Gillisia marina sp. nov., from seashore sand, and emended description of the genus *Gillisia*

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A Gram-staining-negative, strictly aerobic, rod-shaped bacterium, designated CBA3202^T, was isolated from seashore sand on Jeju Island, Republic of Korea. Based on the 16S rRNA gene sequence analysis, strain CBA3202^T was allocated to the genus *Gillisia* (family *Flavobacteriaceae*) and was most closely related to the type strain of *Gillisia mitskevichiae* (99.0 % 16S rRNA gene sequence similarity). Optimal growth occurred at 25 °C and with 3 % NaCl. The only isoprenoid quinone was menaquinone-6 (MK-6), the predominant fatty acids were $C_{16:0}$, iso-$C_{15:1}$ G, iso-$C_{16:0}$ and summed feature 3 (comprising $C_{16:1}\omega6c$ and/or $C_{16:1}\omega7c$), and the DNA G+C content was 34.9 mol%. The polar lipids were phosphatidylethanolamine, two unidentified aminolipids and several unidentified polar lipids. Based on phylogenetic inference and phenotypic data, we conclude that strain CBA3202^T represents a novel species of the genus *Gillisia*, for which the name *Gillisia marina* sp. nov. is proposed. The type strain is CBA3202^T (=KACC 16693^T=KCTC 32030^T=JCM 18402^T). An emended description of the genus *Gillisia* is also provided.

The genus *Gillisia* (family *Flavobacteriaceae* in the phylum *Bacteroidetes*), proposed by Van Trappen *et al.* (2004), currently comprises six species with validly published names: *Gillisia limnaea* (Van Trappen *et al.*, 2004), *Gillisia sandarakina*, *Gillisia illustrilutea*, *Gillisia hiemivivida* (Bowman & Nichols, 2005), *Gillisia mitskevichiae* (Nedashkovskaya *et al.*, 2005) and *Gilisia myxillae* (Lee *et al.*, 2006). Members of the genus *Gillisia* have been isolated from a variety of brackish or marine environments such as a brackish Antarctic lake, marine sponge, sea-ice algae and seawater (Van Trappen, 2011). The taxonomic position of a novel bacterial strain, which was isolated from a marine environment and designated CBA3202^T, was assessed using a polyphasic taxonomy approach, according to the proposed minimal standards for describing new bacterial taxa in the family *Flavobacteriaceae* (Bernardet *et al.*, 2002). The whole-genome sequence of strain CBA3202^T has recently been published (Nam *et al.*, 2012).

Strain CBA3202^T was isolated from sand on a seashore (33° 27′ 38″ N 126° 56′ 07″ E) in Seongsan, Jeju Island, Republic of Korea. A 5 g sample of sand was suspended in distilled water, diluted 10-fold and spread onto marine agar 2216 (MA; Difco). A pure culture was obtained by repeated subcultivation. For long-term preservation, strain CBA3202^T was stored at −80 °C in marine broth 2216 (MB; Difco) supplemented with 20 % (v/v) glycerol. Two reference strains, *G. mitskevichiae* KACC 15410^T and *G. hiemivivida* CIP 108528^T, were obtained from the Korean Agricultural Culture Collection (KACC; Suwon) and Centre de Ressources Biologiques de l'Institut Pasteur (CRBIP; Paris), respectively, and used for comparative purposes.

The chromosomal DNA of strain CBA3202^T was extracted using a G-spin Genomic DNA Extraction kit (iNtRON

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Abbreviations: MP, maximum-parsimony; NJ, neighbour-joining.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CBA3202^T is JQ764565.

