Aquimarina intermedia sp. nov., reclassification of Stanierella latercula (Lewin 1969) as Aquimarina latercula comb. nov. and Gaetbulimicrobium brevivitae Yoon et al. 2006 as Aquimarina brevivitae comb. nov. and emended description of the genus Aquimarina

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A heterotrophic, aerobic, Gram-negative, pigmented and gliding bacterium, strain KMM 6258T, was isolated from the sea urchin Strongylocentrotus intermedius and investigated using a polyphasic taxonomic approach. 16S rRNA gene sequence analysis revealed that the closest relatives of the novel strain are Aquimarina muelleri, Stanierella latercula and Gaetbulimicrobium brevivitae, members of the family Flavobacteriaceae, with sequence similarities of 96.3, 96.4 and 96.2 %, respectively. Phylogenetic evidence, supported by chemotaxonomic and phenotypic data, assigned strain KMM 6258T to the genus Aquimarina as Aquimarina intermedia sp. nov. (type strain KMM 6258T = DSM 17527T = JCM 13506T = LMG 23204T). The reclassification of Stanierella latercula as Aquimarina latercula comb. nov. and Gaetbulimicrobium brevivitae as Aquimarina brevivitae comb. nov. is proposed.

The genus Aquimarina, a member of the family Flavobacteriaceae (Bernardet et al., 2002), accommodates heterotrophic, Gram-negative, aerobic, dark-yellow or brownish-coloured, gliding flavobacteria producing flexirubin-type pigments that have been isolated from seawater samples (Nedashkovskaya et al., 2005). Sufficient phylogenetic divergence, as well as a number of phenotypic differences and distinctive fatty acid contents, allowed the separation of the representatives of the single species of the genus, Aquimarina muelleri, from their nearest neighbour, [Cytophaga] latercula. The latter species was reclassified as Stanierella latercula in the same study.

In the course of a study of cultured bacteria isolated from the sea urchin Strongylocentrotus intermedius, we found a novel reddish-coloured bacterium, strain KMM 6258T. Phylogenetic analysis of the 16S rRNA gene sequence of strain KMM 6258T revealed that the closest relatives of the novel isolate were members of the genera Aquimarina, Stanierella and Gaetbulimicrobium. The novel strain occupied an intermediate position between the three genera. Based on the results of phylogenetic, phenotypic and fatty acid analyses, we propose that strain KMM 6258T be placed in the genus Aquimarina as a separate species and that the nearest neighbours, Stanierella latercula and Gaetbulimicrobium brevivitae, are reclassified as members of the genus Aquimarina.

Strain KMM 6258T was isolated from the sea urchin Strongylocentrotus intermedius collected in Troitsa Bay, Gulf of Peter the Great, Sea of Japan. To isolate the strain, 0.1 ml tissue homogenate was transferred onto plates of marine agar 2216 (Difco). After primary isolation and purification, the novel strain was cultivated at 28 °C on the same medium and stored at −80 °C in marine broth 2216 (Difco) supplemented with 20 % (v/v) glycerol.

The almost-complete 16S rRNA gene sequence of strain KMM 6258T was determined following a previously described procedure (Vancanneyt et al., 2004). This sequence (a continuous stretch of 1476 bp) was aligned with sequences retrieved from EMBL and a phylogenetic tree was constructed by the neighbour-joining method using the BioNumerics software package, version 4.0 (Applied
Maths). Unknown bases were discarded for analyses. Bootstraping analysis was undertaken to test the statistical reliability of the topology of the neighbour-joining tree using 500 bootstrap resamplings of the data (Fig. 1). The tree topology obtained with the neighbour-joining method was evaluated and confirmed by maximum-parsimony analysis using BioNumerics (data not shown). Strain KMM 6258T showed 16S rRNA gene sequence similarities of 99.7, 96.4, 96.3 and 96.2% with its nearest neighbours, [Flexibacter] tractuosus IFO 15980, S. latercula ATCC 23177T, A. muelleri KMM 6020T and G. brevivitae SMK-19T, respectively (Fig. 1). These observations support the reclassification of S. latercula and G. brevivitae in the genus Aquimarina.

DNA was isolated according to the method of Marmur (1961) and G+C content was determined by using the thermal denaturation method (Marmur & Doty, 1962). The G+C content of the DNA of strain KMM 6258T was 37.1 mol%.

