Corallibacter vietnamensis gen. nov., sp. nov., a marine bacterium of the family *Flavobacteriaceae*

Seung Bum Kim,¹ Olga I. Nedashkovskaya,² Natalia V. Zhukova,^{3,4}
Andrey D. Kuchlevskiy^{3,4} and Valery V. Mikhailov²

Correspondence

Olga I. Nedashkovskaya
olganedashkovska@yahoo.com or
olganedashkovska@piboc.dvo.ru

¹Department of Microbiology and Molecular Biology, School of Bioscience and Biotechnology, Chungnam National University, 220 Gung-dong, Yuseong, Daejeon 305-764, Republic of Korea

²Pacific Institute of Bioorganic Chemistry of the Far-Eastern Branch of the Russian Academy of Sciences, Pr. 100 Let Vladivostoku 159, 690022 Vladivostok, Russia

³A. V. Zhirmunsky Institute of Marine Biology of the Far-Eastern Branch of the Russian Academy of Science, Pal'chevskogo St. 17, 690032 Vladivostok, Russia

⁴Far-Eastern Federal University, Sukhanova St. 8, 690950 Vladivostok, Russia

The taxonomic position of two strains of a novel heterotrophic, strictly aerobic, Gram-negative, gliding, dark-orange-pigmented bacterium, designated KMM 6217^T and KMM 6332, was determined. The strains grew at 10–40 °C and with 0.5–8.0% NaCl. They were catalase- and oxidase-positive, produced flexirubin-type pigments and hydrolysed aesculin, gelatin, starch, Tween 80 and DNA. The predominant fatty acids were iso-C_{15:1}, iso-C_{15:0}, C_{15:0}, summed feature 3 (comprising iso-C_{15:0} 2-OH and/or C_{16:1} ω7c) and iso-C_{17:0} 3-OH. The polar lipid profile comprised phosphatidylethanolamine as the major component, three unknown aminolipids and one unknown lipid. The DNA G + C content was 34.7–34.8 mol%. Phylogenetic analysis of the 16S rRNA gene sequence showed that the novel isolates could be placed in the family *Flavobacteriaceae*. The nearest neighbours of strains KMM 6217^T and KMM 6332 were members of the genera *Bizionia*, *Formosa*, *Lacinutrix*, *Meridianimaribacter*, *Mesoflavibacter* and *Winogradskyella*, with sequence similarities to the type strains of species of these genera of 92.8–95.2, 93.7–94.5, 94.1–94.7, 94.9, 94.6 and 93.5–94.4%, respectively. Based on phenotypic, genotypic and phylogenetic data, strains KMM 6217^T and KMM 6332 should be classified as representatives of a novel species in a new genus in the family *Flavobacteriaceae* of the phylum 'Bacteroidetes', for which the name *Corallibacter vietnamensis* gen. nov., sp. nov. is proposed; the type strain is KMM 6217^T (=JCM 17525^T=KCTC 23026^T).

During a study of microbial communities in shallow water, it was observed that a coral (*Palythoa* sp.) collected from Vanfong Bay, South China Sea, Vietnam, harboured diverse cultivable bacteria. It should be noted that the majority of the isolated strains were pigmented and affiliated with the phylum 'Bacteroidetes'. In previous studies, we have described such novel representatives of the classes *Cytophagia* and *Flavobacteria* as *Algoriphagus vanfongensis* and *Leeuwenhoekiella palythoae*, respectively (Nedashkovskaya *et al.*, 2007, 2009a). Here, we report the isolation and taxonomic identification of two novel Gram-negative strains, designated KMM 6217^T and KMM 6332, that were associated with the coral *Palythoa* sp. Comparative phylogenetic analysis based on 16S rRNA gene sequences revealed that strains KMM

6217^T and KMM 6332 formed a distinct lineage in the family *Flavobacteriaceae*. A detailed study of their chemotaxonomic, phenotypic and molecular characteristics was used to determine the precise taxonomic position of the novel isolates.