Biotechnology), and 16S rRNA gene sequencing was conducted as described previously (Roh *et al.*, 2008). SeqMan software (DNASTAR) was used to assemble the nearly full-length 16S rRNA gene sequence (1441 bp). The calculation of pairwise 16S rRNA gene sequence similarity and the identification of phylogenetic neighbours were achieved using the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/) (Kim *et al.*, 2012). The 16S rRNA gene sequences of the novel strain and of related species in the genus *Gillisia* were aligned using the multiple sequence alignment program CLUSTAL X in BioEdit ver. 7.0. Phylogenetic trees were reconstructed using MEGA5 (Tamura *et al.*, 2011) with the neighbour-joining (NJ) (Saitou & Nei, 1987) and maximum-parsimony (MP) (Kluge & Farris, 1969) methods. A bootstrap analysis was performed using a consensus tree based on 1000 randomly generated trees. Fig. 1 shows that strain CBA3202^T fell within the cluster of species of the genus *Gillisia* in both phylogenetic trees with high bootstrap values (99 % and 78 % in the NJ and MP trees, respectively). Strain CBA3202^T was closely related to the type strains of *G. mitskevichiae* (99.0 % 16S rRNA gene sequence similarity), *G. hiemivivida* (97.1 %), *G. myxillae* (96.3 %), *G. limnaea* (96.0 %), *G. illustrilutea* (94.4 %) and *G. sandarakina* (93.8 %), as well as *Salinimicrobium marinum* (93.7 %) and *Salinimicrobium gaetbulicola* (93.6 %). DNA–DNA hybridization was performed using the fluorometric method of Ezaki *et al.* (1989) with a non-treated, black polystyrene microplate (MaxiSorp; FluoroNunc) and photobiotin-labelled DNA probes of strain CBA3202^T. The hybridization temperature was 37 °C and all reactions were performed five times. The highest and lowest of the five values were excluded and the three remaining values were used to generate the DNA–DNA homology value. The DNA–DNA homology values of strain CBA3202^T with *G. mitskevichiae* KACC 15410^T and *G. hiemivivida* CIP 108528^T were 45.3 % and 24.5 %, respectively.

All phenotypic tests described below were performed on strain CBA3202^T and the two reference strains using MA or MB as the basal media unless otherwise indicated. Growth on MA was evaluated at 4, 10, 15, 20, 25, 30, 35 and 40 °C after 2 weeks of incubation. The requirement for and tolerance of NaCl were assessed after 2 weeks of incubation using a medium that contained all of the constituents of MA except NaCl, which was supplemented with 0, 1, 2, 3, 4, 6, 8, 10, 12, 14 or 16 % (w/v) NaCl. Growth was also evaluated on Luria–Bertani agar (LBA; Difco), R2A agar (Difco), tryptic soy agar (TSA; Difco) and nutrient agar (NA; Difco). Growth under anaerobic conditions was determined in an anaerobic chamber (Coy Laboratory Products), in which the atmosphere consisted of $N_2/CO_2/H_2$ (90 : 5 : 5, by vol.). The cell morphology and presence of flagellum were analysed using an electron microscope (Supra 55VP; Carl Zeiss). Before transmission electron microscopy observations, Formvar-coated copper grids with a 200 mesh were floated on a droplet of the sample, which was followed by negative staining with 2 % uranyl acetate for 45 s, two washes with deionized water and air drying. Hydrolysis of aesculin and gelatin was tested according to the protocol of Smibert & Krieg (1994). Tests for Gram staining, catalase and oxidase activities, nitrate reduction, production of indole and H_2S [using Kligler iron agar (Difco)], motility in semi-solid agar, endospore formation and hydrolysis of casein and starch were performed as described by Benson (2002). Gliding motility and production of flexirubin type pigments were assessed according to the method of Bernardet *et al.* (2002). Hydrolysis of Tween 20, Tween 40, Tween 80 and DNA

