Description of *Kushneria aurantia* gen. nov., sp. nov., a novel member of the family *Halomonadaceae*, and a proposal for reclassification of *Halomonas marisflavi* as *Kushneria marisflavi* comb. nov., of *Halomonas indalinina* as *Kushneria indalinina* comb. nov. and of *Halomonas avicenniae* as *Kushneria avicenniae* comb. nov.

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An aerobic, moderately halophilic, Gram-negative, motile, non-sporulating rod-shaped bacterium, designated strain A10T, was isolated from the surface of leaves of the black mangrove *Avicennia germinans* and was subjected to a polyphasic taxonomic study. Strain A10T was able to grow at NaCl concentrations in the range 5–17.5 % (w/v) with optimum growth at 10 % (w/v) NaCl. Growth occurred at temperatures of 20–40 °C (optimal growth at 37 °C) and pH 5.5–8.5 (optimal growth at pH 7.0–8.0). The major respiratory quinone was ubiquinone 9. The major fatty acids were C16:0, C18:1ω7c, C19:0 cyclo ω8c and C12:0 3-OH. The polar lipids were phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine, and unidentified phospholipids, glycolipids and an aminoglycolipid. Phylogenetic analysis based on 16S rRNA gene sequence comparisons revealed that strain A10T is closely related to *Halomonas avicenniae* MW2aT (95.7 % sequence similarity), *Halomonas marisflavi* SW32T (95.2 %) and *Halomonas indalinina* GC2.1T (95.0 %). Strain A10T formed a coherent phylogenetic branch with these three species, separated from other species of *Halomonas* and closely related genera (with 16S rRNA gene sequence similarities below 94.0 %). A complete 23S rRNA gene sequence comparison of strain A10T with closely related species confirmed the phylogenetic position of the novel isolate, forming a branch with the species *Halomonas avicenniae*, *Halomonas indalinina* and *Halomonas marisflavi*, separated from other species of the genera belonging to the family *Halomonadaceae* (showing sequence similarities below 91.7 %). DNA–DNA hybridization studies between strain A10T and *Halomonas avicenniae* MW2aT, *Halomonas marisflavi* DSM 15357T and *Halomonas indalinina* CG2.1T were 21, 17 and 10 %, respectively. These levels of DNA–DNA relatedness were low enough to classify strain A10T as representing a genotypically distinct species. Overall, the phenotypic, genotypic, chemotaxonomic and phylogenetic results demonstrated that strain A10T represents a new genus and species. The name *Kushneria aurantia* gen. nov., sp. nov. is proposed, with strain A10T (=CCM 7415T=CECT 7220T) as the type strain. This is the type species of the new proposed genus, which belongs to the family *Halomonadaceae*. In addition, our data support the placement of the species *Halomonas marisflavi*, *Halomonas indalinina* and *Halomonas avicenniae* within this new genus, as *Kushneria marisflavi* comb. nov., *Kushneria indalinina* comb. nov. and *Kushneria avicenniae* comb. nov., respectively.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain A10T is AM941746, and those for the 23S rRNA gene sequences of strains A10T, *Halomonas avicenniae* MW2aT, *Halomonas indalinina* CG2.1T and *Halomonas marisflavi* DSM 15357T are AM941756, AM941755, AM941754 and AM941753, respectively.

A table showing the 23S rRNA gene amplification and sequencing primers is available as supplementary material with the online version of this paper.
The family Halomonadaceae, in the class Gammaproteobacteria, was proposed by Franzmann et al. (1988) and, at the time of writing, includes a large number of species within seven genera: Halomonas (type genus), Carminonas, Chromohalobacter, Cobetia, Halotalea, Medicinalibacter and Zymobacter (Vreeland et al., 1980; Ventosa et al., 1989; Okamoto et al., 1993; Garriga et al., 1998; Arahal et al., 2002a; Arahal & Ventosa, 2005; Ntougias et al., 2007; Ben Ali Gam et al., 2007). Most genera have a single species, and only Chromohalobacter (9 species) and Halomonas (49 species) have more than one species. The genus Halomonas includes Gram-negative bacteria mainly with a respiratory type of metabolism, and most species that have been isolated from marine or hypersaline environments (salt-terns, saline lakes, saline soils, salted foods, etc.) are halophilic (Arahal & Ventosa, 2005; Arahal et al., 2007; Vreeland, 2005). This genus is very heterogeneous, as reflected by the broad range of phenotypic features of the species that it includes (Mata et al., 2002), or the wide range of G+C content of their DNA (52–68 mol%; Vreeland, 2005). Arahal et al. (2002b) carried out a phylogenetic study of the family Halomonadaceae, based on a comparison of the complete 16S rRNA and 23S rRNA gene sequences, and they established that the genus Halomonas is not monophyletic; it comprises two main clusters of species as well as several species that do not cluster consistently.

