

1 ***Meridianimaribacter flavus* gen. nov., sp. nov., a novel member of the**
2 **family *Flavobacteriaceae* isolated from marine sediment of South**
3 **China Sea**

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12 **Running title:** *Meridianimaribacter flavus* gen. nov., sp. nov.

13

14 The GenBank accession number for the 16S rRNA gene sequence of *Meridianimaribacter flavus*
15 NH57N^T is FJ360684.

16

17 **Summary:** A Gram-staining-negative rod-shaped marine bacterium, designated strain NH57N^T,
18 isolated from sandy sediment in the Mischief Reef of the South China Sea, was characterized
19 based on its physiological and biochemical features, fatty acid profile and phylogenetic position.
20 16S rRNA gene sequence analysis revealed a clear affiliation with the family *Flavobacteriaceae*.
21 Strain NH57N^T showed the closest phylogenetic relationship with members of the genera
22 *Gaetbulibacter*, *Gelidibacter*, *Subsaxibacter*, *Subsaximicrobium* and *Yeosuana*; 16S rRNA gene
23 sequence similarities between strain NH57N^T and the type strains of the related species ranged
24 from 94.9 to 91.2%. Cells of strain NH57N^T were motile by gliding and grew on solid media as
25 yellow colonies at 9-37 °C, at pH 6.5-8.5 and in the presence of 0.5-4.0 % NaCl. The DNA G+C
26 content was 32.7 mol% and the predominant fatty acids were iso-C_{15:1} (22.7%), iso-C_{15:0} (20.7%),
27 iso-C_{17:0} 3-OH (9.5%), iso-C_{16:0} 3-OH (8.3%), C_{15:0} (7.8%) and iso-C_{15:0} 3-OH (5.8%). Based on
28 the physiological and phylogenetic data, and on the fatty acid composition, strain NH57N^T is
29 considered to represent a novel genus and species in the family *Flavobacteriaceae*, for which the
30 name *Meridianimaribacter flavus* gen. nov., sp. nov. is proposed. The type strain is NH57N^T
31 (=CCTCC AB 208318^T=LMG 24839^T=MCCC 1A03544^T).

32

33 The family *Flavobacteriaceae*, proposed by Jooste (1985), is one of the fastest growing families
34 in the phylum *Bacteroidetes*: more than 50 genera have been described, most of them in recent
35 years. The genus *Gaetbulibacter* was created by Jung *et al.* (2005) to accommodate the new
36 species *Gaetbulibacter saemankumensis*, isolated from a tidal flat sediment in the Yellow Sea in
37 Korea (Jung *et al.*, 2005). A second species, *Gaetbulibacter marinus* was subsequently described,
38 from coastal surface seawater, also from the Yellow Sea (Yang & Cho, 2008). Species assigned to
39 this genus are yellow-pigmented, aerobic, rod-shaped, catalase- and alkaline phosphatase-positive,
40 and have the ability to degrade aesculin. Oxidase activity and gliding motility are
41 species-dependent.

42 Strain NH57N^T was isolated recently from the South China Sea. It represents a new lineage
43 closely related to the genus *Gaetbulibacter*. Although NH57N^T shares many physiological and
44 biochemical characteristics with species of the genus *Gaetbulibacter*, it differs from them by

45 narrower pH and salinity ranges for growth, lower G+C content and ability to degrade gelatin. In
46 addition, moderate 16S rRNA gene sequence similarity and differences in cellular fatty acid
47 composition also support the description of strain NH57N^T as a novel genus and species, for
48 which the name *Meridianimaribacter flavus* gen. nov., sp. nov. is proposed.

49 Strain NH57N^T was isolated from a sandy sediment sample with coral granules collected in
50 May 2007 at a water depth of 12 m from the seafloor of a lagoon of the Mischief Reef of the South
51 China Sea (E115.4617°, N9.9217°). This work was part of a taxonomic survey of the broad
52 biodiversity of free-living microbial populations of the South China Sea. In the laboratory,
53 sediment slurry was spread on M2 agar plates, which was then incubated at 25 °C for 1 week. The
54 medium consisted of (l⁻¹ sea water): 5 g CH₃COONa, 0.5 g peptone, 0.5 g yeast extract, 0.5 g
55 glucose, 0.5 g sucrose, 0.5 g starch, 0.05 g trisodium citrate, 0.05 g malic acid, 0.05 g potassium
56 sodium tartrate, 1.0 g NH₄NO₃, 0.2 g NH₄Cl and 15 g agar, adjusted to pH 7.5~7.6. Strain
57 NH57N^T was isolated and subsequently purified on fresh M2 agar four times and stored at -80 °C
58 in M2 broth supplemented with 20% (v/v) glycerol.

