

## Reclassification of *Vibrio fischeri*, *Vibrio logei*, *Vibrio salmonicida* and *Vibrio wodanis* as *Aliivibrio fischeri* gen. nov., comb. nov., *Aliivibrio logei* comb. nov., *Aliivibrio salmonicida* comb. nov. and *Aliivibrio wodanis* comb. nov.

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Four closely related species, *Vibrio fischeri*, *Vibrio logei*, *Vibrio salmonicida* and *Vibrio wodanis*, form a clade within the family *Vibrionaceae*; the taxonomic status and phylogenetic position of this clade have remained ambiguous for many years. To resolve this ambiguity, we tested these species against other species of the *Vibrionaceae* for phylogenetic and phenotypic differences. Sequence identities for the 16S rRNA gene were  $\geq 97.4\%$  among members of the *V. fischeri* group, but were  $\leq 95.5\%$  for members of this group in comparison with type species of other genera of the *Vibrionaceae* (i.e. *Photobacterium* and *Vibrio*, with which they overlap in G+C content, and *Enterovibrio*, *Grimontia* and *Salinivibrio*, with which they do not overlap in G+C content). Combined analysis of the *recA*, *rpoA*, *pyrH*, *gyrB* and 16S rRNA gene sequences revealed that the species of the *V. fischeri* group form a tightly clustered clade, distinct from these other genera. Furthermore, phenotypic traits differentiated the *V. fischeri* group from other genera of the *Vibrionaceae*, and a panel of 13 biochemical tests discriminated members of the *V. fischeri* group from type strains of *Photobacterium* and *Vibrio*. These results indicate that the four species of the *V. fischeri* group represent a lineage within the *Vibrionaceae* that is distinct from other genera. We therefore propose their reclassification in a new genus, *Aliivibrio* gen. nov. *Aliivibrio* is composed of four species: *Aliivibrio fischeri* comb. nov. (the type species) (type strain ATCC 7744<sup>T</sup> = CAIM 329<sup>T</sup> = CCUG 13450<sup>T</sup> = CIP 103206<sup>T</sup> = DSM 507<sup>T</sup> = LMG 4414<sup>T</sup> = NCIMB 1281<sup>T</sup>), *Aliivibrio logei* comb. nov. (type strain ATCC 29985<sup>T</sup> = CCUG 20283<sup>T</sup> = CIP 104991<sup>T</sup> = NCIMB 2252<sup>T</sup>), *Aliivibrio salmonicida* comb. nov. (type strain ATCC 43839<sup>T</sup> = CIP 103166<sup>T</sup> = LMG 14010<sup>T</sup> = NCIMB 2262<sup>T</sup>) and *Aliivibrio wodanis* comb. nov. (type strain ATCC BAA-104<sup>T</sup> = NCIMB 13582<sup>T</sup> = LMG 24053<sup>T</sup>).

The family *Vibrionaceae* consists of a large number of ecologically diverse species (Farmer *et al.*, 2005). Members of this family are relatively easy to culture and common in marine environments, and several species are important animal pathogens. Understanding the ecology and evolution of the *Vibrionaceae* requires a taxonomy that

accurately reflects intrafamilial species relationships; however, a number of groups in the *Vibrionaceae* remain indefinitely resolved (Farmer *et al.*, 2005). One of these groups is the *Vibrio fischeri* group, which contains four species, *Vibrio fischeri* (Beijerinck, 1889), *Vibrio logei* (Harwood *et al.*, 1980), *Vibrio salmonicida* (Egidius *et al.*, 1986) and *Vibrio wodanis* (Lunder *et al.*, 2000). Previous taxonomic studies have shown that these four species are closely related to each other, but there is disagreement about the genus into which these species should be classified (Thyssen & Ollevier, 2005). Some analyses of *V. fischeri*, for example, have identified phenotypic traits (Reichelt & Baumann, 1973) or molecular characters

The GenBank/EMBL/DDBJ accession numbers for the sequences obtained in this study are EF380230–EF380261, EF667054 and EF667055, as detailed in Fig. 1.

GenBank accession numbers for 16S rRNA gene sequences and a table with complete phenotyping data are available as supplementary material with the online version of this paper.

(Baumann & Baumann, 1977) consistent with membership in *Photobacterium*, whereas other studies have placed *V. fischeri* in *Vibrio* based on other phenotypic traits (Hendrie *et al.*, 1970) or on polyphasic analysis of phenotypic and molecular characteristics (Baumann *et al.*, 1980). Recently, Thompson *et al.* (2005) suggested that the *Vibrionaceae* should be divided into four families, with the placement of *V. fischeri*, *V. logei* and *V. wodanis* in an unnamed monophyletic group, sister to the *Vibrionaceae* (which the authors suggest should consist only of genus *Vibrio*) and separate from the family 'Photobacteriaceae'. The ambiguity of the taxonomy and phylogenetic placement of the *V. fischeri* group is reflected in current species naming. *Photobacterium fischeri* and *Photobacterium logei* are synonyms of *V. fischeri* and *V. logei*, although the two species have characteristics more similar to those of *V. wodanis* and *V. salmonicida* than to those of *Photobacterium phosphoreum*, the type species of *Photobacterium*, whereas neither *V. wodanis* nor *V. salmonicida* has been considered to be a member of *Photobacterium*. The conflicting concepts regarding classification of the *V. fischeri* group indicate the need to resolve the relationship of this group relative to other species in the *Vibrionaceae*. The importance of a more definitive resolution of groups currently classified in *Vibrio* is especially evident with the advent of genome sequence analysis and subsequent attempts to interpret differences between pathogenic and non-pathogenic species in a systematic context (e.g. Ruby *et al.*, 2005).

