Phylogenetic Interrelationships of Members of the Genera *Aeromonas* and *Plesiomonas* as Determined by 16S Ribosomal DNA Sequencing: Lack of Congruence with Results of DNA-DNA Hybridizations

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The phylogenetic interrelationships of members of the genera *Aeromonas* and *Plesiomonas* were investigated by using small-subunit ribosomal DNA (rDNA) sequencing. Members of the genus *Aeromonas* formed a distinct line within the gamma subclass of the *Proteobacteria*. *Plesiomonas shigelloides* also clustered within the confines of the gamma subclass of the *Proteobacteria* but exhibited a closer association with members of the family *Enterobacteriaceae* than with members of the family *Aeromonadaceae*. Species of the genus *Aeromonas* exhibited very high levels of overall sequence similarity (ca. 98 to 100%) with each other. Several of the relationships derived from an analysis of the rDNA sequence data were in marked disagreement with the results of chromosomal DNA-DNA pairing experiments. Diagnostic rDNA signatures that have possible value for differentiating most *Aeromonas* species were discerned.

The genus *Aeromonas* was proposed by Kluyver and van Niel in 1936 and comprises a collection of oxidase- and catalase-positive, glucose-fermenting, facultatively anaerobic, gram-negative, rod-shaped bacteria that are resistant to vibriostatic agent 0/129 and generally are motile by means of polar flagella (31). *Aeromonads* are autochthonous to aquatic environments worldwide, but also have been isolated from a variety of raw foods (19-21). Members of this genus tolerate temperatures ranging from 4 to 42°C and are known to cause a diverse spectrum of diseases in both warm- and cold-blooded animals (18). Many mesophilic *Aeromonas* species have been associated with gastrointestinal infections and other human diseases (e.g., septicemia, meningitis, endocarditis, corneal ulcers, wound infections, and peritonitis) (2, 6-8, 12, 15, 16, 18, 20-22, 27-30, 38). *Plesiomonas shigelloides* is widely encountered in natural environments and has been isolated sporadically from clinical specimens (10). The genus *Plesiomonas*, as proposed by Habs and Schubert in 1962, includes strains that do not exhibit some essential features of either the genus *Aeromonas* or the genus *Vibrio* (36). Separation of *Plesiomonas shigelloides* from *aeromonads* is based primarily on differences in flagellation, exoenzyme production, range of carbohydrate fermentation, and DNA base composition (36). Although both the genus *Aeromonas* and the genus *Plesiomonas* currently are placed in the family *Vibrionaceae*, a separate family to accommodate *Aeromonas* species, the *Aeromonadaceae*, has been proposed recently on the basis of the results of 16S rRNA cataloging, 5S rRNA sequencing, and RNA-DNA hybridization studies (9). The nucleic acid data also indicate that *Plesiomonas shigelloides* is more closely related to the family *Enterobacteriaceae* (in particular, the genus *Proteus*) than to the family *Vibrionaceae*.

It is universally acknowledged that the taxonomic history of the genus *Aeromonas* has been one of confusion and controversy (1, 31, 37). In *Bergey's Manual of Systematic*

Bacteriology (31), this genus consists of three mesophilic motile species, *Aeromonas hydrophila* (the type species of the genus), *Aeromonas caviae*, and *Aeromonas sobria*, and the psychrophilic nonmotile organisms *Aeromonas salmonicida* subsp. *salmonicida*, *A. salmonicida* subsp. *achromogenes*, *A. salmonicida* subsp. *mausocida*, and *A. salmonicida* subsp. *smithii*. During the past few years the taxonomy of the genus *Aeromonas* has undergone major revisions because of the use of DNA-DNA pairing study results (22, 32). Extensive investigations, most notably by Brenner and colleagues (15, 16, 22), have demonstrated the presence of at least 12 DNA homology groups within the genus. Currently, the following 12 species are recognized: *A. caviae*, *A. eteropelogenes*, *A. eucrenophila*, *A. hydrophila*, *A. ichthiosmia*, *A. jandaei*, *A. media*, *A. salmonicida*, *A. schubertii*, *A. sobria*, *A. trota*, and *A. veronii*. Although previous studies have clarified the genetic relationships within the genus, there are still serious problems with species identification because of poor correlations between genotypes and phenotypes and a lack of reliable traits for species discrimination and delineation.

16S rRNA (or ribosomal DNA [rDNA] gene) sequencing is one of the most powerful and precise methods for determining the interrelationships of bacteria (41). It has been established that the base sequences of this molecule (or gene) can be used to determine distant as well as close (e.g., intrageneric) genealogical relationships (41). In this study we determined the 16S rDNA sequences of 10 *Aeromonas* species in an attempt to (i) clarify the intrageneric structure of the genus *Aeromonas* and assess the congruence of rDNA sequencing results with results of DNA-DNA pairing and (ii) identify regions or signatures within the rDNA sequences that have value for delineating the various *Aeromonas* genospecies. The 16S rDNA from *Plesiomonas shigelloides* was also sequenced to analyze the relatedness of this organism to *Aeromonas* species and other related organisms belonging to the class *Proteobacteria* (39).

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TABLE 1. Levels of homology for 1,502 nucleotides of 16S rDNAs from members of the genera *Aeromonas* and *Plesiomonas* and some reference species belonging to the class *Proteobacteria*

Organism ^a	% Homology with:											
	<i>A. caviae</i> NCIMB 13016 ^T	<i>A. hydrophila</i> NCIMB 9240 ^T	<i>A. salmonicida</i> NCIMB 1102 ^T	<i>A. sobria</i> NCIMB 12065 ^T	<i>B. abortus</i>	<i>E. coli</i>	<i>M. xanthus</i>	<i>N. gonorrhoeae</i>	<i>Plesiomonas shigelloides</i> NCIMB 9242 ^T	<i>Proteus vulgaris</i>	<i>Serratia marcescens</i>	<i>V. anguillarum</i>
<i>A. caviae</i> NCIMB 13016 ^T	100	99.1	98.3	98.7	83.0	90.5	82.0	83.4	91.2	89.9	90.1	90.5
<i>A. hydrophila</i> NCIMB 9240 ^T		100	98.9	98.7	83.1	90.3	81.7	83.2	90.7	89.7	90.5	90.8
<i>A. salmonicida</i> NCIMB 1102 ^T			100	99.4	83.3	90.3	81.4	82.7	90.9	89.9	90.2	98.5
<i>A. sobria</i> NCIMB 12065 ^T				100	83.0	90.3	81.8	82.8	90.9	90.1	89.9	89.9
<i>B. abortus</i>					100	82.6	82.5	82.3	82.8	82.0	82.6	83.0
<i>E. coli</i>						100	80.5	82.6	93.3	93.5	96.1	89.9
<i>M. xanthus</i>							100	79.9	81.0	81.0	80.2	81.5
<i>N. gonorrhoeae</i>								100	81.9	82.0	82.2	81.6
<i>P. shigelloides</i> NCIMB 9242 ^T									100	93.0	94.9	90.7
<i>Proteus vulgaris</i>										100	93.4	89.8
<i>Serratia marcescens</i>											100	90.4
<i>V. anguillarum</i>												100

^a The sequences of *E. coli* and *Serratia marcescens* were obtained from references 5 and 11, respectively. The sequences of *B. abortus*, *M. xanthus*, *N. gonorrhoeae*, *Proteus vulgaris*, and *V. anguillarum* were obtained from EMBL and GenBank (see text).

