Phylogenetic relationships among marine Alteromonas-like proteobacteria: emended description of the family Alteromonadaceae and proposal of Pseudoalteromonadaceae fam. nov., Colwelliaceae fam. nov., Shewanellaceae fam. nov., Moritellaceae fam. nov., Ferrimonadaceae fam. nov., Idiomarinaceae fam. nov. and Psychromonadaceae fam. nov.

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The phylogenetic relationships among marine Alteromonas-like bacteria of the genera Alteromonas, Pseudoalteromonas, Glaciecola, Thalassomonas, Colwellia, Idiomarina, Oceanimonas, Oceanisphaera, Shewanella, Moritella, Ferrimonas, Psychromonas and several other genera of the ‘Gammaproteobacteria’ were studied. Results of 16S rRNA gene sequence analyses revealed that some members of these genera formed several coherent groups at the family level. Characteristic signature oligonucleotides for studied taxa were defined. Signature positions are divided into three classes: (i) single compensatory mutations, (ii) double compensatory mutations and (iii) mutations affecting nucleotides not paired in the secondary structure. The 16S rRNA gene sequence similarity level within genera was 93 % or above. This value can be a useful additional criterion for genus discrimination. On the basis of this work and previous polyphasic taxonomic studies, the circumscription of the family Alteromonadaceae is limited to the genera Alteromonas and Glaciecola and the creation is proposed of the families Pseudoalteromonadaceae fam. nov. to accommodate bacteria of the genera Pseudoalteromonas and Algicola gen. nov. (formerly Pseudoalteromonas bacteriolytica) and Colwelliaceae fam. nov. to accommodate bacteria of the genera Colwellia and Thalassomonas. Bacteria of the genera Oceanimonas and Oceanisphaera formed a robust cluster and shared common signature oligonucleotides. Because of deep branching and lack of association with any other genus, the following families are proposed that include single genera: Idiomarinaceae fam. nov., Psychromonadaceae fam. nov., Moritellaceae fam. nov., Ferrimonadaceae fam. nov. and Shewanellaceae fam. nov. Finally, this study also revealed that [Hyphomicrobium] indicum should be reclassified as Photobacterium indicum comb. nov.

INTRODUCTION

A large group of Gram-negative marine heterotrophic bacteria of the genera Alteromonas, Pseudoalteromonas, Glaciecola, Thalassomonas, Colwellia, Idiomarina, Shewanella, Moritella, Ferrimonas and Psychromonas that are tentatively considered as Alteromonas-like or Alteromonas-related bacteria belong to the class ‘Gammaproteobacteria’ of the recently proposed phylum Proteobacteria (Garrity & Holt,
species A. tetradoninis has been reclassified as A. haloplanktis subsp. tetradoninis (Akagawa-Matsushita et al., 1993). However, further studies showed that this species had to be retrieved, as Pseudoalteromonas tetradoninis (Ivanova et al., 2001a). In recent years, a number of novel species of marine pseudoalteromonads have been described, such as Pseudoalteromonas antarctica (Bozal et al., 1997) and Pseudoalteromonas pydzienis (Bowman, 1998), which was isolated from Antarctic coastal waters, Pseudoalteromonas bacteriolytica (Sawabe et al., 1998), which was isolated from wounded fronds of Laminaria japonica collected from the Sea of Japan, and Pseudoalteromonas peptidolytica (Venkateswaran & Dohmoto, 2000), which was isolated from sea water. The highly bioactive species Pseudoalteromonas tunicata (Holmström et al., 1998) was isolated from the ascidian Ciona intestinalis residing in coastal waters of western Sweden. More recently, several more species were proposed, including Pseudoalteromonas ulvae (Egan et al., 2001), Pseudoalteromonas issachenkooni (Ivanova et al., 2002a), Pseudoalteromonas ruthenica (Ivanova et al., 2002b), Pseudoalteromonas maricoralis, Pseudoalteromonas flavipulchra (former A. aurantica NCIMB 2033) (Ivanova et al., 2002c), Pseudoalteromonas translucida, Pseudoalteromonas paragorgicola (Ivanova et al., 2002d), Pseudoalteromonas agarivorans (Romanenko et al., 2003a) and Pseudoalteromonas phenolica (Isnansetyo & Kamei, 2003).

Bowman et al. (1998b) described a group of pigmented, psychrophilic, strictly aerobic, heterotrophic organisms isolated from sea-ice cores from eastern Antarctica that formed a distinct branch adjacent to Alteromonas. These bacteria received genus status and consisted of two species, Glaciecola punicea and Glaciecola pallidula. One more species, Glaciecola mesophila, recently enlarged the genus (Romanenko et al., 2003b).

A few years later, the aerobic marine genus Idiomarina was described, which included two species, Idiomarina abyssalis and I. zobellii (Ivanova et al., 2000a). These bacteria were isolated from sea-water samples taken from depths of 4000 and 5000 m, respectively. The species were phenotypically close to bacteria of the genera Alteromonas, Pseudoalteromonas and Marinomonas, but differed from them in their cellular fatty acid profiles and their inability to use carbohydrates as sole sources of carbon and energy. The two species were distinguished by their characteristic morphology: I. zobellii cells were fimbriated, while I. abyssalis cells were enclosed in sheaths. Recently two more species were described, Idiomarina baltica (Brettar et al., 2003) and I. lohiensis (Donachie et al., 2003).

