




# *Aestuariusphingobium litorale* gen. nov., sp. nov., a novel proteobacterium isolated from a water sample of Pearl River estuary

Xin Li · Jia-Ling Li · Xiao-Tong Zhang · Li Duan · Mipeshwaree Devi Asem ·  
Min Xiao · Xiaozhen Mou · Nimaichand Salam · Wen-Jun Li 

Received: 31 December 2018 / Accepted: 19 April 2019 / Published online: 25 April 2019  
© Springer Nature Switzerland AG 2019

**Abstract** Strain SYSU M10002<sup>T</sup> was isolated from a water sample collected from the coastal region of Pearl River estuary, Guangdong Province, southern China. The taxonomic position of the isolate was investigated by polyphasic taxonomic approaches. The isolate was found to be Gram-negative, non-motile, short rods and aerobic. The strain was able to grow at 14–37 °C, pH 6.0–10.0 and in the presence of up to 0.5% (w/v) NaCl. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain SYSU M10002<sup>T</sup> is a member of the family *Sphingomonadaceae*, with high sequence similarity to *Sphingorhabdus buctiana* T5<sup>T</sup> (95.1%). Overall genomic related indices between the genome of strain SYSU M10002<sup>T</sup> and those of related strains were low

to moderate (AAI values < 64.3%; POCP values < 58%), indicating that strain SYSU M10002<sup>T</sup> represents a novel lineage within the family *Sphingomonadaceae*. Strain SYSU M10002<sup>T</sup> contained homospermidine as its polyamine. The major polar lipids were diphosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, sphingoglycolipid, two unidentified phospholipids and an unidentified aminolipid. Ubiquinone Q-9 (44.9%) and Q-10 (43.2%) were the dominant respiratory quinones, along with a minor amount of Q-8 (11.9%). The predominant cellular fatty acids (> 10%) identified were summed feature 3 (C<sub>16:1</sub> ω7c and/or C<sub>16:1</sub> ω6c), summed feature 8 (C<sub>18:1</sub> ω7c) and C<sub>14:0</sub> 2-OH. The genomic DNA G+C content was 64.0%. Based on the analyses of the phenotypic, genotypic and phylogenetic characteristics, strain SYSU M10002<sup>T</sup> is determined to represent a novel species of a novel genus, for which the name *Aestuariusphingobium litorale* gen. nov., sp. nov. is

Xin Li, Jia-Ling Li, and Xiao-Tong Zhang have contributed equally to this work.

**Electronic supplementary material** The online version of this article (<https://doi.org/10.1007/s10482-019-01268-6>) contains supplementary material, which is available to authorized users.

X. Li · J.-L. Li · X.-T. Zhang · L. Duan ·  
M. D. Asem · M. Xiao · N. Salam (✉) · W.-J. Li (✉)  
State Key Laboratory of Biocontrol and Guangdong  
Provincial Key Laboratory of Plant Resources, School of  
Life Sciences, Sun Yat-sen University,  
Guangzhou 510275, People's Republic of China  
e-mail: s.nimaichand@hotmail.com

W.-J. Li  
e-mail: liwenjun3@mail.sysu.edu.cn

X. Mou  
Department of Biological Sciences, Kent State University,  
Kent, OH 44242, USA

W.-J. Li  
College of Fisheries, Henan Normal University,  
Xinxiang 453007, People's Republic of China

proposed. The type strain of the species is SYSU M10002<sup>T</sup> (= KCTC 52944<sup>T</sup> = NBRC 112961<sup>T</sup>).

**Keywords** *Aestuariusphingobium litorale* gen. nov., sp. nov. · Polyphasic taxonomy · Pearl River estuary

## Introduction

The family *Sphingomonadaceae*, proposed by Kosako et al. (2000), belongs to the order *Sphingomonadales* of the *Alphaproteobacteria*. At the time of writing, the family *Sphingomonadaceae* comprises of 22 validly named genera (<https://www.namesforlife.com/10.1601/tx.1165>) that were isolated from various environments, such as freshwater, pristine or contaminated soils, the rhizosphere and clinical specimens (Balkwill et al. 2006; Gich and Overmann 2006; Glöckner et al. 2000; Kim et al. 2007; Takeuchi et al. 2001; Zwart et al. 2002). Members of the family *Sphingomonadaceae* are typically Gram-stain negative, non-spore-forming, ovoid to rod-shaped, non-motile or motile, aerobic [with the exception of the facultative anaerobe *Zymomonas* (Kalnenieks 2006)] and chemoorganotrophic. Members of the family contain sphingoglycolipid as a major polar lipid component, ubiquinone-10 as the main respiratory quinone, C<sub>18:1</sub> ω7c as the predominant fatty acid and homospermidine as the major polyamine. The DNA G+C content is in the range of 59–68.5 mol% (Kosako et al. 2000). In this study, we describe the taxonomic position of strain SYSU M10002<sup>T</sup> by using a polyphasic taxonomic approach.

## Materials and methods

### Isolation and culture conditions

Water samples were collected from a conjunct point of freshwater and seawater, located along the coastal region of Pearl River estuary (113°35'42.00"E, 23°6'35.25"N), Guangdong Province, southern China. The sample was diluted 10<sup>3</sup>-fold with sterile water and 0.2 ml aliquots spread on Reasoner's 2A (R2A; BD) agar plates adjusted to pH 7.0. The isolation plates were incubated at 28 °C for 14 days. Single colonies were picked, purified and cultivated on the same

medium. The strain SYSU M10002<sup>T</sup> was maintained on R2A agar and preserved as glycerol suspensions (20%, v/v) at –80 °C. The basal growth condition of the strain for all experiments was maintained at pH 7.0 and 28 °C, and the biomass for chemical and molecular studies were obtained by cultivating in R2A media, unless otherwise stated.

### Phenotypic characterization

Cell morphology was observed using a light microscope (BH2; Olympus) and a transmission electron microscope (JEM-100CX-II; JEOL), on R2A agar at 28 °C after 3 days of incubation. Samples for transmission electron microscopy were prepared as described by Ming et al. (2012). Gram reaction was tested by the Gram Stain Solution kit (Shanghai Yeasen Biotechnology). Motility was determined by the development of turbidity in a tube containing semi-solid medium (Leifson 1960). Growth temperature range (4, 14, 20, 28, 37, 40, 45, 50, 55, and 60 °C) and NaCl tolerance (up to 5.0%, at intervals of 0.5%, w/v) were observed in R2A agar following 2 weeks of incubation. For pH tolerance, R2A agar was prepared between pH 4.0–10.0 (with an interval of 1.0 pH unit) using the buffer system described by Nie et al. (2012), and growth was observed after 2 weeks of incubation. Oxidase and catalase activities were determined by assessing the oxidation of 1% (w/v) tetramethyl-*p*-phenylenediamine (Kovacs 1956), and the production of gas bubbles on the addition of a drop of 3% H<sub>2</sub>O<sub>2</sub> (v/v), respectively. Hydrolysis of cellulose, gelatin and starch, milk peptonization and coagulation, nitrate reduction, Tweens (20, 40, 60 and 80) degradation and activity of urease were examined as described previously (Gonzalez et al. 1978; Mac Faddin 1976; Tindall et al. 2007). H<sub>2</sub>S production and indole formation were carried out as described by Cui et al. (2007). Additional physiological and biochemical tests were further performed using API 20NE (bioMérieux), API ZYM (bioMérieux), and GenIII MicroPlate systems (Biolog). All the commercial phenotypic tests were performed according to the manufacturer's recommendations.