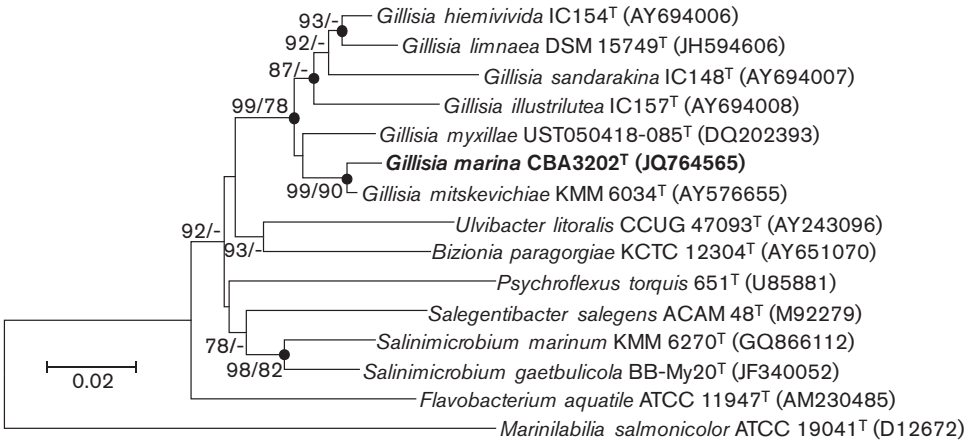

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationships of strain CBA3202^T with the type strains of species of the genus *Gillisia* and representatives of other members of the family *Flavobacteriaceae*. The bootstrap values (>70 %) as calculated using the NJ/MP probabilities are shown at the branching points. Filled circles at the branching points indicate those found also in the MP tree. *Marinilabilia salmonicolor* ATCC 19041^T (family *Marinilabiliaceae*) served as the outgroup. Bar, 0.02 accumulated changes per nucleotide.

Table 1. Differential characteristics of strain CBA3202^T and closely related species in the genus *Gillisia*

Strains: 1, *G. marina* sp. nov. CBA3202^T; 2, *G. mitskevichiae* KACC 15410^T; 3, *G. hiemivivida* CIP 108528^T. All data from this study. In conventional tests, all strains showed optimal growth at 25 °C and with 3 % NaCl and were positive for growth on MA, catalase and oxidase activities and hydrolysis of DNA*, gelatin, starch†, Tween 20†, Tween 40 and Tween 80. All strains were negative for Gram staining, growth on LBA, R2A agar, TSA and NA, anaerobic growth on MA, nitrate reduction, production of indole and H_2S, presence of flexirubin type pigments, flagella, gliding motility, motility in semi-solid agar, endospores and hydrolysis of CM-cellulose, chitin and L-tyrosine*. In API ZYM strips, all strains were positive for alkaline phosphatase, esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase; but negative for lipase (C14), cystine arylamidase, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase*, β-glucosidase*, α-mannosidase and α-fucosidase. In API 50 CH strips, all strains were positive for glycerol, D-glucose, sucrose and raffinose; but negative for erythritol, D-arabinose*, L-sorbose, inositol, D-sorbitol, methyl α-D-mannoside, *N*-acetylglucosamine*, amygdalin, arbutin, xylitol, D-lyxose, D-tagatose, D-fucose and L-arabitol. +, Positive; −, negative; W, weakly positive.

Characteristic	1	2	3
Colony pigmentation	Yellow	Yellow	Orange
Temperature range (°C) for growth	4–30	4–30	10–30 (−2 to 25*)
Growth at 4 °C	W	+	− (+*)
NaCl range (%, w/v) for growth	1–6	1–6 (1–12†)	0–6 (1.2–8.8*)
Hydrolysis of:			
Aesculin	−	+	+ (−*)
Casein	+	+	−
Enzyme activity (API ZYM)			
Esterase (C4)	−	+	+
Trypsin	−	+	−
N-Acetyl-β-glucosaminidase	−	+	−
Assimilation of (API 50CH):			
L-Arabinose	+	−	+
D-Ribose	+	−	+
D-Xylose	+	−	−
L-Xylose	−	−	+
D-Adonitol	−	−	+
Methyl β-D-xyloside	−	−	+
D-Galactose	+	−	+
D-Fructose	+	−	+
D-Mannose	+	+ (−†)	− (+*)
L-Rhamnose	+	+	−
Dulcitol	−	−	+
D-Mannitol	+	−	+
Methyl α-D-glucoside	−	−	+
Aesculin	−	+	+
Salicin	−	−	+
Cellobiose	+	+	−
Maltose	+	−	+
Lactose	−	+ (−†)	+
Melibiose	+	−	−
Trehalose	+	−	+
Inulin	−	+	−
Melezitose	−	−	+
Starch	+	−	+
Glycogen	+	−	+
Gentiobiose	−	−	+
Turanose	+	−	+
L-Fucose	+	−	+
D-Arabitol	−	−	+
Gluconate	+	−	+
2-Ketogluconate	−	−	+
5-Ketogluconate	+	−	+

*Results differ from those reported by Bowman & Nichols (2005).
†Results differ from those reported by Nedashkovskaya *et al.* (2005).

