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 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 (Pinhassi et al., 2009), Marinomonas (Chimetto et al., 2011), Marinospirillum (Satomi et al., 1998), Neptuniibacter (Arahal et al., 2007), Neptunomonas (Hedlund et al., 1999), Nitrincola (Dimitriu et al., 2005), Oceaniserpentilla (Schlösser et al., 2008), Oceano bacter (Satomi et al., 2002), Oleibacter (Teramoto et al., 2011), Oleispira (Yakimov et al., 2003), Pseudospirillum (Satomi et al., 2002), Reinekeana (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
Strain SCSWE24\textsuperscript{T} 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 SCSWE24\textsuperscript{T} formed a deep branch within the family Oceanospirillaceae. Accordingly, the aim of the present work was to determine the exact taxonomic position of strain SCSWE24\textsuperscript{T} 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 seawater 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 DNAMAN (version 5.1; Lynnon Biosoft). Distances (distance options according to Kimura’s two-parameter model) were determined and clustering with the neighbor-joining method (Saitou & Nei, 1987) was performed using a polyphasic characterization including the determination of phenotypic properties and a detailed phylogenetic analysis based on 16S rRNA gene sequences.

A nearly full-length 16S rRNA gene sequence (1504 nt) of strain SCSWE24\textsuperscript{T} 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 mucifloris (FN773233) (Verna et al., 2010), and 92 % similarity to Amphritea japonica JAMM 1866\textsuperscript{5} (Miyazaki et al., 2008b) and ‘Oceanoccus sagamien-sis’ PZ-5 (Park et al., 2011; this name has not been validly published). Phylogenetic analysis of strain SCSWE24\textsuperscript{T} 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 SCSWE24\textsuperscript{T} 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 SCSWE24\textsuperscript{T} and A. japonica JAMM 1866\textsuperscript{5}. 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), Na\textsubscript{2}HPO\textsubscript{4}/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 % CaCl\textsubscript{2}, 2H\textsubscript{2}O, 0.1 % KCl, 0.5 % MgSO\textsubscript{4}, 7H\textsubscript{2}O, 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 SCSWE24\textsuperscript{T} 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 (K\textsubscript{nucl}) units.](https://www.microbiologyresearch.org/assets/images/093709/093709-3.png)
The phenotypic characteristics of strain SCSWE24\(^\mathrm{T}\) are given in the genus and species descriptions and in Table 1.

The fatty acids and polar lipids of strain SCSWE24\(^\mathrm{T}\) and A. japonica JAMM 1866\(^\mathrm{T}\) 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 (Mesbaḥ & Whitman, 1989).

The fatty acid profiles of strain SCSWE24\(^\mathrm{T}\) and A. japonica JAMM 1866\(^\mathrm{T}\) are shown in Table 2, in comparison with those of related strains. The major fatty acids of strain SCSWE24\(^\mathrm{T}\) were summed feature 3 (C\(_{16:1}\)ω7c and/or C\(_{16:1}\)ω6c) 50.4 %) and C\(_{16:0}\) (21.1 %) (Table 2). The fatty acid composition differed a lot from that of ‘O. sagamiensis’ PZ-5 in the presence of C\(_{16:1}\) and C\(_{10:1}\) as well as the absence of C\(_{17:1}\)ω8c, C\(_{11:0}\) 3-OH and iso-C\(_{15:0}\) 2-OH. The fatty acid composition of A. japonica JAMM 1866\(^\mathrm{T}\) was similar, except for the absence of C\(_{12:1}\) 3-OH and the presence of C\(_{14:0}\) (Table 2). The polar lipids of strain SCSWE24\(^\mathrm{T}\) were phosphatidylethanolamine, phosphatidylycerol, an unidentified aminolipid, an unidentified glycolipid and five unidentified phospholipids, while A. japonica JAMM 1866\(^\mathrm{T}\) contained phosphatidylethanolamine, phosphatidylycerol, 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 SCSWE24\(^\mathrm{T}\) was 58.8 mol%, a value higher than those reported for other members of the family (Table 1).

