The genus *Georgenia*, belonging to the family *Bogoriellaceae*, class *Actinobacteria*, was proposed by Altenburger et al. (2002) with *Georgenia muralis* as the type species. At the time of writing, the genus *Georgenia* consists of eight recognized species: *G. muralis* (Altenburger et al., 2002), *G. ruanii* (Li et al., 2007), *G. thermotolerans* (Hamada et al., 2009), *G. soli* (Kämpfer et al., 2010), *G. halophila* (Tang et al., 2010), *G. satyanarayanai* (Srinivas et al., 2012), *G. daeguensis* (Woo et al., 2012) and *G. sediminis* (You et al., 2013). All members of this genus contain anteiso-C15:0 as the predominant cellular fatty acid and MK-8(H4) as the predominant menaquinone and have DNA G+C contents in the range 66.2–73.4 mol% (Srinivas et al., 2012; Woo et al., 2012; You et al., 2013). In the course of our investigation on the biodiversity of bacteria from the western Pacific Ocean, a novel bacterial strain, Y32T, was isolated. On the basis of phenotypic and genotypic evidence, we propose that this strain represents a novel species of the genus *Georgenia*. Strain Y32T was isolated from a deep-sea sediment collected from the western Pacific Ocean (10°54.7’ N 142°19.9’ E; 6310 m water depth) on a modified Zobell 2216E agar (MZ2216E; 1.0 g yeast extract, 5.0 g tryptone, 1 litre of clarified seawater, 15.0 g agar, pH 7.4–7.6). The strain was routinely cultured on MZ2216E agar at 28 °C and maintained as a glycerol suspension (20 %, w/v) at −80 °C. *G. muralis DSM 14418T*, *G. thermotolerans DSM 21501T*, *G. ruanii DSM 17458T* and *G. soli DSM 21838T*, which were obtained from the German Collection of Microorganisms (DSM), and *G. daeguensis JCM 17459T* and *Oceanitalea nanhaiensis JCM 17755T*, from the Japan Collection of Microorganisms (JCM), were used as reference strains for comparative analyses of phenotypic characteristics and fatty acid composition.

Morphological properties of cells were observed by transmission electron microscopy (JEM-1230; JEOL) after growth for 24–36 h on MZ2216E at 35 °C. Colony characteristics were examined by growing colonies on MZ2216E at 35 °C for 2–3 days. Cell motility was studied by...
puncture inoculation in semisolid MZ2216E medium. Growth under anaerobic conditions was determined after incubation in an anaerobic chamber on MZ2216E at 35 °C for about 2 weeks. The temperature range and optimum temperature for growth were determined by incubating the cultures on MZ2216E at 4, 10, 15, 20, 25, 28, 30, 35, 37, 40 and 45 °C. The pH range and optimum pH for growth were established at 35 °C by using MZ2216E that was adjusted to pH 5–12 (at intervals of 1 pH unit) before autostereilization. The tolerance to NaCl and optimum salt concentrations for growth were tested by MZ2216E with different NaCl concentrations (0, 0.5, 1, 3, 3.5, 5, 7, 10, 12 and 15 %) at 35 °C. Catalase activity was determined by assessing the production of bubbles following the addition of a drop of 3 % (v/v) H2O2 and oxidase activity was determined using 1 % (w/v) tetramethyl-p-phenylenediamine. Tests for hydrolysis of cellulose, gelatin, starch, casein, tyrosine and Tweens 20, 60 and 80, milk coagulation and peptonization, utilization of urea and nitrate, reduction of nitrate were performed as described by Xu et al. (2007). Further physiological and biochemical characteristics were determined using the API 20E and API ZYM systems (bioMérieux) and Biolog GP2 MicroPlate panels, according to the instructions of the manufacturers. Strain Y32T formed light-yellow, circular and opaque colonies within 3 days of growth at 35 °C on MZ2216E plates. Cells were Gram-stain-positive, aerobic, motile, non-spore-forming, short rods (about 0.5 μm in width and 0.8–1.3 μm in length) with a single subterminal flagellum at either end (Fig. S1, available in the online Supplementary Material). Growth occurred at 4–40 °C with optimum growth at 30–35 °C. The pH range for growth was 6–11 and optimum pH for growth was 7. Strain Y32T was able to grow with 0–10 % (w/v) NaCl and optimal growth occurred with 3.5 % (w/v) NaCl. Phenotypic characteristics that differentiated strain Y32T from its closest phylogenetic neighbours are listed in Table 1. The detailed physiological and biochemical properties of strain Y32T are given in the species description.