MATERIALS AND METHODS

Bacterial strains and cultivation. The test strains which we examined are listed in Tables 1 and 2. *A. hydrophila* ATCC 7966^T (T = type strain), ATCC 15467, NCIMB 9240^T, and NCIMB 9235, *A. caviae* NCIMB 13016^T and ATCC 15467,

A. eucrenophila NCIMB 74^T, *A. sobria* NCIMB 12065^T and CIP 7433, *A. veronii* ATCC 35624^T and ATCC 35622, and *A. veronii* biogroup *sobria* ATCC 9071 and NCIMB 37 were grown in 10-ml tubes containing nutrient broth (Oxoid) at 30°C. *A. salmonicida* NCIMB 1102^T, NCIMB 1110, and NCIMB 2020 and *Plesiomonas shigelloides* NCIMB

TABLE 2. Levels of homology and numbers of nucleotide differences in the complete 1,502-base 16S rDNA sequence for *Aeromonas* species

Organism(s)	% Homology with and no. of nucleotide differences compared with ^a :												
	<i>A. caviae</i> NCIMB 13016 ^T	<i>A. caviae</i> ATCC 15467	<i>A. eucrenophila</i> NCIMB 74 ^T	<i>A. hydrophila</i> ATCC 7966 ^T and NCIMB 9240 ^T	<i>A. jandaei</i> ATCC 49568 ^T	<i>A. media</i> ATCC 33907 ^T , ATCC 33950, and NCIMB 2237 ^T	<i>A. salmonicida</i> subsp. <i>salmonicida</i> NCIMB 1102 ^T	<i>A. salmonicida</i> subsp. <i>achromogenes</i> NCIMB 1110 ^T	<i>A. schubertii</i> DSM 4882 ^T	<i>A. sobria</i> NCIMB 12065 ^T and CIP 7433 ^T	<i>Aeromonas</i> sp. strain ATCC 35941	<i>A. trota</i> ATCC 49657 ^T	<i>A. veronii</i> ATCC 35624 ^T and ATCC 35622
<i>A. caviae</i> NCIMB 13016 ^T		99.9	98.3	99.1	99.0	99.0	98.3	98.4	97.9	98.7	98.6	99.9	98.6
<i>A. caviae</i> ATCC 15467	2		98.5	98.9	98.9	98.9	98.4	98.3	98.1	98.7	98.7	99.7	98.7
<i>A. eucrenophila</i> NCIMB 74 ^T	25	23		99.1	98.5	99.2	99.3	99.1	98.3	99.1	99.1	98.3	99.0
<i>A. hydrophila</i> ATCC 7966 ^T and NCIMB 9240 ^T	14	16	13		98.3	99.8	98.9	99.1	97.8	98.7	98.7	99.0	98.5
<i>A. jandaei</i> ATCC 49568 ^T	15	17	22	25		98.3	98.3	98.5	98.7	98.8	98.7	98.9	99.5
<i>A. media</i> ATCC 33907 ^T , ATCC 33950, and NCIMB 2237 ^T	15	17	12	3	26		98.7	98.9	97.9	98.7	98.6	98.9	98.5
<i>A. salmonicida</i> subsp. <i>salmonicida</i> NCIMB 1102 ^T	26	24	11	16	25	19		99.9	98.1	99.4	99.4	98.2	98.8
<i>A. salmonicida</i> subsp. <i>achromogenes</i> NCIMB 1110 ^{Tb}	24	26	13	14	23	17	2		97.9	99.4	99.3	98.3	98.7
<i>A. schubertii</i> DSM 4882 ^T	31	29	26	33	20	32	29	31		98.1	98.3	98.0	99.0
<i>A. sobria</i> NCIMB 12065 ^T and CIP 7433 ^T	19	19	14	19	18	20	9	9	28		99.5	98.7	99.1
<i>Aeromonas</i> sp. strain ATCC 35941	21	19	13	19	20	21	9	11	26	8		98.5	99.2
<i>A. trota</i> ATCC 49657 ^T	1	3	26	15	16	16	27	25	30	20	22		98.5
<i>A. veronii</i> ATCC 35624 ^T and ATCC 35622	21	19	15	22	8	23	18	20	15	14	12	22	

^a The values on the upper right are percentages of homology, and the values on the lower left are numbers of nucleotide differences.

^b Identical values were obtained for *A. salmonicida* subsp. *mausocida* NCIMB 2020^T and *Aeromonas* sp. strain CIP 7340 (DNA group 2).

FIG. 1. 16S rRNA primary structures (derived from the 16S rDNA genes) as determined by PCR DNA sequencing. (1), *A. hydrophila* ATCC 7966^T and NCIMB 9240^T; (2), *A. salmonicida* subsp. *salmonicida* NCIMB 1102^T; (3), *A. salmonicida* subsp. *achromogenes* NCIMB 1110^T and *A. salmonicida* subsp. *mausocida* NCIMB 2020^T; (4), *A. caviae* NCIMB 13016^T and NCIMB 9235; (5), *A. media* ATCC 33907^T, NCIMB 2237^T, and ATCC 33950; (6), *A. eucrenophila* NCIMB 74^T; (7), *A. sobria* NCIMB 12065^T and CIP 7433^T; (8), *A. jandaei* ATCC 49568^T; (9), *A. veronii* NCIMB 13015^T and ATCC 35622 and *A. veronii* biogroup *sobria* ATCC 9071 and NCIMB 37; (10), *Aeromonas* sp. strain ATCC 35941 (DNA group 11); (11), *A. schubertii* DSM 4882^T; (12), *A. trota* ATCC 49657^T; (13), *Plesiomonas shigelloides* NCIMB 9242^T. These sequences have been assigned EMBL accession numbers X60404 to X60418. The asterisks indicate positions where nucleotide differences are found in *Aeromonas* species. The numbering system used was the *E. coli* sequence numbering system described by Brosius et al. (5).

