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TAXONOMIC DESCRIPTION
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Marortus luteolus gen. nov., sp. nov., isolated from surface seawater of the East Sea in China

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Abstract

A Gram-stain-negative, motile, aerobic, rod-shaped bacterium with flagella, designated ZX-21^T, was isolated from surface seawater of the East Sea in Zhoushan, China. Growth of strain ZX-21^T was observed at 10–35 °C (optimum, 30 °C), at pH 6.0–8.5 (pH 6.5–7.0) and in the presence of 0.5–8 % (w/v) NaCl (3–4 %). It was positive for oxidase and catalase activity. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain ZX-21^T constituted an independent lineage within the family *Spongiibacteraceae* and was most closely related to *Zhongshania guokunii* (96.83 %). Strain ZX-21^T contained ubiquinone-8 as the sole isoprenoid quinone and summed feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$), summed feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$) and $C_{16:0}$ as the major fatty acids. Phosphatidylglycerol, phosphatidylethanolamine, diphosphatidylglycerol and an unidentified glycolipid were the major cellular polar lipids. The DNA G+C content was 49.1 mol%. Based on its morphological, physiological and chemotaxonomic characteristics, strain ZX-21^{T}is described as a novel species in a novel genus for which the name *Marortus luteolus* gen. nov., sp. nov. (type strain ZX-21^T=MCCC 1K03431^T=KCTC 62160^T) is proposed.

The family *Spongiibacteraceae* belongs to the order *Cellvibrionales*. At the time of writing, this family comprises five genera(*Spongiibacter*, *Zhongshania*, *Dasania*, *Oceanicoccus* and *Sinobacterium*) and 11 species with validly published names. Most isolates of this family were obtained from marine environment.

The genus *Zhongshania* was first proposed by Li *et al.* [1]. At the time of writing, the genus *Zhongshania* comprises four species, *Zhongshania antarctica*, *Zhongshania guokunii* [1], *Zhongshania aliphaticivorans* and *Zhongshania borealis* [2, 3], isolated from marine environments, such as Antarctic coastal attached (fast) ice and surface seawater. Members of the genus *Zhongshania* have cells that are Gram-negative, motile, aerobic, catalase- and oxidase-positive, rod-shaped, and contain $C_{17:1}\omega8c$, summed feature 3 (comprising $C_{16:1}$ $\omega7c$ and/or iso-$C_{15:0}$ 2-OH) and $C_{17:0}$ as the major fatty acids [1]. In this study, strain ZX-21^T was isolated from surface seawater of the East Sea, China. Preliminary analysis of 16S rRNA gene sequences revealed that strain ZX-21^T was most closely related to the genus *Zhongshania*, with moderately high sequence similarities of 96.01–96.83 %. The aim of the present investigation was to determine the taxonomic position of strain ZX-21^T based on analysis of phenotypic, phylogenetic, genomic and chemotaxonomic characteristics. On the basis of the obtained results, ZX-21^T is proposed to be allocated to a novel genus, which is phylogenetically adjacent to the genus *Zhongshania* within the family *Spongiibacteraceae*.

A sample of surface seawater was collected in December 2016 from the East Sea in Zhoushan, China (29° 10′ N 122° 20′ E). One colony, designated ZX-21^T, was isolated by using the standard dilution plating technique at 20 °C on modified marine agar 2216 containing the same ingredients as marine agar 2216 (MA; BD Difco), except that peptone and yeast were reduced to 0.1 g l^{-1} and 0.5 g l^{-1}, respectively [4], and the isolate was purified by repeated streaking. The reference strains, *Z. antarctica* CCCTCC AB 209246^T and *Z. guokunii* CCTCC AB 209247^T, were obtained from the CCTCC (China Centre for Type Culture Collection). Unless otherwise stated, these strains were routinely cultivated on MA or marine broth 2216 (MB; Difco) at 30 °C. Strain ZX-21^T was stored at −80 °C with 25 % (v/v) glycerol and by lyophilization with 20 % (w/v) skimmed milk after 5 days'

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Abbreviations: LC-MS, Liquid Chromatograph Mass Spectrometer; Q-8, ubiquinone-8; TLC, Thin Layer Chromatography.
The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain ZX-21^T is KY857838. The GenBank accession numbers for the whole genome sequence of strain ZX-21^T is PQGG00000000.
One supplementary table and three supplementary figures are available with the online version of this article.

incubation in MB at 30 °C (120 r.p.m.) for long-term preservation.