Recently, we have investigated the microbiota of Avicennia germinans, a mangrove that is able to secrete salt crystals onto the surface of its leaves when the salt concentration is in excess of that normally present in the vascular system (Lugo & Snedaker, 1975). As a result we described two novel bacterial species, Halomonas avicenniae (Soto-Ramirez et al., 2007) and Halobacillus mangrovi (Soto-Ramirez et al., 2008). In the present study, we determined the taxonomic position of strain A10T, a novel organism isolated from the surface of leaves of the black mangrove, which is closely related to the species Halomonas marisflavi (Yoon et al., 2001), and the recently described species Halomonas indalinina (Cabrera et al., 2007) and Halomonas avicenniae (Soto-Ramirez et al., 2007). Our results show that the novel bacterium constitutes a novel species of a new genus, for which we propose the name Kushneria aurantia gen. nov., sp. nov. In addition, we propose the reclassification within this new genus of these three closely related species previously assigned to the genus Halomonas.

Strain A10T was isolated from the surface of leaves of the black mangrove. For isolation, a total of 40 leaves were collected randomly from four A. germinans trees growing near the solar salterns of Cabo Rojo, Puerto Rico. These leaves were placed in sterile bags (Whirl Pak) containing 1% phosphate buffer (pH 7.0) with 15% (w/v) NaCl and mixed. This procedure allowed the suspension of the micro-organisms present in the salt crystals and on the surface of the leaves. Ten millilitres of the mix were then used for serial dilutions. The dilutions were poured onto agar plates containing Sehgal-Gibbons medium at 10% (w/v) NaCl (Sehgal & Gibbons, 1960). The inoculated plates were incubated at 30°C. After 3 days of incubation, colonies were selected and purified by using the quadrant streak plate method.

The following reference strains were used in this study: Halomonas avicenniae MW2aT, Halomonas marisflavi DSM 15357T, Halomonas indalinina CG2.1T and Halomonas elongata ATCC 33173T. They were cultivated in SW10 medium with 10% (w/v) total salts [8.1% NaCl, 0.7% MgCl2, 0.96% MgSO4, 0.036% CaCl2, 0.2% KCl, 0.006% NaHCO3, 0.0026% NaBr, 0.5% yeast extract (Difco)] (Nieto et al., 1989). The pH was adjusted to 7.2 with 1 M KOH. Where necessary, solid media were prepared by adding 2.0% (w/v) Bacto-agar (Difco).

The optimal conditions for growth were determined by growing strain A10T in SW medium at 0, 2.5, 5, 7.5, 10, 12.5, 15, 17.5, 20, 25 and 30% (w/v) total salts as well as in this medium but with only NaCl instead of the salt mixture described above, and at temperatures of 4, 15, 20, 25, 30, 37, 40 and 42°C. The pH range for the isolate was tested in SW10 medium adjusted to the following pH values: 4.0, 5.0, 6.0, 7.0, 8.0, 8.5 and 9.0, with the addition of the appropriate buffering capacity to each medium. The cells were cultivated with constant agitation (180 r.p.m.) and growth was monitored by measuring absorbance at 600 nm. Cells of strain A10T were Gram-negative, non-spor-forming rods. The cells were 2.0–5.0 μm long and 1.0 μm wide at the exponential phase of growth in SW10 medium at 37°C, and were motile. Optimal growth conditions occurred at 10% (w/v) total salts or at 10% (w/v) NaCl, 37°C and a pH of 7.0–8.0. Thus, strain A10T is a moderately halophilic micro-organism (Ventosa et al., 1998).

To characterize the isolate phenotypically, we followed the recommended minimal standards for describing new taxa of the family Halomonadaceae (Arahal et al., 2007). The phenotypic tests included Gram reaction (Dussault, 1955), cell morphology, motility, catalase and oxidase production, as well as other tests included in the species description. Macroscopic properties were determined using the classical characterization of colony appearance. All biochemical tests were carried out at 10% total salts and 37°C, unless stated otherwise. Catalase activity was determined by adding 1% (w/v) H2O2 solution to colonies on SW10 agar medium. The oxidase test was performed using the Dry Slide Assay (Difco). Hydrolysis of starch, gelatin, tyrosine and Tween 80, and production of urease and phosphatase were determined as described by Cowan & Steel (1965), with the addition of 10% total salts to the medium. Citrate utilization was determined on Simmons’ citrate medium supplemented with SW10. Acid production from carbohydrates was determined using a phenol red base supplemented with 1% of the carbohydrate and SW10 medium. Growth under anaerobic conditions was determined by incubating strain A10T in an anaerobic chamber.
in SW10 medium. Further tests for acid production from carbohydrates and enzymes were carried out using API 20 NE and API ID32E (bioMérieux), inoculated according to the manufacturer’s instructions using inoculated fluid at 10 % NaCl and incubation at 37 °C.