The genus Colwellia (Deming et al., 1988) originally included two facultatively anaerobic bacteria, Colwellia psychrerythraea and C. hadaliensis. The first strains of this genus were isolated from water samples taken in the Mariana Trench and near the coast of the United States. The type strain of the species C. psychrerythraea was found to be an obligate barophile. Bowman et al. (1998a) described four novel psychrophilic species of this genus, Colwellia...
demingiae, C. hornerae, C. rossensis and C. psychrotropica, and novel strains of C. psychrerythraea. None of these Antarctic isolates were barophilic and all of them synthesized docosahexaenoic acid (22:6ω3) in amounts of up to 8% of the total cellular content of fatty acids. The type strain of the species *Colwellia maris* was originally assigned to the genus *Vibrio* and was subsequently reclassified (Yumoto et al., 1998).

Another genus, closely related to *Colwellia*, *Thalassomonas*, represented by a single species *Thalassomonas viridans*, was described to accommodate halophilic chemorganotrophic bacteria isolated from oysters cultivated off the Mediterranean coast at Valencia (Spain) (Macián et al., 2001).

Currently, the genus *Shewanella* MacDonell and Colwell 1985 comprises more than 20 species (Shewanella algae, S. amazonensis, S. baltica, S. benthica, S. colwelliana, S. denitrificans, S. fidelis, S. frigidimarina, S. gelidimarina, S. haneldai, S. japonica, S. livingstonensis, S. marinintestina, S. massilia, S. olleyma, S. oneidensis, S. pealeana, S. putrefaciens, S. saira, S. schlegeliana, S. violacea, S. waksmanii and S. woodyi). These species are Gram-negative, facultatively anaerobic and aerobic, readily cultivated gammaproteobacteria mainly associated with aquatic habitats (Jensen et al., 1980; Lee et al., 1981; Weiner et al., 1988; Coyne et al., 1989; Gauthier et al., 1995; Bowman et al., 1997; Leonardo et al., 1999; Venkateswaran et al., 1999; Ivanova et al., 2001b, 2003; Bozal et al., 2002; Satomi et al., 2003). During the last decade, bacteria of this genus have been studied extensively because of their important role in co-metabolic bioremediation of halogenated organic pollutants, destructive souring of crude petroleum and the dissipatory reduction of magnesium and iron oxides and their ability to produce high proportions of polyunsaturated fatty acids (PUFA) (Myers & Nealson, 1988; Petrovskis et al., 1994; Russell & Nichols, 1999).

The closest relatives of *Shewanella* species are bacteria of the genus *Moritella* Urakawa et al. 1998 that are represented by four species, *Moritella marina* (Urakawa et al., 1998), *M. japonica* (Nogi et al., 1998), *M. yayanosii* (Nogi & Kato, 1999) and *M. viscosa* (Benediktsdóttir et al., 2000).

Because of the initial phenotypic misclassification of [*Pseudomonas* doudoroffii as a close relative of *Aeromonas hydrophila* and *Tolunomonas auensis*, its taxonomic status and phylogenetic relationships within the ‘Gammaproteobacteria’ remained unclear until recently. Brown et al. (2001) proposed to create the genus *Oceanimonas* to accommodate the novel phenol-degrading bacterium *Oceanimonas baumannii* as well as *Pseudomonas doudoroffii* (Brown et al., 2001).

The final two genera comprise marine facultatively anaerobic and aerotolerant anaerobic gammaproteobacteria: *Ferrimonas*, represented so far by the species *Ferrimonas balearica* (Rosselló-Mora et al., 1995), and *Psychromonas*, which possesses five species *Psychromonas antarctica* (Mountfort et al., 1998), *Psychromonas kaikoae* (Nogi et al., 2002), *Psychromonas marina* (Kawasaki et al., 2002), *Psychromonas arctica* (Groudieva et al., 2003) and *Psychromonas profunda* (Xu et al., 2003).

This study is a further extension of our investigation of *Alteromonas*-like gammaproteobacteria (Ivanova & Mikhailov, 2001) and aimed to provide a basis of a delineation system based on a comprehensive overview of their phylogenetic relationships (16S rRNA gene sequences), the presence of specific compensatory mutations visible in the secondary structure of the molecule and polyphasic classification strategy. This paper mainly reviews published data with reference to the relevant publications that contain original or cited data on phenotypic and chemotaxonomic characteristics used in the identification of *Alteromonas*-like bacteria.

**METHODS**

16S rRNA gene sequence selection. The core of our analysis were the available 16S rRNA gene sequences of *Alteromonas*-like bacteria and selected relatives of the ‘Gammaproteobacteria’. Sequences were selected within a local database of 81 156 already-aligned sequences (corresponding roughly to release 74, May 2003), of which 56267 had a length of more than 500 nt and 33943 a length of more than 1000 nt. In order to derive proper phylogenetic analyses, only these longer sequences were considered. These sequences included 13780 members of the *Proteobacteria*, among which 6094 were members of the ‘Gammaproteobacteria’. Among these last sequences, about 2550 were identified at the species level (i.e. were not described as unidentified species, clones, bacteria sp., etc.). Large-scale phylogenetic analyses (neighbour-joining; NJ) using these last sequences allowed the identification of genera that had close relationships to the marine bacteria analysed (see the global tree available as Supplementary Fig. A in IJSEM Online). In the final analysis of this work (see below), we retained 24 species of *Pseudoalteromonas* (see Supplementary Fig. B), three species of *Alteromonas* (see Supplementary Fig. C), two species of *Glaciecola*, four species of *Idiomarina*, 18 species of *Shewanella*, five species of *Moritella*, seven species of *Colwellia* (see Supplementary Fig. D), four species of *Psychromonas*, *Ferrimonas balearica* and some neighbouring species; in total, 119 sequences. Selection was according to their placement in the global tree and availability of sequences for type strains. Strain numbers and their corresponding accession numbers are indicated on the phylogenetic trees.