For isolation of strains KMM 6217^T and KMM 6332, 0.1 ml coral tissue homogenate was transferred onto medium containing (1⁻¹) 5.0 g Bacto peptone (Difco), 5.0 g sucrose, 1.0 g glucose, 2.5 g yeast extract (Difco), 0.1 g KH₂PO₄, 0.1 g MgSO₄ and 15.0 g Bacto agar (Difco) in 30% (v/v) natural seawater and 70% (v/v) distilled water, pH 7.2–7.5. After primary isolation and purification, the strains were cultivated at 28 °C on the same medium or on marine agar 2216 (MA) and stored at –80 °C in marine broth 2216 (both from Difco) supplemented with 20% (v/v) glycerol.

An almost complete 16S rRNA gene sequence (1463 nt) of strain KMM 6217^T was determined by following the

The GenBank/EMBL/DBJ accession numbers for the 16S rRNA gene sequences of *Corallibacter vietnamensis* KMM 6217^T and KMM 6332 are HQ257254 and HQ882589, respectively.

A supplementary figure is available with the online version of this paper.

procedure described previously (Kim *et al.*, 1998). DNA of strain KMM 6332 was extracted from 0.1–0.2 g cells (wet wt), using the DNA-extraction protocol of Sambrook *et al.* (1989). PCR was performed in 25 µl of a mixture containing 75 mM Tris/HCl (pH 8.8), 20 mM (NH₄)₂SO₄, 0.01 % Tween 20 and 1.5 mM MgCl₂, 1 mM of a mixture containing 0.25 mM of each dNTP, 0.25 µM of each primer, 10 ng DNA and 1 U *Taq* polymerase (Fermentas). PCR amplification of the 16S rRNA gene was carried out using universal oligonucleotide primers 11F (5'-GTTTGATCMTGGCTC-AG-3') and 1492R (5'-TACGGYTACCTTGTACGACTT-3') as described by Lane (1991). PCR was performed using a GeneAmp PCR System 9700 (Applied Biosystems). Amplification conditions for PCR included an initial denaturation step (94 °C for 3 min), 30 amplification cycles consisting of denaturation (94 °C for 30 s), annealing (56 °C for 30 s) and elongation (72 °C for 1 min 45 s), and a final cycle of 72 °C for 5 min. Reaction products were checked for size and purity on 1 % agarose gel. PCR amplicons were used as templates for sequencing amplification using a BigDye Terminator version 3.1 Cycle sequencing kit (Applied Biosystems). The purified sequencing products were analysed by electrophoresis on a 50 cm capillary array of an ABI Prism 3130 DNA sequencer. Sequences were assembled with SeqScape version 2.6 software (Applied

Biosystems). The obtained sequences were aligned with those of representative members of selected genera of the family *Flavobacteriaceae* by using PHYDIT version 3.2 (<http://plaza.snu.ac.kr/~jchun/phydit/>). Phylogenetic trees were inferred by using suitable programs of the PHYLIP package (Felsenstein, 1993). Phylogenetic distances were calculated from the Jukes–Cantor model (Jukes & Cantor, 1969) and trees were constructed on the basis of the neighbour-joining (Saitou & Nei, 1987) and maximum-likelihood (Felsenstein, 1993) algorithms. A bootstrap analysis was performed with 1000 resampled datasets by using the SEQBOOT and CONSENSE programs of the PHYLIP package.

Phylogenetic analysis indicated that strains KMM 6217^T and KMM 6332 formed a distinct lineage within the family *Flavobacteriaceae* (Fig. 1). The 16S rRNA gene sequence similarity between the two strains was 99.8 %. The closest relatives of the novel isolates were members of recognized species of the genera *Bizionia*, *Formosa*, *Lacinutrix*, *Meridianimaribacter*, *Mesoflavibacter* and *Winogradskyella* with sequence similarities to the type strains of the type species of the genera listed of 92.8–95.2 % (mean 93.9 %), 93.7–94.5 % (mean 94.0 %), 94.1–94.7 % (mean 94.4 %), 94.9 %, 94.6 % and 93.5–94.4 % (mean 93.9 %), respectively. *Bizionia echini* KMM 6177^T and *Meridianimaribacter*

