



**HAL**  
open science

**Pleomorphochaeta caudata gen. nov., sp. nov., an anaerobic bacterium isolated from an offshore oil well, reclassification of Sphaerochaeta multiformis MO-SPC2T as Pleomorphochaeta multiformis MO-SPC2T comb. nov. as the type strain of this novel genus and emended description of the genus Sphaerochaeta**

Boussad Arroua, Anthony Ranchou-Peyruse, Magali Ranchou-Peyruse, Michel Magot, Laurent Urios, Régis Grimaud

► **To cite this version:**

Boussad Arroua, Anthony Ranchou-Peyruse, Magali Ranchou-Peyruse, Michel Magot, Laurent Urios, et al.. Pleomorphochaeta caudata gen. nov., sp. nov., an anaerobic bacterium isolated from an offshore oil well, reclassification of Sphaerochaeta multiformis MO-SPC2T as Pleomorphochaeta multiformis MO-SPC2T comb. nov. as the type strain of this novel genus and emended description of the genus Sphaerochaeta. International Journal of Systematic and Evolutionary Microbiology, 2017, 67 (2), pp.417-424. 10.1099/ijsem.0.001641 . hal-01499133

**HAL Id: hal-01499133**

**<https://hal.science/hal-01499133>**

Submitted on 13 Mar 2024

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# *Pleomorphochaeta caudata* gen. nov., sp. nov., an anaerobic bacterium isolated from an offshore oil well, reclassification of *Sphaerochaeta multiformis* MO-SPC2<sup>T</sup> as *Pleomorphochaeta multiformis* MO-SPC2<sup>T</sup> comb. nov. as the type strain of this novel genus and emended description of the genus *Sphaerochaeta*

Boussad Arroua, Anthony Ranchou-Peyruse, Magali Ranchou-Peyruse, Michel Magot, Laurent Urios\* and Regis Grimaud

## Abstract

A strictly anaerobic Gram-stain-negative bacterium, designated strain SEBR 4223<sup>T</sup>, was isolated from the production water of an offshore Congolese oil field. Cells were non-motile, pleomorphic and had spherical, annular or budding shapes, often exhibiting long stalks. Strain SEBR 4223<sup>T</sup> grew on a range of carbohydrates, optimally at 37 °C and pH 7, in a medium containing 40 g l<sup>-1</sup> NaCl. Predominant fatty acids were C<sub>14</sub>:0, C<sub>14</sub>:0 DMA, C<sub>16</sub>:0 and C<sub>16</sub>:1!7c and the major polar lipids were phosphoglycolipids, phospholipids, glycolipids and diphosphatidylglycerol. The G+C content of the DNA was 28.7 mol%. Phylogenetic analysis, based on the 16S rRNA gene sequence, showed that strain SEBR 4223<sup>T</sup> and *Sphaerochaeta multiformis* MO-SPC2<sup>T</sup> formed a cluster with similarity to other species of the genus *Sphaerochaeta* of less than 86 %. On the basis of the phenotypic characteristics and taxonomic analyses, we propose a novel genus, *Pleomorphochaeta* gen. nov., to accommodate the novel species *Pleomorphochaeta caudata* sp. nov., with SEBR 4223<sup>T</sup> (=DSM 103077<sup>T</sup>=JCM 31 475<sup>T</sup>) as the type strain. We also propose the reclassification of *Sphaerochaeta multiformis* MO-SPC2<sup>T</sup> as *Pleomorphochaeta multiformis* MO-SPC2<sup>T</sup> comb. nov., the type strain of this novel genus and emend description of the genus *Sphaerochaeta*.

Cultivation and molecular studies of the microbial diversity of oil reservoirs has revealed that these environments were inhabited by active and diverse microorganisms. Microbial activity in oil reservoirs has attracted much attention due to it being of both fundamental scientific and applied interest [1–7]. Fermentative bacteria constitute an important microbial community of petroleum reservoirs [8]. They include members of the families *Thermoanaerobiaceae*, *Halanaerobiaceae*, *Thermotogaceae* and *Spirochaetaceae* [1, 9–15]. Although many 16S rRNA gene sequences related to Spirochaetaceae have been retrieved from oil reservoirs [7, 16–20], only one strain belonging to this family, *Sediminispirochaeta smaragdinae* SEBR4228<sup>T</sup>, has been isolated from such ecosystems [21]. Ritalahti and colleagues [22] proposed the novel genus, *Sphaerochaeta*, within the family *Spirochaetaceae*. Members of this genus are Gram-negative, anaerobic, chemoorganotrophic bacteria and

grow by fermenting carbohydrates. At the time of writing, the genus *Sphaerochaeta* contains five species with validly published names: *Sphaerochaeta pleomorpha*, *Sphaerochaeta coccoides*, *Sphaerochaeta multiformis*, *Sphaerochaeta globosa* and *Sphaerochaeta associata* [22–25]. Members of the genus *Sphaerochaeta* were originally characterized by the absence of periplasmic flagella and a lack of motility. These traits have been recognized as discriminating between *Sphaerochaeta* and other genera of the family *Spirochaetaceae* [22, 26, 27]. Consistently, the analysis of the genomes of *S. Buddy*<sup>T</sup> and *S. pleomorpha Grapes*<sup>T</sup> showed the absence of motility and associated signal transduction genes that were present in all sequenced genomes of *Spirochaeta* [26]. *S. globosa Buddy*<sup>T</sup> and *S. pleomorpha Grapes*<sup>T</sup> were globosa isolated from anaerobic dechlorinating consortia that were established from freshwater sediments [22], while *S. coccoides SPN1*<sup>T</sup> and *S. associata GLS2*<sup>T</sup> were isolated from the of the hindgut of the

---

Author affiliation: CNRS/ Université de Pau & des Pays de l'Adour, IPREM UMR 5254, 64000, Pau, France.

\*Correspondence: Laurent Urios, laurent.urias@univ-pau.fr

Keywords: *Pleomorphochaeta*; *Pleomorphochaeta caudata*; *Spirochaetaceae*; *Sphaerochaeta*.

Abbreviations: BICINE, 2-(bis(2-hydroxyethyl)amino) acetic acid; FAME, Fatty acid methyl ester; MES, 2-(N-morpholino) ethanesulfonic acid; MOPS, 3-(N-morpholino) propanesulfonic acid.

