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1 *Parasedimentitalea psychrophila* sp. nov., a psychrophilic 2 bacterium isolated from deep-sea sediment

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17 ;

18 Keywords: *Parasedimentitalea psychrophila*; psychrophile; deep-sea;

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21 The GenBank/EMBL/DDBJ accession number for the 16S rRNA sequence of strain QS115^T
22 is MK801282. The complete genome sequence of QS115^T has been deposited at
23 GenBank/ENA/DDBJ under the accession number CP127247 (Chromosome) and CP127248
24 - CP127250 (Plasmids).

25 Abstract

26 A novel bacterium strain QS115^T was isolated from deep-sea sediment collected from the South
27 China Sea at a depth of 1,151 m. BlastN analyses showed 98.21 % sequence identity between 16S
28 rRNA gene sequences of QS115^T and that of the most closely related strain *Parasedimentitalea*
29 *marina* W43^T. We sequenced strain QS115^T genome and found it shared 82.39 % average nucleotide
30 identity (ANI), 26.3 % digital DNA–DNA hybridization (dDDH), and 85.32 % average amino acid

31 identity (AAI) with the type strain of *P. marina* W43^T. Cells of strain QS115^T were Gram-stain-
32 negative, rod-shaped, and grew optimally at 10 °C, pH 7.5 and 2 % (w/v) NaCl. The principal fatty
33 acids were summed feature 8 (C_{18:1} ω7c/ω6c), major respiratory quinone was ubiquinone-10 and
34 predominant polar lipids were diphosphatidylglycerol, phosphatidylethanolamine, glycerophospholipid,
35 phosphatidylglycerol, and phosphatidylcholine. Polyphasic analyses of physiological, phenotypic
36 characteristics and genomic analyses suggested that strain QS115^T represents a novel species of the
37 genus *Parasedimentitalea*, for which the name *Parasedimentitalea psychrophila* sp. nov. is proposed
38 (type strain QS115^T = MCCC 1K04395^T = JCM 34219^T).

39 Introduction

40 The deep-sea accounts for approximately 75 % volume of the ocean and comprises over 50 % of
41 ocean prokaryotes(1). The deep-sea is an extreme habitat characterized with high hydrostatic pressure
42 and low temperature in general. It is now well-known that high pressure and low temperature had
43 similar impact on microbial cells. For example, they both reduce the fluidity of membrane and induce
44 the synthesis of unsaturated fatty acids in deep-sea microbes(2-4). However, the study of microbial
45 cold- and high-pressure adaptation mechanism has been carried out in limited number of strains, due
46 to the difficulties of cultivation of deep-sea microorganisms.

47 The genus *Parasedimentitalea* was first described in 2019 (5). It belongs to the family
48 *Rhodobacteraceae* and comprises a single species of *P. marina*
49 (<https://lpsn.dsmz.de/genus/parasedimentitalea>). The type strain of *P. marina* W43^T was isolated from
50 deep-sea water of the New Britain Trench at a depth of 4,000 m. It is Gram-stain-negative and
51 contains phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol as dominant
52 polar lipids. The predominant cellular fatty acids are summed feature 8. Strain W43^T was
53 psychrotolerant with optimal temperatures of 16 – 20 °C and abundant genomic features involved in
54 cold adaptation have been identified in its genome(6). The novel candidate strain QS115^T of the genus
55 *Parasedimentitalea* described in this study was isolated from the deep-sea sediment at a depth of
56 1,151 m in the South China Sea. It was psychrophilic and is proposed to represent a new species with
57 the name *Parasedimentitalea psychrophila* sp. nov.

58 Isolation and Ecology

59 Sediment samples were collected using pushcore mounted on HOV “*shen hai yong shi*” at a depth of
60 1,151 m from the South China Sea (19°16' 4.8" N, 114° 21' 1.08" E) in March 2018. Pushcore sample
61 was sliced at 2 cm intervals on board. Surface sediment was suspended and diluted with filtered sea
62 water. A 100 µl aliquot with different dilution (10⁻³, 10⁻⁴ and 10⁻⁵) was plated on Agar Medium

63 (24.4770 g NaCl, 3.9170 g Na₂SO₄, 0.0960 g KBr, 0.0403 g SrCl₂·6H₂O, 4.9810 g MgCl₂·6H₂O,
64 1.2558 g CaCl₂·2H₂O, 0.1920 g NaHCO₃, 0.0260 g H₃BO₃, 0.0039 g NaF, 0.0010 g FePO₄, 0.5 g
65 peptone, 0.1 g yeast extract and 15 g Bacto agar in 1 L distilled water). The plates were incubated at
66 10 °C. Colonies that showed different morphologies were selected and purified by repeated streaking
67 on the same medium. The purified strain QS115^T was routinely conserved in Marine broth 2216
68 medium (MB; BD Difco) with 25 % (v/v) glycerol at -80 °C. The cultivation of strain QS115^T for
69 morphological and biochemical analyses was carried out on MB and MA medium, unless otherwise
70 noted.