Fig. 1. Phylogenetic tree inferred from the 16S rRNA gene sequences of KMM 6217^T and KMM 6332 and related taxa of the family *Flavobacteriaceae*. The tree was constructed using distances based on the Jukes–Cantor model and the neighbour-joining algorithm. Numbers at nodes are bootstrap values (%) calculated using 1000 replicates; only values at nodes representing a genus or above are presented. Asterisks indicate clades at the genus level that were also recovered from the maximum-likelihood tree. Bar, 0.01 substitutions per nucleotide position.

flavus NH57N^T were the nearest neighbours of the two isolates with sequence similarities of 95.1–95.2 and 94.9 %, respectively.

Genomic DNA was isolated following the method of Marmur (1961) and DNA G + C contents were determined by the thermal denaturation method (Marmur & Doty, 1962). The DNA G + C contents of KMM 6217^T and KMM 6332 were 34.8 and 34.7 mol%, respectively. DNA–DNA hybridization experiments were performed spectrophotometrically using the initial renaturation rate method by De Ley *et al.* (1970). The strains under study shared 96 % DNA–DNA hybridization, indicating that the two coral isolates belong to a single species.

For determination of whole-cell fatty acid and polar lipid profiles, strains KMM 6217^T, KMM 6332, *Bizionia paragorgiae* KMM 6029^T, *Formosa algae* KMM 3553^T, *Lacinutrix copepodicola* DJ3^T, *Meridianimaribacter flavus* NH57N^T, *Mesoflavibacter zeaxanthinifaciens* TD-ZX30^T and *Winogradskyella thalassocola* KMM 3907^T were grown at 28 °C for 48 h on MA. Cellular fatty acid methyl esters were prepared according to the standard protocol of the Microbial Identification System (MIDI, version 3.5; Sasser, 1990) and analysed by GC (GC-17A; Shimadzu) equipped with a fused silica capillary column (30 m × 0.25 mm) coated with Supelcowax-10 and SPB-5 phases (Supelco) at 210 °C. Fatty acid methyl esters were identified using equivalent chain-length measurements and by comparing retention times with those of authentic standards. Fatty acid methyl esters were also analysed by GC-MS (Shimadzu QP5050A) equipped with an MDN-5S capillary column (30 m × 0.25 mm) using injector and detector temperatures of 250 °C. The major cellular fatty acid components of strains KMM 6217^T and KMM 6332 were iso-C_{15:1} (29.7–29.9 %), iso-C_{15:0} (20.2–23.3 %), C_{15:0} (9.9–10.6 %), summed feature 3 (comprising C_{16:1}ω7c and iso-C_{15:0} 2-OH; 9.1–10.2 %) and iso-C_{17:0} 3-OH (6.3–6.5 %), which are characteristic for members of the family *Flavobacteriaceae* (Bernardet *et al.*, 2002). The novel strains and their closest relatives showed similar fatty acid profiles; the major constituents included iso-C_{15:1}, iso-C_{15:0} and iso-C_{15:0} 3-OH (Table 1). However, significant differences in cellular fatty acid composition were found between the strains studied and the type strains of their close relatives, thus supporting our suggestion to classify strains KMM 6217^T and KMM 6332 as representatives of a novel species in a new genus. Polar lipids were extracted and analysed as described previously (Nedashkovskaya *et al.*, 2006b). The polar lipid profiles of the coral strains comprised phosphatidylethanolamine as major phospholipid, three unknown aminolipids and one unknown lipid (Supplementary Fig. S1 available in IJSEM Online). In contrast to the novel strains, cell extracts of the *B. paragorgiae* KMM 6029^T and *Meridianimaribacter flavus* NH57N^T, the nearest neighbours of the strains under study, were found to contain only two aminolipids (Supplementary Fig. S1). Isoprenoid quinone composition was characterized by HPLC (Shimadzu LC-10A) using a reversed-phase type Supelcosil LC-18 column (15 cm × 4.6 mm) and acetonitrile/

Table 1. Cellular fatty acid composition (%) of *Corallibacter vietnamensis* gen. nov., sp. nov. and the type strains of closely related species of the family *Flavobacteriaceae*