Analysis of fatty acid methyl esters of strain KMM 6258T and of the type strains of A. muelleri and S. latercula grown on marine agar at 28 °C for 24 h was carried out according to the standard protocol of the Sherlock Microbial Identification System (Microbial ID). The major cellular fatty acids for strain KMM 6258T were: iso-C16 : 1ω5c (32%), iso-C15 : 0 (25.9%), iso-C17 : 1ω9c (12.7%), iso-C15 : 1ω7c (7.8%), iso-C17 : 0ω7c and summed feature 3 (5.0%), comprising C16 : 1ω7c and/or iso-C15 : 02-OH (Table 1).

The absorption spectrum of pigments extracted using acetone/methanol 7 : 2 (v/v) was determined at between 300 and 700 nm with a UV spectrophotometer (CE 7250, CECIL series). Cells of strain KMM 6258T produced reddish-coloured pigments with maximum absorption at 469.8 nm (Table 2).

Phenotypic analysis was performed using methods described previously (Nedashkovskaya et al., 2003a, b). In order to determine susceptibility to antibiotics, the novel strain was grown on marine agar at 28 °C for 48 h. In addition to the antibiotics tested in previous studies, the susceptibility of the strain to chloramphenicol (30 μg), doxycycline (10 μg) and erythromycin (15 μg) was also determined.

![Phylogenetic tree based on 16S rRNA gene sequences of Aquimarina species and of representative members of related genera in the family Flavobacteriaceae, generated using the neighbour-joining method (Saitou & Nei, 1987). Numbers at nodes indicate bootstrap values (expressed as percentages of 500 replications). Bar, 0.05 substitutions per nucleotide position.](image-url)

**Table 1. Fatty acid content of species of the genus Aquimarina**

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<tr>
<td>iso-C13 : 0</td>
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<td>NP</td>
<td>tr</td>
<td>1-5</td>
</tr>
<tr>
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<td>NP</td>
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</tr>
<tr>
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<td>4-5</td>
<td>5-3</td>
<td>1-0</td>
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<tr>
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<td>NP</td>
<td>1-1</td>
<td>NP</td>
</tr>
<tr>
<td>iso-C15 : 0</td>
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<td>20-2</td>
<td>18-4</td>
<td>22-2</td>
</tr>
<tr>
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<td>5-2</td>
</tr>
<tr>
<td>iso-C15 : 5 3-OH</td>
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<td>9-7</td>
</tr>
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<td>NP</td>
<td>NP</td>
</tr>
<tr>
<td>C16 : 0 3-OH</td>
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<td>NP</td>
<td>2-0</td>
<td>1-5</td>
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<tr>
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<td>2-9</td>
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<tr>
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<tr>
<td>C18 : 1ω5c</td>
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<td>Summed feature 3*</td>
<td>5-0</td>
<td>3-7</td>
<td>6-4</td>
<td>6-8</td>
</tr>
</tbody>
</table>

*Summed features consist of one or more fatty acids that could not be separated by the Microbial Identification System. Summed feature 3 comprises iso-C15 : 0 2-OH and/or C16 : 1ω7c.*
The physiological, biochemical and morphological characteristics of strain KMM 6258\textsuperscript{T} are given in the species description and in Table 2. Phenotypic examination revealed many common traits between the novel strain and its closest relatives, \textit{A. muelleri}, \textit{S. latercula} and \textit{G. brevivitae}. However, strain KMM 6258\textsuperscript{T} could be clearly differentiated from these species by its ability to utilize carbohydrates and by a higher DNA G+C content (Table 2). Strain KMM 6258\textsuperscript{T} could also be differentiated from \textit{A. muelleri} by the absence of chitinase activity, the presence of \(\beta\)-galactosidase activity, hydrogen sulfide production and susceptibility to benzylpenicillin. Some features of strain KMM 6258\textsuperscript{T}, including catalase and amylyase activities, the ability to grow in the presence of 10\% NaCl and the absence of agar and chitin hydrolysis, may be helpful for separating the novel strain from \textit{S. latercula}. A combination of phenotypic properties, such as the ability to produce flexirubin-type pigments and hydrogen sulfide, to utilize \(\alpha\)-arabinose, \(\alpha\)-mannonse and sucrose, a lower temperature range for growth and the inability to form acid from carbohydrates, distinguish strain KMM 6258\textsuperscript{T} from \textit{G. brevivitae} (Table 2).

Consequently, on the basis of the combination of significant molecular, genotypic and phenotypic similarities between strain KMM 6258\textsuperscript{T} and \textit{A. muelleri}, we suggest that strain KMM 6258\textsuperscript{T} represents a novel species in the genus \textit{Aquimarina}, for which the name \textit{Aquimarina intermedia} sp. nov. is proposed.

