinone is ubiquinone 10. Predominant cellular fatty acids are $C_{18:1}\omega7c$ and $C_{18:1}$ 11 methyl $\omega7c$. The polar lipids are phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol and three or four unidentified phospholipids. DNA G+C content is 55–68 mol%. The type species is *Ruegeria atlantica*.

Emended description of *Ruegeria atlantica*

Ruegeria atlantica (at.lan′ti.ca. L. fem. adj. *atlantica* pertaining to the Atlantic Ocean as the locality).

The description remains that given by Uchino *et al.* (1998) with the following modifications and additions. Reduces nitrate to nitrogen and requires sea salts for growth. Growth occurs at 10–35 °C (optimum, 25–30 °C). Decomposes aesculin, L-tyrosine, xanthine and hypoxanthine, but not casein, carboxymethylcellulose, gelatin, starch or Tween 80. Positive reaction for β-galactosidase (API 20NE) and negative reactions for arginine dihydrolase and urease. Does not produce acid from glucose or indole from tryptophan. With API ZYM kits, alkaline phosphatase and leucine arylamidase are positive, valine arylamidase and α-glucosidase are weakly positive and esterase (C4) and esterase lipase (C8), lipase (C14), cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase are negative. The cellular fatty acid content is shown in Table 3.

The type strain is 1480^T (=IAM 14463^T=DSM 5823^T).

Description of *Ruegeria lacuscaerulensis* comb. nov.

Ruegeria lacuscaerulensis (la.cus.cae.ru.len′sis. L. masc. n. *lacus* lake; L. adj. *caeruleus* blue; N.L. fem. adj. *lacuscaerulensis* pertaining to the blue lake).

Basonym: *Silicibacter lacuscaerulensis* Petursdottir and Kristjansson 1999.

The description is as given by Petursdottir & Kristjansson (1997) with the following modifications and additions. Reduces nitrate to nitrogen. Does not grow on media supplemented with NaCl only and requires sea salts for growth. Growth occurs at 10–45 °C (optimum, 35–40 °C). Decomposes aesculin, L-tyrosine, Tween 80, xanthine and hypoxanthine, but not carboxymethylcellulose or gelatin. Positive reaction for β-galactosidase (API 20NE) and negative reactions for arginine dihydrolase and urease. Does not produce acid from glucose or indole from tryptophan. With API ZYM kits, alkaline phosphatase, leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and α-glucosidase are positive, esterase (C4), esterase lipase (C8), valine arylamidase and β-galactosidase are weakly positive and lipase (C14), cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-glucuronidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase are negative. The cellular fatty acid content is shown in Table 3.

The type strain is ITI-1157^T (=DSM 11314^T=KCTC 2953^T).

Description of *Ruegeria pomeroyi* comb. nov.

Ruegeria pomeroyi (po.me.roy′i. N.L. masc. gen. n. *pomeroyi* of Pomeroy, named after Lawrence R. Pomeroy, a marine microbial ecologist who first elucidated the role of bacteria in the marine food web).

Basonym: *Silicibacter pomeroyi* González *et al.* 2003.

The description remains that given by González *et al.* (2003) with the following modifications and additions. Does not grow on media supplemented with NaCl only, requires sea salts for growth and hydrolyses Tween 80. Decomposes L-tyrosine, xanthine and hypoxanthine, but not casein or aesculin. Negative reactions for β-galactosidase, arginine dihydrolase and urease. Does not produce acid from glucose or indole from tryptophan. Alkaline phosphatase and leucine arylamidase are positive; esterase lipase (C8) is weakly positive; esterase (C4), lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase are negative in API ZYM kits.

The type strain is DSS-3^T (=ATCC 700808^T=DSM 15171^T).

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