Taxa: 1, *Corallibacter vietnamensis* gen. nov., sp. nov. (strains KMM 6217^T and KMM 6332); 2, *Bizionia paragorgiae* KMM 6029^T; 3, *Formosa algae* KMM 3553^T; 4, *Lacinutrix copepodicola* DJ3^T; 5, *Meridianimaribacter flavus* NH57N^T; 6, *Mesoflavibacter zeaxanthinifaciens* TD-ZX30^T; 7, *Winogradskyella thalassocola* KMM 3907^T. Values of less than 1 % for all strains are not shown. Predominant fatty acids are shown in bold. All data were obtained in this study. –, Not detected.

Fatty acid	1	2	3	4	5	6	7
Straight chain							
C _{14:0}	1.2–1.3	0.4	1.0	0.5	0.5	0.5	0.7
C _{15:0}	9.9–10.6	2.8	27.2	4.0	9.5	8.2	10.2
C _{16:0}	2.7–3.5	1.2	1.0	1.1	2.6	2.0	1.0
Branched							
iso-C _{15:1}	29.7–29.9	17.8	15.3	4.9	37.5	27.9	16.4
anteiso-C _{15:1}	–	2.3	–	3.3	–	3.1	–
iso-C _{15:0}	20.2–23.3	18.0	13.3	11.5	20.4	16.0	10.9
anteiso-C _{15:0}	–	4.5	7.2	20.2	–	2.4	9.6
anteiso-C _{16:1}	0.4–0.5	5.3	–	2.2	–	–	–
iso-C _{16:0}	2.3	7.7	2.3	21.0	3.0	2.9	1.0
anteiso-C _{16:0}	0.3–0.4	1.7	1.0	1.0	1.1	1.4	–
iso-C _{17:1} ω12c	0.9–1.3	2.5	1.4	2.2	–	–	0.6
anteiso-C _{17:0}	–	–	–	1.8	–	–	–
cyclo-C _{17:0}	–	–	7.4	–	–	–	–
Unsaturated							
C _{15:1} ω8c	1.1–1.4	–	1.0	0.3	2.3	2.3	1.4
C _{15:1} ω6c	1.0	1.0	13.0	0.9	–	–	4.5
C _{16:1} ω5c	–	1.1	–	–	–	–	1.0
C _{17:1} ω6c	0.4–0.5	0.7	–	0.4	–	–	2.6
C _{17:1} ω8c	–	–	2.2	–	–	–	–
C _{18:1} ω9c	0.3–0.9	1.0	0.5	–	0.4	0.4	–
Hydroxy							
C _{11:0} 3-OH	–	–	–	–	1.5	–	–
C _{14:0} 3-OH	–	–	1.1	–	–	–	–
iso-C _{15:0} 3-OH	4.0–4.9	1.1	–	0.4	0.8	1.7	8.0
C _{15:0} 3-OH	0.4	–	–	0.4	–	0.4	1.4
iso-C _{16:0} 3-OH	1.8–1.9	7.9	–	4.2	4.2	10.8	7.9
C _{16:0} 3-OH	0.6–1.0	–	–	0.5	–	0.3	1.0
iso-C _{17:0} 3-OH	6.3–6.5	3.4	–	1.3	3.9	5.2	3.4
Summed feature 3*	9.1–10.2	15.1	3.3	7.6	8.1	10.1	9.2

*Summed feature 3 consists of C_{16:1}ω7c and iso-C_{15:0} 2-OH fatty acids which could not be separated by the Microbial Identification System.

2-propanol (65 : 35, v/v) as a mobile phase at a flow rate of 0.5 ml min^{−1}. The column was kept at 40 °C. Menaquinones were detected by monitoring at 270 nm and identified by comparison with known quinones from reference strain *B. paragorgiae* KMM 6029^T. The major respiratory quinone of the novel strains was MK-6.

