Unification of the Genera *Deleya* (Baumann et al. 1983), *Halomonas* (Vreeland et al. 1980), and *Halovibrio* (Fendrich 1988) and the Species *Paracoccus halodenitrificans* (Robinson and Gibbons 1952) into a Single Genus, *Halomonas*, and Placement of the Genus *Zymobacter* in the Family *Halomonadaceae*

S. J. DOBSON* AND P. D. FRANZMANN†

*Antarctic Cooperative Research Centre and Department of Agricultural Science,
University of Tasmania, Hobart, Tasmania 7001, Australia*

We determined the 16S rRNA sequences of the type strains of species belonging to the genera *Deleya* and *Halomonas* for which no sequence data were available previously. We also determined the 16S rRNA sequence of ACAM 21, a representative strain of a biovar of *Halomonas subglaciescola*. The members of the genera *Deleya*, *Halomonas*, and *Halovibrio* and the misnamed organism *Paracoccus halodenitrificans* formed a monophyletic group within the gamma subclass of the *Proteobacteria*. The 16S rRNA sequences of the members of this group contained all of the signature features previously identified as characteristic of the group. The frequency of occurrence of these signature features among other members of the gamma subclass has remained stable during the expansion of the database of rRNA sequences. The levels of 16S rRNA sequence similarity between members of the species belonging to the genera *Deleya*, *Halomonas*, and *Halovibrio* and the misnamed organism *P. halodenitrificans* ranged from 91.5 to 100%; however, the level of sequence similarity for members of well-resolved monophyletic subgroups which might represent separate genera was 98%. At a sequence similarity level of 98% 10 subgroups were resolved, but these groups could not be differentiated on the basis of chemotaxonomic or phenotypic characteristics. In this paper we propose that members of the genera *Deleya*, *Halomonas*, and *Halovibrio* should be placed in a single genus, the genus *Halomonas*, and we emend the description of this genus. The resulting new combinations are *Halomonas aquamarina* (*Deleya aquamarina* Akagawa and Yamasato 1989), *Halomonas variabilis* (*Halovibrio variabilis* Fendrich 1988), *Halomonas venusta* (*Deleya venusta* Baumann et al. 1983), *Halomonas cupida* (*Deleya cupida* Baumann et al. 1983), *Halomonas pacifica* (*Deleya pacifica* Baumann et al. 1983), *Halomonas marina* (*Deleya marina* Baumann et al. 1983), *Halomonas halophila* (*Deleya halophila* Quesada et al. 1984), and *Halomonas salina* (*Deleya salina* Valderrama et al. 1991). We transfer the misnamed organism *P. halodenitrificans* to the genus *Halomonas* as *Halomonas halodenitrificans* comb. nov. (*P. halodenitrificans* Robinson and Gibbons 1952). The genus *Zymobacter* is closely related to the genus *Halomonas*. While the genus *Zymobacter* can be clearly distinguished from the genus *Halomonas*, these two taxa share important genotypic, chemotaxonomic, and phenotypic characteristics. We propose that the genus *Zymobacter* should be transferred to the family *Halomonadaceae* and emend the description of the family *Halomonadaceae*. The 16S rRNA sequence of *Halomonas subglaciescola* ACAM 21 was significantly different from the 16S rRNA sequence of the type strain of *Halomonas subglaciescola* (strain ACAM 12) but was nearly identical to the 16S rRNA sequence of *Halomonas halodurans*.

Species belonging to the genera *Deleya*, *Halomonas*, and *Halovibrio* are heterotrophic, gram-negative, rod-shaped bacteria which have been isolated from seawater, estuarine water, hypersaline soils, and bodies of hypersaline water, including Antarctic lakes and the Dead Sea. These species are aerobic, and some strains have the capacity for facultative anaerobic growth in the presence of nitrate. Members of each genus grow over a wide range of levels of salinity. All of the species require salt for optimal growth and are therefore classified as halophiles according to the definition of Larsen (17). The level of salt required for optimal growth varies among species. For

example, *Deleya halophila* grows optimally in media containing 7.5% marine salts, *Deleya salina* grows optimally in media containing 5% salts, and *Halomonas meridiana* grows optimally in media containing 1 to 3% NaCl (13, 24, 31).

The family *Halomonadaceae* was created to accommodate the genera *Deleya* and *Halomonas* (11). These two genera have similar fatty acid, polar lipid, and respiratory lipoquinone profiles (10). Chemotaxonomic data indicated that the genus *Halovibrio*, which contains a single species, was related to the genera *Deleya* and *Halomonas*, and this was confirmed by the results of a comparison of 16S rRNA sequences (5). An analysis of the 16S rRNA sequences of a representative group of eight species demonstrated that the members of the genera *Deleya* and *Halomonas* do not form monophyletic groups corresponding to their generic assignments (5). Members of all three genera had several signature characteristics in their 16S rRNA sequences which were common to all of them and which defined this group of bacteria within the gamma subclass of the

* Corresponding author. Mailing address: Antarctic CRC and Department of Agricultural Science, University of Tasmania, GPO Box 252C, Hobart, Tasmania 7001, Australia. Electronic mail address: sue.dobson@agsci.utas.edu.au.

† Present address: CSIRO Division of Water Resources, Floreat Park, Western Australia 6014, Australia.

Proteobacteria. The current arrangement of species in the genera *Deleya* and *Halomonas* has no taxonomic validity.

Well-resolved groups which might form the basis of genera could not be identified on a tree derived from the 16S rRNA data. When the formation of genera was considered, the chemotaxonomic differences were too small to describe separate genera. Phenotypic characteristics which defined groups at the generic level could not be identified. As several species belonging to the genera *Deleya* and *Halomonas* were not included in this 16S rRNA study, a formal proposal for unification of these three genera was considered premature. In this study we determined the 16S rRNA sequences of the remaining species belonging to the genera *Deleya* and *Halomonas*. Mellado et al. (18) recently published the 16S rRNA sequences of two strains included in this study, *D. salina* ATCC 495509^T (T = type strain) and *Volcaniella eurihalina* ATCC 49336^T, and proposed that *V. eurihalina* should be transferred to the genus *Halomonas*. Our results and the results of Mellado et al. are compared below.

An analysis of the 16S rRNA sequence of *Paracoccus halodenitrificans* revealed that this species belongs in the family *Halomonadaceae*. However, the lack of meaningful taxonomic structure within the *Halomonadaceae* at the genus level made assignment of a genus name to the misnamed organism *Paracoccus halodenitrificans* an arbitrary and artificial exercise (19). Until the genera in this family are redefined, misnamed and newly described species will remain in a state of limbo.

Strain ACAM 21 is a representative of a biovar of *Halomonas subglaciescola* (9). The members of this biovar were distinguished from the members of the biovar containing type strain ACAM 12 by a lack of motility, a lack of peritrichous flagella, and a cell length of less than 10 µm. Other phenotypic differences between the two biovars included differences in utilization of asparagine, surface growth in thioglycolate broth, and susceptibility to penicillin G. There are significant differences in the proportions of fatty acids in strain ACAM 21 and the type strain of *Halomonas subglaciescola* strain ACAM 12. The levels of the fatty acids 16:0, 16:1 plus 17:0cyc, and 18:1 plus 19:0cyc were 25.4, 19.7, and 52.3% (wt/wt) and 32.0, 28.9, and 28.2% (wt/wt) for strains ACAM 12^T and ACAM 21, respectively, when the organisms were grown under identical conditions (10). The 16S rRNA sequence of strain ACAM 21 was determined to test the hypothesis that the biovar that contains this strain represents a species that is distinct from *Halomonas subglaciescola*.

