A bacterial strain, designated JN14-9ᵀ, was isolated from surface sediment of the Jiulong River and characterized in a taxonomic study using a polyphasic approach. Strain JN14-9ᵀ was Gram-stain-negative, non-motile, rod-shaped and orange-pigmented. It can grow at 15–37 °C (optimum 25–30 °C), at pH 7–8 (optimum pH 7) and in 2–6 % (w/v) NaCl (optimum 3–4 %). Phylogenetic analyses based on 16S rRNA gene sequences showed that strain JN14-9ᵀ formed an independent lineage related to the family Cytophagaceae with low 16S rRNA gene sequence similarities (<92.5 %) to members of other genera with validly published names. The principal fatty acids of strain JN14-9ᵀ were summed feature 3 (C₁₆:₁-ω₆/C₁₆:₁-ω₇c) and iso-C₁₅:₀. The isoprenoid quinone was identified as MK-7. The major polar lipids comprised diphosphatidylglycerol, phosphatidylethanolamine, three aminophospholipids and five unidentified phospholipids. The DNA G+C content was 41.6 mol%. Results of phenotypic, phylogenetic and chemotaxonomic analyses clearly indicate that strain JN14-9ᵀ represents a novel species of a new genus within the family Cytophagaceae, for which the name Jiulongibacter sediminis gen. nov., sp. nov. is proposed. The type strain of the type species is JN14-9ᵀ (=MCCC 1A00733 =KCTC 42153ᵀ).

In the course of a study on dynamic changes and adaptability of freshwater bacteria in a marine environment, an in situ incubation experiment was set up by transferring surface sediment (0–5 cm) from the Jiulong River (Fujian, China) into an enrichment-barrel and then incubating it in estuarine seawater. A number of strains were subsequently isolated and characterized, including ‘Pseudovplanella zhangzhouensis’ JS7-9 (Du et al., 2015a) and Kordia zhangzhouensis JS14SB-1ᵀ (Du et al., 2015b). In the present study, an orange bacterium, designated JN14-9ᵀ, was characterized taxonomically. A preliminary analysis of 16S rRNA gene sequences indicated that strain JN14-9ᵀ was affiliated to the family Cytophagaceae. The family Cytophagaceae, proposed by Stanier (1940), is a member of the order Cytophagales within the phylum Bacteroidetes. At the time of writing, this family comprises 30 identified genera, including the two recently established genera Fluviimonas (Sheu et al., 2013) and Lachihabans (Joung et al., 2014), which were isolated from diverse habitats, such as cotton-waste composts (Joung et al., 2014), warm spring water (Saha & Chakrabarti, 2006), soil (Liu et al., 2008), sediment (Park et al., 2015), and freshwater (Sheu et al., 2013). In this study, strain JN14-9ᵀ is identified as a novel species of a new genus in the family Cytophagaceae.

Strain JN14-9ᵀ was isolated from surface sediment (0–5 cm) of the Jiulong River (24°20’24”N 117°19’11.999”E) (Fujian, China) in June 2013 according to the previously described method (Du et al., 2015a, b). For morphological and biochemical characterization, strain JN14-9ᵀ was cultivated on marine agar 2216 plates (MA; BD Difco). The purified strain was stored at –80°C in water containing 20 % (v/v)
Table 1. Characteristics that differentiate strain JN14-9<sup>T</sup> from the type strains of closely related genera

Strains: 1, JN14-9<sup>T</sup>; 2, Leadbetterella byssophila 4M15<sup>T</sup>; 3, Emticicia oligotrophica GPTS100-15<sup>T</sup>; 4, Fluviimonas palliditellae TQQ6<sup>T</sup>; 5, Lacihabitans soyangensis HME6675<sup>T</sup>. Data for strains 1–3 are from this study except where otherwise indicated; data for strains 4 and 5 are from Joung et al. (2014). API ZYM, API 50 CH and Biolog GN2 tests for all strains were done under the same conditions. All strains contained phosphatidylethanolamine in the polar lipids. +, Positive; –, negative; w, weakly positive; DPG, diphasphatidylglycerol; PL, phospholipids; PC, phosphatidylcholine; AP, aminolipid; APLs, aminophospholipids; APGL, aminophosphoglycolipid; L, polar lipids.