### Chemotaxonomy

Biomass for chemical and molecular studies of strain SYSU M10002<sup>T</sup> were obtained from cultures grown in

R2A broth for 3 days. Extraction and analysis of polyamines were carried out as described earlier (Busse et al. 1997; Busse and Auling 1988). For this, cells were cultivated for 3 days, homogenized in 0.2 M perchloric acid (HClO<sub>4</sub>) and centrifuged. Polyamines in the resultant supernatant were treated with dansyl chloride solution (7.5 µg ml<sup>-1</sup> in acetone), and analyzed by HPLC, using UV–Vis detector L-7420. Polar lipids were extracted, separated and examined by a two-dimensional TLC procedure on silica gel G<sub>60</sub> plates using the method of Minnikin et al. (1979). Respiratory quinones were extracted from lyophilized cells (Collins et al. 1977), purified and analyzed by HPLC (Groth et al. 1996). The fatty acid profile of strain SYSU M10002<sup>T</sup> from cultures grown on R2A agar for 3 days (stationary phase) under optimal growth conditions [Note: Cells of strain SYSU M10002<sup>T</sup> show no growth on tryptic soy agar (Difco)]. Cellular fatty acids were extracted, methylated and analyzed following the instructions of the Sherlock Microbial Identification System (MIDI) version 6.1 and the TSBA6 database (Sasser 2001).

#### Molecular characterization

Genomic DNA extraction, PCR amplification of the 16S rRNA gene and sequencing of the purified products from strain SYSU M10002<sup>T</sup> were carried out as described by Li et al. (2007). The whole genome sequencing of strain SYSU M10002<sup>T</sup> was performed using paired-end sequencing method with Hiseq X platform (Illumina, San Diego, CA, USA) at Magi-gene Company (Guangzhou, China). Reads of each data set were filtered, and high-quality paired-end reads were assembled using Spades (Harrison and Strulo 2000). Contigs with length greater than 500 bp were kept for gene prediction by applying Prodigal (Hyatt et al. 2010). The predicted coding sequences of the genome were translated and annotated in the COG, KEGG, GO and Pfam databases (Ashburner et al. 2000; Finn et al. 2016; Kanehisa et al. 2017; Tatusov et al. 2001). The phylogenetic relationship of strain SYSU M10002<sup>T</sup> was determined after BLAST searches (Altschul et al. 1990) of the 16S rRNA gene sequences in NCBI and the EzBioCloud server (Yoon et al. 2017) databases. Analysis of the sequence data was performed by using the software package BioEdit (Hall 1999) and MEGA 7 (Kumar et al. 2016), after multiple alignments of the data by CLUSTAL X

software package (Thompson et al. 1997). Phylogenetic analyses were carried out with three tree-making algorithms: neighbour-joining (NJ; Saitou and Nei 1987), maximum-likelihood (ML; Felsenstein 1981) and maximum-parsimony (MP; Fitch 1971) methods. Evolutionary distances in the NJ and ML dendrograms were calculated by the Kimura two-parameter model (Kimura 1983) and the topologies of the phylogenetic trees were evaluated by the bootstrap analysis of Felsenstein (1985) with 1000 replicates. Genome trees based on the concatenated sequences of 29 protein marker genes were generated using RAxML (Stamatakis 2014). Overall genomic relatedness indices between strain SYSU M10002<sup>T</sup> and related strains in the family *Sphingomonadaceae* were calculated based on the percentage of conserved proteins (POCP; Qin et al. 2014) and Average amino acid identities (AAI; Hua et al. 2018).

## Results and discussion

#### Phenotypic characteristics

Cells of strain SYSU M10002<sup>T</sup> were Gram-stain negative, non-motile and aerobic. Colonies on R2A agar following incubation for 3 days were circular, opaque, convex and yellow. Cells of strain SYSU M10002<sup>T</sup> were short rods, with size measuring approximately 0.8–1.8 µm in length and 0.4–0.6 µm in width (Fig. S1). Strain SYSU M10002<sup>T</sup> grew at 14–37 °C, pH 6.0–10.0 and in the presence of up to 0.5% NaCl. Optimum growth was observed at 28 °C, pH 6.0–7.0 and in the absence of NaCl. The strain was positive for oxidase and catalase activities, but negative for H<sub>2</sub>S production, indole formation, milk coagulation and peptonization, nitrate reduction and urease activity tests. Strain SYSU M10002<sup>T</sup> could hydrolyse Tween 60, but not cellulose, gelatin, starch or Tweens 20, 40 or 80. The strain utilized D-turanose, N-acetyl-D-glucosamine, N-acetyl-β-D-mannosamine, L-fucose and glucuronamide as sole carbon source, but not L-alanine, D-fucose, D-galactose, D-galacturonic acid, D-glucose, stachyose, acetic acid, acetoacetic acid, N-acetyl-D-galactosamine, N-acetyl neuraminic acid, γ-amino-butyric acid, D-arabitol, L-arginine, D-aspartic acid, L-aspartic acid, bromosuccinic acid, D-cellobiose, citric acid, dextrin, formic acid, D-fructose, D-fructose-6-PO<sub>4</sub>, L-galactonic acid lactone, gelatin,

