Marinoscillum gen. nov., a member of the family 'Flexibacteraceae', with *Marinoscillum pacificum* sp. nov. from a marine sponge and *Marinoscillum furvescens* nom. rev., comb. nov.

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A novel strain, designated MRN461^T, was isolated from a marine sponge in Micronesia. The 16S rRNA gene sequence of the isolate showed 95.6 % similarity with that of '*Microscilla furvescens*' IFO (now NBRC) 15994. Phylogenetic analysis revealed that the isolate and '*Microscilla furvescens*' IFO 15994 formed a distinct phyletic line within the family 'Flexibacteraceae'. Cells of strain MRN461^T were Gram-negative, long filamentous rods, motile by gliding. Growth was observed at 15–40 °C (optimum, 33 °C), at pH 5.0–9.5 (optimum, pH 7.5) and in the presence of 0.5–7.0 % sea salts (optimum, 2.5 %). The major isoprenoid quinone was MK-7. The dominant fatty acids were summed feature 3 (comprising iso-C_{15:0} 2-OH and/or C_{16:1}ω7c; 34.8 %), C_{16:1}ω5c (21.6 %) and iso-C_{16:1} (19.8 %). The DNA G+C content was 41.5 mol%. On the basis of evidence from our polyphasic taxonomic study, strain MRN461^T is classified within a novel genus and species in the family 'Flexibacteraceae', for which the name *Marinoscillum pacificum* gen. nov., sp. nov. is proposed. The type strain of *Marinoscillum pacificum* is strain MRN461^T (=KCCM 42325^T =JCM 14064^T). The misclassified species '*[Microscilla] furvescens*' is transferred to the new genus as *Marinoscillum furvescens* (ex Lewin 1969) nom. rev., comb. nov., with LMG 13023^T (=DSM 4134^T =ATCC 23129^T =NBRC 15994^T) as the type strain.

Although widely distributed in coastal marine ecosystems, the heterotrophic, Gram-negative bacteria, non-motile or motile by gliding, that belong to the phylum *Bacteroidetes* (previously *Cytophaga*–*Flavobacterium*–*Bacteroides*) have been poorly investigated (Nedashkovskaya *et al.*, 2003). On the basis of 16S rRNA gene sequence analysis, Nakagawa *et al.* (2002) demonstrated the considerable heterogeneity of strains previously allocated to the genera *Flexibacter*, *Microscilla* and *Flexithrix*. They proposed that all *Flexibacter* and *Microscilla* species except for the type species *Flexibacter flexilis* and *Microscilla marina* should be reclassified on the basis of their genomic and phenotypic characteristics. For instance, *[Flexibacter] sancti*, *[Flexibacter] filiformis* and *[Flexibacter] japonensis* were transferred to the genus *Chitinophaga* (Kämpfer *et al.*, 2006) and '*[Microscilla] arenaria*' was reclassified as *Flammeovirga arenaria* (Takahashi *et al.*, 2006). However, *[Flexibacter] tractuosus*, '*[Microscilla] furvescens*' and

'*[Microscilla] sericea*' have not yet been reclassified and remain incorrectly classified. In the present study, we report the results of a polyphasic taxonomic investigation of the novel strain MRN461^T isolated from a marine sponge. The isolate represents a novel genus in the family 'Flexibacteraceae' together with '*[Microscilla] furvescens*' (Lewin, 1969; Lewin & Lounsbury, 1969).

Strain MRN461^T was isolated from an unidentified marine sponge inhabiting the coast of the tropical Weno Island, Chuuk State, Federated States of Micronesia. A small piece of sponge was homogenized, diluted with sterile seawater and spread on marine agar 2216 (MA; Difco). Inoculated plates were cultivated at 25 °C for 2 days and morphologically distinct colonies were purified. Strain MRN461^T and '*[Microscilla] furvescens*' LMG 13023 were cultivated at 25 °C on MA for biochemical and physiological characterization and stored at –80 °C in marine broth (MB; Difco) supplemented with 20 % (v/v) glycerol (Bae *et al.*, 2007).

The 16S rRNA gene was amplified by direct PCR and sequenced using an ABI 3100 automatic DNA sequencer according to the manufacturer's instructions. Comparison of the 16S rRNA gene sequence with sequences retrieved

The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain MRN461^T is DQ660388.