In this study, we tested the ability of genetic loci and phenotypic traits to distinguish members of the *V. fischeri* group from representatives of *Vibrio*, *Photobacterium* and other genera in the family. Based on the results, which demonstrate that the *V. fischeri* group is distinct phylogenetically and phenotypically from other genera in the *Vibrionaceae*, we propose the establishment of a new genus, *Aliivibrio* gen. nov., for the members of the *V. fischeri* group.

First, we determined the extent of 16S rRNA gene sequence identity between members of the *V. fischeri* group and representative species of *Enterovibrio*, *Grimontia*, *Photobacterium*, *Salinivibrio* and *Vibrio*. Despite the importance of the 16S rRNA gene for bacterial taxonomy, special care is required for its use in taxonomy of the *Vibrionaceae*. Several loop regions of the 16S rRNA gene have highly variable sequences and are difficult to align objectively; at the same time, stem regions are sometimes invariant between different species. To minimize difficulties associated with 16S rRNA gene sequence comparison, we used direct optimization (Wheeler, 1996) for alignment of sequences, as implemented by the program POY (version 4.0 beta build 1822) (Varón *et al.*, 2007). The direct optimization method iteratively evaluates alignments in a phylogenetic context, and this method of analysis results in a more rigorously tested alignment; it is therefore an improvement over other alignment programs and is especially useful for sequences like those of 16S rRNA

genes in which insertion–deletion events are common and result in length differences among homologous sequences. For the analysis, we compared 16S rRNA gene sequences of 92 type strains from the *Vibrionaceae*, including the type strains of the four species of the *V. fischeri* group. Accession numbers of the 16S rRNA gene sequences used here can be found in Supplementary Table S1, available in IJSEM Online. For direct optimization, gap and nucleotide change costs were set to 1 [using the command transform((all, tcm:(1,1)))]. Ten initial trees were built using random addition sequence [build()]. Each starting tree was subjected to branch swapping, alternating subtree pruning-grafting and tree bisection-reconnection [swap()]. After 20 replicates, the shortest three trees were kept [select(best:3)] and submitted to tree fusing [fuse()] and to 100 iterations of parsimony ratcheting, with reweighting 20% of the characters [transform((all, static\_approx))] by 5 and keeping up to five trees [perturb(iterations:100, ratchet:(0.2,5), swap(trees:5))]. Tree fusing and ratcheting independently found the same shortest phylogenetic hypothesis and alignment, with an alignment length of 1397 characters (the resulting alignment and tree are available from the authors on request). After the alignment was constructed, pairwise distances between 16S rRNA gene sequences were calculated in PAUP\* (Swofford, 2003) using the Kimura two-parameter model.

High sequence identities, of 97.4% or more, were obtained for the 16S rRNA genes of the *V. fischeri* group (Table 1), indicating a close relationship among these strains. In contrast, 16S rRNA gene sequence identities between type strains of species in the *V. fischeri* group and other species of the *Vibrionaceae* were consistently lower. Specifically, sequence identities for *V. fischeri* ATCC 7744<sup>T</sup> and *P. phosphoreum* ATCC 11040<sup>T</sup> (the type species of *Photobacterium*) and *Vibrio cholerae* ATCC 14035<sup>T</sup> (the type species of *Vibrio*) are 95.4 and 94.6%, respectively. The 16S rRNA gene sequence identities of *V. fischeri* ATCC 7744<sup>T</sup> to type strains of type species of other genera in the family were below 92.3%. These results indicate that, based on 16S rRNA gene sequence identities, the four species of the *V. fischeri* group form a group within the *Vibrionaceae* that is distinct from other species in the family.

As a complement to analysis of the 16S rRNA gene, we next carried out a multilocus sequence analysis to gain insight into the phylogenetic relationship between the *V. fischeri* group and species of *Enterovibrio*, *Grimontia*, *Photobacterium*, *Salinivibrio* and *Vibrio*. Emphasis was placed on species of *Vibrio* and *Photobacterium*, to which the members of the *V. fischeri* group exhibit greater similarity. For the analysis, we used sequences of three housekeeping genes, *recA*, *rpoA* and *pyrH*, recommended by Thompson *et al.* (2005) for *Vibrionaceae* phylogeny, together with *gyrB*, the sequence of which discriminates species in the *Vibrionaceae* (Ast & Dunlap, 2005), and sequences of the 16S rRNA gene. These genes were analysed simultaneously using type strains of 43 representative species of the *Vibrionaceae*, including representatives of the five

**Table 1.** Pairwise comparison of 16S rRNA gene sequence identities between representative strains of the Vibrionaceae

Values are percentage similarity. Values in bold are sequence identities among strains of *Aliivibrio* gen. nov. 16S rRNA gene sequence identity comparisons for the full set of 92 strains examined in this study are available from the authors on request.