was tested as described by Gonzalez *et al.* (1978) and hydrolysis of CM-cellulose was assessed using a published method (Percival Zhang *et al.*, 2006). Hydrolysis of chitin (2.5 g l^{-1}) and L-tyrosine (0.5 g l^{-1}) was determined from the appearance of clear zones around colonies. The enzyme activities and utilization of various substrates as sole carbon sources were assessed using API ZYM and API 50 CH test strips with the AUX medium (bioMérieux), respectively, according to the manufacturer's instructions. The API ZYM and API 50CH strips were incubated at 25 °C for 4 h and 2 weeks, respectively. The cells of strain CBA3202^T were strictly aerobic, Gram-staining-negative and catalase- and oxidase-positive rods. Detailed phenotypic data are presented in the species description and in Table 1. Strain CBA3202^T could be distinguished from *G. mitskevichiae* KACC 15410^T by hydrolysis of aesculin and esterase (C4), trypsin and *N*-acetyl-β-glucosaminidase activities and from *G. hiemivivida* CIP 108528^T by the colony pigmentation, growth at 4 °C, growth with 0 % NaCl, hydrolysis of aesculin and casein and esterase (C4) activity.

Table 2. Fatty acid compositions of strain CBA3202^T and closely related species of the genus *Gillisia*

Strains: 1, *Gillisia marina* sp. nov. CBA3202^T; 2, *G. mitskevichiae* KACC 15410^T; 3, *G. hiemivivida* CIP 108528^T. All data from this study. Fatty acids amounting to <1 % of the total fatty acids in all strains are not shown. TR, Trace (<1 %); −, not detected.

Fatty acid	1	2	3
Straight-chain saturated			
$C_{10:0}$	1.3	−	−
$C_{14:0}$	TR	TR	1.2
$C_{16:0}$	16.8	16.7	9.8
$C_{18:0}$	7.0	7.1	2.0
$C_{15:0}$ 2-OH	3.2	2.3	4.5
$C_{16:0}$ 3-OH	1.2	1.9	2.8
$C_{17:0}$ 2-OH	5.9	4.6	6.0
$C_{17:0}$ 3-OH	TR	TR	3.4
Straight-chain unsaturated			
$C_{17:1}\omega6c$	3.6	2.7	3.3
$C_{17:1}\omega8c$	1.1	TR	2.1
Branched saturated			
iso-$C_{15:0}$	4.1	6.1	6.8
anteiso-$C_{15:0}$	5.0	5.8	5.3
iso-$C_{16:0}$	8.2	8.0	3.7
iso-$C_{15:0}$ 3-OH	1.0	TR	1.8
iso-$C_{16:0}$ 3-OH	2.2	3.3	1.4
iso-$C_{17:0}$ 3-OH	7.2	6.3	11.8
Branched unsaturated			
iso-$C_{15:1}$ G	10.1	8.0	10.5
anteiso-$C_{15:1}$ A	2.0	1.8	1.9
iso-$C_{16:1}$ G	2.0	−	−
iso-$C_{16:1}$ H	−	2.4	1.5
anteiso-$C_{17:1}\omega9c$	2.1	1.5	2.1
Summed feature 3*	7.9	13.4	13.5
Summed feature 9*	3.3	2.7	3.9

*Summed feature 3 comprised $C_{16:1}\omega6c$ and/or $C_{16:1}\omega7c$; summed feature 9 comprised iso-$C_{17:1}\omega9c$ and/or $C_{16:0}$ 10-methyl.