MATERIALS AND METHODS

Microorganisms. *Halomonas halodurans* DSM 5160^T was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany. *Halomonas subglaciescola* ACAM 21, *Halomonas eurihalina* ACAM 353^T (= ATCC 49336^T), *Deleya venusta* ACAM 346^T (= DSM 4743^T), *Deleya pacifica* ACAM 345^T (= DSM 4742^T), and *Deleya cupida* ACAM 343^T (= DSM 4740^T) were obtained from the Australian Collection of Antarctic Microorganisms, University of Tasmania, Hobart, Tasmania, Australia. *D. salina* ATCC 49509^T was kindly supplied by A. Ventosa.

Halomonas subglaciescola ACAM 21 was grown in artificial organic lake water peptone broth (9). The other strains were grown in a marine broth supplemented with glucose (0.5% [wt/vol] peptone, 0.1% [wt/vol] yeast extract, and 1.0% [wt/vol] glucose in seawater that had been filtered [pore size, 0.45 µm]). All strains were grown at 25°C.

Determination of 16S rRNA sequences. The methods used to extract DNAs from the strains and to amplify the 16S rRNA gene by the PCR have been described previously (4). The sequence data for *Halomonas halodurans*, *Halomonas subglaciescola* ACAM 21, *D. venusta*, and *D. salina* were obtained by directly sequencing the isolated 16S ribosomal DNA PCR products. Most of these sequence data were obtained by manual sequencing, as described previously (4). A small amount of the sequence data for these strains was obtained by directly sequencing the isolated 16S ribosomal DNA PCR products with a PRISM Ready Reaction dideoxy termination cycle sequencing kit (Perkin Elmer); for electro-

phoresis and data collection we used an Applied Biosystems model 373A DNA sequencer (Perkin Elmer). The 16S ribosomal DNA PCR products of *D. pacifica*, *D. cupida*, and *Halomonas eurihalina* which we isolated were cloned by using the pGEM-T vector system (Promega). Plasmid DNA was isolated by using the Wizard DNA purification system (Promega). A sample of an isolated plasmid was digested with restriction enzyme *PvuII* (Promega), and the products of this digestion were electrophoresed in an agarose minigel (27) to make sure that the cloned insertion was the correct size. Plasmids containing a fragment of the correct size were sequenced by using a PRISM Ready Reaction dideoxy termination cycle sequencing kit (Perkin Elmer) and an Applied Biosystems model 373A DNA sequencer (Perkin Elmer) for electrophoresis and data collection.

16S rRNA data analysis. The 16S rRNA sequences which we obtained were manually aligned with previously determined sequences of members of the *Halomonadaceae* and some members of the gamma subclass of the *Proteobacteria*, including the nearest relatives of the *Halomonadaceae*. The strains and GenBank accession numbers for the previously determined 16S rRNA sequences are as follows (for sequences which are not present in the GenBank database the Ribosomal Database Project code is given): *Paracoccus halodenitrificans* DSM 735^T, L04942; *Halomonas halmophila* ATCC 19717^T, M59153; *Halomonas elongata* DSM 2851^T, M93355; *Halomonas subglaciescola* ACAM 12^T, M93358; *Halomonas meridiana* ACAM 246^T, M93356; *D. halophila* DSM 4770^T, M93353; *Deleya aquamarina* DSM 30161^T, M93352; *Deleya marina* DSM 4741^T, M93354; *Halovibrio variabilis* DSM 3051^T, M93357; *Chromohalobacter marismortui* ATCC 17056^T, X87219; *C. marismortui* str. A-65, X87220; *Zymobacter palmae* IAM 14233^T, D14555; *Oceanospirillum minutulum* ATCC 19193^T, osp.minutulu; *Oceanospirillum linum* ATCC 11336^T, M22365; *Marinomonas vaga* ATCC 27119^T, mrm.vaga; *Pseudomonas aeruginosa* ATCC 25330, M34133; *Pseudomonas nautica* IAM 12929, D11189, D11238, and D11287; and *Alteromonas haloplanktis* ATCC 14393^T, X67024. Most of the 16S rRNA sequences of the reference species and the previously determined sequences of members of the *Halomonadaceae* were obtained from the Ribosomal Database Project (22); the exceptions were the sequences of *C. marismortui* ATCC 17056^T and str. A-65, which were obtained from GenBank. Almost-complete sequences were available for all of the reference species except *Pseudomonas nautica*, for which only a partial sequence (440 bases) was available. Consequently, *Pseudomonas nautica* was not included in most analyses.

The sequences which we determined were examined for the presence of the 19 signature characteristics of the *Halomonadaceae* (5). Trees were constructed by using distance and parsimony methods. Programs in the PHYLIP version 3.4 package were used for the distance analysis (6). Jukes-Cantor evolutionary distances were calculated by using the DNADIST program, and a tree was constructed from these distances by using the FITCH program and the global rearrangement option. PAUP version 3.1 was used for the parsimony analysis (29). Most-parsimonious trees were obtained by the branch-and-bound method. Levels of similarity between sequences were determined from the mean distances calculated with PAUP version 3.1. A bootstrap analysis (2,000 replications) was performed for both the distance and parsimony methods. The parsimony bootstrap analysis was performed by using a heuristic search method because of computing limitations.

The database of 16S rRNA sequences continues to expand rapidly (22). A total of 38 16S rRNA sequences of organisms belonging to the gamma subclass of the *Proteobacteria* were present in the database when the 19 signature characteristics of the members of the *Halomonadaceae* were identified (5). The ribosomal database now contains 433 6S rRNA sequences of organisms belonging to this subclass. The 395 new sequences were examined for the presence of the signature characteristics of the *Halomonadaceae* to determine whether the rare occurrence of the full set of signature characteristics observed previously has been maintained. All of the 16S rRNA sequences in the database were examined to determine whether they contained the rarest of the signature characteristics, a cytosine residue at position 486 (*Escherichia coli* numbering).

Nucleotide sequence accession numbers. The sequence data determined in this study have been deposited in the GenBank database under the following accession numbers: *Halomonas subglaciescola* ACAM 21, L42614; *D. cupida* DSM 4740^T, L42615; *D. pacifica* DSM 4742^T, L42616; *D. salina* ATCC 49509^T, L42617; *D. venusta* DSM 4743^T, L42618; *Halomonas halodurans* DSM 5160^T, L42619; and *V. eurihalina* ATCC 49336^T, L42620.

