**Sphaerochaeta multiformis** sp. nov., an anaerobic, psychrophilic bacterium isolated from subseafloor sediment, and emended description of the genus *Sphaerochaeta*

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An anaerobic, psychrophilic bacterium, strain MO-SPC2T, was isolated from a methanogenic microbial community in a continuous-flow bioreactor that was established from subseafloor sediments collected from off the Shimokita Peninsula of Japan in the north-western Pacific Ocean. Cells were pleomorphic: spherical, annular, curved rod, helical and coccoid cell morphologies were observed. Motility only occurred in helical cells. Strain MO-SPC2T grew at 0–17 °C (optimally at 9 °C), at pH 6.0–8.0 (optimally at pH 6.8–7.2) and in 20–40 g NaCl l⁻¹ (optimally at 20–30 NaCl l⁻¹). The strain grew chemo-organotrophically with mono-, di- and polysaccharides. The major end products of glucose fermentation were acetate, ethanol, hydrogen and carbon dioxide. The abundant polar lipids of strain MO-SPC2T were phosphatidyglycolipids, phospholipids and glycolipids. The major cellular fatty acids were C₁₄ : ₀, C₁₆ : ₀ and C₁₆ : ₁ω₉. Isoprenoid quinones were not detected. The G+C content of the DNA was 32.3 mol%. 16S rRNA gene-based phylogenetic analysis showed that strain MO-SPC2T was affiliated with the genus *Sphaerochaeta* within the phylum *Spirochaetes*, and its closest relatives were *Sphaerochaeta pleomorpha* GrapesT (88.4 % sequence identity), *Sphaerochaeta globosa* BuddyT (86.7 %) and *Sphaerochaeta coccoides* SPN1T (85.4 %). Based on phenotypic characteristics and phylogenetic traits, strain MO-SPC2T is considered to represent a novel species of the genus *Sphaerochaeta*, for which the name *Sphaerochaeta multiformis* sp. nov. is proposed. The type strain is MO-SPC2T (=JCM 17281T=DSM 23952T). An emended description of the genus *Sphaerochaeta* is also proposed.

The genus *Sphaerochaeta*, a recently proposed genus within the phylum *Spirochaetes*, forms a sister clade of the genus *Spirochaeta* (Ritalahti et al., 2012). To date, three isolates of the genus *Sphaerochaeta* have been isolated and given validly published names: *Sphaerochaeta globosa* BuddyT, *Sphaerochaeta pleomorpha* GrapesT and *Sphaerochaeta coccoides* SPN1T (Abt et al., 2012; Ritalahti et al., 2012). *Sphaerochaeta globosa* BuddyT and *Sphaerochaeta pleomorpha* GrapesT were isolated from anaerobic dechlorinating consortia that were established from freshwater sediments (Ritalahti et al., 2012), while *Sphaerochaeta coccoides* SPN1T was isolated from the hindgut contents of the termite...
Neotermes castaneus (Dröge et al., 2006). Spherical morphologies and the lack of motility distinguish the members of Sphaerochaeta from other members of the phylum Spirochaetes, which all share the helical morphology and motility conferred by axial periplasmic flagella (Caro-Quintero et al., 2012; Ritalahti et al., 2012; Charon et al., 2012). Whole-genome sequence analyses confirmed that isolates of Sphaerochaeta lack the suite of motility and associated signal transduction gene cassettes (Caro-Quintero et al., 2012; Abt et al., 2012). Therefore, investigations of isolates of the genus Sphaerochaeta can provide new insights into the ecology, physiology, pathogenicity and evolution of the phylum Spirochaetes.