For the cellular fatty acid analysis, strain CBA3202^T, *G. mitskevichiae* KACC 15410^T and *G. hiemivivida* CIP 108528^T were cultivated on MA at 20 °C and harvested at the same physiological age, i.e. after 144, 69 and 144 h, respectively. Cells were collected from the third sector when tiny colonies were visible on the fourth sector (Sasser, 1990). Fatty acids were saponified, methylated and extracted using the standard protocol of MIDI (Sherlock Microbial Identification System, version 6.2). The fatty acids were analysed by GC (model 6890; Hewlett Packard) and identified using the TSBA6 database of the Microbial Identification System (Sasser, 1990). The fatty acid compositions of strain CBA3202^T and the two reference strains are shown in Table 2. $C_{16:0}$ was a predominant fatty acid in all strains. Major amounts of iso-$C_{15:1}$ G, iso-$C_{16:0}$ and summed feature 3 (comprising $C_{16:1}\omega6c$ and/or $C_{16:1}\omega7c$) were also present. Strain CBA3202^T differed from the two reference strains by presence of $C_{10:0}$ and iso-$C_{16:1}$ G and absence of iso-$C_{16:1}$ H. The polar lipids of strain CBA3202^T and the two reference strains were extracted and analysed by TLC on silica gel glass plates (Merck), which were sprayed with 5 % ethanolic molybdophosphoric acid, ninhydrin, molybdenum blue and α-naphthol-sulfuric acid, according to the method of Minnikin *et al.* (1984). The detected polar lipids of strain CBA3202^T and the two reference strains were phosphatidylethanolamine, two unidentified aminolipids and several unidentified polar lipids. No glycolipids were detected. The polar lipid profiles of the three strains only differed in the presence of a few unidentified polar lipids (Fig. 2). The respiratory quinones of strain CBA3202^T were characterized by HPLC according to the protocols of Collins (1985) and Wu *et al.* (1989), using menaquinone-6 (MK-6) extracted from *Gillisia myxillae* UST050418-085^T as a reference. Strain CBA3202^T had MK-6 as the only isoprenoid quinone. MK-6 is the only or major respiratory quinone in all species of the genus *Gillisia* and all members of the family *Flavobacteriaceae* (Bernardet, 2011). The DNA G+C content of strain CBA3202^T was 34.9 mol% as determined on the basis of the whole-genome sequence (Nam *et al.*, 2012). This value was within the range reported for species of the genus *Gillisia* (32–37.8 mol%; Bowman & Nichols, 2005; Lee *et al.*, 2006; Nedashkovskaya *et al.*, 2005; Van Trappen *et al.*, 2004).

In conclusion, strain CBA3202^T is closely related to members of the genus *Gillisia*, but it can be clearly differentiated from the type strains of species of the genus *Gillisia*. Based on the results of the polyphasic approach, strain CBA3202^T is described below as a novel species within the genus *Gillisia*, for which the name *Gillisia marina* sp. nov. is proposed. In addition, an emended description of the genus *Gillisia* is proposed on the basis of new data obtained in this study.

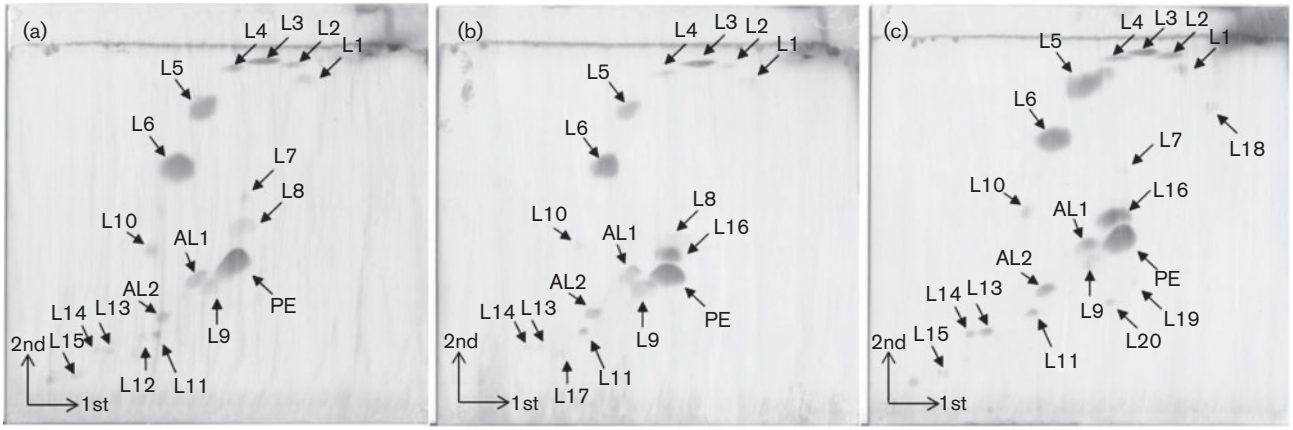

Fig. 2. Two-dimensional thin-layer chromatogram of the total polar lipids of strain CBA3202^T (a), *G. mitskevichiae* KACC 15410^T (b) and *G. hiemivivida* CIP 108528^T (c) detected with 5 % ethanolic molybdophosphoric acid. PE, Phosphatidylethanolamine; AL1–2, unidentified aminolipids; L1–20, unidentified polar lipids.

