Emended descriptions of the genus *Lewinella* and of *Lewinella cohaerens*, *Lewinella nigricans* and *Lewinella persica*, and description of *Lewinella lutea* sp. nov. and *Lewinella marina* sp. nov.

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Two strains, MKG-38\(^T\) and FYK2402M69\(^T\), were isolated from a marine sediment sample and a sea snail, respectively, both collected on the Pacific coast of Japan. Phylogeny of these new isolates based on 16S rRNA gene sequences indicated that they are members of the genus *Lewinella*. Morphological, physiological and biochemical properties of these two isolates, together with the type strains of the three previously described species of the genus *Lewinella*, were characterized. The new isolates were Gram-negative, aerobic, rod-shaped, chemo-organotrophic and able to degrade starch and CM-cellulose. A comparative polyphasic study showed that these two isolates represent two novel species of the genus *Lewinella*, for which the names *Lewinella marina* sp. nov. (type strain, MKG-38\(^T\) = NBRC 102633\(^T\) = NCIMB 14312\(^T\)) and *Lewinella lutea* sp. nov. (type strain, FYK2402M69\(^T\) = NBRC 102634\(^T\) = NCIMB 14313\(^T\)) are proposed. Emended descriptions of the genus *Lewinella* (Sly *et al.*, 1998) and of *Lewinella cohaerens*, *Lewinella nigricans* and *Lewinella persica* are also proposed.

Lewin (1970) described three novel marine species of the genus *Herpetosiphon*, namely *Herpetosiphon cohaerens*, *Herpetosiphon nigricans* and *Herpetosiphon persicus*, based on phenotypic characteristics. Later, 16S rRNA gene sequences of these strains were determined and a novel genus, *Lewinella*, in the family ‘Saprospiraceae’ was created for these strains (Sly *et al.*, 1998). In this paper, we describe the characterization of two strains isolated on the Pacific coast of Japan; strain MKG-38\(^T\) was isolated from a marine sediment sample collected at Kamogawa, whilst strain FYK2402M69\(^T\) was isolated from a sea snail (*Nodilittorina trochoïdes*) collected from Mikurajima. Polyphasic characterization of the new isolates, together with the type strains of previously described species of the genus *Lewinella*, was performed. Based on their phenotypic traits and phylogenetic positions, we propose that the two new isolates represent novel species of the genus *Lewinella*.

Strains MKG-38\(^T\) and FYK2402M69\(^T\) formed light-orange and dark-orange colonies, respectively, on half-strength marine agar (HSMA) plates (Marine Agar 2216; Difco) after 3–4 days incubation at 25 °C. Strains were maintained on HSMA plates and stored at −80 °C in artificial seawater (ASW; Naigai Chemicals) containing 20% (v/v) glycerol.

For 16S rRNA gene sequencing, template DNA was prepared by using Prepman Ultra (Applied Biosystems). The 16S rRNA gene was amplified by using a universal set of primers (27f and 1492r) and sequenced directly, using a BigDye Terminator v3.1 cycle sequencing kit and an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). By BLAST search (Altschul *et al.*, 1990) against the sequences available in GenBank/DBJ, *Lewinella persica* (accession no. AF039295) was identified as the closest relative of strains MKG-38\(^T\) and FYK2402M69\(^T\). Thus, pairwise comparison of the 16S rRNA gene sequences of strains MKG-38\(^T\) and FYK2402M69\(^T\) with those of type strains of species of the genus *Lewinella* and other related genera of the family ‘Saprospiraceae’ were performed by using the Needleman–Wunsch alignment algorithm (Needleman & Wunsch, 1970; http://www.ebi.ac.uk/emboss/align/). Strains MKG-38\(^T\) and FYK2402M69\(^T\) shared sequence similarity of 86.4–92.7% with the members of the genus *Lewinella*, whereas they shared <85% sequence similarity.
with members of other related genera (see Supplementary Table S1, available in IJSEM Online). The 16S rRNA gene sequences of the strains shown in Fig. 1 were aligned by using the CLUSTAL_X program (Thompson et al., 1997), and a phylogenetic tree was constructed by the neighbour-joining method (Saitou & Nei, 1987). The robustness of the tree topology was calculated from bootstrap analysis using 1000 resamplings of the sequences (Felsenstein, 1985). Strains MKG-38<sup>T</sup> and FYK2402M69<sup>T</sup> clustered with the members of the genus *Lewinella*. *L. persica* was the closest relative of both strains, and the grouping was supported by high bootstrap values (Fig. 1).