408	:	*****	*****
(1)	GUGAAGAAGGCCUUCGGGUUGUAAAGCACUUUCAGCGAGGAGGAAAGGUUGAUGCCUAAUACGUAUACUGUGACGUUACUCGCGAGAAGAAGCACCGGC		
(2)	GUGAAGAAGGCCUUCGGGUUGUAAAGCACUUUCAGCGAGGAGGAAAGGUUGGCGCCUAAUACGUGUACUACUGUGACGUUACUCGCGAGAAGAAGCACCGGC		
(3)	GUGAAGAAGGCCUUCGGGUUGUAAAGCACUUUCAGCGAGGAGGAAAGGUUGGCGCCUAAUACGUGUACUACUGUGACGUUACUCGCGAGAAGAAGCACCGGC		
(4)	GUGAAGAAGGCCUUCGGGUUGUAAAGCACUUUCAGCGAGGAGGAAAGGUUGGCGCCUAAUACGUGUACUACUGUGACGUUACUCGCGAGAAGAAGCACCGGC		
(5)	GUGAAGAAGGCCUUCGGGUUGUAAAGCACUUUCAGCGAGGAGGAAAGGUUGGCGCCUAAUACGUGUACUACUGUGACGUUACUCGCGAGAAGAAGCACCGGC		
(6)	GUGAAGAAGGCCUUCGGGUUGUAAAGCACUUUCAGCGAGGAGGAAAGGUUGGCGCCUAAUACGUGUACUACUGUGACGUUACUCGCGAGAAGAAGCACCGGC		
(7)	GUGAAGAAGGCCUUCGGGUUGUAAAGCACUUUCAGCGAGGAGGAAAGGUUGGCGCCUAAUACGUGUACUACUGUGACGUUACUCGCGAGAAGAAGCACCGGC		
(8)	GUGAAGAAGGCCUUCGGGUUGUAAAGCACUUUCAGCGAGGAGGAAAGGUUGGCGCCUAAUACGUGUACUACUGUGACGUUACUCGCGAGAAGAAGCACCGGC		
(9)	GUGAAGAAGGCCUUCGGGUUGUAAAGCACUUUCAGCGAGGAGGAAAGGUUGGCGCCUAAUACGUGUACUACUGUGACGUUACUCGCGAGAAGAAGCACCGGC		
(10)	GUGAAGAAGGCCUUCGGGUUGUAAAGCACUUUCAGCGAGGAGGAAAGGUUGGCGCCUAAUACGUGUACUACUGUGACGUUACUCGCGAGAAGAAGCACCGGC		
(11)	GUGAAGAAGGCCUUCGGGUUGUAAAGCACUUUCAGCGAGGAGGAAAGGUUGGCGCCUAAUACGUGUACUACUGUGACGUUACUCGCGAGAAGAAGCACCGGC		
(12)	GUGAAGAAGGCCUUCGGGUUGUAAAGCACUUUCAGCGAGGAGGAAAGGUUGGCGCCUAAUACGUGUACUACUGUGACGUUACUCGCGAGAAGAAGCACCGGC		
(13)	GUGAAGAAGGCCUUCGGGUUGUAAAGCACUUUCAGCGAGGAGGAAAGGUUGGCGCCUAAUACGUGUACUACUGUGACGUUACUCGCGAGAAGAAGCACCGGC		
(14)	GUGAAGAAGGCCUUCGGGUUGUAAAGCACUUUCAGCGAGGAGGAAAGGUUGGCGCCUAAUACGUGUACUACUGUGACGUUACUCGCGAGAAGAAGCACCGGC		
508	:		
(1)	UAAUCUCCGUGCCAGCAGCCGCGGUAAUACGAGGAGGUGCAAGCGUAAUACGGAUUAUCUGGGCGUAAAGCGCACGAGCGGCGUUGGAUAGUUAAGUAGUGUA		
(2)	UAAUCUCCGUGCCAGCAGCCGCGGUAAUACGAGGAGGUGCAAGCGUAAUACGGAUUAUCUGGGCGUAAAGCGCACGAGCGGCGUUGGAUAGUUAAGUAGUGUA		
(3)	UAAUCUCCGUGCCAGCAGCCGCGGUAAUACGAGGAGGUGCAAGCGUAAUACGGAUUAUCUGGGCGUAAAGCGCACGAGCGGCGUUGGAUAGUUAAGUAGUGUA		
(4)	UAAUCUCCGUGCCAGCAGCCGCGGUAAUACGAGGAGGUGCAAGCGUAAUACGGAUUAUCUGGGCGUAAAGCGCACGAGCGGCGUUGGAUAGUUAAGUAGUGUA		
(5)	UAAUCUCCGUGCCAGCAGCCGCGGUAAUACGAGGAGGUGCAAGCGUAAUACGGAUUAUCUGGGCGUAAAGCGCACGAGCGGCGUUGGAUAGUUAAGUAGUGUA		
(6)	UAAUCUCCGUGCCAGCAGCCGCGGUAAUACGAGGAGGUGCAAGCGUAAUACGGAUUAUCUGGGCGUAAAGCGCACGAGCGGCGUUGGAUAGUUAAGUAGUGUA		
(7)	UAAUCUCCGUGCCAGCAGCCGCGGUAAUACGAGGAGGUGCAAGCGUAAUACGGAUUAUCUGGGCGUAAAGCGCACGAGCGGCGUUGGAUAGUUAAGUAGUGUA		
(8)	UAAUCUCCGUGCCAGCAGCCGCGGUAAUACGAGGAGGUGCAAGCGUAAUACGGAUUAUCUGGGCGUAAAGCGCACGAGCGGCGUUGGAUAGUUAAGUAGUGUA		
(9)	UAAUCUCCGUGCCAGCAGCCGCGGUAAUACGAGGAGGUGCAAGCGUAAUACGGAUUAUCUGGGCGUAAAGCGCACGAGCGGCGUUGGAUAGUUAAGUAGUGUA		
(10)	UAAUCUCCGUGCCAGCAGCCGCGGUAAUACGAGGAGGUGCAAGCGUAAUACGGAUUAUCUGGGCGUAAAGCGCACGAGCGGCGUUGGAUAGUUAAGUAGUGUA		
(11)	UAAUCUCCGUGCCAGCAGCCGCGGUAAUACGAGGAGGUGCAAGCGUAAUACGGAUUAUCUGGGCGUAAAGCGCACGAGCGGCGUUGGAUAGUUAAGUAGUGUA		
(12)	UAAUCUCCGUGCCAGCAGCCGCGGUAAUACGAGGAGGUGCAAGCGUAAUACGGAUUAUCUGGGCGUAAAGCGCACGAGCGGCGUUGGAUAGUUAAGUAGUGUA		
(13)	UAAUCUCCGUGCCAGCAGCCGCGGUAAUACGAGGAGGUGCAAGCGUAAUACGGAUUAUCUGGGCGUAAAGCGCACGAGCGGCGUUGGAUAGUUAAGUAGUGUA		
(14)	UAAUCUCCGUGCCAGCAGCCGCGGUAAUACGAGGAGGUGCAAGCGUAAUACGGAUUAUCUGGGCGUAAAGCGCACGAGCGGCGUUGGAUAGUUAAGUAGUGUA		
