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Reanalysis of 5S rRNA Sequence Data for the Vibrionaceae with the Clustan Program Suite

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Abstract. A pooled data set of Gram-negative eubacterial, mainly Vibrionaceae, 5S rRNA sequences from MacDonell and Colwell [15] and from Erdmann et al. [10] was processed by single, average, and complete linkage procedures, as generated by the CLUSTAN program suite [23]. The hierarchical structures produced broadly resembled those for the published data: the resolution of the new genus *Shewanella* MacDonnell and Colwell 1985 [15] and an expanded definition for *Photobacterium* are supported. The new phenon representing *Listonella* [15] was not resolved, but the related species *Vibrio mimicus* and *V. cholerae* were linked by one procedure. It is suggested that recognition of synonymy between *Photobacterium angustum* and *P. leiognathi* and the incorporation of *V. anguillarum*, *V. damsela*, and *V. pelagius* into the new genus *Listonella* be held in abeyance pending supporting evidence. It is concluded that this widely available program produces analyses of 5S rRNA sequences of quality comparable to more specialized packages.

Ribosomal RNA has become an increasingly accepted vehicle for phylogenetic classification. This has been due largely to the 16S ribosomal cataloging technique [11]. This classification scheme relies, firstly, upon the existence in molecules of a neutral evolutionary process [13] and, secondly, assumes the presence of isochronic molecular clocks [22]. The ribosomal RNA molecule was selected over other molecules for its ubiquity and its relative isolation from selective evolutionary pressure [20]. The structural conservation of this molecule has been demonstrated by the functionality of hybrid ribosomes reassembled from lesser subunits of distantly related species of bacteria [6, 26]. The 16S ribosomal subunit has been chosen by some workers as a suitable vehicle for analysis in acknowledgment of susceptibility of the much shorter 5S rRNA molecule to "perturbations" and "convergent evolution" (homoplasy) [24]. Heterogeneity of 5S rRNA sequences has also been shown within strains of *Escherichia coli* [8]. The cataloguing technique, however, is an indirect assay, which relies upon selective enzymic fragmentation of the molecule and assumes that larger sequence fragments are site specific and occur only once in each molecule [24]. The shorter length of the 5S rRNA subunit, about 120 bases, compared with about 1600 in the 16S molecule, allows for a more rapid analysis of comparative sequence data.

The mutational clock of the 5S rRNA molecule has been shown by Lane et al. [14] to be slower than that of the genome. It follows that the resolution of the molecule varies in accordance with the rate of evolution and age in different groups.

Phylogenetic analyses have been produced by a variety of minimum length (parsimony) procedures, matrix methods, and by an ancestral sequence method [22]. The statistical relevance of resultant classifications has been addressed by some [7, 12, 21]; all procedures generated confidence limits that encourage conservative interpretation of the data. On the basis that strong data should generate the same classification irrespective of procedure, a commercial matrix-generating program, CLUSTAN [23], was tested for its ability to resolve, from 5S rRNA sequence data, the phylogenetic structure of the Vibrionaceae, in context with other known and unknown strains. Sequences for different strains of *Escherichia coli* and *Vibrio cholerae* were included to form cluster nuclei and gauge intraspecific variance.

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Table 1. Sequence and strain sources