Morphological, physiological and biochemical traits of strains MKG-38<sup>T</sup> and FYK2402M69<sup>T</sup>, *Lewinella cohaerens* NBRC 102661<sup>T</sup>, *L. nigricans* NBRC 102662<sup>T</sup> and *L. persica* NBRC 102663<sup>T</sup> were characterized. The morphology of Gram-stained and unstained cells was observed under a light microscope, and that of cells negatively stained with 1 % (w/v) phosphotungstic acid was observed under a transmission electron microscope (H7600; Hitachi).

For most of the tests, cells grown on HSMA plates for 3–5 days at 25 °C were used unless stated otherwise. Gram reactions were performed as described by Cowan & Steel (1993). The method described by Buck (1982) was also used for the differentiation of Gram-positive and Gram-negative bacteria. Gliding motility was examined in hanging drops of Marine Broth 2216 (MB; Difco) and on HSMA plates (Perry, 1973). Catalase activity was tested by mixing cells from colonies grown on HSMA plates with 3 % (v/v) hydrogen peroxide on a glass slide, whilst oxidase activity was tested by spotting the cells onto a cytochrome oxidase strip (Nissui Pharmaceuticals). Absorption spectra (260–700 nm) of acetone extracts of cells were recorded spectrophotometrically to examine the presence of carotenoid-type pigments. Bathochromic shift tests with 20 % (w/v) KOH were performed to test for the presence of flexirubin-type pigments (Fautz & Reichenbach, 1980). Growth at different temperatures (4, 10, 15, 20, 25, 30, 35, 37, 40, 42 and 45 °C) was assessed on HSMA plates, whilst growth at different pH values was examined on HSMA plates or in MB with the final pH adjusted with either HCl (pH 5 and 6) or NaOH (pH 8–10). Growth at high NaCl concentrations [4–8 % (w/v) NaCl] was tested in MB. Requirement of NaCl for growth was tested in one-fifth strength Luria–Bertani (LB) medium containing 0, 1 or 2 % (w/v) NaCl (Khan et al., 2007). Utilization of different nitrogen sources [sodium nitrate (7.8 g l<sup>−1</sup>), ammonium sulphate (7.8 g l<sup>−1</sup>), sodium glutamate (20 g l<sup>−1</sup>) and peptone (10 g l<sup>−1</sup>) and peptone (10 g l<sup>−1</sup>)] was tested in medium N (1.0 g glucose and 0.2 g NaHCO<sub>3</sub> dissolved in 1 l ASW, pH 7.0). Methods described in detail elsewhere (Cowan & Steel, 1993; Lewin & Lounsbery, 1969; Smibert & Krieg, 1981; Khan et al., 2007) were used to test the abilities of the strains to hydrolyse agar, carrageenan (type I; Sigma), casein, cellulose, chitin, CM-cellulose (High Viscosity; Sigma), DNA, gelatin, starch and Tweens 20, 40 and 80. Indole production from tryptophan, acid production from glucose, hydrolysis of urea, aesculin and gelatin, and reduction of nitrate and nitrite by these strains were tested with the API 20NE system (bioMérieux), whilst their abilities to acidify different carbon sources were tested with the API 50CH system (bioMérieux) according to the
manufacturer’s instructions, except that inocula for API 20NE were prepared in ASW, whereas those for API 50CH were made in a 1:1 mixture of CHB medium (bioMérieux) and ASW. The API strips were incubated at 25 °C for 3–4 days before test results were scored.

For fatty acid analysis, strains were grown on Marine Agar (MA; Difco) for 3–4 days at 25 °C. Fatty acid profiles were determined by using the Sherlock microbial identification system (MIDI) according to the manufacturer’s protocol (Sasser, 1990). Respiratory quinones were analysed by using the protocol of Nakagawa & Yamasato (1993). Exponentially growing cells of strains MKG-38T and FYK2402M69T in MB were used to prepare genomic DNA as described by Minamisawa (1990); the HPLC method of Mesbah et al. (1989) was used to determine the G+C content of the DNA samples.

Morphological, physiological and chemotaxonomic characteristics of the strains are summarized in Tables 1 and 2 in the genus and species descriptions. Although strains MKG-38T and FYK2402M69T formed a monophyletic clade with L. persica (Fig. 1), low 16S rRNA gene sequence similarities (<92.7 %) and differences in phenotypic characteristics indicated clearly that strains MKG-38T and FYK2402M69T cannot be assigned to any of the previously described species in the genus Lewinella. Therefore, the names Lewinella marina sp. nov. and Lewinella lutea sp. nov. are proposed to include strains MKG-38T and FYK2402M69T, respectively.