59 The 16S rRNA gene was amplified from a single colony by PCR with Taq DNA polymerase
60 (MBI) and two universal primers (Lane, 1991). The purification of the PCR product was carried
61 out according to the protocol of the TIANquick midi purification kit (TIANGEN). Sequencing of
62 the 16S rRNA gene was performed with an Applied Biosystems automatic sequencer
63 (ABI3730XL). The 16S rRNA gene sequence (1470bp) of strain NH57N^T was blasted in NCBI.
64 The closely related sequences were retrieved and aligned. Then, evolutionary distances were
65 calculated according to the Kimura two-parameter model (Kimura, 1980) with the DNAMAN
66 software (Version 6). Phylogenetic trees were constructed by using the Neighbor-Joining (Saitou
67 & Nei, 1987) method, and evaluated by bootstrap analysis based on 1000 replicates.

68 Phylogenetic analysis of 16S rRNA gene sequences revealed that strain NH57N^T is a member of
69 the family *Flavobacteriaceae* and forms a distinct lineage with species of the genera
70 *Gaetbulibacter*, *Gelidibacter*, *Subsaxibacter*, *Subsaximicrobium* and *Yeosuana* (Fig. 1). The
71 nearest neighbours of this strain are *Gaetbulibacter marinus* IMCC1914^T and *G. saemankumensis*
72 SMK-12^T, with 16S rRNA gene sequence similarities of 94.9% and 94.2%, respectively. Although

73 the branching of the tested strain with *Gaetbulibacter* species in the phylogenetic tree was not
74 supported strongly by bootstrap analysis (Fig.1), the trees generated with the distance matrix using
75 the Jukes-Cantor and Maximum-Likelihood models showed the same topology (data not shown).
76 Sequence similarities between strain NH57N^T and other related type strains were 93.4-91.2%. The
77 relatively low similarity values to members of the most closely related genera warrant
78 classification of strain NH57N^T as the representative of a novel genus.

79 Cell morphology and motility were observed by phase-contrast light microscopy (model 50i;
80 Nikon) and transmission electron microscopy (model JEM-1230; JEOL), using cells from the
81 early exponential phase grown on M2 agar. Gliding motility was determined as described by
82 Bowman (2000). Growth was tested at pH 3-10 using 5 ml HLB medium supplemented with 200
83 µl M2 medium, adjusted with NaOH and HCl solutions. HLB was modified from Luria-Bertani
84 (LB) medium (Sambrook *et al.*, 1989), with the concentration of NaCl increased to 30 g l⁻¹.
85 Growth was tested at 4-45°C and in the presence of 0.5-25% (w/v) NaCl on M2 agar. The
86 requirement for NaCl was studied on M2 agar devoid of NaCl obtained by replacing sea water by
87 distilled water. Oxidase reaction was tested by using oxidase reagent (bioMérieux). Catalase
88 activity was tested using a 3% H₂O₂ solution. Hydrolysis of starch and Tweens 20, 40 and 80 were
89 determined as described by Cowan & Steel (1965). Casein hydrolysis was tested by the method of
90 Smibert & Krieg (1994). Other physiological and biochemical tests were performed with the API
91 20E and API 20NE systems (bioMérieux) and the inoculum was prepared by suspending cells in a
92 3% (w/v) NaCl solution. The API ZYM system (bioMérieux) was used to determine the activity of
93 19 enzymes. The oxidation of 95 carbon sources was determined by using the GN2 MicroPlate
94 (BIOLOG) as described previously (Ivanova *et al.*, 1998). Cupules were inoculated with 150 µl
95 liquid culture, which NaCl concentration was increased to 2.4%. All above –mentioned tests were
96 conducted at 30°C.