Phylogenetic analyses. Phylogenetic dendrograms were reconstructed according to three different methods: NJ (BIONJ), maximum-likelihood (ML) (using the Global option) and maximum-parsimony (MP). For the NJ analysis, a matrix distance was calculated according to Kimura’s two-parameter correction. Bootstraps were done using 1000 replications, BIONJ and Kimura’s two-parameter correction. BIONJ was according to Gascuel (1997), ML and MP were from PHYLIP (Phylogeny Inference Package, version 3.573c; distributed by J. Felsenstein, Department of Genetics, University of Washington, Seattle, USA). Preliminary phylogenetic analyses were done using the most conserved parts of the sequences. Phylogenetic dendrograms were drawn using NIPLOT (Perrière & Gouy, 1996).

Domains used to construct final phylogenetic trees excluded positions likely to show homoplasy or that were difficult to align. When bacterial
sequences from different genera are used to determine phylogenetic relationships, domains used to construct a phylogenetic tree should be examined extremely carefully, since positions that can be properly aligned decrease and homoplasmy increases with the depth of the phylogenetic tree. For that reason, a detailed phylogenetic tree that analyses the position of a species within a genus is usually different from that used to position this genus within its class or phylum. As a result, the result presented in this paper (and the trees available as supplementary material) should be taken with caution near their leaves: the analysis has been done to position the genera in the ‘\textit{Gammaproteobacteria}’, not to position the different species within a genus. These trees define which species belong to a genus, but not, with consistency, which are sister species. For Fig. 1, the domains used corresponded to positions 95–175, 193–442, 454–819 and 845–1393 of the sequence of \textit{Aeromonas allosaccharophila} CECT 4199$^\text{T}$ (S39232). The topology shown is that of the bootstrap analysis, as it as been demonstrated that this topology is often better than that of a simple tree (Berry & Gascuel, 1996).

Operational genetic unit (OGU) analysis. We have also analysed how phylogenetic clusters could be interpreted in terms of groups of sequences sharing a fixed percentage of similarity. Similarly to OTU (operational taxonomic unit), we defined an OGU as a group of sequences in which every sequence has a similarity above a cut-off level with at least one sequence of this group and similarities under this level with every sequence not included in the group (simple linkage method). Percentages of 16S rRNA gene sequence similarities between each pair of sequences were calculated by parsing the result of a stand-alone \textsc{blast} analysis of these sequences on themselves, with the options no filter and \textit{W} = 7. All non-overlapping high-scoring-segment pairs (see \textsc{blast} documentation at NCBI) were taken into account to calculate the percentages of similarity (Supplementary Table A in IJSEM Online). Table 1 shows the numbers of OGUs obtained when the cut-off was increased, and OGUs obtained at 93 % (at which a clear plateau was obtained corresponding largely to genus delimitation) of similarity are indicated in Supplementary Fig. E. In order to perform this comparison, it is necessary to compare only sequences that overlap completely (no missing 5’ or 3’ end); for this reason, similarity values were calculated using positions 137–1335 corresponding to \textit{Aeromonas allosaccharophila} CECT 4199$^\text{T}$ (S39232).

Signature nucleotides. For each cluster revealed by the phylogenetic analysis, we have searched for single nucleotides that may be present in every sequence of a specific cluster and absent in any other sequence. This was done using an alignment of the sequences (manually checked) of the \textit{Alteromonas}-like bacteria with that of \textit{Escherichia coli}. A specific program (SeqAm; S. Flavier and R. Christen, unpublished) has been written for that purpose. Every signature nucleotide found was then positioned on the secondary structure of the 16S rRNA molecule of \textit{E. coli} (obtained from http://www.rna.icmb.utexas.edu/members/). This analysis allowed the interpretation of the signatures found in terms of single or double compensatory mutations in helices of the secondary structure. Compensatory mutations are two nucleotides that stabilize a stem in the secondary structure (such as G–C) and that are mutated (for example to C–G) in specific taxa. It must be stated that, if transitions such as G–C to A–T (through the stable intermediate G–T) are common, transversion mutations such as G–C to C–G are comparatively rare and are thus particularly strong signatures. The types of mutations (not involved in stems, simple mutations, double mutations) are indicated in Table 1.

RESULTS AND DISCUSSION

Phylogenetic analyses

Any phylogenetic method has its ‘Felsenstein’ zone, i.e. can retrieve the wrong tree (even if the bootstrap percentages are high) when the dataset does not fulfil implicit hypotheses on which the method is based. As a result, it is considered that a proper phylogenetic analysis should include the comparison of the results of different methods by combining three algorithms (NJ, MP and ML). Branches in Fig. 1 are labelled to indicate which branches were found by all analyses. Labelled branches are thus particularly ‘secure’, which does not mean that unlabelled branches are wrong, simply that we cannot be sure that they represent the evolutionary history of the gene.

