Puniceibacterium antarcticum gen. nov., sp. nov., isolated from seawater

Chang Liu,[1,2]† Xi-Ying Zhang,[1,2]† Hai-Nan Su,[1,2] Ming-Yang Zhou,[1,2] Bo Chen,[3] Hai Li,[1,2] Xiu-Lan Chen,[1,2] Dian-Li Zhao,[1,2] Bai-Cheng Zhou,[1,2] Mei Shi[1,2] and Yu-Zhong Zhang[1,2]

Correspondence
Mei Shi
clubstone@sdu.edu.cn

[1]State Key Laboratory of Microbial Technology, Shandong University, Jinan 250100, PR China

[2]Marine Biotechnology Research Center, Shandong University, Jinan 250100, PR China

[3]SOA Key Laboratory for Polar Science, Polar Research Institute of China, Shanghai 200136, PR China

A Gram-reaction-negative, aerobic, non-flagellated, rod-shaped bacterium, designated strain SM1211^T, was isolated from Antarctic seawater. The isolate grew at 4–35 °C and with 0–10 % (w/v) NaCl. It could produce bacteriochlorophyll *a*, but did not reduce nitrate to nitrite or hydrolyse DNA. Phylogenetic analysis of 16S rRNA gene sequences revealed that strain SM1211^T constituted a distinct phylogenetic line within the family *Rhodobacteraceae* and was closely related to species in the genera *Litorimicrobium*, *Leisingera*, *Seohaeicola* and *Phaeobacter* with 95.1–96.0 % similarities. The predominant cellular fatty acid was $C_{18:1}\omega7c$. The major polar lipids were phosphatidylethanolamine, phosphatidylglycerol, phosphatidylcholine, an unidentified aminolipid and two unidentified phospholipids. The genomic DNA G+C content of strain SM1211^T was 60.7 mol%. Based on the phylogenetic, chemotaxonomic and phenotypic data obtained in this study, strain SM1211^T is considered to represent a novel species in a new genus within the family *Rhodobacteraceae*, for which the name *Puniceibacterium antarcticum* gen. nov., sp. nov. is proposed. The type strain of *Puniceibacterium antarcticum* is SM1211^T (=CCTCC AB 2013147^T=KACC 16875^T).

The genera *Seohaeicola* and *Litorimicrobium* in the family *Rhodobacteraceae* of the class *Alphaproteobacteria* were respectively proposed by Yoon *et al.* (2009) and Jin *et al.* (2011). At the time of writing, the two genera both contain only a single species, *Seohaeicola saemankumensis* and *Litorimicrobium taeanense*, the type strains of which were both isolated from different coastal areas of the Yellow Sea, Korea (Yoon *et al.*, 2009; Jin *et al.*, 2011). The genus *Phaeobacter* in the family *Rhodobacteraceae* was initially proposed by Martens *et al.* (2006) and currently comprises six recognized species: *Phaeobacter gallaeciensis* (formerly *Roseobacter gallaeciensis*) (type species, Ruiz-Ponte *et al.*, 1998; Martens *et al.*, 2006), *Phaeobacter inhibens* (Martens *et al.*, 2006), *Phaeobacter daeponensis* (Yoon *et al.*, 2007), *Phaeobacter arcticus* (Zhang *et al.*, 2008), *Phaeobacter caeruleus* (Vandecandelaere *et al.*, 2009) and *Phaeobacter leonis* (Gaboyer *et al.*, 2013), all originating from diverse marine environments. In a study to screen bacteria from different Antarctic samples, a pinkish-coloured strain, SM1211^T, was isolated from a seawater sample. This new isolate was found to be closely related to species in the genera *Litorimicrobium*, *Seohaeicola* and *Phaeobacter* based on 16S rRNA gene sequence analysis. In the present study, strain SM1211^T was taxonomically characterized using a polyphasic approach and, on the basis of the results, it was proposed to represent a novel species in a new genus in the family *Rhodobacteraceae*.

The Antarctic surface seawater sample was collected from the sea area (62° 12′ 16.95″ S 58° 56′ 19.08″ W) adjacent to the Chinese Antarctic Great Wall Station (Fildes Peninsula, King George Island, West Antarctic) during the 26th Chinese National Antarctic Research Expedition in October 2010. Strain SM1211^T was isolated by direct spreading of the sample onto marine agar 2216 (Difco) plates incubated at 15 °C for 3 weeks. The strain was routinely cultivated in TYS broth [0.5 % tryptone (Oxoid), 0.1 % yeast extract (Oxoid) and artificial seawater] or on TYS agar (0.5 % tryptone, 0.1 % yeast extract, 1.5 % agar

†These authors contributed equally to this work.

Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SM1211^T is JX070673.

Four supplementary figures are available with the online version of this paper.

and artificial seawater) at 20 °C and preserved at −80 °C in TYS supplemented with 20 % glycerol. Artificial seawater was prepared using Sigma sea salts (3 %). *Phaeobacter gallaeciensis* DSM 17395^T (obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany) and *Phaeobacter arcticus* 20188^T (kindly provided by Yong Yu from SOA Key Laboratory for Polar Science, Polar Research Institute of China), used as reference strains in fatty acid and polar lipid analyses and some phenotypic tests, were routinely cultivated on TYS agar or in TYS broth at 20 °C.

Genomic DNA of strain SM1211^T was extracted using a commercial DNA isolation kit (Bioteke). The 16S rRNA gene was PCR-amplified from the genomic DNA using primers 27F and 1492R (Lane, 1991). PCR products were cloned into pMD 18-T vectors (TakaRa) and sequenced using an Applied Biosystems 3730 DNA Sequencer. The obtained 16S rRNA gene sequence was compared with those in GenBank and those of type strains of species with validly published names in the Eztaxon-e database (http://eztaxon-e.ezbiocloud.net/; Kim *et al.*, 2012) using BLASTN (Altschul *et al.*, 1997). Pairwise sequence similarity values were obtained through the EzTaxon-e server. Sequence alignment and phylogenetic analysis were performed using MEGA version 5 (Tamura *et al.*, 2011). Phylogenetic trees were generated by using the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) methods. Bootstrap analyses based on 1000 replications (Felsenstein, 1985) were performed to evaluate the topologies of the resulting trees.

The nearly full-length 16S rRNA gene sequence of strain SM1211^T (1427 bp) was obtained. Sequence comparison showed that strain SM1211^T shared highest 16S rRNA gene sequence similarity with *Phaeobacter gallaeciensis* BS107^T (96.0 %) and high sequence similarities (<96.0 but >95 %) with type strains of 13 species from nine genera in the family *Rhodobacteraceae*, including *Phaeobacter inhibens* T5^T (95.8 %), *Phaeobacter arcticus* 20188^T (95.6 %), *Litorimicrobium taeanense* G4^T (95.5 %), *Seohaeicola saemankumensis* SD-15^T (95.4 %), *Citreicella marina* CK-I3-6^T (95.2 %), *Phaeobacter daeponensis* TF-218^T (95.2 %), *Roseobacter denitrificans* OCh 114^T (95.2 %), *Roseobacter litoralis* OCh 149^T (95.2 %), *Leisingera nanhaiensis* NH52F^T (95.1 %), *Sulfitobacter porphyrae* SCM-1^T (95.1 %), *Citreicella thiooxidans* CHLG 1^T (95.0 %) and *Thalassococcus halodurans* UST050418-052^T (95.0 %). In the neighbour-joining tree based on 16S rRNA gene sequences (Fig. 1), strain SM1211^T formed a distinct phylogenetic branch, adjacent to the one formed by *Litorimicrobium taeanense* and *Leisingera nanhaiensis*, within a cluster occupied by *Litorimicrobium taeanense*, *Leisingera nanhaiensis*, four species of the genus *Phaeobacter* (*Phaeobacter gallaeciensis*, *Phaeobacter inhibens*, *Phaeobacter arcticus* and *Phaeobacter leonis*) and *Seohaeicola saemankumensis*. In the maximum-parsimony and maximum-likelihood trees (Fig. 2 and Fig. S1, available in the online Supplementary Material), strain SM1211^T clustered only with *Litorimicrobium taeanense*, *Leisingera nanhaiensis* and *Seohaeicola saemankumensis* and formed a distinct internal branch close to *Litorimicrobium taeanense* and *Leisingera nanhaiensis*.

Cellular fatty acids of strain SM1211^T and the reference strains *Phaeobacter gallaeciensis* DSM 17395^T and *Phaeobacter arcticus* 20188^T were analysed using GC following the instructions of the Sherlock Microbial Identification System (version 4.5, TSBA 40 4.10 database). For the analysis, the three strains were all cultivated in TYS broth at 20 °C for 3 days. Polar lipids were extracted following the methods of Komagata & Suzuki (1987) and analysed using two-dimensional TLC with the following spraying reagents: ethanolic molybdophosphoric acid (total lipids), ninhydrin (aminolipids) and Zinzadze reagent (phospholipids) (Collins & Jones, 1980). Determination of the genomic DNA G+C content was carried out using the thermal denaturation temperature method (Marmur & Doty, 1962) with a Beckman DU800 spectrophotometer. Genomic DNA for the analysis was prepared following the procedure of Marmur (1961).