**Table 1** Key taxonomic characteristics differentiating strain SYSU M10002<sup>T</sup> from its closely related phylogenetic neighbours of the family *Sphingomonadaceae*

| Characteristic                  | 1  | 2  | 3   | 4  | 5                                  | 6  |
|---------------------------------|--|--|---|--|------------------------------------|--|
| Pigmentation (colony colour)    | Yellow   | Light-yellow   | Cream   | Yellow, orange, or brown                                     | Whitish-brown, or yellow           | Yellowish orange, transparent                              |
| Cell morphology                 | Short rod  | Rod  | Rod   | Rod, or wedge  | Rod                                | Rod  |
| Motility                        | –  | –  | –   | ND   | +                                  | –  |
| Optimum growth temperature (°C) | 28   | 25   | 30  | 30–35  | ND                                 | 18–24  |
| Optimum growth pH               | 6.0–7.0  | 9.0  | 6.0   | 7.0–7.5  | ND                                 | 7.0–8.0  |
| Nitrate reduction               | –  | +  | +   | –  | ND                                 | –  |
| Hydrolysis of                   |  |  |   |  |                                    |  |
| Aesculin                        | +  | +  | +   | –  | w                                  | ND   |
| Gelatin                         | –  | –  | –   | +  | ND                                 | ND   |
| Tween 20                        | –  | +  | –   | ND   | ND                                 | ND   |
| Tween 80                        | –  | +  | –   | +  | ND                                 | ND   |
| Enzyme activities               |  |  |   |  |                                    |  |
| Catalase                        | +  | +  | –   | +  | +                                  | +  |
| $\beta$ -Glucosidase            | –  | –  | +   | ND   | +                                  | +  |
| $\beta$ -Galactosidase          | –  | +  | –   | ND   | +                                  | +  |
| Assimilation of                 |  |  |   |  |                                    |  |
| Glucose                         | +  | –  | –   | –  | +                                  | +  |
| L-Arabinose                     | –  | –  | +   | –  | +                                  | –  |
| D-Mannose                       | +  | –  | –   | –  | ND                                 | –  |
| Mannitol                        | –  | –  | –   | ND   | w                                  | –  |
| Maltose                         | +  | –  | –   | +  | +                                  | –  |
| Polyamine                       | Homospermidine   | Spermidine   | Spermidine, putrescine  | Spermidine   | Spermidine                         | Spermidine   |
| Major fatty acids               | Summed feature 3, summed feature, C <sub>14:0</sub> 2-OH | C <sub>18:1</sub> $\omega$ 7c, C <sub>16:1</sub> $\omega$ 7c | Spermidine, putrescine, C <sub>17:1</sub> $\omega$ 6c, summed feature 3, C <sub>18:1</sub> $\omega$ 7c, C <sub>15:0</sub> 2-OH, C <sub>17:1</sub> $\omega$ 8c | C <sub>18:1</sub> $\omega$ 7c, C <sub>17:1</sub> $\omega$ 6c | Summed feature 3, summed feature 8 | Summed feature 8, summed feature 3, C <sub>14:0</sub> 2-OH |

**Table 1** continued

| Characteristic         | 1                    | 2    | 3                         | 4               | 5    | 6                               |
|------------------------|----------------------|------|---------------------------|-----------------|------|---------------------------------|
| Major ubiquinones      | Q-9, Q-10            | Q-10 | Q-10                      | Q-10            | Q-10 | Q-10                            |
| Major phospholipids    | DPG, PC, PE, PG, SGL | PE   | PG, PE, DPG, PDE, SGL, PC | PG, PE, PDE, PC | ND   | DPG, PG, PE, PMME, PDE, PC, SGL |
| DNA G+C content (mol%) | 64.0                 | 58.5 | 66.6                      | 64.8            | 63.9 | 55.7                            |

Taxa: 1, *SYSU M10002<sup>T</sup>* (data from this study); 2, *Sphingorhabdus buctiana* T5<sup>T</sup> (Chen et al. 2018); 3, *Novosphingobium fontis* BCRC 80924<sup>T</sup> (Sheu et al. 2017); 4, *Blastomonas natatoria* LMG 17322<sup>T</sup> (Sly and Cahill 1997); 5, *Sphingopyxis macrogoltabida* DSM 8826<sup>T</sup> (Takeuchi et al. 1993); 6, *Sphingorhabdus planktonica* DSM 25081<sup>T</sup> (Jogler et al. 2013)

+, positive; –, negative; w, weakly positive; ND, not determined or data not available at relevant literature

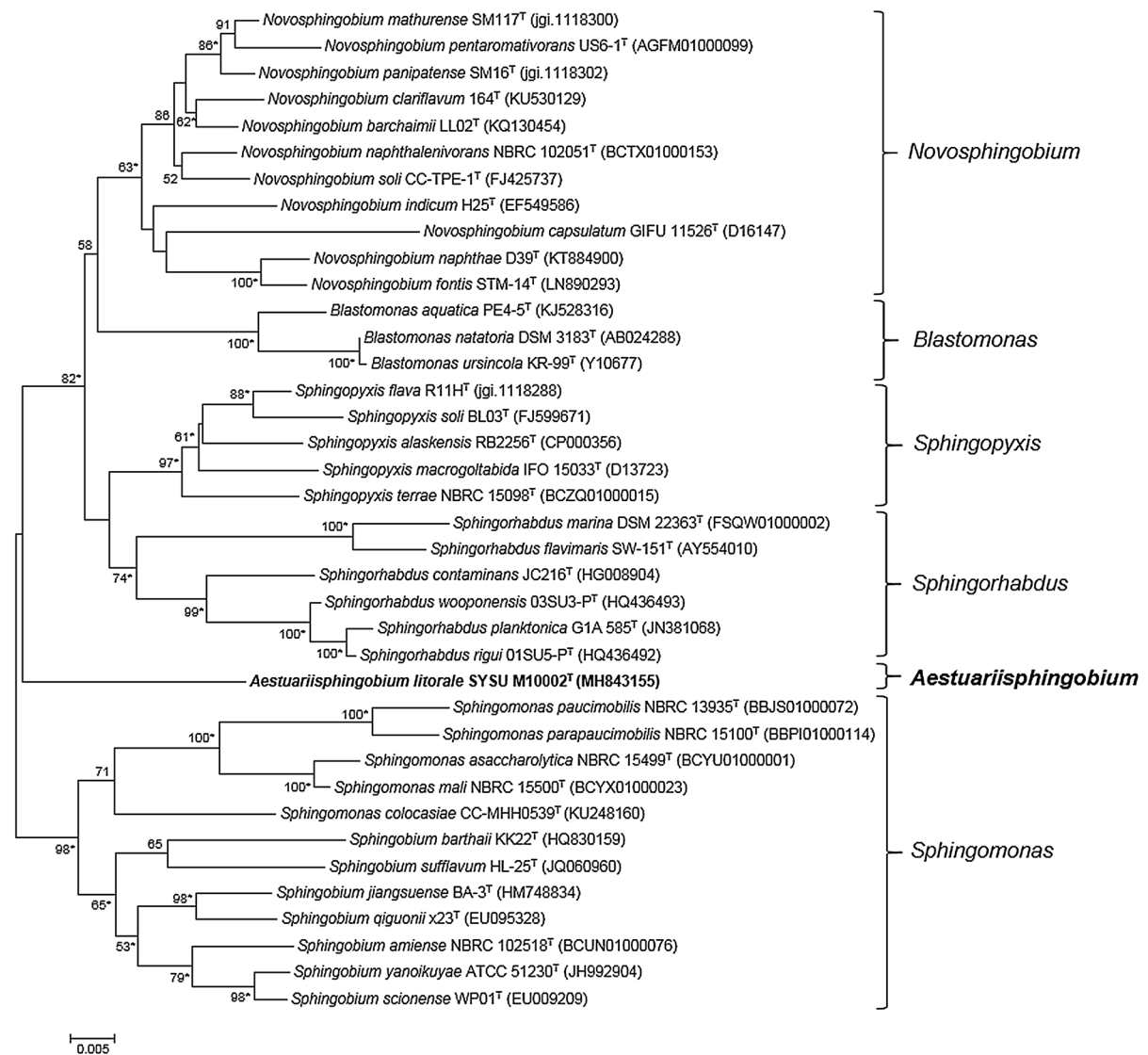
DPG, diphosphatidylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; SGL, sphingoglycolipid; PDE, phosphatidylmethylethanolamine; PMME, phosphatidylmonomethylethanolamine