Emended description of the genus *Gillisia* Van Trappen *et al.* 2004

The description of the genus is as given by Van Trappen *et al.* (2004), with the following amendments. β-Galactosidase activity is species-dependent. The predominant fatty acids may include $C_{16:0}$ depending on the culture conditions. The major polar lipids in the type strains of the three analysed species are phosphatidylethanolamine and two unidentified lipids.

Description of *Gillisia marina* sp. nov.

Gillisia marina (ma.ri′na. L. fem. adj. *marina* of the sea, marine).

Cells are Gram-staining-negative, strictly aerobic, non-motile rods approximately 0.4–0.6 μm in diameter and 1.0–1.8 μm in length, devoid of flagella and endospores. Colonies are circular, yellow-pigmented and approximately 0.5–1 mm in diameter after 7 days on MA at 25 °C. Growth occurs at 4–30 °C (optimum, 25 °C) and in the presence of 1–6 % (w/v) NaCl (optimum, 3 %). No growth is observed on LBA, R2A agar, TSA or NA. Catalase- and oxidase-positive. Nitrate is not reduced. Flexirubin type pigments, indole and H_2S are not produced. Casein, DNA, gelatin, starch, Tween 20, Tween 40 and Tween 80 are hydrolysed, but aesculin, CM-cellulose, chitin and L-tyrosine are not. In the API ZYM strip, alkaline phosphatase, esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase activities are present; esterase (C4), lipase (C14), cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities are absent. In the API 50 CH strips the following substrates are assimilated: glycerol, L-arabinose, D-ribose, D-xylose, D-galactose, D-glucose, D-fructose, D-mannose, L-rhamnose, D-mannitol, cellobiose, maltose, melibiose, sucrose, trehalose, raffinose, starch, glycogen, turanose, L-fucose, gluconate and 5-ketogluconate; the following substrates are not assimilated: erythritol, D-arabinose, L-xylose, D-adonitol, methyl β-D-xyloside, L-sorbose, dulcitol, inositol, D-sorbitol, methyl α-D-mannoside, methyl α-D-glucoside, *N*-acetylglucosamine, amygdalin, arbutin, aesculin, salicin, lactose, inulin, melezitose, xylitol, gentiobiose, D-lyxose, D-tagatose, D-fucose, D-arabitol, L-arabitol and 2-ketogluconate. The polar lipids comprise phosphatidylethanolamine, two unidentified aminolipids and several unidentified polar lipids. The predominant fatty acids are $C_{16:0}$, iso-$C_{15:1}$ G, iso-$C_{16:0}$ and summed feature 3 (comprising $C_{16:1}\omega6c$ and/or $C_{16:1}\omega7c$).

The type strain is CBA3202^T (=KACC 16693^T=KCTC 32030^T=JCM 18402^T), isolated from seashore sand on Jeju Island, Republic of Korea. The DNA G+C content of the type strain is 34.9 mol%.

Acknowledgements

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References

Benson, H. J. (2002). *Microbiological Applications: aLlaboratory Manual in General Microbiology.* New York: McGraw-Hill.

Bernardet, J.-F. (2011). Family I. *Flavobacteriaceae* Reichenbach 1992. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 4, pp. 106–111. Edited by N. R. Krieg, W. Ludwig, W. B. Whitman, B. P. Hedlund, B. J. Paster, J. T. Staley, N. Ward, D. Brown & A. Parte. New York: Springer.

Bernardet, J.-F., Nakagawa, Y., Holmes, B. & Subcommittee on the taxonomy of Flavobacterium and Cytophaga-like bacteria of the International Committee on Systematics of Prokaryotes (2002). Proposed minimal standards for describing new taxa of the family *Flavobacteriaceae* and emended description of the family. *Int J Syst Evol Microbiol* **52**, 1049–1070.