97 Susceptibility to antibiotics was tested on M2 agar at 30 °C for 2 days by using the following
98 discs (OXOID): ceftriaxone (30 µg), cephadrine (30 µg), chloramphenicol (30 µg), gentamicin (10
99 µg), erythromycin (15 µg), cefoperazone (75 µg), ciprofloxacin (5 µg), clindamycin (2 µg),
100 doxycycline hydrochloride (30 µg), neomycin (10 µg), tetracycline (30 µg), cephalixin (30 µg),

101 ampicillin (10 µg), furazolidone (15 µg), metronidazole (5 µg), cephalosin (30 µg), lincomycin (2
102 µg), minocycline (30 µg), norfloxacin (10 µg), kanamycin (30 µg), vancomycin (30 µg),
103 trimethoprim (25 µg), piperacillin (100 µg), ofloxacin (5 µg), rifampicin (5 µg), carbenicillin (100
104 µg), polymyxin B (300 U), streptomycin (10 µg), oxacillin (1 µg) and penicillin G (10 U). The
105 physiological and biochemical characteristics of strain NH57N^T are given in the genus and species
106 descriptions and in Table 1.

107 For cellular fatty acid analysis, strain NH57N^T was harvested from MA plates after cultivation
108 for 3 days at 30°C. As we know that *Gaetbulibacter* species had also been grown on MA for 3
109 days; growth temperature was 30°C for *G. saemankumensis* (Jung *et al.*, 2005) and 25°C for *G.*
110 *marinus* (Yang & Cho, 2008). The fatty acids were extracted according to the standard protocol of
111 the Microbial Identification System (MIDI, Sherlock). Analysis of the fatty acid methyl esters was
112 performed on a GC (6850, Agilent), and peaks were identified with the MIDI software (Version
113 6.0). The main cellular fatty acids were branched-chain unsaturated and saturated, and
114 straight-chain saturated, fatty acids, namely iso-C_{15:1} (22.7%), iso-C_{15:0} (20.7%), iso-C_{17:0} 3-OH
115 (9.5%), iso-C_{16:0} 3-OH (8.3%), C_{15:0} (7.8%) and iso-C_{15:0} 3-OH (5.8%). High levels of
116 branched-chain and 3-hydroxy C₁₅-C₁₇ fatty acids are typical for members of the family
117 *Flavobacteriaceae* (Bowman *et al.*, 1998). So, the presence of significant amounts of iso-C_{17:0}
118 3-OH and branched-chain fatty acids supports the placement of strain NH57N^T in the family
119 *Flavobacteriaceae*.

120 The DNA G+C content was 32.7 mol% as determined by reverse HPLC according to the
121 method of Tamaoka & Komagata (1984).

122 Separation of the tested strain from members of related genera was supported by the
123 comparison of their phenotypic and genotypic characteristics (Tables). Strain NH57N^T exhibited
124 narrower pH and salinity ranges for growth. In addition, it could be distinguished from its closest
125 relatives *Gaetbulibacter marinus* and *G. saemankumensis* by the hydrolysis of gelatin and
126 susceptibility to ampicillin. Ability to produce oxidase, inability to generate acid from glucose and
127 requirement of Na⁺ ions for growth distinguished the strain studied from members of the genus
128 *Gelidibacter*. Weak degradation of starch and failure to hydrolyse casein and Tween 80 separated

129 the strain from *Subsaxibacter* and *Subsaximicrobium* species. The difference of more than 10
130 mol% in DNA G+C content readily distinguished strain NH57N^T from *Yeosuana aromativorans*
131 GW1-1^T. Moreover, strain NH57N^T was clearly differentiated from members of all
132 phylogenetically related genera by differences in the presence and proportions of several fatty
133 acids (Table 2).

134 Overall, the low similarity of 16S rRNA gene sequence, together with differences in
135 chemotaxonomic, physiological and biochemical properties, indicate that isolate NH57N^T should
136 be classified as a novel genus and species, for which the name *Meridianimaribacter flavus* gen.
137 nov., sp. nov. is proposed.