<table>
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<td>2–7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2–5&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>0.3–0.4&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Light pink&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Pale orange</td>
<td>Orange</td>
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<td>Esterase lipase (C8)</td>
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<td>D-Ribose, inositol, D-mannitol</td>
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<td>N-Acetyl-D-galactosamine, D-alanine, D-fucose, myo-inositol, α-ketoglutaric acid,</td>
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<td>L-Ornithine, L-threonine</td>
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<td>Dextrin, D-gluconic acid</td>
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<td>D-Raffinose, acetic acid, D-fructose, N-acetyl-D-glucosamine</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>D-Galactose, D-fructose, D-arabitol,</td>
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<td>–</td>
<td>+</td>
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<td>+</td>
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<td>α-D-Glucose, sucrose</td>
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<td>+</td>
<td>+</td>
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<tr>
<td>D-Mannose, methyl β-D-glucoside, α-D-glucose 1-phosphate, turanose, D-glucose 6-</td>
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<td>+</td>
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glycerol. Unless otherwise noted, for all characterizations described hereafter, strain JN14-9T was grown on MA or in marine broth 2216 (MB; BD Difco) at 28 °C.

Cell morphology was observed by transmission electron microscopy (JEM-1230; JEOL) (Fig. S1, available in the online Supplementary Material). Motility was observed by using the methods of Dong & Cai (2001). Gram staining was performed using a Gram stain kit (Hangzhou Tianhe Microorganism Reagent Co.) according to the manufacturer’s instructions. Catalase activity was determined by adding a drop of 3 % H2O2 to colonies. Oxidase activity was tested for by using tetramethyl-p-phenylenediamine.

Lipase (TWEEN 80) activity, and hydrolysis of starch, casein, gelatin, skimmed milk, urea and DNA were tested according to standard methods (Dong & Cai, 2001). Anaerobic growth was tested on MA in an anaerobic jar with the Anoxomat Mark II Anaerobic System (Marterieux), and the GN2 and subsequently deposited in the GenBank database.

The genomic DNA G+C content of strain JN14-9T was 41.6 mol% based on the genome sequence, which was similar to those of closely related genera (35.6–42.2 mol%) (Joung et al., 2014). The 16S rRNA gene sequence was obtained from the genome sequence, and then deposited in the GenBank database. 16S rRNA gene sequence similarities were determined using the EzTaxon-e server (Kim et al., 2012), and sequences of related taxa were obtained from the GenBank database. Phylogenetic trees were reconstructed using MEGA software package version 5.2.2 (Tamura et al., 2011) with three independent methods: neighbour-joining (NJ) (Saitou & Nei, 1987), maximum-likelihood (ML) (Felsenstein, 1981), and minimum-evolution (ME) (Rzhetsky & Nei, 1992). Each treeing method used the same options of Kimura’s two-parameter model, the combination of transversions and transversions, uniform rates, complete deletion, and bootstrap values based on 1000 replications. In addition, the ML analysis was conducted using the Nearest-Neighbour-Interchange (NNI) ML heuristic method and otherwise default parameters; the ME analysis was

Table 1. cont.