The Genbank/ EMBL/ DDBJ accession number for the 16S rRNA gene sequence of strain SEBR 4223<sup>T</sup> is KU714929.

termite *Neotermes castaneus* and from aggregates of the psychrotolerant archaeon *Methanosarcina mazei* JL01, respectively [25, 28]. *S. multiformis* MO-SPC2<sup>T</sup> was isolated from seafloor sediment samples collected from off the Shimokita Peninsula, Japan [24]. The more pronounced pleomorphic character of strain MO-SPC2<sup>T</sup> and the motility of its helical cells distinguished this strain from other members of the genus *Sphaerochaeta*. Here, we describe a novel anaerobic fermentative strain of a species of *Spirochaetaceae*, designated SEBR 4223<sup>T</sup>, isolated from water produced from the Emeraude offshore oil field in the Congo (well number BB325).

Strain SEBR 4223<sup>T</sup> was isolated from water samples collected at the well-head in sterile 250 ml glass bottles and stored at 4 C until inoculation, as described previously [29]. The temperature of the water produced was 35 C, the pH was 6.7 and the NaCl concentration was 32 g l<sup>-1</sup>. Enrichment and isolation were performed under anoxic conditions, as described by Magot and collaborators [21]. Enrichment cultures were initiated by inoculating 2 ml of a production water sample into serum bottles containing 18 ml of SEM medium and incubated without shaking at 37 C. Strain SEBR 4223<sup>T</sup> was purified by successive streaking on SEM agar plates (2.5 %, w/v; Noble agar, Difco) as described previously [13, 21]. After 1 week of incubation at 37 C on agar plates, strain SEBR 4223<sup>T</sup> produced round, convex, opaque and non-pigmented colonies with regular edges. The purity of the strain was confirmed from the morphological homogeneity of the colonies on agar plates and by sequencing the 16S rRNA gene of 10 colonies. After isolation of the strain, all cultivations were performed in basal medium containing (l<sup>-1</sup>): 0.5 g NH<sub>4</sub>Cl, 0.1 g KH<sub>2</sub>PO<sub>4</sub>, 4 g MgCl<sub>2</sub>·6H<sub>2</sub>O, 1 g CaCl<sub>2</sub>·2H<sub>2</sub>O, 40 g NaCl, 0.15 g cysteine hydrochloride, 1 ml trace mineral element solution [30], 0.5 g yeast extract and 1 ml resazurin solution (0.1 %, w/v). The medium was autoclaved at 121 C for 30 min and then cooled at room temperature under a stream of N<sub>2</sub>/CO<sub>2</sub> (80 : 20, v/v). Prior to inoculation, the medium was supplemented with the following sterilized solutions (l<sup>-1</sup>): 2 g NaHCO<sub>3</sub> (flushed with N<sub>2</sub>/CO<sub>2</sub>, 80 : 20, v/v), 0.15 g Na<sub>2</sub>S·9H<sub>2</sub>O (flushed with N<sub>2</sub>) and 2 ml filtered (0.22 µm) vitamin solution [31]. The pH was adjusted to 7.3 with an anoxic 5 M NaOH solution. Finally, the medium was dispensed under a stream of N<sub>2</sub>/CO<sub>2</sub> (80 : 20, v/v) into Hungate tubes or 50 ml glass serum bottles. Strain SEBR 4223<sup>T</sup> was routinely cultivated with 10 mM glucose as the carbon source.

Genomic DNA was purified using a PowerSoil DNA isolation kit (MoBio Laboratories). The 16S rRNA gene was amplified by PCR and subsequently sequenced by the Qiagen Sequencing Service (Hilden, Germany) using the universal primer pair, 8F (5'-AGAGTTTGATCCTGGCTCAG-3') [32] and 1387R (5'-GGGCGWGTGTACAAGGC-3') [33]. A 1400 bp fragment of the 16S rRNA gene was obtained and searched against the 16S rRNA gene sequence database, EzTaxon ([www.ezbiocloud.net](http://www.ezbiocloud.net); [34]). The retrieved sequences were

aligned with CLUSTAL W [35] and phylogenetic trees were reconstructed using the software package MEGA 6 [36]. Phylogenetic distances (corrected according to Kimura's two-parameter model; [37]) were calculated and clustering was performed with the neighbour joining method [38]. Bootstrap-resampling analysis with 1000 replicates [39] was performed for the neighbour joining as well as the maximum-likelihood and maximum parsimony methods to obtain confidence for tree topologies.

Bacterial growth was assessed by measuring the optical density at 600 nm using a spectrophotometer (spectronic 401, Spectronic Instruments). All tests for growth were performed in triplicate in the dark, with two successive transfers, and confirmed by microscopic observations. Growth at various temperatures, pH values and NaCl concentrations were performed in basal medium containing 10 mM glucose and 0.05 % (w/v) yeast extract. The temperature range for growth was determined between 10 and 60 C. For studies of pH, the following buffers were used at 30 mM: MES for pH 5.2 to 6.0, MOPS for pH 6.8 to 8.0 and Bicine for pH 8.5 to 9.0. The salt concentration range for growth was determined by varying the NaCl concentration from 0 to 100 g l<sup>-1</sup> (w/v) and maintaining the NaCl/MgCl<sub>2</sub> molar ratio at 25. Oxidase activity was determined by using an oxidase reagent kit (bioMerieux). Catalase activity was detected by formation of bubbles by cells resuspended in 9 % (v/v) H<sub>2</sub>O<sub>2</sub>. Substrate utilization tests were performed using exponentially growing pre-cultures as inoculum (10 %, v/v) in basal medium with 40 g l<sup>-1</sup> NaCl at 37 C. Substrates were added to the medium from sterile solutions at a final concentration of 10 mM. The utilization of sulfate (20 mM), thiosulfate (20 mM), nitrate (20 mM), nitrite (1 mM), ferric citrate (5 mM) and elemental sulfur (0.1 %, w/v) as terminal electron acceptors was tested in basal medium containing glucose, acetate, lactate and propionate as electron donors and carbon sources. Reduction of sulfur compounds was measured using the method of Cline [40] and nitrite was determined by following the Griess reaction. The ability to grow in oxic conditions was tested in basal medium with glucose without a reducing agent at 37 C, pH 7 and 40 g l<sup>-1</sup> of NaCl.

Transmission electron micrographs of negative staining and thin sections were performed with an HT 7700 Hitachi electron microscope at the Centre de Microscopie Electronique Appliquee a la Biologie (Toulouse). During the exponential growth and stationary phases, cells were collected and negative staining was performed with 2 % (w/v) aqueous uranyl solution.