A table listing differentiating characteristics of the two *Marinoscillum* species is available as supplementary material with the online version of this paper.

from GenBank and phylogenetic analyses were conducted according to the procedure described by Kwon *et al.* (2005). The 16S rRNA gene sequence of the isolate was a continuous stretch of 1495 bp, and a total of 1321 unambiguously aligned positions were compared with closely related sequences. Strain MRN461^T showed 95.6 % 16S rRNA gene sequence similarity with '[*Microscilla*] *furvescens*' IFO (now NBRC) 15994 (=LMG 13023), 90.0–91.5 % with members of the genera *Fabibacter* and *Roseivirga* and <90 % sequence similarity with all other related taxa. The neighbour-joining phylogenetic tree confirmed that strain MRN461^T and '[*Microscilla*] *furvescens*' IFO 15994 occupy a distinct phyletic line, but the relationship with other species was not supported by bootstrap analysis (Fig. 1). The maximum-likelihood tree showed essentially the same topology (data not shown). Consequently, strains MRN461^T and '[*Microscilla*] *furvescens*' IFO 15994 represent two different species in a novel genus.

As determined by HPLC using a Symmetry reversed-phase C₁₈ column (Stackebrandt & Liesack, 1993), the DNA G + C content of strain MRN461^T was 41.5 mol%, a value in line with that reported for '[*Microscilla*] *furvescens*' LMG 13023 (44 mol%; Lewin, 1969).

Analysis of the fatty acid methyl esters of strain MRN461^T and '[*Microscilla*] *furvescens*' LMG 13023 was carried out by using the MIDI/Hewlett Packard Microbial Identification System (MIS) (Sasser, 1990) according to the manufacturer's instructions using cells grown on MA for 3 days at 25 °C. The major fatty acids of strain MRN461^T were summed feature 3 (comprising iso-C_{15:0} 2-OH and/or C_{16:1}ω7c; 34.8 %), C_{16:1}ω5c (21.6 %) and iso-C_{15:0} (19.8 %) and those of '[*Microscilla*] *furvescens*' LMG 13023 were C_{16:1}ω5c (28.5 %), summed feature 3 (22.2 %) and iso-C_{15:1} G (8.5 %) (Table 1). The fatty acid profiles of the two strains were similar but differed in the respective proportions of iso-C_{15:0} and iso-C_{15:1} G. The proportions of C_{16:1}ω5c and summed feature 3 in the two

strains distinguished them from members of related genera. In accordance with the other members of the family 'Flexibacteraceae', the major respiratory quinone of both strains was MK-7, as determined by HPLC analysis according to the method of Collins (1985).

Unless otherwise stated, the phenotypic characterization of strain MRN461^T and '[*Microscilla*] *furvescens*' LMG 13023 was conducted according to previously described methods (Bae *et al.*, 2005; Lee *et al.*, 2008; Sohn *et al.*, 2004). The presence of NaCl alone in the medium did not support growth of strain MRN461^T or '[*Microscilla*] *furvescens*' LMG 13023. We therefore tested the requirement for three other seawater components: CaCl₂, KCl and MgCl₂. All combinations of these components at concentrations of 0.18 % CaCl₂·2H₂O, 0.055 % KCl and 0.59 % MgCl₂·6H₂O were added to modified ZoBell 2216e medium containing 5 g yeast extract, 1 g peptone, 30 g NaCl and 0.01 g FePO₄ per litre of distilled water. A commercial sea salts preparation (Sigma) was also used to test the salt tolerance range of strain MRN461^T and '[*Microscilla*] *furvescens*' LMG 13023. The degradation of starch and casein was tested according to Smibert & Krieg (1994). Bacterial suspensions used to inoculate the API 20E and API 20NE (bioMérieux) and Microlog GN2 (Biolog) systems were prepared in a 2.5 % sea salt solution. The physiological, biochemical and morphological characteristics of strain MRN461^T and '[*Microscilla*] *furvescens*' LMG 13023 are given below in the genus and species descriptions, in Table 2 and in Supplementary Table S1 (available in IJSEM Online).