Cell morphology was observed using transmission electron microscopy (JEM-1230, JEOL) for strain ZX-21^T that had been grown on MA at 30 °C for 3 days. The Gram reaction was tested by the method described by Claus [5]. The motility was tested by inoculation in semi-solid MB (MB medium with 0.5 % agar, w/v). The temperature range for growth was determined at 4–50 °C (4, 10, 15, 20, 25, 28, 30, 35, 37, 40, 45 and 50 °C). The pH range for growth in MB was measured from pH 5.0 to 10.0 with an interval of 0.5 at 30 °C. Biological buffers (MES for pH 5.0–5.5, MOPS for pH 6.0–7.5, Tricine for pH 8.0–8.5 and CAPSO for pH 9.0–10.0) were supplemented in MB at a concentration of 40 mm. The NaCl concentration range for growth (0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 8.0 and 10.0 %, w/v) was tested by using NaCl-free MB medium (prepared according to the MB formula, but without NaCl). Anaerobic growth was examined in an anaerobic jar (MGC) with AnaeroPack (MGC) on modified MA supplemented with sodium thiosulfate (20 mm), sodium sulfite (5 mm), sodium sulfate (20 mm), sodium nitrite (5 mm), sodium nitrate (20 mm) or L-arginine (5.0 g l^{-1}) as potential electron acceptors for 30 days.

Unless stated otherwise, biochemical and physiological tests were performed in MB or on MA at 30 °C. Catalase and oxidase activities were individually tested by using 3 % (v/v) hydrogen peroxide solution and oxidase reagent (bioMérieux). Hydrolysis of Tweens 20, 40, 60 and 80 were determined according to Sun *et al.*[6]. Hydrolysis of starch, gelatin, casein and CM-cellulose were tested as described by Dong and Cai [7]. Degradation of L-tyrosine was tested on MA supplemented with 0.5 % (w/v) L-tyrosine. Other biochemical properties, enzyme activities and utilization of carbohydrates were tested using API ZYM and API 20NE strips (bioMérieux) according to the manufacturers' instructions. Antibiotic susceptibility tests were determined on MA plates at 30 °C with antibiotic discs containing one of the following antibiotics (μg/disc, unless stated otherwise): chloramphenicol (30), cefradine (30), kanamycin (30), erythromycin (10), ofloxacin (5), penicillin (10 IU), rifampicin (5), streptomycin (10), tetracycline (30) and vancomycin (30). The antibiotic circles were measured and the susceptibility to antibiotics was determined according to the diameter of the antibiotic circles (susceptible >5 mm, intermediate 2–5 mm, resistant <2 mm). *Z. antarctica* CCTCC AB 209246^T and *Z. guokunii* CCTCC AB 209247^T were tested in parallel under identical conditions.

For chemotaxonomic studies, cell mass of strain ZX-21^T and the two reference strains used for the analysis of respiratory quinones, fatty acids and polar lipids was cultured in MB at 30 °C (120 r.p.m.) until exponential phase (about 5 days). Respiratory quinones were extracted from freeze-dried cells using chloroform/methanol (2 : 1) and purified by TLC as described by Komagata and Suzuki [8]. The purified product was analysed by LC-MS (Agilent) [9]. Cellular fatty acid methyl esters were extracted from freeze-dried cells as described by Kuykendall *et al.* [10] and analysed according to the instructions of the Microbial Identification System (MIDI, Microbial ID). The polar lipids were extracted as described by Sun *et al.* [11] and separated by two-dimensional TLC (silica-gel plates 60 F254, Merck 5554), then identified by following the standard procedures [12]. Four kinds of spray reagents were used to detect the corresponding lipids, including molybdophosphoric acid for total lipids, molybdenum blue for phosphorus-containing lipids, ninhydrin reagent for lipids containing free aminolipids and α-naphthol reagent for glycolipids.