The predominant fatty acid of strain SM1211^T was $C_{18:1}\omega7c$ (80.8 %), similar to that for closely related species in the genera *Phaeobacter*, *Litorimicrobium*, *Seohaeicola* and *Leisingera*, but the absence of ECL 11.799 and the presence of $C_{12:1}$ 3-OH distinguished the new isolate (Table 1). Polar lipids of strain SM1211^T comprised major amounts of phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), an unidentified aminolipid (AL1) and two unidentified phospholipids (PL1 and PL2), and minor amounts of an unidentified lipid (L1), an unidentified aminophospholipid (APL) and two unidentified phospholipids (PL3, PL4) (Fig. S2). PC, PE, PG and AL1 were also found as predominant components in *Phaeobacter gallaeciensis* DSM 17395^T and *Phaeobacter arcticus* 20188^T, but the latter two both contained minor to moderate amounts of an unidentified lipid (L2), which was not detected in strain SM1211^T (Fig. S2). The genomic DNA G+C content of strain SM1211^T was 60.7 mol%, an intermediate value among those of closely related species (Table 2).

Cell morphology was examined by transmission electron microscopy (JEM-100CX II) with cells grown in TYS broth at 20 °C for 3 days and negatively stained with a 1.0 % phosphotungstic acid solution. Colony morphology was observed after 3–10 days of incubation on TYS agar at 20 °C. Growth at different temperatures (4, 10, 15, 20, 25, 30, 35, 37, 40, 45 °C) and pH [pH 4.0–10.0, at 0.5 pH-unit intervals, buffered with MES (pH 4.0–6.0), MOPS (pH 6.5–7.0), Tris (pH 7.5–8.5) or CHES (pH 9.0–10.0)] was determined in TYS broth. The NaCl concentration range for growth was tested at 20 °C in a medium containing 0.5 % tryptone (Oxoid), 0.1 % yeast extract (Oxoid) and distilled water with 0–6.5 % (at 0.5 % intervals) or 7–15 % (at 1 % intervals) NaCl. Anaerobic growth was observed in TYS broth [supplemented with