71 16S rRNA Gene Phylogeny

72 Strain QS115^T was collected by centrifugation (12,000 rpm, 10 min) at the logarithmic phase,
73 genomic DNA of QS115^T was extracted using TIANamp Bacteria DNA Kit (TIANGEN Biotech Co.,
74 LTD). The 16S rRNA gene was amplified by PCR with the universal primers 27F (5'-
75 AGTTTGATCATGGCTCAG-3') and 1492R (5'-GGYTACCTTGTTACGACTT-3'). The amplified
76 16S rRNA gene was sequenced by company (Beijing Ruibio Biotech Co., Ltd), and a nearly complete
77 16S rRNA gene sequence (1,346 bp) of strain QS115^T was obtained and has been deposited in the
78 Genbank database under the accession number MK801282. Sequence similarity was determined by
79 Blast against the EzBioCloud database (<http://www.ezbiocloud.net/>) (7), and the result suggested that
80 strain QS115^T shared the highest similarity with *Parasedimentitalea marina* W43^T (98.21 % of 16S
81 rRNA sequence identity), followed by *Zongyalingia marina* DSW4-44^T (98.07 %). Phylogenetic
82 trees were constructed with MEGA X (8) using maximum-likelihood(9), neighbor-joining (10) and
83 maximum-parsimony (11) methods. The topologies of the phylogenetic trees were evaluated by
84 bootstrap analyses based on 1,000 replications. As shown in **Figure 1**, strain QS115^T robustly
85 branched with *P. marina* W43^T, and together they formed a cluster with *Z. marina* DSW4-44^T and *Z.*
86 *huanghaiensis* CY05^T. The phylogenetic topologies of these closely related strains were also
87 confirmed using maximum-likelihood and minimum-parsimony methods (**Figure S1 and S2**,
88 **Supporting Information**).

89 Genomic Features and Phylogenomic Analysis

90 The genome sequence was determined by BGI-Qingdao (Qingdao, China) using the BGISEQ-500
91 platform. More than 1.05 Gb clean data were generated to reach approximately 219-fold depth of
92 coverage. The resulting complete genome of strain QS115^T contained 5,005,898 bp with a G + C
93 content of 57.71 %, consisted of a circular chromosome of 4,839,724 bp and three circular plasmids
94 ranging from 16,332 bp to 91,805 bp in length. A total of 4,819 protein-coding genes, 83 tRNA genes
95 and 21 rRNA genes were identified. The genome of strain QS115^T was deposited at the Genbank

96 database under the accession number CP127247 for chromosome and CP127248 - CP127250 for
97 plasmids.

98 A genome-based phylogenetic tree was reconstructed based on an up-to-date bacterial core gene set
99 (UBCG) consisting of 120 single-copy core genes via GTDB-Tk (v 1.7.0) (12). The whole-genome-
100 based phylogenetic tree showed that strain QS115^T formed a distinct branch with *P. marina* W43^T,
101 and together with the branch of *Z. marina* DSW4-44^T and *Z. huanghaiensis* CY05^T (**Figure 2**). The
102 genome-based similarity between strain QS115^T and closely related species was evaluated by two
103 indices at DNA level: average nucleotide identities (ANI) (13) and in silico digital DNA-DNA
104 hybridization (dDDH) (14), and two indices at protein level: average amino acid identities (AAI) (15)
105 and percent of conserved proteins (POCP) (16) (**Table S1**). The ANI values between strain QS115^T
106 and *P. marina* W43^T, *Z. marina* DSW4-44^T and *Z. huanghaiensis* CY05^T were 82.39 %, 77.62 % and
107 77.56 % identical, respectively. The in silico dDDH value was calculated by the Genome-to-Genome
108 Distance Calculator 3.0. (17) [the recommended results from formula 2 (identities/high-scoring
109 segment pairs length), <http://ggdc.dsmz.de/ggdc.php>], the results indicated that strain QS115^T shared
110 26.3 %, 21.5 %, and 21.4 % DNA relatedness with *P. marina* W43^T, *Z. marina* DSW4-44^T and *Z.*
111 *huanghaiensis* CY05^T, respectively. The AAI percentages between strain QS115^T and the reference
112 strains were 85.32 %, 81.17 % and 80.93 % identical, respectively, and the POCP values between
113 them ranged from 68.83 % to 70.70 %. The threshold values for bacterial species discrimination were
114 95-96 % for ANI(18), 70% for DDH(14), 65-95% for AAI(15), and 50-80 % for POCP(19).
115 Therefore, the genome-based similarity between strain QS115^T and closely related species were below
116 the threshold value for bacterial species discrimination with respect to all four indices analyzed,
117 indicating a novel genomic species of the genus *Parasedimentitalea*.

