**Luteibaculum oceani** gen. nov., sp. nov., a carotenoid-producing, lipolytic bacterium isolated from surface seawater, and emended description of the genus *Owenweeksia* Lau et al. 2005

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A yellow-pigmented, Gram-staining-negative, strictly aerobic, rod-shaped, non-flagellated, non-spore-forming, lipolytic and gliding marine bacterium designated strain CC-AMWY-103B¹ was isolated from surface seawater collected at Kending, Taiwan. The strain shared the highest 16S rRNA gene sequence similarity of 99.4 % with *Owenweeksia hongkongensis* JCM 12287T and *Brumimicrobium mesophilum* YH207¹, and established a distinct phylectic lineage associated with the members of the family *Cryomorphaceae*. The polar lipid profile of strain CC-AMWY-103B¹ consisted of phosphatidylethanolamine, ten unidentified lipids and four unidentified aminolipids. The major fatty acids (>5 % of the total) were iso-C₁₅ : 0, iso-C₁₅ : 1ω5c, G, C₁₅ : 1ω8c and C₁₅ : 1ω7c. The DNA G + C content was 44.2 ± 0.3 mol%. The predominant respiratory quinone was menaquinone-6 (MK-6) and the major polyamine was spermidine. Based on its genetic, phylogenetic, phenotypic and chemotaxonomic distinctiveness, strain CC-AMWY-103B¹ is proposed to represent a distinct member of the family *Cryomorphaceae*, for which the name *Luteibaculum oceani* gen. nov., sp. nov. is proposed; the type strain of *Luteibaculum oceani* is CC-AMWY-103B¹ (=JCM 18817T=BCRC 80551T). An emended description of the genus *Owenweeksia* is also proposed.

Representatives of the phylum *Bacteroidetes* are widespread in various marine habitats and are specialized in degrading high-molecular-mass organic compounds (Fernández-Gómez et al., 2013). In the order *Flavobacteriales* of the phylum *Bacteroidetes*, members of the family *Flavobacteriaceae* are characterized as carotenoid-producers (Hameed et al., 2012; Shindo et al., 2007). However, carotenoid production in neighbouring families within the order *Flavobacteriales* is yet to be explored. In this study, via a polyphasic taxonomic approach (Vandamme et al., 1996), we investigated the taxonomic position of a novel carotenoid-producing strain that shared low (<89.5 %) 16S rRNA gene sequence similarity with known members of the order *Flavobacteriales*.

During an investigation pertaining to carotenoid-producing bacteria inhabiting oceanic surface water, a yellow-pigmented strain, designated CC-AMWY-103B¹, was isolated from surface seawater that had been collected at coastal Kending, Taiwan (21.935004' N 120.821419' E). The seawater sample was subjected to the standard dilution-to-extinction plating method using marine agar 2216 (MA; BD Difco) and incubation at 30 °C for 48–96 h. A single colony of strain CC-AMWY-103B¹ was isolated, purified and preserved as 20 % glycerol stocks. Taxonomic investigations were carried out according to previously published guidelines (Tindall et al., 2010). The whole-genome-sequenced strain *Owenweeksia hongkongensis* JCM 12287T (Lau et al., 2005; Riedel et al., 2012) was used as a reference for direct comparative analysis. Both strains were cultured on MA or in marine broth (MB; BD Difco) for 48 h at 30 °C for taxonomic analysis, unless specified otherwise.