138 **Description of *Meridianimaribacter* gen. nov.**

139 *Meridianimaribacter* (Me.ri.di.a.ni.ma.ri.bac'ter. L. adj. *meridianus*, of or belonging to the south,
140 southern, meridional; L. n. *mare*, the sea; N.L. masc. n. *bacter*, a rod; N.L. masc. n.
141 *Meridianimaribacter*, a rod of the southern sea, isolated from the the South China Sea)

142 Cells are strictly aerobic, Gram-staining-negative, non-spore-forming, rod-shaped and motile by
143 gliding. Yellow colonies are formed on M2 plates. The major cellular fatty acids are straight-chain
144 saturated, branched-chain saturated and unsaturated fatty acids. Oxidase- and catalase- positive.
145 The DNA G+C content of the type strain of the type species is 32.7 mol%. As determined by 16S
146 rRNA gene sequence analysis, the genus *Meridianimaribacter* is a member of the family
147 *Flavobacteriaceae*, phylum *Bacteroidetes*. The type species is *Meridianimaribacter flavus*.

148 **Description of *Meridianimaribacter flavus* sp. nov.**

149 *Meridianimaribacter flavus* (*fla.vus*. L. masc. adj. *flavus* yellow, the colour of colonies).

150 In addition to the characteristics of the genus, cells are 1.15-2.3 µm in length and 0.3-0.55 µm in
151 diameter, surrounded by an unidentified extracellular compound (Fig. 2). Growth occurs at
152 9-37 °C (optimum, 30-37 °C), at pH 6.5-8.5 (optimum, pH 7.8) and with 0.5-4.0% NaCl (optimum,
153 0.5-2.0%). NaCl is essential for growth. Colonies on M2 are circular, smooth, glistening,
154 moist-appearing, convex with entire margins and 1-2 mm in diameter after 3 days of incubation at
155 30 °C. Gelatin is hydrolyzed. Starch and Tween 40 are weakly hydrolyzed. Agar, casein and
156 Tweens 20 and 80 are not hydrolyzed. Nitrate is not reduced. Acetoin is produced, but H₂S and
157 indole are not produced. In API 20E and 20NE strips, β-glucosidase, esculinase and

158 β -galactosidase activities are present, but urease, arginine dihydrolase, ornithine decarboxylase,
159 tryptophan deaminase and lysine decarboxylase activities are absent. In the API ZYM strip,
160 alkaline phosphatase, esterase lipase (C8), leucine arylamidase, valine arylamidase,
161 α -chymotrypsin, acid phosphatase and naphthol-AS-BI-phosphohydrolase activities are present;
162 weak esterase (C4), cystine arylamidase, trypsin and α -glucosidase activities are present; and
163 lipase (C14), α -galactosidase, β -glucuronidase, *N*-acetyl- β -glucosaminidase, α -mannosidase and
164 α -fucosidase activities are absent. The following carbon substrates are oxidized in GN2
165 MicroPlate: α -cyclodextrin, dextrin, *N*-acetyl-D-galactosamine, D-cellobiose, gentiobiose,
166 α -D-glucose, m-inositol, D-mannose, D-trehalose, acetic acid, γ -hydroxy butyric acid, α -keto
167 valeric acid, propionic acid and L-proline. The following carbon substrates are weakly oxidized:
168 lactulose, D-raffinose, sucrose, α -keto butyric acid and D, L-lactic acid. All other substrates in the
169 GN2 MicroPlate are not oxidized. Susceptible to trimethoprim, ofloxacin, carbenicillin,
170 cephadrine, doxycycline hydrochloride, chloramphenicol, ciprofloxacin, cefoperazone,
171 erythromycin, clindamycin, ceftriaxone, cephalixin, furazolidone, ampicillin, lincomycin,
172 minocycline, norfloxacin, tetracycline, piperacillin, penicillin G, rifampicin and vancomycin, but
173 resistant to kanamycin, polymyxin B, cephalosporin, metronidazole, streptomycin, oxacillin,
174 gentamicin and neomycin. The predominant fatty acids (>5% of the total fatty acids) are iso-C_{15:1},
175 iso-C_{15:0}, iso-C_{17:0} 3-OH, iso-C_{16:0} 3-OH, C_{15:0}, iso-C_{15:0} 3-OH and summed feature 3 (comprising
176 C_{16:1 ω 7c} and/or iso-C_{15:0} 2-OH). The DNA G+C content is 32.7 mol%. Other phenotypic
177 characteristics are given in Table 1.