118 Morphological and Physiological Characteristics

119 Strain QS115^T formed smooth, white colonies with regular edges on MA medium after 20 days'
120 incubation at 10 °C. Phase-contrast microscopy (BX53 with a DP80 digital camera, Olympus) and
121 transmission electron microscopy (JEM-1230, JEOL Ltd.) observation showed that QS115^T cells were
122 Gram-stain-negative and polar flagellated rods (**Figure 3**), with a size of 3.5 - 4.5 µm in length and
123 0.5 - 1.0 µm in width. The motility of the cells was determined using semi-solid agar and the puncture
124 method. Cells of strain QS115^T could diffuse on the semi-solid agar and grow along the puncture line,
125 while strain W43^T couldn't.

126 The optimal growth conditions of strain QS115^T were analyzed in MB medium. The tolerance of
127 temperature was tested at 4, 10, 15, 20, 25, 30, 37 and 50 °C. The pH range [pH 5.0 - 9.0 in 0.5 unit
128 increments, were adjusted using 10 mM MES (pH 5.0 - 6.0), PIPES (pH 6.5 - 7.5) and HEPES (pH

129 8.0 - 9.0) buffers, respectively] and tolerance to NaCl (0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0,
130 7.0 and 10.0 %, w/v) were determined as previously described (20). All experiments were carried out
131 in triplicate unless stated otherwise. Growth was recorded by measuring optical density at 600 nm
132 (OD_{600nm}) using a UV-Vis spectrophotometer (Cary60, Agilent Technologies) as previously
133 described(21). Growth rates were calculated as described by Li *et al* (22). Marine broth with the
134 addition of 10 mM NaNO₃ was prepared in an anaerobic glove box for the evaluation of growth under
135 anaerobic condition. The inoculated anaerobic medium was transferred into the Hungate tubes and
136 placed at 10 °C for 30 days (23). No growth was observed for strain QS115^T and any of the reference
137 strains after 30 days' incubation under anaerobic condition with NaNO₃ as electron acceptor.

138 Growth of strain QS115^T was observed at temperatures ranging from 4 °C to 20 °C, with an optimal
139 growth temperature at 10 °C (**Table 1 and Figure S3, Supporting Information**). It was noted that all
140 three reference strains analyzed were able to grow at 25 °C with an optimal growth temperature over
141 20 °C (**Table 1**). The difference suggested that the psychrophilic strain QS115^T was better adapted to
142 the low-temperature environment. The pH and NaCl range for growth of strain QS115^T were pH 7.0
143 to 8.0 (optimum, pH 7.5) and 1.0 - 5.0 % NaCl (optimum, 2.0 %) (**Table 1 and Figure S3**).
144 Compared to the reference strains, the strain QS115^T has a lower tolerance to NaCl and preference for
145 neutral pH.

146 Oxidase and catalase activity, and hydrolysis of hypoxanthine, L-tyrosine, starch, casein, xanthine,
147 xylan and Tween 80 were tested on MA medium. Strain QS115^T was negative for catalase and
148 oxidase activities. It was capable of utilizing xylan, xanthine, and Tween 80, but failed for hydrolysis
149 of hypoxanthine and L-tyrosine. However, the difference was the closely reference strain *P. marina*
150 W43^T cannot utilize xanthine, *Z. huanghaiensis* CY05^T could not utilize any of the five substances
151 mentioned above, *Z. marina* DSW4-44^T could utilize them except xylan. Carbon utilization was tested
152 on basal agar medium supplemented with yeast extract (23.60 g NaCl, 0.64 g KCl, 4.53 g
153 MgCl₂·6H₂O, 5.94 g MgSO₄·7H₂O, 1.30 g CaCl₂·2H₂O, 0.20 g NaNO₃, 0.20 g NH₄Cl, 15 g Bacto
154 agar, 0.05 g yeast extract, 1 L distilled water) containing 0.2 % carbon source(24, 25). Strain QS115^T
155 could utilize pyruvic acid as carbon source, but not betaine, lysine or proline, which differed it from
156 *P. marina* W43^T. Biolog assay suggested that L-malic acid, TWEEN 20, β-hydroxy-D, L butyric acid,
157 sodium butyrate, propionic acid and acetic acid could be utilized by *P. marina* W43^T but not strain
158 QS115^T. Biochemical tests were carried out using API 20NE and API ZYM strips (bioMérieux)
159 according to the manufacturer's instructions. The strain QS115^T differed from phylogenetically
160 related taxa by nitrate reduction, denitrification, presence of arginine dihydrolase, urease, β-
161 galactosidase and protease, and assimilation of D-glucose, sodium citrate and phenylacetic acid. The

162 morphological, physiological and biochemical characteristics of the strain QS115^T and closely related
163 species were presented in **Table 1**.