608	:	* * *	
(1)	AAGCCCCGGGCUAACCCUGGGAUUGCAUUUAAAACUGUCCAGCUAGAGUCUUGUAGAGGGGGGUGAGAAUCCAGGUGUAGCGGUGAAAUGCGUAGAGAU		
(2)	AAGCCCCGGGCUAACCCUGGGAUUGCAUUUAAAACUGUCCAGCUAGAGUCUUGUAGAGGGGGGUGAGAAUCCAGGUGUAGCGGUGAAAUGCGUAGAGAU		
(3)	AAGCCCCGGGCUAACCCUGGGAUUGCAUUUAAAACUGUCCAGCUAGAGUCUUGUAGAGGGGGGUGAGAAUCCAGGUGUAGCGGUGAAAUGCGUAGAGAU		
(4)	AAGCCCCGGGCUAACCCUGGGAUUGCAUUUAAAACUGUCCAGCUAGAGUCUUGUAGAGGGGGGUGAGAAUCCAGGUGUAGCGGUGAAAUGCGUAGAGAU		
(5)	AAGCCCCGGGCUAACCCUGGGAUUGCAUUUAAAACUGUCCAGCUAGAGUCUUGUAGAGGGGGGUGAGAAUCCAGGUGUAGCGGUGAAAUGCGUAGAGAU		
(6)	AAGCCCCGGGCUAACCCUGGGAUUGCAUUUAAAACUGUCCAGCUAGAGUCUUGUAGAGGGGGGUGAGAAUCCAGGUGUAGCGGUGAAAUGCGUAGAGAU		
(7)	AAGCCCCGGGCUAACCCUGGGAUUGCAUUUAAAACUGUCCAGCUAGAGUCUUGUAGAGGGGGGUGAGAAUCCAGGUGUAGCGGUGAAAUGCGUAGAGAU		
(8)	AAGCCCCGGGCUAACCCUGGGAUUGCAUUUAAAACUGUCCAGCUAGAGUCUUGUAGAGGGGGGUGAGAAUCCAGGUGUAGCGGUGAAAUGCGUAGAGAU		
(9)	AAGCCCCGGGCUAACCCUGGGAUUGCAUUUAAAACUGUCCAGCUAGAGUCUUGUAGAGGGGGGUGAGAAUCCAGGUGUAGCGGUGAAAUGCGUAGAGAU		
(10)	AAGCCCCGGGCUAACCCUGGGAUUGCAUUUAAAACUGUCCAGCUAGAGUCUUGUAGAGGGGGGUGAGAAUCCAGGUGUAGCGGUGAAAUGCGUAGAGAU		
(11)	AAGCCCCGGGCUAACCCUGGGAUUGCAUUUAAAACUGUCCAGCUAGAGUCUUGUAGAGGGGGGUGAGAAUCCAGGUGUAGCGGUGAAAUGCGUAGAGAU		
(12)	AAGCCCCGGGCUAACCCUGGGAUUGCAUUUAAAACUGUCCAGCUAGAGUCUUGUAGAGGGGGGUGAGAAUCCAGGUGUAGCGGUGAAAUGCGUAGAGAU		
(13)	AAGCCCCGGGCUAACCCUGGGAUUGCAUUUAAAACUGUCCAGCUAGAGUCUUGUAGAGGGGGGUGAGAAUCCAGGUGUAGCGGUGAAAUGCGUAGAGAU		
(14)	AAGCCCCGGGCUAACCCUGGGAUUGCAUUUAAAACUGUCCAGCUAGAGUCUUGUAGAGGGGGGUGAGAAUCCAGGUGUAGCGGUGAAAUGCGUAGAGAU		
707	:	*	
(1)	CUGGAGGAAUACCGGUGGCGAAGGCGGCCCCUGGACAAAGACUGACGUCAGGUGCGAAAGCGUGGGGAGCAAACAGGAUUAAGAUACCCUGGUAGUCCA		
(2)	CUGGAGGAAUACCGGUGGCGAAGGCGGCCCCUGGACAAAGACUGACGUCAGGUGCGAAAGCGUGGGGAGCAAACAGGAUUAAGAUACCCUGGUAGUCCA		
(3)	CUGGAGGAAUACCGGUGGCGAAGGCGGCCCCUGGACAAAGACUGACGUCAGGUGCGAAAGCGUGGGGAGCAAACAGGAUUAAGAUACCCUGGUAGUCCA		
(4)	CUGGAGGAAUACCGGUGGCGAAGGCGGCCCCUGGACAAAGACUGACGUCAGGUGCGAAAGCGUGGGGAGCAAACAGGAUUAAGAUACCCUGGUAGUCCA		
(5)	CUGGAGGAAUACCGGUGGCGAAGGCGGCCCCUGGACAAAGACUGACGUCAGGUGCGAAAGCGUGGGGAGCAAACAGGAUUAAGAUACCCUGGUAGUCCA		
(6)	CUGGAGGAAUACCGGUGGCGAAGGCGGCCCCUGGACAAAGACUGACGUCAGGUGCGAAAGCGUGGGGAGCAAACAGGAUUAAGAUACCCUGGUAGUCCA		
(7)	CUGGAGGAAUACCGGUGGCGAAGGCGGCCCCUGGACAAAGACUGACGUCAGGUGCGAAAGCGUGGGGAGCAAACAGGAUUAAGAUACCCUGGUAGUCCA		
(8)	CUGGAGGAAUACCGGUGGCGAAGGCGGCCCCUGGACAAAGACUGACGUCAGGUGCGAAAGCGUGGGGAGCAAACAGGAUUAAGAUACCCUGGUAGUCCA		
(9)	CUGGAGGAAUACCGGUGGCGAAGGCGGCCCCUGGACAAAGACUGACGUCAGGUGCGAAAGCGUGGGGAGCAAACAGGAUUAAGAUACCCUGGUAGUCCA		
(10)	CUGGAGGAAUACCGGUGGCGAAGGCGGCCCCUGGACAAAGACUGACGUCAGGUGCGAAAGCGUGGGGAGCAAACAGGAUUAAGAUACCCUGGUAGUCCA		
(11)	CUGGAGGAAUACCGGUGGCGAAGGCGGCCCCUGGACAAAGACUGACGUCAGGUGCGAAAGCGUGGGGAGCAAACAGGAUUAAGAUACCCUGGUAGUCCA		
(12)	CUGGAGGAAUACCGGUGGCGAAGGCGGCCCCUGGACAAAGACUGACGUCAGGUGCGAAAGCGUGGGGAGCAAACAGGAUUAAGAUACCCUGGUAGUCCA		
(13)	CUGGAGGAAUACCGGUGGCGAAGGCGGCCCCUGGACAAAGACUGACGUCAGGUGCGAAAGCGUGGGGAGCAAACAGGAUUAAGAUACCCUGGUAGUCCA		
(14)	CUGGAGGAAUACCGGUGGCGAAGGCGGCCCCUGGACAAAGACUGACGUCAGGUGCGAAAGCGUGGGGAGCAAACAGGAUUAAGAUACCCUGGUAGUCCA		