Phenotypic analysis of strains KMM 6217^T and KMM 6332 was performed by using previously described methods

Table 2. Differential phenotypic characteristics of *Corallibacter vietnamensis* gen. nov., sp. nov. and closely related members of the family *Flavobacteriaceae*

Taxa: 1, *Corallibacter vietnamensis* gen. nov., sp. nov. (strains KMM 6217^T and KMM 6332); 2, *Bizionia* (data for seven species); 3, *Formosa* (two species); 4, *Lacinutrix* (three species); 5, *Meridianimaribacter flavus* NH57N^T; 6, *Mesoflavibacter zeaxanthinifaciens* TD-ZX30^T; 7, *Winogradskyella* (eight species). All taxa are positive for oxidase, catalase and alkaline phosphatase activities, hydrolysis of gelatin and carbohydrate utilization. All taxa are negative for organic growth factor requirement, nitrate reduction and indole production. v, Variable reaction; ND, not determined; data in parentheses are for type strains of type species. Data are from Asker *et al.* (2007), Bercovich *et al.* (2008), Bowman & Nichols (2005), Ivanova *et al.* (2004), Kim & Nedashkovskaya (2010), Lau *et al.* (2005), Nedashkovskaya *et al.* (2005a, b, 2006a, 2008, 2009b, 2010), Pinhassi *et al.* (2009), Romanenko *et al.* (2009), Wang *et al.* (2010) and this study.

Characteristic	1	2	3	4	5	6	7
Cell size (µm)	0.4–0.5 × 1.4–3.2	0.2–0.5 × 1.4–5.0	0.4–0.9 × 0.8–1.8	0.4–0.8 × 0.7–2.5	0.3–0.5 × 1.1–2.3	0.3–0.4 × 1.7–5.3	0.2–0.7 × 0.8–7.3
Colony colour*	DO	YO	Y	Y	Y	Y	Y
Gliding motility	+	–	+	–	+	+	+
Salinity range for growth (%)	0.5–8.0	1–17	0–8	0–10	0.5–4.0	1–10	1–9
Growth temperature range (°C)	10–40	–2 to 39	4–34	–2 to 25	9–37	16–44	4–44
Flexirubin-type pigment production	+	–	–	–	–	–	–
Arginine dihydrolase activity	–	v (–)	–	–	–	+	–
β-Galactosidase activity	–	–	v (–)	v (–)	+	+	–
Acid production from carbohydrates	+	–	+	–	ND	+	+
Citrate utilization	+	v (–)	–	–	–	–	–
Acetoin production	–	–	–	–	+	–	v (–)
Degradation of:							
Casein	–	v (+)	–	v (–)	–	+	v (–)
Starch	+	–	v	+	+	+	v (–)
Tween 80	+	v (+)	–	v (+)	–	+	v (–)
DNA G + C content (mol%)	34.3–34.8	34–45	34–36	34–37	32.7	32.0	32–36

*DO, Dark orange; YO, yellow–orange; Y, yellow.

(Nedashkovskaya *et al.*, 2003, 2004). API 20E, API 20NE and API ZYM galleries (bioMérieux) were also used according to the manufacturer's instructions except that the galleries were incubated at 28 °C.

The physiological, biochemical and morphological characteristics of the novel strains are listed in the species description and in Table 2. Strains KMM 6217^T and KMM 6332 differed from all close relatives by production of flexirubin-type pigments and citrate utilization (Table 2). Properties such as gliding motility, acid production from carbohydrates and hydrolysis of starch can be helpful for distinguishing the novel isolates from members of the genus *Bizionia*. The inability of the novel isolates to produce β -galactosidase and acetoin may be used to discriminate them from their other nearest neighbour, *Meridianimaribacter flavus*. The new genus status of the coral strains was further supported by differences in phenotypic characteristics between the novel isolate and its close relatives (Table 2). For example, strains belonging to the genus *Lacinutrix* demonstrated no gliding motility or acid production from carbohydrates, in contrast to the coral epibionts. Strains KMM 6217^T and KMM 6332 can be distinguished from *Mesoflavibacter zeaxanthinifaciens*, another close neighbour, by the absence of arginine dihydrolase, β -galactosidase and caseinase activities.