Strain MRN461^T and '[*Microscilla*] *furvescens*' LMG 13023 shared many characteristics with members of closely related genera within the family 'Flexibacteraceae' such as the presence of MK-7 as the major respiratory quinone, the presence of oxidase and catalase activities and the absence of flexirubin-type pigments. However, the results of the phylogenetic analysis and the level of 16S rRNA gene sequence similarity as well as differences in cell morpho-

Fig. 1. Rooted neighbour-joining tree based on nearly complete 16S rRNA gene sequences (1323 unambiguously aligned positions) showing relationships between strain MRN461^T and '[*Microscilla*] *furvescens*' IFO 15994 (=LMG 13023) and related taxa. The 16S rRNA gene sequences of *Hymenobacter gelipurpurascens* Txg1^T and *Microscilla marina* IFO 16560^T served as an outgroup. Bootstrap values >700 of 1000 replicates are shown (upper, Jukes–Cantor neighbour-joining; lower, maximum-likelihood-parsimony). Bar, 0.01 substitutions per nucleotide position.

Table 1. Fatty acid compositions of strain MRN461^T and '[*Microscilla*] *furvenscens*' LMG 13023 and related taxa in the family 'Flexibacteraceae'

Taxa: 1, strain MRN461^T; 2, '[*Microscilla*] *furvenscens*' LMG 13023; 3, *Fabibacter halotolerans* UST030701-097^T; 4, genus *Roseivirga* (three species). Data were taken from this study, Lau *et al.* (2006), Nedashkovskaya *et al.* (2005a, 2005b) and Yoon *et al.* (2005). Values are percentages of total fatty acids. –, Not detected; tr, traces (<1%); ECL, equivalent chain-length. Fatty acids amounting to <0.5% of the total fatty acids in all strains are not shown. Strain MRN461^T and '[*Microscilla*] *furvenscens*' LMG 13023 were grown on MA for 3 days at 25 °C; *Fabibacter* and *Roseivirga* strains were grown under different culture conditions.

Fatty acid	1	2	3	4
Straight-chain				
C _{14:0}	1.5	1.1	–	–
C _{15:0}	tr	0.9	–	–
C _{16:0}	2.6	3.5	–	–
Branched				
iso-C _{13:0}	–	–	1.6	0.7–5.2
iso-C _{14:0}	–	–	4.7	1.9
iso-C _{15:0}	19.8	8.5	18.3	18.6–33.5
iso-C _{15:1}	–	–	14.2	12.5–20.2
iso-C _{15:1} G	tr	3.2	–	–
iso-C _{16:0}	1.9	3.2	1.2	1.2–2.0
iso-C _{16:1}	–	–	1.2	2.0
iso-C _{17:0}	tr	1.0	0.5	1.0
anteiso-C _{15:0}	–	–	2.5	2.4–13.1
anteiso-C _{15:1}	–	–	tr	1.8–2.4
Unsaturated				
C _{16:1} ω5c	21.6	28.5	–	–
C _{17:1} ω6	tr	1.8	–	–
iso-C _{17:1} ω9c	–	–	–	1.1–10.1
Hydroxy				
C _{14:0} 2-OH	3.9	2.6	–	–
C _{15:0} 2-OH	tr	1.5	1.9	3.2
C _{16:0} 2-OH	tr	1.2	–	–
C _{17:0} 2-OH	–	–	1.3	2.0–10.1
C _{15:0} 3-OH	–	–	1.3	–
C _{16:0} 3-OH	1.2	1.9	1.2	1.6–1.8
C _{17:0} 3-OH	tr	1.0	–	–
iso-C _{14:0} 3-OH	–	–	1.1	–
iso-C _{15:0} 3-OH	–	–	12.5	3.0–5.6
iso-C _{16:0} 3-OH	tr	1.3	12.7	2.0–10.1
iso-C _{17:0} 3-OH	4.1	9.8	9.3	7.7–18.3
Summed features*				
3	34.8	22.2	13.7	1.0–5.5
4	tr	1.0	–	–
Unknown ECL 14.959	1.3	1.1	–	–

*Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 3 contained iso-C_{15:0} 2-OH and/or C_{16:1}ω7c. Summed feature 4 contained anteiso-C_{17:1} B and/or iso-C_{17:1} I.

logy and in degradation ability for some high-molecular-mass compounds distinguished the two strains from other members of the family 'Flexibacteraceae' and suggested that they represent two different species in a novel genus (Fig. 1, Table 2 and Supplementary Table S1). Consequently, strain MRN461^T and '[*Microscilla*] *furvenscens*' LMG 13023 should be classified in a new genus in the family 'Flexibacteraceae', for which the name *Marinoscillum* gen. nov. is proposed. The novel species *Marinoscillum pacificus* gen. nov., sp. nov. and *Marinoscillum furvenscens* nom. rev., comb. nov. are proposed to accommodate strain MRN461^T and '[*Microscilla*] *furvenscens*' LMG 13023, respectively.