Genomic DNA of strain ZX-21^T was extracted using the bacterial genomic DNA fast extraction kit (DongSheng Biotech). The 16S rRNA gene was amplified by PCR using the bacterial domain-specific primer pair 27F (5′-GAGAG TTTGATCCTGGCTCAG-3′) and 1492R (5′-GTCGTAACAAGGTAGCCGTA-3′). PCR products were cloned into vector pMD 19 T (TaKaRa) and sequenced to determine the almost-complete sequence of the 16S rRNA gene. Then, the almost-complete 16S rRNA gene sequence of strain ZX-21^T was identified by using the EzTaxon-e server [13]. Phylogenetic analysis was performed in ARB release 6.0.2 [14] in the All-Species Living Tree Project database (LTPs128) [15] using the maximum-likelihood method. Based on the obtained EzTaxon-e and phylogenetic resolution results, further trees were reconstructed using the MEGA 5.0 program package [16] while multiple sequences were aligned by using CLUSTAL_W [17]. Phylogenetic trees were reconstructed using the neighbour-joining (NJ) [18], maximum-parsimony (MP) [19] and maximum-likelihood (ML) [20] methods. Bootstrap analysis (1000 replications) was used to evaluate the topology of the trees. Evolutionary distances were calculated for the three methods according to Kimura's two-parameter model [21].

The genome of ZX-21^T was sequencing using the Illumina HiSeq 4000 system at the Beijing Genomics Institute (Shenzhen, China). Genomic DNA was sheared randomly to reconstruct three read libraries by a Bioruptor ultrasonicator (Diagenode) and physico–chemical methods. The sequencing generated approximate 1 Gb clean data (approximately 260-fold genome coverage). The *de novo* assembly of the reads was performed by using ABySS 2.0.2[22]. The assembly *k*-value was tested from 32 to 64 to find the optimal *k*-value using the abyss-pe script. The quality of microbial genomes was assessed using the bioinformatic tool CheckM [23]. The DNA G+C content was obtained from the genomic sequences.

Cells of strain ZX-21^T were Gram-stain-negative, aerobic, non-spore-forming, motile, rod-shaped (0.3–0.8 μm wide and 1.5–2.5 μm long) and had more than one flagellum (Fig. S1). Colonies were buff, convex, smooth, circular with entire margins and 0.2–0.8 mm in diameter after incubation on MA at 30 °C for 5 days. The temperature range for growth was 10–35 °C (optimum, 30 °C). Growth occurred at pH 6.0–8.5 (optimum, pH 6.5–7.0) and with 0.5–8.0 % (w/v)

NaCl (optimum at 3.0–4.0 %). No growth was observed without NaCl or at 4 °C after 1 month's incubation. Strain ZX-21^T was positive for oxidase, catalase and hydrolysis of Tweens 20, 40, 60 and 80. The isolate was susceptible to (μg/disc, unless stated otherwise): chloramphenicol (30), kanamycin (30), ofloxacin (5), penicillin (10 IU), rifampicin (5), streptomycin (10), and was resistant to cefradine (30), erythromycin (10), tetracycline (30) and vancomycin (30). Detailed physiological and biochemical characteristics are given in the species description, Table 1 1–3, 24and Table S1 (available in the online version of this article).