To determine the range of substrates used as carbon and energy sources, the classical medium of Koser (1923) as modified by Ventosa et al. (1982) was used, containing (l−1): 75 g NaCl, 2 g KCl, 0.2 g MgSO4.7H2O, 1 g KNO3, 1 g (NH4)2HPO4, 0.5 g KH2PO4 and 0.05 g yeast extract (Difco). Substrates were added as filter-sterilized solutions to give a final concentration of 1 g l−1, except for carbohydrates, which were used at 2 g l−1. When the substrate was an amino acid, it was tested as carbon, nitrogen and energy sources, and the basal medium was therefore prepared without KNO3 and (NH4)2HPO4. Additional nutritional features of the isolate were determined using Biolog MicroPlates. Strain A10T was grown on SW10 medium at 37 °C for 48 h, and suspended in 10 % (w/v) NaCl solution, within the density range specified by the manufacturer with a Biolog photometer model 21101. Immediately after suspending the cells in the saline solution, the suspensions were transferred into sterile multichannel pipetter reservoirs (Biolog) and the Biolog GN Microplates were inoculated with 125 μl of the cell suspension per well by means of an 8-channel repeating pipetter. The inoculated Biolog plates were incubated at 37 °C for 7 days and the results were read with a MicroPlate Reader using Microlog 3.59 computer software to perform automated reading. Antibiotic susceptibility was determined according to the conventional Kirby-Bauer method (Bauer et al., 1966). Other characterization methods used have been described in detail by Ventosa et al. (1982) and Quesada et al. (1984). The phenotypic characteristics of strain A10T are given in Table 1 and in the species description.

Chromosomal DNA was isolated and purified according to the method described by Marmur (1961). The 16S rRNA gene was amplified using the universal primers 16F27 and 16R1488 as described by Mellado et al. (1995). An almost complete 16S rRNA nucleotide sequence of strain A10T was determined by NBT-Newbiotechnics (Sevilla, Spain) using an automated DNA sequencer model 3130XL (Applied Biosystems) and was compared with 16S rRNA reference gene sequences retrieved from GenBank and EMBL databases by BLAST search. Subsequent sequence analysis was conducted using the ARB program package (Ludwig et al., 2004). Following the recommendations of Ludwig et al. (1998), alternative treeing methods (maximum-parsimony, neighbour-joining and maximum-likelihood) were used. A comparison using 16S rRNA gene sequences from databases revealed that the 16S rRNA gene sequence (1428 bp) of strain A10T displayed a high level of similarity to those of Halomonas species. The closest relatives were

### Table 1. Characteristics that differentiate strain A10T from the type strains of Halomonas marisflavi, Halomonas indalinina, Halomonas avicenniae and Halomonas elongata

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Strain: 1, A10T (Kushneria aurantia gen. nov., sp. nov.); 2, Halomonas marisflavi KCCM 80003T; 3, Halomonas indalinina CG2.1T; 4, Halomonas avicenniae MW2aT; 5, Halomonas elongata ATCC 33173T. Data are from Yoon et al. (2001), Cabrera et al. (2007), Soto-Ramírez et al. (2007), Arahal &amp; Ventosa (2005) and this study.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<tr>
<td>Cell morphology</td>
<td>Rods</td>
<td>Rods or oval cells</td>
<td>Short rods</td>
<td>Rods or oval cells</td>
<td>Short rods</td>
</tr>
<tr>
<td>Colony pigmentation</td>
<td>Orange</td>
<td>Yellow</td>
<td>Orange</td>
<td>Orange</td>
<td>Cream</td>
</tr>
<tr>
<td>NaCl range (%, w/v)</td>
<td>5–17.5</td>
<td>0.5–27</td>
<td>3–25</td>
<td>0–25</td>
<td>0–20</td>
</tr>
<tr>
<td>NaCl optimum (%, w/v)</td>
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<td>0.5–12</td>
<td>7.5–10</td>
<td>5</td>
<td>3–8</td>
</tr>
<tr>
<td>pH range</td>
<td>5.5–8.5</td>
<td>5.0–10.0</td>
<td>5.0–9.0</td>
<td>5.0–9.0</td>
<td>5.0–10.0</td>
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<tr>
<td>Temperature range (°C)</td>
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<td>4–37</td>
<td>15–40</td>
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<td>4–45</td>
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<tr>
<td>Nitrate reduction</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>H2S production</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
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<td>Acid production from:</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>l-Arabinose</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>D-Fructose</td>
<td>+</td>
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<td>+</td>
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</tr>
<tr>
<td>Maltose</td>
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<td>+</td>
<td>−</td>
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<td>+</td>
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<tr>
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<td></td>
<td></td>
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<td></td>
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<tr>
<td>Aesculin</td>
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<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Urea</td>
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<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>61.7</td>
<td>59.0</td>
<td>60.9</td>
<td>61.5</td>
<td>60.5</td>
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</tbody>
</table>