Phylogenetic analyses based on 16S rRNA gene sequences

As discussed below, it would have been best to be able to compare the results of analyses using different genes. However, this was not possible as only 16S rRNA gene sequences are available for representatives of the group of \textit{bacteria} analysed (see below). Phylogenetic analyses of 16S rRNA gene sequences of the \textit{Alteromonas}-like bacteria revealed that, despite their close phenetic similarity, bacteria of the genera \textit{Alteromonas}, \textit{Pseudoalteromonas}, \textit{Glaciecola}, \textit{Thalassomonas}, \textit{Colwellia} and \textit{Idiomarina} did not form a clade but several phylogenetically distinct lineages that were consistently recovered from several phylogenetic analyses. The topology shown in Fig. 1 is that of the bootstrap tree from the NJ method. Only branches with percentages indicated should be interpreted as consistent. Some other branches were retrieved by two methods only when all sequences were used (for example \textit{Pseudoalteromonas}; see Supplementary Fig. B). This is sometimes a problem with character-based methods (ML or MP) when the number of sequences analysed approaches the number of significant characters; reducing the number of sequences analysed allowed us to find a monophyletic taxon with all three methods (Supplementary Figures B–D in IJSEM Online). Finally, phylogenetic analyses based on a single gene reveal the history of the gene, not always that of the species. 16S rRNA gene sequences are, however, peculiar as they pertain to a true multigene family; their presence usually in multiple copies and the phenomenon of gene conversion (Cilia \textit{et al.}, 1996) render them less sensitive to lateral transfer (no positive selective pressure and unlikely genetic drift) and they are thus particularly appropriate for phylogenetic analyses above the species level (Cilia \textit{et al.}, 1996). It is clear, however, that the precise position of each genus within the phylum \textit{Proteobacteria} will become clearer as more and more housekeeping gene sequences become available, a goal that has not yet been reached for \textit{Alteromonas}-like bacteria.
Table 1. Nature of signature sites detected among the various taxa

Signature sites are shown in bold; other columns indicate the nucleotide present in each OGU. OGUs are numbered as in Fig. 1: 1, *Pseudoalteromonas*; 2, *Oceanimonas*; 3, *Aeromonas*; 4, *Vibrio*; 6, *Psychromonas*; 7, *Idiomarina*; 8, *Salinivibrio* + *Vibrio aspartigenicus*; 9, *Moritella* and *Idiomarina*; 10, *Colwellia*; 10b, *Thalassomonas* + *Colwellia hornerae*; 11, 13 and 15, *Alteromonas/Glaciecola*; 12, outgroup; 16, 17 and 19, *Pseudomonas/Halomonas*. No indication is given for clusters not detailed in the manuscript. Types: 1, double compensatory mutations; 2, single compensatory mutation; 3, non-paired nucleotide. Dashes indicate deletions in some sequences at this position.

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**Housekeeping genes**

Other housekeeping genes such as *gyrB*, *rpoB* and *recA* would have been useful, but the sequences of too many genera are still missing (*gyrB*, four entries for proteobacteria; *rpoB*, 352 entries for ‘Gammaproteobacteria’ and, for the *Alteromonadales*, only five sequences of *Shewanella*, two complete, three partial 100 nt; *recA*, 546 sequences for ‘Gammaproteobacteria’, for the *Alteromonadales*, no sequence, for vibrios, 216 entries). Finally, the 16S–23S intergenic spacer region is much too divergent for use in phylogenetic analyses at the family level.

**Signature nucleotide positions**

The use of signature nucleotide positions. Signature nucleotides are nucleotide residues that are found explicitly in all currently described species of a proposed taxon and not in other taxa. Signatures sites may be particularly strong when they consist of compensatory mutations involved in maintaining the secondary structure of the molecule (see Methods). Taking into account signature sites is only an improvement in phylogenetic analyses, as no phylogenetic method can yet take into account that different positions should be weighted differently according to the selective pressure that applies at every position. It is possible to use weights, but we do not yet know how to define what weight to apply according to the position in the structure.

Taxon identification with signature positions. Confirmation of the branching order of the *Alteromonas*-like bacteria was sought by searching for signature nucleotide positions (Table 1). The signature patterns must be considered tentative since, with a significant increase in the number of sequences in any phylogenetic lineage, the number of signature nucleotides may decrease because of possible new mutations (and/or sequencing errors overlooked).

Careful examination of Table 1 suggested that some mutations were compensatory (for example, a G–C pair instead of an A–T pair). We then added the *E. coli* sequence in our alignment and mapped all of the signatures on the secondary structure of the 16S rRNA molecule. Since all *Alteromonas*-like bacteria belong to the ‘Gammaproteobacteria’ and hence are related to *E. coli*, there is little variation in the secondary structure, except for the variable parts of helices indicated with blue dashed lines on Fig. 2. This analysis showed that signature positions can be divided into three classes: (i) single compensatory mutations, for example a G–C pair mutating to G–U, (ii) double compensatory mutations (transition), for example A–T to G–C, and finally (iii) mutations affecting nucleotides not paired in the secondary structure (indicated in Table 1). Observations of single or double compensatory mutations are rather strong arguments that the signatures observed are not the result of errors in sequences. The last type of mutations is quite interesting: since these mutations are conserved for a large cluster of species, it means that there is rather strong selective pressure on these nucleotides, suggesting that they might be part of interactions involving the structures of the rRNA at the tertiary or quaternary level (within 16S rRNA or between 16S and 23S rRNA).

**Simple linkage sequence aggregations**

Grouping sequences in OGUs. Finally, we investigated a classical classification system, i.e. grouping together sequences that share a percentage of similarity above a given threshold. The main problem is to decide the cut-off level for aggregation. We found, as expected, that the sequences studied were distributed in decreasing numbers of OGUs as the cut-off percentage chosen for aggregation decreased (Supplementary Fig. E). Interestingly, a plateau was observed for 93–94 % 16S rRNA gene sequence similarity (see Methods). We then used 93 % similarity to label OGUs in the tree of Fig. 1.