Colonies of strain CC-AMWY-103B¹ were examined for morphological features such as appearance, size, shape and texture. The presence of endospores was determined by phase-contrast microscopy (model A3000; Zeiss) after malachite green staining (Smibert & Krieg, 1994) of cells grown on MA for 7 days. Cell morphology, including the presence of flagella, was determined by placing cells (1–2 days old) on a carbon-coated copper grid and staining with 0.2 % uranyl acetate for 5–10 s followed by brief...
air-drying and observation under a transmission electron microscope (JEOL JEM-1400). Gram-staining was performed according to Murray et al. (1994). Gliding motility was investigated by using phase-contrast microscopy (model A3000; Zeiss) of a hanging-drop preparation from an MB culture (Bernardet et al., 2002). The presence of flexirubin-type pigments was investigated as described by Reichenbach (1992) and Bernardet et al. (2002). Growth under anaerobic conditions was tested using MA or MA supplemented with 0.1% KNO₃ by incubating culture plates in an anaerobic chamber (COY). Activities of catalase and oxidase and hydrolysis of starch (0.2%), egg yolk (1.0%) and Tween 20 and 80 (both 1.0%) were tested on MA according to Smibert & Krieg (1994). Degradation of casein (1.0% skimmed milk), colloidal chitin (1.0%), CM-cellulose (1.0%) and xylan (1.0%) was tested on MA. Degradation was revealed by the formation of a clear zone around colonies either directly or after flooding with appropriate stains (Teather & Wood, 1982). Hydrolysis of L-tyrosine (0.5%) was tested on MA (Cowan & Steel, 1993). DNase activity was assessed using DNase test agar (HiMedia) supplemented with 3.2% sea salts. The requirement for NaCl was tested on R2A agar (BD Difco) supplemented with 0–10% NaCl (at 1% intervals). The pH range for growth was determined in MB that was adjusted before sterilization to pH 4.0–12.0 (at 1.0 pH unit intervals) using appropriate buffers. Growth at 10, 20, 25, 30, 37, 40, 45, 50 and 55 °C was tested on MA after 72 h of incubation. Carbon source utilization was determined using the GN2 MicroPlate (Biolog). Nitrate reduction, production of indole, D-glucose fermentation, activities of arginine dihydrolase, urease and p-nitrophenyl-β-D-galactopyranosidase, hydrolysis of aesculin and gelatin and growth on D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetylglucosamine, maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid and hydrolysis of casein, xylan, CM-cellulose, chitin, DNA, starch and Tween 80.

Table 1. Differential phenotypic characteristics of strain CC-AMWY-103Bᵀ and O. hongkongensis JCM 12287ᵀ

Strains: 1, CC-AMWY-103Bᵀ; 2, O. hongkongensis JCM 12287ᵀ. Data are from this study except the DNA G+C content of the reference strain (taken from Lau et al., 2005). +, Positive; −, negative; w, weak reaction. Both strains possessed MK-6 as the predominant respiratory quinone. Both strains were positive for growth on MA, activities of oxidase, alkaline phosphatase, esterase (C4), leucine, valine and cystine arylamidases, acid phosphatase and naphthol-AS-Bl-phosphohydrolase and hydrolysis of gelatin and ascinul. Both strains were negative for activities of lipase (C14), z-chymotrypsin, z-galactosidase, β-galactosidase, β-glucuronidase, z-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, z-mannosidase, z-fucosidase, arginine dihydrolase, urease and p-nitrophenyl-β-D-galactopyranosidase, production of indole, fermentation of D-glucose, assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetylglucosamine, maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid and hydrolysis of casein, xylan, CM-cellulose, chitin, DNA, starch and Tween 80.

Genomic DNA of strain CC-AMWY-103Bᵀ was isolated by using an UltraClean microbial genomic DNA isolation kit (MO BIO) by following the manufacturer’s instructions. The partial 16S rRNA gene was amplified by PCR according to Shahina et al. (2013). Gene sequencing was performed by using the ABI Big Dye terminator kit (Heiner et al., 1998) and an automatic DNA sequencer (ABI Prism 310; Applied Biosystems) (Watts & MacBeath, 2001). Sequence fragments were then assembled using the Fragment Assembly System program from the Wisconsin GCG, 1995). Sequence similarity values were computed using BLAST searches (Altschul et al., 1990) and the EzTaxon-e server (Kim et al., 2012). Sequence data were analysed by using MEGA 5 (Tamura et al., 2011), after multiple alignment by CLUSTAL_X (Thompson et al., 1997). A distance matrix method (distance options according to
Kimura’s two-parameter model; Kimura, 1980) including clustering by the neighbour-joining method (Saitou & Nei, 1987), a discrete character-based maximum-parsimony method (Fitch, 1971) and the maximum-likelihood method (Felsenstein, 1981) were used. The topologies of trees were evaluated by using the bootstrap resampling method based on 1000 replications (Felsenstein, 1985).