Strain	1	2	3	4	5	6	7	8	9	10	11	12	13	14
<b>Aliivibrio gen. nov.</b>														
1. <i>A. fischeri</i> ATCC 7744 <sup>T</sup>	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2. <i>A. logei</i> ATCC 29985 <sup>T</sup>	<b>98.0</b>	–	–	–	–	–	–	–	–	–	–	–	–	–
3. <i>A. salmonicida</i> ATCC 43839 <sup>T</sup>	<b>97.4</b>	<b>99.4</b>	–	–	–	–	–	–	–	–	–	–	–	–
4. <i>A. wodanis</i> ATCC BAA-104 <sup>T</sup>	<b>98.5</b>	<b>98.6</b>	<b>98.1</b>	–	–	–	–	–	–	–	–	–	–	–
<b>Enterovibrio</b>														
5. <i>E. corallii</i> LMG 22228 <sup>T</sup>	92.2*	91.9	91.7	92.5	–	–	–	–	–	–	–	–	–	–
6. <i>E. norvegicus</i> LMG 19839 <sup>T</sup>	92.5	92.2	91.9	92.7	96.8	–	–	–	–	–	–	–	–	–
<b>Grimontia</b>														
7. <i>G. hollisae</i> ATCC 33564 <sup>T</sup>	91.1*	91.7	91.5	91.8	95.9	95.3	–	–	–	–	–	–	–	–
<b>Photobacterium</b>														
8. <i>P. phosphoreum</i> ATCC 11040 <sup>T</sup>	95.4*	95.2	95.0	95.5	93.0	93.7	92.5	–	–	–	–	–	–	–
9. <i>P. profundum</i> JCM 10084 <sup>T</sup>	95.0	95.1	94.9	95.9	93.3	93.2	93.6	96.0	–	–	–	–	–	–
<b>Salinivibrio</b>														
10. <i>S. costicola</i> subsp. <i>alcaliphilus</i> ATCC BAA-952 <sup>T</sup>	90.7	90.4	90.2	91.1	93.6	93.3	91.9	91.0	91.3	–	–	–	–	–
11. <i>S. costicola</i> subsp. <i>costicola</i> ATCC 33508 <sup>T</sup>	90.4*	90.2	89.9	90.8	93.5	93.3	91.8	90.8	91.1	99.5	–	–	–	–
<b>Vibrio</b>														
12. <i>V. cholerae</i> ATCC 14035 <sup>T</sup>	94.6*	93.3	93.0	93.7	93.0	92.6	91.6	93.0	92.9	90.2	90.0	–	–	–
13. <i>V. harveyi</i> ATCC 14126 <sup>T</sup>	94.7	95.2	95.0	95.6	93.2	93.5	92.8	94.2	94.6	90.8	90.5	94.4	–	–
14. <i>V. splendidus</i> ATCC 33125 <sup>T</sup>	96.0	96.4	96.1	96.0	92.6	92.8	91.8	94.3	93.9	90.9	90.8	94.1	96.3	–

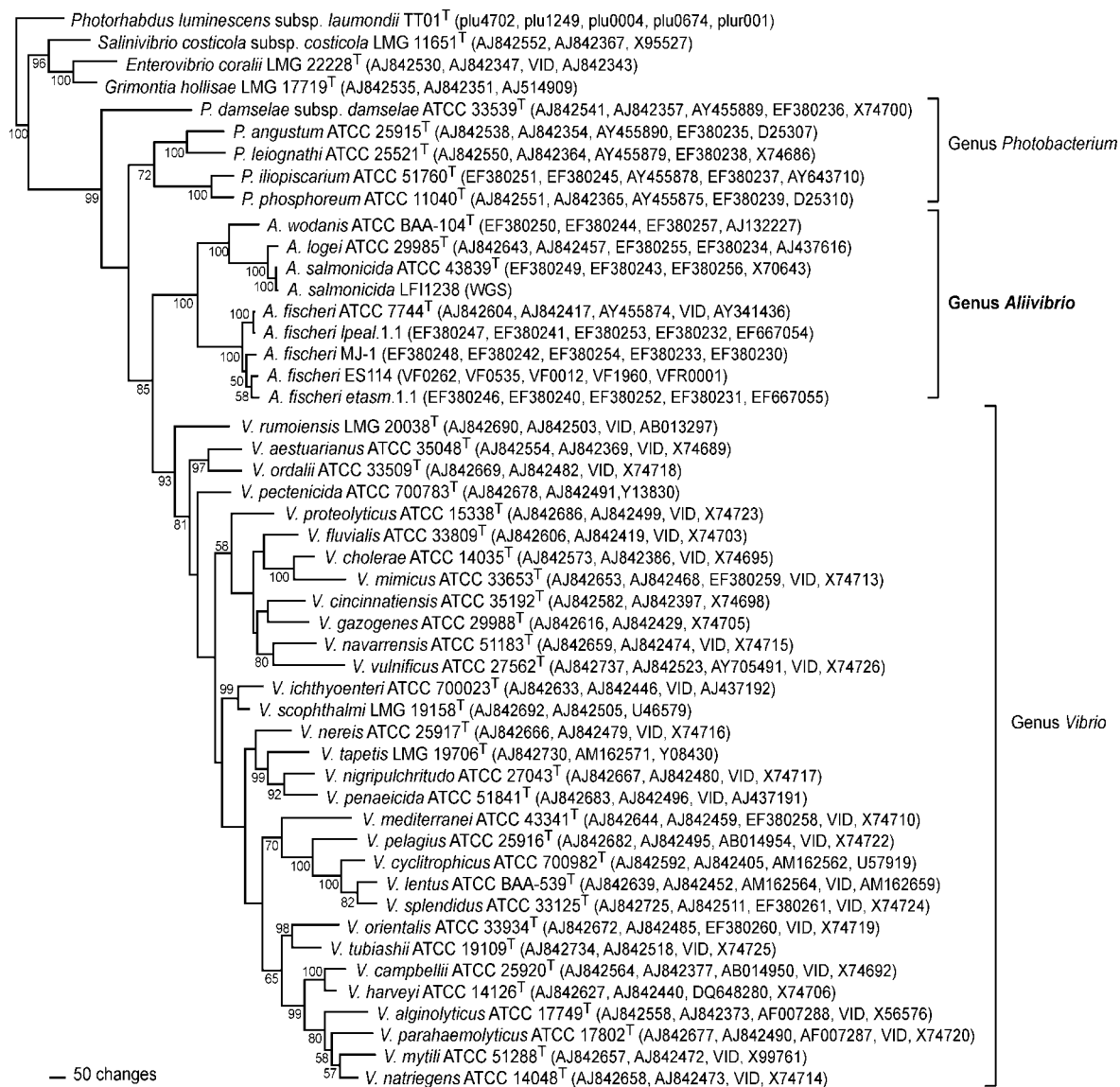
\*16S rRNA gene identities between *A. fischeri* (the type species of *Aliivibrio*) and type species of other genera of the Vibrionaceae.