Originally, members of the genus Lewinella were described to be 2–3 μm in length (Sly et al., 1998). Strains MKG-38T and FYK2402M69T, however, were smaller than 2 μm in size (see Supplementary Fig. S1, available in IJSEM Online, and novel species descriptions below). In addition, members of the genus Lewinella have been described to be unable to degrade starch and cellulose (Sly et al., 1998). In contrast to the description, all strains studied were able to degrade starch, and strains MKG-38T and FYK2402M69T were able to degrade CM-cellulose. Gliding motility has previously been observed in members of the genus Lewinella (Sly et al., 1998). However, in the present study, no gliding motility was observed in any of the strains studied. Thus, an emended description of the genus Lewinella is proposed. We also emend the species descriptions of L. cohaerens, L. nigricans and L. persica.

Table 1. Differentiating characteristics of the novel isolates and other species of the genus Lewinella

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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</thead>
<tbody>
<tr>
<td>Cell shape</td>
<td>Short to medium rods</td>
<td>Short to elongated rods</td>
<td>Short to elongated rods</td>
<td>Short to elongated rods</td>
<td>Short to elongated rods; clumps are formed</td>
</tr>
<tr>
<td>Pigment</td>
<td>Dull orange</td>
<td>Dark orange</td>
<td>Light orange</td>
<td>Diffusible black</td>
<td>Dark orange</td>
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<tr>
<td>CM-cellulose</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Casein</td>
<td>(+)</td>
<td>( +)</td>
<td>(+)</td>
<td>(+)</td>
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<td>Growth at:</td>
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<tr>
<td>pH 5.0</td>
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<tr>
<td>pH 10.0</td>
<td>+</td>
<td>+</td>
<td>(+)</td>
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<tr>
<td>4 °C</td>
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<td>35 °C</td>
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<td>42 °C</td>
<td>+</td>
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<td>Growth in 7 % NaCl</td>
<td>+</td>
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<td>Cellular fatty acids (% of total)</td>
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<tr>
<td>C15:0</td>
<td>tr</td>
<td>tr</td>
<td>13</td>
<td>6</td>
<td>tr</td>
</tr>
<tr>
<td>i-C17:1o9c</td>
<td>23</td>
<td>9</td>
<td>tr</td>
<td>2</td>
<td>10</td>
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<tr>
<td>Summed feature 3*</td>
<td>23</td>
<td>53</td>
<td>tr</td>
<td>9</td>
<td>30</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>61</td>
<td>56</td>
<td>45†</td>
<td>53†</td>
<td>53†</td>
</tr>
</tbody>
</table>

*Summed feature 3 comprises i-C15:0 2-OH and/or C16:1o7c.
†DNA G+C content data for L. cohaerens, L. nigricans and L. persica are from Sly et al. (1998).
The type species of the genus is *Lewinella* et al. 1998

**Lewinella cohaerens** (co.hae’rens. L. part. adj. cohaerens coherent, uniting together).

The species possesses the following characteristics in addition to those described for the genus. Cells are rod-shaped, 1.2–2.0 μm in length and 0.3–0.5 μm in width, often elongated to a long filament of 5–15 μm in length. Colonies on MA are light orange. Growth occurs at 10–30 °C (optimally at 25 °C). Growth does not occur at 4 °C or at 35 °C or higher. Growth occurs at pH 6–8 (optimally at pH 7). Weak growth is observed at pH 9 and 10. Growth does not occur at pH 5. Growth occurs with 2–4 % (w/v) NaCl; weak growth is observed with 5 % (w/v) NaCl. Growth does not occur with 0, 1 or 6 % (w/v) NaCl or higher. Positive for degradation of agar, carrageenan, cellulose, chitin, CM-cellulose and urea. β-Galactosidase and β-glucosidase are positive, but arginine dihydrolase is negative. Casamino acids and peptone are utilized as nitrogen sources, but ammonium sulphate, sodium glutamate and sodium nitrate are not. Acid is produced from D-cellobiose, D-glucose, D-lactose, D-lyxose, D-turanose, aesculin and gentiobiose. Acid is produced from starch, D-arabinose, D-cellobiose, D-galactose, D-gluco- and D-glucopyranosides. Major cellular fatty acids are i-C15:0, i-C15:1 and C15:0. The DNA G+C content is 45 mol%.

The type strain is II-2T (=NBRC 102661T=NCIMB 12855T). Isolated from beach sand at Biarritz, France.