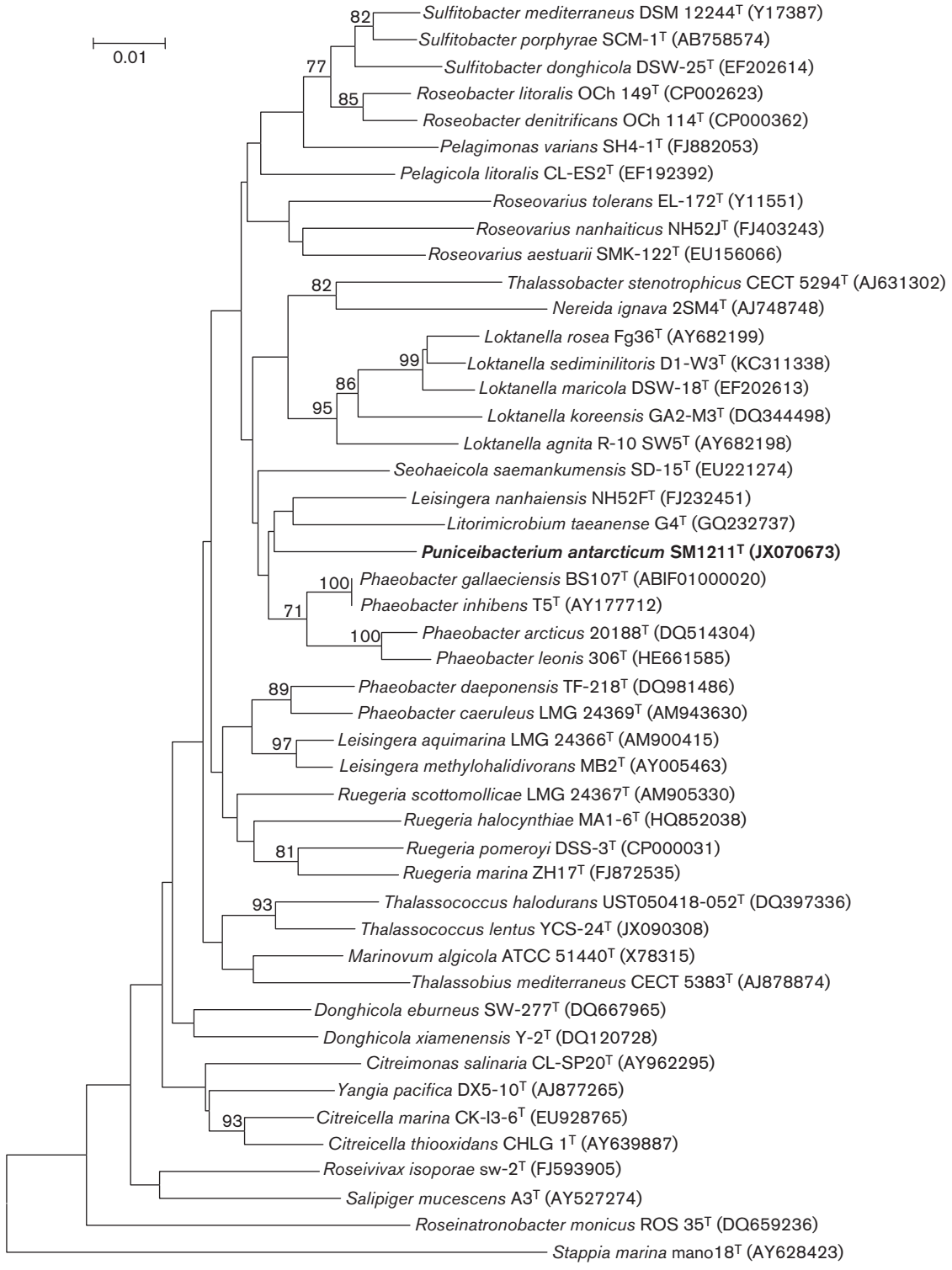

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the positions of strain SM1211^T (in bold type) and other related species. Bootstrap values, based on 1000 replications, above 70 % are shown at nodes. Bar, 0.01 substitutions per nucleotide position.

0.1 % (w/v) potassium nitrate, 0.05 % (w/v) cysteine hydrochloride and 0.05 % (w/v) sodium sulfide] in Hungate tubes.

The Gram reaction was examined according to the Hucker staining method (Murray *et al.*, 1994). Catalase activity was evaluated by bubble production in 3 % (v/v) hydrogen