178 The type strain, NH57N^T (=CCTCC AB 208318^T=LMG 24839^T=MCCC 1A03544^T), was
179 isolated from a sandy sediment sample of the South China Sea at a water depth of 12 m.

180

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186 **References**

- 187 **Bercovich, A., Vazquez, S. C., Yankilevich, P. & 8 other authors. (2008).** *Bizionia*
188 *argentinensis* sp. nov., isolated from surface marine water in Antarctica. *Int J Syst Evol Microbiol*
189 **58**, 2363–2367.
- 190 **Bowman, J. P. (2000).** Description of *Cellulophaga algicola* sp. nov., isolated from the surfaces
191 of Antarctic algae, and reclassification of *Cytophaga uliginosa* (ZoBell and Upham 1944)
192 Reichenbach 1989 as *Cellulophaga uliginosa* comb. nov. *Int J Syst Evol Microbiol* **50**,
193 1861–1868.
- 194 **Bowman, J. P., McCammon, S. A., Lewis, T., Skerratt, J. H., Brown, J. L., Nichols, D. S. &**
195 **McMeekin, T. A. (1998).** Description of *Psychroflexus torquis* gen. nov., sp. nov., a psychrophilic
196 species from Antarctic sea ice, and reclassification of *Flavobacterium gondwanense* (Dobson et al.
197 1993) as *Psychroflexus gondwanense* gen. nov., comb. nov. *Microbiology* **144**, 1601–1609.
- 198 **Bowman, J. P. & Nichols, D. S. (2005).** Novel members of the family *Flavobacteriaceae* from
199 Antarctic maritime habitats including *Subsaximicrobium wynnwilliamsii* gen. nov., sp. nov.,
200 *Subsaximicrobium saxinquilinus* sp. nov., *Subsaxibacter broadyi* gen. nov., sp. nov., *Lacinutrix*
201 *copepodicola* gen. nov., sp. nov., and novel species of the genera *Bizionia*, *Gelidibacter* and
202 *Gillisia*. *Int J Syst Evol Microbiol* **55**, 1471–1486.
- 203 **Cowan, S. T. & Steel, K. J. (1965).** *Manual for the Identification of Medical Bacteria*. London:
204 Cambridge University Press.
- 205 **Ivanova, E. P., Kiprianova, E. A., Mikhailov, V. V. & 8 other authors. (1998).** Phenotypic
206 diversity of *Pseudoalteromonas citrea* from different marine habitats and emendation of the
207 description. *Int J Syst Bacteriol* **48**, 247–256.
- 208 **Jooste, P. J. (1985).** *The taxonomy and significance of Flavobacterium–Cytophaga strains from*
209 *dairy sources*. PhD thesis, University of the Orange Free State, Bloemfontein, South Africa.
- 210 **Jung, S. Y., Kang, S. J., Lee, M. H., Lee, S. Y., Oh, T. K. & Yoon, J. H. (2005).** *Gaetbulibacter*
211 *saemankumensis* gen. nov., sp. nov., a novel member of the family *Flavobacteriaceae* isolated
212 from a tidal flat sediment in Korea. *Int J Syst Evol Microbiol* **55**, 1845–1849.
- 213 **Kimura, M. (1980).** A simple method for estimating evolutionary rates of base substitutions
214 through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.

215 **Kwon, K. K., Lee, H. S., Jung, H. B., Kang, J. H. & Kim, S. J. (2006).** *Yeosuana aromativorans*
216 gen. nov., sp. nov., a mesophilic marine bacterium belonging to the family *Flavobacteriaceae*,
217 isolated from estuarine sediment of the South Sea, Korea. *Int J Syst Evol Microbiol* **56**, 727-732.

218 **Lane, D. J. (1991).** 16S/23S rRNA sequencing. In *Nucleic Acid Techniques in Bacterial*
219 *Systematics*, pp. 115–175. Edited by E. Stackebrandt & M. Goodfellow. Chichester: Wiley.

220 **Nedashkovskaya, O. I., Kim, S. B., Han, S. K. & 7 other authors (2004).** *Algibacter lectus* gen.
221 nov., sp. nov., a novel member of the family *Flavobacteriaceae* isolated from green algae. *Int J*
222 *Syst Evol Microbiol* **54**, 1257–1261.