*Data from this study.*
Halomonas avicenniae MW2aT, Halomonas marisflavi DSM 15357T and Halomonas indalinina CG2.1T, with 95.7, 95.2 and 95.0 % sequence similarities, respectively. Sequence similarity with respect to other Halomonas species was equal to or less than 92.6 %; the similarities obtained when strain A10T was compared with other species of the genera Halomonas, Chromohalobacter, Modicisalibacter, Halotalea, Zymobacter, Carnimonas or Cobetia were 92.6–90.7, 93.6–92.0, 92.5, 91.3, 91.1, 91.0 and 90.9 %, respectively. The phylogenetic tree obtained by using the maximum-parsimony method showed strain A10T within a cluster constituted by the species Halomonas avicenniae MW2aT, Halomonas marisflavi SW32T and Halomonas indalinina CG2.1T (Fig. 1). The tree topology was similar when we used other algorithms (neighbour-joining and maximum-likelihood). Strain A10T and these three Halomonas species were phylogenetically related to Chromohalobacter species (94.0–92.0 % sequence similarity) and to other species of Halomonas (93.8–90.7 %). The similarity values with respect to the genera Modicisalibacter, Halotalea, Zymobacter, Carnimonas and Cobetia were 92.8–92.5 %, 92.8–91.3 %, 93.4–91.1 %, 91.8–91.0 % and 92.6–90.9 %, respectively.

In order to confirm the phylogenetic position of these species, we carried out a phylogenetic study based on a comparison of 23S rRNA gene sequences. The 23S rRNA gene was amplified using primers designed in this study and others described by Arahal et al. (2002b). An almost complete 23S rRNA nucleotide sequence was determined by NBT-Newbiotechnics (Sevilla, Spain) using an automated DNA sequencer model 3130XL (Applied Biosystems). 23S rRNA gene amplification and sequencing primers used are shown in Supplementary Table S1 (available in IJSEM Online). The 23S rRNA gene sequences of strain A10T, Halomonas avicenniae MW2aT, Halomonas marisflavi DSM 15357T and Halomonas indalinina CG2.1T were compared with reference 23S rRNA gene sequences retrieved from the GenBank and EMBL databases by BLAST search. Subsequently, sequence analysis was performed using the ARB program package (Ludwig et al., 2004). Alternative treeing methods (neighbour-joining, maximum-parsimony and maximum-likelihood) were used for these analyses. Similar to the 16S rRNA gene sequence analysis, the 23S rRNA gene sequence (2914 bp) of strain A10T displayed a high level of similarity to those of Halomonas avicenniae MW2aT, Halomonas indalinina CG2.1T and Halomonas marisflavi DSM 15357T, with 94.5, 93.6 and 93.5 % gene sequence similarities, respectively. The sequence similarities of strain A10T and other Halomonas species were 90.2–87.6 %, whereas when we compared the sequence of strain A10T with those of other related genera the values were 90.1–89.3 % with Chromohalobacter, 89.8 % with Cobetia, 88.8 % with Zymobacter and 88.3 % with Carnimonas. The phylogenetic tree obtained using the maximum-parsimony method showed that strain A10T fell within a cluster constituted by the species Halomonas marisflavi DSM 15357T, Halomonas indalinina CG2.1T and Halomonas avicenniae MW2aT (Fig. 2). When we used the neighbour-joining or maximum-likelihood algorithms the trees obtained were very similar to each other, confirming the stability of the tree topologies. These data confirm that strain A10T and the species Halomonas marisflavi, Halomonas indalinina and Halomonas avicenniae constitute a robust and stable phylogenetic branch within the family Halomonadaceae, sufficiently separated from other species of the genera of
this family as to warrant their placement in a new genus. In fact, when we compared the 23S rRNA gene sequences of strain A10T and those of \textit{Halomonas marisflavi} DSM 15357\textsuperscript{T}, \textit{Halomonas indalinina} CG2.1\textsuperscript{T} and \textit{Halomonas avicenniae} MW2a\textsuperscript{T} with respect to other species of the most closely related genera, we obtained the following similarity values: 91.5–87.6\% for other species of the genus \textit{Halomonas}, 91.7–89.3\% for species of \textit{Chromohalobacter}, 91.7–89.8\% for \textit{Cobetia}, 90.4–88.8\% for \textit{Zymobacter} and 89.8–88.3\% for \textit{Carnimonas}.