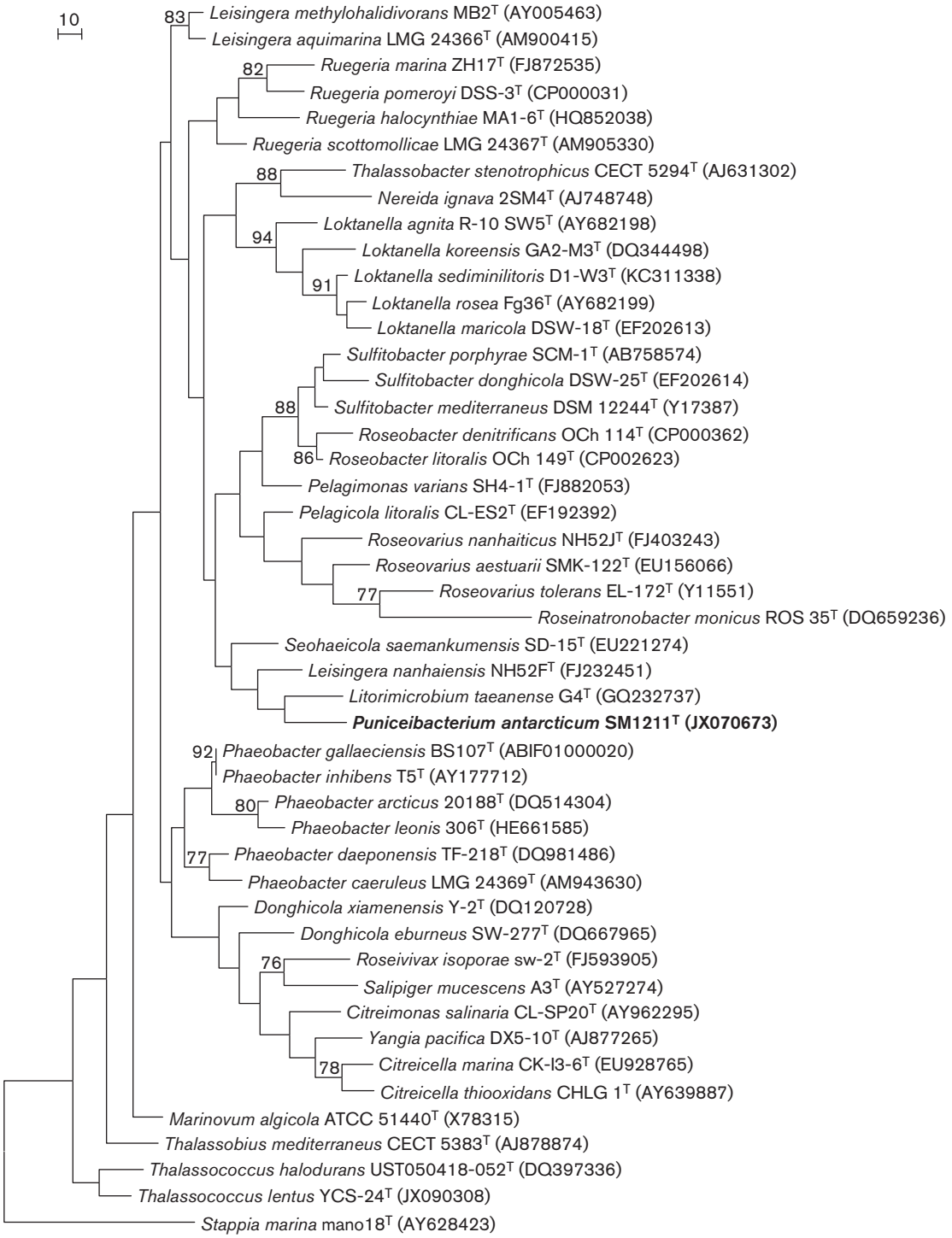

Fig. 2. Maximum-parsimony phylogenetic tree based on 16S rRNA gene sequences showing the positions of strain SM1211^T (in bold type) and other related species. Bootstrap values, based on 1000 replications, above 70 % are shown at nodes. Bar, branch length corresponding to 10 character-state changes.

peroxide solution while oxidase activity was examined using Bactident Oxidase strips (Merck). DNA hydrolysis was tested with DNase test agar (Oxoid). Hydrolysis of starch, casein and Tweens 20, 40, 60 and 80 was determined on TYS agar based on the methods described by Smibert & Krieg (1994). Antibiotic susceptibility tests were performed on TYS agar using the disc-diffusion method with the following antibiotic-impregnated discs

Table 1. Fatty acid contents (%) of strain SM1211^T and the type strains of closely related species in the genera *Phaeobacter*, *Litorimicrobium*, *Leisingera* and *Seohaeicola*

Strains: 1, SM1211^T (this study); 2, *Phaeobacter gallaeciensis* DSM 17395^T (this study); 3, *Phaeobacter arcticus* 20188^T (this study); 4, *Litorimicrobium taeanense* G4^T (Jin *et al.*, 2011); 5, *Leisingera nanhaiensis* NH52F^T (Sun *et al.*, 2010); 6, *Seohaeicola saemankumensis* SD-15^T (Yoon *et al.*, 2009). Fatty acids amounting to <1 % in all strains are not shown. Major fatty acids (>5 %) in each strain are shown in bold type. −, Not detected or reported.

Fatty acid	1	2	3	4	5	6
$C_{16:0}$	**6.6**	**11.1**	**9.9**	**5.8**	**3.0**	**8.0**
$C_{18:0}$	1.6	2.0	0.8	1.3	0.6	−
$C_{10:0}$ 3-OH	−	3.2	3.2	−	3.9	−
$C_{12:0}$ 3-OH	0.5	2.5	0.1	−	2.9	−
$C_{12:1}$ 3-OH	4.0	−	−	**9.8**	−	−
$C_{16:0}$ 2-OH	−	1.6	1.8	**7.3**	**5.4**	−
$C_{18:1}\omega7c$	**80.8**	**64.5**	**66.1**	**59.0**	**71.9**	**68.0**
11-Methyl $C_{18:1}\omega7c$	3.8	**9.2**	**11.3**	**11.3**	**3.0**	**12.9**
Cyclo $C_{19:0}\omega8c$	−	−	−	−	−	**5.4**
Summed feature 3*	1.4	0.3	0.7	−	0.6	−
ECL 11.799†	−	3.8	4.3	**5.4**	**5.7**	**5.7**

*Summed feature 3 contains $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH.
†ECL, equivalent chain-length.

