**Sinobacterium caligoides** gen. nov., sp. nov., a new member of the family *Oceanospirillaceae* isolated from the South China Sea, and emended description of *Amphritea japonica*

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A taxonomic study was carried out on strain SCSWE24T, isolated from a seawater sample collected from the South China Sea. Cells of strain SCSWE24T were Gram-negative, rod-shaped, non-motile, moderately halophilic and capable of reducing nitrate to nitrite. Growth was observed at salinities from 1.5 to 4.5 % and at 4–37 °C; it was unable to degrade gelatin. The dominant fatty acids (>15 %) were summed feature 3 (C16:1ω7c and/or C16:1ω6c; 50.4 %) and C16:0 (21.1 %). The G+C content of the chromosomal DNA was 58.8 mol%. 16S rRNA gene sequence comparisons showed that strain SCSWE24T was most closely related to an uncultured bacterium clone Tun3b.F5 (98 %; GenBank accession no. FJ169216), and showed 92 % similarity to an endosymbiont bacterium from the bone-eating worm *Osedax mucofloris* (clone Omu 9 c4791; FN773233). Levels of similarity between strain SCSWE24T and type strains of recognized species in the family *Oceanospirillaceae* were less than 93 %; the highest similarity was 92 %, to both *Amphritea japonica* JAMM 1866T and ‘*Oceanicoccus sagamiensis*’ PZ-5. Phylogenetic analyses based on 16S rRNA gene sequences showed that strain SCSWE24T was most closely related to an uncultured bacterium clone Tun3b.F5 (98 %; GenBank accession no. FJ169216), and showed 92 % similarity to an endosymbiont bacterium from the bone-eating worm *Osedax mucofloris* (clone Omu 9 c4791; FN773233). Levels of similarity between strain SCSWE24T and type strains of recognized species in the family *Oceanospirillaceae* were less than 93 %; the highest similarity was 92 %, to both *Amphritea japonica* JAMM 1866T and ‘*Oceanicoccus sagamiensis*’ PZ-5. Phylogenetic analyses based on 16S rRNA gene sequences showed that strain SCSWE24T formed a distinct evolutionary lineage within the family *Oceanospirillaceae*. Strain SCSWE24T was distinguishable from members of phylogenetically related genera by differences in several phenotypic properties. On the basis of the phenotypic and phylogenetic data, strain SCSWE24T represents a novel species of a new genus, for which the name *Sinobacterium caligoides* gen. nov., sp. nov. is proposed. The type strain of *Sinobacterium caligoides* is SCSWE24T (=CCTCC AB 209289T = LMG 25705T = MCCC 1F01088T). An emended description of *Amphritea japonica* is also provided.

The family *Oceanospirillaceae* contains the genera *Oceanospirillum* (the type genus; Skerman et al., 1980), *Amphritea* (Gärtner et al., 2008), *Balneatrix* (Dauga et al., 1993), *Bermanella* (Pinhasi et al., 2009), *Marinomonas* (Chimetto et al., 2011), *Marinospirillum* (Satomi et al., 1998), *Neptuniibacter* (Arahali et al., 2007), *Neptunomonas* (Hedlund et al., 1999), *Nitrincola* (Dimitriu et al., 2005), *Oceaniserpentilla* (Schlösser et al., 2008), *Oceanobacter* (Satomi et al., 2002), *Oleibacter* (Teramoto et al., 2011), *Oleispira* (Yakimov et al., 2003), *Pseudospirillum* (Satomi et al., 2002), *Reinekea* (Romanenko et al., 2004) and *Thalassolituus* (Yakimov et al., 2004). Members of the family are motile by polar flagella and aerobic; their metabolism is strictly respiratory except for that of *Neptunomonas*, which gives weak fermentation reactions (Brenner et al., 2005). Members of *Balneatrix* are found in

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SCSWE24T is HQ686140.