The G$+C$ content of the genomic DNA was determined from the mid-point value ($T_m$) of the thermal denaturation profile (Marmur & Doty, 1962) using the equation of Owen & Hill (1979), as previously described in detail (Ventosa \textit{et al.}, 1999). The DNA G$+C$ content of strain A10T was 61.7\%, which is within the broad range described for species belonging to the genus \textit{Halomonas}, 91.7–93.9\% for species of \textit{Chromohalobacter}, 91.7–98.9\% for \textit{Cobetia}, 90.4–88.8\% for \textit{Zymobacter} and 89.8–88.3\% for \textit{Carnimonas}.

The DNA–DNA hybridization studies were performed by using the competition procedure of the membrane method (Johnson, 1994), described in detail by Arahal \textit{et al.} (2001a, b). The hybridization temperature used was 56.9\degree C, which is within the limit of validity for the filter method (De Ley & Tijtgat, 1970) and the percentage hybridization was calculated according to Johnson (1994). The experiments were carried out in triplicate. The levels of DNA–DNA relatedness between strain A10\textsuperscript{T} and \textit{Halomonas avicenniae} MW2a\textsuperscript{T}, \textit{Halomonas marisflavi} DSM 15357\textsuperscript{T} and \textit{Halomonas indalinina} CG2.1\textsuperscript{T} were 21, 17 and 10\%, respectively. These levels of DNA–DNA relatedness were low enough to classify strain A10\textsuperscript{T} as representing a genotypically distinct species (Stackebrandt & Goebel, 1994; Stackebrandt \textit{et al.}, 2002).

Analysis of quinones and polar lipids was carried out by the Identification Service of the DSMZ (Braunschweig, Germany). The polar lipids detected for strain A10\textsuperscript{T} were phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine, three different phospholipids of unknown structure, two glycolipids and an aminoglycolipid. This pattern was essentially the same as those found for \textit{Halomonas avicenniae} MW2a\textsuperscript{T}, \textit{Halomonas marisflavi} DSM 15357\textsuperscript{T} and \textit{Halomonas indalinina} CG2.1\textsuperscript{T}. Strain A10\textsuperscript{T} contained ubiquinone 9 (Q9) as the major respiratory quinone (95\%), as well as ubiquinone 8 (Q8) (5\%) and traces of ubiquinone 10 (Q10). The major respiratory quinones detected for \textit{Halomonas avicenniae} MW2a\textsuperscript{T} were Q9 (99\%) and traces of Q8 and Q10, for \textit{Halomonas marisflavi} DSM 15357\textsuperscript{T} Q9 (92\%), Q8 (6\%) and Q10 (2\%) and for \textit{Halomonas indalinina} CG2.1\textsuperscript{T} Q9 (93\%), Q8 (4\%) and Q10 (3\%). Thus, the polar lipids and respiratory quinones of strain A10\textsuperscript{T} were almost identical to those of the other three species; however, Q9 is typically also found in \textit{Halomonas elongata} ATCC 33173\textsuperscript{T} (Table 2). However, this is a differential feature of the new species of \textit{Halomonas}.

Fatty acids were analysed using GC (Kämpfer & Kroppenstedt, 1996; Miller, 1982) at the BCCM/LMG, Gent, Belgium. Cells were cultured on SW10 medium at pH 7.0 and 37\degree C for 24 h. The predominant fatty acids of strain A10\textsuperscript{T} were C\textsubscript{16:0}, C\textsubscript{18:1\textit{v9c}}, C\textsubscript{19:0\textit{cyclo}}\textit{v8c} and C\textsubscript{12:0\textit{3-OH}}. This composition was very similar to those that we obtained under the same conditions for \textit{Halomonas avicenniae} MW2a\textsuperscript{T}, \textit{Halomonas marisflavi} DSM 15357\textsuperscript{T} and \textit{Halomonas indalinina} CG2.1\textsuperscript{T}, but some differences were observed with respect to \textit{Halomonas elongata} ATCC 33173\textsuperscript{T} (Table 2). However, this is a differential feature of the new species of \textit{Halomonas}.