In addition to the three isolates mentioned above, some other cultures belonging to the genus Sphaerochaeta have been reported. An anaerobic isolate, strain ACE-P, obtained from the anoxic hypolimnion of Ace Lake, Antarctica (Franzmann & Dobson, 1992), shows similar morphological and physiological properties to the members of Sphaerochaeta. Based on 16S rRNA gene sequence identity, strain ACE-P is closely related to the genus Sphaerochaeta (Fig. S1, available in the online Supplementary Material) (Franzmann & Rohde, 1992; Ritalahti et al., 2012). Toffin et al. (2004) reported enrichment cultures derived from marine subsurface sediments collected from the Nankai Trough site 1173 of the Ocean Drilling Program that contained spirochaetes. The 16S rRNA gene sequences of these spirochaetes (GenBank accession numbers AY485798, AY485800 and AY485802) were closely related to that of Sphaerochaeta globosa BuddýT (sequence identities 97.3–98.4 %). In addition, many 16S rRNA gene sequences related to Sphaerochaeta have been retrieved from a variety of anoxic environments (Fig. S1), such as animal intestines (Lerse et al., 2002; Ley et al., 2008; Scupham et al., 2010), methanogenic waste/wastewater treatment bioreactors (e.g. Briones et al., 2007; Fernandez et al., 2000; Riviére et al., 2009), dechlorinating microbial consortia (Chung et al., 2008; Gu et al., 2004), oil reservoir/contaminated field/production water (Acosta-González et al., 2013; Grabowski et al., 2005; Kobayashi et al., 2012), leachate sediment (Liu et al., 2011) and an extreme cryogenic brine ecosystem in Antarctica (Murray et al., 2012). These findings suggest that members of Sphaerochaeta are widely distributed in global anoxic environments.

Recently, we have isolated a bacterium, designated strain MO-SPC2T, from subseaﬂoor sediment samples collected from off the Shimokita Peninsula of Japan in the northwestern Paciﬁc Ocean (site C9001, water depth 1180 m) (Imachi et al., 2011). Enrichment and isolation of the strain were achieved using a continuous-ﬂow bioreactor approach and serial dilutions in liquid medium, respectively. Here, we report details of the isolation procedure and the morphological, physiological and genetic properties of strain MO-SPC2T. Based on the taxonomic characterization, we propose that strain MO-SPC2T is a member of a novel species within the genus Sphaerochaeta. The basal medium used in this study was prepared with the following components (1–1): 0.53 g NH4Cl, 0.1 g KH2PO4, 4 g MgCl2·6H2O, 1 g CaCl2·2H2O, 25 g NaCl, 2 g NaHCO3, 0.15 g Na2S·9H2O, 0.15 g cystine hydrochloride, 1 ml trace element solution (Imachi et al., 2008), 1 ml vitamin solution (Imachi et al., 2009) and 1 ml resazurin solution (1 mg ml−1). The final pH was 7.2 at 25 °C. The primary enrichment culture was incubated at 10 °C under anaerobic conditions. For the roll tube isolation method, solid medium was prepared by adding purified agar (agar noble; Difco) to the basal medium at a final concentration of 20 g l−1. After isolation of the strain, all cultivations were performed in 50 ml glass serum bottles or 25 ml glass tubes containing 20 or 10 ml medium, respectively, at 10 °C under an atmosphere of N2/CO2 (80:20, v/v) without shaking. The incubation vessels were sealed with butyl rubber stoppers and aluminium crimp seals (Nichiden Rika Glass). Growth substrates (see below) were added to the medium containers from neutralized and sterilized stock solutions prior to inoculation.

Growth was determined by OD600 measurements (spectrophotometer DR2500; Hach). Substrate utilization tests were performed using exponentially growing pre-cultures (10 % inoculum, v/v) at 10 °C for over 1 month. Effects of temperature, pH and NaCl concentration on growth of strain MO-SPC2T were determined in basal medium containing 10 mM maltose plus 0.1 % (w/v) yeast extract (Difco). To determine the temperature range for growth, a temperature gradient incubator with bio-photorecorder (model TVS126MA; Advantec) was used. The temperature was set at 5, 9, 11, 13, 15, 17, 19 and 21 °C. Growth at 2 and 0 °C was tested with low-temperature incubators. To evaluate the pH range for growth, the medium was adjusted to pH 5.5, 6.0, 6.5, 6.8, 7.0, 7.2, 7.5, 8.0, 8.2 and 8.5 with 1 M HCl or 1 M NaOH solutions. The pH of the medium was monitored every 4 days during growth using a portable pH meter (Horiba Twin pH B-212), and the pH was readjusted by using HCl or NaOH if the initial pH had changed significantly. NaCl requirement was determined by varying the NaCl concentration in the basal medium from 0 to 80 g l−1 (w/v). All growth tests were performed in triplicate culture vessels without shaking in the dark.