**Lewinella nigricans** (ni’gri.cans. L. part. adj. nigricans blackening).

The species possesses the following characteristics in addition to those described for the genus. Cells are rod-shaped, 1.5–3.0 μm in length and 0.3–0.6 μm in width, often elongated to a filament of 5–25 μm in length. Colonies on MA produce black pigments. Growth occurs at 15–37 °C (optimally at 25–30 °C). Growth does not occur at 4, 10 or 40 °C or higher. Growth occurs at pH 6–8 (optimally at pH 7). Weak growth is observed at pH 9 and 10. Growth does not occur at pH 5. Growth occurs with 2–4 % (w/v) NaCl; weak growth is observed with 5 % (w/v) NaCl. Growth does not occur with 0 or 1 % (w/v) NaCl or higher. Positive for degradation of casein, DNA, gelatin, starch and Tweens 20, 40 and 80. Negative for degradation of agar, carrageenan, cellulose, chitin, CM-cellulose and urea. β-Galactosidase and β-glucosidase are positive, but arginine dihydrolase is negative. Casamino acids and peptone are utilized as nitrogen sources, but ammonium sulphate, sodium glutamate and sodium nitrate are not. Acid is produced from D-cellobiose, D-glucose, D-lactose, D-lyxose, D-turanose, ascin and gentiobiace. Acid production is weak from amygdalin, arbutin, D-fucose, D-melezitose, D-tatagost, L-fucose and methyl-β-D-glucopyrano-side. Major cellular fatty acids are i-C15:0, i-C15:1 and C15:0. The DNA G+C content is 45 mol%.

The type strain is II-2T (=NBRC 102661T=NCIMB 12855T). Isolated from beach sand at Biarritz, France.

**Emended description of the genus *Lewinella* Sly et al. 1998**

*Lewinella* (Le.wi.net’la. L. dim. ending -ella; N.L. fem. dim. n. *Lewinella* named after Professor Ralph Lewin, who first isolated these organisms).

Cells are Gram-negative, aerobic, chemo-organotrophic, asporogenic rods that are 0.7–3.0 μm in length and 0.3–0.7 μm in width; often elongated to a filament of 5–25 μm in length. Carotenoid-type pigments are produced; flexirubin-type pigments are not. Oxidase- and catalase-positive. Major respiratory quinone is MK-7. Major cellular fatty acids are i-C15:0, either i-C15:1 or i-C17:1ω9c and either C15:0 or summed feature 3 (i-C15:0 2-OH and/or C16:1ω7c). Members of the genus are marine bacteria that require NaCl for growth. Nitrate and nitrite are not reduced. Indole is not produced from tryptophan. The DNA G+C content is 45–61 mol%.

The type species of the genus is *Lewinella cohaerens*.
D-melezitose, D-raffinose, D-sucrose, D-trehalose, D-turanose, D-xylose, ascinul, glycogen, L-arabinose, L-rhamnose, salicin and xylitol. Acid production is weak from amygdalin, arbutin, D-lyxose, D-fucose, D-mannose, L-fucose, methyl-α-D-glucopyranoside, methyl-α-D-mannopyranoside, methyl-β-D-xylopyranoside and N-acetylglucosamine. Major fatty acids are i-C15:0, i-C15:1 and summed feature 3 (i-C15:0 2-OH and/or C16:1 ω7c). The DNA G+C content is 53 mol%.

The type strain is SS-2T (=NBRC 102662T=NCIMB 1420T). Isolated from beach sand near Lagos, Nigeria.

**Emended description of Lewinella persica (Lewin 1970) Sly et al. 1998**

*Lewinella persica* [per’si.ca. L. adj. persica Persian (of peach), i.e. peach-coloured).

The species possesses the following characteristics in addition to those described for the genus. Cells are rod-shaped, 1.5–3.0 μm in length and 0.3–0.5 μm in width, often elongated to a filament of 5–17 μm in length. Cells adhere to each other, forming clumps. Colonies on MA are dark orange. Growth occurs at 4–30 °C (optimally at 25 °C). Growth does not occur at 35 °C or higher. Growth occurs at pH 6–8 (optimum at pH 7). Weak growth is observed at pH 9 and 10. Growth does not occur at pH 5. Growth occurs with 2–4% (w/v) NaCl; weak growth is observed with 5% (w/v) NaCl. Growth does not occur with 0, 1 or 6% (w/v) NaCl or higher. Positive for degradation of casein, DNA, gelatin, starch and Tweens 20, 40 and 80. Negative for degradation of agar, carrageenan, cellulose, chitin and urea. β-Galactosidase and β-glucosidase are positive, but arginine dihydrolase is negative. Casamino acids and peptone are used as nitrogen sources, but ammonium sulphate, sodium glutamate and sodium nitrate are not. Acid is produced from D-galactose, D-glucose, D-fructose, D-mannose, D-ribose, D-sucrose, erythritol, D-fucose, methyl-α-D-glucopyranoside and methyl-β-D-xylpyranoside. Major cellular fatty acids are i-C15:0, summed feature 3 (i-C15:0 2-OH and/or C16:1 ω7c) and i-C17:1 ω9c. The DNA G+C content is 61 mol%.