For ultrathin sectioning, 2.5 % (w/v) glutaraldehyde, 0.1 M cacodylate and 0.04 % (w/v) ruthenium red were added to the medium to fix the cells overnight at 10 C. After harvesting by centrifugation, cells were embedded in 2 % (w/v) low melting point agarose. Pieces of agarose were washed in

0.2 M cacodylate, 0.04 % (w/v) ruthenium red solution and post-fixed with 1 % (v/v) osmium tetroxide for 1 h at room temperature. Agarose pieces were dehydrated in a series of ethanol solutions, impregnated with Embed 812 resin for 48 h and cut with an ultramicrotome. The ultrathin sections were observed at an acceleration voltage of 80 kV. For scanning electron microscopy, cells were prefixed overnight in 2 % (w/v) glutaraldehyde and 0.1 M cacodylate buffer at 4 C. After washing in water, cells were adhered to poly-lysine (0.5 %) coated glass slides for 1 h at room temperature, rinsed with distilled water, dehydrated in a series of ethanol solutions and critical-point dried in a Leica EM CPD 300. Cells were coated with platinum using a platinum metallizer (Leica EM Med 020) and observed with a MEB ESEM Quanta 250 FEG field emission scanning electron microscope operated at 5 kV.

Strain SEBR 4223<sup>T</sup> was cultured in basal medium supplemented with 10 mM glucose and 0.1 % (w/v) yeast extract. Cells were harvested during the exponential phase and sent to the Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany. The peptidoglycan was isolated according to Schumann [41] and analyzed by gas chromatography coupled with mass spectrometry, as described [42]. Polar lipids were extracted using the method of Bligh and Dyer [43], separated by two dimensional silica gel TLC and analyzed according to the

procedures described by Tindall et al. [44]. FAMEs were prepared from 40 mg cells, as described by Miller [45] and Kuykendall [46] and identified using the Sherlock Microbial Identification System (MIDI, Microbial ID, Newark, DE 19 711 USA). The G+C content of the DNA was determined by reversed-phase HPLC [47]. G+C values were calculated from the ratio of deoxyguanosine and thymidine, according to the method of Mesbah et al. [48].

In exponential phase cultures, cells of strain SEBR 4223<sup>T</sup> appeared pleomorphic. Morphologies such as cocci (0.4–1.1 µm in diameter, Fig. 1a), annular cells with a central hole (0.8–1.14 µm, Fig. 1d, f) and chains of cocci (Fig. S1, available in the online Supplementary Material) were observed less frequently. The dominant morphotypes were spherical cells (0.5–0.95 µm) with protrusions (Fig. 1b) or long stalks (Fig. 1c). The morphology of cells in stationary phase cultures was much less heterogeneous with a predominance of the spherical morphotype. Similar cell morphologies were observed with *S. multiformis* MO-SPC2<sup>T</sup> [24]. TEM of a thin section of strain SEBR 4223<sup>T</sup> revealed features of a Gram-stain-negative cell wall (Fig. 1f). TEM showed a smaller structure, a bud produced on a mother cell (Fig. 1e), suggesting that cells grew by budding [49]. Motility was not observed during any stage of growth and no periplasmic flagella-like structure was observed with electron microscopy. Diaminopimelic acid was not detected in the

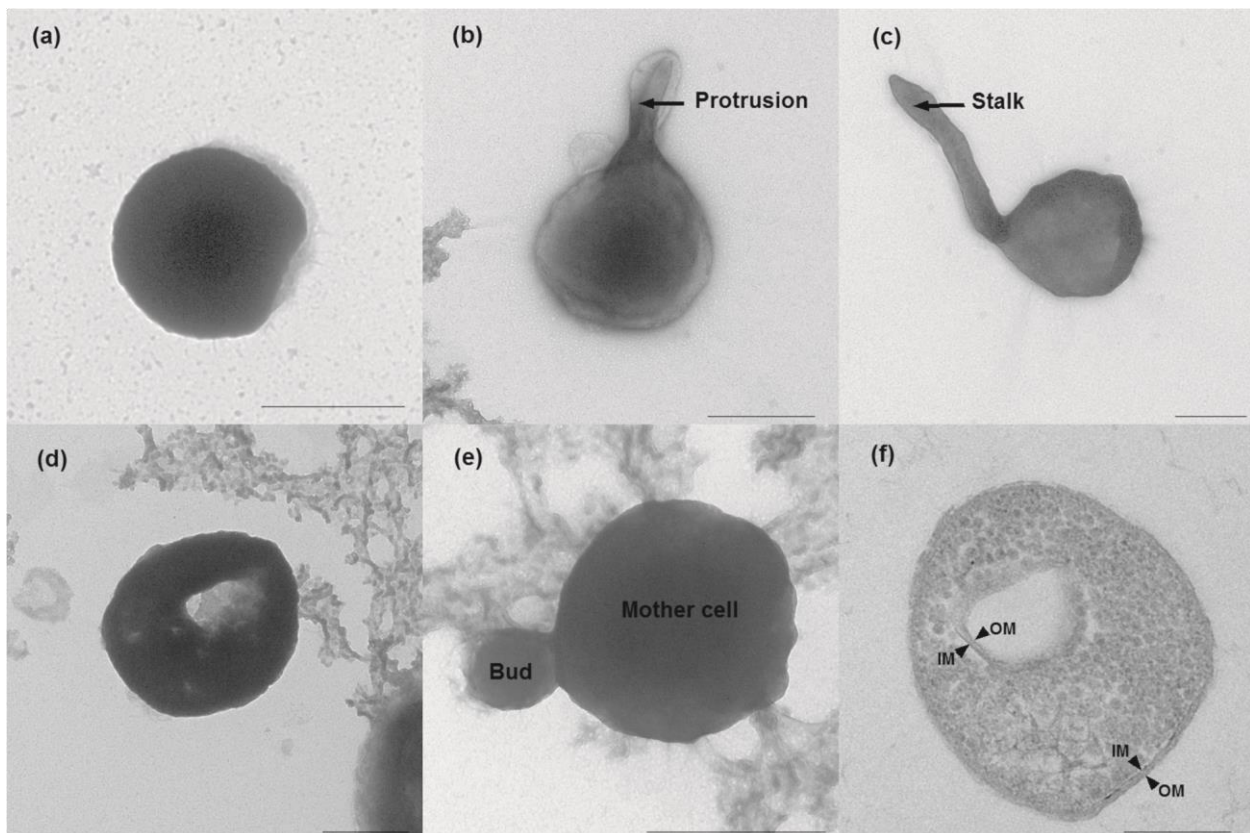


Fig. 1. Cells of strain SEBR 4223<sup>T</sup> visualized by TEM. a–e), negatively stained cells showing: (a) spherical shape, (b) a protrusion, (c) a long stalk, (d) an annular shape and (e) a bud. (f) Thin-section image of an annular cell. Arrowheads indicate the outer membrane (OM) and the inner membrane (IM). Bars, 0.5 µm.

cell wall of strain SEBR 4223<sup>T</sup>. Trace amounts of ornithine were detected.