Cytochrome oxidase activity was determined by spreading cell pellets on oxidase test paper (Nissui Pharmaceutical). Catalase activity was determined by O2-bubble production following the addition of 3 % (v/v) H2O2 solution to a cell pellet (Barrow & Feltham, 1993).

Cell morphology was examined under a fluorescent microscope (Olympus BX51F) equipped with a colour CCD camera system (Olympus DP72). The Gram-staining reaction was performed by Hucker’s method (Doetsch, 1981). Transmission electron micrographs of negatively stained cells and thin sections were obtained as described by Zillig et al. (1990). Briefly, cells were collected during the mid-exponential growth phase at 10 °C and negatively stained with 1 % (w/v) neutral phosphotungstic acid for
observation with a JEOL JEM-1210 electron microscope at an acceleration voltage of 120 kV. For ultrathin sectioning, 2.5% (w/v) glutaraldehyde was added to the maltose-yeast extract medium to fix the cells overnight at 10 °C. Specimens were then washed in filtered seawater and post-fixed with 2% osmium tetroxide in filtered seawater for 2 h at 4 °C. The samples were stained en bloc with 1% aqueous uranyl acetate for 2 h at room temperature. After rinsing with distilled water, the samples were dehydrated in a graded ethanol series and the specimen was embedded overnight in Epon 812 resin (TAAB) and cut with an ultramicrotome (Ultracut S; Leica). The ultrathin sections were stained in 2% (w/v) uranyl acetate and lead stain solution (Sigma-Aldrich) and subjected to transmission electron microscopy using a JEOL JEM-1210 at an acceleration voltage of 100 kV. For scanning electron microscopic images, cells were prefixed overnight in 2.5% (w/v) glutaraldehyde in the maltose-yeast extract medium at 10 °C. After washing in 0.1 M PBS (pH 7.4), cell suspensions were adhered to the poly-L-lysine- (Sigma) coated glass slide for 30 min at room temperature. Following this treatment, the cells were fixed in 2% (w/v) osmium tetroxide dissolved in PBS. After the samples were rinsed with distilled water, conductive staining was performed by incubating in 0.2% aqueous tannic acid (pH 6.8) for 30 min; the samples were then washed with distilled water and treated with 1% aqueous osmium tetroxide for 1 h. After that, the samples were dehydrated in a graded ethanol series and critical-point dried in a JEOL JCPD-5 instrument. The samples were coated with osmium using an osmium plasma coater (POC-3; Meiwafosis) and observed with a JEOL JSM-6700F field emission scanning electron microscope operated at 5 kV.

For analyses of isoprenoid quinones, polar lipids and cellular fatty acid methyl esters (FAMEs), cells of strain MO-SPC2^T were harvested from cultures grown with 10 mM glucose plus 0.5% (w/v) yeast extract. The type strains of the three species of the genus *Sphaerochaeta*, *Sphaerochaeta pleomorpha* DSM 22778^T, *Sphaerochaeta globosa* DSM 22777^T and *Sphaerochaeta cocoides* DSM 17374^T, were purchased from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany) and subjected to polar lipid and FAME analyses for comparison. Cells of the reference strains were grown in 1:10-diluted basal medium containing 10 mM glucose plus 0.5% (w/v) yeast extract. The reference strains were cultured at the reported optimum growth temperatures (Dröge et al., 2006; Ritalahti et al., 2012). Polar lipids and isoprenoid quinones were extracted from lyophilized cells (approx. 50 mg) according to the procedures described by Minnikin et al. (1984). Polar lipids were separated by two-dimensional TLC and visualized by spraying with the appropriate detection reagents (Minnikin et al., 1984; Komagata & Suzuki, 1987). The fatty acids of strain MO-SPC2^T and the reference strains were obtained from cells by saponification, methylation and extraction according to a protocol provided with the Sherlock Microbial Identification System (MIDI, 1999). To determine double-bond positions in unsaturated fatty acids, dimethyl disulfide derivatives were added to the FAMEs (Christie, 1997). The fatty acid compositions were determined using a Finnigan TRACE DSQ GC-MS system (Thermo Scientific) equipped with a Trace TR-5MS column (Thermo Scientific) with a helium flow of 1.5 ml min^-1^ and an oven temperature increasing from 140 °C (5 min) to 260 °C (5 min) at a rate of 4 °C min^-1^.