Summed features represent two or three fatty acids that cannot be separated by GC with the MIDI system. Summed feature 3, C<sub>16:1</sub> ω7c and/or C<sub>16:1</sub> ω6c, Summed feature 8, C<sub>18:1</sub> ω7c and/or C<sub>18:1</sub> ω6c

gentiobiose, D-gluconic acid, D-glucuronic acid, D-glucose-6-PO<sub>4</sub>, glycerol, glycyl-L-proline, L-glutamic acid, L-histidine, α-hydroxy-butyric acid, β-hydroxy-D,L-butyric acid, p-hydroxy-phenylacetic acid, α-keto-butyric acid, α-keto-glutaric acid, inosine, L-lactic acid, D-lactic acid methyl ester, α-D-lactose, D-malic acid, L-malic acid, D-maltose, D-mannitol, D-mannose, D-melibiose, 3-methyl glucose, β-methyl-D-glucoside, methyl pyruvate, myo-inositol, mucic acid, pectin, propionic acid, L-pyroglytamic acid, quinic acid, D-raffinose, L-rhamnose, D-saccharic acid, D-salicin, D-serine, L-serine, D-sorbitol, sucrose, D-trehalose, or Tween 40. In the API ZYM system, strain SYSU M10002<sup>T</sup> was found to be positive for the activities of alkaline phosphatase, acid phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, chymotrypsin, naphthol-AS-BI phosphohydrolase and α-glucosidase, but not cystine arylamidase, trypsin, β-glucosidase, β-fucosidase, α-galactosidase, β-galactosidase, β-glucuronidase, N-acetyl-β-glucosaminidase, lipase (C14) or α-mannosidase. In the API 20NE system, it could hydrolyse aesculin and assimilate glucose, arabinose, mannose, N-acetyl-D-glucosamine, maltose, gluconate, capric acid and malic acid. Differentiating characteristics of strain SYSU M10002<sup>T</sup> from members of related genera of the family *Sphingomonadaceae* are listed in Table 1. The detailed physiological characteristics of strain SYSU M10002<sup>T</sup> are given in the genus and species descriptions below.

**Chemotaxonomic characteristics**

The respiratory quinones of strain SYSU M10002<sup>T</sup> were identified as ubiquinone Q-9 (44.9%), Q-10 (43.2%) and Q-8 (11.9%). Strain SYSU M10002<sup>T</sup> contained homospermidine as its polyamine. The polar lipids of the strain comprised of diphosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, sphingoglycolipid, two unidentified phospholipids and an unidentified aminolipid (Fig. S2). The major cellular fatty acids (> 10%) detected for strain SYSU M10002<sup>T</sup> were summed feature 3 (C<sub>16:1</sub> ω7c and/or C<sub>16:1</sub> ω6c, 37.4%), summed feature 8 (C<sub>18:1</sub> ω7c, 27.9%) and C<sub>14:0</sub> 2-OH (10.5%). A detailed fatty acid profile of strain SYSU M10002<sup>T</sup> is provided in Table S1.



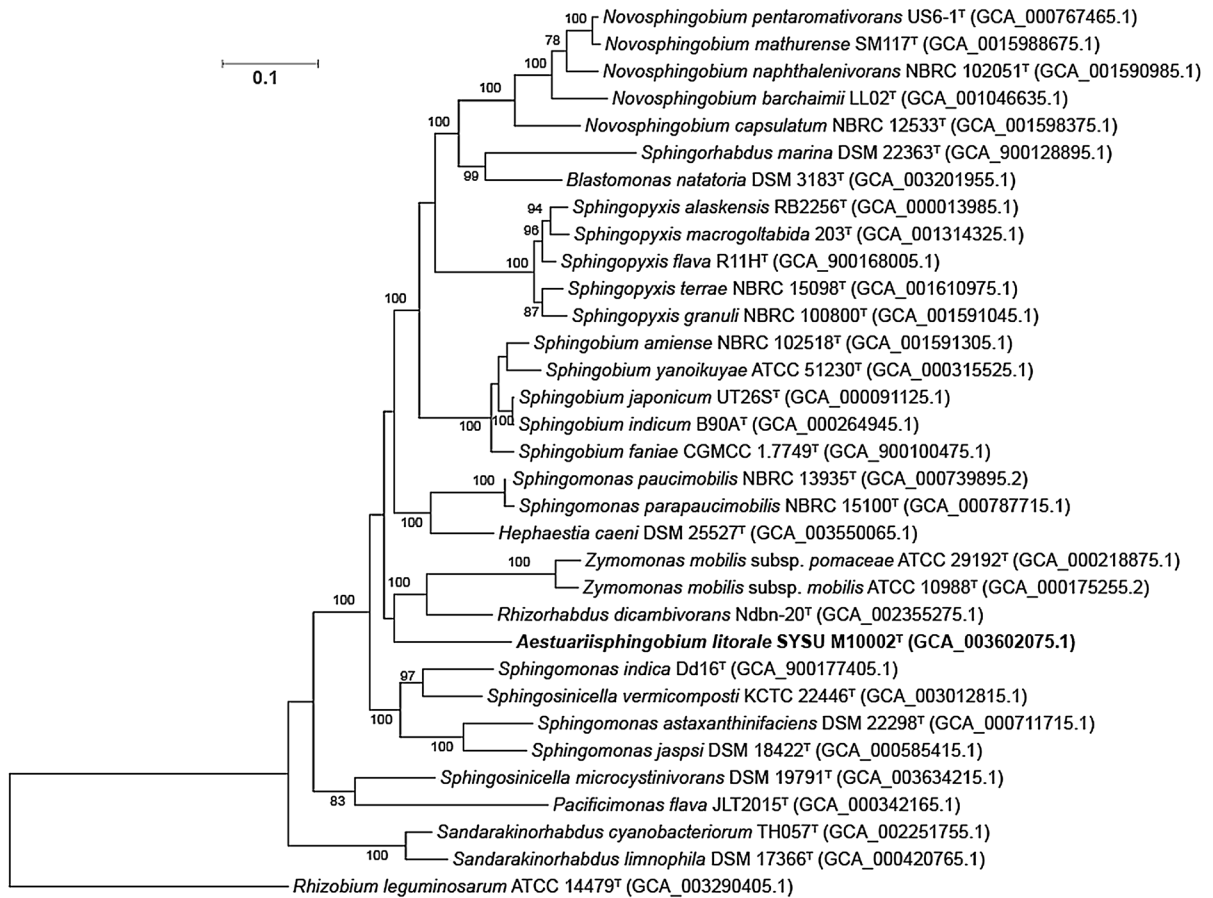
**Fig. 1** Unrooted neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationships between strain SYSU M10002<sup>T</sup> and closely related members of the family *Sphingomonadaceae*. Asterisks indicate nodes that were