RESULTS

The amounts of the 16S rRNA gene sequence determined for the seven strains which we examined ranged from 91.8 to 99.1% (average, 96.5%) in the region from position 29 to position 1521 (*E. coli* numbering). The 16S rRNA sequences of *D. cupida*, *D. pacifica*, *D. venusta*, *Halomonas halodurans*, and *Halomonas subglaciescola* ACAM 21 all contained the 19 signature features previously identified as characteristic features of the family *Halomonadaceae* in the gamma subclass of the *Proteobacteria*. Table 1 shows the levels of similarity for mem-

TABLE 1. Levels of 16S rRNA sequence similarity for members of the *Halomonadaceae* and some close relatives in the gamma subclass of the *Proteobacteria*

Organism	% 16S rRNA sequence similarity																		
	<i>Halovibrio variabilis</i>	<i>Deleya aquamaria</i>	<i>Halomonas meridiana</i>	<i>Deleya venusta</i>	<i>Halomonas subglaciexcola</i>	<i>Halomonas subglaciexcola</i>	<i>Halomonas subglaciexcola</i>	<i>Halomonas subglaciexcola</i>	<i>Halomonas subglaciexcola</i>	<i>Halomonas subglaciexcola</i>	<i>Halomonas subglaciexcola</i>	<i>Halomonas subglaciexcola</i>	<i>Halomonas subglaciexcola</i>	<i>Halomonas subglaciexcola</i>	<i>Halomonas subglaciexcola</i>	<i>Halomonas subglaciexcola</i>	<i>Halomonas subglaciexcola</i>	<i>Halomonas subglaciexcola</i>	<i>Halomonas subglaciexcola</i>
<i>Deleya aquamaria</i>	96.8																		
<i>Halomonas meridiana</i>	96.8	100.0																	
<i>Deleya venusta</i>	96.5	98.0	97.8																
<i>Halomonas subglaciexcola</i> ACAM 12 ^T	94.4	95.4	95.1	95.7															
<i>Halomonas subglaciexcola</i> ACAM 21	94.9	95.7	95.5	95.9	98.2														
<i>Halomonas halodurans</i>	95.0	96.0	95.7	96.1	98.2	99.9													
<i>Paracoccus halodenitrificans</i>	91.5	93.6	93.2	94.1	93.8	94.3	94.3												
<i>Deleya cupida</i>	92.6	93.6	93.3	94.5	94.1	93.8	93.8	94.7											
<i>Deleya pacifica</i>	93.2	94.8	94.4	95.0	94.4	94.3	94.3	94.3	95.6	96.5									
<i>Deleya marina</i>	92.3	93.6	93.4	93.7	93.1	93.6	93.7	93.1	94.3	94.3	94.9								
<i>Deleya halophila</i>	93.8	94.0	93.7	94.2	94.5	94.6	94.6	94.6	94.8	94.2	94.9	96.0							
<i>Deleya salina</i>	93.8	94.1	93.7	94.2	94.8	94.7	94.9	94.6	95.0	96.5	94.0	94.8	99.2						
<i>Halomonas eurihalina</i>	93.1	94.2	93.8	94.0	95.1	94.6	94.6	94.6	93.7	94.3	95.8	93.1	96.4	96.7					
<i>Halomonas elongata</i>	92.9	94.0	93.7	93.9	94.9	94.2	94.2	93.9	95.0	96.6	93.8	93.8	96.8	97.2	97.6				
<i>Halomonas halnophila</i>	92.2	94.0	93.5	93.7	94.8	94.3	94.3	93.9	94.2	95.5	93.2	92.5	91.9	91.2	90.3	90.7			
<i>Zymobacter palmae</i>	89.3	90.0	89.6	91.1	90.2	88.8	88.8	88.8	89.3	89.7	90.2	90.2	90.2	90.2	89.8	89.2	90.5		
<i>Oceanospirillum minutulum</i>	89.0	90.6	90.4	90.4	88.8	88.8	88.8	88.8	89.3	89.7	90.2	90.2	90.2	90.2	89.8	89.2	89.1	89.8	
<i>Oceanospirillum linum</i>	88.7	89.5	89.1	90.4	89.3	89.5	89.5	89.8	89.4	89.3	88.4	88.4	88.9	88.9	88.7	88.7	88.7	88.7	89.7
<i>Marinomonas vaga</i>	87.2	88.0	87.5	88.5	88.5	88.1	88.4	87.5	88.2	89.1	88.4	88.4	88.8	88.1	88.4	88.2	88.2	89.1	89.9
<i>Pseudomonas aeruginosa</i>	87.4	88.4	88.2	88.5	87.9	87.8	87.8	87.9	88.9	89.8	88.1	88.2	88.1	88.1	88.3	88.0	87.6	88.2	89.0
<i>Pseudomonas nautica</i>	87.6	87.2	87.8	87.8	88.3	88.1	88.1	87.2	87.8	87.4	87.4	87.4	88.5	87.0	86.9	86.7	86.1	88.7	88.7
<i>Alteromonas haloplanktis</i>	85.4	86.4	86.1	87.2	86.2	86.1	86.2	84.3	84.9	85.1	86.6	85.4	85.3	85.3	85.6	85.3	85.3	85.9	86.1
<i>Chromohalobacter marismortui</i> ATCC 17056 ^T	92.2	93.7	93.2	93.8	93.0	93.2	93.2	93.4	93.2	94.1	92.0	93.3	93.5	93.5	93.7	93.8	93.3	87.0	87.7
<i>Chromohalobacter marismortui</i> str. A-65	93.2	93.9	93.7	93.8	93.4	93.5	93.5	93.1	93.1	94.1	93.1	93.9	93.9	94.1	94.1	94.2	91.0	88.2	87.6
																	85.7	85.7	96.9

Chromohalobacter marismortui ATCC 17056^T

bers of the *Halomonadaceae* and the nearest relatives of this family.

The level of similarity of the 16S rRNA sequences of *Halomonas eurihalina* ATCC 49336^T determined by Mellado et al. (18) and in this study was 99.5%. The 0.5% difference between the two sequences represents experimental error or microheterogeneity in the 16S rRNA gene, which may be present in more than one copy in the genome, or a combination of both of these factors. The level of similarity of the 16S rDNA sequences of *D. salina* ATCC 49509^T determined by Mellado et al. (18) and in this study was 96.3%, which was much lower than the value that might be expected as a result of experimental error. A comparison of primary and secondary structural features did not indicate substantial errors in either sequence. At most points at which the sequences varied in helical sections of the secondary structure, there was a concomitant variation in the paired bases of the helix to maintain canonical base pairing. Where canonical base pairing was not maintained or where the variation occurred in a loop section, both sequence variations also occurred in other species belonging to the *Halomonadaceae*, with a few exceptions. These exceptions were a deletion at position 98, a G at position 1243, and a G at position 1286 (*E. coli* numbering) which occurred in the sequence of Mellado et al. (18) but were not present in the sequence of any other species belonging to the *Halomonadaceae*. The low level of similarity between the 16S rRNA sequences determined by Mellado et al. and by us suggests that two different strains were sequenced. Both strains belonged to the *Halomonadaceae*. The strain used in this study was closely related to *D. halophila* (level of 16S rRNA sequence similarity, 99.2%), whereas the strain used in the study of Mellado et al. (18) was equidistant from *Halomonas elongata*, *D. aquamarina*, and *D. halophila* (levels of 16S rRNA sequence similarity, 96.3, 96.2, and 96.7%, respectively). Further investigation is required to confirm which strain is the authentic type strain.