The G+C contents of the DNA was determined by reversed-phase HPLC with a DNA-GC kit (Yamasu Shoyu) after digestion of the DNA with nuclease P1.

Procedures for DNA extraction, PCR amplification, cloning and sequencing were performed as described previously (Imachi et al., 2011). The nearly full-length 16S rRNA gene of the isolate was amplified with the general bacterial primer pair 8F/UN1490R, and comparative 16S rRNA gene sequence similarity and phylogenetic analyses were performed as described previously (Imachi et al., 2006). To obtain confidence for the tree topologies, bootstrap-resampling analysis with 1000 replicates was performed for the neighbour-joining, maximum-par-simony and maximum-likelihood methods by using the MEGA program package (Tamura et al., 2011).

Carbohydrates, short-chain fatty acids and alcohols were determined by HPLC using a Rezox ROA-Organic Acid Aminex HPX-87H column (Phenomenex; eluent 0.01 M H_2SO_4; column temperature, 50 °C), a UV-Vis detector (Shimadzu SPD-10Avp) and a refractive index detector (Shimadzu RID-10A). Molecular hydrogen and carbon dioxide were measured by gas chromatography (GL Science model GC-3200; thermal conductivity detector; packing material, ShinCarbon; column temperature, 100 °C).

To obtain pure cultures of anaerobic micro-organisms from the methanogenic bioreactor, 2 ml samples of the enrichment sediment slurry were inoculated into basal medium containing various substrates in 50 ml serum vials. Of these, we found that coccoid cells dominated in a yeast extract (0.1%, w/v) medium supplemented with ampicillin and vancomycin, which was incubated at 10 °C. To identify the microbes present in the enrichment culture, a bacterial 16S rRNA gene clone library was established (Imachi et al., 2011). The analysis indicated that all eight cloned bacterial 16S rRNA gene fragments were identical and were related to sequences of the genus *Sphaerochaeta*. To isolate the bacterium, the roll-tube isolation technique was tested twice, but no colony formation was observed after a 3-month incubation period. Purification of the bacterium was performed by serial dilution in basal medium containing 0.1% yeast extract. After two successive transfers, the purified strain, designated strain MO-SPC2^T, was obtained. The purity of strain MO-SPC2^T was demonstrated by the failure to grow micro-organisms other than strain MO-SPC2^T in the following media at 10, 25 and 55 °C: (i) thioglycollate medium (Difco) containing approximately 150 kPa H_2/CO_2 (in the headspace) plus...
Cells of MO-SPC2T are pleomorphic (Figs 1 and S2). In the exponential growth phase, cocci between 1.1 and 2.7 μm in diameter dominated, but other cell morphologies were also observed. Other morphologies included spherical cells with an indentation (1.1–2.1 μm; Fig. 1b), annular cells (0.5–0.6 × 1.5–1.6 μm) with a central hole, 0.4–0.5 μm in diameter (Fig. 1c, d), curved rod-shaped cells (0.9–1.6 × 2.7–5.9 μm; Fig. 1e, f), helical cells (0.4–0.9 × 10–25 μm with a wavelength of 2.2–3.2 μm; Fig. 1g) and chains of cocci (0.9–1.6 × 4–14 μm, individual cells 0.5–0.9 × 0.6–1.7 μm; Fig. 1h). In stationary-phase cultures, the spherical morphotype dominated. Interestingly, the helical cells showed motility (Video S1), although electron microscopic observation did not find periplasmic flagella-like structures. A thread-like connection was observed between dividing cells (Fig. 1i). Thin-section images of annular cells showed a continuous cell membrane on the inside and outside of the cells (Fig. 1j–l). Thin-section images also depicted a typical Gram-negative bacterial cell-wall structure (Fig. 1k, l), an observation corroborated by the Gram-staining test. Scanning electron micrographs revealed that fibre structures like pili and fimbriae were present on the cell surface and inside the cavities of annular cells (Fig. S2). Based on microscopic observations of morphological change, we propose a cell life cycle of strain MO-SPC2T characterized by various morphological stages (Fig. 1m).