223 **Nedashkovskaya, O. I., Vancanneyt, M., Cleenwerck, I., Snauwaert, C., Kim, S. B., Lysenko,**
224 **A. M., Shevchenko, L. S., Lee, K. H., Park, M. S. & other authors (2006a).** *Arenibacter*
225 *palladensis* sp. nov., a novel marine bacterium isolated from the green alga *Ulva fenestrata*, and
226 emended description of the genus *Arenibacter*. *Int J Syst Evol Microbiol* **56**, 155–160.

227 **Nedashkovskaya, O. I., Kim, S. B., Vancanneyt, M., Snauwaert, C., Lysenko, A. M., Rohde,**
228 **M., Frolova, G. M., Zhukova, N. V., Mikhailov, V. V., Bae, K. S., Oh, H. W. & Swings, J.**
229 **(2006b).** *Formosa agariphila* sp. nov., a budding bacterium of the family *Flavobacteriaceae*
230 isolated from marine environments, and emended description of the genus *Formosa*. *Int J Syst*
231 *Evol Microbiol* **56**, 161-167.

232 **Nedashkovskaya, O. I., Vancanneyt, M., Kim, S. B., Hoste, B., & Bae, K. S. (2007).** *Algibacter*
233 *mikhailovii* sp. nov., a novel marine bacterium of the family *Flavobacteriaceae*, and emended
234 description of the genus *Algibacter*. *Int J Syst Evol Microbiol* **57**, 2147-2150.

235 **Saitou, N. & Nei, M. (1987).** The neighbor-joining method: a new method for reconstructing
236 phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

237 **Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989).** *Molecular Cloning: a Laboratory Manual*,
238 2nd ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

239 **Smibert, R. M. & Krieg, N. R. (1994).** Phenotypic characterization. In *Methods for General and*
240 *Molecular Bacteriology*, pp. 607–654. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N.
241 R. Krieg. Washington, DC: American Society for Microbiology.

242 **Tamaoka, J. & Komagata, K. (1984).** Determination of DNA base composition by reverse-phase
243 high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.

244 **Yang, S.-J. & Cho, J.-C. (2008).** *Gaetbulibacter marinus* sp. nov., isolated from coastal seawater,
245 and emended description of the genus *Gaetbulibacter*. *Int J Syst Evol Microbiol* **58**, 315-318.
246

247 **Table 1.** Phenotypic characteristics that differentiate strain NH57N^T from closely related members of the family *Flavobacteriaceae*

248 Taxa: 1, strain NH57N^T (data from this study); 2, *Gaetbulibacter* (n=2; Jung *et al.*, 2005; Yang & Cho, 2008); 3, *Gelidibacter* (n=4; Kwon *et al.*, 2006; Bowman &
 249 Nichols, 2005); 4, *Subsaxibacter broadyi* P7^T (Bowman & Nichols, 2005; Kwon *et al.*, 2006;) ; 5, *Subsaximicrobium* (n=2; Bowman & Nichols, 2005; Kwon *et al.*,
 250 2006); 6, *Yeosuana aromativorans* GW1-1^T (Kwon *et al.*, 2006); 7, *Algibacter* (n=2; Nedashkovskaya *et al.*, 2004, 2007); 8, *Bizionia* (n=6; Kwon *et al.*, 2006;
 251 Bercovich *et al.*, 2008; Bowman & Nichols, 2005); 9, *Formosa* (n=2; Nedashkovskaya *et al.*, 2006b). n= the number of type strains in each genus is given in
 252 parentheses. +, Positive; -, negative; W, weakly positive; ND, no data available; V, variable. All taxa are positive for catalase and negative for indole and H₂S
 253 production.
 254