supported in trees generated with maximum-likelihood and maximum-parsimony methods. Bootstrap values ( $\geq 50\%$ ) based on 1000 resamplings are given at the nodes. Bar, 0.005 substitutions per nucleotide position

### Molecular characteristics

The DNA G+C content of strain SYSU M10002<sup>T</sup> was calculated at 64.0% (genome). Pairwise comparison with the almost-complete 16S rRNA gene sequence of strain SYSU M10002<sup>T</sup> showed sequence similarities of less than 96% with members of the family *Sphingomonadaceae*. The highest 16S rRNA gene sequence identity of 95.1% is determined with *Sphingorhabdus buctiana* T5<sup>T</sup>. Besides the low sequence

identity, strain SYSU M10002<sup>T</sup> also formed a separate clade distinct from the members of the genera *Novosphingobium*, *Blastomonas*, *Sphingopyxis*, *Sphingorhabdus* or *Sphingomonas* in all three phylogenetic trees (Fig. 1; Figs. S3 and S4). These findings indicated that the topology of the strain SYSU M10002<sup>T</sup> is stable and that the strain should be affiliated to the family *Sphingomonadaceae*. A similar finding was observed with the RAxML tree generated with related members of the family



**Fig. 2** RAxML phylogenomic tree showing the phylogenetic relationships between strain SYSU M10002<sup>T</sup> and closely related members of the family *Sphingomonadaceae*. Bootstrap values

(≥ 70%) based on 1000 resamplings are given at the nodes. Bar, 0.1 substitutions per nucleotide position

*Sphingomonadaceae* (Fig. 2). Strain SYSU M10002<sup>T</sup> showed low to moderately high POCP and AAI values with related members of the family *Sphingomonadaceae* (Table 2). Other general features of the genome of strain SYSU M10002<sup>T</sup> are listed in Table S2.

### Discussion

On the basis of 16S rRNA gene sequences identity and chemotaxonomic features, strain SYSU M10002<sup>T</sup> can be affiliated to the family *Sphingomonadaceae*. While strain SYSU M10002<sup>T</sup> exhibited many characteristics similar to members of the family *Sphingomonadaceae* in having ovoid to rod-shaped morphology, optimum growth temperature of about 30 °C, optimum growth pH of about 7 and DNA G+C content within the range

of the family, it could, however, be differentiated physiologically and phenotypically from the other closely related genera (Table 1). For example, strain SYSU M10002<sup>T</sup> could tolerate pH 10 which is obviously different from the other closely related genera. Unlike the other closely related genera, the major polyamine in strain SYSU M10002<sup>T</sup> is homospermidine. Further, on pairwise comparison with the closely related phylogenetic neighbor *S. buctiana* T5<sup>T</sup>, despite showing high 16S rRNA gene sequence similarity to *S. buctiana* T5<sup>T</sup>, strain SYSU M10002<sup>T</sup> differed from it on the basis of several distinct chemotaxonomic characteristics, such as (1) fatty acid profile [summed feature 3 (C<sub>16:1</sub> ω7c and/or C<sub>16:1</sub> ω6c), summed feature 8 (C<sub>18:1</sub> ω7c) and C<sub>14:0</sub> 2-OH for strain SYSU M10002<sup>T</sup>, and C<sub>18:1</sub> ω7c and C<sub>16:1</sub> ω7c as major fatty acids for *Sphingorhabdus buctiana* T5<sup>T</sup>]; and (2) respiratory quinone [ubiquinone Q-9

**Table 2** Overall genome relatedness indices between strain SYSU M10002<sup>T</sup> and related members of the family *Sphingomonadaceae*