Based on the results of phylogenetic, genotypic and phenotypic analyses presented in this work, *Corallibacter vietnamensis* gen. nov., sp. nov. is proposed with KMM 6217^T (=JCM 17525^T=KCTC 23026^T) as the type strain.

Description of *Corallibacter* gen. nov.

Corallibacter (Co.ral.li.bac'ter. L. n. *corallium* coral; N.L. masc. n. *bacter* a rod; N.L. masc. n. *Corallibacter* a rod from a coral, referring to the isolation of the first strains from the coral *Palythoa* sp.).

Cells are Gram-negative, rod-shaped and strictly aerobic. Do not form endospores. Non-diffusible pigments are produced. Chemo-organotroph. Cytochrome oxidase-, catalase- and alkaline phosphatase-positive. The predominant cellular fatty acids are branched-chain saturated and unsaturated, and straight-chain unsaturated fatty acids iso-C_{15:1}, iso-C_{15:0}, C_{15:0}, summed feature 3 (comprising iso-C_{15:0} 2-OH and/or C_{16:1} ω7c) and iso-C_{17:0} 3-OH. Polar lipid profile comprises phosphatidylethanolamine as the major component, three unknown aminolipids and one unknown lipid. The major respiratory quinone is MK-6. The DNA G+C content of the type species is 34–35 mol%. 16S rRNA gene sequence analysis indicates that the genus *Corallibacter* is a member of the family *Flavobacteriaceae* within the phylum 'Bacteroidetes'. The type species is *Corallibacter vietnamensis*.

Description of *Corallibacter vietnamensis* sp. nov.

Corallibacter vietnamensis (vi.et.na.men'sis. N.L. masc. adj. *vietnamensis* of or belonging to Vietnam, the country of origin of the type strain).

Main characteristics are those given for the genus. In addition, cells are motile by gliding, 0.4–0.5 µm in width and 1.4–3.2 µm in length. On MA, colonies are 2–3 mm in diameter, circular with entire edges and dark orange in colour. Growth occurs with 0.5–8.0 % NaCl, at 10–40 °C and at pH 4.0–10.0. Optimal growth is observed with 2.0–4.0 % NaCl and at pH 5.5–7.5. Flexirubin-type pigments are produced. Arginine dihydrolase, ornithine decarboxylase, lysine decarboxylase and tryptophan deaminase activities are absent. Aesculin, gelatin, starch, DNA, and Tweens 40 and 80 are hydrolysed, but agar, casein, Tween 20, urea and chitin are not. Forms acid from glucose and maltose, but not from L-arabinose, cellobiose, fructose, D-galactose, lactose, raffinose, L-rhamnose, L-sorbose, sucrose, DL-xylose, N-acetylglucosamine, citrate, glycerol, inositol or mannitol. L-Arabinose, mannose and citrate are utilized, but lactose, sucrose, inositol, mannitol and sorbitol are not. According to API ZYM, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, cystine arylamidase, valine arylamidase, acid and alkaline phosphatases, naphthol-AS-BI-phosphohydrolase, α -glucosidase and N-acetylglucosaminidase activities are present, but not trypsin, α -chymotrypsin, β -glucuronidase, α -galactosidase, β -galactosidase, β -glucosidase, α -mannosidase or α -fucosidase activities. Indole, hydrogen sulfide and acetoin are not produced. Susceptible to carbenicillin, chloramphenicol, erythromycin and lincomycin; resistant to ampicillin, benzylpenicillin, doxycycline, gentamicin, kanamycin, neomycin, oleandomycin, polymyxin, streptomycin and tetracycline. Other biochemical and physiological characteristics are listed in Table 2.

The type strain is KMM 6217^T (=JCM 17525^T=KCTC 23026^T), isolated from the coral *Palythoa* sp., collected from Vanfong Bay, South China Sea, Vietnam. The DNA G+C content of the type strain is 34.8 mol%.