Fig. 2. Maximum-parsimony phylogenetic tree, based on 23S rRNA gene sequences of strain A10\textsuperscript{T} and closely related species. Sequence accession numbers used are shown in parentheses. Bootstrap values greater than 50\% are indicated at branch points. \textit{Zymobacter palmae} ATCC 51623\textsuperscript{T} was used as an outgroup. Bar, 2\% sequence divergence.
Table 2. Cellular fatty acid compositions (%) of strain A10T and closely related Halomonas species

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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<td>C16:0</td>
<td>40.4</td>
<td>37.4</td>
<td>40.7</td>
<td>33.4</td>
<td>25.6</td>
</tr>
<tr>
<td>C18:1</td>
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<td>1.2</td>
<td>1.0</td>
<td>2.1</td>
<td>0.6</td>
</tr>
<tr>
<td>C18:0</td>
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<td>1.6</td>
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</tr>
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<td>C18:2</td>
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<td>0.8</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C18:3</td>
<td>0.4</td>
<td>3.2</td>
<td>3.6</td>
<td>3.2</td>
<td>–</td>
</tr>
<tr>
<td>C18:1 9c</td>
<td>8.8</td>
<td>11.3</td>
<td>12.1</td>
<td>11.0</td>
<td>15.3</td>
</tr>
<tr>
<td>C18:1 3-OH</td>
<td>1.9</td>
<td>0.4</td>
<td>0.7</td>
<td>0.5</td>
<td>–</td>
</tr>
<tr>
<td>C18:0 3-OH</td>
<td>3.5</td>
<td>2.6</td>
<td>4.2</td>
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</tr>
<tr>
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<td>37.4</td>
<td>40.7</td>
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<tr>
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<td>28.4</td>
<td>16.6</td>
<td>20.0</td>
<td>25.7</td>
<td>25.6</td>
</tr>
<tr>
<td>C18:0 Cyclo</td>
<td>1.1</td>
<td>1.2</td>
<td>1.0</td>
<td>2.1</td>
<td>0.6</td>
</tr>
<tr>
<td>C19:0 Cyclo</td>
<td>11.8</td>
<td>22.3</td>
<td>12.9</td>
<td>15.7</td>
<td>10.6</td>
</tr>
</tbody>
</table>

*Included in summed feature 3 (C18:1ω7c and/or iso-C15:0 2-0H).

Table 3 shows the differential features between the four species of Kushneria gen. nov. Some features that serve to differentiate this new genus from other genera of the Halomonadaceae are shown in Table 3.

Description of Kushneria gen. nov.

Kushneria (Kush.ne’ri.a. N.L. fem. n. Kushneria from the name Kushner, honouring Dr Donn J. Kushner, a Canadian microbiologist who carried out pioneering studies on halophilic micro-organisms).


quinone is Q9. Major fatty acids are C16:0, C18:1ω7c, C19:0 cyclo ω8c and C12:0 3-0H. Polar lipids are phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine and unidentified phospholipids and glycolipids. The DNA G+C content is 59.0–61.7 mol%. The genus Kushneria is a member of the family Halomonadaceae, phylogenetically related to Chromohalobacter and Halomonas. The type species is Kushneria aurantia.

Genus with respect to other genera of the Halomonadaceae (Table 3).

Overall, the phylogenetic data based on both the 16S rRNA and 23S rRNA gene sequence comparisons, the chemotaxonomic and genotypic results and the phenotypic features show clearly that strain A10T constitutes a novel species and genus, separated from the genus Halomonas and other genera belonging to the Halomonadaceae, for which we propose the name Kushneria aurantia gen. nov., sp. nov. In addition, on the basis of the results reported we also propose to transfer the three species Halomonas marisflavi, Halomonas indalinina and Halomonas avicenniae to this new genus as Kushneria marisflavi comb. nov., Kushneria indalinina comb. nov. and Kushneria avicenniae comb. nov., respectively. The species of this new genus have all the 16S rRNA signature nucleotides characteristic of the family Halomonadaceae (Ben Ali Gam et al., 2007). Table 1 shows the differential features between the four species of Kushneria gen. nov. Some features that serve to differentiate this new genus from other genera of the family Halomonadaceae are shown in Table 3.

Description of Kushneria aurantia sp. nov.

Kushneria aurantia (au.ran.ti.a. N.L. adj. aurantius orange; N.L. fem. adj. aurantia orange-pigmented).