**Discrimination of genera.** The results revealed that there was a good correlation at the similarity level between genera, signature sites and OGUs, though there were a few exceptions. One exception was the genus *Pseudoalteromonas* in its current state, but this problem was solved as proposed below, by creating a separate genus, *Algicola*, for [Pseudoalteromonas] *bacterioltyica*. A second exception was the genus *Glaciecola*. Bacteria of this genus shared many signature nucleotides with *Alteromonas* species. This finding might be a strong argument for the unification of these two genera. Indeed, these bacteria are close genetically (similar G+C content of the DNA) and share many phenotypic and chemotaxonomic characteristics. They were, however, distributed in different OGUs, which is an argument to keep distinct genera. *Vibrio* and *Photobacterium* were grouped in a single OGU (with the exception of ‘*Vibrio* aspartigenicus’). The taxonomic status of these genera seems far from clear, since detailed phylogenetic analyses (not shown) suggest that the two genera are in fact intermingled; a possible solution would be to reduce them to a single genus. For these taxa, data on housekeeping genes are clearly required. *Oceanimonas* and *Oceanisphaera* were in a single OGU, but we were not able to find signature nucleotides for this cluster. A close examination of the only sequences available for two of the three species suggested the presence of obvious sequencing
Fig. 2. Locations of characteristic signature nucleotides of Alteromonas-like and related taxa localized in the secondary structure of the small-subunit rRNA (E. coli). Nucleotides are colour-coded as in Fig. 1, which allows the visualization of each cluster where mutations are localized. Blue dashed lines: parts of the molecule where the secondary structure may change in the various taxa and that were not assessed for the presence of signature sites. The original cartoon was taken from http://www.rna.icmb.utexas.edu (accession no. J01695, November 1999; cosmetic changes July 2001).
errors (a different nucleotide at a position otherwise conserved in all other sequences). The availability of more and better sequences should solve this problem. Finally, *Thalassomonas* and *Colwellia* were also placed in a single OGU. Analysis of sequence signatures suggested splitting the genus *Colwellia* into two genera. A more detailed analysis of this clade is required.

**Formal rules for genus identification**

So far, there is no formal rule for delimitation of a taxon above the species level. The general position is to define a genus when there is a robust branch that clearly delineates a clade in phylogeny and when these organisms present distinct shared phenotypes. This is often problematic when members of a group have adapted to very different ecological niches, resulting in divergent phenotypes. Our analyses in terms of aggregative OGUs sharing a minimal level of 16S rRNA gene similarity, comparison with the phylogeny and sequence signatures suggest that it might be possible to use useful criteria for taxon delineation above the species level; this would extend the present criteria used at the species level: percentage of DNA–DNA association and 97 % 16S rRNA gene sequence similarity (Stackebrandt & Goebel, 1994). Aggregative clustering at the 93 % similarity level and the presence of site signatures may be used as a general criterion to help to decide at which level in a tree a genus can be defined. If such an approach was shown to be possible for the genera analysed in this study, studies of different groups (e.g. *Actinobacteria*, *Cyanobacteria*, *Archaea*) are required before we can make a definitive proposal. Presently, we were not able to use lower percentages of aggregation to try to define families, since the OGUs obtained were largely inconsistent with the phylogeny. A possible reason might be that domains that are too divergent for that level of taxonomy exist in the complete rRNA sequences. Restriction of the analysis to conserved domains might be a solution. We are presently investigating the use of more sophisticated clustering algorithms, restricting to more conserved domains as well as studying different groups of bacteria in order to propose a generalization of the procedure.

In conclusion, the following monophyletic groups can be distinguished.

**Group I, including Pseudoalteromonas species and [Pseudoalteromonas] bacteriolytica**

The *Pseudoalteromonas* cluster, encompassing more than 30 species, was relatively heterogeneous, with interstrain 16S rRNA gene sequence similarity values ranging from 90 to 99-9 % (see Supplementary Table A). The results obtained are consistent with previous detailed phylogenetic analysis (Gauthier et al., 1995) and confirmed that species of the genus consisted of several monophyletic taxa. One of them comprises a closely related group of so-called non-pigmented species (currently includes 15 species, including the type strain of the genus, *Pseudoalteromonas haloplanktis*, *Pseudoalteromonas nigrifaciens* and *Pseudoalteromonas distincta* can produce melanin-like pigments depending on culture medium) with high interstrain similarity values of 98–99-9 %. Other species in the genus are pigmented, synthesizing a variety of pigments (prodigiosin-like, carotenoids and some other pigments), and could be split into six clusters: (i) *Pseudoalteromonas citrea* and *Pseudoalteromonas aurantica*, (ii) *Pseudoalteromonas rutenica*, (iii) *Pseudoalteromonas rubra*, *Pseudoalteromonas luteoviolacea*, *Pseudoalteromonas peptidolytica*, *Pseudoalteromonas piscicida*, *Pseudoalteromonas flavipulchra* and *Pseudoalteromonas mariscaloris*, (iv) *Pseudoalteromonas tunicata* and *Pseudoalteromonas ulvae*, (v) *Pseudoalteromonas denitrificans* and (vi) *[Pseudoalteromonas] bacteriolytica* (see detailed tree; Supplementary Fig. B). This last species branched deeply and was a sister species to all other species. The deep branching (low similarity levels for nucleotides of 16S rRNA down to 90-3 %), the lack of sequence signature, the lack of association with other species of the genus, low DNA–DNA hybridization values (3–5 %) and some characteristic phenotypic traits (bacteriolytic activity, requirement for organic growth factors, different pattern of carbohydrate utilization) indicated that this bacterium should be placed in a separate genus. Therefore, we propose to create a new family, *Pseudoalteromonadaceae* fam. nov., which comprises two genera, *Pseudoalteromonas* and *Algicola* gen. nov., which contains *Algicola bacteriolytica* comb. nov. as its type species.