(Oxoid) (μg per disc): penicillin G (10), ampicillin (10), chloramphenicol (30), erythromycin (15), gentamicin (10), kanamycin (30), vancomycin (30), novobiocin (5) and colistin sulphate (10). Production of bacteriochlorophyll *a* was determined by spectrophotometric analysis of acetone/methanol (7 : 2, v/v) extracts (Martens *et al.*, 2006). Cells for the analysis were grown at 20 °C for 3 days. Additional enzyme activities and biochemical properties were examined using API ZYM and API 20NE strips (bioMérieux) following the manufacturer's instructions except that cells to inoculate the API strips were suspended in artificial seawater.

Cells of strain SM1211^T were Gram-reaction-negative rods which were devoid of flagella (Fig. S3). Growth of strain SM1211^T under anaerobic conditions was not observed. The strain formed pink- to red-pigmented colonies on TYS agar whereas *Phaeobacter gallaeciensis* DSM 17395^T and *Phaeobacter arcticus* 20188^T respectively formed brown and light yellow colonies on the same agar. The absorption spectrum of the acetone/methanol (7 : 2, v/v) extract of strain SM1211^T showed a specific peak at 768 nm (Fig. S4), indicating that the strain had the ability to produce bacteriochlorophyll *a* (Suyama *et al.*, 1999; Biebl *et al.*, 2005). Other phenotypic characteristics of strain SM1211^T are given in the genus and species descriptions below. Characteristics allowing differentiation of strain SM1211^T from closely related species in the genera *Phaeobacter*, *Litorimicrobium*, *Leisingera* and *Seohaeicola* are given in Table 2.

Results from phylogenetic analyses of 16S rRNA gene sequences and chemotaxonomic and phenotypic characterization indicated that strain SM1211^T should be assigned to a new genus as a representative of a novel species, for which the name *Puniceibacterium antarcticum* gen. nov., sp. nov. is proposed.

Description of *Puniceibacterium* gen. nov.

Puniceibacterium (Pu.ni.ce.i.bac.te′ri.um. L. adj. *puniceus* pinkish red; L. neut. n. *bacterium* a rod; N.L. neut. n. *Puniceibacterium* a pinkish-red rod).

Cells are Gram-reaction-negative, aerobic rods. Catalase- and oxidase-positive. Nitrate is not reduced to nitrite. The predominant cellular fatty acid is $C_{18:1}\omega7c$. The major polar lipids include PE, PG, PC, an unidentified aminolipid and two unidentified phospholipids. The genus belongs phylogenetically to the family *Rhodobacteraceae* of the class *Alphaproteobacteria*. The type species is *Puniceibacterium antarcticum*.

Description of *Puniceibacterium antarcticum* sp. nov.

Puniceibacterium antarcticum (ant.arc′ti.cum. L. neut. adj. *antarcticum* from the opposite of the North, of the Antarctic).

The description is as for the genus plus the following characteristics. Cells are non-flagellated rods (0.5–0.8 × 0.8–1.3 μm). Colonies are pink- to red-pigmented, circular (1.0–2.0 mm in diameter) and convex with smooth surface after incubation for 3–7 days at 20 °C on TYS agar. Grows at 4–35 °C (optimum, 25 °C), at pH 5.0–9.0 (optimum, pH 7.0) and with 0–10 % (w/v) NaCl (optimum, 2–3 % NaCl). Can produce bacteriochlorophyll *a*. Hydrolyses Tween 20, but not DNA, Tweens 40, 60 or 80 or starch. In API 20NE tests, cells are positive for acid production from glucose and assimilation of D-glucose, arabinose, mannose, mannitol, maltose, malate and citrate, but negative for indole production, arginine dihydrolase, urease, aesculin hydrolysis, gelatinase, β-galactosidase and assimilation of *N*-acetylglucosamine, gluconate, caprate, adipate and phenylacetate. Using API ZYM strips, activities of alkaline phosphatase, acid phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, naphthol-AS-BI-phosphohydrolase and α-glucosidase (weakly) are detected, but activities of lipase (C14), trypsin, α-chymotrypsin, α-galactosidase, cystine arylamidase, β-galactosidase, β-glucosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase are not detected. Susceptible to penicillin G, ampicillin, chloramphenicol, erythromycin, gentamicin, kanamycin and vancomycin, but resistant to colistin sulphate and novobiocin. Besides the predominant fatty acid $C_{18:1}\omega7c$, also contains $C_{16:0}$, $C_{12:1}$ 3-OH and 11-methyl $C_{18:1}\omega7c$ (3.8 %) as minor fatty acids.