164 Chemotaxonomic Characteristics

165 Cells of strain QS115^T and closely related species (*P. marina* W43^T, *Z. huanghaiensis* CY05^T and *Z.*
166 *marina* DSW4-44^T) grown to exponential phase were collected for the analysis of fatty acids. Fatty
167 acids were prepared by Beijing Biogenro (Beijing Biogenro Biotechnology Co., LTD). Cellular fatty
168 acids were identified and quantified by gas chromatograph (Agilent Technologies 56890N) with the
169 standard Microbial Identification System (MIDI Calibration Mix 1). Similar to the most closely
170 related species, the major fatty acids of strain QS115^T were summed feature 8 (C_{18:1} ω7c/ω6c), but
171 with a relatively lower proportion (54.4 % in strain QS115^T versus 89.4 % in strain W43^T) (**Table 2**).
172 Meanwhile, strain QS115^T had a higher amount of C_{16:0}, C_{10:0} 3-OH, C_{16:0} 2-OH, C_{16:1} 2-OH and
173 summed feature 3 (C_{16:1} ω7c/ω6c).

174 Polar lipids were extracted from 100 mg of freeze-dried cellular material, separated by two-
175 dimensional thin-layer chromatography (TLC) and then identified according to a previously described
176 method (26). Diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol,
177 phosphatidylcholine and glycopospholipid were detected in strain QS115^T (**Figure S4, Supporting**
178 **Information**). The composition of polar lipids in strain QS115^T was similar to *P. marina* W43^T
179 except for glycopospholipid, which was not reported in this strain. By means of a high-performance
180 liquid chromatography-mass spectrometry system (HPLC-MS, Agilent), the isoprenoid quinone was
181 measured. The major respiratory quinone of strain QS115^T was ubiquinone 10, which was in
182 accordance with the properties of the family *Rhodobacteraceae*.

183 Description of *Parasedimentitalea psychrophila* sp. nov.

184 *Parasedimentitalea psychrophila* sp. nov. [psy.chro'phi.la. Gr. adj. psychros cold; N.L. adj. philus
185 (from Gr. adj. philos) loving; N.L. fem. adj. psychrophila referring to loving low temperatures].

186 Cells are Gram-stain-negative, mobile, rod-shaped with approximately 0.5 - 1.0 μm in width and 3.5 -
187 4.5 μm in length, aerobic, and oxidase- and catalase-negative. Colonies are smooth with regular
188 edges, and 0.5 - 1.0 mm in diameter after 20 day's culture on MA medium. Growth occurs at 4 - 20
189 °C (optimum 10 °C), from pH 7.0 - 8.0 (optimum pH 7.5), and in the presence of 1.0 - 5.0 % (w/v)
190 NaCl (optimum 2.0 %). Positive for hydrolysis of xylan, xanthine and Tween 80. Negative for
191 hydrolysis of hypoxanthine, L-tyrosine, casein, DNA, and starch. Strain QS115^T can utilize pyruvic
192 acid as the sole carbon source for growth, but not lysine, succinic acid, betaine, proline, and histidine.

193 In the test of API 20NE, strain QS115^T cannot utilize D-glucose, L-arabinose, D-mannose, D-
194 mannitol, N-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid,
195 trisodium citrate, and phenylacetic acid. Positive for β-galactosidase, but negative for reduction of
196 nitrate to nitrite, reduction of denitrification, indole production, D-glucose fermentation, arginine
197 dihydrolase, urease, β-glucosidase or gelatin hydrolysis. In the test of API ZYM, strain QS115^T is
198 positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, and acid
199 phosphatase; weak positive for lipase (C14), valine arylamidase, and naphthol-AS-BI-
200 phosphohydrolase; negative for cystine arylamidase, trypsin, α-chymotrypsin α-galactosidase, β-
201 galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, n-acetyl-β-glucosaminidase, α-
202 mannosidase, and α-fucosidase. The major ubiquinone is ubiquinone 10. The major fatty acids are
203 summed feature 8 (C_{18:1} ω7c/ω6c). The predominant polar lipids are diphosphatidylglycerol,
204 phosphatidylethanolamine, glycerophospholipid, phosphatidylglycerol and phosphatidylcholine. The
205 DNA G + C content of the type strain is 57.71 %.