Acknowledgements

This research was supported by grants from the Presidium of the Russian Academy of Sciences 'Molecular and Cell Biology', the Presidium of the Far-Eastern Branch of the Russian Academy of Sciences no. 069-III-A-06-183227 and the Government of Russian Federation for the state support of scientific investigations conducted under the guidance of the leading researchers at the Russian education institutions of the high professional education, agreement no. 11.G34.31.0010.

References

- Asker, D., Beppu, T. & Ueda, K. (2007). *Mesoflavibacter zeaxanthinifaciens* gen. nov., sp. nov., a novel zeaxanthin-producing marine bacterium of the family *Flavobacteriaceae*. *Syst Appl Microbiol* **30**, 291–296.
- Bercovich, A., Vazquez, S. C., Yankilevich, P., Coria, S. H., Foti, M., Hernández, E., Vidal, A., Ruberto, L., Melo, C. & other authors (2008). *Bizionia argentinensis* sp. nov., isolated from surface marine water in Antarctica. *Int J Syst Evol Microbiol* **58**, 2363–2367.
- 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 wynmwilliamsii* 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.

De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.

Felsenstein, J. (1993). PHYLIP (phylogenetic inference package), version 3.5c. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.

Ivanova, E. P., Alexeeva, Y. V., Flavier, S., Wright, J. P., Zhukova, N. V., Gorshkova, N. M., Mikhailov, V. V., Nicolau, D. V. & Christen, R. (2004). *Formosa algae* gen. nov., sp. nov., a novel member of the family *Flavobacteriaceae*. *Int J Syst Evol Microbiol* **54**, 705–711.

Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, vol. 3, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.

Kim, S. B. & Nedashkovskaya, O. I. (2010). *Winogradskyella pacifica* sp. nov., a marine bacterium of the family *Flavobacteriaceae*. *Int J Syst Evol Microbiol* **60**, 1948–1951.

Kim, S. B., Falconer, C., Williams, E. & Goodfellow, M. (1998). *Streptomyces thermocarboxydovorans* sp. nov. and *Streptomyces thermocarboxydus* sp. nov., two moderately thermophilic carboxydophilic species from soil. *Int J Syst Bacteriol* **48**, 59–68.

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

Lau, S. C. K., Tsoi, M. M. Y., Li, X., Plakhotnikova, I., Dobretsov, S., Lau, K. W. K., Wu, M., Wong, P. K., Pawlik, J. R. & Qian, P. Y. (2005). *Winogradskyella poriferorum* sp. nov., a novel member of the family *Flavobacteriaceae* isolated from a sponge in the Bahamas. *Int J Syst Evol Microbiol* **55**, 1589–1592.

Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208–218.

Marmur, J. & Doty, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J Mol Biol* **5**, 109–118.

Nedashkovskaya, O. I., Kim, S. B., Han, S. K., Lysenko, A. M., Rohde, M., Zhukova, N. V., Falsen, E., Frolova, G. M., Mikhailov, V. V. & Bae, K. S. (2003). *Mesonina algae* gen. nov., sp. nov., a novel marine bacterium of the family *Flavobacteriaceae* isolated from the green alga *Acrosiphonia sonderi* (Kütz) Kornm. *Int J Syst Evol Microbiol* **53**, 1967–1971.

Nedashkovskaya, O. I., Kim, S. B., Han, S. K., Rhee, M. S., Lysenko, A. M., Falsen, E., Frolova, G. M., Mikhailov, V. V. & Bae, K. S. (2004). *Ulvibacter litoralis* gen. nov., sp. nov., a novel member of the family *Flavobacteriaceae* isolated from the green alga *Ulva fenestrata*. *Int J Syst Evol Microbiol* **54**, 119–123.

Nedashkovskaya, O. I., Kim, S. B., Han, S. K., Snauwaert, C., Vancanneyt, M., Swings, J., Kim, K. O., Lysenko, A. M., Rohde, M. & other authors (2005a). *Winogradskyella thalassocola* gen. nov., sp. nov., *Winogradskyella epiphytica* sp. nov. and *Winogradskyella eximia* sp. nov., marine bacteria of the family *Flavobacteriaceae*. *Int J Syst Evol Microbiol* **55**, 49–55.