Species	Strain source	[Ref]
Aeromonas hydrophila	ATCC 9071	[15]
A. media	ATCC 33097	[15]
A. salmonicida	ATCC 27013	[15]
Azotobacter vinelandii	NCIB 8789	[10]
Escherichia coli	MRE600 (1) 1	[10]
E. coli	MRE600 (2) 1	[10]
E. coli	MRE600 2	[10]
E. coli	A19 1	[10]
E. coli	A19 2	[10]
E. coli	CA265 (1)	[10]
E. coli	CA265 (2)	[10]
Photobacterium angustum	ATCC 25915	[15]
P. leiognathi	ATCC 25521	[15]
P. phosphoreum	8625	[10]
Plesiomonas shigelloides	ATCC 14029	[15]
Proteus vulgaris	Unknown	[15]
Pseudomonas aeruginosa	CLEB 481	[15]
Ps. fluorescens	ATCC 13525	[15]
Shewanella putrifaciens	ATCC 8071	[10]
S. benthica	UM140, W145	[10]
Vibrio alginolyticus	ATCC 17749	[15]
V. anguillarum	ATCC 19264	[15]
V. carchariae	ATCC 35084	[15]
V. cholerae	ATCC 14033	[15]
V. cholerae	E8498	[15]
V. cincinnatiensis	ATCC 35912	[15]
V. damsela	ATCC 33539	[15]
V. diazotrophicus	ATCC 33466	[15]
V. fischeri	ATCC 7744	[15]
V. fluvialis	ATCC 33812	[15]
V. gazogenes	ATCC 29988	[15]
V. harveyi	392	[10]
V. logei	ATCC 15382	[15]
V. marinus	ATCC 15381	[15]
V. metschnikovii	ATCC 7708	[15]
V. mimicus	ATCC 33655	[15]
V. natriegens	ATCC 14048	[15]
V. parahaemolyticus	ATCC 17802	[15]
V. pelagius	ATCC 25916	[15]
V. proteolyticus	ATCC 15338	[15]
V. psychroerythrus	ATCC 27364	[15]
V. vulnificus	ATCC 27562	[15]
Unidentified symbiont	*Calyptogena magnifica*	[10]
Unidentified symbiont	*Odontella regia*	[15]
Unidentified symbiont	*Odentella sinensis*	[15]
Unidentified symbiont	*Riftia pachyptila*	[10]
Unidentified symbiont	*Solemya velum*	[10]
Unidentified	BNL-1	[15]

Materials and Methods

5S rRNA sequence data (Table 1) from Erdmann et al. [10] and Mac Donell and Colwell [15] were pooled and synchronously aligned; the species are shown in Table 1. Unmatched or ambiguous data were scored as spaces. The bases adenine, guanine, cytosine, and uracil were numerically coded 1–4, respectively. Spaces were coded as zero. The data were analyzed by the CLUSTAN program suite (release 2.1) [23] on a Digital Equipment KL 10 computer. The Jaccard similarity coefficient was selected to operate under single, average, and complete linkage protocols.

Results

The dendrograms (Figs. 1–3) were produced by single, average, and complete linkage procedures, respectively. These show the homogeneity of the data. By complete linkage, all clusters were linked with a similarity of 78%; by average and single linkage, the similarities were 88% and 92%, respectively. *Vibrio* species, with few exceptions, were resolved by all analyses into a discrete cluster, though the arrangement of species within the group fluctuated between analyses. *Vibrio parahaemolyticus, V. natriegens*, and *V. pelagius* formed a tight sub-cluster independent of cluster method. Stable sub-clusters were also formed by *V. carchariae*, *V. diazotrophicus*, and *V. proteolyticus*. The doublets—*V. metschnikovii* with *V. fluvialis* and *V. cincinnatiensis* with *V. gazogenes*—were maintained throughout. The remaining species—*V. harveyi*, *V. vulnificus*, *Aeromonas salmonicida*, *V. alginolyticus*, *V. anguillarum*, *V. cholerae*, *V. damsela*, and *V. mimicus*—were not associated with any single species. The last five of these were linked with different species in each analysis. *Vibrio cholerae* was linked to *V. mimicus* only by average linkage. Under single and complete linkage *V. cholerae* was associated most closely with *V. harveyi* and *V. vulnificus*, respectively. *Vibrio harveyi*, by all but average linkage, was grouped with the *V. carchariae* subcluster. *Vibrio vulnificus* under single linkage was most similar to *A. salmonicida*. By average linkage it was grouped into the cluster formed by *V. cholerae* and *V. mimicus*. The species which constitute the proposed new genus *Listonella*—*V. anguillarum*, *V. damsela*, and *V. pelagius*—were never linked to each other by any protocol.

All cluster protocols resolved an identical cluster of luminescent species. The species of *Photobacterium* maintained an identical hierarchical structure. *Photobacterium angustum* and *P. leiognathi* exhibited total sequence homology and were then linked by *P. phosphoreum*. The two *Vibrio* species, *V. fischeri* and *V. logei*, remained as stable outliers to the cluster. The photobacterial group was most closely associated with the vibrio cluster only by single linkage. By other procedures the vibrio cluster was only distantly associated with the other groups.