Characteristic	1	2	3	4	5	6	7	8	9
Gliding motility	+	V	+	+	+	-	+	-	+
Production of acetoin	+	ND	ND	ND	ND	ND	-	†	-
Urease activity	-	-	-	-	-	-	-	V	-
Oxidase activity	+	V	-	ND	ND	-	+	+	+
Nitrate reduction	-	V	-	-	-	-	V	-	V
Requirement for:									
Na ⁺	+	+	-	+	+	+	+	+	V
Oxygen	+	V	+	+	+	+	V	+	V
Temperature (°C)*	9-37 (30-37)	3-40 (25)	0-37 (15-18)	-2-20	-2-25	23-39 (33-36)	4-37 (23)	4-36 (23-25)	4-34
pH*	6.5-8.5 (7.8)	5.5-11	ND	ND	ND	5-8 (7)	ND	ND	5-10
NaCl (%)*	0.5-4.0 (0.5-2.0)	0.5-7	0-15	0.5-7.3 (1.8-2.3)	0.5-12	0.5-4.0 (2.0)	1-6	1-17.6	0-8
Degradation of:									
Agar	-	‡	-	-	-	-	+	-	V
Casein	-	V	V	-	+	-	-	+	-
Gelatin	+	-	V	+	+	+	+	+	+
Starch	W	V	V	-	+	-	V	-	V

Tween 80	-	-	V	+	+	ND	-	+‡	-
Aesculin	+	+	+	W	+	ND	+	-	+
Acid from glucose	-	V	+	-	-	-	V	-	+
Susceptibility to:									
Ampicillin	+	-	ND	ND	ND	ND	-	ND	V
Penicillin G	+	+	ND	ND	ND	ND	-	ND	-
DNA G+C content (mol%)	32.7	34.7-38.1	36-42	35	38-41	51.4	31-35.1	34-45	34-36

255 *Ranges with optima shown in parentheses.

256 †Only the type strain of *Bizionia paragorgiae* was negative.

257 ‡Only the type strain of *Bizionia argentinensis* was negative

259 **Table 2.** Cellular fatty acid composition (%) of strain NH57NT and closely related members of
 260 the family Flavobacteriaceae

261 Taxa: 1, strain NH57N^T (data from this study); 2, *Gaetbulibacter* (n=2; Yang & Cho, 2008); 3,
 262 *Gelidibacter* (n=4; Bowman & Nichols, 2005); 4, *Subsaximicrobium* (n=2; Bowman & Nichols,
 263 2005); 5, *Subsaxibacter broadyi* P7^T (two strains analysed; data from Bowman & Nichols, 2005);
 264 6, *Yeosuana aromativorans* GW1-1^T (Kwon *et al.*, 2006); 7, *Algibacter* (n=2; Nedashkovskaya *et*
 265 *al.*, 2004, 2007); 8, *Bizionia* (n=6; Bowman & Nichols, 2005; Bercovich *et al.*, 2008); 9, *Formosa*
 266 (n=2; Nedashkovskaya *et al.*, 2006b). n= the number of type strains in each genus is given in
 267 parentheses. For taxa 2, 3, 4, 5, 7, 8 and 9, values shown are the range of percentage for all species
 268 in the genus or for all strains of the species.br, branched (in which branching positions have not
 269 been determined) fatty acids; tr, trace (< 0.3%); ND, not detected. Fatty acids amounting to <
 270 0.3% of the total fatty acids in all strains listed were omitted.