Description of *Marinoscillum* gen. nov.

Marinoscillum [Ma.ri.no.scil'lum. L. adj. *marinus* marine; L. neut. n. *oscillum* a swing; N.L. neut. n. *Marinoscillum* a marine swing(-like organism)].

Cells are aerobic, Gram-negative, flexible thread-like rods and motile by gliding. The cell mass is orange- or apricot-coloured. Mesophilic. Oxidase and catalase activities are present. The dominant respiratory quinone is MK-7 and the dominant fatty acids are C_{16:1}ω5c and summed feature 3 (comprising iso-C_{15:0} 2-OH and/or C_{16:1}ω7c). The DNA G + C content is 41–44 mol%. As determined by 16S rRNA gene sequence analysis, the genus is a member of the family 'Flexibacteraceae' in the order 'Sphingobacteriales'. The type species is *Marinoscillum pacificum*.

Description of *Marinoscillum pacificum* sp. nov.

Marinoscillum pacificum (pa.ci'fi.cum. L. neut. adj. *pacificum* pacific, used to refer to the Pacific Ocean).

In addition to the description of the genus, the following properties are displayed. Cells are usually 0.2–0.5 µm in diameter and 10 to >100 µm in length. Cells form apricot-coloured colonies on MA. Growth occurs at 15–40 °C (optimum, 33 °C), at pH 5–9.5 (optimum, pH 7.5) and in the presence of 0.5–7.0% (w/v) sea salts (optimum, 2.5%). Nitrate is not reduced to nitrite. Agar, starch and urea are not hydrolysed. H₂S is not produced. Requires Na⁺ and Mg²⁺ ions for growth. In the API 20E and 20NE systems, β-glucosidase and tryptophan deaminase activities are present, but protease (gelatin hydrolysis) and urease activities are absent. Acid is not produced from glucose. In the Microlog GN2 microplate, α-cyclodextrin, dextrin, L-arabinose, D-fructose, lactulose, maltose, D-galactose, D-mannose, melibiose, methyl β-D-glucoside, sucrose, glucuronamide, L-alanine, L-alanyl glycine, glucose 1-phosphate, *cis*-aconitic acid, citric acid, α-ketobutyric acid, α-ketoglutaric acid, DL-lactic acid, succinic acid, succinamic acid, L-glutamic acid, glycyl L-glutamic acid, L-pyroglytamic acid, cellobiose, gentiobiose, α-D-lactose, raffinose, L-rhamnose, D-sorbitol, trehalose, turanose, hydroxy-L-proline, L-proline, L-fucose, D-galacturonic acid, L-aspartic acid, γ-aminobutyric acid and D-mannitol are utilized. The other substrates in the Microlog GN2 microplate are not

Table 2. Differential characteristics of strain MRN461^T and '[*Microscilla*] *furvescens*' LMG 13023 and related taxa in the family 'Flexibacteraceae'

Taxa: 1, strain MRN461^T; 2, '[*Microscilla*] *furvescens*' LMG 13023; 3, *Fabibacter halotolerans* UST030701-097^T; 4, genus *Roseivirga* (three species). Data were taken from this study, Lewin (1969), Lau *et al.* (2006), Nedashkovskaya *et al.* (2003, 2005a, b, 2008) and Yoon *et al.* (2005). +, Positive reaction; –, negative reaction; v, variable; (+), weakly positive; All taxa are positive for oxidase and catalase activities and possess MK-7 as the major respiratory quinone. All taxa are negative for H₂S production, nitrate reduction, degradation of agar, cellulose, chitin and urea and production of flexirubin-type pigments.