By average and complete linkage procedures,

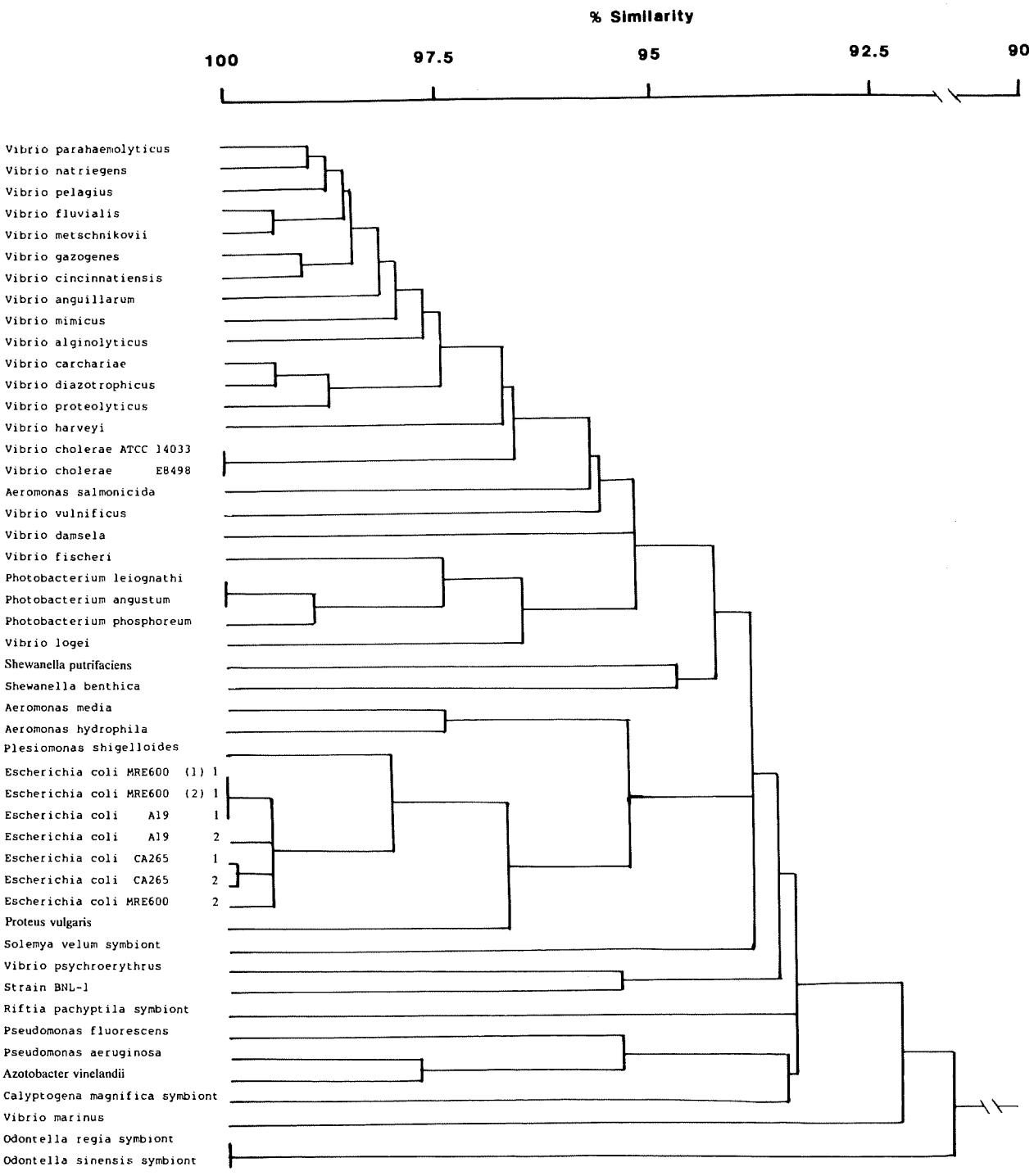

Fig. 1. Hierarchical tree for 5S rRNA sequences as rendered by CLUSTAN under single linkage regimen.

the *Shewanella* species *S. putrifaciens* and *S. benthica* were most closely related to the luminescent group. Under single linkage these species linked with the combined *Vibrio/Photobacterium* cluster. The enterobacterial species *Escherichia coli*, *Plesiomonas shigelloides*, and *Proteus vulgaris* maintained a constant spatial relationship under all of the cluster protocols. *Escherichia coli* always linked with *Pl. shigelloides* before *Pr. vulgaris*. The *Aeromonas* species *A. hydrophila* and *A. media* in all