There are other differences between our 16S rRNA similarity results and those of Mellado et al. (18). The 16S rRNA similarity values obtained for *Halomonas elongata*, *Halomonas meridiana*, *Halomonas subglaciescola*, *D. aquamarina*, *D. halophila*, and *Halovibrio variabilis* in the two studies are similar, but the values obtained for *D. marina*, and *Halomonas halmophila* are significantly different. The levels of 16S rRNA similarity between *Halomonas halophila* and the six species listed above were 1.6 to 1.9% higher in our study than in the study of Mellado et al. (18). When *D. marina* was studied, the levels of 16S rRNA similarity between this species and four of the species listed above were 3.3 to 3.8% higher in this study than in the study of Mellado et al. (18) and the levels of 16S rRNA similarity between this species and the other two species were 0.8 to 1.3% lower. As the sequences which were used in the two studies were the same, the differences must have arisen from differences in the sequence alignments. All of the values given in this paper are values which were generated in this study.

The trees obtained by the distance and parsimony methods are shown in Fig. 1 and 2, respectively. *D. cupida*, *D. pacifica*, *D. venusta*, and *Halomonas halodurans* fell within the *Halomonadaceae*. *D. venusta* was closely related to *D. aquamarina*, which is consistent with DNA-rRNA hybridization data (15). *Halomonas halodurans* and strain ACAM 21 had almost identical sequences.

There were two equally most-parsimonious trees which varied only in the internal arrangement of the *Halomonas eurihalina*-*Halomonas elongata*-*Halomonas halmophila* cluster. The branching structures of the distance and parsimony trees were similar; however, several of the groups of species oc-

curred in less than 90% of the bootstrap consensus trees obtained with the two methods (Fig. 3). This indicated that there was some instability in the arrangement of the species. *Halomonas meridiana*, *Halomonas halodurans*, and *D. salina* were not included in the bootstrap analyses described in this paper since in previous analyses these species grouped with *D. aquamarina*, strain ACAM 21, and *D. salina*, respectively, in 100% of the bootstrap trees, and we believed that excluding them would not significantly alter the results. However, their exclusion did affect the result. In the previous bootstrap parsimony analyses the group containing *Halovibrio variabilis*, *D. aquamarina*, *Halomonas meridiana*, *D. venusta*, *Halomonas subglaciescola* ACAM 12^T, strain ACAM21, and *Halomonas halodurans* appeared less frequently, occurring in 66% of the bootstrap trees (not shown).

The frequency of the signature characteristics of members of the *Halomonadaceae* in the 433 16S rRNA sequences of members of the gamma subclass of the *Proteobacteria* currently available was similar to the frequency which occurred in the 38 sequences available 3 years ago (Table 2). Four of the signature characteristics are relatively common and occur in 18 to 24% of 16S rRNA sequences of members of the gamma subclass; however, only 4.6% of the sequences contained 4 of the 19 signature characteristics, illustrating that in combination the signature characteristics provide an extremely effective way to describe the *Halomonadaceae* (Table 3). Only 3.8% of the sequences contained between 7 and 10 of the signature characteristics, and there was only one species, *Z. palmae*, which contained more than 10 (this species contained 15 of the signature characteristics).

The rarest signature characteristic is a cytosine residue at position 486. When all available sequences were examined 3 years ago, a C occurred at position 486 in only one other prokaryote, *Listonella anguillarum* (5). An examination of all prokaryotic sequences currently available revealed a C at position 486 in only the following six sequences: *Listonella anguillarum*, *Legionella hackeliae*, *Z. palmae*, *Isosphaera pallida*, *Magnetobacterium bavaricum*, and *Staphylococcus schleiferi* subsp. *schleiferi* sequences. The last three species do not belong to the gamma subclass of the *Proteobacteria*. The 16S rRNA sequence of *Legionella hackeliae* contains only two other signature characteristics associated with the members of the *Halomonadaceae*, and the 16S rRNA sequence of *Listonella anguillarum* contains none of the other signature characteristics, although only a partial sequence is available for this species. *Z. palmae* is the only one of the six species which exhibits a significant level of similarity to members of the *Halomonadaceae* in terms of signature characteristics.

DISCUSSION

The genus *Halomonas* was described to accommodate a group of moderately halophilic strains isolated from a saltern (33). In the initial study of the strains assigned to the genus *Halomonas*, the researchers did not compare them with the marine *Alcaligenes* strains which later formed the genus *Deleya*. Similarly, in the studies which were the basis of the description of the genus *Deleya* the researchers did not compare the *Deleya* strains with *Halomonas elongata* which at that time was the sole species in the genus *Halomonas* (3). Following the initial descriptions of these genera, additional new species were assigned to them, primarily on the basis of phenotypic characteristics and DNA G+C contents. Creation of the genus *Halovibrio* was based on phenotypic distinction from previously described genera, including the genus *Halomonas* (7).

The species belonging to the genera *Deleya*, *Halomonas*, and

FIG. 1. Distance tree containing the members of the genera *Deleya*, *Halomonas*, and *Halovibrio*, the misnamed organism *Paracoccus halodenitrificans*, and some members of the gamma subclass of the *Proteobacteria*. Bar = 0.01 distance calculated by the Jukes-Cantor equation. a, b, and c are short internodes connecting the main branches of the monophyletic group containing the members of the genera *Deleya*, *Halomonas*, and *Halovibrio*. Abbreviations: D., *Deleya*; H., *Halomonas*; Hv., *Halovibrio*; Z., *Zymobacter*; O., *Oceanospirillum*; Ps., *Pseudomonas*; M., *Marinomonas*; A., *Alteromonas*; P., *Paracoccus*.

Halovibrio and the misclassified organism *Paracoccus halodenitrificans* form a coherent monophyletic group in trees derived from 16S rRNA sequence comparisons. This is evident not only from the levels of similarity obtained for the members of this group when they are compared with their nearest relatives, *Z. palmae* and *O. minutulum*, but also from the extensive set of signature characteristics associated with this group of species.

We needed to consider whether there are monophyletic subgroups within the set of species belonging to the genera *Deleya*, *Halomonas*, and *Halovibrio* and *Paracoccus halodenitrificans* which might deserve recognition as separate genera. The description of a genus requires not only that a group of species be phylogenetically coherent as inferred from genotypic data but that there be chemotaxonomic or phenotypic features characteristic of the group which differentiate it from closely related genera. As the Ad Hoc Committee on Approaches to Taxonomy within the *Proteobacteria* states, "The first step in the identification of bacteria is the assignment of organisms to genera. It is completely impracticable to define genera solely on the basis of phylogenetic data" (20).