FIG. 1—Continued

9242^T were cultivated in the same medium at 20 and 25°C, respectively. *A. jandaei* ATCC 49568^T, *A. trota* ATCC 49657^T and ATCC 49659, and *A. schubertii* DSM 4882^T were grown in Trypticase soy broth (Oxoid) at 30°C, whereas *A. media* ATCC 33907^T, ATCC 33950, and NCIMB 2237^T and strain ATCC 35941 were grown at 22 to 25°C. Cultures were harvested by centrifugation in late exponential phase and were washed in deionized water.

Preparation of DNA. Harvested cells (ca. 0.1 to 0.2 g) were diluted with 100 µl of lysis solution (4 M guanidinium

thiocyanate, 25 mM sodium citrate, 0.1 M 2-mercaptoethanol, 0.5% sarcosyl, 1% sodium dodecyl sulfate, 0.1% anti-foam A; pH 7.5) and then vortexed for 1 min. After 300 µl of TES (50 mM Tris-HCl, 5 mM EDTA, 50 mM sodium chloride) was added, the mixture was extracted three times by using 500 µl of 70% phenol–20% water–10% chloroform. The DNA was precipitated in a solution containing 30 µl of 2 M sodium acetate (pH 4.5) and 1 ml of ethanol and was purified by using a DNA GeneClean kit (Bio 101, Inc.) following the manufacturer's instructions.