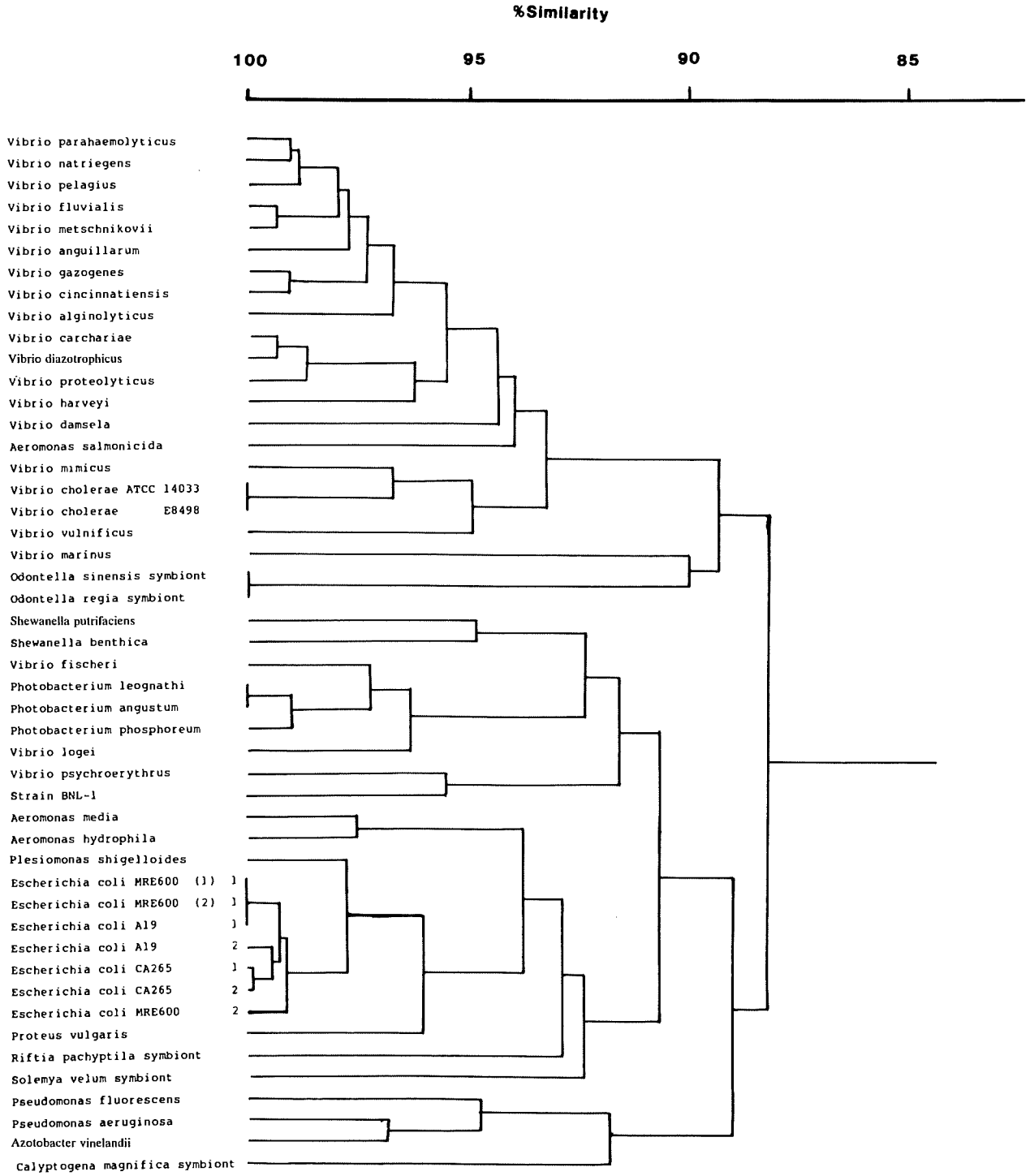

Fig. 2. Hierarchical tree for 5S rRNA sequences as rendered by CLUSTAN under average linkage regimen.

cases associated most closely to each other and then with the enterobacterial group. *Aeromonas salmonicida* was not linked to the other *Aeromonas* species by any analysis method. It was found always associated with the vibrio group.