There is a level of DNA-DNA hybridization (70%) which is widely accepted as a basis for defining bacterial species (34), but no level of 16S rRNA sequence similarity has been arrived at for defining bacterial genera. This is because the ages of

genera over the full breadth of evolutionary history are different and a single figure is not relevant for such a diverse range of organisms (28). It is also because of the practical requirement for data other than genotypic data to define genera. When the species belonging to the *Halomonadaceae* on the distance tree (Fig. 1) were examined, 10 distinct groups formed at a level of 16S rRNA similarity of 98.0%. Five of these groups each contained a single species. The groups contained the following organisms: group 1, *Halovibrio variabilis*; group 2, *D. aquamarina*, *Halomonas meridiana*, and *D. venusta*; group 3, *Halomonas subglaciescola*, strain ACAM 21, and *Halomonas halodurans*; group 4, *Paracoccus halodenitrificans*; group 5, *D. cupida*; group 6, *D. pacifica*; group 7, *D. marina*; group 8, *D. halophila* and *D. salina*; group 9, *Halomonas eurihalina* and *Halomonas elongata*; and group 10, *Halomonas halophila*. At a level of similarity of 97% the separation of some species into distinct groups is not clear-cut. Groups 8, 9, and 10 begin to merge as the level of similarity between *Halomonas elongata* and *D. salina* is 97.2%; however, the levels of similarity among this group of five species are as low as 95.7%. In addition, the level of similarity between *D. aquamarina* and *Halovibrio variabilis* is 96.8%. It is unlikely that a difference of 0.2% is meaningful simply because a value falls below the 97.0% level. At lower levels of similarity the formation of distinct groups be-

FIG. 2. Most-parsimonious tree containing the members of the genera *Deleya*, *Halomonas*, and *Halovibrio*, the misnamed organism *Paracoccus halodenitrificans*, and some members of the gamma subclass of the *Proteobacteria*. Bar = 50 nucleotides. A second equally most-parsimonious tree differed from the one shown only in the arrangement of species in the *Halomonas eurihalina*-*Halomonas elongata*-*Halomonas halmophila* cluster. For abbreviations see the legend to Fig. 1.

comes even less clear. At a level of similarity of 96% groups 5 and 6 merge and group 6 merges with group 8. *D. pacifica* is equidistant between *D. cupida* and *D. salina* at a level of similarity of 96.5%. However, the level of similarity between *D. salina* and *Halomonas eurihalina* is 96.7%, but the level of similarity between *Halomonas eurihalina* and *D. cupida* is only 94.3%.

TABLE 2. Frequency of occurrence of signature characteristics of members of the genera *Deleya*, *Halomonas*, and *Halovibrio* and the misnamed organism *Paracoccus halodenitrificans* in other members of the gamma subclass of the *Proteobacteria*

Position(s)	Sequence or nucleotide	Frequency (%) in ^a :	
		1995	1992
76-93	6-bp stem	7.4	10.5
484	A	3.2	2.6
486	C	0.5	0
640	G	7.6	7.9
660 ^b	A-	9.0	2.6
745	U	20.6	18.4
668-	A-	11.3	13.2
738	U	10.6	13.2
669-	A-	13.4	15.8
737	U	13.4	15.8
776	U	11.8	2.6
1124	U	19.2	7.9
1297	U	23.6	18.4
1298	C	3.5	10.5
1423	A	3.0	10.5
1424	C	18.2	13.2
1439	U	2.3	5.3
1462	A	2.1	5.3
1464	C	4.8	5.3

^a In 1995, 433 sequences of members of the gamma subclass of the *Proteobacteria* (other than members of the genera *Deleya*, *Halomonas*, and *Halovibrio* and the misnamed organism *Paracoccus halodenitrificans*) were available from the Ribosomal Database Project when the manuscript was prepared. In 1992, 38 such sequences were available.

^b The dash indicates base pairing with the next nucleotide listed.

When the overall arrangement of species within the *Halomonadaceae* is considered, it appears that there are three subgroups containing the following organisms: subgroup i, *Halomonas halmophila*, *Halomonas elongata*, *Halomonas eurihalina*, *D. salina*, *D. halophila*, *D. pacifica*, *D. cupida*, and *Paracoccus halodenitrificans*; subgroup ii, *Halomonas subglaciescola* ACAM 21 and ACAM 12^T, *Halomonas halodurans*, *D. venusta*, *D.*

FIG. 3. Bootstrap consensus trees derived by parsimony (a) and distance (b) methods. The numbers shown are the percentages of groups which occurred on bootstrap trees (2,000 replications). For abbreviations see the legend to Fig. 1.

TABLE 3. Percentages of members of the gamma subclass of the *Proteobacteria* that contain different numbers of the signature characteristics of the group containing species belonging to the genera *Deleya*, *Halomonas*, and *Halovibrio* and the misnamed organism *Paracoccus halodenitrificans*

No. of signature characteristics in the 16S rRNA sequence	% of members of the gamma subclass that contain this no. of signature characteristics ^a
1.....	25
2.....	21
3.....	8.3
4.....	4.6
5.....	4.8
6.....	3.0
7.....	0.9
8.....	1.2
9.....	0.7
10.....	0.7
11.....	0
12.....	0
13.....	0
14.....	0
15.....	0.2
16.....	0
17.....	0
18.....	0
19.....	0

^a Excluding members of the genera *Deleya*, *Halomonas*, and *Halovibrio* and the misnamed organism *Paracoccus halodenitrificans*, all of which contain the 19 signature characteristics.

aquamarina, *Halomonas meridiana*, and *Halovibrio variabilis*; and subgroup iii, *D. marina*. However, in this arrangement there are some species which are as closely related to species belonging to other subgroups as they are to members of their own subgroup. For example, *D. halophila* is as closely related to *D. marina* as it is to *D. cupida*. Levels of similarity for species belonging to subgroup i are as low as 93.8%, and levels of similarity for species belonging to subgroup ii are as low as 94.4%, whereas some intersubgroup levels of similarity are higher than these values. For example, the levels of similarity between *D. venusta* and *D. pacifica* and between *D. marina* and *D. pacifica* are 95.0 and 94.9%, respectively. Several groups of species in the bootstrap consensus tree derived from the distance analysis occurred on less than 75% of the trees, indicating that there was some instability in the arrangement of species in the trees. The lack of resolution of subgroups within this set of species (except at high levels of sequence similarity) is associated with short internodal distances between the junction of the three subgroups and the branch points of species within the subgroups (Fig. 1, internodes a through c).

All species belonging to the genera *Deleya*, *Halomonas*, and *Halovibrio* and the misnamed organism *Paracoccus halodenitrificans* contain the same major fatty acids (10, 30). While the proportions of these fatty acids vary among species, the differences among some members of different subgroups are too small to be useful for delineating genera (5). When differences are so small, it is not possible to accurately predict the subgroup to which an unknown organism may belong on the basis of its fatty acid profile. For example, *D. cupida* and *D. pacifica* are in different subgroups (level of 16S rRNA sequence similarity; 96.5%), and the differences in the proportions of the major fatty acids 16:0, 16:1 plus 17cyc, and 18:1 plus 19cyc of these organisms are 3.6, 1.9, and 0.4%, respectively. *D. venusta*

and *D. aquamarina* are in the same subgroup (level of 16S rRNA sequence similarity, 98.0%), and the differences in the proportions of the major fatty acids 16:0, 16:1 plus 17cyc, and 18:1 plus 19cyc are 1.5, 2.3, and 2.1%, respectively. *D. aquamarina* and *Halomonas meridiana* are members of the same rRNA species complex and exhibit 100% 16S rRNA sequence similarity; the differences in the proportions of fatty acids 16:0, 16:1 plus 17cyc, and 18:1 plus 19cyc in these organisms are similar to the differences in the proportions found in inter- and intrasubgroup comparisons (1.9, 0.7, and 2.7%, respectively).