Characteristic	1	2	3	4
Cell morphology	Long thread	Long thread	Short curved rod	Rod
Pigmentation	Apricot	Orange	Pink	Orange/pink
Gliding motility	+	+	+	v
Temperature range for growth (°C)	15–40	15–45	12–30	4–44
NaCl range for growth (%)	0.4–5.4	0.4–9.3	0–12.0	0–16.0
Hydrolysis of:				
Starch	–	–	(+)	–
Gelatin	–	+	–	+
DNA	–	+	+	v
Tween 80	–	–	+	v
Production of:				
Indole	+	–	–	–
Acetoin	+	–	+	v
Acid from glucose	–	–	+	–
Utilization of:				
Inositol	–	+	–	–
Lactose	–	–	+	–
D-Mannose	+	+	–	–
Mannitol	+	–	–	–
Sorbitol	+	–	–	–
DNA G + C content (mol%)	41.5	44	42.5	40.1–43.7

utilized. The dominant fatty acids are summed feature 3 (comprising iso-C_{15:0} 2-OH and/or C_{16:1}ω7c), C_{16:1}ω5c, iso-C_{15:0} and iso-C_{17:0} 3-OH. The full fatty acid composition is given in Table 1. The DNA G + C content of the type strain is 41.5 mol%.

The type strain is MRN461^T (=KCCM 42325^T =JCM 14064^T), isolated from an unidentified marine sponge near the tropical Weno Island, Chuuk State, Federated States of Micronesia.

Description of *Marinoscillum furvescens* (ex Lewin 1969) nom. rev., comb. nov.

Marinoscillum furvescens (fur.ves'cens. L. adj. *furvus* pitch black, dark; L. part. adj. *furvescens* growing dark, dusky).

Basonym: '*Microscilla furvescens*' Lewin 1969.

In addition to the descriptions of the genus and of '[*Microscilla*] *furvescens*' (Lewin, 1969), the following properties are displayed. Cells are usually 0.2–0.5 µm in diameter and 10 to >100 µm in length. Cells form orange-coloured colonies on MA. Growth occurs at 15–45 °C (optimum, 33.5 °C), at pH 6.5–9 (optimum, pH 7–7.5) and in the presence of 0.5–12 % (w/v) sea salts (optimum,

2.5 %). Nitrate is not reduced to nitrite. Agar, starch and urea are not hydrolysed. H₂S is not produced. Requires Na⁺ and either Mg²⁺ or Ca²⁺ ions for growth. In the API 20E and 20NE systems, β-glucosidase and protease (gelatin hydrolysis) activities are present, but urease activity is absent. Indole is not produced. Acid is not produced from glucose. In the Microlog GN2 microplate, α-cyclodextrin, dextrin, L-arabinose, D-fructose, lactulose, maltose, D-galactose, D-mannose, melibiose, methyl β-D-glucoside, sucrose, glucuronamide, L-alanine, L-alanyl glycine, glucose 1-phosphate, *cis*-aconitic acid, citric acid, α-ketobutyric acid, α-ketoglutaric acid, DL-lactic acid, succinic acid, succinamic acid, L-glutamic acid, glycyl L-glutamic acid, L-pyrroglutamic acid, N-acetyl-D-glucosamine, erythritol, myo-inositol, D-psicose, methyl pyruvate, L-serine, alaninamide, L-asparagine, L-threonine, inosine, DL-α-glycerol phosphate, acetic acid, D-gluconic acid, β-hydroxybutyric acid, propionic acid, *p*-hydroxyphenylacetic acid, α-keto-valeric acid and D-saccharic acid are utilized. The other substrates in the Microlog GN2 microplate are not utilized. The dominant fatty acids are C_{16:1}ω5c, summed feature 3 (comprising iso-C_{15:0} 2-OH and/or C_{16:1}ω7c), iso-C_{17:0} 3-OH and iso-C_{15:1}. The full fatty acid composition is given in Table 1. The DNA G + C content of the type strain is 44 mol%.

The type strain is LMG 13023^T (=DSM 4134^T =ATCC 23129^T =NBRC 15994^T), isolated from sand in Samoa.

Note added in proof

According to Ludwig *et al.* (2008), the genera *Fabibacter*, *Reichenbachia* and *Roseivirga* are members of the family 'Flammeovirgaceae'. It is therefore likely that the new genus *Marinoscillum* is also a member of the 'Flammeovirgaceae' rather than the 'Flexibacteraceae'.

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