The nearly complete 16S rRNA gene sequence (1389 nt) of strain ZX-21^T was obtained (accession number KY857838). On the basis of the EzTaxon service results (16S rRNA gene sequence alignment), strain ZX-21^T exhibited the highest similarity value to *Z. guokunii* (96.83 %), and was related closely to another three species belonging to the genus *Zhongshania: Z. borealis* (96.69 %), *Z. antarctica* (96.26 %) and *Z. aliphaticivorans* (96.01 %). Strain ZX-21^T exhibited less than 96.0 % sequence similarity to type strains of genera *Spongiibacter* (92.94–94.38 %) and *Dasania* (91.86 %) within the family *Spongiibacteraceae*. The All-Species Living Tree indicated that the genus *Zhongshania* forms a monophyletic clade and strain ZX-21^T represents a separate branch next to the cluster comprising the *Zhongshania* species (Fig. S2). In the phylogenetic tree based on the ML method, strain ZX-21^T constituted an independent lineage within the family *Spongiibacteraceae* with 100 % bootstrap support (Fig. 1). Similar results were obtained using NJ and MP algorithms. The genome completeness of strain ZX-21^T was 100 % with 1.8 % contamination. Genome sequences estimated to be ≥95 % complete, and with ≤5 % contamination, were considered as excellent reference genomes for deeper analyses [23]. The genome sequence of strain ZX-21^T comprised 49 contigs. The N50 and L50 values were 13 4147 and 10, respectively. The number of predicted genes was 3890. The GenBank accession number for the whole genome sequence of strain ZX-21^T is PQGG00000000. The G+C content of strain ZX-21^T was 49.1 %, which was lower than those of other members of the genus *Zhongshania*: *Z. borealis* (53.6 %), *Z. antarctica* (51.5 %), *Z. aliphaticivorans* (52.2 %) and *Z. guokunii* (51.8 %) [1–3].

The chemotaxonomic data supported that strain ZX-21^T had something in common with the family *Spongiibacteraceae*. Respiratory quinone composition analysis showed that the only isoprenoid quinone in strain ZX-21^T was Q-8, which is same as the type species in the family *Spongiibacteraceae*.

Fatty acid analysis of strain ZX-21^T revealed that summed feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$; 22.8 %), summed feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$; 21.0 %) and $C_{16:0}$ (8.9 %) were the major cellular fatty acids (Table 2). The cellular fatty acid profiles of strain ZX-21^T and the other type strains

Table 1. Comparisons between strain ZX-21^T and the type strains of genus *Zhongshania* and *S. marinus*

Strain: 1, ZX-21^T (this study); 2, *Z. guokunii* CCTCC AB 209247^T (this study); 3, *Z. antarctica* CCTCC AB 209246^T (this study); 4, *Z. aliphaticivorans* SM-2^T (data from Lo *et al.* [2]); 5, *Z. borealis* CL-AS9^T (Jang *et al.* [3]); 6, *Spongiibacter marinus* DSM 17750^T (Graeber *et al.* [24]). +, Positive; -, negative; w, weakly positive; ND, no data available.

Characteristic	1	2	3	4	5	6
Isolation source	Surface seawater	Surface seawater	Antarctic fast ice	Sea-tidal flat	Coastal seawater	Marine sponge
Colony colour	Buff	Ivory	Ivory	Ivory	Creamy	Beige
Catalase activity	+	+	+	+	+	w
Temperature range for growth (optimum)(°C)	10–35 (30)	4–35 (30)*	4–35 (30)*	10–37 (25–30)	4–30–(20–25)	10–40 (20–30)
pH range for growth (optimum)	6.0–8.5 (6.5–7.0)	5.5–9.0 (6.5–7.5)*	5.5–9.0 (7.0–7.5)*	5.5–8.5 (7.0–7.5)	5.8–9.3 (6.6–8.0)	6.5–9.5 (7.0–9.0)
NaCl tolerance (optimum) (%, w/v)	0.5–8.0 (3.0–4.0)	0–7.5 (2.5–3.5)†	0–9.0 (0.5–3.0)†	0–11.0 (2.0)	1.0–8.0 (3.0–4.0)	1.0–7.0 (3.0)
Hydrolysis of:						
Starch	–	–	–	+	–	–
Tween 40	+	+	+	ND	+	+
Tween 80	+	+	–	+	+	+
Enzyme activities: (API 20NE):						
D-Glucose	+	+	–	+	+	–
Maltose	–	–	+	–	w	–
Malic acid	+	+	w	+	w	–
Trisodium citrate	–	+	–	–	–	+
Phenylacetic acid	–	+	–	–	w	w
Major polar lipids	PG, DPG, PE	PG, DPG, PE	PG, DPG, PE	PG, DPG, PE	PG, DPG	ND
DNA G+C content (mol%)	49.1	51.8*	51.5*	52.2	53.6	69.1

*Data from Li *et al.* [1].

†DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol.