Two supplementary figures are available with the online version of this paper.
Strain SCSWE24T was isolated from a seawater sample collected from the South China Sea. Bacteria were isolated by the plate-screening method on laboratory-prepared marine agar (MA; 5.0 g tryptone, 1.0 g yeast extract and 10 g agar per litre aged seawater, pH 7.6–7.8) incubated at 28 °C for 7 days. This medium was also used for morphological and biochemical characterization. The strain was stored at −80 °C in marine broth 2216E (MB; Difco) supplemented with 20 % (v/v) glycerol. Comparative 16S rRNA gene sequence analysis indicated that strain SCSWE24T formed a deep branch within the family Oceanospirillaceae. Accordingly, the aim of the present work was to determine the exact taxonomic position of strain SCSWE24T using a polyphasic characterization including the determination of phenotypic properties and a detailed phylogenetic analysis based on 16S rRNA gene sequences.

Seawater was sampled with Niskin bottles attached to a CTD (conductivity, temperature and depth) sensor in August 2007 during the summer cruise of the Innovative Research Team on the ship Dong-Fang-Hong Er-Hao. The sampling site, numbered Y56, was at 15° 01.340’N 112° 59.861’E; the water depth at the site is 450 m. The water sample was supplemented with 20 % (v/v) glycerol and stored at −20 °C for preservation. Isolation was performed 1 month later in the laboratory. Aliquots of 100 μl were spread onto MA plates and incubated at 28 °C for 7 days.

Genomic DNA was prepared according to the method of Su et al. (2007). The 16S rRNA gene was amplified by PCR using primers 27F and 1492R (DeLong, 1992). Sequences of related taxa were obtained from the GenBank database. Phylogenetic analysis was performed using MEGA version 4 (Tamura et al., 2007) after multiple alignment of data by DNA MAN (version 5.1; Lynnon Biosoft). Distances (distance options according to Kimura’s two-parameter model) were determined and clustering with the neighbour-joining method (Saitou & Nei, 1987) was performed using bootstrap values based on 1000 replications.

A nearly full-length 16S rRNA gene sequence (1504 nt) of strain SCSWE24T was determined. It showed 98 % similarity to an uncultured bacterium clone Tun3b.F5 (GenBank accession no. FJ169216), 92 % similarity to a sequence from an endosymbiotic bacterium in the bone-eating worm Oseadax muclovis (FN773233) (Verna et al., 2010), and 92 % similarity to Amphitrite japonica JAMM 1866T (Miyazaki et al., 2008b) and ‘Oceanoccus sagamensis’ PZ-5 (Park et al., 2011; this name has not been validly published). Phylogenetic analysis of strain SCSWE24T indicated that it belonged to a new genus of the family Oceanospirillaceae, with bootstrap support of 100 % by the neighbour-joining method (Fig. 1) and by minimum-evolution analysis. The two algorithms yielded essentially the same tree topology (data not shown).

General cell morphology was studied under an Olympus inverted microscope using 3-day-old cultures on MA. For electron microscopy, cells of strain SCSWE24T taken from agar plates were stained with 2 % phosphotungstic acid and examined in a JEM2100 transmission electron microscope (JEOL) (Fig. S1, available at IJSEM Online). The following tests were performed on strain SCSWE24T and A. japonica JAMM 1866T. The Gram reaction and activities of catalase, oxidase, lipase (TWEEN 80) and amylase were examined according to Dong & Cai (2001). Growth in MB was evaluated at 0, 4, 10, 16, 20, 25, 28, 30, 37, 40 and 45 °C at pH 3.0–10.0 (at intervals of 1.0 pH unit). The pH of MB was adjusted prior to sterilization using the following buffers: citric acid/sodium citrate (pH 3.0–6.0), Na2HPO4/citric acid (pH 7.0–8.0) and lysine/NaOH (pH 9.0–10.0). Verification of the pH after autoclaving revealed only minor changes. Tolerance of NaCl was tested using distilled water supplemented with 0.5 % tryptone and 0.1 % yeast extract and containing 0, 0.3, 0.5–6.5 % (w/v) NaCl (at intervals of 0.5 %) and 8, 9, 10, 15 and 20 % (w/v) NaCl. Anaerobic growth was examined using the Shelllab anaerobic system. Other biochemical tests were carried out using API 20NE, API 50CH and API ZYM strips (bioMérieux) according to the manufacturer’s instructions except that artificial seawater (0.1 % CaCl2, 2H2O, 0.1 % KCl, 0.5 % MgSO4, 7H2O, 2.5 % NaCl; Lau et al., 2005) was used to prepare bacterial suspensions. Antibiotic susceptibility tests were performed by the disc diffusion method (Drew et al., 1972) on MA at 28 °C for 3–10 days.