The type strain is SM1211^T (=CCTCC AB 2013147^T=KACC 16875^T), isolated from Antarctic seawater. The genomic G+C content of the type strain is 60.7 mol%.

Table 2. Differential characteristics between strain SM1211^T and closely related species in the genera *Phaeobacter*, *Litorimicrobium*, *Leisingera* and *Seohaeicola*

Strains: 1, SM1211^T (this study); 2, *Phaeobacter gallaeciensis* DSM 17395^T (this study); 3, *Phaeobacter arcticus* 20188^T (this study); 4, *Litorimicrobium taeanense* G4^T (Jin *et al.*, 2011); 5, *Leisingera nanhaiensis* NH52F^T (Sun *et al.*, 2010); 6, *Seohaeicola saemankumensis* SD-15^T (Yoon *et al.*, 2009). +, Positive; −, negative; W, weakly positive; ND, no data. DPG, diphosphatidylglycerol; L, unidentified lipid; PL, unidentified phospholipid; AL, unidentified aminolipid; APL, unidentified aminophospholipid.

Characteristic	1	2	3	4	5	6
Colony colour	Pink to red	Brown	Yellow	Creamy white	Beige	Pale yellow
Flagellum	−	+	+	−	−	−
Ranges for growth:						
Temperature (°C)	4–35	15–37*	0–25†	15–35	4–37	4–40
NaCl (%, w/v)	0–10.0	0.6–11.7*	2.0–9.0†	16.0	0.6–6.0	0.5–7.0
Production of bacteriochlorophyll *a*	+	−	−	ND	−	−
Nitrate reduction	−	−	−	+	−	+
Aesculin hydrolysis	−	−	−	+	−	−
API ZYM results						
α-Glucosidase	W	+	−	−	−	−
α-Galactosidase	−	W	−	W	−	−
Valine arylamidase	+	+	+	+	+	−
Cystine arylamidase	−	−	W	W	+	−
Susceptibility to:						
Polymyxin B	+	+	+	−	+	−
Tetracycline	+	+	+	−	−	+
Ampicillin	+	+	+	−	+	+
Colistin sulphate	−	+	+	ND	ND	ND
Polar lipids	PC, PG, PE, L, PLs, AL, APL	PC, PG, PE, Ls, PLs, ALs, APL	PG, PC, PE, Ls, PL, ALs	PC, PG, DPG, L, PL, AL	PG, PE, L, PL, AL	PC, PG, PE, L, PLs
DNA G+C content (mol%)	60.7	58.0*	59.6†	62.4	60.5	63.4
Isolation source	Antarctic seawater	Seawater from scallop larval cultures, Spain	Sediment of the Arctic Ocean	Sand beach, Yellow Sea, Korea	Sandy sediment, South China Sea	A tidal flat, Yellow Sea, Korea

*Data from Ruiz-Ponte *et al.* (1998).
†Data from Zhang *et al.* (2008).

Acknowledgements

The work was supported by the National Natural Science Foundation of China (grants 31290231, 31025001, 31170055, 41106161 and 41176130), the Hi-Tech Research and Development Program of China (grants 2011AA090703, 2012AA092103 and 2012AA092105), the China Ocean Mineral Resources R & D Association (COMRA) Special Foundation (grants DY125-15-T-05 and DY125-15-R-03), the Special Fund of China for Marine-scientific Research in the Public Interest (grant 201005032-6) and the 26th Chinese National Antarctic Research Expedition (project no. 09/10GW01).

References

Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25**, 3389–3402.

Biebl, H., Allgaier, M., Tindall, B. J., Koblizek, M., Lünsdorf, H., Pukall, R. & Wagner-Döbler, I. (2005). *Dinoroseobacter shibae* gen. nov., sp. nov., a new aerobic phototrophic bacterium isolated from dinoflagellates. *Int J Syst Evol Microbiol* **55**, 1089–1096.

Collins, M. D. & Jones, D. (1980). Lipids in the classification and identification of coryneform bacteria containing peptidoglycans based on 2,4-diaminobutyric acid. *J Appl Bacteriol* **48**, 459–470.

Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368–376.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

Fitch, W. M. (1971). Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.

