Reclassification of *Alteromonas distincta* Romanenko *et al.* 1995 as *Pseudoalteromonas distincta* comb. nov.

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The 16S rRNA gene of *Alteromonas distincta* KMM 638\(^\top\) was amplified, cloned and sequenced. The nucleotide sequence was aligned with sequences of representative strains of *Alteromonas, Moritella, Pseudoalteromonas* and *Shewanella*. Results of phylogenetic analysis, using neighbour-joining and Fitch–Margoliash methods, clearly indicated that this species should be assigned to the genus *Pseudoalteromonas*. On the basis of polyphasic data obtained from previous work and this study, it is proposed that the species *Alteromonas distincta* be reclassified as *Pseudoalteromonas distincta* comb. nov. with type strain KMM 638\(^\top\) ( = ATCC 700518\(^\top\)).

**Keywords:** reclassification of *Pseudoalteromonas distincta*, *Alteromonas distincta*

Marine *Pseudomonas*-like bacteria comprise several related genera, including *Alteromonas* (Baumann & Baumann, 1981; Baumann et al., 1984), *Marinomonas* (Gauthier & Brettmayer, 1992), *Pseudoalteromonas* (Gauthier et al., 1995), *Moritella* (Gauthier et al., 1995), *Marinobacter* (Gauthier et al., 1992), *Psychrobacter* (Bowman et al., 1996), *Colwellia* (Deming et al., 1988), *Shewanella* (MacDonnell & Colwell, 1985) and *Halomonas* (Dobson & Franzmann, 1996). Classification of these aerobic heterotrophic micro-organisms from the marine environment remains difficult and absent of robust chemotaxonomic markers. Fortunately, phylogenetic information, based on 16S rRNA gene sequences, is useful in classifying and identifying micro-organisms belonging to previously poorly defined taxa.

In 1995, Romanenko and colleagues created a new species, *Alteromonas distincta*, into which was placed a bacterium isolated from a marine sponge collected at a depth of 350 m near the Komandorskie Islands, Russia. Using phenotypic and genotypic criteria, these investigators were able to separate *A. distincta* from all validly described species of the genus *Alteromonas*. In an independent phylogenetic study based on 16S rRNA sequences, Gauthier et al. (1995) proposed that members of the genus *Alteromonas* be divided into two groups: *Alteromonas*, including the type species *Alteromonas macleodii*, and a cluster of species corresponding to the ‘*Alteromonas haloplanktis*’ cluster of De Vos et al. (1989), for which the new genus *Pseudoalteromonas* was proposed. However, the taxonomic position of *A. distincta* was not clear, since it was not included in the study of Gauthier et al. (1995). The present study was undertaken to determine the appropriate genus assignment for the species.

*A. distincta* KMM 638\(^\top\) ( = ATCC 700518\(^\top\)) (the species is represented by a single strain) was grown at 25 °C on Marine agar 2216 (Difco Laboratories) and stored at −80 °C in Marine broth (Difco) supple-
mented with 30% (v/v) glycerol. Genomic DNA was extracted employing the method of Gauthier et al. (1995). PCR, cloning and sequencing of the 16S rRNA gene were carried out using the Taq DyeDeoxy Terminator Cycle Sequencing kit (Applied Biosystems) and an Applied Biosystems 373A DNA sequencer, as described previously by Chun & Goodfellow (1995). The resultant 16S rRNA gene sequence was aligned manually against sequences obtained from the GenBank database. Phylogenetic trees were constructed using the Fitch–Margoliash (Fitch & Margoliash, 1967) and neighbour-joining (Saitou & Nei, 1987) methods. Evolutionary distance matrices were generated according to Jukes & Cantor (1969). The PHYLIP package (Felsenstein, 1993) was used for all analyses. The resultant unrooted tree topology was evaluated in bootstrap analyses (Felsenstein, 1985), using the neighbour-joining method based on 1000 resamplings.