Exhibits the following properties in addition to those given in the genus description. Cells are 1.0 × 2.0–5.0 μm in size and occur singly or in pairs. Does not produce exopoly-saccharide and does not accumulate poly-hydroxyalkanoate. Colonies on SW10 medium after 2 days incubation at 37 °C are 0.5–1.0 mm in diameter, circular, smooth, regular and convex with an entire margin, with an orange pigmentation. Moderately halophilic, requires NaCl; grows at NaCl concentrations in the range 5–17.5 % (w/v), with an optimum at 10 % (w/v) NaCl. Growth occurs at temperatures of 20–40 °C (optimally at 37 °C) and at pH 5.5–8.5 (optimally at pH 7.0–8.0). Respiration with fumarate, nitrate and nitrite is negative. Gelatin and aesculin are hydrolysed. Positive for methyl red, phosphatase and Simmons’ citrate tests. Starch, casein, Tween 80, tyrosine and DNA are not hydrolysed. Negative for H₂S production, Voges–Proskauer, indole production, nitrate and nitrite reduction, urease, arginine dihydrolase, phenylalanine deaminase, lysine decarboxylase, α-galactosidase, α-maltosidase, N-acetyl-β-glucosaminidase and L-aspartic acid arylamidase but positive for ornithine decarboxylase, β-galactosidase, α-galactosidase and β-gluconidase. Acid is produced from D-glucose, D-galactose, D-fructose, maltose, D-mannose and xylose, but not from arabinose, glycerol, mannitol, trehalose or sorbitol. Does not grow on MacConkey agar with 10 % (w/v) salts. The following compounds are used as sole carbon and energy sources: D-fuctose, D-glucose, DL-malate, D-ribose, D-tartrate and D-xylose. The following compounds are not used as sole carbon and energy sources: ascin, benzoate, ethanol, fumarate, hippurate, D-lactose, melezitose, salicin and starch. L-Cysteine is used as sole carbon, nitrogen and energy sources. The following compounds are not used as sole carbons, nitrogen and energy sources: L-isoleucine, DL-lysine, L-methionine, L-tryptophan and L-valine. The following compounds are used (oxidized) as sole carbon and energy sources (Biolog): dextrin, glycerogen, adonitol, L-arabinose, D-arabitol, cellulose, i-erythritol, D-fructose, D-galactose, gentiobiose, α-D-glucose, maltose, D-mannitol, D-mannose, D-psicose, D-sorbitol, trehalose, turanose, xylitol, succinic acid monomethyl ester, acetic acid, cis-aconitic acid, citric acid, D-galacturonic acid, α-ketoglutaric acid, D-lactic acid, quinic acid, D-saccharic acid, succinic acid, bromosuccinic acid, succinic acid, L-glutamic acid,
Table 3. Characteristics that differentiate Kushneria gen. nov. from other genera of the family Halomonadaceae

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Kushneria marisflavi</th>
<th>Halomonas</th>
<th>Chromohalobacter</th>
<th>Halomonas</th>
<th>Modicisalibacter</th>
<th>Halotalea</th>
<th>Carnimonas</th>
<th>Zymobacter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell morphology</td>
<td>Rods</td>
<td>Rods</td>
<td>Straight rods</td>
<td>Rods</td>
<td>Slightly curved rods</td>
<td>Rods</td>
<td>Straight or slightly curved rods</td>
<td>Rods</td>
</tr>
<tr>
<td>NaCl requirement</td>
<td>Moderately halophilic</td>
<td>Moderately halophilic</td>
<td>Slightly halophilic</td>
<td>Moderately halophilic</td>
<td>Moderately halophilic</td>
<td>Non-halophilic</td>
<td>Halotolerant</td>
<td>Halotolerant</td>
</tr>
<tr>
<td>Oxygen requirement</td>
<td>Aerobic</td>
<td>Aerobic</td>
<td>Aerobic</td>
<td>Aerobic</td>
<td>Aerobic</td>
<td>Aerobic</td>
<td>Aerobic</td>
<td>Facultatively anaerobic</td>
</tr>
<tr>
<td>Oxidase</td>
<td>++</td>
<td>*</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Major fatty acids</td>
<td>C16 : 0, C18 : 1</td>
<td>C16 : 0, C18 : 1</td>
<td>C16 : 0, C19 : 0 cyclo, C16 : 1, C17 : 0 cyclo, C16 : 0, C19 : 0 cyclo</td>
<td>C16 : 0, C18 : 1</td>
<td>C16 : 0, C19 : 0 cyclo</td>
<td>C16 : 0, C19 : 0 cyclo</td>
<td>C16 : 0, C18 : 1, 7 c, 7 c, 7 c, 7 c, C18 : 1</td>
<td>C16 : 0, C19 : 0 cyclo, C12 : 0 3-OH</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>59.0–61.7</td>
<td>52–68</td>
<td>62.1–64.9</td>
<td>62.1–64.9</td>
<td>53.7</td>
<td>64.4</td>
<td>55.4–56.2</td>
<td></td>
</tr>
</tbody>
</table>

*Some species are oxidase-negative.