Strain SEBR 4223<sup>T</sup> was oxidase- and catalase- negative, and was unable to grow under oxic conditions. Strain SEBR 4223<sup>T</sup> grew between 20 and 42 C and optimally at 37 C. The optimum pH for growth was 7 and growth occurred between pH 5.2 and 8.5. The NaCl range for growth was 10–60 g l<sup>-1</sup> (0.17–1.02 M), with optimum concentration being 40 g l<sup>-1</sup> (0.68 M). Strain SEBR 4223<sup>T</sup> was a chemoorganotroph with a fermentative metabolism, and used mono-, diand trisaccharides as sources of carbon and energy for growth. It grew on L-arabinose, D-fructose, D-galactose, D-glucose, D-mannose, D-sorbitol, D-xylose, D-ribose, cellobiose, lactose, maltose, raffinose, sucrose, trehalose, pyruvate, mannitol and xylitol. No growth occurred with D-arabinose, D-arabinogalactan, L-rhamnose, xylene, acetate, butyrate, propionate, lactate, malate, citrate, succinate, fumarate, ethanol, methanol, adonitol, myo-inositol, glycerol, glycogen, casamino acids, peptone, yeast extract and H<sub>2</sub>/CO<sub>2</sub> (80 : 20, v/v). Yeast extract was required for growth and could not be replaced by vitamins. The doubling time in the presence of glucose and yeast extract (37 C, pH 7 and 40

g l<sup>-1</sup> NaCl) was estimated to be about 6 h. Sulfate, thiosulfate, nitrate, nitrite, ferric citrate and elemental sulfur were not reduced in basal medium amended with glucose, acetate, lactate and propionate as electron donors and carbon sources. Growth of strain SEBR 4223<sup>T</sup> was completely inhibited by nitrite and ferric citrate.

Phylogenetic analysis, based on the 16S rRNA gene, showed that strain SEBR 4223<sup>T</sup> formed a stable cluster with *S. multiformis* MO-SPC2<sup>T</sup> (91.1 % similarity), which was supported by a 100 % bootstrap value (Fig. 2). Lower sequence similarities (<86 %) were found with the other type species of the genus *Sphaerochaeta*: *S. pleomorpha* Grapes<sup>T</sup> (85.3 %), *S. globosa* Buddy<sup>T</sup> (84.7 %), *S. coccoides* SPN1<sup>T</sup> (83.1 %) and *S. associata* GLS2<sup>T</sup> (86 %). The DNA G+C content of strain SEBR 4223<sup>T</sup> was 28.7 mol%. The major cellular fatty acids were C<sub>14:0</sub>, C<sub>14:0</sub>DMA, C<sub>16:0</sub> and C<sub>16:1</sub> ! 7c (Tables 1 and 2). Interestingly, C<sub>14:0</sub> accounted for about 42 and 64 % of the fatty acid contents in strains SEBR 4223<sup>T</sup> and MO-SPC2<sup>T</sup>, respectively, while it represented from 13 to 26 % in other members of the genus *Sphaerochaeta*. In addition, the C<sub>14:0</sub>/C<sub>16:0</sub> ratios were 3.4 and 4.8

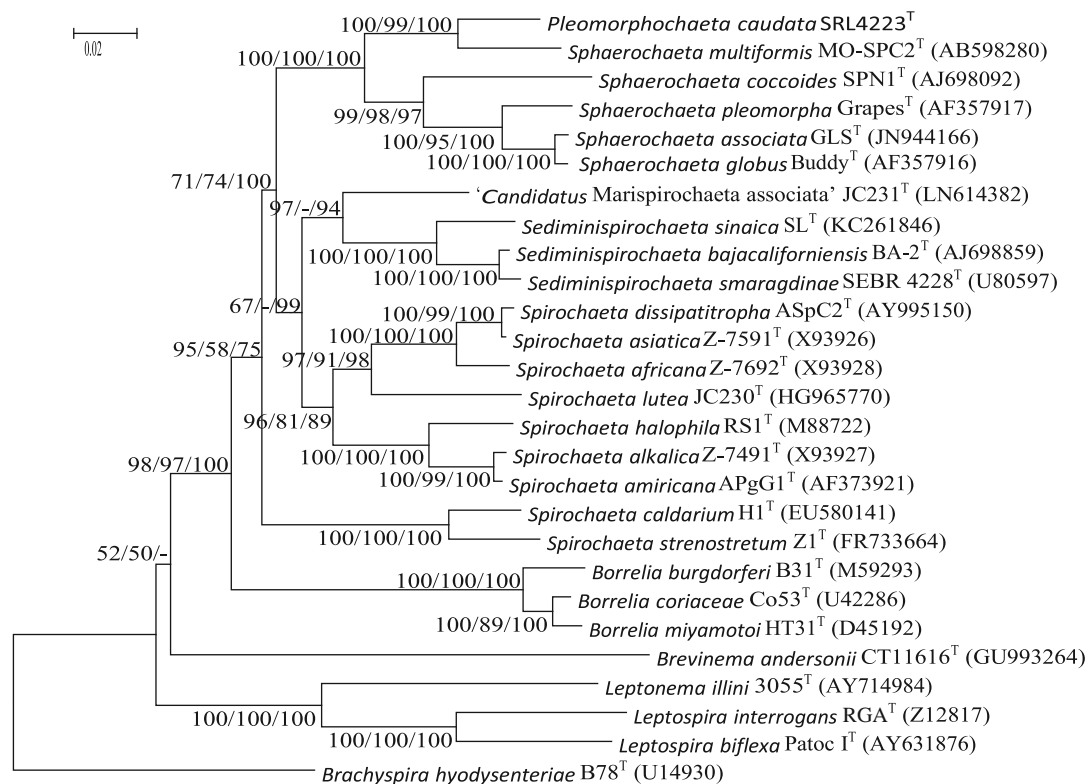


Fig. 2. Phylogenetic tree of Spirochaetaceae, based on a 16S rRNA gene sequence subset, showing the position of strain SEBR 4223<sup>T</sup>. The tree was based upon 1231 positions and reconstructed by the neighbour-joining method. Bootstrap values greater than 50 % are shown. They were obtained with the neighbour-joining/maximum-likelihood/maximum-parsimony methods based on 1000 replicates. Bar, 0.02 changes per nucleotide position.