Strain MO-SPC2T was weakly oxidase-positive and catalase-negative, and was unable to grow under aerobic conditions, i.e. no growth was observed in the maltose-yeast extract medium in the absence of reducing agents such as sulfide and cysteine. Strain MO-SPC2T could utilize the following substrates as sole energy and carbon sources (final concentration 10 mM unless otherwise indicated): cellobiose, D-glucose, maltose, D-ribose, trehalose, D-xyllose, pectin (0.5 %, w/v), skimmed milk (0.5 %, w/v), xylan (0.5 %, w/v) and yeast extract (0.1 %, w/v). Yeast extract stimulated growth of strain MO-SPC2T, but was not required for growth. Major end products of glucose fermentation were acetate, ethanol, hydrogen and carbon dioxide. Pyruvate was observed as an additional end product during maltose fermentation. The following substrates did not support growth: D- and L-arabinose, D-fructose, D-galactose, glycerol, myo-inositol, lactose, D-mannitol, D-mannose, raffinose, D-rhamnose, ribitol, D-sorbitol, L-sorbos, sucrose, xylitol, lactate, malate, pyruvate, succinate, methanol (5 mM), ethanol (5 mM), arabinogalactan (0.5 %, w/v), Casamino acids (0.5 %, w/v), glycogen (0.5 %, w/v), inulin (0.5 %, w/v), starch (0.5 %, w/v) and H₂/CO₂ (80:20, v/v). In addition, strain MO-SPC2T did not reduce the following substances in maltose-yeast extract medium: sulfate (1 mM), thiosulfate (1 mM), elemental sulfur (0.1 %, w/v), nitrite (1 mM), nitrate (1 mM), fumarate (5 mM) and ferric nitrolriacetate (1 mM).

Strain MO-SPC2T grew between 0 and 17 °C and the optimum growth temperature was 9 °C. The pH range for growth of the strain was pH 6.0–8.0, with optimum growth at pH 6.8–7.2. The optimum NaCl concentration for growth was 20–30 g NaCl l⁻¹. Growth occurred at 40 g NaCl l⁻¹, but not at concentrations exceeding 50 g NaCl l⁻¹. No growth occurred in the absence of NaCl. Based on the growth curves with OD₆₀₀, the doubling time in maltose-yeast extract medium was approximately 1.8 h.

**Fig. 1.** Micrographs of strain MO-SPC2T grown in maltose-yeast extract medium and a cell life cycle of the strain inferred from microscopic observations. (a–i) Transmission electron micrographs of negatively stained cells. (j–l) Thin-section images of an annular cell. The arrowheads in (k) and (l) indicate the inner and outer membranes. Bars, 0.1 μm (k, l), 0.5 μm (a–d, f, i, j), 1 μm (e) and 2 μm (g, h). (m) Life cycle of strain MO-SPC2T inferred from morphological change of cells by microscopic observation.
extract medium (9 °C, pH 6.8 and 25 g NaCl l⁻¹) was estimated to be about 12 h.

The polar lipid analysis showed that the isolate had phosphatidylglycolipids, phospholipids and glycolipids (Fig. S3). The major cellular fatty acids were C₁₄:₀, C₁₆:₀ and C₁₆:₁ω₉ (Tables 1 and S1). Isoprenoid quinones were not detected. The G+C content of the total DNA was 32.3 mol%. A nearly full-length 16S rRNA gene sequence of strain MO-SPC²₅ was determined (1466 bp). Comparative 16S rRNA gene sequence analysis showed that strain MO-SPC²₅ was affiliated with the genus Sphaerochaeta, but the strain was distinct from the recognized species of the genus (Figs 2 and S1). The closest cultured relative of strain MO-SPC²₅ was strain ACE-P (sequence identity 98.8 %), isolated from anoxic bottom water of Ace Lake, Antarctica (Franzmann & Dobson, 1992). The most closely related members of species with validly published names were Sphaerochaeta pleomorpha GrapesT (88.4 %), Sphaerochaeta globosa BuddyT (86.7 %) and Sphaerochaeta coccoides SPN1T (85.4 %).