L-proline, L-prolylglutamic acid, L-serine, L-threonine, urocanic acid, inosine, uridine, thymidine, 2,3-butanediol, glycerol and D-glucose 6-phosphate. The following compounds are not used (oxidized) as sole carbon and energy sources (Biolog): z-cyclodextrin, Tween 40, Tween 80, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, L-fucose, myo-inositol, z-D-lactose, lactulose, D-melibiose, methyl z-D-glucoside, D-raffinose, L-rhamnose, sucrose, pyruvic acid methyl ester, formic acid, D-galactonic acid lactone, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, z-hydroxybutyric acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, p-hydroxyphenylacetic acid, itaconic acid, z-ketobutyric acid, 3-ketovaleric acid, malonic acid, propionic acid, sebacic acid, glucuronamide, alaminamide, D-alanine, L-alanine, L-alanyl-glycine, L-asparagine, L-aspartic acid, glycerol and D-aspartic acid, L-glutamine, L-glutamic acid, L-histidine, hydroxy-L-proline, L-leucine, L-ornithine, L-phenylalanine, D-serine, DL-carnitine, γ-aminoobutyric acid, phenylethylamine, putrescine, 2-aminoethanol, DL-β-glycerol phosphate and z-D-glucose 1-phosphate. Resistant to (μg per disc, unless specified otherwise): bacitracin (10 U), cephalothin (30), erythromycin (15), kanamycin (30), nalidixic acid (30) and streptomycin (10). Susceptible to chloramphenicol (30), novobiocin (30), penicillin G (10 U), polymyxin B (300 U), rifampicin (5), sulfamethazole/trimethoprim (23.75/1.25) and vancomycin (30). The DNA G+C content of the type strain is 61.7 mol%.

The type strain, A10T (=CCM 7415T = CECT 7220T), was isolated from the leaf surface of A. germinans (black mangrove).

Description of Kushneria marisflavi comb. nov.

Kushneria marisflavi (ma.ris.flav’i. L. gen. neut. n. maris of the sea; L. neut. adj. flavum yellow; L. gen. neut. n. marisflavi of the Yellow Sea, Korea).


The description is the same as that for Halomonas marisflavi (Yoon et al. 2001), with the addition that the polar lipids are phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine, aminoglycolipid and an unidentified phospholipid and a glycolipid.

The type strain is SW32T (= DSM 15357T = JCM 10873T = KCCM 80003T).

Description of Kushneria indalinina comb. nov.

Kushneria indalinina (in.da.li.ni’na. N.L. n. indalo -inis a prehistoric magical symbol; L. suff. -inis-a-un suffix used in the sense of ‘belonging to’; N.L. fem. adj. indalinina pertaining to the Indalo, the symbol of the province of Almeria, Spain, from where the type strain was isolated).

Basonym: Halomonas indalinina Cabrera et al. 2007.

The description is the same as that for Halomonas indalinina (Cabrera et al., 2007), with the addition that...
the polar lipids are phosphatidylglycerol, diphasphatidylglycerol, phosphatidylethanolamine, aminoglycolipid and an unidentified phospholipid and a glycolipid. The major respiratory quinone is Q9.

The type strain is CG2.1T (=CECT 5902T= CIP 109528T= LMG 23625T).

**Description of Kushneria avicenniae comb. nov.**

Kushneria avicenniae [a.vi.cn.ni’ae. N.L. n. Avicennia scientific name of a botanical genus which encompasses Avicennia germinans (the black mangrove); N.L. gen. n. avicenniae of Avicennia, isolated from leaves of Avicennia germinans].


The description is the same as that for Halomonas avicenniae (Soto-Ramírez et al. 2007), with the addition that the polar lipids are phosphatidylglycerol, diphasphatidylglycerol, phosphatidylethanolamine, aminoglycolipid and an unidentified phospholipid and a glycolipid. The major respiratory quinone is Q9.

The type strain is MW2aT (=CECT 7193T= CIP 109711T= CCM 7396T).

**Acknowledgements**

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**References**