206 The type strain, QS115^T (= MCCC 1K04395^T = JCM 34219^T), was isolated from a deep-sea sediment
207 sample (1,151 m) collected from the South China Sea (119°16' 4.8" N, 114° 21' 1.08" E). The
208 GenBank accession numbers for the 16S rRNA gene and genome sequences of strain QS115^T are
209 MK801282 and CP127247- CP127250, respectively.

210 **Author statements**

211 **1.1 Author contributions**

212 A.-Q.L.: conceptualization, investigation, formal analysis, validation, visualization, writing-original
213 draft preparation. C.Z.: conceptualization, investigation. D.-H.L.: formal analysis, visualization. X.-
214 Q.Q.: investigation, project administration. L.M.: supervision. L.-F.W.: supervision. X.-G.L.:
215 supervision, writing-review and editing. W.-J.Z.: conceptualization, writing-original draft preparation,
216 writing-review and editing.

217 **1.2 Conflicts of interest**

218 The authors declare that there are no conflicts of interest.

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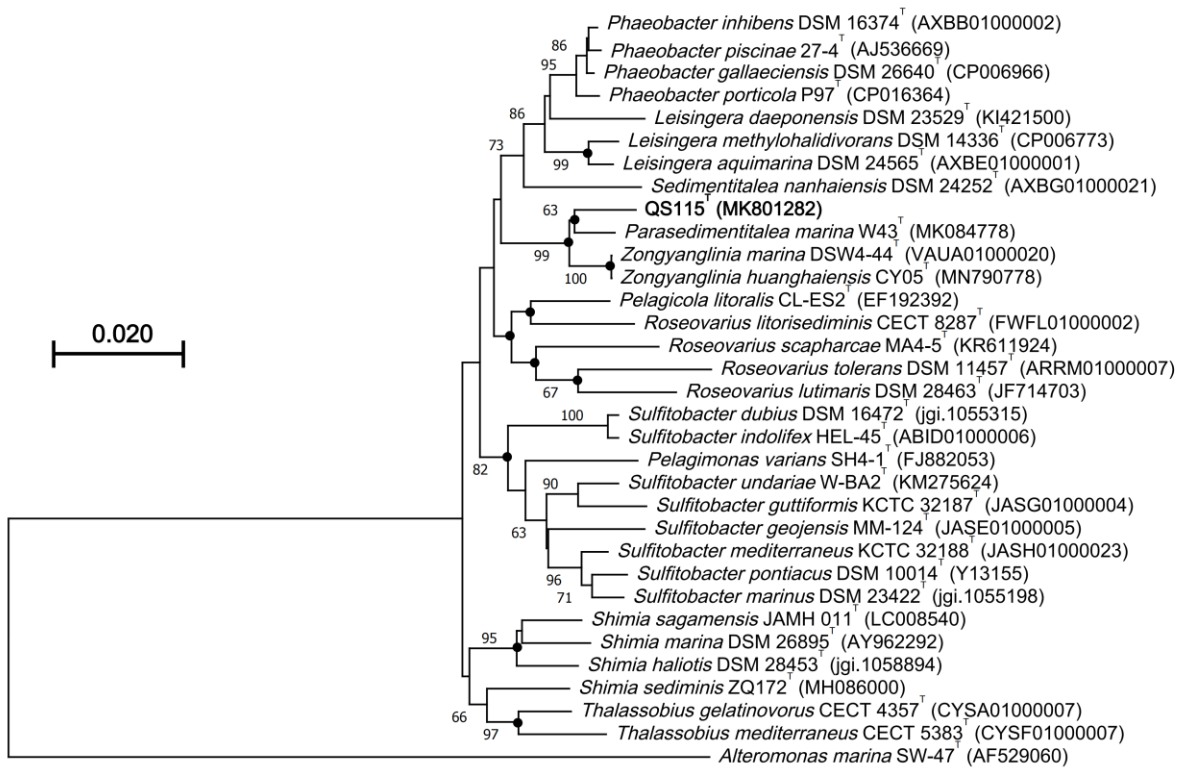
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227 References