Based on its morphological, physiological and phylogenetic traits, strain MO-SPC²₅ is considered to represent a novel species within the genus Sphaerochaeta. The new isolate MO-SPC²₅ has several phenotypic traits in common with members of the genus Sphaerochaeta, e.g. anaerobic growth, chemo-organotrophic metabolism with sugars and polysaccharides and fermentative products, the major fatty acid composition and growth pH range (Table 1). However, there are distinctive phenotypic differences between strain MO-SPC²₅ and other species of the genus Sphaerochaeta: (i) strain MO-SPC²₅ exhibits a more pronounced pleomorphic growth pattern than other members

### Table 1. Differential phenotypic characteristics of strains MO-SPC²₅ and ACE-P and type strains of species of the genus Sphaerochaeta

<table>
<thead>
<tr>
<th>Characteristic</th>
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<th>3</th>
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<th>5</th>
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<tr>
<td>Cell morphology</td>
<td>Pleomorphic (coccus, annular, curved rod, helical)</td>
<td>Pleomorphic (coccus, annular, filamentous)*</td>
<td>Pleomorphic*</td>
<td>Coccus</td>
<td>Coccus</td>
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<tr>
<td>Motile</td>
<td>+†</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Optimum temperature (range) (°C)</td>
<td>9 (0–17)</td>
<td>12–13 (&lt;19)‡</td>
<td>20–25 (15–30)</td>
<td>30 (20–37)</td>
<td>30 (15–40)</td>
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<tr>
<td>Optimum pH (range)</td>
<td>6.8–7.2 (6.0–8.0)</td>
<td>7.4</td>
<td>6.5–7.5</td>
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<td>++</td>
<td>+</td>
<td>–</td>
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<td>DNA G+C content (mol% or %)</td>
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<td>32.3a</td>
<td>29.3 ± 0.4a</td>
<td>46.2b</td>
<td>48.9b</td>
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<tr>
<td>Substrate utilization</td>
<td>Cellobiose</td>
<td>+</td>
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<td></td>
<td>D-Galactose</td>
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<td></td>
<td>Glucose</td>
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<td>Sucrose</td>
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<td>Starch</td>
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<td>+</td>
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<td></td>
<td>Growth products from oligosaccharides‡</td>
<td>Ac, EtOH, H₂, CO₂</td>
<td>Ac, EtOH, Su, La, Py, H₂, CO₂</td>
<td>Ac, Fo, EtOH</td>
<td>Ac, Fo, EtOH</td>
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<tr>
<td>Isolation source</td>
<td>Marine subsurface sediment</td>
<td>Anoxic lake bottom water</td>
<td>Anoxic river sediment</td>
<td>Anoxic river sediment</td>
<td>Lower termite hindgut</td>
</tr>
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</table>

*These strains did not display helical morphology. For *Sphaerochaeta pleomorpha* Grapes⁷, the cells were basically cocci, but they formed different sizes and shapes.

†Motility is observed only in helical cells.

‡Growth was observed at 1.7 °C, which was the temperature of the isolation source in situ.

§Determined in this study.

।a, Determined by HPLC (mol%); b, obtained from genome information (%).