Extraction of genomic DNA and PCR amplification of the 16S rRNA gene were performed as described by Li et al. (2007). The 16S rRNA gene sequence of strain Y32T was determined by capillary Sanger sequencing and was compared with 16S rRNA gene sequences of valid species from GenBank via the BLAST program and the EzTaxon-e server (Kim et al., 2012). All sequence alignments were analysed with the MEGA 5 software package (Tamura et al., 2011). Phylogenetic trees were reconstructed by using three different methods, namely the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and minimum-evolution (Rzhetsky & Nei, 1992) methods. Phylogenetic distances were calculated using the

**Table 1. Differential phenotypic characteristics between strain Y32T and its closest phylogenetic neighbours**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
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</thead>
<tbody>
<tr>
<td>Motility</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Morphology</td>
<td>Short rods</td>
<td>Rod–coccus cycle</td>
<td>Rods</td>
<td>Short rods</td>
<td>Short rods</td>
<td>Coccoid</td>
<td>Short rods</td>
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<tr>
<td>Size (μm)</td>
<td>Length</td>
<td>0.8–1.3</td>
<td>0.8–2.0</td>
<td>1.0</td>
<td>0.5–0.8</td>
<td>1.5–2.5</td>
<td>0.8–1.2</td>
</tr>
<tr>
<td></td>
<td>Width</td>
<td>0.5</td>
<td>0.8</td>
<td>0.5–0.8</td>
<td>0.3–0.5</td>
<td>0.5–0.8</td>
<td>0.8–1.2</td>
</tr>
<tr>
<td>Colony colour</td>
<td>Light yellow</td>
<td>Yellow</td>
<td>Light yellow</td>
<td>Yellow</td>
<td>Yellow</td>
<td>Light yellow</td>
<td>Yellow</td>
</tr>
<tr>
<td>Growth temperature (°C)</td>
<td>Range</td>
<td>4–40</td>
<td>10–40</td>
<td>10–45</td>
<td>4–45</td>
<td>10–45</td>
<td>4–45</td>
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<tr>
<td></td>
<td>Optimum</td>
<td>30–35</td>
<td>35–37</td>
<td>35</td>
<td>35–37</td>
<td>35</td>
<td>37</td>
</tr>
<tr>
<td>Growth pH</td>
<td>Range</td>
<td>6–11</td>
<td>6–10</td>
<td>5–12</td>
<td>5–12</td>
<td>5–10</td>
<td>6–12</td>
</tr>
<tr>
<td></td>
<td>Optimum</td>
<td>7</td>
<td>7–8</td>
<td>8–9</td>
<td>7–8</td>
<td>8</td>
<td>8–9</td>
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<td>NaCl range (%)</td>
<td>0–10</td>
<td>0–7</td>
<td>0–10</td>
<td>0–10</td>
<td>0–7</td>
<td>0–10</td>
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<tr>
<td>API ZYM results:</td>
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<td>–</td>
<td>–</td>
<td>–</td>
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<td>Trypsin</td>
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<td>Acid phosphatase</td>
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<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>w</td>
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<td></td>
<td>β-Glucuronidase</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>β-Glucosidase</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
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<td>–</td>
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<tr>
<td></td>
<td>N-Acetyl-β-glucosaminidase</td>
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<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>DNA G+C content (mol%)</td>
<td>71.2</td>
<td>69.7</td>
<td>73.0</td>
<td>66.2</td>
<td>72.9</td>
<td>ND</td>
<td>62.3</td>
</tr>
</tbody>
</table>

Georgenia subflava sp. nov.
Kimura two-parameter substitution model (Kimura, 1980). Bootstrap values based on 1000 replications were used to evaluate the topology of the phylogenetic trees (Felsenstein, 1985). DNA–DNA hybridization was performed according to the method of De Ley et al. (1970) and incorporated the modifications as described by Huss et al. (1983), using a Perkin-Elmer Lambda 35 spectrophotometer equipped with a Peltier temperature-controlling programmer.