Table 1. Differential phenotypic characteristics of strain SEBR 4223<sup>T</sup> and type strains of species of the genus *Sphaerochaeta*

Strains: 1, SEBR 4223<sup>T</sup> (present study); 2, *S. multiformis* MO-SPC2<sup>T</sup> (unless otherwise stated, data from [24]); 3, *S. pleomorpha* Grapes<sup>T</sup> [22]; 4, *S. globosa* Buddy<sup>T</sup> [22]; 5, *S. coccoides* SPN1<sup>T</sup> [28]; 6, *S. associata* GLS2<sup>T</sup> [25]. +, Positive; (+) weakly positive; –, negative; ND, no data available.

Characteristic	1	2	3	4	5	6
Cell morphology	Pleomorphic (spherical, annular, protrusion and stalk shapes)	Pleomorphic (coccus, annular, curved rod, helical)	Pleomorphic (spherical, elongated or crescent shapes)	Pleomorphic (spherical and hook-like protrusion)	Coccus	Pleomorphic (spherical, ovoid, annular)
Motility	–	+*	–	–	–	–
Temperature optimum (C)	37	9	20–25	30	30	30–34
Temperature range (C)	20–40	0–17	15–30	20–37	15–40	20–40
pH optimum	7.0	6.8–7.2	6.5–7.5	6.5–7.5	7.4	6.8–7.5
pH range	5.2–8.5	6.0–8.0	ND	ND	5.5–9.5	5.7–8.2
NaCl optimum (g l <sup>-1</sup> )	40	20–30	1	1	1	1–2
NaCl range (g l <sup>-1</sup> )	10–60	ND	ND	ND	ND	ND
Major fatty acids	C <sub>14:0</sub> , C <sub>16:0</sub> , C <sub>14:0</sub> DMA, C <sub>16:1</sub> !7c	C <sub>14:0</sub> , C <sub>16:0</sub> , C <sub>16:1</sub> !9c	C <sub>14:0</sub> , C <sub>16:0</sub> , C <sub>16:1</sub> !7c, br- C <sub>17:1</sub>	C <sub>14:0</sub> , C <sub>16:0</sub> , C <sub>16:1</sub> !7c, br-C <sub>17:1</sub>	C <sub>14:0</sub> , C <sub>16</sub> :0, iso- C <sub>16:0</sub> §	C <sub>14:0</sub> , C <sub>16:0</sub> , C <sub>16:1</sub>
DNA G+C content (mol%)	28.7†	32.3†	46.2‡	48.9‡	50.6‡	47.2†
Substrate utilization						
Cellobiose	+	+	–	–	+	+
Lactose	+	–	–	+	–	(+)
D-Galactose	+	–	+	+	–	+
Sucrose	+	–	+	+	–	(+)
Raffinose	+	–	+	+	–	+
D-Fructose	+	–	+	+	ND	–
Pyruvate	+	–	ND	ND	–	–
Isolation source	Oil field	Marine subsurface sediment	Anoxic river sediment	Anoxic river sediment	Lower termite hindgut	Aggregates of Methanosarcina mazei JL01

\*Motility observed in helical cells only.

†Determined by HPLC (mol%).

‡Obtained from genome information (%).

§Data from Miyazaki et al. [24].

in strains SEBR 4223<sup>T</sup> and MO-SPC2<sup>T</sup>, respectively, while in other members of the genus *Sphaerochaeta* this ratio was between 0.7 and 1.5 (Table 2). Polar lipid analysis showed that strain SEBR 4223<sup>T</sup> contained phosphoglycolipids, phospholipids and glycolipids as do other members of the genus *Sphaerochaeta*. The absence of phosphoglycolipid 5 (PGL5, Miyazaki and colleagues' nomenclature [24]) seemed to be specific to strains SEBR 4223<sup>T</sup> and MOSPC2<sup>T</sup>. Diphosphatidylglycerol was detected in strain SEBR 4223<sup>T</sup>, while this polar lipid was absent from other members of the genus *Sphaerochaeta*.

Genetic and phenotypic examinations (Table 1) revealed that the novel isolate, SEBR 4223<sup>T</sup>, had common traits with its closest relative, *S. multiformis* MO-SPC2<sup>T</sup>, which distinguished them from other members of *Sphaerochaeta*. (1) Phylogenetic analysis demonstrated that strain SEBR 4223<sup>T</sup> and its closest relative, strain MO-SPC2<sup>T</sup>, formed a

stable cluster with a similarity to other species of the genus *Sphaerochaeta* of less than 86 %. (2) The DNA G+C contents of strains SEBR 4223<sup>T</sup> (28.7 mol%) and MO-SPC2<sup>T</sup> (32.3 mol%) were much lower than those reported for other members of the genus *Sphaerochaeta* (46.2–50.6 mol%). (3) Cells of strains SEBR 4223<sup>T</sup> and MO-SPC2<sup>T</sup> exhibited a more pronounced pleomorphic growth pattern and shared morphological features (cocci, annular cells with a central hole and chains of cocci). (4) Strains SEBR 4223<sup>T</sup> and MOSPC2<sup>T</sup> grow optimally at 25 and 40 g l<sup>-1</sup> NaCl, respectively, while other type species of the genus *Sphaerochaeta* grow optimally at 1–2 g l<sup>-1</sup> NaCl. (5) C<sub>14:0</sub> is the most abundant fatty acid in strains SEBR 4223<sup>T</sup> and MO-SPC2<sup>T</sup> whereas in other members of *Sphaerochaeta* the proportion of C<sub>14:0</sub> is much lower and never the major fatty acid. (6) Phosphoglycolipid PGL5 was not detected in strains SEBR 4223<sup>T</sup> and MO-SPC2<sup>T</sup>, while it is present in other species of the genus *Sphaerochaeta*. (7) The peptidoglycan of strain

SEBR 4223<sup>T</sup> contains ornithine, while *S. pleomorpha* Grapes<sup>T</sup> and

Table 2. Fatty acid compositions, as percentages of the totals, of strain SEBR 4223<sup>T</sup> and type strains of species of the genus

*Sphaerochaeta*

Strains: 1, SEBR 4223<sup>T</sup> (present study); 2, *S. multiformis* MO-SPC2<sup>T</sup> [24]; 3, *S. pleomorpha* Grapes<sup>T</sup> [22]; 4, *S. globosa* Buddy<sup>T</sup> [22]; 5, *S. coccoides* SPN1<sup>T</sup> [24]; 6, *S. associata* GLS2<sup>T</sup> [25]. Fatty acids amounting to <1 % of the total fatty acids are not shown.