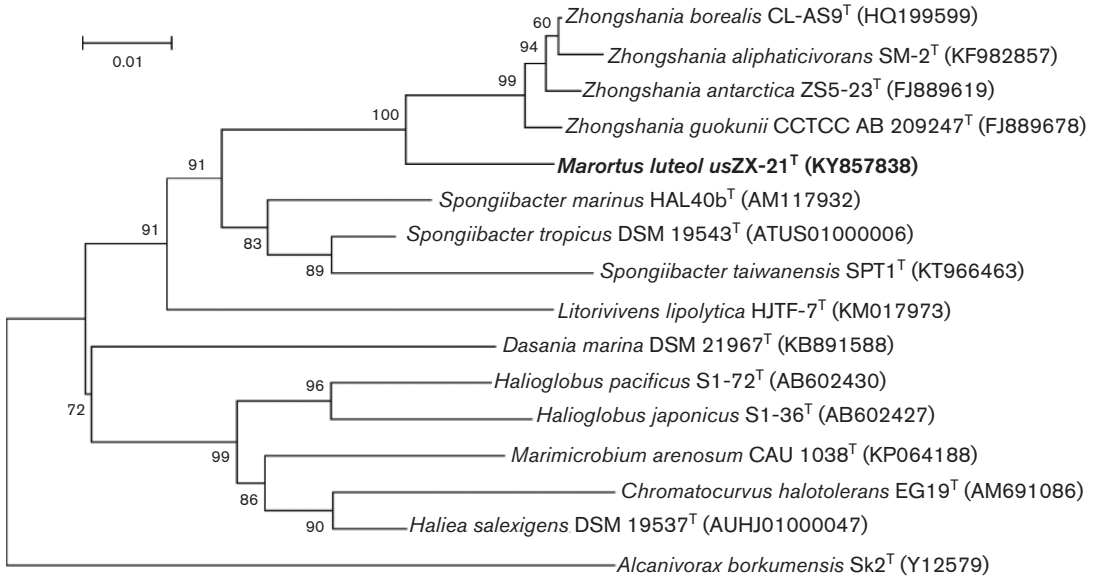

Fig. 1. Maximum-likelihood phylogenetic tree using Kimura's two-parameter model based on 16S rRNA gene sequences, showing the phylogenetic relationships of strain ZX-21^T and its related taxa. Bootstrap values (>50 %) based on 1000 replications are shown at branch nodes. Solid circles indicate that the corresponding nodes were also recovered in the trees generated by using the neighbour-joining and maximum-parsimony trees. Bar, 0.01 substitutions per nucleotide position.

were very different. In detail, two fatty acids, $C_{17:0}$ and $C_{17:1}\omega 8c$, were the major fatty acids of other strains belonging to the genus *Zhongshania* and the type strain of the genus *Spongiibacter*: *Z. guokunii* (16.3 and 46.5 %, respectively), *Z. antarctica* (15.8 and 41.6 %, respectively), *Z. aliphaticivorans* (9.4 and 31.0 %, respectively), *Z. borealis* (7.2 and 22.1 %, respectively) and *S. marinus* (9.6 and 51.7 %, respectively), but they were strikingly lower in strain ZX-21^T (1.8 and 6.8 %, respectively). In addition, many branched fatty acids and hydroxylated fatty acids were found in strain ZX-21^T, but they were not found in other strains in the genus *Zhongshania* or the proportions in those strains were much lower than in strain ZX-21^T. For example, iso-$C_{13:0}$ was found in ZX-21^T (2.9 %), but not detected in other strains in the genus *Zhongshania* and the proportions in *S. marinus* (0.5 %) were lower than that in ZX-21^T. The percentages of iso-$C_{15:0}$ and iso-$C_{11:0}$ 3-OH in strain ZX-21^T (3.8 and 6.1 %, respectively) were higher than those found in the type strains of the genus *Zhongshania* ($\leq$1.2 % and $\leq$2.9 %, respectively) [1–3, 24].