| Strains   | Genome accession | POCP (%) | AAI (%) |
|---|------------------|----------|---------|
| <i>Novosphingobium barchaimii</i> LL02 <sup>T</sup>                     | GCA_001046635.1  | 43.1     | 61.2    |
| <i>Novosphingobium capsulatum</i> NBRC 12533 <sup>T</sup>               | GCA_001598375.1  | 48.6     | 62.1    |
| <i>Novosphingobium mathurense</i> SM117 <sup>T</sup>                    | GCA_900168325.1  | 47.8     | 61.8    |
| <i>Novosphingobium naphthalenivorans</i> NBRC 102051 <sup>T</sup>       | GCA_001590985.1  | 43.4     | 61.2    |
| <i>Novosphingobium pentaromativorans</i> US6-1 <sup>T</sup>             | GCA_000767465.1  | 42.5     | 60.6    |
| <i>Blastomonas natatoria</i> DSM 3183 <sup>T</sup>                      | GCA_003201955.1  | 52.7     | 62.6    |
| <i>Hephaestia caeni</i> DSM 25527 <sup>T</sup>                          | GCA_003550065.1  | 49.4     | 63.9    |
| <i>Pacificimonas flava</i> JLT2015 <sup>T</sup>                         | GCA_000342165.1  | 47.7     | 57.8    |
| <i>Rhizorhabdus dicambivorans</i> Ndbn-20 <sup>T</sup>                  | GCA_002355275.1  | 46.0     | 62.6    |
| <i>Sandarakinorhabdus cyanobacteriorum</i> TH057 <sup>T</sup>           | GCA_002251755.1  | 50.9     | 59.4    |
| <i>Sandarakinorhabdus limnophila</i> DSM 17366 <sup>T</sup>             | GCA_000420765.1  | 52.3     | 59.3    |
| <i>Sphingobium amiense</i> NBRC 102518 <sup>T</sup>                     | GCA_001591305.1  | 49.0     | 63.6    |
| <i>Sphingobium faniae</i> CGMCC 1.7749 <sup>T</sup>                     | GCA_900100475.1  | 47.0     | 63.8    |
| <i>Sphingobium indicum</i> B90A <sup>T</sup>                            | GCA_000264945.2  | 50.4     | 64.2    |
| <i>Sphingobium japonicum</i> UT26S <sup>T</sup>                         | GCA_000091125.1  | 49.3     | 63.7    |
| <i>Sphingobium yanoikuyae</i> ATCC 51230 <sup>T</sup>                   | GCA_000315525.1  | 46.3     | 62.9    |
| <i>Sphingomonas astaxanthinifaciens</i> DSM 22298 <sup>T</sup>          | GCA_000711715.1  | 50.2     | 61.6    |
| <i>Sphingomonas indica</i> Dd16 <sup>T</sup>                            | GCA_900177405.1  | 51.9     | 63.7    |
| <i>Sphingomonas jaspsi</i> DSM 18422 <sup>T</sup>                       | GCA_000585415.1  | 49.5     | 62.3    |
| <i>Sphingomonas paucimobilis</i> NBRC 13935 <sup>T</sup>                | GCA_000739895.2  | 47.1     | 63.2    |
| <i>Sphingomonas sanxanigenens</i> DSM 19645 <sup>T</sup>                | GCA_000512205.2  | 43.0     | 63.9    |
| <i>Sphingopyxis alaskensis</i> RB2256 <sup>T</sup>                      | GCA_000013985.1  | 57.9     | 64.0    |
| <i>Sphingopyxis flava</i> R11H <sup>T</sup>                             | GCA_900168005.1  | 50.8     | 63.5    |
| <i>Sphingopyxis granuli</i> NBRC 100800 <sup>T</sup>                    | GCA_001591045.1  | 52.8     | 63.5    |
| <i>Sphingopyxis macrogoltabida</i> 203 <sup>T</sup>                     | GCA_001314325.1  | 48.6     | 64.1    |
| <i>Sphingopyxis terrae</i> NBRC 100800 <sup>T</sup>                     | GCA_001610975.1  | 55.9     | 63.6    |
| <i>Sphingosinicella microcystinivorans</i> DSM 19791 <sup>T</sup>       | GCA_003634215.1  | 49.4     | 60.0    |
| <i>Sphingosinicella vermicomposti</i> KCTC 22446 <sup>T</sup>           | GCA_003012815.1  | 51.9     | 64.2    |
| <i>Zymomonas mobilis</i> subsp. <i>mobilis</i> ATCC 10988 <sup>T</sup>  | GCA_000175255.2  | 37.7     | 59.7    |
| <i>Zymomonas mobilis</i> subsp. <i>pomaceae</i> ATCC 29192 <sup>T</sup> | GCA_000218875.1  | 38.4     | 60.0    |

(44.9%), Q-10 (43.2%) and Q-8 (11.9%) for SYSU M10002<sup>T</sup> and ubiquinone Q-10 for *S. buctiana* T5<sup>T</sup>], and genomic DNA G+C content (64.0% for strain SYSU M10002<sup>T</sup> and 58.5 mol% for *S. buctiana* T5<sup>T</sup>) (Table 1). Observation of the POCP and AAI also indicate that strain SYSU M10002<sup>T</sup> was distinct from the related genera by low to moderate values ranging from 37 to 58 POCP values and 57–65 AAI values (Table 2). Based on the results of these differentiating characteristics (Tables 1 and 2) and phylogenetic analyses, strain SYSU M10002<sup>T</sup> is considered to represent a novel species in a new genus of the family *Sphingomonadaceae*, for which

the name *Aestuariusphingobium litorale* gen. nov., sp. nov. is proposed.

#### Description of *Aestuariusphingobium* gen. nov.

*Aestuariusphingobium* [Aes.tu.a.ri.i.sphin.go'bi.um. L. neut. n. *aestuarium* an estuary; N.L. neut. n. *Sphingobium*, a bacterial genus; N.L. neut. n. *Aestuariusphingobium*, a *Sphingobium*-like organism from an estuary].

Cells are Gram-negative, non-motile, strictly aerobic, and short rods. Colonies are irregular, opaque,



convex and yellow. No pigments are produced. Oxidase-positive and catalase-positive. The main respiratory isoprenologues are Q-9 and Q-10. Major polyamine is homospermidine. Major cellular fatty acids are summed feature 3, summed feature 8 and C<sub>14:0</sub> 2-OH. The known polar lipid profile comprises diphosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol and sphingoglycolipid. The genomic DNA G+C content of the type strain of the type species is about 64.0 mol%. The type species is *Aestuariusphingobium litorale*.

### Description of *Aestuariusphingobium litorale* sp. nov.

*Aestuariusphingobium litorale* (li.to.ra'le. L. neut. adj. *litorale*, of or belonging to the coast).

Displays the following properties in addition to those described for the genus. Cells measure 0.8–1.8 µm in length and 0.4–0.6 µm in width after 4 days of growth at 28 °C on R2A agar plates. Growth occurs at 14–37 °C (optimum, 28 °C), pH 6.0–10.0 (optimum, pH 6.0–7.0) and in the presence of up to 0.5% NaCl. Negative results for H<sub>2</sub>S production, indole formation, nitrate reduction, milk coagulation and peptonization, and urease activity. Hydrolyses Tween 60, but not cellulose, gelatin, starch, or Tweens 20, 40 or 80.

The type strain SYSU M10002<sup>T</sup> (= KCTC 52944<sup>T</sup> = NBRC 112961<sup>T</sup>) was isolated from a water sample collected along the coast of Pearl River estuary, Guangdong Province, southern China. The genomic DNA G+C content of the type strain is 64.0%. The GenBank accession number for the 16S rRNA gene sequence of strain SYSU M10002<sup>T</sup> is MH843155. The draft genome has been deposited under accession number RAGX00000000.

**Acknowledgements** This research was supported by the Natural Science Foundation of China (No. 31528001). X. Mou was supported by the Ohio Board of Regents (sub-award no. 60049296). W.-J. Li was also supported by Guangdong Province Higher Vocational Colleges & Schools Pearl River Scholar Funded Scheme (2014).

**Authors' contribution** N.S. and W.J.L. conceived the study. X.L., J.L.L., X.T.Z., N.S., L.D. and M.D.A. performed research. N.S., X.M. and M.X. analyzed data. X.L., N.S. and W.J.L. wrote the paper. All authors approved the manuscript.

### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical statement** This article does not contain any studies with human participants or animals performed by any of the authors.

### References

- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. *J Mol Biol* 215:403–410. [https://doi.org/10.1016/s0022-2836\(05\)80360-2](https://doi.org/10.1016/s0022-2836(05)80360-2)
- Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppiq JT, Harris MA, Hill DP, Issel-Tarver L, Kasarskis A, Lewis S, Matese JC, Richardson JE, Ringwald M, Rubin GM, Sherlock G (2000) Gene Ontology: tool for the unification of biology. The gene ontology consortium. *Nat Genet* 25:25–29. <https://doi.org/10.1038/75556>
- Balkwill DL, Fredrickson JK, Romine MF (2006) *Sphingomonas* and related genera. In: Dworkin M, Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt E (eds) The prokaryotes: volume 7: *Proteobacteria: Delta, Epsilon* Subclass. Springer, New York, pp 605–629. [https://doi.org/10.1007/0-387-30747-8\\_23](https://doi.org/10.1007/0-387-30747-8_23)
- Busse J, Auling G (1988) Polyamine pattern as a chemotaxonomic marker within the *Proteobacteria*. *Syst Appl Microbiol* 11:1–8. [https://doi.org/10.1016/S0723-2020\(88\)80040-7](https://doi.org/10.1016/S0723-2020(88)80040-7)
- Busse H-J, Bunka S, Hensel A, Lubitz W (1997) Discrimination of members of the family *Pasteurellaceae* based on polyamine patterns. *Int J Syst Evol Microbiol* 47:698–708. <https://doi.org/10.1099/00207713-47-3-698>
- Chen H, Piao A-L, Tan X, Nogi Y, Yeo J, Lu H, Feng Q-Q, Lv J (2018) *Sphingorhabdus buctiana* sp. nov., isolated from fresh water, and reclassification of *Sphingopyxis contaminans* as *Sphingorhabdus contaminans* comb. nov. *Antonie Van Leeuwenhoek* 111:323–331. <https://doi.org/10.1007/s10482-017-0954-z>
- Collins MD, Pirouz T, Goodfellow M, Minnikin DE (1977) Distribution of menaquinones in actinomycetes and corynebacteria. *J Gen Microbiol* 100:221–230
- Cui H-L, Lin Z-Y, Dong Y, Zhou P-J, Liu S-J (2007) *Halorubrum litoreum* sp. nov., an extremely halophilic archaeon from a solar saltern. *Int J Syst Evol Microbiol* 57:2204–2206. <https://doi.org/10.1099/ijs.0.65268-0>
- Felsenstein J (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* 17:368–376. <https://doi.org/10.1007/bf01734359>
- Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39:783–791. <https://doi.org/10.2307/2408678>
- Finn RD, Coghill P, Eberhardt RY, Eddy SR, Mistry J, Mitchell AL, Potter SC, Punta M, Qureshi M, Sangrador-Vegas A, Salazar GA, Tate J, Bateman A (2016) The Pfam protein