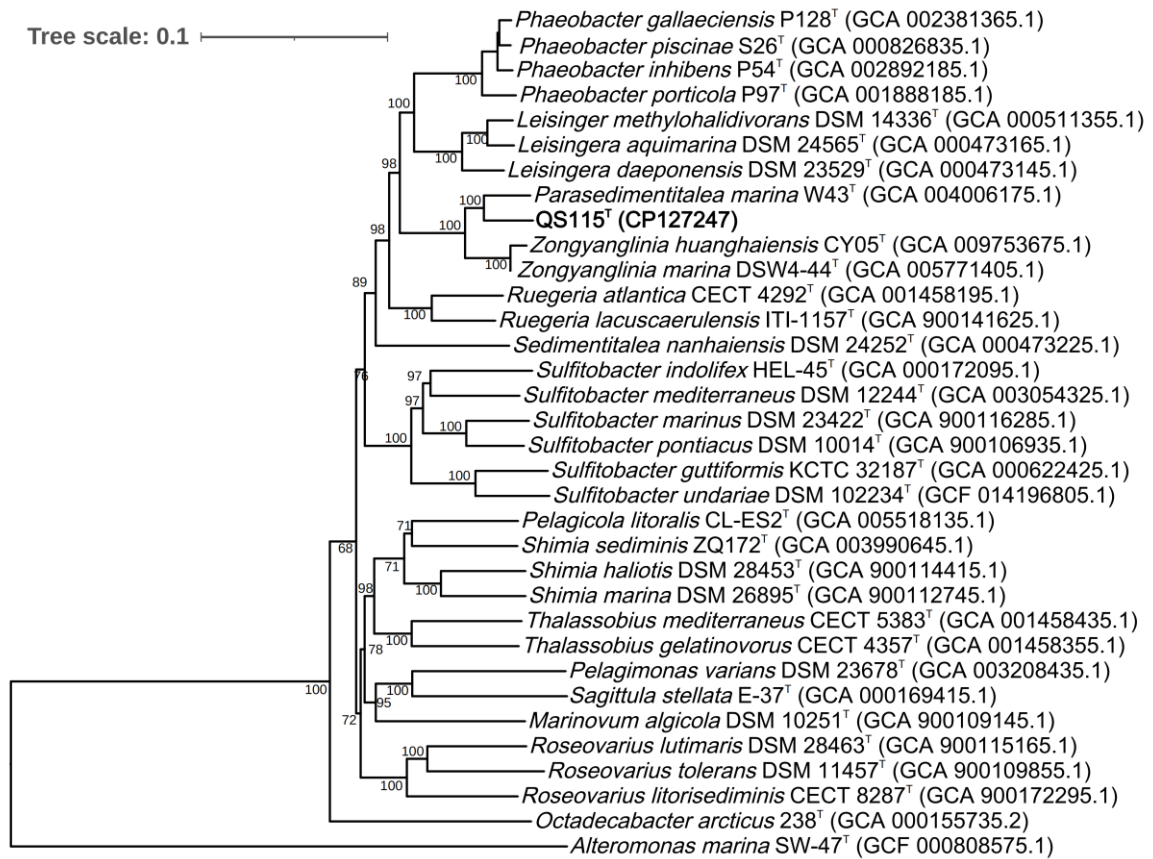
- 228 1. Aristegui J, Gasol JM, Duarte CM, Herndl GJ. Microbial oceanography of the dark ocean's
229 pelagic realm. *Limnology and Oceanography*. 2009;54(5):1501-29.
- 230 2. Los DA, Murata N. Membrane fluidity and its roles in the perception of environmental signals.
231 *Biochimica Et Biophysica Acta-Biomembranes*. 2004;1666(1-2):142-57.
- 232 3. Winter R, Jeworrek C. Effect of pressure on membranes. *Soft Matter*. 2009;5(17):3157-73.
- 233 4. Brooks NJ, Ces O, Templer RH, Seddon JM. Pressure effects on lipid membrane structure and
234 dynamics. *Chemistry and Physics of Lipids*. 2011;164(2):89-98.
- 235 5. Ding WZ, Liu P, Xu YP, Fang JS, Cao JW. Polyphasic taxonomic analysis of *Parasedimentitalea*
236 *marina* gen. nov., sp. nov., a psychrotolerant bacterium isolated from deep sea water of the New Britain
237 Trench. *Fems Microbiology Letters*. 2019;366(22).
- 238 6. Zhang HG, Fang JS, Zhang HC, Cao JW. Complete genome sequence of a psychrotolerant and
239 piezotolerant bacterium *Parasedimentitalea marina* W43(T), isolated from deep sea water of the New
240 Britain trench. *Marine Genomics*. 2022;61.
- 241 7. Yoon SH, Ha SM, Kwon S, Lim J, Kim Y, Seo H, et al. Introducing EzBioCloud: a taxonomically
242 united database of 16S rRNA gene sequences and whole-genome assemblies. *International Journal of*
243 *Systematic and Evolutionary Microbiology*. 2017;67(5):1613-7.
- 244 8. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary
245 Genetics Analysis Version 6.0. *Molecular Biology and Evolution*. 2013;30(12):2725-9.
- 246 9. Felsenstein J. Evolutionary Trees from DNA-Sequences - A Maximum-Likelihood Approach.
247 *Journal of Molecular Evolution*. 1981;17(6):368-76.
- 248 10. Saitou N, Nei M. The Neighbor-joining Method - A new method for reconstructing Phylogenetic
249 Trees. *Molecular Biology and Evolution*. 1987;4(4):406-25.
- 250 11. Fitch WM. Citation-classic-toward defining the course of evolution-minimum change for a
251 specific tree topology. *Current Contents/Agriculture Biology & Environmental Sciences*. 1987(27):14-.
- 252 12. Chaumeil PA, Mussig AJ, Hugenholtz P, Parks DH. GTDB-Tk: a toolkit to classify genomes with
253 the Genome Taxonomy Database. *Bioinformatics*. 2020;36(6):1925-7.
- 254 13. Xu LL, Liu A, Zhang YJ. *Zongyanglinia huanghaiensis* gen. nov., sp. nov., a novel denitrifying
255 bacterium isolated from the yellow sea, and transfer of *Pelagicola marinus* to *Zongyanglinia* gen. nov. as