Furthermore, the high level of 16S rRNA gene sequence similarity of \textit{S. latercula} and \textit{G. brevivitae} with members of the genus \textit{Aquimarina} (96.2–96.4\% ) supports the placement of these two species in the genus \textit{Aquimarina} (Stackebrandt & Goebel, 1994). Taken together with the phylogenetic data, the similarity in fatty acid content of all the strains tested (Table 1) supports the transfer of \textit{Stanierella latercula} and \textit{Gaetbulimicrobium brevivitae} to the genus \textit{Aquimarina} as \textit{Aquimarina latercula} comb. nov. and \textit{Aquimarina brevivitae} comb. nov., respectively. Since gliding motility and catalase production, both characteristics of other members of the genus \textit{Aquimarina}, were not observed among cells of \textit{S. latercula} in this and previous studies (Lewin, 1969; Reichenbach, 1989; Nedashkovskaya et al., 2005), \textit{Aquimarina intermedia} sp. nov. is proposed.

### Table 2. Differential characteristics of species in the genus \textit{Aquimarina}

Species: 1, \textit{Aquimarina intermedia}; 2, \textit{Aquimarina} (\textit{Gaetbulimicrobium} \textit{brevivitae}) \textit{3}, \textit{Aquimarina} (\textit{Stanierella} \textit{latercula}); 4, \textit{Aquimarina muelleri}. All strains were positive for the following: respiratory type of metabolism, oxidase and alkaline phosphatase activities, hydrolysis of casein, gelatin and Tweens 20, 40 and 80, susceptibility to ampicillin, chloramphenicol, lincomycin and oleandomycin and resistance to chloramphenicol, lincomycin and oleandomycin and resistance to benzylpenicillin. All strains were negative for the following tests: nitrate reduction; cellulose (carboxymethyl cellulose and filter paper) and urea degradation and indole and susceptibility to benzylpenicillin. Some features of strain KMM 6258\textsuperscript{T}, including catalase and amylyase activities, the ability to grow in the presence of 10\% NaCl and the absence of agar and chitin hydrolysis, may be helpful for separating the novel strain from \textit{S. latercula}. A combination of phenotypic properties, such as the ability to produce flexirubin-type pigments and hydrogen sulfide, to utilize \(\alpha\)-arabinose, \(\alpha\)-mannonse and sucrose, a lower temperature range for growth and the inability to form acid from carbohydrates, distinguish strain KMM 6258\textsuperscript{T} from \textit{G. brevivitae} (Table 2).

Consequently, on the basis of the combination of significant molecular, genotypic and phenotypic similarities between strain KMM 6258\textsuperscript{T} and \textit{A. muelleri}, we suggest that strain KMM 6258\textsuperscript{T} represents a novel species in the genus \textit{Aquimarina}, for which the name \textit{Aquimarina intermedia} sp. nov. is proposed.

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### Emended description of the genus \textit{Aquimarina}


Rod-shaped and strictly aerobic cells. Gram-negative. Do not form endospores. Produce non-diffusible carotenoid and/or flexirubin-type pigments. Cells of some species can move by means of gliding. Chemoorganotroph. Cytochrome oxidase- and alkaline phosphatase-positive. The predominant cellular fatty acids are branched-chain saturated and unsaturated and straight-chain unsaturated fatty acids iso-C\textsubscript{15:0}, iso-C\textsubscript{15:1}, iso-C\textsubscript{16:0} 3-OH, iso-C\textsubscript{17:1} \textit{b}9c, iso-C\textsubscript{17:0} 3-OH and summed feature 3 (comprising C\textsubscript{16:0} \textit{b}7 and/or iso-C\textsubscript{15:0} 2-OH). The main respiratory quinone is MK-6. 16S rRNA gene sequence analysis indicates that the genus \textit{Aquimarina} is a member of the family \textit{Flavobacteriaceae}, phylum \textit{Bacteroidetes}. The type species is \textit{Aquimarina muelleri}.