**Fig. 1.** Neighbour-joining tree showing the phylogenetic positions of strain SCSWE24T and representatives of some other related taxa, based on 16S rRNA gene sequences. Bootstrap values (expressed as percentages of 1000 replications) are shown at branch points. Bar, 0.01 nucleotide substitution rate (Knucl) units.
The phenotypic characteristics of strain SCSWE24T are given in the genus and species descriptions and in Table 1.

The fatty acids and polar lipids of strain SCSWE24T and A. japonica JAMM 1866T were analysed. After growth in MB at 28 °C for 3 days, fatty acids were extracted, saponified and esterified and the fatty acid methyl esters were analysed by GC (Agilent Technologies 7890A). The TBSA6 database was used according to the instructions of the MIDI System (Sasser, 1997). Polar lipid analysis was carried out by the Identification Service of the DSMZ (Braunschweig, Germany). Respiratory quinones were also analysed at the DSMZ on an LDC Analytical HPLC (Thermo Separation Products) fitted with a reversed-phase column (Macherey-Nagel; 2 × 125 mm, 3 μm, RP18) using methanol/heptane (9:1, v/v) as the eluent. The G+C content of the chromosomal DNA was determined using a reversed-phase HPLC method (Mesbah & Whitman, 1989).

The fatty acid profiles of strain SCSWE24T and A. japonica JAMM 1866T are shown in Table 2, in comparison with those of related strains. The major fatty acids of strain SCSWE24T were summed feature 3 (C16 : 1v7c and/or C16 : 1v9c; 50.4 %) and C16 : 0 (21.1 %) (Table 2). The fatty acid composition differed a lot from that of 'O. sagamiensis' PZ-5 in the presence of C16 : 1 and C10 : 1 as well as the absence of C17 : 10c, C11 : 0 3-0H and iso-C15 : 0 2-0H. The fatty acid composition of A. japonica JAMM 1866T was similar, except for the absence of C12 : 1 3-0H and the presence of C14 : 0 (Table 2). The polar lipids of strain SCSWE24T were phosphatidylethanolamine, phosphatidylglycerol, an unidentified aminolipid, an unidentified glycolipid and five unidentified phospholipids, while A. japonica JAMM 1866T contained phosphatidylethanolamine, phosphatidylglycerol, an unidentified glycolipid, four unidentified phospholipids and two unidentified lipids (Fig. 2). The major respiratory quinone was ubiquinone 8. The DNA G+C content of strain SCSWE24T was 58.8 mol%, a value higher than those reported for other members of the family (Table 1).

Phenotypically, strain SCSWE24T differed considerably from other members of the family. It has no flagella, while others have one or more flagella; the cells are non-motile rods. In addition, strain SCSWE24T can grow at 30 °C but not with 6 % NaCl, which differentiates it from members of the genera Amphritea, Neptuniibacter and Neptunomonas (Table 1). The biggest difference from 'O. sagamiensis' PZ-5 was its shorter doubling time (3 days for strain SCSWE24T; 4 weeks for 'O. sagamiensis' PZ-5). The low levels of 16S rRNA gene sequence similarity between strain SCSWE24T and all other members of the family Oceanospirillaceae, together with the differential phenotypic properties shown in Table 1, suggest that strain SCSWE24T represents a novel species of a new genus within the family Oceanospirillaceae, for which the name Sinobacterium caligoides gen. nov., sp. nov. is proposed. The results of our polar lipid analysis also necessitate an emended description of Amphritea japonica.