	1	2	3	4	5	6
Straight chain						
C <sub>14:0</sub>	42.3	64.7	26.4	13.4	21.7	10.5
C <sub>15:0</sub>	–	–	–	–	1.7	–
C <sub>16:0</sub>	8.8	19.1	16.9	15.2	26	14.3
C <sub>18:0</sub>	–	–	–	2.3	2.0	–
Branched chain						
C <sub>14:0</sub> DMA	19.8	–	–	–	–	2.7
iso C <sub>14:0</sub>	–	–	–	–	7.3	–
C <sub>16:0</sub> DMA	2.2	–	–	–	–	20.1
C <sub>16:0</sub> ALDE	–	–	–	–	–	2.1
anteiso C <sub>15:0</sub>	–	–	–	–	7.1	–
iso C <sub>16:0</sub>	–	–	–	–	31.1	–
iso C <sub>18:0</sub>	–	–	–	–	1.1	–
Unsaturated straight chain						
C <sub>13:1</sub>	4.5	–	–	–	–	–
C <sub>14:1</sub>	–	–	3.1	–	–	–
C <sub>14:1</sub> !7c	–	1.2	–	–	–	–
C <sub>16:1</sub>	–	–	–	–	–	24.4
C <sub>16:1</sub> !2c	–	–	2.8	–	–	–
C <sub>16:1</sub> !7c	9.8	3.4	33.7	13.1	–	–
C <sub>16:1</sub> !9c	3.9	11.3	5.1	5.1	1.0	–
C <sub>18:1</sub>	–	–	–	–	–	2.9
C <sub>18:1</sub> !7c	–	–	–	10.7	–	–
C <sub>18:1</sub> !9c	–	–	1.1	8.4	–	–
Hydroxy C <sub>14:</sub>						
o3OH	3.4	–	–	–	–	2.0
C <sub>16:0</sub> 3OH	2.2	–	–	–	–	16.9
Branched monoenoic acids						
br-15:1 B	–	–	2.4	3.7	–	–
iso-17:1!7c	–	–	5.1	–	–	–
br-17:1 A	–	–	33.7	15.8	–	–
br-17:1 B	–	–	–	1.2	–	–
br-17:1 C	–	–	–	1.0	–	–
br-17:1 D	–	–	1.1	1.0	–	–
br-19:1 C	–	–	–	2.1	–	–
br-19:1 D	–	–	–	3.0	–	–

*S. globosa* Buddy<sup>T</sup> do not have the ornithine peptidoglycan cross-link.

Furthermore, some characteristics differentiate strain SEBR 4223<sup>T</sup> from *S. multiformis* MO-SPC2<sup>T</sup> (Table 1). (1) The 16S rRNA gene sequence divergence between strains SEBR 4223<sup>T</sup> and MO-SPC2<sup>T</sup> is greater than 3 % (91.1 % similarity) (2) Motility is observed for the helical cells of strain MO-SPC2<sup>T</sup> but not in strain SEBR 4223<sup>T</sup> (3) Strain SEBR 4223<sup>T</sup> is a mesophilic bacterium, whereas strain MO-SPC2<sup>T</sup> is an obligate psychrophile (4) Strain SEBR 4223<sup>T</sup> is oxidase-negative, whereas MO-SPC2<sup>T</sup> is weakly oxidase-positive (5) These strains also differ in the range of substrates used, in the composition of fatty acids and polar lipids (Tables 1 and 2).

Consequently, on the basis of these phenotypic and genotypic properties, we propose that the novel isolate, SEBR 4223<sup>T</sup>, belongs to a novel species of a novel genus, *Pleomorphochaeta caudata* gen. nov., sp. nov. Additionally, the phylogenetic data and the common phenotypic characteristics (Table 1) of strains SEBR 4223<sup>T</sup> and MO-SPC2<sup>T</sup> clearly support the reclassification of *S. multiformis* MO-SPC2<sup>T</sup> as *Pleomorphochaeta multiformis* comb. nov. and its designation as the type strain of this novel genus.

## EMENDED DESCRIPTION OF THE GENUS SPHAEROCHAETA RITALAHTI ET AL.

(2012), EMEND. ABT ET AL. (2012) AND EMEND. MIYAZAKI ET AL. (2014)

The reclassification of *Sphaerochaeta multiformis* MOSPC2<sup>T</sup> as *Pleomorphochaeta multiformis* MO-SPC2<sup>T</sup> comb. nov. involves the removal of the emended description from Miyazaki et al. [24]. Thus the description of the genus is as given by Ritalahti et al. [22] and subsequently amended by Abt et al. [23].

## DESCRIPTION OF PLEOMORPHOCHAETA GEN. NOV.

*Pleomorphochaeta* [ple.o.mor.pho.chae.ta. Gr. adv. pleon more; Gr. n. morphe shape, form; Gr. fem. n. chait^e (Latin transliteration chaeta), long flowing hair; Pleomorphochaeta cells with multiple morphologies with a derivation from spirochaetal ancestry].

Cells are pleomorphic. Spherical, annular and coccoid cell morphologies are observed. Cells have a Gram-stainnegative cell wall. Chemoheterotrophic anaerobes and fermentative growth is observed. The DNA G+C content is 28.7–32.3 %. C<sub>14:0</sub> is the major fatty acid. The phylogenetic position is in the family *Spirochaetaceae*, order *Spirochaetales*, class *Spirochaetia* of the phylum

*Spirochaetes. Pleomorphochatea multiformis* MO-SPC2<sup>T</sup> is the type species.

## DESCRIPTION OF PLEOMORPHOCHATEA MULTIFORMIS COMB. NOV.

Basonym: *Sphaerochaeta multiformis* MO-SPC2<sup>T</sup> [24].

Description is as that given by Miyazaki et al. [24].

The type strain is strain MO-SPC2<sup>T</sup> (=JCM 17 281<sup>T</sup>=DSM 23 952<sup>T</sup>), which was isolated from marine subsurface sediments collected off the Shimokita Peninsula in Japan, in the north-western Pacific Ocean.

## DESCRIPTION OF PLEOMORPHOCHAETA CAUDATA SP. NOV.

*Pleomorphochaeta caudata* (cau.da†ta. N. L. fem. adj. caudata having a tail).