- 808
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- (1) CGCCGUAACGAUGUCGAUUGGAGGCGUGUCCUUGAGACGUGGCUUCCGGAGCUAACCGGUUAAUCCGACCGCCUGGGGAGUACGGCCGCAAGGUUAA
(2) CGCCGUAACGAUGUCGAUUGGAGGCGUGUCCUUGAGACGUGGCUUCCGGAGCUAACCGGUUAAUCCGACCGCCUGGGGAGUACGGCCGCAAGGUUAA
(3) CGCCGUAACGAUGUCGAUUGGAGGCGUGUCCUUGAGACGUGGCUUCCGGAGCUAACCGGUUAAUCCGACCGCCUGGGGAGUACGGCCGCAAGGUUAA
(4) CGCCGUAACGAUGUCGAUUGGAGGCGUGUCCUUGAGACGUGGCUUCCGGAGCUAACCGGUUAAUCCGACCGCCUGGGGAGUACGGCCGCAAGGUUAA
(5) CGCCGUAACGAUGUCGAUUGGAGGCGUGUCCUUGAGACGUGGCUUCCGGAGCUAACCGGUUAAUCCGACCGCCUGGGGAGUACGGCCGCAAGGUUAA
(6) CGCCGUAACGAUGUCGAUUGGAGGCGUGUCCUUGAGACGUGGCUUCCGGAGCUAACCGGUUAAUCCGACCGCCUGGGGAGUACGGCCGCAAGGUUAA
(7) CGCCGUAACGAUGUCGAUUGGAGGCGUGUCCUUGAGACGUGGCUUCCGGAGCUAACCGGUUAAUCCGACCGCCUGGGGAGUACGGCCGCAAGGUUAA
(8) CGCCGUAACGAUGUCGAUUGGAGGCGUGUCCUUGAGACGUGGCUUCCGGAGCUAACCGGUUAAUCCGACCGCCUGGGGAGUACGGCCGCAAGGUUAA
(9) CGCCGUAACGAUGUCGAUUGGAGGCGUGUCCUUGAGACGUGGCUUCCGGAGCUAACCGGUUAAUCCGACCGCCUGGGGAGUACGGCCGCAAGGUUAA
(10) CGCCGUAACGAUGUCGAUUGGAGGCGUGUCCUUGAGACGUGGCUUCCGGAGCUAACCGGUUAAUCCGACCGCCUGGGGAGUACGGCCGCAAGGUUAA
(11) CGCCGUAACGAUGUCGAUUGGAGGCGUGUCCUUGAGACGUGGCUUCCGGAGCUAACCGGUUAAUCCGACCGCCUGGGGAGUACGGCCGCAAGGUUAA
(12) CGCCGUAACGAUGUCGAUUGGAGGCGUGUCCUUGAGACGUGGCUUCCGGAGCUAACCGGUUAAUCCGACCGCCUGGGGAGUACGGCCGCAAGGUUAA
(13) CGCCGUAACGAUGUCGAUUGGAGGCGUGUCCUUGAGACGUGGCUUCCGGAGCUAACCGGUUAAUCCGACCGCCUGGGGAGUACGGCCGCAAGGUUAA
(14) CGCGUAAACGAUGUCGAUUGGAGGCGUGUCCUUGAGGCGUGGCUUCCGGAGCUAACCGGUUAAUCCGACCGCCUGGGGAGUACGGCCGCAAGGUUAA
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- (1) AACUCAAAUGAAUUGACGGGGGCCCGCACAAAGCGGUGGAGCAUGUGGUUAAUUCGAUGCAACGCGAAGAACCUUACCUGGCCUUGACAUGUCUGGAAUC
(2) AACUCAAAUGAAUUGACGGGGGCCCGCACAAAGCGGUGGAGCAUGUGGUUAAUUCGAUGCAACGCGAAGAACCUUACCUGGCCUUGACAUGUCUGGAAUC
(3) AACUCAAAUGAAUUGACGGGGGCCCGCACAAAGCGGUGGAGCAUGUGGUUAAUUCGAUGCAACGCGAAGAACCUUACCUGGCCUUGACAUGUCUGGAAUC
(4) AACUCAAAUGAAUUGACGGGGGCCCGCACAAAGCGGUGGAGCAUGUGGUUAAUUCGAUGCAACGCGAAGAACCUUACCUGGCCUUGACAUGUCUGGAAUC
(5) AACUCAAAUGAAUUGACGGGGGCCCGCACAAAGCGGUGGAGCAUGUGGUUAAUUCGAUGCAACGCGAAGAACCUUACCUGGCCUUGACAUGUCUGGAAUC
(6) AACUCAAAUGAAUUGACGGGGGCCCGCACAAAGCGGUGGAGCAUGUGGUUAAUUCGAUGCAACGCGAAGAACCUUACCUGGCCUUGACAUGUCUGGAAUC
(7) AACUCAAAUGAAUUGACGGGGGCCCGCACAAAGCGGUGGAGCAUGUGGUUAAUUCGAUGCAACGCGAAGAACCUUACCUGGCCUUGACAUGUCUGGAAUC
(8) AACUCAAAUGAAUUGACGGGGGCCCGCACAAAGCGGUGGAGCAUGUGGUUAAUUCGAUGCAACGCGAAGAACCUUACCUGGCCUUGACAUGUCUGGAAUC
(9) AACUCAAAUGAAUUGACGGGGGCCCGCACAAAGCGGUGGAGCAUGUGGUUAAUUCGAUGCAACGCGAAGAACCUUACCUGGCCUUGACAUGUCUGGAAUC
(10) AACUCAAAUGAAUUGACGGGGGCCCGCACAAAGCGGUGGAGCAUGUGGUUAAUUCGAUGCAACGCGAAGAACCUUACCUGGCCUUGACAUGUCUGGAAUC
(11) AACUCAAAUGAAUUGACGGGGGCCCGCACAAAGCGGUGGAGCAUGUGGUUAAUUCGAUGCAACGCGAAGAACCUUACCUGGCCUUGACAUGUCUGGAAUC
(12) AACUCAAAUGAAUUGACGGGGGCCCGCACAAAGCGGUGGAGCAUGUGGUUAAUUCGAUGCAACGCGAAGAACCUUACCUGGCCUUGACAUGUCUGGAAUC
(13) AACUCAAAUGAAUUGACGGGGGCCCGCACAAAGCGGUGGAGCAUGUGGUUAAUUCGAUGCAACGCGAAGAACCUUACCUGGCCUUGACAUGUCUGGAAUC
(14) AACUCAAAUGAAUUGACGGGGGCCCGCACAAAGCGGUGGAGCAUGUGGUUAAUUCGAUGCAACGCGAAGAACCUUACCUGGCCUUGACAUGUCUGGAAUC
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- (1) CUGCAGAGAUGCGGGAGUGCCUUCGGGAAUCAGAACACAGGUGCUGCAUGGCUGUCGUCAGCUCGUGUCGUGAGAUGUUGGGUUAAGUCCCGCAACGAGC
(2) CUGUAGAGAUAACGGGAGUGCCUUCGGGAAUCAGAACACAGGUGCUGCAUGGCUGUCGUCAGCUCGUGUCGUGAGAUGUUGGGUUAAGUCCCGCAACGAGC
(3) CUGCAGAGAUGCGGGAGUGCCUUCGGGAAUCAGAACACAGGUGCUGCAUGGCUGUCGUCAGCUCGUGUCGUGAGAUGUUGGGUUAAGUCCCGCAACGAGC
(4) CUGCAGAGAUGCGGGAGUGCCUUCGGGAAUCAGAACACAGGUGCUGCAUGGCUGUCGUCAGCUCGUGUCGUGAGAUGUUGGGUUAAGUCCCGCAACGAGC
(5) CUGUAGAGAUAACGGGAGUGCCUUCGGGAAUCAGAACACAGGUGCUGCAUGGCUGUCGUCAGCUCGUGUCGUGAGAUGUUGGGUUAAGUCCCGCAACGAGC
(6) CUGCAGAGAUGCGGGAGUGCCUUCGGGAAUCAGAACACAGGUGCUGCAUGGCUGUCGUCAGCUCGUGUCGUGAGAUGUUGGGUUAAGUCCCGCAACGAGC
(7) CUGUAGAGAUAACGGGAGUGCCUUCGGGAAUCAGAACACAGGUGCUGCAUGGCUGUCGUCAGCUCGUGUCGUGAGAUGUUGGGUUAAGUCCCGCAACGAGC
(8) CUGCAGAGAUAACGGGAGUGCCUUCGGGAAUCAGAACACAGGUGCUGCAUGGCUGUCGUCAGCUCGUGUCGUGAGAUGUUGGGUUAAGUCCCGCAACGAGC
(9) CUGCAGAGAUAACGGGAGUGCCUUCGGGAAUCAGAACACAGGUGCUGCAUGGCUGUCGUCAGCUCGUGUCGUGAGAUGUUGGGUUAAGUCCCGCAACGAGC
(10) CUGUAGAGAUAACGGGAGUGCCUUCGGGAAUCAGAACACAGGUGCUGCAUGGCUGUCGUCAGCUCGUGUCGUGAGAUGUUGGGUUAAGUCCCGCAACGAGC
(11) CUGUAGAGAUAACGGGAGUGCCUUCGGGAAUCAGAACACAGGUGCUGCAUGGCUGUCGUCAGCUCGUGUCGUGAGAUGUUGGGUUAAGUCCCGCAACGAGC
(12) CUGUAGAGAUAACGGGAGUGCCUUCGGGAAUCAGAACACAGGUGCUGCAUGGCUGUCGUCAGCUCGUGUCGUGAGAUGUUGGGUUAAGUCCCGCAACGAGC
(13) CUGCAGAGAUGCGGGAGUGCCUUCGGGAAUCAGAACACAGGUGCUGCAUGGCUGUCGUCAGCUCGUGUCGUGAGAUGUUGGGUUAAGUCCCGCAACGAGC
(14) UUCAGAGAUGGAUUGGUGCCUUCGGGAAUCUGAGACAGGUGCUGCAUGGCUGUCGUCAGCUCGUGUUGAUAUUGGGUUAAGUCCCGCAACGAGC
- 1108
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* *
- (1) GCAACCCUGUCCUUGUUGCCAGCAGUAAUGGUGGGAACUCAAGGAGACUGCCGGUGAUAAACCGGAGGAAGGUGGGGAUGACGUCAAGUCAUCAUG
(2) GCAACCCUGUCCUUGUUGCCAGCAGUAAUGGUGGGAACUCAAGGAGACUGCCGGUGAUAAACCGGAGGAAGGUGGGGAUGACGUCAAGUCAUCAUG
(3) GCAACCCUGUCCUUGUUGCCAGCAGUAAUGGUGGGAACUCAAGGAGACUGCCGGUGAUAAACCGGAGGAAGGUGGGGAUGACGUCAAGUCAUCAUG
(4) GCAACCCUGUCCUUGUUGCCAGCAGUAAUGGUGGGAACUCAAGGAGACUGCCGGUGAUAAACCGGAGGAAGGUGGGGAUGACGUCAAGUCAUCAUG
(5) GCAACCCUGUCCUUGUUGCCAGCAGUAAUGGUGGGAACUCAAGGAGACUGCCGGUGAUAAACCGGAGGAAGGUGGGGAUGACGUCAAGUCAUCAUG
(6) GCAACCCUGUCCUUGUUGCCAGCAGUAAUGGUGGGAACUCAAGGAGACUGCCGGUGAUAAACCGGAGGAAGGUGGGGAUGACGUCAAGUCAUCAUG
(7) GCAACCCUGUCCUUGUUGCCAGCAGUAAUGGUGGGAACUCAAGGAGACUGCCGGUGAUAAACCGGAGGAAGGUGGGGAUGACGUCAAGUCAUCAUG
(8) GCAACCCUGUCCUUGUUGCCAGCAGUAAUGGUGGGAACUCAAGGAGACUGCCGGUGAUAAACCGGAGGAAGGUGGGGAUGACGUCAAGUCAUCAUG
(9) GCAACCCUGUCCUUGUUGCCAGCAGUAAUGGUGGGAACUCAAGGAGACUGCCGGUGAUAAACCGGAGGAAGGUGGGGAUGACGUCAAGUCAUCAUG
(10) GCAACCCUGUCCUUGUUGCCAGCAGUAAUGGUGGGAACUCAAGGAGACUGCCGGUGAUAAACCGGAGGAAGGUGGGGAUGACGUCAAGUCAUCAUG
(11) GCAACCCUGUCCUUGUUGCCAGCAGUAAUGGUGGGAACUCAAGGAGACUGCCGGUGAUAAACCGGAGGAAGGUGGGGAUGACGUCAAGUCAUCAUG
(12) GCAACCCUGUCCUUGUUGCCAGCAGUAAUGGUGGGAACUCAAGGAGACUGCCGGUGAUAAACCGGAGGAAGGUGGGGAUGACGUCAAGUCAUCAUG
(13) GCAACCCUGUCCUUGUUGCCAGCAGUAAUGGUGGGAACUCAAGGAGACUGCCGGUGAUAAACCGGAGGAAGGUGGGGAUGACGUCAAGUCAUCAUG
(14) GCAACCCUUAUCCUUGUUGCCAGC GAUUCGGUCGGGAACUCAAGGAGACUGCCGGUGAUAAACCGGAGGAAGGUGGGGAUGACGUCAAGUCAUCAUG