The genus *Volcaniella* was created on the basis of phenotypic characteristics, including morphology, motility, oxidase test results, the ability to produce acid from glucose and other carbohydrates, and the ability to grow on different substrates as sole sources of carbon, nitrogen, and energy (23). The high level of 16S rRNA similarity between *Halomonas eurihalina* and *Halomonas elongata* (99.1%), which resulted in the transfer of *V. eurihalina* to the genus *Halomonas* (18), illustrates that these phenotypic characteristics can no longer be considered appropriate markers to describe genera in this group of organisms. *Halovibrio variabilis* has a vibroid morphology, and yet it forms a monophyletic group with *D. aquamarina*, *Halomonas meridiana*, and *D. venusta*. *Halovibrio variabilis* grows optimally in media containing 9.4% NaCl, whereas *Halomonas meridiana* grows optimally in media containing 1 to 3% NaCl. While many phenotypic characteristics are not useful for describing genera in this group, they are very useful for describing and differentiating species.

The species which currently belong to the genera *Deleya*, *Halomonas*, and *Halovibrio* and the misnamed organism *Paracoccus halodenitrificans* form a well-defined monophyletic group containing 10 well-resolved subgroups at a level of 16S rRNA sequence similarity of 98.0%; however, resolution of the subgroups is lost at lower levels of similarity. These 10 subgroups cannot be distinguished on the basis of their fatty acid or polar lipid profiles, and all members of all subgroups contain ubiquinone 9 as their major respiratory lipoquinone. The phenotypic characteristics used to define genera within this group of species have consistently failed to delineate phylogenetically meaningful taxa. As we cannot identify chemotaxonomic and phenotypic characteristics that distinguish the genotypic subgroups, these organisms must be included in a single genus.

At some point in the future it might be possible to identify chemotaxonomic or phenotypic characteristics which can be used to differentiate the 10 genotypic subgroups; however, this possibility does not justify perpetuation of the current confusing taxonomy. According to Rule 44 of the *International Code of Nomenclature of Bacteria* (16), when genera are unified, the genus name used should be the name associated with the type species having the earliest legitimate name. Consequently, we propose that the genera *Deleya*, *Halomonas*, and *Halovibrio* should be united in a single genus, the genus *Halomonas*, and that *Paracoccus halodenitrificans* should be transferred to the genus *Halomonas*. The new name combinations are as follows: *Halomonas aquamarina* (*Deleya aquamarina* Akagawa and Yamamoto 1989), *Halomonas variabilis* (*Halovibrio variabilis* Fendrich 1988), *Halomonas venusta* (*Deleya venusta* Baumann et al. 1983), *Halomonas halodenitrificans* (*Paracoccus halodenitrificans* Robinson and Gibbons 1952), *Halomonas cupida* (*Deleya cupida* Baumann et al. 1983), *Halomonas pacifica* (*Deleya pacifica* Baumann et al. 1983), *Halomonas marina* (*Deleya marina* Baumann et al. 1983), *Halomonas halophila* (*Deleya halophila* Quesada et al. 1984), and *Halomonas salina* (*Deleya salina* Valderrama et al. 1991). The species descriptions are unchanged. An emended description of the genus *Halomonas* is given below.

A total of 19 signature characteristics were previously associated with the monophyletic group of organisms now proposed as members of the genus *Halomonas*. The frequency of these signature characteristics among the other members of the gamma subclass of the *Proteobacteria* is similar to the frequency found when the signatures were identified by using a much smaller database of sequences. It might be possible to create a more stringent set of signature characteristics if the four characteristics which occur most frequently (at positions 745, 1124, 1297, and 1424 [*E. coli* numbering] [Table 2]) were not included. We do not recommend this, however, because at any time during the development of the database the frequency of occurrence of particular signature characteristics will be biased by the inclusion of some taxa for which there are many sequences and other taxa for which there may be a single sequence present.

Mellado et al. (18) determined the 16S rRNA sequences of two strains of *C. marismortui*. The levels of 16S rRNA sequence similarity between these two strains and the members of the genus *Halomonas* range from 92.0 to 94.2% (Table 1). The G+C contents of the DNAs of the *C. marismortui* strains were 62 to 65 mol%, values which are within the range of values for the genus *Halomonas*. *C. marismortui* contained the 19 16S rRNA signature characteristics associated with the genus *Halomonas* (18). Mellado et al. (18) concluded that there are enough phenotypic differences between *C. marismortui* and the "*Halomonas-Deleya* complex" to warrant classification of *C. marismortui* as a member of a separate genus. The genus *Chromohalobacter* can be distinguished from the genus *Halomonas* by the presence of yellow and blue pigments (32). When other phenotypic traits were examined, *C. marismortui* was quite similar to the members of the genus *Halomonas* (including members of the genus *Deleya*) with which it was compared. *C. marismortui* was differentiated from *D. cupida* only by differences in the ability to grow on some substrates as sole sources of carbon, nitrogen, and energy (32). The recently described genus *Pseudoalteromonas* includes both pigmented and nonpigmented species (12). *Halomonas subglaciescola* has been observed to form yellow colonies when it is grown on media containing alanine and (in the case of some strains) hydroxy-L-proline (9). A decision concerning whether *C. marismortui* represents a separate genus within the *Halomonadaceae* should await further data, including the determination of its lipid profile and identification of its major respiratory lipoquinone(s).

The genus *Zymobacter*, which contains a single species, *Z. palmae*, is the closest relative of the genera *Halomonas* and *Chromohalobacter*. The levels of 16S rRNA sequence similarity between *Z. palmae* and members of the genus *Halomonas* range from 89.3 to 92.5% (average, 90.8%). The 16S rRNA sequence of *Z. palmae* contains 15 of the 19 signature characteristics which have been associated with the genus *Halomonas*, including the rare cytosine residue at position 486. *Z. palmae* is fermentative, producing ethanol from sugars, and was isolated from palm sap (21). The G+C content of its DNA is 55.8 mol%, and it contains ubiquinone 9. The major elements of its fatty acid profile are similar to those of the members of the genus *Halomonas*; however, the levels of 16:0 in the profiles of the members of the genus *Halomonas* range from 15.5 to 32.0%, whereas the level in *Z. palmae* is 51%, and *Z. palmae* contains <0.5% 16:1, whereas this fatty acid is present at levels ranging from 2.1 to 32.0% in members of the genus *Halomonas*. The latter comparison is more distinct if the proportions of 16:1 and 17cyc are added together. Conversion of a monounsaturated fatty acid to its cyclopropane fatty acid derivative is sensitive to various culture conditions. It is

thought that adding the two moieties provides a more stable characteristic (14). The fatty acid profile of *Z. palmae* contains <0.5% 16:1 plus 17cyc, while the fatty acid profiles of the members of the genus *Halomonas* contain 7.7 to 43.1% 16:1 plus 17cyc. While *Z. palmae* was not isolated from a saline environment, it is highly osmotolerant and can grow in the presence of 50% (wt/vol) maltose (21). The genus *Zymobacter* is clearly distinct from the genus *Halomonas*, but the level of similarity between these two genera indicates that they belong to the same family. Therefore, we propose that the genus *Zymobacter* should be included in the family *Halomonadaceae*, and the description of the family *Halomonadaceae* is emended accordingly below. The 15 signature characteristics common to both genera are retained as descriptive characteristics of members of the family *Halomonadaceae*, and the remaining four signature characteristics are included in the description of the genus *Halomonas* within the *Halomonadaceae*.