16S rRNA gene sequence analysis revealed that strain CC-AMWY-103B\textsuperscript{T} shared the highest pairwise sequence similarity with both \textit{O. hongkongensis} JCM 12287\textsuperscript{T} and \textit{Brumimicrobium mesophilicum} YH207\textsuperscript{T} (89.4%) (Bowman et al., 2003) followed by \textit{Crocinitomix catalasitica} IFO 15977\textsuperscript{T} (88.3%), \textit{Lishizhenia caseinilytica} UST040201-001\textsuperscript{T} (88.1%) and \textit{Wandonia haliotis} Haldis-1\textsuperscript{T} (87.8%); other strains showed \leq 87.7% similarity. In the neighbour-joining phylogenetic tree (Fig. 1), strain CC-AMWY-103B\textsuperscript{T} segregated clearly from the clade ‘\textit{Brumimicrobiaceae}’ and formed a phyletic lineage associated with \textit{O. hongkongensis} JCM 12287\textsuperscript{T} and \textit{Phaeocystidibacter luteus} PG2S01\textsuperscript{T} (Zhou et al., 2013) with 42% bootstrap support of the node. Similarly, in the maximum-parsimony and maximum-likelihood trees (not shown), strain CC-AMWY-103B\textsuperscript{T} was clearly segregated from members of the families ‘\textit{Brumimicrobiaceae}’ and \textit{Flavobacteriaceae}, and established a phyletic lineage adjacent to representatives of the family \textit{Cryomorphaceae}.

For cellular fatty acid analysis, fatty acid methyl esters of strain CC-AMWY-103B\textsuperscript{T} and the reference strain were extracted and analysed by reversed-phase HPLC according to Scherer & Kneifel (1983) with slight modifications to saponification, methylation and extraction as described previously (Kämpfer & Kroppenstedt, 1996) and analysed by GC (model 7890A; Agilent). Peaks were automatically integrated and fatty acid names and percentages were determined using the Microbial Identification standard software package (MIDI version 6) (Sasser, 1990) by using the RTSBA6 database.

The fatty acid profiles of the two strains displayed both qualitative and quantitative differences (Table 2). The major fatty acids (>5% of total fatty acids) of strain CC-AMWY-103B\textsuperscript{T} were iso-C15:0 (42.9%), iso-C15:1 G (28.5%), C15:1ω5c (7.1%), iso-C17:0 3-OH (5.5%) and C15:1ω8c (5.3%). Strain CC-AMWY-103B\textsuperscript{T} and \textit{O. hongkongensis} JCM 12287\textsuperscript{T} shared common predominant branched unsaturated (iso-C15:0) and branched mono-unsaturated (iso-C15:1 G) fatty acids. However, strain CC-AMWY-103B\textsuperscript{T} differed clearly from the reference strain by possessing C15:1ω5c, C15:1ω8c and iso-C17:0 3-OH in major amounts while lacking major amounts of summed features 3 (C16:1ω6c and/or C16:1ω7c) and 9 (10-methyl C16:1 and/or iso-C17:1ω9c), which were found in significant amounts in \textit{O. hongkongensis} JCM 12287\textsuperscript{T}.