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Polar lipids

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<tr>
<td>APGL</td>
<td>–</td>
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<td>L</td>
<td>–</td>
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</table>

DNA G+C content (mol%)

| DNA G+C content (mol%) | 41.6 | 40.4† | 35.6† | 42.2 | 37.7 |

*Data from: a, Weon et al. (2005); b, Saha & Chakrabarti (2006).
†Data were obtained from the genome sequences.
A complete 16S rRNA gene sequence (1508 bp) of strain JN14-9<sup>T</sup> was most closely related to Lachitabitan ssoyangensis HME6675<sup>T</sup> (92.3 % 16S rRNA gene sequence similarity), Leadbetterella byssophila 4M15<sup>T</sup> (90.6 %), Emticicia ginsengi Gsoil 085<sup>T</sup> (90.6 %), Emticicia oligoraphica GPTSA100-15<sup>T</sup> (90.3 %), Fluviimonas pallidilutea TQQ6<sup>T</sup> (90.1 %) and Emticicia sediminis JBR12<sup>T</sup> (87.4 %). These values are significantly lower than the threshold value of 95 % that is generally used to delineate a potential new genus (Yarza et al., 2014). Phylogenetic analyses based on 16S rRNA gene sequences showed that strain JN14-9<sup>T</sup> formed an independent lineage related to the family Cytophagaceae in the NJ tree (Fig. 1). The ML and ME trees were nearly identical in topology to the NJ tree, and therefore were condensed in the NJ tree.
The compositions of fatty acids for strain JN14-9<sup>T</sup> and other reference strains are listed in Table 2. The major fatty acids of strain JN14-9<sup>T</sup> were summed feature 3 (C<sub>16:1ω6c</sub>/C<sub>16:1ω7c</sub>, 39.6 %) and iso-C<sub>15:0</sub> (31.0 %); small amounts of C<sub>16:1ω5c</sub> (5.9 %), iso-C<sub>17:0</sub> 3-OH (5.2 %), C<sub>16:0</sub> (2.6 %) and C<sub>16:0</sub> 3-OH (2.4 %) were also present (Table 2). The fatty acids profile of strain JN14-9<sup>T</sup> was similar to those of other reference strains (Joung et al., 2014), except for the presence of C<sub>16:0</sub> 3-OH, summed feature 4, and C<sub>18:0</sub> in strain JN14-9<sup>T</sup> and absence of them in other reference strains. The respiratory quinone of strain JN14-9<sup>T</sup> was identified as MK-7, which was accordant with those of type strains of other closely related recognized species of the family Cytophagaceae. The polar lipids of strain JN14-9<sup>T</sup> comprised diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), three aminophospholipids (APLs) and five unidentified phospholipids (Fig. S2). The type strains of two type species of related genera, F. pallidilutea TQQ6<sup>T</sup> and E. olistrophica GPTSA100-15<sup>T</sup>, had the same types of polar lipids which included PE, DPG, phosphatidylcholine, APL and PL (Sheu et al., 2013). The polar lipids of Leadbetterella byssohiffa 4M15<sup>T</sup> were PE, APL, PL and aminophosphoglycolipid (Weon et al., 2005). The polar lipids of Lacihabitans soyangensis HME6675<sup>T</sup> comprised PE, aminolipid, APL and polar lipids (Joung et al., 2014).

It is now generally accepted that 95 % 16S rRNA gene sequence similarity has been widely used for prokaryotic genus definition (Yarza et al., 2014). In this study, strain JN14-9<sup>T</sup> formed an independent lineage related to the family Cytophagaceae with low 16S rRNA gene sequence similarities (<92.5 %) to members of other genera with validly published names. Moreover, strain JN14-9<sup>T</sup> can be readily distinguished from related species by fatty acid compositions, polar lipid profile and other characteristics. Therefore, combined with phylogenetic, physiological and biochemical characteristics, strain JN14-9<sup>T</sup> is considered to represent a novel species of a new genus in the family Cytophagaceae, for which the name *Jiulongibacter sediminis* gen. nov. sp. nov. is proposed.

### Description of *Jiulongibacter* gen. nov.

*Jiulongibacter* (ji’u.long.i.bacter.N.L. masc. n. *bacter* a rod; N.L. masc. n. *Jiulongibacter* a rod from the Jiulong River).