### Description of \textit{Aquimarina intermedia} sp. nov.

\textit{Aquimarina intermedia} (in.ter.me’d.i.a. L. fem. adj. \textit{intermedia} intermediate, referring to the level of 16S rRNA gene sequence similarity with its closest relatives).
The main characteristics are the same as those given for the genus. In addition, cells range from 0.4 to 0.5 µm in width and from 2.1 to 3.2 µm in length and are motile by means of gliding. On marine agar, colonies are 2–3 mm in diameter, circular, shiny with entire edges and reddish-pigmented. Growth is observed at 4–36 °C. Optimal temperature for growth is 25–28 °C. Growth occurs in 1–10 % NaCl, with an optimum of 2–5 % NaCl. β-Galactosidase activity is present. Flexirubin-type pigments are produced. Decomposes casein, gelatin, starch, DNA and Tweens 20, 40 and 80. Does not degrade agar, cellulose (carboxymethylcellulose and filter paper) or chitin. Does not form acid from L-arabinose, D-cellobiose, L-fucose, D-galactose, D-glucose, D-lactose, D-maltose, D-melibiose, L-rafﬁnose, L-rhamnose, L-sorbose, sucrose, DL-xyllose, N-acetylgluconamide, citrate, adonitol, dulcitol, glycerol, inositol or mannitol. Utilizes L-arabinose, D-glucose, D-mannose and sucrose but not D-lactose, mannitol, inositol, sorbitol, malonate or citrate. Nitrate is not reduced. Hydrogen sulﬁde is produced. The results for indole and acetoain (Voges–Proskauer reaction) production are negative. Susceptible to ampicillin, benzylpenicillin, carbenicillin, chloramphenicol, doxycycline, erythromycin, lincomycin, oleandomycin and tetracycline. Resistant to gentamicin, kanamycin, neomycin, polymyxin B and streptomycin. The dominant fatty acids are iso-C17:0 3-OH (32–6 %), iso-C15:0 (25–9 %), iso-C17:1ω9c (12–7 %), iso-C15:1ω8c (7–8 %), iso-C15:0 3-OH (7–0 %) and summed feature 3 (5–0 %), comprising C16:1ω7 and/or iso-C15:0 2-OH. The G+C content of the DNA is 37.1 mol%.

The type strain, strain KMM 6258T (=DSM 17527T=JCM 13506T=LMG 23204T), was isolated from the sea urchin Strongylocentrotus intermedius collected in Troitsa Bay, Gulf of Peter the Great, Sea of Japan.

**Description of Aquimarina latercula** (Lewin 1969) comb. nov.

*Aquimarina latercula* (la.ter’cu.la. L. masc. dim. n. laterculus a small brick; N.L. fem. adj. latercula brick-like, brick-red colour).


The description is as given for the genus and by Nedashkovskaya et al. (2005), with the addition that the strain produces flexirubin-type pigments. Gliding motility and catalase activity are not observed. Does not utilize L-arabinose, D-glucose, D-lactose, D-mannose, sucrose, inositol, mannitol, sorbitol, malate or citrate. The predominant fatty acids are iso-C17:0 3-OH (30–5 %), iso-C15:0 (18–4 %), iso-C15:1ω8c (7–3 %), iso-C15:0 3-OH (7–0 %), summed feature 3 (6–4 %), comprising C16:1ω7 and/or iso-C15:0 2-OH, C15:0 (5–3 %) and iso-C17:1ω9c (4–7 %), and The G+C content of the DNA is 34 mol%.

The type strain, LMG 1343T (=ATCC 23177T=NCIMB 1399T=CIP 104806T), was isolated from the outflow of a marine aquarium in La Jolla, California, USA.

**Description of Aquimarina brevivitae** (Yoon et al. 2006) comb. nov.

Aquimarina brevivitae (bre.vi.va’tae. L. adj. brevis short; L. gen. n. vitae of life; N.L. gen. n. brevivitae of short life, referring to the short-lived cultures of the type strain).

Basonym: *Gaetbulimicrobium brevivitae* Yoon et al. 2006.

The description is as given for *Gaetbulimicrobium brevivitae* by Yoon et al. (2006).

The type strain is SMK-19T (=DSM 17196T=KCTC 12390T).

**Acknowledgements**

This research was supported by grants from the Russian Foundation for Basic Research (RFBR) no. 05-04-48211 and no. 06-04-48578, RFBR-Far Eastern Branch Russian Academy of Sciences (FEB RAS) no. 06-04-96067, FEB RAS Micro-organisms of the Russian Far East and State Contracts 'Scientific Schools' from the Federal Agency for Science and Innovations of the Russian Federation, M.V., L.C. and J.S. acknowledge the Belgian Federal Public Planning Service – Science Policy.

**References**


Aquimarina intermedia sp. nov.