Cells are pleomorphic, non-motile, without periplasmic flagella and Gram-stain-negative. Strictly anaerobic, catalase- and oxidase-negative, chemoorganotrophic and fermentative. Cells have ornithine but not diaminopimelic acid peptidoglycan cross-links. The optimum temperature for growth is 37 °C with growth occurring between 20 and 42 °C. The optimum NaCl concentration for growth is 40 g l<sup>-1</sup> and the NaCl concentration range for growth is 10–60 g l<sup>-1</sup>. The optimum pH for growth is 7 and growth occurs between pH 5.2 and 8.5. The following substrates are utilized: L-arabinose, D-fructose, D-galactose, D-glucose, D-mannose, D-sorbitol, D-xylose, D-ribose, cellobiose, lactose, maltose, raffinose, sucrose, trehalose, pyruvate, mannitol and xylitol. No growth occurs with D-arabinose, D-arabinogalactan, L-rhamnose, xylene, acetate, butyrate, propionate, lactate, malate, citrate, succinate, fumarate, ethanol, methanol, adonitol, myo-inositol, glycerol, glycogen, casamino acids, peptone, yeast extract and H<sub>2</sub>/CO<sub>2</sub> (80 : 20, v/v). The dominant cellular fatty acids are C<sub>14</sub>:0, C<sub>14</sub>:0 DMA, C<sub>16</sub>:0 and C<sub>16</sub>:1 !7c. The polar lipids are phosphoglycolipids, phospholipids, glycolipids and diphosphatidylglycerol.

The type strain, SEBR 4223<sup>T</sup> (=DSM 103077<sup>T</sup>=JCM 31 475<sup>T</sup>), was isolated from a production water sample of well number BB325 of the Emeraude off shore oil field in Congo. The G+C content of the genomic DNA is 28.7 mol%.

### Funding information

Total S. A. is acknowledged for its financial support. B. A. was beneficiary of a fellowship from the Region Aquitaine (project APE02358).

### References

- Magot M, Ollivier B, Patel BK. Microbiology of petroleum reservoirs. *Antonie van Leeuwenhoek* 2000;77:103–116.
- Head IM, Jones DM, Larter SR. Biological activity in the deep subsurface and the origin of heavy oil. *Nature* 2003;426:344–352.
- Huang H, Bowler BFJ, Zhang Z, Oldenburg TBP, Larter SR. Influence of biodegradation on carbazole and benzocarbazole distributions in oil columns from the Liaohe basin, NE China. *Org Geochem* 2003;34:951–969.
- Hallmann C, Schwark L, Grice K. Community dynamics of anaerobic bacteria in deep petroleum reservoirs. *Nat Geosci* 2008;1:588–591.
- Youssef N, Elshahed MS, McInerney MJ. Microbial processes in oil fields: culprits, problems, and opportunities. *Adv Appl Microbiol* 2009;66:141–251.
- Gray ND, Sherry A, Hubert C, Dolfig J, Head IM. Methanogenic degradation of petroleum hydrocarbons in subsurface environments remediation, heavy oil formation, and energy recovery. *Adv Appl Microbiol* 2010;72:137–161.
- Nazina TN, Shestakova NM, Pavlova NK, Tatarkin YV, Ivoilov VS et al. Functional and phylogenetic microbial diversity in formation waters of a low-temperature carbonate petroleum reservoir. *Int Biodeterior Biodegradation* 2013;81:71–81.
- Ollivier B, Cayol JL. The fermentative, iron-reducing, and nitrate-reducing microorganisms. In: Ollivier B and Magot M (editors). *Petroleum Microbiology*. Washington, DC: American Society for Microbiology; 2005. pp. 71–88.
- Davey ME, Wood WA, Key R, Nakamura K, Stahl DA. Isolation of three species of *Geotoga* and *Petrogoga*: Two new genera, representing a new lineage in the bacterial line of descent distantly related to the “*Thermotogales*”. *Syst Appl Microbiol* 1993;16:191–200.
- Miranda-Tello E, Fardeau ML, Thomas P, Ramirez F, Casalot L et al. *Petrogoga mexicana* sp. nov., a novel thermophilic, anaerobic and xylanolytic bacterium isolated from an oil-producing well in the Gulf of Mexico. *Int J Syst Evol Microbiol* 2004;54:169–174.
- Cayol JL, Ollivier B, Patel BK, Ravot G, Magot M et al. Description of *Thermoanaerobacter brockii* subsp. *lactiethylicus* subsp. nov., isolated from a deep subsurface French oil well, a proposal to reclassify *Thermoanaerobacter finnii* as *Thermoanaerobacter brockii* subsp. *finnii* comb. nov., and an emended description of *Thermoanaerobacter brockii*. *Int J Syst Bacteriol* 1995;45:783–789.
- Ravot G, Magot M, Fardeau ML, Patel BK, Prensier G et al. *Thermotoga elfii* sp. nov., a novel thermophilic bacterium from an African oil-producing well. *Int J Syst Bacteriol* 1995;45:308–314.
- Ravot G, Magot M, Ollivier B, Patel BK, Ageron E et al. *Haloanaerobium congolense* sp. nov., an anaerobic, moderately halophilic, thiosulfate- and sulfur-reducing bacterium from an African oil field. *FEMS Microbiol Lett* 1997;147:81–88.
- Fardeau ML, Ollivier B, Patel BK, Magot M, Thomas P et al. *Thermotoga hypogea* sp. nov., a xylanolytic, thermophilic bacterium from an oil-producing well. *Int J Syst Bacteriol* 1997;47:1013–1019.
- Fardeau ML, Magot M, Patel BK, Thomas P, Garcia JL et al. *Thermoanaerobacter subterraneus* sp. nov., a novel thermophile isolated from oilfield water. *Int J Syst Evol Microbiol* 2000;50: 2141–2149.
- Grabowski A, Nercessian O, Fayolle F, Blanchet D, Jeanthon C. Microbial diversity in production waters of a low-temperature biodegraded oil reservoir. *FEMS Microbiol Ecol* 2005;54:427–443.
- Tang YQ, Li Y, Zhao JY, Chi CQ, Huang LX et al. Microbial communities in long-term, water-flooded petroleum reservoirs with different in situ temperatures in the Huabei oilfield, China. *PLoS One* 2012;7:e33535.
- Kobayashi H, Endo K, Sakata S, Mayumi D, Kawaguchi H et al. Phylogenetic diversity of microbial communities associated with the crude-oil, large-insoluble-particle and formation-water components of the reservoir fluid from a non-flooded high-temperature petroleum reservoir. *J Biosci Bioeng* 2012;113:204–210.
- Gao P, Tian H, Li G, Sun H, Ma T. Microbial diversity and abundance in the Xinjiang Luliang long-term water-flooding petroleum reservoir. *Microbiologyopen* 2015;4:332–342.