The 16S rRNA gene sequence of strain Y32T was a continuous stretch of 1486 bp. Using the EzTaxon-e database, strain Y32T showed highest 16S rRNA gene sequence similarity to G. muralis DSM 14418T (97.8 %), followed by G. thermotolerans DSM 21501T (97.4 %), G. daequensis JCM 17459T (97.2 %), O. nanhaiensis JCM 17755T (97.2 %), G. ruanii DSM 17458T (97.0 %) and G. soli DSM 21838T (97.0 %). 16S rRNA gene sequence similarities between strain Y32T and the type strains of other type species investigated were less than 97.0 %. In the phylogenetic trees reconstructed using the neighbour-joining, maximum-likelihood and minimum-evolution algorithms, strain Y32T fell within the clade comprising Georgenia species (Fig. 1; Figs S2 and S3). In addition, strain Y32T showed relatively low DNA–DNA relatedness to G. muralis DSM 14418T (46.2 %; reciprocal reaction, 45.2 %), G. thermotolerans DSM 21501T (45.5 %; reciprocal reaction, 47.6 %), G. daequens JCM 17459T (33.5 %; reciprocal reaction, 29.2 %), O. nanhaiensis JCM 17755T (36.4 %; reciprocal reaction, 32.8 %), G. ruanii DSM 17458T (31.6 %; reciprocal reaction, 30.7 %) and G. soli DSM 21838T (30.2 %; reciprocal reaction, 28.4 %). The values were well below the threshold value (70 %) recommended by Wayne et al. (1987) for the definition of members of a species. The phylogenetic analysis results and DNA–DNA relatedness values thus indicated that strain Y32T may represent a novel species of the genus Georgenia.

For fatty acid analyses, all strains were grown on triptische soy agar (TSA; Difco) at 28 °C for 2 days. Fatty acids were saponified, methylated and extracted using the standard MIDI protocol (Sherlock Microbial Identification System, version 6.1), then analysed by GC (GC6850; Agilent) and identified by using the TSBA6 database of the Microbial Identification System (Sasser, 1990). Polar lipids were extracted according to the procedures described by Minnikin et al. (1984) and separated by two-dimensional TLC on plates of silica gel 60 F254 (Merck). Chloroform/methanol/water (65 : 25 : 4 by vol.) was used for the first dimension and chloroform/methanol/acetic acid/water (40 : 7 : 5 : 2 by vol.) for the second dimension, as described by Minnikin et al. (1977). Total lipids were detected by spraying the plate with 10 % ethanolic molybdophosphoric acid and phospholipids were detected by spraying with molybdenum blue reagent. Aminoacids and glycolipids were detected by spraying with ninhydrin and m-naphthal-sulphuric acid reagent, respectively. Phosphatidylglycerol and phosphatidylglycerol were confirmed by spraying with periodate-Schiff reagent. Isoprenoid quinones of strain Y32T were analysed using HPLC as described by Collins et al. (1977), and G. muralis DSM 14418T was used as a reference strain. The isoprenoid quinones were eluted by a mixture of methanol/2-propanol (2 : 1, v/v), using a flow rate of 1 ml min−1 at 40 °C and detected by UV absorbance at 240 and 275 nm. A purified cell-wall preparation of strain Y32T was obtained and hydrolysed as described by Schleifer & Kandler (1972). Amino acids in cell-wall hydrolysates were analysed by HPLC using precolumn derivatization with o-phthalaldehyde (Tang et al., 2009). G. muralis DSM 14418T and G. thermotolerans DSM 21501T were used as the reference strains for analysis of cell-wall amino acids. The cell-wall sugars were analysed as described by Lechevalier & Lechevalier (1980). The DNA G + C content was determined by the method of Mesbah et al. (1989).