Gaboyer, F., Tindall, B. J., Ciobanu, M.-C., Duthoit, F., Le Romancer, M. & Alain, K. (2013). *Phaeobacter leonis* sp. nov., an alphaproteobacterium from Mediterranean Sea sediments. *Int J Syst Evol Microbiol* **63**, 3301–3306.

Jin, H. M., Lee, H. J., Kim, J. M., Park, M. S., Lee, K. & Jeon, C. O. (2011). *Litorimicrobium taeanense* gen. nov., sp. nov., isolated from a sandy beach. *Int J Syst Evol Microbiol* **61**, 1392–1396.

Kim, O.-S., Cho, Y.-J., Lee, K., Yoon, S.-H., Kim, M., Na, H., Park, S.-C., Jeon, Y. S., Lee, J.-H. & other authors (2012). Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int J Syst Evol Microbiol* **62**, 716–721.

Komagata, K. & Suzuki, K. (1987). Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–207.

Lane, D. J. (1991). 16S/23S rRNA sequencing. In *Nucleic Acid Techniques in Bacterial Systematics*, pp. 115–175. Edited by E. Stackebrandt & M. Goodfellow. Chichester: Wiley.

Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from micro-organisms. *J Mol Biol* **3**, 208–218.

Marmur, J. & Doty, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J Mol Biol* **5**, 109–118.

Martens, T., Heidorn, T., Pukall, R., Simon, M., Tindall, B. J. & Brinkhoff, T. (2006). Reclassification of *Roseobacter gallaeciensis* Ruiz-Ponte *et al.* 1998 as *Phaeobacter gallaeciensis* gen. nov., comb. nov., description of *Phaeobacter inhibens* sp. nov., reclassification of *Ruegeria algicola* (Lafay *et al.* 1995) Uchino *et al.* 1999 as *Marinovum algicola* gen. nov., comb. nov., and emended descriptions of the genera *Roseobacter*, *Ruegeria* and *Leisingera*. *Int J Syst Evol Microbiol* **56**, 1293–1304.

Murray, R. G. E., Doetsch, R. N. & Robinow, C. F. (1994). Determinative and cytological light microscopy. In *Methods for General and Molecular Bacteriology*, pp. 21–41. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.

Ruiz-Ponte, C., Cilia, V., Lambert, C. & Nicolas, J. L. (1998). *Roseobacter gallaeciensis* sp. nov., a new marine bacterium isolated from rearings and collectors of the scallop *Pecten maximus*. *Int J Syst Bacteriol* **48**, 537–542.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Smibert, R. M. & Krieg, N. R. (1994). Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607–654. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.

Sun, F., Wang, B., Liu, X., Lai, Q., Du, Y., Li, G., Luo, J. & Shao, Z. (2010). *Leisingera nanhaiensis* sp. nov., isolated from marine sediment. *Int J Syst Evol Microbiol* **60**, 275–280.

Suyama, T., Shigematsu, T., Takaichi, S., Nodasaka, Y., Fujikawa, S., Hosoya, H., Tokiwa, Y., Kanagawa, T. & Hanada, S. (1999). *Roseateles depolymerans* gen. nov., sp. nov., a new bacteriochlorophyll *a*-containing obligate aerobe belonging to the β-subclass of the *Proteobacteria*. *Int J Syst Bacteriol* **49**, 449–457.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* **28**, 2731–2739.

Vandecandelaere, I., Segaert, E., Mollica, A., Faimali, M. & Vandamme, P. (2009). *Phaeobacter caeruleus* sp. nov., a blue-coloured, colony-forming bacterium isolated from a marine electroactive biofilm. *Int J Syst Evol Microbiol* **59**, 1209–1214.

Yoon, J.-H., Kang, S.-J., Lee, S.-Y. & Oh, T.-K. (2007). *Phaeobacter daeponensis* sp. nov., isolated from a tidal flat of the Yellow Sea in Korea. *Int J Syst Evol Microbiol* **57**, 856–861.

Yoon, J.-H., Kang, S.-J., Lee, S.-Y., Oh, K.-H. & Oh, T.-K. (2009). *Seohaeicola saemankumensis* gen. nov., sp. nov., isolated from a tidal flat. *Int J Syst Evol Microbiol* **59**, 2675–2679.

Zhang, D.-C., Li, H.-R., Xin, Y.-H., Liu, H.-C., Chi, Z.-M., Zhou, P.-J. & Yu, Y. (2008). *Phaeobacter arcticus* sp. nov., a psychrophilic bacterium isolated from the Arctic. *Int J Syst Evol Microbiol* **58**, 1384–1387.