Cells are Gram-stain-negative, strictly aerobic, non-motile, rod-shaped, oxidase- and catalase-positive. Flexirubin-type

### Table 2. Cellular fatty acid compositions of strain JN14-9<sup>T</sup> and type strains of type species of closely related genera

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<th>Fatty acid</th>
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<td>C&lt;sub&gt;18:0&lt;/sub&gt;</td>
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<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<tr>
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<td>TR</td>
<td>1.4</td>
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<td>22.3</td>
<td>19.2</td>
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<td>iso-C&lt;sub&gt;14:0&lt;/sub&gt;</td>
<td>TR</td>
<td>1.2</td>
<td>ND</td>
<td>3.0</td>
<td>1.4</td>
</tr>
<tr>
<td>iso-C&lt;sub&gt;17:1&lt;/sub&gt;G</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>1.5</td>
<td>2.9</td>
</tr>
<tr>
<td>Unsaturated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Summed feature 3†</td>
<td>39.6</td>
<td>44.3</td>
<td>41.9</td>
<td>17.3</td>
<td>31.3</td>
</tr>
<tr>
<td>C&lt;sub&gt;16:1ω5c&lt;/sub&gt;</td>
<td>5.9</td>
<td>2.4</td>
<td>7.3</td>
<td>16.4</td>
<td>5.9</td>
</tr>
<tr>
<td>C&lt;sub&gt;16:1ω6c&lt;/sub&gt;</td>
<td>1.8</td>
<td>4.1</td>
<td>3.4</td>
<td>1.2</td>
<td>4.4</td>
</tr>
<tr>
<td>C&lt;sub&gt;17:1ω6c&lt;/sub&gt;</td>
<td>TR</td>
<td>3.0</td>
<td>TR</td>
<td>TR</td>
<td>TR</td>
</tr>
<tr>
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<td></td>
</tr>
<tr>
<td>iso-C&lt;sub&gt;17:0&lt;/sub&gt;3-OH</td>
<td>5.2</td>
<td>5.0</td>
<td>4.9</td>
<td>7.3</td>
<td>5.9</td>
</tr>
<tr>
<td>iso-C&lt;sub&gt;15:0&lt;/sub&gt;3-OH</td>
<td>1.3</td>
<td>1.8</td>
<td>2.7</td>
<td>1.3</td>
<td>ND</td>
</tr>
<tr>
<td>C&lt;sub&gt;16:0&lt;/sub&gt;3-OH</td>
<td>2.4</td>
<td>1.1</td>
<td>2.2</td>
<td>3.1</td>
<td>ND</td>
</tr>
<tr>
<td>C&lt;sub&gt;15:0&lt;/sub&gt;2-OH</td>
<td>TR</td>
<td>1.2</td>
<td>1.5</td>
<td>TR</td>
<td>TR</td>
</tr>
<tr>
<td>Summed feature 4†</td>
<td>1.7</td>
<td>ND</td>
<td>ND</td>
<td>1.5</td>
<td>2.9</td>
</tr>
<tr>
<td>Summed feature 9†</td>
<td>1.5</td>
<td>2.6</td>
<td>TR</td>
<td>TR</td>
<td>ND</td>
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</tbody>
</table>

†Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 4 comprises anteiso-C<sub>17:1</sub>B/iso-C<sub>17:1</sub>I.

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pigments and poly-β-hydroxybutyrate are absent. The major fatty acids are summed feature 3 (C₁₆:1ω6c/C₁₆:1ω7c) and iso-C₁₅:0. The major polar lipids are diphosphatidylglycerol, aminophospholipid and phosphatidylethanolamine. The respiratory quinone is MK-7. The DNA G+C content of the type strain of the type species is 41.6 mol%. The genus is a member of the family Cytophagaceae (phylum Bacteroidetes) according to 16S rRNA gene sequence analysis.

The type species is Jiulongibacter sediminis.

**Description of Jiulongibacter sediminis sp. nov.**

*Jiulongibacter sediminis* (se.di.mi’nis. L. gen. n. sediminis of the sediment, the source of the type strain).