well-characterized genera in the family. Because inclusion of additional taxa improves phylogenetic analysis (Graybeal, 1998; Hillis, 1998), we also included sequence data for *V. fischeri* strains ES114 (from the light organ of the sepiolid squid *Euprymna scolopes*; Ruby *et al.*, 2005), MJ-1 (from the light organ of the monocentrid fish *Monocentris japonicus*; Ruby & Nealson, 1976), *etasm.1.1* (from the light organ of the sepiolid squid *Euprymna tasmanica*; this study) and *lpeal.1.1* (from the accessory nidamental gland of the loliginid squid *Loligo pealei*; this study) and for *V. salmonicida* strain LFI1238 ([http://www.sanger.ac.uk/Projects/V\\_salmonicida/](http://www.sanger.ac.uk/Projects/V_salmonicida/)). As outgroup in the analysis, we used *Photobacterium luminescens* subsp. *laumondii* TT01<sup>T</sup> (Duchaud *et al.*, 2003), giving a total of 49 strains. PCR amplifications were done as described previously (Thompson *et al.*, 2005; Ast & Dunlap, 2005) except for *pyrH*, for which primer BPRA2966Rv (5'-GAATCGGCATTTTATGGTACAG-3') was used instead of *pyrH*-02-R. Sequencing was done by staff of the University of Michigan Sequencing Core using dye-terminator cycle sequencing on a Perkin-Elmer ABI 3730 or 3700 DNA Analyzer. Gene sequences from the five loci were concatenated and used for direct optimization analysis. Gaps and nucleotide changes were set to 1 for the 16S rRNA gene, as they were in the analysis of percentage sequence identity of the 16S rRNA gene. For the four protein-coding genes, initial gap costs were set to 2, with extension gaps and nucleotide change costs set to 1. Analysis proceeded as described above for the 16S rRNA

gene (except for the use of 35 replicates of builds and swapping instead of the 20 replicates used in the 16S rRNA gene analysis and 20% of fragments, instead of characters, were reweighted in the ratchet). A single, shortest hypothesis was found after fusing and ratcheting (see legend to Fig. 1 for tree statistics). Confidence values were obtained with 10 000 jackknife resampling replicates (Farris *et al.*, 1996) using TNT (Goloboff *et al.*, 2005).

The analysis revealed a distinct separation, with high jackknife support values, between species of the *V. fischeri* group and members of other genera in the Vibrionaceae (Fig. 1). The *V. fischeri* group forms a clade closely related to *Photobacterium* and *Vibrio* but clearly separate from both these genera and more distantly related to other genera of the Vibrionaceae. We note parenthetically here also that these relationships accord with the overlap in G+C contents between the *V. fischeri* group and *Photobacterium* and *Vibrio* and the lack of overlap in these values between the *V. fischeri* group and *Enterovibrio*, *Grimontia* and *Salinivibrio* (see Table 2). These results, which are consistent with the percentage identities in 16S rRNA gene sequences (Table 1), indicate that the members of the *V. fischeri* group form a phylogenetic lineage of the Vibrionaceae that is distinct from *Photobacterium*, *Vibrio* and other genera in the family.

To test this result, we next asked whether the members of the *V. fischeri* group could be distinguished from other genera of the Vibrionaceae by phenotypic criteria. A panel



**Fig. 1.** Phylogenetic resolution of *Aliivibrio* gen. nov. from other genera of the *Vibrionaceae*. For the analysis, sequences of five genes, *gyrB*, *rpoA*, *recA*, *pyrH* and the 16S rRNA gene, were concatenated and then aligned, giving a total of 4868 aligned characters (1539 phylogenetically informative characters). The analysis resulted in a single most-parsimonious tree. Tree length=9824, consistency index=0.480, retention index=0.570. Jackknife values are reported at nodes. See text for methodological details. Accession numbers for sequences used in the analysis are given in parentheses. Sources of sequences other than GenBank/EMBL/DBJ are indicated as follows: VID, MLSA identification page for *Vibrionaceae* (<http://www.taxvibrio.lncc.br>); WGS, *A. salmonicida* LFI1238 whole-genome sequencing project ([http://www.sanger.ac.uk/Projects/V\\_salmonicida/](http://www.sanger.ac.uk/Projects/V_salmonicida/)). For *A. fischeri* ES114 and *P. luminescens* subsp. *laumondii* TTO1<sup>T</sup>, locus tags assigned during whole-genome sequencing projects are given (see NC\_006840 and NC\_006841, and NC\_005126, respectively, at <http://www.ncbi.nlm.nih.gov/sites/entrez?db=Genome>).