**Description of Sinobacterium gen. nov.**

**Sinobacterium** (Si.no.bac.te.ri.um. M.L. n. Sina China; L. neut. n. bacterium a small staff or rod and, in biology, a bacterium; N.L. neut. n. Sinobacterium a bacterium from China).

Cells are rod shaped, non-motile, Gram-negative, oxidase-positive, catalase-positive and capable of reducing nitrate to nitrite, but not denitrification. The dominant fatty acids are summed feature 3 (C16 : 1v9c and/or C16 : 1v9c) and C16 : 0. The major polar lipids are phosphatidylethanolamine,
Table 2. Cellular fatty acid contents of strain SCSWE24T and members of related genera

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*Summed features are groups of two or three fatty acids that cannot be separated by GLC with the MIDI System. Summed feature 1 contains C16 : 07c and/or iso-C15 : 0 2-OH; summed feature 2 contains one or more of C19 : 0 cyclo and unknown ECL 18.846; summed feature 3 contains C16 : 07c and/or C16 : 106c; summed feature 8 contains C18 : 107c and/or C18 : 106c.

phosphatidylglycerol, an unidentified aminolipid, an unidentified glycolipid and five unidentified phospholipids. The major respiratory quinone is ubiquinone 8 (Q-8). The DNA G+C content of the type strain of the type species is 58.8 mol%. The type species is Sinobacterium caligoides.

Description of Sinobacterium caligoides sp. nov.

Sinobacterium caligoides (L. n. caligo fog; L. suff. -ides looking like; N.L. neut. adj. caligoides looking like fog, referring to the colony shape).

Displays the following features in addition to those listed in the genus description. Cells are 1.5–2.5 μm long and 0.4–0.8 μm wide. Positive for urease, but negative for indole production. On MA, forms moist, white–grey colonies with irregular edges, 2–5 mm in diameter after 72 h of incubation at 28 °C, non-pigmented and deeper in colour at the centre (Fig. S2). Moderately halophilic; grows in 1.5–4.5% NaCl (optimum 2.0–4.5%). Grows at 4–37 °C (optimum 20–30°C). Unable to ferment glucose. In API ZYM tests, positive for acid and alkaline phosphatases, leucine arylamidase, esterase (C4), esterase lipase (C8), α-glucosidase and β-galactosidase, weakly positive for valine arylamidase, lipase (C14), naphthol-AS-BI-phosphohydrolase, α-galactosidase, α-chymotrypsin and β-glucosidase and negative for cystine arylamidase, N-acetyl-β-glucosaminidase, trypsin, α-fucosidase, α-mannosidase and β-glucuronidase.

No carbon source can be utilized in the API 50CH test. Acid is not produced from carbohydrates. Sensitive to (per disc; Oxo) chloromycetin (30 μg), rifampicin (5 μg), trimethoprim/sulfamethoxazole (25 μg), gentamicin (10 μg), polymyxin (300 μg), ciprofloxacin (5 μg), kanamycin (30 μg) and furazolidone (15 μg). Resistant to tetracycline (30 μg), oxacillin (1 μg), cefoperazone (75 μg), vancomycin (30 μg), ampicillin (10 μg), minocycline (30 μg), cephalaxin (30 μg) and doxycycline (30 μg). The complete fatty acid composition of the type strain is given in Table 2. Table 1 shows characteristics used to distinguish the type strain from members of related genera.

The type strain, SCSWE24T (=CCTCC AB 209289T =LMG 25705T =MCCC 1F01088T), was isolated from a water sample from a 450 m-deep area of the South China Sea.

Emended description of Amphritea japonica Miyazaki et al. 2008

The description is as given by Miyazaki et al. (2008b) with the following amendment. Polar lipids are phosphatidylethanolamine, phosphatidylglycerol, an unidentified glycolipid, four unidentified phospholipids and two unidentified lipids.
Acknowledgements

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References