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the position of strain Y32T and its closest neighbours in the family Bogoriellaceae. The 16S rRNA gene sequence of Microbacterium lacticum DSM 20427T was used as an outgroup. Numbers at nodes indicate percentages of bootstrap support (>50 %) based on 1000 replications. Bar, 1 substitution per 100 nt.
The menaquinone system of strain Y32<sup>T</sup> contained MK-8(H<sub>4</sub>) (87.5 %) and MK-7(H<sub>4</sub>) (7.8 %). This matched the quinone system of *G. muralis* DSM 14418<sup>T</sup> (Fig. S4). The peptidoglycan of strain Y32<sup>T</sup> contained alanine, glutamic acid and lysine, which was similar to the results for *G. muralis* DSM 14418<sup>T</sup> and *G. thermotolerans* DSM 21501<sup>T</sup>, and was characteristic of the genus *Georgenia* (peptidoglycan type A4<sub>d</sub> diamino acid). The major polar lipids were diphosphatidylethanolamine (12.1 %) and iso-C<sub>14</sub>:0 (10.2 %). Strain Y32<sup>T</sup> and its closest phylogenetic neighbours all shared anteiso-C<sub>15</sub>:0 as the major fatty acid (Table S1). However, the proportions of iso-C<sub>15</sub>:0 and iso-C<sub>14</sub>:0 of strain Y32<sup>T</sup> were clearly higher than those of the reference strains, which could be used to discriminate strain Y32<sup>T</sup> from its closest neighbours. Galactose was detected as the cell-wall sugar. The DNA G+C content of strain Y32<sup>T</sup> was 71.2 mol%, which was in the range of 66.2–73.4 mol% reported for the genus *Georgenia* (Srinivas et al., 2012; Woo et al., 2012; You et al., 2013).

Based on the results of phenotypic, genotypic and phylogenetic analyses, strain Y32<sup>T</sup> is considered to represent a novel species of the genus *Georgenia*, for which the name *Georgenia subflava* sp. nov. is proposed.

**Description of Georgenia subflava** sp. nov.

*Georgenia subflava* (sub.fla’va. L. fem. adj. subflava yellowish).

Cells are Gram-stain-positive, aerobic, motile, non-spore-forming, short rods (about 0.5 μm in width and 0.8–1.3 μm in length) with a single subterminal flagellum at either end. Colonies on M2216E medium are light yellow, circular and opaque after incubation at 35 °C for 48 h. Growth occurs at 4–40 °C with optimum growth at 30–35 °C. The pH range for growth is 6–11 and the optimum pH for growth is 7. Growth occurs in the presence of 0–10 % (w/v) NaCl with the optimum in 3.5 % (w/v) NaCl. Catalase-positive and oxidase-negative. Positive for nitrate reduction and casein hydrolysis; negative for H<sub>2</sub>S production, indole production, citrate utilization, activity of urease, gelatinase, lysine decarboxylase and ornithine decarboxylase, and fermentation of glucose, sorbitol, rhamnose, melibiose, mannitol, inositol, sucrose, amygdalin and arabinose. In the Biolog GP2 MicroPlate, positive for oxidation of dextrin, glycerol, D-glucosamine, D-fructose, sucrose, turanose, acetic acid, propionic acid, L-glutamic acid, adenosine, 2′-deoxyadenosine, inosine, thymidine, uridine, adenosine-5′-monophosphate, thymidine-5′-monophosphate and uridine-5′-monophosphate; negative for oxidation of N-acetyld-galactosamine, D-ribose, sucrose, rhamnose, fructose, D-ribose, trehalose, D-xylene, pyruvic acid methyl ester, pyruvic acid, succinic acid monomethyl ester and glycerol; weakly positive for oxidation of N-acetyld-glucosamine, D-fructose, sucrose, turanose, acetic acid, propionic acid, L-glutamic acid, adenosine, 2′-deoxyadenosine, inosine, thymidine, uridine, adenosine-5′-monophosphate, thymidine-5′-monophosphate and uridine-5′-monophosphate; negative for oxidation of the remaining Biolog GP2 substrates. The predominant menaquinones are MK-8(H<sub>4</sub>) and MK-7(H<sub>4</sub>). Major cellular fatty acids are anteiso-C<sub>15</sub>:0, iso-C<sub>15</sub>:0 and iso-C<sub>14</sub>:0. The polar lipids are diphosphatidylethanolamine, phosphatidylethanolamine, phosphatidylglycerol and phosphatidylglycerol. The cell-wall sugar is galactose.

The type strain, Y32<sup>T</sup> (=LMG 28101<sup>T</sup>=CGMCC 5.12782<sup>T</sup>=JCM 19765<sup>T</sup>=MCCC 1A09955<sup>T</sup>), was isolated from a deep-sea sediment collected from the western Pacific. The DNA G+C content of the type strain is 71.2 mol%.

**Acknowledgements**

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**References**