**Group II, including Idiomarina species, Colwellia species and Thalassomonas viridans**

Group II appeared as a clade that comprised two subclades strongly supported by bootstrap (Fig. 1 and Supplementary Fig. D): one cluster including all *Colwellia* species and *Thalassomonas viridans* and a second cluster including *Idiomarina* species. Species of the genus *Colwellia* failed to share common signature nucleotides, except when the deeply branched sequence of *Colwellia hornerae* was removed from the analysis. It is possible that some errors in sequences might be responsible for the failure to find characteristic signatures; on the other hand, the deep branching of *C. hornerae* and peculiar characteristic phenotypic traits (sensitivity to vibriostatic agent O/129, the lack of sequence signature, the lack of the ability to produce chitinase, different pattern of carbohydrate utilization) and distinct cellular fatty acid composition, e.g. significantly high proportions of 15:1(n-8), 15:0 and i16:0 fatty acids (Bowman et al., 1998a), are indications that this species may need to be recognized as representing a separate genus. A detailed study of this cluster is necessary.

Because of the weak bootstrap percentage uniting *Idiomarina* with the other genera, it should be retained as a separate taxon. In this context, we propose to create the new families *Colwelliaceae* fam. nov., restricted to bacteria of the genera *Colwellia* and *Thalassomonas*, and *Idiomarinaceae* fam. nov. Further sequences for novel species or
different genes may help to improve certainty in this part of
the tree.

**Group III, including Alteromonas and Glaciecola species**

This clade included the genus *Alteromonas* and two species of *Glaciecola*. We therefore propose to restrict the recently proposed family *Alteromonadaceae* (Ivanova & Mikhailov, 2001) to only these two genera, since the taxonomic placement of bacteria of the genera *Pseudoalteromonas*, *Idiomarina* and *Colwellia* within the family appears not to be appropriate (Fig. 1 and Supplementary Fig. C).

**Group IV, including Aeromonas, Oceanisphaera and Oceanimonas species**

*Oceanisphaera* and *Oceanimonas* species formed a loosely supported clade (only two methods) and, with *Aeromonas* species, a loosely supported clade was also revealed (two methods also, although different ones). For the reasons mentioned above, taxonomic affiliation on the family level remains unclear and the recognition of a family should await more sequences.

**Group V, including Vibrio, Salinivibrio and Photobacterium species**

These genera formed a robust cluster that constitutes the family *Vibrioaceae*. Importantly, it included the sequences of *Allomonas enterica* and *Hyphomicrobium indicum*; the grouping of the genus *Allomonas* (and also the genus *Listonella*) within the genus *Vibrio* still awaits nomenclatural clarification. Since these sequences could result from sequencing of contaminants, one should await the appearance of at least a second sequence before adjusting their taxonomy (as also suggested by Thompson et al., 2003).

**Isolated genera**

Bacteria of the four genera *Shewanella*, *Moritella*, *Psychromonas* and *Ferrimonas* (Fig. 1) did not form a monophyletic clade with other genera included in this study, at least when using 16S rRNA gene sequences as support of phylogenetic information. The *Shewanella* clade, encompassing 22 species, was rather heterogeneous, with 16S rRNA gene sequence similarity values ranging from 93 to 99.9%. Notably, a single signature nucleotide, 858 (C), was identified; this small number may result from errors in some sequences. Our results confirmed previous observations (Venkateswaran et al., 1999; Nogi & Kato, 2002) that a few monophyletic clusters are constantly recovered for *Shewanella* species.

Bacteria of the genera *Moritella*, *Psychromonas* and *Ferrimonas* clustered separately at the family level (supported by specific signature nucleotides; Table 1) and could not be grouped with any other taxa.

Thus, phylogenetic classification performed in this study was consistent with phenotypic and polyphasic classifications and has led to the grouping of these bacteria into several taxa on the family level as follows: *Alteromonadaceae* emend., including genera *Alteromonas* and *Glaciecola*, *Pseudoalteromonadaceae* fam. nov., including genera *Pseudoalteromonas* and *Algicola* gen. nov., *Colwelliaceae* fam. nov., comprising *Colwellia* and *Thalassomonas*, and the monogenic families *Idiomarinaceae* fam. nov., *Ferrimonadaceae* fam. nov., *Shewanellaceae* fam. nov. and *Psychromonadaceae* fam. nov.

The sources, habitats and differential characteristics of the proposed taxa are summarized in Table 2. Bacteria of the family *Alteromonadaceae* are aerobic, slightly halophilic organisms, distinct in the inability to hydrolyse chitin and agar, but able to utilize a range of carbohydrates. Bacteria of these genera can be distinguished by pigmented colonies, G+C content of their DNA and psychrophily. The numerous species of the family *Pseudoalteromonadaceae* are diverse in their phenotypic traits and difficult even in tentative classification. However, bacteria of the newly proposed genus *Algicola* can be easily differentiated from other pseudoalteromonads by the lack (or weak activity) of catalase, limited temperatures for growth (from 15 to 35 °C) and presence of bacteriolytic activity (Sawabe et al., 1998). Though the bacteria of the two families *Alteromonadaceae* and *Pseudoalteromonadaceae* have similar cellular fatty acid compositions, the different ratio of major cellular fatty acids allows separation at the genus level (Svetashev et al., 1995; Ivanova et al., 2000b). Bacteria of the family *Colwelliaceae* are both aerobic and facultatively anaerobic, obligatorily marine organisms that require sodium ions for growth and include pigmented and non-pigmented species; many of those hydrolyse chitin, gelatin, starch and Tween 80. Bacteria of the genus *Colwellia* are different from those of *Thalassomonas* in their ability to reduce nitrate to nitrite and psychrophily. Bacteria of the family *Idiomarinaceae* differ from *colwellias* and *thalassomonas* by the ability to tolerate high concentrations of NaCl and limited ability to utilize carbohydrates. The bacteria of the genera *Colwellia*, *Thalassomonas* and *Idiomarina* have characteristic patterns of cellular fatty acids (Bowman et al., 1997; Russell & Nichols, 1999; Macián et al., 2001). Bacteria of the families *Shewanellaceae* and *Moritellaceae* consist of both aerobic and facultatively anaerobic organisms that require sea water or sodium ions for growth and can be distinguished by halophilicity and by the ability to synthesize either eicosapentaenoic (20:5ω3) or docosahexaenoic (22:6ω3) acid, respectively.