FIG. 1.—Continued

PCR and sequence determination. 16S rDNA polymerase chain reaction (PCR) amplification was performed as described by Bottger (4), except for the antisense primer having the sequence 5' AGAGUUGAUAUGGCUCAG at the 5' end of the 16S rDNA gene (positions 8 to 27 in the *Escherichia coli* sequence), by using a temperature of 55°C for primer annealing. The nucleotide sequences of PCR products were determined by using the dideoxynucleotide chain termination method (35) and Sequenase version 2.0 (US Biochemicals). The sequencing primers which we used

were those described by Lane et al. (23) and Bottger (4), except that we used an additional sequencing primer (5' CTCTCAGACCAGCTAGGGA) for the region around position 300 (complementary to positions 283 to 301 in the *E. coli* numbering system). The nucleotide fragments generated in the chain elongation reactions were separated on 55-cm wedge-shaped (0.2 to 0.6 mm) 6% acrylamide–7 M urea gels at 55°C by using an LKB MacroPhor 2010 sequencing unit operated at 50 W per gel.

Analysis of sequence data. The sequences which we deter-

FIG. 1—Continued

mined were aligned, and homology values were calculated by using the Beckman Microgenie program (33). Nucleotide substitution rates (K_{nuc} values) were determined (17), and phylogenetic trees were produced by the distance matrix method using the algorithm of Fitch and Margoliash (13, 14), and the neighbor-joining method (34). The *E. coli* 16S rRNA sequence and the numbering system which we used were the sequence and system described by Brosius et al. (5).

Nucleotide sequence accession numbers. The sequence data have been deposited in the EMBL Data Library under accession numbers X60404 and X60418. The sequences of reference species were obtained from EMBL and GenBank. The accession numbers for these sequences were as follows: *Brucella abortus*, X13695; *Myxococcus xanthus*, M34114; *Neisseria gonorrhoea*, X07714; *Proteus vulgaris*, X07652; and *Vibrio anguillarum*, X16895.

RESULTS

The 16S rDNA sequences of 20 strains of *Aeromonas* species and the type strain of *Plesiomonas shigelloides* were determined by PCR DNA sequencing. The derived 16S rRNA primary sequences are shown in Fig. 1 and comprised a continuous stretch of 1,502 nucleotides (ranging from

positions 9 to 1,507 in the *E. coli* numbering system), representing approximately 98% of the total primary structure.

The 16S rDNA sequences of *Plesiomonas shigelloides* and four representative *Aeromonas* species were compared with the sequences of seven reference species belonging to the class *Proteobacteria* (39). Calculated percentages of similarity and derived K_{nuc} values are shown in Table 1. Figure 2 shows an unrooted phylogenetic tree that was constructed by using the distant matrix method and the Fitch-Margoliash algorithm.

In order to investigate the intragenetic relationships of aeromonads, we aligned the rDNA sequences of 20 strains (including the type strains of 10 described species and representative strains of DNA homology groups). The percentages of sequence similarity based on a comparison of 1,502 nucleotides are shown in Table 2. We observed very high levels of sequence similarity (ca. 97.8 to 100%, corresponding to 0 to 32 base differences) between the *Aeromonas* strains. An inspection of the rDNA sequence alignments revealed that most genospecies possessed characteristic signatures in region V3 at positions 457 to 476 (*E. coli* numbering) (Fig. 1). The secondary structure of this region in *A. hydrophila* is shown in Fig. 3. Figure 4 shows an

FIG. 2. Evolutionary tree showing the relationships of the genera *Aeromonas* and *Plesiomonas* to some representative members of the class *Proteobacteria* (α , β , γ , and δ subgroups), as determined by the distance matrix method. *Pr.*, *Proteus*; *P.*, *Plesiomonas*; *S.*, *Serratia*.

unrooted tree that was constructed from a matrix of derived K_{nuc} values by using the neighbor-joining method.

DISCUSSION

It is evident from our phylogenetic analysis that the genera *Aeromonas* and *Plesiomonas* cluster in the gamma subdivision of the *Proteobacteria*. This relationship is supported by the presence of the signature oligonucleotides CUAUA CCG, YCACAYYG (Y = pyrimidine), CUAACUYYG, and UCACACCAUG, which are present at positions ca. 170, 315, 510, and 1410 (*E. coli* numbering system), respectively, in all members of the gamma subgroup of the *Proteobacteria*

(39). The genus *Aeromonas* formed a distinct line that was separate from *V. anguillarum* and the representatives of the family *Enterobacteriaceae* which we examined. In this scheme *Plesiomonas shigelloides* exhibited a closer affinity with members of the *Enterobacteriaceae* than with members of the *Aeromonadaceae*. Both of these findings agree with previous conclusions of Colwell et al. (9) that were based on RNA-DNA pairing, 16S rRNA cataloging, and 5S rRNA sequencing data.

The 13 *Aeromonas* genospecies which we examined were closely related on the basis of their 16S rDNA sequences (Table 2). Some of the relationships that were derived from a comparative analysis of the 16S rDNA sequence data (Fig. 3 and Table 2) were markedly different from the results of chromosomal DNA-DNA pairing studies (22, 32). The 16S rDNA sequence of *A. hydrophila* subsp. *hydrophila* ATCC 7966^T exhibited only three nucleotide differences with the sequence of *A. media* ATCC 33907^T, indicating that these species are genealogically closely related. However, these taxa have been reported to belong to two separate DNA hybridization groups (groups 1 and 5B) (22). In order to check these findings, duplicate type strains of *A. hydrophila* subsp. *hydrophila* and *A. media* were obtained from the National Collection of Industrial and Marine Bacteria (strains NCIMB 9240^T and NCIMB 2237^T), and a second strain of *A. media* (strain ATCC 33950) was examined. Full 16S rDNA sequence determinations for these additional strains confirmed the original sequence data. The 16S rDNA sequences of *A. hydrophila* subsp. *anaerogenes* ATCC 15467^T and NCIMB 9235 exhibited two and no base differences, respectively, with the sequence of the type strain of *A. caviae* (strain NCIMB 13016). These data are in accord with previously reported high levels of chromosomal DNA relatedness between these taxa (15) and suggest that these

FIG. 3. Secondary structure of the region of *A. hydrophila* 16S rRNA around position 460. Variable nucleotides are in boldface, and the corresponding specific signatures are shown in Fig. 1.