The closest relative of the genus *Halomonas* after *Z. palmae* is *O. minutulum*. The levels of 16S rRNA sequence similarity between *O. minutulum* and the members of the genus *Halomonas* range from 88.8 to 90.6% (average, 89.4%). The level of 16S rRNA similarity between *O. minutulum* and *Z. palmae* is 89.8%. *O. minutulum* contains only 10 of the signature characteristics associated with the *Halomonadaceae* and does not contain the rare cytosine residue at position 486. The major respiratory lipoquinone in *O. minutulum* is ubiquinone 8, and *O. minutulum* contains 3-OH 14:1 in its fatty acid profile, which has not been found in any species belonging to the genus *Halomonas* or in *Z. palmae* (26). The chemotaxonomic and genotypic differences between *O. minutulum* and the genera *Halomonas* and *Zymobacter* indicate that *O. minutulum* should not be transferred to the family *Halomonadaceae*.

The results of comparisons of levels of 16S rRNA sequence similarity and DNA-DNA hybridization values for some *Fibrobacter* strains and some *Bacillus* strains suggested that it was unlikely that two strains that exhibited less than 99% 16S rRNA sequence similarity would have a DNA-DNA hybridization value of 70% or greater in these groups (2, 8). Data for members of the *Halomonadaceae* are limited. The level of 16S rRNA similarity between *Halomonas eurihalina* and *Halomonas elongata* is 99.1%, and the level of DNA-DNA hybridization of these taxa is 7% (31). The level of 16S rRNA similarity between *Halomonas subglaciescola* ACAM 21 and ACAM 12^T was 98.2%. On the basis of this value, the phenotypic differences between strain ACAM 21 and *Halomonas subglaciescola* ACAM 12^T, and differences in the proportions of the major fatty acids of these organisms, it is doubtful that ACAM 21 is a member of *Halomonas subglaciescola*. DNA-DNA hybridization data will be required to confirm this hypothesis. The 16S rRNA sequences of strain ACAM 21 and *Halomonas halodurans* were almost identical. In the terminology of Fox et al. (8), these strains belong to the same rRNA species complex or rRNA superspecies. Determining whether strain ACAM 21 is a member of *Halomonas halodurans* will require determining the level of DNA-DNA hybridization between the two organisms.

Emended description of the genus *Halomonas* Vreeland, Litchfield, Martin, and Elliot 1980. Gram-negative, straight or curved, rod-shaped cells. Slight or moderate halophiles; optimal growth occurs in the presence of 0.3 to 2.0 M NaCl. May tolerate NaCl concentrations up to 5.5 M. Aerobic, but some strains are capable of anaerobic growth in the presence of nitrate. Motile by means of peritrichous or polar flagella or nonmotile. Chemoorganotrophic. The major respiratory lipoquinone is ubiquinone 9, and the major fatty acids are 16:1 plus 17:0cyc, 16:0, and 18:1 plus 19:0cyc. The 16S rRNA sequences

of members of this genus contain four signature characteristics (C at position 1424, U at position 1439, A at position 1462, C at position 1464 [*E. coli* numbering]) in addition to the 15 signature characteristics associated with the family *Halomonadaceae*.

Emendation of the description of the family *Halomonadaceae* Franzmann, Wehmeyer, and Stackebrandt 1988. Gram-negative, straight or curved, rod-shaped cells. Members are either slight or moderate halophiles or osmotolerant, growing in the presence of high concentrations of sugars. Aerobic or facultatively anaerobic. The major respiratory lipoquinone is ubiquinone 9, and the major fatty acids are 18:1 plus 19:0cyc and 16:0. The family is a member of the gamma subclass of the *Proteobacteria*, and its members have 15 signature characteristics in their 16S rRNA sequences, including a very rare cytosine residue at position 486. The 15 signature characteristics are as follows: a 6-bp stem at positions 76 to 93, A at position 484, C at position 486, G at position 640, A at position 660, A at position 668, A at position 669, U at position 737, U at position 738, U at position 745, U at position 776, U at position 1124, U at position 1297, C at position 1298, and A at position 1423 (*E. coli* numbering). The family comprises three genera, *Halomonas*, *Chromohalobacter*, and *Zymobacter*.