- families database: towards a more sustainable future. *Nucleic Acids Res* 44:D279–D285. <https://doi.org/10.1093/nar/gkv1344>
- Fitch WM (1971) Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* 20:406–416. <https://doi.org/10.2307/2412116>
- Gich F, Overmann J (2006) *Sandarakinorhabdus limnophila* gen. nov., sp. nov., a novel bacteriochlorophyll a-containing, obligately aerobic bacterium isolated from freshwater lakes. *Int J Syst Evol Microbiol* 56:847–854. <https://doi.org/10.1099/ijs.0.63970-0>
- Glöckner FO, Zaichikov E, Belkova N, Denissova L, Pernthaler J, Pernthaler A, Amann R (2000) Comparative 16S rRNA analysis of lake bacterioplankton reveals globally distributed phylogenetic clusters including an abundant group of *Actinobacteria*. *Appl Environ Microbiol* 66:5053–5065. <https://doi.org/10.1128/aem.66.11.5053-5065.2000>
- Gonzalez C, Gutierrez C, Ramirez C (1978) *Halobacterium vallismortis* sp. nov., an amylolytic and carbohydrate-metabolizing, extremely halophilic bacterium. *Can J Microbiol* 24:710–715
- Groth I, Schumann P, Weiss N, Martin K, Rainey FA (1996) *Agrococcus jenensis* gen. nov., sp. nov., a new genus of actinomycetes with diamminobutyric acid in the cell wall. *Int J Syst Evol Microbiol* 46:234–239. <https://doi.org/10.1099/00207713-46-1-234>
- Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl Acids Symp Ser* 41:91–98
- Harrison PG, Strulo B (2000) SPADES—a process algebra for discrete event simulation. *J Logic Comput* 10:3–42. <https://doi.org/10.1093/logcom/10.1.3>
- Hua Z-S, Qu Y-N, Zhu Q, Zhou E-M, Qi Y-L, Yin Y-R, Rao Y-Z, Tian Y, Li Y-X, Liu L, Castelle CJ, Hedlun BP, Shu W-S, Knight R, Li W-J (2018) Genomic inference of the metabolism and evolution of the archaeal phylum *Aigarchaeota*. *Nat Commun* 9:2832. <https://doi.org/10.1038/s41467-018-05284-4>
- Hyatt D, Chen G-L, LoCascio PF, Land ML, Larimer FW, Hauser LJ (2010) Prodigal: prokaryotic gene recognition and translation initiation site identification. *BMC Bioinformatics* 11:119. <https://doi.org/10.1186/1471-2105-11-119>
- Jogler M, Chen H, Simon J, Rohde M, Busse H-J, Klenk H-P, Tindall BJ, Overmann J (2013) Description of *Sphingorhabdus planktonica* gen. nov., sp. nov. and reclassification of three related members of the genus *Sphingopyxis* in the genus *Sphingorhabdus* gen. nov. *Int J Syst Evol Microbiol* 63:1342–1349. <https://doi.org/10.1099/ijs.0.043133-0>
- Kalnenieks U (2006) Physiology of *Zymomonas mobilis*: some unanswered questions. In: Poole RK (ed) *Advances in microbial physiology*, vol 51. Academic Press, Cambridge, pp 73–117. [https://doi.org/10.1016/s0065-2911\(06\)51002-1](https://doi.org/10.1016/s0065-2911(06)51002-1)
- Kanehisa M, Furumichi M, Tanabe M, Sato Y, Morishima K (2017) KEGG: new perspectives on genomes, pathways, diseases and drugs. *Nucleic Acids Res* 45:D353–D361. <https://doi.org/10.1093/nar/gkw1092>
- Kim MK, Schubert K, Im W-T, Kim K-H, Lee S-T, Overmann J (2007) *Sphingomonas kaistensis* sp. nov., a novel alphaproteobacterium containing *putfLM* genes. *Int J Syst Evol Microbiol* 57:1527–1534. <https://doi.org/10.1099/ijs.0.64579-0>
- Kimura M (1983) *The neutral theory of molecular evolution*. Cambridge University Press, Cambridge
- Kosako Y, Yabuuchi E, Naka T, Fujiwara N, Kobayashi K et al (2000) Proposal of *Sphingomonadaceae* fam. nov., consisting of *Sphingomonas* Yabuuchi et al. 1990, *Erythrobacter* Shiba and Shimidu 1982, *Erythromicrobium* Yurkov et al. 1994, *Porphyrobacter* Fuerst et al. 1993, *Zymomonas* Kluyver and van Niel 1936, and *Sandaracinobacter* Yurkov et al. 1997, with the type genus *Sphingomonas* Yabuuchi et al. 1990. *Microbiol Immunol* 44:563–575. <https://doi.org/10.1111/j.1348-0421.2000.tb02535.x>
- Kovacs N (1956) Identification of *Pseudomonas pyocyanea* by the oxidase reaction. *Nature* 178:703–704. <https://doi.org/10.1038/178703a0>
- Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol* 33:1870–1874. <https://doi.org/10.1093/molbev/msw054>
- Leifson E (1960) *Atlas of bacterial flagellation*. Academic Press, New York
- Li W-J, Xu P, Schumann P, Zhang Y-Q, Pukall R, Xu L-H, Stackebrandt E, Jiang C-L (2007) *Georgenia ruanii* sp. nov., a novel actinobacterium isolated from forest soil in Yunnan (China), and emended description of the genus *Georgenia*. *Int J Syst Evol Microbiol* 57:1424–1428. <https://doi.org/10.1099/ijs.0.64749-0>
- Mac Faddin JF (1976) *Biochemical tests for identification of medical bacteria*. Williams & Wilkins Co., Baltimore
- Ming H, Nie G-X, Jiang H-C, Yu T-T, Zhou E-M, Feng H-G, Tang S-K, Li W-J (2012) *Paenibacillus frigidiresistens* sp. nov., a novel psychrotroph isolated from a peat bog in Heilongjiang, Northern China. *Antonie van Leeuwenhoek* 102:297–305. <https://doi.org/10.1007/s10482-012-9738-7>
- Minnikin DE, Collins MD, Goodfellow M (1979) Fatty acid and polar lipid composition in the classification of *Cellulomonas*, *Oerskovia* and related taxa. *J Appl Bacteriol* 47:87–95. <https://doi.org/10.1111/j.1365-2672.1979.tb01172.x>
- Nie G-X, Ming H, Li S, Zhou E-M, Cheng J, Tang X, Feng H-G, Tang S-K, Li W-J (2012) *Amycolatopsis dongchuanensis* sp. nov., an actinobacterium isolated from soil. *Int J Syst Evol Microbiol* 62:2650–2656. <https://doi.org/10.1099/ijs.0.038125-0>
- Qin Q-L, Xie B-B, Zhang X-Y, Chen X-L, Zhou B-C, Zhou J, Oren A, Zhang Y-X (2014) A proposed genus boundary for the prokaryotes based on genomic insights. *J Bacteriol* 196:2210–2215. <https://doi.org/10.1128/jb.01688-14>
- Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 4:406–425
- Sasser M (2001) Identification of bacteria by gas chromatography of cellular fatty acids. [http://www.microbialid.com/PDF/TechNote\\_101.pdf](http://www.microbialid.com/PDF/TechNote_101.pdf)
- Sheu S-Y, Liu L-P, Young C-C, Chen W-M (2017) *Novosphingobium fontis* sp. nov., isolated from a spring. *Int J Syst Evol Microbiol* 67:2423–2429. <https://doi.org/10.1099/ijsem.0.001973>

- Sly LI, Cahill MM (1997) Transfer of *Blastobacter natatorius* (Sly 1985) to the genus *Blastomonas* gen. nov. as *Blastomonas natatoria* comb. nov. *Int J Syst Evol Microbiol* 47:566–568. <https://doi.org/10.1099/00207713-47-2-566>
- Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30:1312–1313. <https://doi.org/10.1093/bioinformatics/btu033>
- Takeuchi M, Kawai F, Shimada Y, Yokota A (1993) Taxonomic study of polyethylene glycol-utilizing bacteria: emended description of the genus *Sphingomonas* and new descriptions of *Sphingomonas macrogoltabidus* sp. nov., *Sphingomonas sanguis* sp. nov. and *Sphingomonas terrae* sp. nov. *Syst Appl Microbiol* 16:227–238. [https://doi.org/10.1016/S0723-2020\(11\)80473-X](https://doi.org/10.1016/S0723-2020(11)80473-X)
- Takeuchi M, Hamana K, Hiraishi A (2001) Proposal of the genus *Sphingomonas* sensu stricto and three new genera, *Sphingobium*, *Novosphingobium* and *Sphingopyxis*, on the basis of phylogenetic and chemotaxonomic analyses. *Int J Syst Evol Microbiol* 51:1405–1417. <https://doi.org/10.1099/00207713-51-4-1405>
- Tatusov RL, Natale DA, Garkavtsev IV, Tatusova TA, Shakhavram UT, Rao BS, Kiryutin B, Galperin MY, Fedorova ND, Koonin EV (2001) The COG database: new developments in phylogenetic classification of proteins from complete genomes. *Nucleic Acids Res* 29:22–28. <https://doi.org/10.1093/nar/29.1.22>
- Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL-X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* 25:4876–4882. <https://doi.org/10.1093/nar/25.24.4876>
- Tindall BJ, Sikorski J, Smibert RA, Krieg NR (2007) Phenotypic characterization and the principles of comparative systematics. In: Reddy CA, Beveridge TJ, Breznak JA, Marzluf GA, Schmidt TM, Snyder LR (eds) *Methods for general and molecular microbiology*, 3rd edn. American Society of Microbiology, Washington DC, pp 330–393. <https://doi.org/10.1128/9781555817497.ch15>
- Yoon S-H, Ha S-M, Kwon S, Lim J, Kim Y, Seo H, Chun J (2017) Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *Int J Syst Evol Microbiol* 67:1613–1617. <https://doi.org/10.1099/ijsem.0.001755>
- Zwart G, Crump BC, Kamst-van Agterveld MP, Hagen F, Han S-K (2002) Typical freshwater bacteria: an analysis of available 16S rRNA gene sequences from plankton of lakes and rivers. *Aquat Microb Ecol* 28:141–155

**Publisher's Note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.