FIG. 4. Phylogenetic intrarelations in the genus *Aeromonas* as determined by a continuous 1,502-nucleotide 16S rDNA sequence comparison, using the neighbor-joining method.

organisms should be placed in a single species. Surprisingly, we found that the 16S rDNA of the type strain of *A. trota*, strain ATCC 49657, was also almost identical to the 16S rDNA of *A. caviae* NCIMB 13016^T; we detected only a single nucleotide difference (position 649, *E. coli* numbering) between these species. The 16S rDNA sequence of a second strain of *A. trota* (strain ATCC 49659) was determined and was found to have only one base difference (position 476) with the sequence of the type strain of *A. caviae*, thereby confirming the close genetic relatedness of these species. This result is completely at variance with the low levels of chromosomal DNA homology (30% at optimal reassociation) between *A. trota* and *A. caviae* reported by Carnahan et al. (7). The three strains of *A. salmonicida* which we examined exhibited very high levels of sequence similarity. The 16S rDNA sequences of *A. salmonicida* subsp. *achromogenes* and *A. salmonicida* subsp. *mausocida* were identical and exhibited only two base differences with the sequence of *A. salmonicida* subsp. *salmonicida*. These findings are consistent with the close genetic relatedness of these subspecies as

shown by DNA-DNA pairing data (3, 24). The 16S rDNA of *Aeromonas* sp. strain CIP 7430 was also found to be identical to the 16S rDNAs of *A. salmonicida* subsp. *achromogenes* and *A. salmonicida* subsp. *mausocida*. However, strain CIP 7430 has been placed in a different group (DNA group 2) than *A. salmonicida* (DNA group 3) on the basis of DNA-DNA pairing data (22).

A. eucrenophila (DNA group 6), *A. sobria* (DNA group 7), and unnamed *Aeromonas* species strain ATCC 35941 (enteric group 77, DNA group 11) (22) formed quite separate lines on the basis of the 16S rDNA sequence analysis results and clearly represent distinct genospecies. The 16S rDNA sequence of the type strain of *A. veronii* (DNA group 10) (16, 22) was identical to the 16S rDNA sequence of *Aeromonas* sp. strain ATCC 9071 (*A. veronii* biogroup *sobria*, DNA group 8) (22). Additional strains belonging to *A. veronii* (strain ATCC 35622) and *A. veronii* biogroup *sobria* (strain NCIMB 37) were examined, and the 16S rDNA sequence results were confirmed. However, *A. veronii* biogroup *sobria* (DNA group 8) was reported by Popoff et al. (32) to be

FIG. 5. Two diagnostic areas in the region of the 16S rDNA around position 160 which distinguish the *Aeromonas* "type" group (A) from the "Schubertii" cluster (B). The nucleotide in parentheses occurs only in *A. eucrenophila*.

distinct from, but relatively closely related to, *A. sobria* on the basis of the results of DNA-DNA pairing (ca. 60 to 65% at optimal reassociation). The sequence data are consistent with the inclusion of *A. veronii* and *A. veronii* biogroup *sobria* (DNA group 8) in a single species (2, 16, 22) and show that *A. veronii* biogroup *sobria* is not closely related to *A. sobria* (strains NCIMB 13016^T and CIP 7433^T).

A. jandaei and *A. schubertii* clearly represent distinct species on the basis of 16S rDNA sequence analysis results. These two species, together with *A. veronii*, seemed to form a separate subbranch or affiliation within the genus (Fig. 3). Support for this possible loose association of *A. jandaei*, *A. schubertii*, and *A. veronii* comes from the presence of paired signature triplets in the V2 region of the 16S rRNA (positions 154 to 156 and 167 to 165, *E. coli* numbering), which distinguishes this "Schubertii" branch from all other aeromonads (Fig. 5).

The results of chromosomal DNA-DNA pairing studies clarified the genetic interrelationships of aeromonads, but in many species there is still a poor correlation between phenotype and genotype. In this study we investigated whether small-subunit rDNA sequences could be used to discriminate among the various *Aeromonas* genospecies and to evaluate systematically congruence with DNA-DNA pairing data. Most of the genospecies which we examined had unique 16S rDNA sequences. Some of the relationships that were derived from the rDNA sequence analysis correlated well with the results of DNA-DNA pairing, but others were in marked disagreement. Bacterial species are defined on the basis of the results of chromosomal DNA hybridization; strains of the same species exhibit 70% or greater relatedness under optimal reassociation conditions, with a difference between the heterologous melting temperature and the homologous melting temperature of 0 to 5°C (40). This working definition, which has been established by extensive studies on a very wide range of bacteria, is nevertheless empirically derived, and values should not be treated as absolute or fixed. Although there is no bacterial species

definition based on sequence similarities of rRNAs (or their genes) (and absolute values for species delineation are not possible because of different rates of sequence divergence), there is good agreement between relationships or groups derived from rRNA sequencing data and relationships or groups derived from DNA-DNA pairing data (e.g., the genera *Enterococcus*, *Leuconostoc*, *Serratia*) (11, 25, 26). To our knowledge, this is the first report of a serious lack of agreement between the results of these approaches. Some of the discrepancies between rRNA and DNA-DNA pairing data may be due to a lack of synchronization between the two chronometers (i.e., the high overall conservation of the rRNA molecule may preclude the differentiation of closely related genotypes or splitting that is too fine when DNA-DNA pairing is used). A likely example of this phenomenon is *A. salmonicida* and its subspecies and DNA group 2, which exhibited high levels of rDNA sequence relatedness (no to two base differences) but were distinct on the basis of DNA-DNA pairing data (ca. 50 to 60% optimal reassociation).

In other cases the lack of agreement is far more pronounced and requires another explanation. For example, *A. caviae* and the recently described species *A. trota* have been reported to exhibit low levels of chromosomal DNA relatedness (30%), but the 16S rDNAs of these taxa exhibit only a single base difference. A similar situation exists in *A. hydrophila* and *A. media*, which are genomically different (22) but exhibit a high level of 16S rDNA homology (three base differences). In contrast, *A. sobria* (DNA group 7) and strain ATCC 9071 (*A. veronii* biogroup *sobria*, DNA group 8), which are relatively closely related on the basis of DNA-DNA reassociation data (60 to 65%), form well-separated 16S rDNA phylogenetic lines. The possibility of mislabeling of strains or contamination within our laboratory was ruled out by performing rDNA sequencing experiments with duplicate strains and/or different strains obtained from separate culture collections. Over the past 10 years 16S rRNA (and more recently rDNA) sequencing has emerged as the most powerful method for elucidating bacterial phylogeny (41). In addition, this technique is proving to be valuable in providing signatures to assist in the delineation and identification of bacterial species. In view of the increasing use of this approach in bacterial systematics and our findings, it is essential that the congruence between the groups derived from rDNA sequencing and the groups derived from chromosomal DNA-DNA hybridizations be investigated comprehensively for a wide range of bacterial taxa.

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