of 46 standardized biochemical tests, discriminatory for species of the *Vibrionaceae* (Carson *et al.*, 2006; see references cited in Supplementary Table S2 for test methods), was used. Several of these tests were found to distinguish the *V. fischeri* group from other genera in the *Vibrionaceae* (Table 2). Furthermore, a panel of 13 tests

was identified that differentiated species within the *V. fischeri* group from each other and from species of *Photobacterium* and *Vibrio*, the two genera most closely related to the *V. fischeri* group (Table 3). For this species-level analysis, strains used in the multilocus phylogenetic analysis (Fig. 1), together with type strains of six

**Table 2.** Traits that differentiate *Aliivibrio* gen. nov. from other genera of *Vibrionaceae*

Genera: 1, *Aliivibrio*; 2, *Enterovibrio*; 3, *Grimontia*; 4, *Photobacterium*; 5, *Salinivibrio*; 6, *Vibrio*. Data for *Enterovibrio* and *Salinivibrio* were obtained from Thompson *et al.* (2002) and Huang *et al.* (2000). +,  $\geq 75\%$  of species positive; -,  $< 25\%$  of species positive; d, different reactions given by different species.

Trait	1	2	3	4	5	6
Resistance to 150 $\mu\text{g}$ O/129	-	+	-	-	-	-
Gelatinase activity	-	-	-	-	+	d
Voges-Proskauer test (acetoin)	-	-	-	+	+	-
Arginine dihydrolase	-	+	-	+	+	d
Indole production	-	+	+	-	-	d
D-Alanine utilization	-	-	+	-	+	+
DNA G+C content (mol%)	38–42	49.4–50.5	47.1–47.9	40–44	49–50.5	38.8–50.6

*Photobacterium* species and 32 *Vibrio* species and additional strains of *V. fischeri*, were examined. Discriminatory tests were identified using the GBEST algorithm, part of the PIBWin suite of tools for probabilistic identification of bacteria (Bryant, 1991, 2004). All taxa could be differentiated by at least two tests with a test difference  $\geq 70\%$  (Willcox *et al.*, 1973) (Table 3; complete results for all 46 tests can be found in Supplementary Table S2). The results of these phenotypic comparisons are consistent with the differences revealed by the 16S rRNA gene sequence identity comparison and by the multilocus phylogenetic analysis of the *recA*, *rpoA*, *pyrH*, *gyrB* and 16S rRNA genes.

Based on these results, we propose the establishment of a new genus, *Aliivibrio* gen. nov., to accommodate *Vibrio fischeri*, *Vibrio logei*, *Vibrio salmonicida* and *Vibrio wodanis*, and we propose the reclassification of these species as *Aliivibrio fischeri* comb. nov. (the type species), *Aliivibrio logei* comb. nov., *Aliivibrio salmonicida* comb. nov. and *Aliivibrio wodanis* comb. nov., respectively.

### Description of *Aliivibrio* gen. nov.

*Aliivibrio* (A.li.i.vib'ri.o. L. n. *alius* other, another; N.L. masc. n. *Vibrio* a bacterial genus name; N.L. masc. n. *Aliivibrio* the other *Vibrio*).

Gram-negative, motile, rod-shaped cells with one or more sheathed flagella. Conforms to the description of the family *Vibrionaceae*. Some strains are luminous. Oxidase-positive, fermentative and can utilize glucose as a sole carbon source; sensitive to the vibriostatic agent O/129 at 10  $\mu\text{g}$ . Grow on media with 1% (w/v) NaCl but not with 10% (w/v) NaCl. Species are arginine dihydrolase-negative, do not hydrolyse gelatin, do not form acetoin (Voges-Proskauer test) and are sensitive to novobiocin at 5  $\mu\text{g}$ . *Aliivibrio fischeri* ferments gentiobiose and is urease-positive; all other species of the genus are negative for these two characteristics. All species utilize acetate as a sole carbon source except *A. fischeri*. Species other than *A. salmonicida* have yellow–orange cell-associated pigment. DNA G+C content is between 38 and 42 mol%. Found in the marine

environment, often associated with animals; some species are mutualistic symbionts or pathogens of marine animals. Member of the *Gammaproteobacteria*. The type species is *Aliivibrio fischeri*.

### Description of *Aliivibrio fischeri* (Beijerinck 1889) comb. nov.

Basonym: *Vibrio fischeri* (Beijerinck 1889) Lehmann and Neumann 1896.

Other synonym: *Photobacterium fischeri* (Beijerinck 1889) Reichelt and Baumann 1973.

The description is the same as that given for *Photobacterium fischeri* by Reichelt & Baumann (1973) with the following additions. Negative for the Voges-Proskauer (acetoin) test, indole production, gelatinase and agarolysis. Urease-positive. Resistant to carbenicillin (100  $\mu\text{g}$ ) and ampicillin (10  $\mu\text{g}$ ). The type strain is ATCC 7744<sup>T</sup> = CAIM 329<sup>T</sup> = CCUG 13450<sup>T</sup> = CIP 103206<sup>T</sup> = DSM 507<sup>T</sup> = LMG 4414<sup>T</sup> = NCIMB 1281<sup>T</sup>.

### Description of *Aliivibrio logei* (Harwood *et al.* 1980) comb. nov.

Basonym: *Photobacterium logei* (*ex* Bang *et al.* 1978) Harwood *et al.* 1980.