In addition to the characteristics listed for the genus, cells are 0.5–0.6 μm in width and 2.8–3.0 μm in length. Colonies are orange, circular, convex and smooth with entire margins, and approximately 2 mm in diameter on MA agar after 3 days at 28°C. Growth occurs at 15–37°C (optimum 25–30°C), at pH 7–8 (optimum pH 7) and in the presence of 2–6% NaCl (w/v) (optimum, 3–4%). Cannot hydrolyse starch, casein, gelatin, skimmed milk, urea or DNA. In the API ZYM tests, positive for alkaline phosphatase, esterase lipase (C₈), elastase (C₉), leucine aminopeptidase, valine aminopeptidase, cystine aminopeptidase, trypsin, α-chymotrypsin, acid phosphatase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, α-mannosidase, lipase (C₁₁₄), and N-acetyl-β-glucosaminidase; weakly positive for naphthol-AS-BI-phosphoamidase; negative for α-fucosidase. In API 50CH tests, acid is produced from L-arabinose, D-xylose, methyl β-D-xylopyranoside, D-galactose, D-glucose, D-mannose, L-rhamnose, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, amygdalin, arbutin, ascorbic acid, citrate, salicin, D-cellobiose, maltose, lactose (bovine origin), melibiose, sucrose, trehalose, D-melezitose, D-raffinose, gentiobiose and D-turanose, but not from glycerol, erythritol, D-arabinose, D-ribose, L-xyllose, D-adonitol, D-fructose, L-sorbitose, dulcitol, inositol, D-mannitol, D-sorbitol, N-acetylglucosamine, inulin, starch, glycopyrogen, xylobiose, D-lyxose, D-lactitose, D-fucose, L-fucose, D-arabinose, L-arabinose, potassium gluconate, potassium 3-ketogluronate. In Biolog GN2 microplate tests, positive results for utilization of α-cyclodextrin, dextrin, N-acetyl D-glucosamine, cellobiose, D-fructose, D-galactose, gentiobiose, α-D-glucose, α-D-lactose, lactulose, maltose, D-mannose, melibiose, β-methyl D-glucoside, D-raffinose, sucrose, trehalose, turanose, succinic acid monomethyl ester, acetic acid, D-glucuronic acid, D-lactic acid, α-D-glucose 1-phosphate and D-glucose 6-phosphate; weakly positive result for utilization of glycerol, D-psicose, L-rhamnose, D-sorbitol, D-galacturonic acid, D-glucuronic acid, glucuronolamin, α-glutamic acid, glycol L-glutamic acid, L-proline, glycerol and DL-α-glycerol phosphate; and negative result for utilization of Tween 40, Tween 80, N-acetyl-D-galactosamine, adonitol, L-arabinose, D-arabitol, L-rhamnitol, D-glucosylglycerol, L-fucose, N-myo-inositol, D-mannitol, N-acetyl-D-glucosamine, D-glucosamine, L-proline, glycyl L-aspartic acid, D-glutamic acid, D-lysine, glycyl L-proline, D-leucine, L-ornithine, D-phenylalanine, L-lysine, α-onosamine, L-mannosamine, adonitol, L-serine, L-threonine, DL-carmitine, α-aminobutyric acid, urocanic acid, inosine, uridine, thymine, phénylalanine, putrescine, 2-aminoethanol and 2,3-butanediol. In addition to the major fatty acids listed for the genus, significant amounts of C₁₁₅₀C₁₀, iso-C₁₇₀, 3-OH, C₁₆₀ and C₁₆₅₀, 3-0H are also present. The complete fatty acid profile of the type strain is given in Table 2. In addition to diphosphatidyglycerol, three aminophospholipids and phosphatidylethanolamine, several unidentified phospholipids are also present.

The type strain is JN14-9T (=MCCC 1A00733T =KCTC 42153T), isolated from surface sediment of the Jiulong River in Fujian province, China. The DNA G+C content of the type strain is 41.6 mol%.

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**References**