Nedashkovskaya, O. I., Kim, S. B., Lysenko, A. M., Frolova, G. M., Mikhailov, V. V. & Bae, K. S. (2005b). *Bizionia paragorgiae* gen. nov., sp. nov., a novel member of the family *Flavobacteriaceae* isolated from the soft coral *Paragorgia arborea*. *Int J Syst Evol Microbiol* **55**, 375–378.

Nedashkovskaya, O. I., Kim, S. B., Vancanneyt, M., Snauwaert, C., Lysenko, A. M., Rohde, M., Frolova, G. M., Zhukova, N. V., Mikhailov, V. V. & other authors (2006a). *Formosa agariphila* sp. nov., a budding bacterium of the family *Flavobacteriaceae* isolated from marine environments, and emended description of the genus *Formosa*. *Int J Syst Evol Microbiol* **56**, 161–167.

Nedashkovskaya, O. I., Kim, S. B., Zhukova, N. V., Kwak, J., Mikhailov, V. V. & Bae, K. S. (2006b). *Mesonina mobilis* sp. nov., isolated from seawater, and emended description of the genus *Mesonina*. *Int J Syst Evol Microbiol* **56**, 2433–2436.

Nedashkovskaya, O. I., Kim, S. B., Kwon, K. K., Shin, D. S., Luo, X., Kim, S. J. & Mikhailov, V. V. (2007). Proposal of *Algoriphagus vanfongensis* sp. nov., transfer of members of the genera *Hongiella* Yi and Chun 2004 emend. Nedashkovskaya *et al.* 2004 and *Chimaericella* Tiago *et al.* 2006 to the genus *Algoriphagus*, and emended description of the genus *Algoriphagus* Bowman *et al.* 2003 emend. Nedashkovskaya *et al.* 2004. *Int J Syst Evol Microbiol* **57**, 1988–1994.

Nedashkovskaya, O. I., Kwon, K. K., Yang, S. H., Lee, H. S., Chung, K. H. & Kim, S. J. (2008). *Lacinutrix algicola* sp. nov. and *Lacinutrix mariniflava* sp. nov., two novel marine alga-associated bacteria and emended description of the genus *Lacinutrix*. *Int J Syst Evol Microbiol* **58**, 2694–2698.

Nedashkovskaya, O. I., Vancanneyt, M., Kim, S. B., Zhukova, N. V., Han, J. H. & Mikhailov, V. V. (2009a). *Leeuwenhoeekiella palythoae* sp. nov., a new member of the family *Flavobacteriaceae*. *Int J Syst Evol Microbiol* **59**, 3074–3077.

Nedashkovskaya, O. I., Vancanneyt, M., Kim, S. B. & Zhukova, N. V. (2009b). *Winogradskyella echinorum* sp. nov., a marine bacterium of the family *Flavobacteriaceae* isolated from the sea urchin *Strongylocentrotus intermedius*. *Int J Syst Evol Microbiol* **59**, 1465–1468.

Nedashkovskaya, O. I., Vancanneyt, M. & Kim, S. B. (2010). *Bizionia echini* sp. nov., isolated from a sea urchin. *Int J Syst Evol Microbiol* **60**, 928–931.

Pinhassi, J., Nedashkovskaya, O. I., Hagström, Å. & Vancanneyt, M. (2009). *Winogradskyella rapida* sp. nov., isolated from protein-enriched seawater. *Int J Syst Evol Microbiol* **59**, 2180–2184.

Romanenko, L. A., Tanaka, N., Frolova, G. M. & Mikhailov, V. V. (2009). *Winogradskyella arenosi* sp. nov., a member of the family *Flavobacteriaceae* isolated from marine sediments from the Sea of Japan. *Int J Syst Evol Microbiol* **59**, 1443–1446.

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

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

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

Wang, B., Sun, F., Du, Y., Liu, X., Li, G., Lai, Q., Luo, J. & Shao, Z. (2010). *Meridianimaribacter flavus* gen. nov., sp. nov., a member of the family *Flavobacteriaceae* isolated from marine sediment of the South China Sea. *Int J Syst Evol Microbiol* **60**, 121–127.