Carotenoids of strain CC-AMWY-103B\textsuperscript{T} and \textit{O. hongkongensis} JCM 12287\textsuperscript{T} were extracted by using methanol and analysed by UV–visible spectrophotometry according to Hameed et al. (2012). The polyamines of strain CC-AMWY-103B\textsuperscript{T} and \textit{O. hongkongensis} JCM 12287\textsuperscript{T} were extracted and analysed by reversed-phase HPLC according to Scherer & Kneifel (1983) with slight modifications.
(Shahina et al., 2013). Respiratory quinones of strain CC-AMWY-103B\textsuperscript{T} and \textit{O. hongkongensis} JCM 12287\textsuperscript{T} were extracted according to Minnikin et al. (1984) and analysed by reversed-phase HPLC according to Collins (1985) with minor modifications (Shahina et al., 2013). Polar lipids of strain CC-AMWY-103B\textsuperscript{T} and \textit{O. hongkongensis} JCM 12287\textsuperscript{T} were extracted and analysed by two-dimensional TLC (Embley & Wait, 1994). For the determination of G+C content, genomic DNA of strain CC-AMWY-103B\textsuperscript{T} was prepared by thermal denaturation followed by enzymic digestion into nucleosides as described previously (Mesbah et al., 1989). The resultant nucleoside mixture was separated and quantified by reversed-phase HPLC with minor modifications as given by Shahina et al. (2013). UV–visible spectrophotometry of a crude carotenoid extract from strain CC-AMWY-103B\textsuperscript{T} showed a zeaxanthin-like absorption spectrum, with \( \lambda_{\text{max}} \) 451 nm and associated shoulder peaks (Fig. S2). In contrast, carotenoids isolated from \textit{O. hongkongensis} JCM 12287\textsuperscript{T} exhibited a spectrum that was similar to that of monomeric carotenoids, having \( \lambda_{\text{max}} \) 469 nm and associated shoulder peaks (Shindo et al., 2007). Based on these preliminary data, it is assumed that strain CC-AMWY-103B\textsuperscript{T} produced zeaxanthin in predominant amounts, whereas \textit{O. hongkongensis} JCM 12287\textsuperscript{T} is possibly capable of synthesizing monomeric carotenoids in major amounts. The polyamine profile of strain CC-AMWY-103B\textsuperscript{T} consisted of a major amount of spermidine (79.6%) and a minor amount of putrescine (20.3%). In contrast, \textit{O. hongkongensis} JCM 12287\textsuperscript{T} possessed the triamine \textit{syn}-homospermidine as the major polyamine (96.5%) and contained trace amounts of putrescine (2.6%) and spermidine (0.8%). Strain CC-AMWY-103B\textsuperscript{T} showed major amounts of menaquinone MK-6 (96.6%) and minor amounts of MK-5 (3.3%) as respiratory quinones, a profile that was qualitatively and quantitatively similar to that of \textit{O. hongkongensis} JCM 12287\textsuperscript{T}. The DNA G+C content of strain CC-AMWY-103B\textsuperscript{T} was 44.2 mol%, a value significantly higher than that of \textit{O. hongkongensis} JCM 12287\textsuperscript{T} (Table 1). TLC patterns of total polar lipids of strain CC-AMWY-103B\textsuperscript{T} and \textit{O. hongkongensis} JCM 12287\textsuperscript{T} are shown in Fig. 2. Strain CC-AMWY-103B\textsuperscript{T} contained phosphatidylethanolamine, ten unidentified lipids and four unidentified aminolipids, of which four lipids (L1, L4, L5 and L7) and two aminolipids (AL1 and AL2) were present in major amounts; the others were present in moderate to trace amounts. The predominance of L1, L4 and L7 was also observed in \textit{O. hongkongensis} JCM 12287\textsuperscript{T}. However, there were clear qualitative and quantitative differences in the other polar lipids produced by these strains. Taken together, the phylogenetic, chemotaxonomic (quinones, polar lipids, fatty acids, polyamines and G+C content) and biochemical features presented here support the classification of strain CC-AMWY-103B\textsuperscript{T} as a distinct representative of the family \textit{Cryomorphaeaceae}, order \textit{Flavobacteriales}. Thus, strain CC-AMWY-103B\textsuperscript{T} is proposed to represent a novel genus and species of the family \textit{Cryomorphaeaceae}, for which the name \textit{Luteibaculum oceani} gen. nov., sp. nov. is proposed. On the basis of new data obtained in this study, an emended description of the genus \textit{Owenweeksia} is also proposed.

### Emended description of the genus \textit{Owenweeksia} Lau et al. 2005

The description of the genus is as given by Lau et al. (2005), under consideration of the emended description of the type species provided by Zhou et al. (2013), with the following amendments. The triamine \textit{syn}-homospermidine is a major polyamine; moderate amounts of putrescine and spermidine are also present. Five unidentified lipids and an unidentified phospholipid are present in major amounts.

### Description of \textit{Luteibaculum} gen. nov.

\textit{Luteibaculum} (Lu.te.i.ba.cu.lum. L. adj. \textit{luteus} yellow; L. neut. n. \textit{baculum} a stick, in bacteriology a bacterium; N.L. neut. n. \textit{Luteibaculum} a yellow-coloured bacterium).