271

Fatty acid	1	2	3	4	5	6	7	8	9
iso-C _{13:0}	0.5	0.6-2.0			ND-tr			tr-3.0	
anteiso-C _{13:0}					ND-tr			0.3-0.4	
C _{14:0}	0.3		0.3-0.6	tr-0.5	0.6-0.7			0.4-0.8	
iso-C _{14:0}	1.2	ND-1.0	0.4-2.8	tr-0.7	0.4-0.6			1.2-2.5	
iso-C _{14:0} 3-OH	0.4								
br-C _{14:1}			0.3-1.7	0.3-0.5	0.4-0.5			0.7-1.1	
C _{15:0}	7.8	1.3-3.9	2.4-5.3	0.6-1.1	0.7-1	5.3	7-13.4	2.3-6.0	8.7-15.5
anteiso-C _{15:0}	1.3	4-10.8	10.5-17.7	9.9-17.1	14.3-14.5	14.9	4.6-7.2	10.1-20.8	1.6-4.7
iso-C _{15:0}	20.7	20.6-24.3	3.4-8.8	7.4-10.4	6.3-6.4	21.7	11.3-12.5	3.1-17.3	12.7-17.2
iso-C _{15:0} 3-OH	5.8	4.8-8.6	2.2-6.2	2.4-5.4	1.9-2.3		5.8-9.4	1.2-9.3	6.7-10.5
anteiso-C _{15:0} 3-OH			0.3-10.6	3.6-8.7	5.2			3.4-22.9	
C _{15:0} 2-OH	0.9	0.6-1.6					ND-2.4	ND-2.1	1.5-1.8
C _{15:0} 3-OH	1.3		tr-1.5	ND-0.8	ND-0.5		ND-1.3	tr-2.0	2.3-4.0
C _{15:1} ω6c			2.7-4.2	tr-1.0	1.3-2.0		1.7-10.9	1.2-3.0	6.0-8.5
anteiso-C _{15:1}	0.4	1.4-3.4	11.8-16.6	5.9-14.7	9.9-15.5		ND-2.1	3.6-14.0	ND-1.0
iso-C _{15:1}	22.7	12.5-32.1	5.3-11.4	6.7-13.4	6.2-6.7	14.8	13-13.4	2.1-18.1	6.5-11.4
Summed feature 3*	9.2	4.5-10.4	4.3-9.5	9.5-13.4	8.3-13.1	10.9	22.2	2.6-6.3	5.9-15.8
C _{16:0}	1.4	0.9-1.3	1.2-2.2	1.9-2.7	1.6-2		ND-2.3	1.2-3.4	ND-1.6
C _{16:0} 10-methyl						4.7	ND-2.1		
C _{16:0} 3-OH	1.1	ND-1.2					ND-1.4		tr-2.1
iso-C _{16:0}	0.9	ND-1.2	1.4-4.4		1.5-1.7			1.1-5.8	ND-2.1
iso-C _{16:0} 3-OH	8.3	2.0-5.5	4.1-12.2	8.8-13.7	5.0-6.0			1.3-8.5	3.1-8.9
C _{16:1} ω5c			ND-1.1	ND-0.5	1.1-1.2			0.4-0.8	

br-C _{16:1}			1.4-10.3	1.9-4.7	3.5-7.7		1.1-5.4	
iso-C _{16:1}	0.4					ND-2.0		ND-2.5
anteiso C _{17:0}	0.3						4.2	
C _{17:0} 2-OH	0.3	1.0-2.4				ND-2.4	ND-1.8	ND-1.5
C _{17:0} 3-OH	0.5							
iso-C _{17:0} 3-OH	9.5	7.8-16.0	ND-3.1	1.0-3.4		9.1-13.0	0.4-9.2	8.5-10.7
anteiso-C _{17:0} 3-OH			1.0-11.3	2.7-5.3	2.3-3.2		0.5-2.9	
anteiso-C _{17:1} ω _{9c}			1.9-3.4	2.1-3.8	4.0-7.4		1.5-4.6	
iso-C _{17:1} ω _{9c}		0.3-2.4	1.1-2.3	3.5-5.1	2.3-2.6		1.7-15.1	tr-1.4
C _{17:1} ω _{6c}	0.3	tr-1.1						1.4-3.5
C _{18:0}			0.5-1.2	1.2-2.1	ND-0.5		0.5-1.1	
C _{18:1} ω _{9c}					8.2-18.7			

272 *Summed features are groups of two or three fatty acids that could not be separated by GC using
273 the MIDI system. Summed feature 3 comprised iso-C_{15:0} 2-OH and/or C_{16:1}ω_{7c}.
274

275 **Legends to Figures:**

276 **Fig. 1.** Neighbour-joining phylogenetic tree based on the 16S rRNA gene sequences of strain
277 NH57N^T and representative members of related genera in the family *Flavobacteriaceae*. Bootstrap
278 values above 50% (1000 replicates) are indicated at the nodes. *Capnocytophaga ochracea* ATCC
279 27872T was used as an outgroup. GenBank accession numbers of 16S rRNA gene sequences are
280 shown in parentheses. Bar, 0.05 substitutions per nucleotide position.

281 **Fig. 2.** Cell morphology of strain NH57N^T grown on M2 agar for 24h at 30°C: (a) phase contrast
282 micrograph, Bar, 10 µm; (b-c), transmission electron micrographs of negatively stained cells (b:
283 bar, 0.5µm and c: bar, 1 µm)

284
285

0.05