The polar lipid profile of strain ZX-21^T contained phosphatidylethanolamine (PE), phosphatidylglycerol (PG), diphosphatidylglycerol (DPG) and one uncharacterized glycolipid (GL2) as major polar lipids. In addition, strain ZX-21^T also contained one additional unidentified glycolipid, two unidentified phospholipids and three uncharacterized lipids as minor components (Fig. S3). PG and DPG are the major polar lipids found in most members of the family *Spongiibacteraceae*. Also, PE is a major polar lipid for members of the genus *Zhongshania* except *Z. borealis* (Table 1) [2, 3]. The chemotaxonomic results also showed clear differences in the polar lipid profiles among strain ZX-21^T and the reference strains. An unidentified glycolipid (GL2) was detected in strain ZX-21^T as one of the major polar lipids, which was not found in the reference strains (Fig. S3). Two unidentified phospholipids (PL1 and PL2) and two unidentified lipids (L2 and L3) were present in strain ZX-21^T in moderate or minor amounts, while they were not detected in the reference strains, which made it easy for us to clearly distinguish ZX-21^T from the reference strains. In addition, one unidentified aminolipid (AL2) and one unidentified lipids (L5) were present in the reference strains, while it was absent from strain ZX-21^T.

Besides the cellular fatty acid, polar lipid and phylogenetic analyses, strain ZX-21^T could be definitively differentiated from the members of the family *Spongiibacteraceae* based on several physiological and phenotypic characteristics, such as NaCl or temperature range and optimum, hydrolysis of Tweens 20, 40, 60 and 80, and enzyme activities in API tests (Tables 1 and S1). For instance, strain ZX-21^T could not grow at 4 °C or without NaCl, while most of its close relatives were able to grow at 4 °C or without NaCl. Especially, the colony colour of all members of genus *Zhongshania* and Spongiibacter was ivory, creamy or beige, but the colony colour of strain ZX-21^T was buff. In addition, the cells of all members of genera *Zhongshania* and *Spongiibacter* were rod-shaped with a single polar flagellum, but more flagella were observed in the isolate of strain ZX-21^T [1–3, 24].

As the phylogentic analysis using all algorithms (NJ, MP, ML) showed that the isolate formed a tight phylogenic

Table 2. Cellular fatty acid profile (%) of strain ZX-21^T and the type strains of related species *Z. guokunii* CCTCC AB 209247^T and *Z. antarctica* CCTCC AB 209246^T

Strains: 1, ZX-21^T; 2, *Z. guokunii* CCTCC AB 209247^T; 3, *Z. antarctica* CCTCC AB 209246^T. All data were obtained from this study. Fatty acids that represented <0.5 % in all strains were omitted. Fatty acids that represented >10 % are in bold. –, Not detected.

Fatty acid	1	2	3
Straight chain			
$C_{10:0}$	0.8	–	0.2
$C_{11:0}$	0.4	2.0	1.3
$C_{12:0}$	1.7	0.4	0.4
$C_{14:0}$	5.4	0.7	0.7
$C_{16:0}$	8.9	4.4	6.7
$C_{17:0}$	1.8	**16.3**	**15.8**
$C_{18:0}$	1.6	1.0	0.9
Branched			
iso-$C_{13:0}$	2.9	–	–
iso-$C_{15:0}$	3.8	–	–
iso-$C_{17:0}$	1.5	–	–
Hydroxy			
$C_{10:0}$ 3-OH	4.0	1.3	1.0
$C_{11:0}$ 3-OH	2.4	8.1	4.0
$C_{12:0}$ 3-OH	1.4	0.2	0.1
iso-$C_{11:0}$ 3-OH	6.1	0.5	0.2
Unsaturated			
$C_{15:1}\omega6c$	0.2	1.6	0.6
$C_{17:1}\omega8c$	6.8	**46.5**	**41.6**
Summed features*			
3	**22.8**	9.8	**13.9**
8	**21.0**	4.8	8.6

*Summed features represent groups of two fatty acids that could not be separated by GLC with the MIDI system. Summed feature 3 contained $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$; summed feature 8 contained $C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$.