The type strain is MKG-38T (=NBRC 102633T=NCIMB 14312T). Isolated from a marine sediment sample of Kamogawa city, Japan.

**Description of Lewinella lutea sp. nov.**


The species possesses the following characteristics in addition to those described for the genus. Cells are short rods, 0.6–1.0 μm in length and 0.3–0.5 μm in width, often elongated to a filament of 5–20 μm in length. Colonies on MA are dark orange. Growth occurs at 10–35 °C (optimally at 25 °C). Growth does not occur at 4 or 37 °C or higher. Growth occurs at pH 6–8 (optimally at pH 7). Growth does not occur at pH 4 or 5. Weak growth is observed at pH 9 and 10. Growth occurs with 2–4% (w/v) NaCl; weak growth is observed with 5% (w/v) NaCl. Growth does not occur with 6% (w/v) NaCl or higher. Positive for degradation of CM-cellulose, DNA, gelatin, starch and Tweens 20, 40 and 80. Casein degradation activity is weak. Negative for degradation of agar, carrageenan, chitin, cellulose and urea. β-Galactosidase and β-glucosidase are positive, but arginine dihydrolase is negative. Casamino acids and peptone are utilized as nitrogen sources, but ammonium sulphate, sodium glutamate and sodium nitrate are not. Acid is produced from amygdalin, arbutin, D-ribose, D-fructose, D-mannose, D-lactose, D-xylose, D-maltose, D-melibiose, D-mannose, D-melezitose, D-fructose, D-sucrose, D-trehalose, D-turanose, D-xylose, gentiobiose, aesculin, L-arabinose, L-fucose, L-rhamnose, methyl-α-D-glucopyranoside, methyl-β-D-mannopyranoside, methyl-β-D-xylpyranoside, N-acetylglucosamine and salicin. Acid production is weak from starch, D-fucose, D-ribose, D-tagatose, L-sorbose, L-xylose and inulin. The major cellular fatty acids are i-C15:0, summed feature 3 (i-C15:0 2-OH and/or C16:1 ω7c) and i-C17:1 ω9c. The DNA G+C content is 61 mol%.

The type strain is SS-2T (=NBRC 102662T=NCIMB 1420T). Isolated from a marine sediment sample of Kamogawa city, Japan.

**Description of Lewinella marina sp. nov.**


The species possesses the following characteristics in addition to those described for the genus. Cells are short rods, 0.6–1.0 μm in length and 0.4–0.5 μm in width. Colonies on MA are semi-dry and dull orange. Growth occurs at 4–42 °C (optimally at 25–30 °C). Growth does not occur at 45 °C. Growth occurs at pH 6–10 (optimally at pH 7–8). Weak growth is observed at pH 5. Growth is observed with 1–7% (w/v) NaCl [optimally with 2–4% (w/v) NaCl]. Positive for degradation of DNA, CM-cellulose, gelatin, starch and Tweens 20, 40 and 80. Casein degradation activity is weak. Negative for degradation of agar, carrageenan, cellulose, chitin and urea. β-Galactosidase and β-glucosidase are positive, but arginine dihydrolase is negative. Casamino acids and peptone are utilized as nitrogen sources, but ammonium sulphate, sodium glutamate and sodium nitrate are not. Acid is produced from amygdalin, arbutin, D-ribitol, D-cellobiose, D-fructose, D-galactose, D-glucose, D-lactose, D-lyxose, D-maltose, D-melibiose, D-mannose, D-melezitose, D-fructose, D-sucrose, D-trehalose, D-turanose, D-xylose, gentiobiose, aesculin, L-arabinose, L-fucose, L-rhamnose, methyl-β-D-glucopyranoside, methyl-α-D-mannopyranoside, methyl-β-D-xylpyranoside, N-acetylglucosamine and salicin. Acid production is weak from starch, D-fructose, D-melezitose and L-fucose. The major cellular fatty acids are summed feature 3 (i-C15:0 2-OH and/or
C16:1ω7c, i-C15:0 and i-C17:1ω9c. The DNA G+C content is 56 mol%.

The type strain is FYK2402M69^T (=NBRC 102634^T=NCIMB 14313^T). Isolated from a marine snail collected from Mikurajima island, Japan.

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References