Bowman, J. P. & Nichols, D. S. (2005). Novel members of the family *Flavobacteriaceae* from Antarctic maritime habitats including *Subsaximicrobium wynnwilliamsii* gen. nov., sp. nov., *Subsaximicrobium saxinquilinus* sp. nov., *Subsaxibacter broadyi* gen. nov., sp. nov., *Lacinutrix copepodicola* gen. nov., sp. nov., and novel species of the genera *Bizionia*, *Gelidibacter* and *Gillisia*. *Int J Syst Evol Microbiol* **55**, 1471–1486.

Collins, M. D. (1985). Isoprenoid quinone analysis in classification and identification. In *Chemical Methods in Bacterial Systematics*, pp. 267–287. Edited by M. Goodfellow & D. E. Minnikin. London: Academic Press.

Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in micro-dilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Evol Microbiol* **39**, 224–229.

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.

Kim, O. S., Cho, Y. J., Lee, K., Yoon, S. H., Kim, M., Na, H., Park, S. C., Jeon, Y. S., Lee, J. H. & other authors (2012). Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int J Syst Evol Microbiol* **62**, 716–721.

Kluge, A. G. & Farris, F. S. (1969). Quantitative phyletics and the evolution of anurans. *Syst Zool* **18**, 1–32.

Lee, O. O., Lau, S. C., Tsoi, M. M., Li, X., Plakhotnikova, I., Dobretsov, S., Wu, M. C., Wong, P. K. & Qian, P. Y. (2006). *Gillisia myxillae* sp. nov., a novel member of the family *Flavobacteriaceae*, isolated from the marine sponge *Myxilla incrustans*. *Int J Syst Evol Microbiol* **56**, 1795–1799.

Minnikin, D. E., O'Donnell, A. G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, A. & Parlett, J. H. (1984). An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* **2**, 233–241.

Nam, Y. D., Lee, H. W., Lee, M., Yim, K. J., Kim, K. N., Roh, S. W. & Kim, D. (2012). Draft genome sequence of *Gillisia* sp. strain CBA3202, a novel member of the genus *Gillisia*, which belongs to the family *Flavobacteriaceae*. *J Bacteriol* **194**, 3739.

Nedashkovskaya, O. I., Kim, S. B., Lee, K. H., Mikhailov, V. V. & Bae, K. S. (2005). *Gillisia mitskevichiae* sp. nov., a novel bacterium of the family *Flavobacteriaceae*, isolated from sea water. *Int J Syst Evol Microbiol* **55**, 321–323.

Percival Zhang, Y. H., Himmel, M. E. & Mielenz, J. R. (2006). Outlook for cellulase improvement: screening and selection strategies. *Biotechnol Adv* **24**, 452–481.

Roh, S. W., Sung, Y., Nam, Y. D., Chang, H. W., Kim, K. H., Yoon, J. H., Jeon, C. O., Oh, H. M. & Bae, J. W. (2008). *Arthrobacter soli* sp. nov., a novel bacterium isolated from wastewater reservoir sediment. *J Microbiol* **46**, 40–44.

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

Sasser, M. (1990). *Identification of bacteria by gas chromatography of cellular fatty acids*, MIDI Technical Note 101. Newark, DE: MIDI.

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

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* **28**, 2731–2739.

Van Trappen, S. (2011). Genus XXIV. *Gillisia* Van Trappen *et al.* 2004. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 4, pp. 221–226. Edited by N. R. Krieg, W. Ludwig, W. B. Whitman, B. P. Hedlund, B. J. Paster, J. T. Staley, N. Ward, D. Brown & A. Parte. New York: Springer.

Van Trappen, S., Vandecandelaere, I., Mergaert, J. & Swings, J. (2004). *Gillisia limnaea* gen. nov., sp. nov., a new member of the family *Flavobacteriaceae* isolated from a microbial mat in Lake Fryxell, Antarctica. *Int J Syst Evol Microbiol* **54**, 445–448.

Wu, C., Lu, X., Qin, M., Wang, Y. & Ruan, J. (1989). Analysis of menaquinone compound in microbial cells by HPLC. *Microbiology* [*English translation of Microbiology (Beijing)*] **16**, 176–178.