**Emended description of Alteromonadaceae Ivanova and Mikhailov 2001**

*Alteromonadaceae* (Al.te.ro.mo.na.da´ceae. N.L. fem. n. *Alteromonas* type genus of the family; -aceae ending to denote a family; N.L. fem. pl. n. *Alteromonadaceae* the *Alteromonas* family).

Gram-negative, rod-shaped bacteria. Motile. Do not form
Table 2. Differential characteristics of marine gammaproteobacteria of the families *Alteromonadaceae*, *Pseudoalteromonadaceae*, *Colwelliaceae*, *Idiomarinaceae*, *Shewanellaceae*, *Moritellaceae*, *Ferrimonadaceae* and *Psychromonadaceae*

ND, No data available; W, weak reaction; V, reaction is different depending on strain. All taxa show polar flagellation.

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<td>Chitin</td>
<td>–</td>
<td>–</td>
<td>V</td>
<td>–</td>
<td>+</td>
<td>ND</td>
<td>V</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Agar</td>
<td>–</td>
<td>–</td>
<td>V</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td>–</td>
<td>V</td>
<td>ND</td>
</tr>
<tr>
<td>Starch</td>
<td>+</td>
<td>V</td>
<td>V</td>
<td>+</td>
<td>–</td>
<td>ND</td>
<td>V</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Gelatin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>V</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Utilization:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-Glucose</td>
<td>+</td>
<td>–</td>
<td>V</td>
<td>–</td>
<td>+</td>
<td>V</td>
<td>ND</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>D-Fructose</td>
<td>+</td>
<td>–</td>
<td>V</td>
<td>–</td>
<td>+</td>
<td>ND</td>
<td>–</td>
<td>V</td>
<td>ND</td>
</tr>
<tr>
<td>D-Mannose</td>
<td>V</td>
<td>–</td>
<td>V</td>
<td>+</td>
<td>–</td>
<td>ND</td>
<td>V</td>
<td>V</td>
<td>ND</td>
</tr>
<tr>
<td>L-Rhamnose</td>
<td>+</td>
<td>+</td>
<td>V</td>
<td>ND</td>
<td>–</td>
<td>V</td>
<td>–</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Sucrose</td>
<td>+</td>
<td>+</td>
<td>V</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>ND</td>
</tr>
<tr>
<td>Cellulose</td>
<td>+</td>
<td>+</td>
<td>V</td>
<td>–</td>
<td>–</td>
<td>V</td>
<td>ND</td>
<td>–</td>
<td>ND</td>
</tr>
<tr>
<td>Lactose</td>
<td>+</td>
<td>+</td>
<td>V</td>
<td>–</td>
<td>–</td>
<td>V</td>
<td>–</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Glycerol</td>
<td>+</td>
<td>V</td>
<td>V</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>V</td>
</tr>
<tr>
<td>Major fatty acids</td>
<td>16-0, 16:1ω7</td>
<td>18-1ω7</td>
<td>18-1ω7</td>
<td>18-1ω7</td>
<td>22-6ω3</td>
<td>17-1ω8</td>
<td>18-1ω7</td>
<td>18-1ω7</td>
<td>18-1ω7</td>
</tr>
<tr>
<td></td>
<td>16-1ω7</td>
<td>18-1ω7</td>
<td>18-1ω7</td>
<td>18-1ω7</td>
<td>22-6ω3</td>
<td>17-1ω8</td>
<td>18-1ω7</td>
<td>18-1ω7</td>
<td>18-1ω7</td>
</tr>
<tr>
<td>Known habitats§</td>
<td>S</td>
<td>S</td>
<td>S, Inv, A</td>
<td>A</td>
<td>S</td>
<td>Inv</td>
<td>S</td>
<td>S</td>
<td>B</td>
</tr>
<tr>
<td>G+C content of DNA (mol%)</td>
<td>44-48</td>
<td>40-46</td>
<td>37-50</td>
<td>44-46</td>
<td>35-46</td>
<td>48-4</td>
<td>48-50</td>
<td>54</td>
<td>39-52</td>
</tr>
</tbody>
</table>
| *Colonies on TSI medium produce a black iron precipitate.*
| †A, Aerobic; AN, anaerobic; F, facultatively anaerobic.
| §According to the original description, *Pseudoalteromonas tunicata* is facultatively anaerobic.
| §A, Algae; B, benthic sediments; Em, estuarine mud; F, fish; Inv, invertebrates; S, sea water; Si, sea ice.
endospores or microcysts. Chemo-organotrophs. Oxygen is used as the electron acceptor. Aerobic or facultatively anaerobic. Usually do not denitrify. Arginine dihydrolase is absent. Require Na⁺ ions for growth. In most species, the major isoprenoid quinone is Q8. The major fatty acids are 16:0, 16:1o7 and 16:1o7. Members of the family have been isolated from coastal, open and deep-sea waters and invertebrates from marine environments. The family is a member of the ‘Gammaproteobacteria’ with the following nucleotide sequence characteristics: 304 (A), 734 (A), 736 (T), 770 (T), 809 (A). The family comprises the type genus Alteromonas Baumann et al. 1972 emend. Gauthier et al. 1995 and the genus Glaciecola Bowman et al. 1998.

Description of Pseudoalteromonadaceae fam. nov.

Pseudoalteromonadaceae (Pseud.alter.o.mo.na.da’ce.ae. N.L. fem. n. Pseudoalteromonas type genus of the family; -aceae ending to denote a family; N.L. fem. pl. n. Pseudoalteromonadaceae the Pseudoalteromonas family).