¶For *Sphaerochaeta coccoides* SPN1T, end products were measured from maltose. For the other strains, they were detected in glucose medium. Ac, Acetate; EtOH, ethanol; Fo, formate; La, lactate; Py, pyruvate; Su, succinate.
of *Sphaerochaeta*; (ii) all species with validly published names are mesophiles, whereas strain MO-SPC2T is an obligate psychrophile; (iii) all species of *Sphaerochaeta* are non-marine microorganisms, whereas strain MO-SPC2T absolutely requires NaCl for growth and is a typical marine organism; and (iv) the DNA G+C content of strain MO-SPC2T is significantly lower than the values reported for other members of the genus *Sphaerochaeta*. In addition, the motility of helical cells distinguishes strain MO-SPC2T from known members of the genus *Sphaerochaeta*, which have no motility (Video S1). Preparative studies have demonstrated clearly that periplasmic flagella are essential for motility of spirochaetes (Charon et al., 2012; Charon & Goldstein, 2002). Nevertheless, no periplasmic flagella-like structures were observed in the new isolate (Fig. 1g). The rotation of strain MO-SPC2T is slower and irregular compared with the helical motility observed in other spirochaetes (e.g. supplementary video 3 in Charon et al., 2012). So far, the absence of periplasmic flagella and motility has been recognized as a significant property distinguishing the genus *Sphaerochaeta* from other spirochaete genera. Hence, strain MO-SPC2T may represent an evolutionary transitional state between the genera *Spirochaeta* and *Sphaerochaeta*. It is known that helical morphology and motility are key features of pathogenic spirochaetes (Charon et al., 2012; Caro-Quintero et al., 2012). Therefore, our new isolate may provide new insights into understanding the relationship between morphological change, motility and pathogenicity of spirochaetes and implications for the clinical epidemiology and treatment of such spirochaete-caused diseases.

As shown in Table 1, strains MO-SPC2T and ACE-P have many common phenotypic characteristics. These strains also share morphological features except for the helical cell stage, which was not reported for strain ACE-P (Franzmann & Dobson, 1992). The physiological and phylogenetic data demonstrate that strains ACE-P and MO-SPC2T share similarities; however, more detailed comparative analyses, including DNA–DNA hybridization and genome comparisons, are needed to determine whether the two isolates belong to the same species.

On the basis of these phenotypic and genotypic properties, we propose strain MO-SPC2T as the type strain of the novel species *Sphaerochaeta multiformis* sp. nov. within the genus *Sphaerochaeta*. Additionally, an emended description of the genus *Sphaerochaeta* is proposed, because the morphology, motility, obligately psychrophilic lifestyle and lower DNA G+C content of strain MO-SPC2T are new characteristics among members of the genus.


The description of the genus is as given by Ritalahti et al. (2012) and subsequently amended by Abt et al. (2012), with the following further modifications. Helical morphology is observed in strain MO-SPC2T, whereas strain MO-SPC2 T is a typical mesophile. Therefore, our new isolate may provide new insights into understanding the relationship between morphological change, motility and pathogenicity of spirochaetes...
and mesophilic species exist. DNA G+C content is 32–51 mol%.

**Description of Sphaerochaeta multiformis sp. nov.**


Strictly anaerobic and psychrophilic organism. Cells are pleomorphic. Motility occurs in helical cells. Gram-negative cell-wall structure. The optimal temperature for growth is 9 °C. Growth occurs between 0 and 17 °C, but not above 19 °C. NaCl is required for growth, but no growth occurs at >50 g NaCl l⁻¹. Optimum growth occurs in the presence of 20–30 g NaCl l⁻¹. The optimum pH for growth is pH 6.8–7.2. Growth occurs at pH 6.0 and 8.0, but not below pH 5.5 or above pH 8.5. Cells are catalase-negative and weakly positive for cytochrome oxidase. The following substrates are utilized: cellulose, glucose, maltose, ribose, trehalose, xyllose, pectin, skimmed milk, xylan and yeast extract. The following substrates are not utilized: D- and L-arabinose, D-fructose, D-galactose, glycerol, myo-inositol, lactose, D-mannitol, D-mannose, raffinose, D-xylohexose, ribitol, D-sorbitol, L-sorbose, sucrose, xylitol, lactate, malate, pyruvate, succinate, methanol, ethanol, arabino-galactan, Casamino acids, glycogen, inulin and starch. Isopropenyl quinones are absent. The dominant cellular fatty acids are C₁₄:₀, C₁₆:₀ and C₁₆:₁ω₉c. The polar lipids are phosphatidylglycolipids, phospholipids and glycolipids.

The type strain is strain MO-SPC2ᵀ (=JCM 17281ᵀ=DSM 23952ᵀ), which was isolated from marine subsurface sediments collected off the Shimokita Peninsula of Japan, in the north-western Pacific Ocean (site C9001, 41° 10.6389’ N 142° 12.081’ E, water depth 1180 m). The G+C content of the genomic DNA of the type strain is 32.3 mol% (determined using HPLC).

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