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REFERENCES

- Akagawa, M., and K. Yamasato. 1989. Synonymy of *Alcaligenes aquamarinus*, *Alcaligenes faecalis* subsp. *homari*, and *Deleya aesta*: *Deleya aquamarina* comb. nov. as the type species of the genus *Deleya*. Int. J. Syst. Bacteriol. **39**: 462–466.
- Amann, R. L., C. Lin, R. Key, L. Montgomery, and D. Stahl. 1992. Diversity among *Fibrobacter* isolates: towards a phylogenetic classification. Syst. Appl. Microbiol. **15**:23–31.
- Baumann, L., R. D. Bowditch, and P. Baumann. 1983. Description of *Deleya* gen. nov. created to accommodate the marine species *Alcaligenes aestus*, *A. pacificus*, *A. cupidus*, *A. venustus*, and *Pseudomonas marina*. Int. J. St. Bacteriol. **33**:793–802.
- Dobson, S. J., R. R. Colwell, T. A. McMeekin, and P. D. Franzmann. 1993. Direct sequencing of the polymerase chain reaction-amplified 16S rRNA gene of *Flavobacterium gondwanense* sp. nov. and *Flavobacterium salegens* sp. nov., two new species from a hypersaline Antarctic lake. Int. J. Syst. Bacteriol. **43**:77–83.
- Dobson, S. J., T. A. McMeekin, and P. D. Franzmann. 1993. Phylogenetic relationships between some members of the genera *Deleya*, *Halomonas*, and *Halovibrio*. Int. J. Syst. Bacteriol. **43**:665–673.
- Felsenstein, J. 1989. PHYLIP—phylogeny inference package (version 3.2). Cladistics **5**:164–166.
- Fendrich, C. 1988. *Halovibrio variabilis* gen. nov., *Pseudomonas halophila* sp. nov. and a new halophilic aerobic coccoid eubacterium from Great Salt Lake, Utah, USA. Syst. Appl. Microbiol. **11**:36–43.
- Fox, G. E., J. D. Wisotzky, and P. Jurtschuk, Jr. 1992. How close is close: 16S rRNA sequence identity may not be sufficient to guarantee species identity. Int. J. Syst. Bacteriol. **42**:166–170.
- Franzmann, P. D., H. R. Burton, and T. A. McMeekin. 1987. *Halomonas subglaciescola*, a new species of halotolerant bacteria isolated from Antarctica. Int. J. Syst. Bacteriol. **37**:27–34.
- Franzmann, P. D., and B. J. Tindall. 1990. A chemotaxonomic study of members of the family *Halomonadaceae*. Syst. Appl. Microbiol. **13**:142–147.
- Franzmann, P. D., U. Wehmeyer, and E. Stackebrandt. 1989. *Halomonadaceae* fam. nov., a new family of the class *Proteobacteria* to accommodate the genera *Halomonas* and *Deleya*. Syst. Appl. Microbiol. **11**:16–19.
- Gauthier, G., M. Gauthier, and R. Christen. 1995. Phylogenetic analysis of the genera *Alteromonas*, *Shewanella*, and *Moritella* using genes coding for small-subunit rRNA sequences and division of the genus *Alteromonas* into two genera, *Alteromonas* (emended) and *Pseudoalteromonas* gen. nov., and proposal of twelve new species combinations. Int. J. Syst. Bacteriol. **45**: 755–761.
- James, S. R., S. J. Dobson, P. D. Franzmann, and T. A. McMeekin. 1990. *Halomonas meridiana*, a new species of extremely halotolerant bacteria isolated from Antarctic saline lakes. Syst. Appl. Microbiol. **13**:270–278.
- Jantzen, E., and K. Bryn. 1985. Whole-cell and lipopolysaccharide fatty acids and sugars of Gram-negative bacteria, p. 145–172. In M. Goodfellow and D. E. Minnikin (ed.), Chemical methods in bacterial systematics. Academic Press, Inc., London.
- Kerstens, K. 1992. The genus *Deleya*, p. 3189–3197. In A. Balows, H. G. Trüper, M. Dworkin, W. Harder, and K.-H. Schleifer (ed.), The prokaryotes. A handbook on the biology of bacteria: ecophysiology, isolation, identification, applications, 2nd ed., vol. 4. Springer-Verlag, New York.
- Lapage, S. P., P. H. A. Sneath, E. F. Lessel, V. B. D. Skerman, H. P. R. Seeliger, and W. A. Clarke (ed.). 1992. International code of nomenclature of bacteria. 1990 revision. American Society for Microbiology, Washington, D.C.
- Larsen, H. 1986. Halophilic and halotolerant microorganisms—an overview and historical perspective. FEMS Microbiol. Rev. **39**:3–7.
- Mellado, E., E. R. B. Moore, J. J. Nieto, and A. Ventosa. 1995. Phylogenetic inferences and taxonomic consequences of 16S ribosomal DNA sequence comparison of *Chromohalobacter marismortui*, *Volcaniella eurihalina*, and *Deleya salina* and reclassification of *V. eurihalina* as *Halomonas eurihalina* comb. nov. Int. J. Syst. Bacteriol. **45**:712–716.
- Miller, J. M., S. J. Dobson, P. D. Franzmann, and T. A. McMeekin. 1994. Reevaluating the classification of *Paracoccus halodentificans* with sequence comparisons of 16S ribosomal DNA. Int. J. Syst. Bacteriol. **44**:360–361.
- Murray, R. G. E., D. J. Brenner, R. R. Colwell, P. De Vos, M. Goodfellow, P. A. D. Grimont, N. Pfennig, E. Stackebrandt, and G. A. Zavarin. 1990. Report of the Ad Hoc Committee on Approaches to Taxonomy within the *Proteobacteria*. Int. J. Syst. Bacteriol. **40**:213–215.
- Okamoto, T., H. Taguchi, K. Nakamura, H. Ikenaga, H. Kuraishi, and K. Yamasato. 1993. *Zymobacter palmae* gen. nov., sp. nov., a new ethanol-fermenting peritrichous bacterium isolated from palm sap. Arch. Microbiol. **160**:333–337.
- Olsen, G. J., N. Larsen, and C. R. Woese. 1991. The Ribosomal RNA Database Project. Nucleic Acids Res. **19**(Suppl.):2017–2021.
- Quesada, E., M. J. Valderrama, V. Bejar, A. Ventosa, M. C. Gutierrez, F. Ruiz-Berraquero, and A. Ramos-Cormenzana. 1990. *Volcaniella eurihalina* gen. nov., sp. nov., a moderately halophilic nonmotile gram-negative rod. Int. J. Syst. Bacteriol. **40**:261–267.
- Quesada, E., A. Ventosa, F. Ruiz-Berraquero, and A. Ramos-Cormenzana. 1984. *Deleya halophila*, a new species of moderately halophilic bacteria. Int. J. Syst. Bacteriol. **34**:287–292.
- Robinson, J., and N. E. Gibbons. 1952. The effects of salts on the growth of *Micrococcus halodentificans* n. sp. Can. J. Bot. **30**:147–154.
- Sakane, T., and A. Yokota. 1994. Chemotaxonomic investigation of heterotrophic, aerobic and microaerophilic spirilla, the genera *Aquaspirillum*, *Magnetospirillum* and *Oceanospirillum*. Syst. Appl. Microbiol. **17**:128–134.
- Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
- Stackebrandt, E. 1992. Unifying phylogeny and phenotypic diversity, p. 19–47. In A. Balows, H. G. Trüper, M. Dworkin, W. Harder, and K.-H. Schleifer (ed.), The prokaryotes. A handbook on the biology of bacteria: ecophysiology, isolation, identification, applications, 2nd ed., vol. 1. Springer-Verlag, New York.
- Swofford, D. L. 1990. PAUP: phylogenetic analysis using parsimony, version 3.1. Illinois Natural History Survey, Champaign.
- Urakami, T., H. Araki, H. Oyanagi, K.-I. Suzuki, and K. Komagata. 1990. *Paracoccus aminophilus* sp. nov. and *Paracoccus aminovorans* sp. nov., which utilize *N,N*-dimethylformamide. Int. J. Syst. Bacteriol. **40**:287–291.
- Valderrama, M. J., E. Quesada, V. Bejar, A. Ventosa, M. C. Gutierrez, F. Ruiz-Berraquero, and A. Ramos-Cormenzana. 1991. *Deleya salina* sp. nov., a moderately halophilic gram-negative bacterium. Int. J. Syst. Bacteriol. **41**: 377–384.
- Ventosa, A., M. C. Gutierrez, M. T. Garcia, and F. Ruiz-Berraquero. 1989. Classification of “*Chromobacterium marismortui*” in a new genus, *Chromohalobacter* gen. nov., as *Chromohalobacter marismortui* comb. nov., nom. rev. Int. J. Syst. Bacteriol. **39**:382–386.
- Vreeland, R. H., C. D. Litchfield, E. L. Martin, and E. Elliot. 1980. *Halomonas elongata*, a new genus and species of extremely salt-tolerant bacteria. Int. J. Syst. Bacteriol. **30**:485–495.
- Wayne, L. G., D. J. Brenner, R. R. Colwell, P. A. D. Grimont, O. Kandler, M. I. Krichevsky, L. H. Moore, W. E. C. Moore, R. G. E. Murray, E. Stackebrandt, M. P. Starr, and H. G. Trüper. 1987. Report of the Ad Hoc Committee on Reconciliation of Approaches to Bacterial Systematics. Int. J. Syst. Bacteriol. **37**:463–464.