20. You J, Wu G, Ren F, Chang Q, Yu B et al. Microbial community dynamics in Baolige oilfield during MEOR treatment, revealed by Illumina MiSeq sequencing. *Appl Microbiol Biotechnol* 2016;100: 1469–1478.
21. Magot M, Fardeau ML, Arnauld O, Lanau C, Ollivier B et al. *Spirochaeta smaragdinae* sp. nov., a new mesophilic strictly anaerobic spirochete from an oil field. *FEMS Microbiol Lett* 1997;155:185–191.
22. Ritalahti KM, Justicia-Leon SD, Cusick KD, Ramos-Hernandez N, Rubin M et al. *Sphaerochaeta globosa* gen. nov., sp. nov. and *Sphaerochaeta pleomorpha* sp. nov., free-living, spherical spirochaetes. *Int J Syst Evol Microbiol* 2012;62:210–216.
23. Abt B, Han C, Scheuner C, Lu M, Lapidus A et al. Complete genome sequence of the termite hindgut bacterium *Spirochaeta coccoides* type strain (SPN1<sup>T</sup>), reclassification in the genus *Sphaerochaeta* as *Sphaerochaeta coccoides* comb. nov. and emendations of the family Spirochaetaceae and the genus *Sphaerochaeta*. *Stand Genomic Sci* 2012;6:194–209.
24. Miyazaki M, Sakai S, Ritalahti KM, Saito Y, Yamanaka Y et al. *Sphaerochaeta multiformis* sp. nov., an anaerobic, psychrophilic bacterium isolated from subseafloor sediment, and emended description of the genus *Sphaerochaeta*. *Int J Syst Evol Microbiol* 2014;64:4147–4154.
25. Troshina O, Oshurkova V, Suzina N, Machulin A, Ariskina E et al. *Sphaerochaeta associata* sp. nov., a spherical spirochaete isolated from cultures of *Methanosarcina mazei* JL01. *Int J Syst Evol Microbiol* 2015;65:4315–4322.
26. Caro-Quintero A, Ritalahti KM, Cusick KD, Löffler FE, Konstantinidis KT. The chimeric genome of *Sphaerochaeta*: nonspiral spirochetes that break with the prevalent dogma in spirochete biology. *MBio* 2012;3:e00025-12.
27. Charon NW, Cockburn A, Li C, Liu J, Miller KA et al. The unique paradigm of spirochete motility and chemotaxis. *Annu Rev Microbiol* 2012;66:349–370.
28. Dröge S, Fröhlich J, Radek R, König H. *Spirochaeta coccoides* sp. nov., a novel coccoid spirochete from the hindgut of the termite *Neotermes castaneus*. *Appl Environ Microbiol* 2006;72:392–397.
29. Bernard FP, Connan J, Magot M. Indigenous microorganisms in connate water of many oil fields: a new tool in exploration and production techniques. In: Proceedings of the 67th Annual Technical Conference and Exhibition of the Society of Petroleum Engineers, Paper SPE 24811. Richardson, TX: SPE; 1992. pp. 1–10.
30. Balch WE, Fox GE, Magrum LJ, Woese CR, Wolfe RS. Methanogens: reevaluation of a unique biological group. *Microbiol Rev* 1979;43:260–296.
31. Pfennig N, Widdel F, Truper HG. The dissimilatory sulfate-reducing bacteria. In: Starr MP, Stolp H, Truper HG, Balows A and Schlegel HG (editors). *The Prokaryotes*, vol. 1. Berlin: Springer; 1981. pp. 926–940.
32. Lane DJ. 16S/23S rRNA sequencing. In: Stackebrandt E and Goodfellow M (editors). *Nucleic Acid Techniques in Bacterial Systematics*. Chichester: Wiley; 1991. pp. 115–175.
33. Marchesi JR, Sato T, Weightman AJ, Martin TA, Fry JC et al. Design and evaluation of useful bacterium-specific PCR primers that amplify genes coding for bacterial 16S rRNA. *Appl Environ Microbiol* 1998;64:795–799.
34. Kim OS, Cho YJ, Lee K, Yoon SH, Kim M et al. Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int J Syst Evol Microbiol* 2012;62:716–721.
35. Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* 1994;22:4673–4680.
36. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* 2013;30:2725–2729.
37. Kimura M. *The Neutral Theory of Molecular Evolution*. Cambridge: Cambridge University Press; 1983.
38. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 1987;4:406–425.
39. Felsenstein J. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 1985;39:783–791.
40. Cline JD. Spectrophotometric determination of hydrogen sulfide in natural waters. *Limnol Oceanogr* 1969;14:454–458.
41. Schumann P. Peptidoglycan structure. *Method Microbiol* 2011;38: 101–129.
42. Jeske O, Schüler M, Schumann P, Schneider A, Boedeker C et al. Planctomycetes do possess a peptidoglycan cell wall. *Nat Commun* 2015;6:7116.
43. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* 1959;37:911–917.
44. Tindall BJ, Sikorski J, Smibert RM, Kreig NR. Phenotypic characterization and the principles of comparative systematics. In: Reddy CA, Beveridge TJ, Breznak JA, Marzluf G, Schmidt TM et al. (editors). *Methods for General and Molecular Microbiology*, 3rd ed. Washington, DC: ASM Press; 2007. pp. 330–393.
45. Miller LT. A single derivatization method for bacterial fatty acid methyl esters including hydroxy acids. *J Clin Microbiol* 1982;16: 584–586.
46. Kuykendall LD, Roy MA, O'Neill JJ, Devine TE. Fatty acids, antibiotic resistance, and deoxyribonucleic acid homology groups of *Bradyrhizobium japonicum*. *Int J Syst Bacteriol* 1988; 38:358–361.
47. Tamaoka J, Komagata K. Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* 1984;25:125–128.
48. Mesbah M, Premachandran U, Whitman WB. Precise measurement of the G+C content of deoxyribonucleic acid by highperformance liquid chromatography. *Int J Syst Bacteriol* 1989;39: 159–167.
49. Hirsch P. Budding bacteria. *Annu Rev Microbiol* 1974;28:391–440.