lineage with the members of the genus *Zhongshania* with 100 % bootstrap support, we now discuss why the isolate should represent a new genus, not a new species of *Zhongshania*. First, although forming a tight lineage with the genus *Zhongshania*, the novel strain represents a separate branch next to the monophyletic cluster containing all type strains of the currently described *Zhongshania* species. The most important reason is that the fatty acid comparison showed that strain ZX-21^T and strains of the genus *Zhongshania* have quite different compositions. Major fatty acids are different. In particular, the branched fatty acids of strain ZX-21^T are not identified in strains of the genus *Zhongshania*. In addition, there are clear differences in polar lipid profiles among strain ZX-21^T and the reference strains. As for phenotypic properties, the major difference is that more flagella were observed in the isolate of strain ZX-21^T. These results suggest that ZX-21^T should be a new genus, not a new species of the genus *Zhongshania*.

The low level of 16S rRNA gene sequence similarity between strain ZX-21^T and all other members of the family *Spongiibacteraceae*, together with the biochemical and physiological tests, chemotaxonomic results, and the phenotypic characteristics, suggest that strain ZX-21^T represents a novel species of a new genus within the family *Spongiibacteraceae*, for which the name *Marortus luteolus* gen. nov., sp. nov. is proposed.

DESCRIPTION OF *MARORTUS* GEN. NOV.

Marortus (Ma.r or′tus. L. n. *mare* sea; L. past. part. *ortus* risen; N.L. masc. n. *Marortus* risen from the sea).

Cells are Gram-stain-negative, aerobic, non-spore-forming and rod-shaped. Motile with one or more than one flagellum. Catalase- and oxidase-positive. Nitrates are reduced to nitrites. NaCl is needed for growth. The major respiratory quinone is Q-8. The major fatty acids are summed feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$), summed feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$) and $C_{16:0}$. The principal polar lipids include PE, PG, DPG and one uncharacterized glycolipid (GL2). Phylogenetically, the genus belongs to the family *Spongiibacteraceae* of the order *Cellvibrionales*. The type species is *Marortus luteolus*.

DESCRIPTION OF *MARORTUS LUTEOLUS* SP. NOV.

Marortus luteolus (lu.te′o.lus L. masc. adj. *luteolus* yellow, the colour of colonies or pigment that the bacterium produces).

Description is as for the genus plus the following characteristics. Cells are 0.3–0.8 μm wide and 1.5–2.5 μm long with more than one flagellum. Colonies are buff, convex, smooth, circular with entire margins and 0.2–0.8 mm in diameter after incubation on MA at 30 °C for 5 days. Growth occurs at 10–35 °C (optimum, 30 °C), but not above 37 °C or lower than 4 °C. Growth occurs at pH 6.0–8.5 (optimum, pH 6.5–7.0) and with 0.5–8 % NaCl (3–4 %). It cannot grow when pH is lower than pH 5.5 or higher than pH 9.0. NaCl is necessary for growth, but it cannot grow with 10 % NaCl. Nitrate can be reduced to nitrite, but not to nitrogen. Tweens 20, 40, 60 and 80 are hydrolysed, but starch, casein, tyrosine, CM-cellulose and gelatin are not. In API ZYM tests, alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and *N*-acetyl-β-glucosaminidase activities are positive; and valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, α-mannosidase and α-fucosidase activities are negative. In API 20NE tests, reduction of nitrates to nitrites, fermentation of D-glucose, hydrolysis of aesculin, activity of arginine dihydrolase, urease and β-galactosidase, and assimilation of D-glucose, D-mannose, D-mannitol, potassium gluconate, capric acid, adipic acid and malic acid are positive, but negative results are observed for reduction of nitrates to

nitrogen, production of indole, hydrolysis of gelatin, and assimilation of L-arabinose, *N*-acetyl- glucosamine, maltose, trisodium citrate and phenylacetic acid. The only respiratory quinone is Q-8. The major fatty acids are summed feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$), summed feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$) and $C_{16:0}$. The principal polar lipids are PE, PG, DPG and GL2.

The type strain is ZX-21^T (=MCCC 1K03431^T=KCTC 62160^T), isolated from surface seawater of the East Sea, China. The DNA G+C content is 49.1 mol% (by genome, GenBank accession number: PQGG00000000).

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Conflicts of interest
The authors declare that are no conflicts of interest.

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