- 256 *Zongyanglinia marinus* comb. nov. Antonie Van Leeuwenhoek International Journal of General and
257 Molecular Microbiology. 2021;114(2):137-49.
- 258 14. Chun J, Rainey FA. Integrating genomics into the taxonomy and systematics of the Bacteria and
259 Archaea. International Journal of Systematic and Evolutionary Microbiology. 2014;64:316-24.
- 260 15. Luo CW, Rodriguez LM, Konstantinidis KT. MyTaxa: an advanced taxonomic classifier for
261 genomic and metagenomic sequences. Nucleic Acids Research. 2014;42(8).
- 262 16. Qin QL, Xie BB, Zhang XY, Chen XL, Zhou BC, Zhou JZ, et al. A Proposed Genus Boundary for
263 the Prokaryotes Based on Genomic Insights. Journal of Bacteriology. 2014;196(12):2210-5.
- 264 17. Meier-Kolthoff JP, Auch AF, Klenk HP, Goker M. Genome sequence-based species delimitation
265 with confidence intervals and improved distance functions. BMC Bioinformatics. 2013;14.
- 266 18. Richter M, Rossello-Mora R. Shifting the genomic gold standard for the prokaryotic species
267 definition. Proceedings of the National Academy of Sciences of the United States of America.
268 2009;106(45):19126-31.
- 269 19. Wirth JS, Whitman WB. Phylogenomic analyses of a clade within the roseobacter group suggest
270 taxonomic reassignments of species of the genera *Aestuariivita*, *Citreicella*, *Loktanella*, *Nautella*,
271 *Pelagibaca*, *Ruegeria*, *Thalassobius*, *Thiobacimonas* and *Tropicibacter*, and the proposal of six novel
272 genera. International Journal of Systematic and Evolutionary Microbiology. 2018;68(7):2393-411.
- 273 20. Lai QL, Cao JW, Yuan J, Li FY, Shao ZZ. *Celeribacter indicus* sp nov., a polycyclic aromatic
274 hydrocarbon-degrading bacterium from deep-sea sediment and reclassification of *Huaishuia halophila* as
275 *Celeribacter halophilus* comb. nov. International Journal of Systematic and Evolutionary Microbiology.
276 2014;64:4160-7.
- 277 21. Dai J, Tang HZ, Li XG, Santini CL, Cui WP, Liu N, et al. Resazurin as an indicator of reducing
278 capacity for analyzing the physiologic status of deep-sea bacterium *Photobacterium phosphoreum* ANT-
279 2200. Journal of Oceanology and Limnology. 2021;39(1):297-305.
- 280 22. Li XG, Lin J, Bai SJ, Dai J, Jiao ZX, Tang HZ, et al. *Crassaminicella thermophila* sp. nov., a
281 moderately thermophilic bacterium isolated from a deep-sea hydrothermal vent chimney and emended
282 description of the genus *Crassaminicella*. International Journal of Systematic and Evolutionary
283 Microbiology. 2021;71(11).
- 284 23. Jiao Z-X, Li X-G, Zhang H-H, Xu J, Bai S-J, Dai J, et al. *Crassaminicella indica* sp. nov., a novel
285 thermophilic anaerobic bacterium isolated from a deep-sea hydrothermal vent. International journal of
286 systematic and evolutionary microbiology. 2023;73(3).
- 287 24. Choi YS, Oh JS, Roh DH. *Pelagicola marinus* sp. nov. isolated from deep-sea water. International
288 Journal of Systematic and Evolutionary Microbiology. 2019;69(12):3961-6.
- 289 25. Choi DH, Cho BC. *Citreimonas salinaria* gen. nov., sp nov., a member of the Roseobacter clade
290 isolated from a solar saltern. International Journal of Systematic and Evolutionary Microbiology.
291 2006;56:2799-803.
- 292 26. da Costa MS, Albuquerque L, Nobre MF, Wait R. The Identification of Polar Lipids in
293 Prokaryotes. In: Rainey F, Oren A, editors. Methods in Microbiology, Vol 38: Taxonomy of Prokaryotes.
294 Methods in Microbiology. 382011. p. 165-81.