The pseudomonad group (*Pseudomonas*

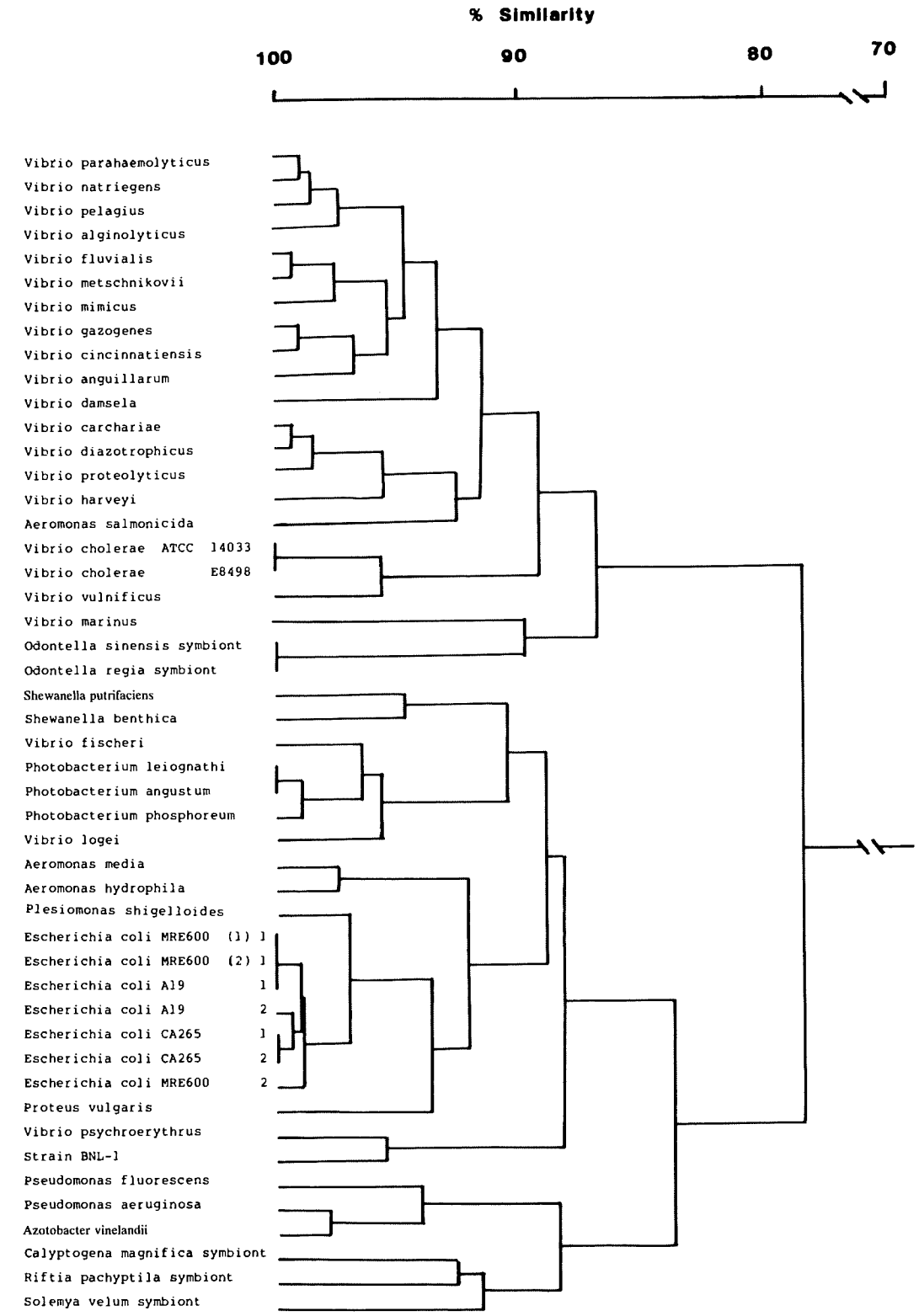

Fig. 3. Hierarchical tree for 5S rRNA sequences as rendered by CLUSTAN under complete linkage regimen.