Phenotypically, strain SCSWE24\(^\mathrm{T}\) 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 SCSWE24\(^\mathrm{T}\) can grow at 30 °C but not with 6 % NaCl, which differentiates it from members of the genera Amphritea, Neptunibacter and Neptunomonas (Table 1). The biggest difference from ‘O. sagamiensis’ PZ-5 was its shorter doubling time (3 days for strain SCSWE24\(^\mathrm{T}\); 4 weeks for ‘O. sagamiensis’ PZ-5). The low levels of 16S rRNA gene sequence similarity between strain SCSWE24\(^\mathrm{T}\) and all other members of the family Oceanospirillaceae, together with the differential phenotypic properties shown in Table 1, suggest that strain SCSWE24\(^\mathrm{T}\) 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.rium. 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 (C\(_{16:1}\)ω7c and/or C\(_{16:1}\)ω6c) and C\(_{16:0}\). The major polar lipids are phosphatidylethanolamine,

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**Table 1. Characteristics that differentiate SCSWE24\(^\mathrm{T}\) from members of related genera**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell shape</td>
<td>Rods</td>
<td>Coccolid or amorphous</td>
<td>Rods</td>
<td>Rods</td>
<td>Rods</td>
<td>Spirilla</td>
</tr>
<tr>
<td>Cell length (μm)</td>
<td>1.5–2.5</td>
<td>0.6–0.8</td>
<td>0.5–2.0</td>
<td>1.5–2.3</td>
<td>1.0–1.8</td>
<td>2.5–40</td>
</tr>
<tr>
<td>Motility</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Flagella</td>
<td>–</td>
<td>Single polar</td>
<td>Single polar or bipolar</td>
<td>–/Single polar</td>
<td>Single polar or subterminal</td>
<td>Bipolar tufts</td>
</tr>
<tr>
<td>Growth in/at:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 °C</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+/–</td>
<td>+</td>
</tr>
<tr>
<td>6 % NaCl</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+/–</td>
<td>ND</td>
</tr>
<tr>
<td>Catalase</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Gelatinase</td>
<td>–</td>
<td>ND</td>
<td>+/–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Lipase</td>
<td>W</td>
<td>ND</td>
<td>+</td>
<td>–</td>
<td>+/–</td>
<td>ND</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+/–</td>
<td>–</td>
</tr>
<tr>
<td>DNA G + C content (mol%)</td>
<td>58.8</td>
<td>49.8</td>
<td>46.7–52.2</td>
<td>46.6–54.2</td>
<td>43.6–48.2</td>
<td>46</td>
</tr>
</tbody>
</table>

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phosphatidyglycerol, an unidentified aminolipid, an unidentified glycolipid and five unidentified phospholipids. The major respiratory quinone is ubiquinone 8 (Q-8). The respiratory quinones contain C16 : 1 \( \mu \) and/or C18 : 1 \( \mu \); summed feature 2 contains C16 : 1 \( \mu \); summed feature 3 contains C16 : 1 \( \mu \) and/or C18 : 1 \( \mu \); summed feature 8 contains C16 : 1 \( \mu \) and/or C18 : 1 \( \mu \).

Table 2. Cellular fatty acid contents of strain SCSWE24\(^T\) and members of related genera

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>C10 : 0</td>
<td>2.6</td>
<td>–</td>
<td>tr</td>
<td>tr</td>
<td>tr</td>
<td>5.4</td>
<td></td>
</tr>
<tr>
<td>C12 : 0</td>
<td>1.3</td>
<td>–</td>
<td>–</td>
<td>2</td>
<td>–</td>
<td>5.7</td>
<td></td>
</tr>
<tr>
<td>C14 : 0</td>
<td>11.8</td>
<td>–</td>
<td>–</td>
<td>tr</td>
<td>1.07</td>
<td>4.2</td>
<td></td>
</tr>
<tr>
<td>C16 : 0</td>
<td>21.1</td>
<td>15.4</td>
<td>29</td>
<td>16.1</td>
<td>36.2</td>
<td>15.6</td>
<td></td>
</tr>
<tr>
<td>C18 : 0</td>
<td>2.1</td>
<td>–</td>
<td>tr</td>
<td>tr</td>
<td>1.0</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>C12 : 1</td>
<td>–</td>
<td>–</td>
<td>tr</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>C14 : 1</td>
<td>–</td>
<td>–</td>
<td>tr</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>C16 : 1</td>
<td>16.2</td>
<td>–</td>
<td>–</td>
<td>36.9</td>
<td>35.9</td>
<td>39.5</td>
<td></td>
</tr>
<tr>
<td>C18 : 1</td>
<td>–</td>
<td>–</td>
<td>22</td>
<td>48.5</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>C20 : 1</td>
<td>–</td>
<td>–</td>
<td>tr</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>C10 : 0 3-OH</td>
<td>3.3</td>
<td>2.8</td>
<td>5</td>
<td>5.0</td>
<td>7.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C11 : 0 3-OH</td>
<td>7.5</td>
<td>–</td>
<td>7.5</td>
<td>–</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C12 : 1 3-OH</td>
<td>–</td>
<td>–</td>
<td>3.2</td>
<td>3</td>
<td>–</td>
<td></td>
<td></td>
</tr>
<tr>
<td>iso-C15 : 0 2-OH</td>
<td>19.0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Summed features