Other synonym: *Vibrio logei* (Harwood *et al.* 1980) Baumann *et al.* 1981.

The description is the same as that given for *Photobacterium logei* by Bang *et al.* (1978) with the following additions. Indole-negative. Resistant to carbenicillin (100  $\mu\text{g}$ ) and ampicillin (10  $\mu\text{g}$ ) and sensitive to novobiocin (5  $\mu\text{g}$ ). The type strain is ATCC 29985<sup>T</sup> = CCUG 20283<sup>T</sup> = CIP 104991<sup>T</sup> = NCIMB 2252<sup>T</sup>.

### Description of *Aliivibrio salmonicida* (Egidius *et al.* 1986) comb. nov.

Basonym: *Vibrio salmonicida* Egidius *et al.* 1986.

**Table 3.** Phenotypic traits that differentiate species of *Aliivibrio* gen. nov., *Photobacterium* and *Vibrio*

Tests: 1, arginine dihydrolase; 2, growth on 10% NaCl; 3, indole production; 4,  $\alpha$ -D-galactosidase (4-nitrophenyl  $\alpha$ -D-galactopyranoside); 5,  $\gamma$ -glutamyl transpeptidase (L-glutamic acid 5-(4-nitroanilide)); 6, aesculin hydrolysis; 7,  $\alpha$ -ketoglutarate utilization (as sole carbon and energy source); 8, galactose utilization; 9, gluconate utilization; 10, propionate utilization; 11, sucrose utilization; 12, ampicillin resistance (10  $\mu$ g); 13, lysine decarboxylase. For the five tested *A. fischeri* strains (ATCC 7744<sup>T</sup>, ES114, MJ-1, *lpeal.1.1* and *etasm.1.1*), the percentage of strains giving a positive result is reported. +, Positive; -, negative; ND, not determined. Each test was performed twice, with the same result obtained each time. For test methods see Carson *et al.* (2006) or Supplementary Table S2.

Strain(s)	1	2	3	4	5	6	7	8	9	10	11	12	13
<i>A. fischeri</i> (5 strains)	0	0	0	0	80	100	0	80	0	0	20	100	40
<i>A. logei</i> ATCC 29985 <sup>T</sup>	-	-	-	-	+	+	+	+	+	-	-	+	+
<i>A. salmonicida</i> ATCC 43839 <sup>T</sup>	-	-	-	-	-	-	-	+	+	-	+	+	-
<i>A. wodanis</i> ATCC BAA-104 <sup>T</sup>	-	-	+	-	-	-	-	-	-	-	+	-	-
<i>P. angustum</i> ATCC 25915 <sup>T</sup>	-	-	-	-	-	ND	+	+	-	-	-	+	+
<i>P. damsela</i> subsp. <i>damsela</i> ATCC 33539 <sup>T</sup>	+	-	-	-	-	-	-	+	-	-	-	+	+
<i>P. damsela</i> subsp. <i>piscicida</i> ATCC 51736 <sup>T</sup>	+	-	-	+	-	-	-	-	-	-	-	-	-
<i>P. iliopiscarium</i> ATCC 51760 <sup>T</sup>	+	-	-	-	-	-	-	+	+	-	-	-	+
<i>P. leiognathi</i> ATCC 25521 <sup>T</sup>	+	-	-	-	-	-	-	-	+	-	-	-	-
<i>P. phosphoreum</i> ATCC 11040 <sup>T</sup>	+	-	-	+	-	-	+	+	-	-	-	-	-
<i>V. aestuarianus</i> ATCC 35048 <sup>T</sup>	+	-	+	+	-	-	+	+	+	-	+	-	-
<i>V. alginolyticus</i> ATCC 17749 <sup>T</sup>	-	+	+	-	+	-	+	-	+	+	+	+	+
<i>V. anguillarum</i> ATCC 19264 <sup>T</sup>	+	-	+	-	+	-	-	+	+	-	+	+	-
<i>V. campbellii</i> ATCC 25920 <sup>T</sup>	-	-	+	-	+	+	+	-	-	-	-	+	+
<i>V. cincinnatiensis</i> ATCC 35912 <sup>T</sup>	-	+	-	+	-	+	-	-	+	-	+	-	ND
<i>V. cyclitrophicus</i> ATCC 700982 <sup>T</sup>	+	-	-	+	+	+	+	+	+	+	+	-	-
<i>V. fluvialis</i> ATCC 33809 <sup>T</sup>	+	-	+	-	+	+	+	+	+	+	+	-	-
<i>V. gazogenes</i> ATCC 29988 <sup>T</sup>	-	+	-	+	-	+	-	-	-	+	+	-	-
<i>V. harveyi</i> ATCC 14126 <sup>T</sup>	+	-	+	+	+	+	+	+	+	+	+	+	+
<i>V. ichthyenteri</i> ATCC 700023 <sup>T</sup>	-	-	-	-	-	+	-	-	+	-	-	-	-
<i>V. lentus</i> ATCC BAA-539 <sup>T</sup>	+	-	+	-	-	+	+	-	-	-	-	-	-
<i>V. mediterranei</i> ATCC 43341 <sup>T</sup>	+	-	+	+	+	+	+	+	-	+	+	-	-
<i>V. mimicus</i> ATCC 33653 <sup>T</sup>	-	+	+	-	+	-	+	+	+	-	-	-	+
<i>V. mytili</i> ATCC 51288 <sup>T</sup>	+	+	-	+	-	+	-	-	+	+	+	-	-
<i>V. natriegens</i> ATCC 14048 <sup>T</sup>	-	+	-	+	+	+	+	+	+	+	+	-	-
<i>V. navarrensis</i> ATCC 51183 <sup>T</sup>	-	-	+	-	+	+	+	-	+	+	+	+	-
<i>V. nereis</i> ATCC 25917 <sup>T</sup>	+	-	+	-	+	-	+	-	+	+	+	-	-
<i>V. nigripulchritudo</i> ATCC 27043 <sup>T</sup>	-	-	+	+	+	+	+	+	-	+	-	-	-
<i>V. ordalii</i> ATCC 33509 <sup>T</sup>	-	-	-	-	+	-	-	-	-	-	+	+	-
<i>V. orientalis</i> ATCC 33934 <sup>T</sup>	+	-	+	-	+	-	-	-	+	-	+	-	-
<i>V. parahaemolyticus</i> ATCC 17802 <sup>T</sup>	-	+	+	-	+	+	+	+	+	+	-	+	+
<i>V. pectenicida</i> ATCC 700783 <sup>T</sup>	+	-	-	-	-	-	-	-	-	-	-	+	-
<i>V. pelagius</i> ATCC 25916 <sup>T</sup>	-	-	-	+	+	+	-	+	+	+	+	-	-
<i>V. penaeicida</i> ATCC 51841 <sup>T</sup>	-	-	-	+	-	-	+	+	+	+	-	-	-
<i>V. proteolyticus</i> ATCC 15338 <sup>T</sup>	+	+	+	-	+	-	+	-	+	+	+	+	-
<i>V. rumoiensis</i> LMG 20038 <sup>T</sup>	+	+	-	-	+	-	+	+	+	-	+	-	-
<i>V. scophthalmi</i> LMG 19158 <sup>T</sup>	-	-	-	-	-	+	-	+	+	+	+	-	-
<i>V. splendidus</i> ATCC 33125 <sup>T</sup>	+	-	+	+	-	+	+	+	-	+	-	-	-
<i>V. tapetis</i> LMG 19706 <sup>T</sup>	-	-	-	-	-	-	+	-	-	-	-	-	-
<i>V. tasmaniensis</i> LMG 20012 <sup>T</sup>	+	-	+	-	-	+	-	-	+	-	-	-	-
<i>V. tubiashii</i> ATCC 19109 <sup>T</sup>	+	-	+	+	+	+	-	+	+	+	+	-	-
<i>V. vulnificus</i> ATCC 27562 <sup>T</sup>	-	-	+	+	-	+	+	+	+	+	-	-	+