Cells are Gram-staining-negative, strictly aerobic, non-spor-forming, chemoheterotrophic and mesophilic, typically rod-shaped with rounded ends, non-flagellated and motile by means of gliding. Oxidase-positive and catalase-negative. Flexirubin-type pigments are absent. The major

### Table 2. Whole-cell fatty acid profiles of strain CC-AMWY-103B\textsuperscript{T} and \textit{O. hongkongensis} JCM 12287\textsuperscript{T}

<table>
<thead>
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<th>Fatty acid</th>
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<td></td>
</tr>
<tr>
<td>C\textsubscript{16}:0</td>
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<tr>
<td>Branched saturated</td>
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<td></td>
</tr>
<tr>
<td>iso-C\textsubscript{15}:0</td>
<td>42.9</td>
<td>43.9</td>
</tr>
<tr>
<td>iso-C\textsubscript{16}:0</td>
<td>1.1</td>
<td>TR</td>
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<tr>
<td>Unsaturated</td>
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<td></td>
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<tr>
<td>C\textsubscript{15}:0\textsubscript{c}</td>
<td>7.1</td>
<td>4.0</td>
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<tr>
<td>C\textsubscript{16}:0\textsubscript{c}</td>
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<td>Branched monounsaturated</td>
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<td>iso-C\textsubscript{15}:1 G</td>
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</tr>
<tr>
<td>iso-C\textsubscript{17}:0 3-OH</td>
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<tr>
<td>Summed feature 9</td>
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</table>

*Summed features represent groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 3 consists of C\textsubscript{16}:0\textsubscript{c} and/or C\textsubscript{16}:1\textsubscript{c}; summed feature 9 consists of 10-methyl C\textsubscript{16}:1 and/or iso-C\textsubscript{17}:1\textsubscript{c}.
isoprenoid quinone is MK-6. Predominant fatty acids (>25% of the total) are iso-C_{15:0} and iso-C_{15:1} G. The polar lipid profile includes four unidentified lipids and two unidentified aminolipids in predominant amounts. Spermidine is the major polyamine. As determined by 16S rRNA gene sequence analysis, the genus is a novel member of the family Cryomorphaceae. The type species is Luteibaculum oceani.

### Description of Luteibaculum oceani sp. nov.

*Luteibaculum oceani* (o.ce.Ë’a’ni. L. gen. n. oceani of the ocean).

Displays the following properties in addition to those described for the genus. Cells are 1.4–1.8 µm long and 0.3–0.5 µm in diameter. On MA, after 1–2 days of incubation at 30 °C, colonies are small, circular, convex and yellow, 0.5–1.0 mm in diameter. Carotenoid pigments are present. Growth occurs at 20–35 °C (optimum, 30 °C), at pH 6.0–8.0 (optimum, pH 7.0) and in the presence of 1–5% NaCl (optimum, 2%). L-Tyrosine, Tween 20 and egg yolk are hydrolysed, whereas chitin, starch, Tween 80, casein, CM-cellulose, xylan and DNA are not. In the GN2 MicroPlate, α-cyclodextrin, methyl β-D-glucoside, trehalose, L-glutamic acid and L-pyroglutamic acid are utilized weakly, while lactulose, maltose, quinic acid and L-ornithine are utilized strongly; the remaining substrates are not utilized. In the API 20 NE strip, positive for nitrate reduction and hydrolysis of aesculin and gelatin; negative for p-nitrophenyl-β-D-galactopyranosidase, arginine dihydrolase and urease, tryptophan deaminase, arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase, production of H_{2}S, acetoin and indole, utilization of citrate and fermentation/oxidation of D-glucose, D-mannitol, inositol, D-sorbitol, L-rhamnose, sucrose, melibiose, amygdalin and L-arabinose. In the API 50CH strip, acid is produced from potassium 5-ketogluconate only. The major fatty acids (>5% of total) are iso-C_{15:0}, iso-C_{15:1} G, C_{15:0} 10c, iso-C_{17:0} 3-OH and C_{15:0} 10c. The polar lipid profile includes phosphatidylethanolamine, ten unidentified lipids and four unidentified aminolipids. The polyamine profile includes minor amounts of putrescine. The quinone profile includes trace amounts of MK-5.

The type strain is CC-AMWY-103B^{T} (=JCM 18817^{T}=BCRC 80551^{T}), which was isolated from surface seawater collected at the seashore of Kending, Taiwan. The DNA G+C content of the type strain is 44.2 mol%.

### Acknowledgements

The authors thank the editor and anonymous reviewers for their constructive comments on this manuscript. M. S. thanks the Ministry of Economic Affairs and National Chung Hsing University, Taiwan,
for awarding a doctoral scholarship. This work was supported in part by the Ministry of Education, Taiwan, ROC under the ATU plan.

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