A 16S rRNA gene sequence (1451 nt) was obtained for the type strain of *A. distincta*. From the unrooted evolutionary tree shown in Fig. 1, it is concluded that *A. distincta* belongs to the genus *Pseudoalteromonas*. The relationship is based on data from different tree-making algorithms and the 100% bootstrap value. Furthermore, the tree is very similar to that of Gauthier et al. (1995). *A. distincta* was recovered within a monophyletic clade, *Pseudoalteromonas*, containing the non-pigmented species, *Pseudoalteromonas antarctica*, *P. haloplanktis* subsp. *haloplanktis*, *P. haloplanktis* subsp. *tetrodonis*, *P. haloplanktis* subsp. *nigrifaciens* and *P. haloplanktis undina*. Comparison of the deduced primary structure of the 16S rRNA of *A. distincta* revealed a distant relationship with *A. macleodii* (89-4% sequence similarity), but closer relatedness with pseudalteromonads, ranging from 94.5% (*Pseudoalteromonas dentrificans*) to 99.7% (*P. nigrifaciens*).

*A. distincta* possesses several features that are different from other previously described species of the genus *Pseudoalteromonas* and a table with differential characteristics has been published elsewhere (Ivanova et al., 1998). Notably, the bacterium can be distinguished by the presence of both monotrichous cells and cells possessing two to five polar and lateral flagella. The species *P. dentrificans* has been found to have occasional cells with a tuft of two or three flagella (Enger et al., 1987). On nutrient media, strain KMM 638T produces an intracellular and extracellular diffusible, melanin-like pigment of the species belonging to the non-pigmented clade. It is worth noting that only *A. distincta* and *P. nigrifaciens* produce melanin (Ivanova et al., 1996).

Although pseudalteromonads utilize a wide range of compounds as a carbon source, strain KMM 638T is unable to utilize glucose, sucrose, lactose, cellobiose, maltose, mannitol, N-acetylglucosamine, D-xylene, L-arabinose, D-mannose or L-rhamnose. In addition, the number of complex substrates utilized by *A. distincta* is relatively small (Romanenko et al., 1995; Ivanova et al., 1998). DNA–DNA hybridizations carried out by Romanenko et al. (1995) and in this study showed the genetic uniqueness of *A. distincta*, when compared with other members of the genus *Pseudoalteromonas*. That is, DNA relatedness values of *A. distincta* and *P. undina*, *P. haloplanktis* subsp. *tetrodonis*, *P. haloplanktis* subsp. *nigrifaciens*, *P. espejiana* and *P. espejiana* were 16–41%, and the corresponding value for *A. macleodii* was 10% (Table 1).

Given the polyphasic data from the earlier study (Romanenko et al., 1995) and presented here, it is proposed that *A. distincta* Romanenko et al. (1995) be assigned to the genus *Pseudoalteromonas* as *Pseudoalteromonas distincta* comb. nov.
**Table 1.** DNA relatedness among strains examined in this study

<table>
<thead>
<tr>
<th>Species</th>
<th>G + C content (mol %)</th>
<th>Hybridization (%)</th>
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<tr>
<td></td>
<td></td>
<td><em>P. distincta</em> ATCC 700518&lt;sup&gt;T&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>P. distincta</em> ATCC 700518&lt;sup&gt;T&lt;/sup&gt;</td>
<td>43.8</td>
<td>100</td>
</tr>
<tr>
<td><em>P. nigrifaciens</em> IAM 13010&lt;sup&gt;T&lt;/sup&gt;</td>
<td>41.7</td>
<td>31</td>
</tr>
<tr>
<td><em>P. espejiana</em> IAM 12640&lt;sup&gt;T&lt;/sup&gt;</td>
<td>41.4</td>
<td>31</td>
</tr>
<tr>
<td><em>P. haloplanktis</em> subsp. <em>haloplanktis</em> ATCC 14393&lt;sup&gt;T&lt;/sup&gt;</td>
<td>42.7</td>
<td>30</td>
</tr>
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</table>

**Description of *Pseudoalteromonas distincta* (Romanenko, Mikhailov, Lysenko and Stepanenko) comb. nov.**

The description of *Pseudoalteromonas distincta* comb. nov. is identical to that of Romanenko *et al.* (1995), with the addition that the species with four to seven lateral flagella be included. The description is based on a single strain, which is the type strain KMM 638<sup>T</sup> (= ATCC 700518<sup>T</sup>).

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**References**