The description is the same as that given for *Vibrio salmonicida* by Egidius *et al.* (1986) with the following additions. Negative for indole production and agarolysis. Resistant to carbenicillin (100  $\mu$ g) and ampicillin (10  $\mu$ g) and sensitive to novobiocin (5  $\mu$ g). The type strain is ATCC 43839<sup>T</sup> = CIP 103166<sup>T</sup> = LMG 14010<sup>T</sup> = NCIMB 2262<sup>T</sup>.

#### Description of *Aliivibrio wodanis* (Lunder *et al.* 2000) comb. nov.

Basonym: *Vibrio wodanis* Lunder *et al.* 2000.

The description is the same as that given for *Vibrio wodanis* by Lunder *et al.* (2000) with the following additions.

Sensitive to carbenicillin (100 µg) and novobiocin (5 µg). The type strain is ATCC BAA-104<sup>T</sup> = NCIMB 13582<sup>T</sup> = LMG 24053<sup>T</sup>.

## Acknowledgements

We thank Dr Natalie Moltschanivskyj, School of Aquaculture, University of Tasmania, for providing specimens of *Euprymna tasmanica*. DNA sequencing was carried out by the staff of the University of Michigan Sequencing Core. This work was supported by grant DEB 0413441 from the National Science Foundation and by grant 01/628 from the Fisheries Research & Development Corporation in Australia.