Gram-negative, rod-shaped bacteria. Motile by means one or several flagella (sometimes coated); some species have lateral or bipolar flagella. Some species produce capsules. Chemo-organotrophs. Require Na⁺ ions for growth; some strains are capable of growing in media containing 15% NaCl. Aerobic or facultatively anaerobic. Usually do not denitrify. Arginine dihydrolase is absent. In most species, the major isoprenoid quinone is Q8. The major fatty acids are 16:0, 16:1o7 and 18:1o7. Members of the family have been isolated from coastal, open and deep-sea waters, sediments, marine invertebrates, fish and algae from marine environments. The family is a member of the ‘Gammaproteobacteria’ with the following nucleotide sequence characteristics: 733 (A), 744 (T), 833 (C), 852 (T), 853 (T). The family comprises the type genus Pseudoalteromonas Gauthier et al. 1995 and the genus Algicola gen. nov.

Description of Algicola gen. nov.

Algicola (Algi.co’la. L. n. alga -ae a seaweed; L. suff. -cola from L. n. inocula an inhabitant, dweller; N.L. fem. n. Algicola inhabitant of algae).


Description of Algicola bacteriolytica (Sawabe et al. 1998) comb. nov.


Description is identical to that given by Sawabe et al. (1998). The DNA G+C content of the type strain is 46-0 mol%. The type strain is IAM 14595T (=ATCC 700679 =CIP 105725).
(A), 830 (T), 856 (A). The type and only genus is *Idiomarina*
Ivanova et al. 2000.

**Description of Colwelliaceae fam. nov.**

*Colwelliaceae* (Colwel.li.a.ce.ae. N.L. fem. n. *Colwellia* type
genus of the family; - *aceae* ending to denote a family; N.L.
fem. pl. n. *Colwelliaceae* the *Colwellia* family).

Gram-negative, curved rod-shaped bacteria. Motile. Some
species are non-motile. Do not form endospores or micro-
cysts. Require Na\(^+\) ions for growth. Chemo-organotrophs.
Facultatively anaerobic. The major fatty acids are 15:1o8,
15:0, 16:0, 16:1o7 and 16:0. Produce PUFA. The family
is a member of the ‘*Gammaproteobacteria*’ with the follow-
ing nucleotide sequence characteristics: 579 (T), 762 (A).
Genera belonging to the family are the type genus *Colwellia*
Deming et al. 1988 and the genus *Thalassomonas* Macián
et al. 2001.

**Description of Ferrimonadaceae fam. nov.**

*Ferrimonadaceae* (Fer.ri.mo.na.da.ce.ae. N.L. fem. n.
*Ferrimonas* type genus of the family; -aceae ending to
denote a family; N.L. fem. pl. n. *Ferrimonadaceae* the
*Ferrimonas* family).

Gram-negative, rod-shaped bacteria. Motile. Do not form
endospores or microcysts. Chemo-organotrophs. Faculta-
tively anaerobic. Nitrate is reduced to nitrite. Require Na\(^+\)
ions for growth. The major fatty acids are 15:0, 16:1o9
and 17:1o9. The family is a member of the ‘*Gammaproteo-
bacteria*’. The type genus is *Ferrimonas* Rosselló-Mora et al.
1995.

**Description of Psychromonadaceae fam. nov.**

*Psychromonadaceae* (Psy.chro.mo.na.da.ce.ae. N.L. fem. n.
*Psychromonas* type genus of the family; -aceae ending to
denote a family; N.L. fem. pl. n. *Psychromonadaceae* the
*Psychromonas* family).

Gram-negative, rod- to oval-shaped bacteria. Motile. Do not
form endospores or microcysts. Chemo-organotrophs.
Aerotolerant anaerobes. Some species do not require Na\(^+\)
ions for growth. Arginine dihydrolase is absent. In most
species, the major isoprenoid quinone is Q8. The major
fatty acids are 16:0 and 16:1o7. Members of the family
have been isolated from coastal, open and deep-sea waters
and invertebrates from marine environments. The family
is a member of the ‘*Gammaproteobacteria*’ with the follow-
ing nucleotide sequence characteristics: 385 (T), 811
(A), 842 (A), 845 (T), 1336 (T). The type and only genus
is *Psychromonas* Mountfort et al. 1998.

**Description of Photobacterium indicum**

*Photobacterium indicum* (Johnson and Weisrock 1969) comb. nov.


The description is identical to that given by Johnson &
Weisrock (1969). The type strain is MBIC 3157\(^T\) [= ATCC
19614\(^T\) = DSM 5151\(^T\) = IFO (now NBRC) 14233\(^T\)].

**ACKNOWLEDGEMENTS**

The authors are grateful to the Editor Dr J. Bowman, Dr J. Euzéby and
two anonymous referees. This study was partially supported by funds
from the Australian Research Council (ARC), grant 2-2.16 from the
Ministry for Industry, Science and Technologies of the Russian
Federation, grant #02-04-49517 from the Russian Foundation for Basic
Research and grant #03-1-0-05-005 from the Far-Eastern Branch of the
Russian Academy of Sciences. This work was also supported by funds
from the European Commission for the AQUA-CHIP project (QLK4-
2000-00764). The authors are solely responsible for the content of this
publication. It does not represent the opinion of the European
Commission. The European Commission is not responsible for any use
that might be made of data appearing therein.

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Reichelt and Baumann 1973 and *Alteromonas tetraodonis* Simidu


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Antarctic species with the ability to produce eicosapentaenoic acid
(20:5o3) and grow anaerobically by dissimilatory Fe(III) reduction.

Bowman, J. P., Gosink, J. J., McCammon, S. A., Lewis, T. E., Nichols,

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