- Summed feature 1 contains C16 : 1\(\mu\)7c and/or iso-C15 : 0 2-OH
- Summed feature 2 contains one or more of C19 : 1\(\mu\)6c, C19 : 0 cyclo and unknown ECL 18.846
- Summed feature 3 contains C16 : 1\(\mu\)7c and/or C16 : 1\(\mu\)6c
- Summed feature 8 contains C18 : 1\(\mu\)7c and/or C18 : 1\(\mu\)6c

*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 : 1\(\mu\)7c and/or iso-C15 : 0 2-OH; summed feature 2 contains one or more of C19 : 1\(\mu\)6c, C19 : 0 cyclo and unknown ECL 18.846; summed feature 3 contains C16 : 1\(\mu\)7c and/or C16 : 1\(\mu\)6c; summed feature 8 contains C18 : 1\(\mu\)7c and/or C18 : 1\(\mu\)6c.

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.

Strains: 1, SCSWE24\(^T\); 2, ‘O. sagamiensis’ PZ-5 (data from Park et al., 2011); 3, A. japonica JAMM 1866\(^T\) (data from this study); 4, Amphritea atlantica M41\(^T\) (Gartner et al., 2008); 5, Neptuniibacter caesariensis MED92\(^T\) (Arakah et al., 2007); 6, Marinospirillum celere v1c_Sn-red\(^T\) (Namsaraev et al., 2009); 7, Marinomonas ostreisangi UST010306-043\(^T\) (Lau et al., 2006).

**Fig. 2.** Polar lipids of strain SCSWE24\(^T\) (a) and A. japonica JAMM 1866\(^T\) (b). PE, Phosphatidylethanolamine; PG, phosphatidylglycerol; AL1, unidentified aminolipid; PL1–5, unidentified phospholipids; GL1, unidentified glycolipid; L1–2, unidentified lipids.

**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 \( \mu \)m long and 0.4–0.8 \( \mu \)m wide. Positive for urease, but negative for indole production. On MA, forms moist, white–grey colonies with irregular edges, 2–5 \( \mu \)m 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), \( \alpha \)-glucosidase and \( \beta \)-galactosidase, weakly positive for valine arylamidase, lipase (C14), naphthol-AS-BI-phosphohydrolase, \( \alpha \)-galactosidase, \( \alpha \)-chymotrypsin and \( \beta \)-glucosidase and negative for cystine arylamidase, N-acetyl-\( \beta \)-glucosaminidase, trypsin, \( \alpha \)-fucosidase, \( \alpha \)-mannosidase and \( \beta \)-glucuronidase. No carbon source can be utilized in the API 50CH test. Acid is not produced from carbohydrates. Sensitive to (per disc; Oxoid) chloromycetin (30 \( \mu \)g), rifampicin (5 \( \mu \)g), trimethoprim/sulfamethoxazole (25 \( \mu \)g), gentamicin (10 \( \mu \)g), polymyxin (300 \( \mu \)g), ciprofloxacin (5 \( \mu \)g), kanamycin (30 \( \mu \)g) and furazolidone (15 \( \mu \)g). Resistant to tetracycline (30 \( \mu \)g), oxacillin (1 \( \mu \)g), cefoperazone (75 \( \mu \)g), vancomycin (30 \( \mu \)g), ampicillin (10 \( \mu \)g), minomycin (30 \( \mu \)g), cephalin (30 \( \mu \)g) and doxycycline (30 \( \mu \)g). 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, SCSWE24\(^T\) (=CCTCC AB 209289\(^T\) =LMG 25705\(^T\) =MCCC 1F01088\(^T\)), 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