## References

- Ast, J. C. & Dunlap, P. V. (2005). Phylogenetic resolution and habitat specificity of members of the *Photobacterium phosphoreum* species group. *Environ Microbiol* **7**, 1641–1654.
- Bang, S. S., Baumann, P. & Neilson, K. H. (1978). Phenotypic characterization of *Photobacterium logei* (sp. nov.), a species related to *P. fischeri*. *Curr Microbiol* **1**, 285–288.
- Baumann, L. & Baumann, P. (1977). Biology of the marine enterobacteria: genera *Beneckea* and *Photobacterium*. *Annu Rev Microbiol* **31**, 39–61.
- Baumann, P., Baumann, L., Bang, S. S. & Woolkalis, M. J. (1980). Reevaluation of the taxonomy of *Vibrio*, *Beneckea*, and *Photobacterium*: abolition of the genus *Beneckea*. *Curr Microbiol* **4**, 127–132.
- Beijerinck, M. W. (1889). *Le Photobacterium luminosum*, bactérie lumineuse de la Mer du Nord. *Arch Neerl Sci Exactes Nat* **23**, 401–405 (in French).
- Bryant, T. N. (1991). Software for the development and evaluation of probabilistic identification matrices. *Comput Appl Biosci* **7**, 189–193.
- Bryant, T. N. (2004). PIBWin – software for probabilistic identification. *J Appl Microbiol* **97**, 1326–1327.
- Carson, J., Higgins, M. J., Wilson, T. K., Gudkovs, N. & Bryant, T. N. (2006). *Aquatic Animal Health Subprogram. Vibrios of Aquatic Animals: Development of a National Standard Diagnostic Technology. Final report.* Project 01/628. Hobart: Fisheries Research & Development Corporation.
- Duchaud, E., Rusniok, C., Frangeul, L., Buchrieser, C., Givaudan, A., Taourit, S., Bocs, S., Boursaux-Eude, C., Chandler, M. & other authors (2003). The genome sequence of the entomopathogenic bacterium *Photorhabdus luminescens*. *Nat Biotechnol* **21**, 1307–1313.
- Egidius, E., Wiik, R., Andersen, K., Hoff, K. A. & Hjeltnes, B. (1986). *Vibrio salmonicida* sp. nov., a new fish pathogen. *Int J Syst Bacteriol* **36**, 518–520.
- Farmer, J. J., III, Janda, J. M., Brenner, F. W., Cameron, D. N. & Birkhead, K. M. (2005). Genus I. *Vibrio* Pacini 1854, 411<sup>AL</sup>. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2, part B, pp. 494–546. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.
- Farris, J. S., Albert, V. A., Källersjö, M., Lipscomb, D. & Kluge, A. G. (1996). Parsimony jackknifing outperforms neighbor-joining. *Cladistics* **12**, 99–124.
- Goloboff, P. A., Farris, J. S. & Nixon, K. C. (2005). TNT: tree analysis using new technology. Version 1.0. [http://www.zmuc.dk/public/phylogeny/TNT/Read\\_Mel.htm](http://www.zmuc.dk/public/phylogeny/TNT/Read_Mel.htm)
- Graybeal, A. (1998). Is it better to add taxa or characters to a difficult phylogenetic problem? *Syst Biol* **47**, 9–17.
- Harwood, C. S., Bang, S. S., Baumann, P. & Neilson, K. H. (1980). *Photobacterium logei* sp. nov., nom. rev.; *Beneckea nereida* sp. nov., nom. rev.; and *Beneckea gazogenes* sp. nov., nom. rev. *Int J Syst Bacteriol* **30**, 655.
- Hendrie, M., Hodgkiss, W. & Shewan, J. M. (1970). The identification, taxonomy and classification of luminous bacteria. *J Gen Microbiol* **64**, 151–169.
- Hillis, D. M. (1998). Taxonomic sampling, phylogenetic accuracy, and investigator bias. *Syst Biol* **47**, 3–8.
- Huang, C. Y., Garcia, J. L., Patel, B. K., Cayol, J. L., Baresi, L. & Mah, R. A. (2000). *Salinivibrio costicola* subsp. *vallismortis* subsp. nov., a halotolerant facultative anaerobe from Death Valley, and emended description of *Salinivibrio costicola*. *Int J Syst Evol Microbiol* **50**, 615–622.
- Lunder, T., Sørum, H., Holstad, G., Steigerwalt, A. G., Mowinckel, P. & Brenner, D. J. (2000). Phenotypic and genotypic characterization of *Vibrio viscosus* sp. nov. and *Vibrio wodanis* sp. nov. isolated from Atlantic salmon (*Salmo salar*) with 'winter ulcer'. *Int J Syst Evol Microbiol* **50**, 427–450.
- Reichelt, J. L. & Baumann, P. (1973). Taxonomy of the marine, luminous bacteria. *Arch Microbiol* **94**, 283–330.
- Ruby, E. G. & Neilson, K. H. (1976). Symbiotic association of *Photobacterium fischeri* with the marine luminous fish *Monocentris japonica*, a model of symbiosis based on bacterial studies. *Biol Bull* **151**, 574–586.
- Ruby, E. G., Urbanowski, M., Campbell, J., Dunn, A., Faini, M., Gunsalus, R., Lostroh, P., Lupp, C., McCann, J. & other authors (2005). Complete genome sequence of *Vibrio fischeri*: a symbiotic bacterium with pathogenic congeners. *Proc Natl Acad Sci U S A* **102**, 3004–3009.
- Swofford, D. L. (2003). PAUP\*: Phylogenetic Analysis Using Parsimony (\*and Other Methods), version 4.0B10. Sunderland, MA: Sinauer Associates.
- Thompson, F. L., Hoste, B., Thompson, C. C., Goris, J., Gomez-Gil, B., Huys, L., De Vos, P. & Swings, J. (2002). *Enterovibrio norvegicus* gen. nov., sp. nov., isolated from the gut of turbot (*Scophthalmus maximus*) larvae: a new member of the family Vibrionaceae. *Int J Syst Evol Microbiol* **52**, 2015–2022.
- Thompson, F. L., Gevers, D., Thompson, C. C., Dawyndt, P., Naser, S., Hoste, B., Munn, C. B. & Swings, J. (2005). Phylogeny and molecular identification of vibrios on the basis of multilocus sequence analysis. *Appl Environ Microbiol* **71**, 5107–5115.
- Thyssen, A. & Ollevier, F. (2005). Genus II. *Photobacterium* Beijerinck 1889, 401<sup>AL</sup>. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2, part B, pp. 546–552. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.
- Varón, A., Vinh, L. S., Bomash, I. & Wheeler, W. C. (2007). POY 4.0 Beta 1822. New York: American Museum of Natural History.
- Wheeler, W. (1996). Optimization alignment: the end of multiple sequence alignment in phylogenetics? *Cladistics* **12**, 1–9.
- Willcox, W. R., Lapage, S. P., Bascomb, S. & Curtis, M. A. (1973). Identification of bacteria by computer: theory and programming. *J Gen Microbiol* **77**, 317–330.