297

298 **Figure 1.** Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic
 299 relationship of QS115^T and related taxa. Bootstrap values (expressed percentages of 1,000
 300 replications) greater than 60 % are given at the nodes. Filled circles indicate nodes recovered
 301 reproducibly with maximum-likelihood and maximum-parsimony trees. The sequences of
 302 *Alteromonas marina* SW-47^T was used as outgroup and GenBank accession numbers are given in
 303 parentheses. Bar, 0.020 substitutions per nucleotide position.



304

305 **Figure 2.** Neighbour-joining tree based on genome sequences comparisons showing the phylogenetic
 306 relationship of QS115^T and related taxa. Bootstrap values (expressed percentages of 1,000
 307 replications) greater than 60 % are given at the nodes. The sequences of *Alteromonas marina* SW-47^T
 308 was used as an outgroup number are given in parentheses. Bar, 0.1 substitutions per nucleotide
 309 position.



310

311 **Figure 3.** Transmission-electron microscopy image of a cell of strain QS115^T. Bar: 1 μm.

312 **Table 1.** Differential characteristics of QS115^T and type strains of closely related species.

313 Strains: 1, QS115^T; 2, *Parasedimentitalea marina* W43^T; 3, *Zongyanglinia huanghaiensis* CY05^T; 4,
 314 *Zongyanglinia marina* DSW4-44^T. All strains and species were analyzed under the same laboratory
 315 conditions unless otherwise stated. “+”, positive; “W”, weakly positive; “-”, negative.

Characteristic	1	2	3	4
Motility	+	- ^a	- ^b	- ^c
Growth conditions				
Temperature Range (optimum, °C)	4-20 (10)	10-25 (20)	10-25 (20)	15-25 (25)
NaCl Range (optimum, w/v %)	1-5 (2)	1-4 (3)	1-5 (3)	2-4 (3)
pH Range (optimum)	7.0-8.0 (7.5)	6.0-7.5 (7.0)	6.5-8.0 (7.0)	6.0-8.5 (6.5)
Carbon utilization:				
Betaine	-	+	W	-
Lysine	-	+	-	-
Proline	-	+	+	-

Pyruvic Acid	+	-	-	+
Macromolecule utilization:				
Hypoxanthine	-	-	-	+
L-tyrosine	-	-	-	+
Tween 80	+	+	-	+
Xanthine	+	-	-	+
Xylan	+	+	-	-
Biolog GEIII:				
D-Trehalose	-	-	-	+
D-Cellobiose	-	-	-	+
Glycerol	-	-	-	+
L-Malic Acid	-	+	-	-
β -Hydroxy-D, L Butyric Acid	-	+	-	-
Propionic Acid	-	+	-	-
Acetic Acid	-	+	-	-
Sodium Butyrate	-	+	+	-
API 20NE:				
Denitrification	-	+	-	-
Arginine Dihydrolase	-	+	-	-
Urease	-	+	-	-
Gelatin Hydrolysis	-	+	-	-
β -Galactosidase	+	+	-	+
D-Glucose	-	-	+	-
Trisodium Citrate	-	-	+	-
Phenylacetic Acid	-	-	+	-
G+C content (%)	57.71	55.9^a	54.2^b	54.3^c

316 Data from the following studies: a. Ding *et al.* (2020); b. Xu *et al.* (2021); c. Choi *et al.* (2019);

317

318 **Table 2.** Fatty acid compositions (%) of strain QS115^T and closely related species.

319 Strains: 1, QS115^T; 2, *Parasedimentitalea marina* W43^T; 3, *Zongyanglinia huanghaiensis* CY05^T; 4,

320 *Zongyanglinia marina* DSW4-44^T. All data from this study unless otherwise stated. “-”: no data

321 available. Fatty acids that represented <1 % in all strains were omitted. TR, Traces (<1 %).

Fatty acid	1	2	3	4
Saturated fatty acids				
C _{16:0}	1.5	1.2	1.3	2.9
C _{18:0}	-	TR	TR	1.5
Unsaturated fatty acids				
C _{18:1} ω7c 11-methyl	-	-	-	1.3
Hydroxy fatty acids				
C _{10:0} 3OH	6.5	3.5	3.0	5.3
C _{16:0} 2OH	3.7	2.8	2.5	8.7
C _{16:1} 2OH	2.3	1.3	TR	-
C _{18:1} 2OH	TR	TR	TR	-
Others				
Sum in feature 3	4.1	TR	TR	-
Sum in feature 8	54.4	89.4	91.0	65.3

322