fluorescens, *Ps. aeruginosa*, and *Azotobacter vinelandii*) was constant in structure irrespective of cluster protocol. The symbionts from *Solemya velum*, *Calyptogena magnifica*, and *Riftia pachyptila* were clustered by complete linkage into a separate group most closely associated with the pseudomonads, but by average and single linkage only the symbiont from *Calyptogena* maintained this association. The other symbionts, from *Odontella regia* and *O. sinensis*, were most closely associated with *Vibrio marinus*. This triplet formed usually at the margin of the vibrios, except by single linkage, where no association with any group was evident. The sequences for *V. psychroerythrus* and strain BNL-1 were most closely associated with the photobacterial and *Shewanalla* groups by average linkage. By single linkage there was a distant association with the combined vibrio and enterobacterial groups. Under complete linkage the association was also distant but with the enterobacterial and photobacterial groups.

The variance which was encountered within and between strains of *E. coli* was not seen in either of the two strains of *V. cholerae* or between *Photobacterium angustum* and *P. leiognathi*.

Discussion

The cluster hierachy of major taxonomic groups as rendered by CLUSTAN under single linkage was grossly in accord with the 16S rRNA cataloguing scheme of Woese et al. [25], despite its formulation from catalogs of only luminescent species. It was consistent also with the rRNA/DNA hybridization scheme of Baumann and Schubert [5]. The most notable deviation of the current scheme from those above was the inclusion of *Aeromonas salmonicida* in the vibrio group. *Aeromonas salmonicida* was shown phenotypically to be significantly different from other *Aeromonas* species [1, 19]. The taxonomic status of this species was reviewed by Austin and Allen-Austin [2], and its uncertain classification was restated. DNA hybridization studies [16], however, would seem to support the traditional scheme. The placement of *A. salmonicida* within *Vibrio* is consequently attributed to procedural "noise" or homoplasy.

The failure of *V. mimicus* and *V. cholerae* to associate in all cases despite their demonstrated close DNA pairing [9], and likewise the failure of *V. parahaemolyticus* and *V. alginolyticus* to associate directly despite an average DNA homology of 65% [5], under any regimen for this analysis or those of MacDonell and Colwell [15], strongly suggest the susceptibility of the 5S rRNA molecule to noise and homoplasy as indicated by Woese [24]. When these features of 5S rRNA are considered, it can be seen that it is beyond the scope of this molecule to independently discern relationships amongst the constituent species of the Vibrionaceae. Consequently, without support from simultaneous analysis of sequence data from *V. ordalii*, (*V. anguillarum* biogroup II), which has 75% DNA homology with *V. anguillarum* [5], the possibility that the sub-cluster generated by MacDonell and Colwell [15], and subsequently described as *Listonella*, was not an artifact has not been addressed. Additional work should also be directed toward a comparison of DNA homologies from within *Listonella* and *Vibrio*. The assignment of the species *V. anguillarum*, *V. damsela*, and *V. pelagius* into the new genus *Listonella* MacDonnel and Colwell 1985 [15] is not supported. It is recommended that the combinations *Vibrio anguillarum*, *Vibrio damsela*, and *Vibrio pelagius* be maintained, and that the genus *Listonella* should meanwhile be held in abeyance pending its evaluation by comparative procedures.

The association of *V. fischeri* and *V. logei* with *Photobacterium* in the analyses was consistent with the results of MacDonnell and Colwell [15]. Additional molecular evidence supporting this relationship has been presented [3, 4, 17]. Phenotypically, "*Vibrio*" *fischeri* has been shown to resemble *Photobacterium* species more closely than it did *Vibrio harveyii* [17] despite the presence of sheathed polar flagella.

The species *Shewanella benthica* and *S. putrifaciens* were likewise linked in all analyses. Consequently, this analysis supports the proposal [15] for the genus *Shewanella*. The synonymy of *P. angustum* and *P. leiognathi* [15] cannot be justified solely on the basis of 5S rRNA sequence homology. The results may simply reflect the only recent divergence of the two species; Reichelt et al. [18], in DNA/DNA hybridization analysis of these species, found only 57% homology. Lane et al. [14] found similar high 5S rRNA homology for more ancient species of *Thiobacillus* with low DNA homology.

The CLUSTAN program package produced results similar to the more specialized but less widely available phylogenetic programs. Inaccuracies of the resultant phylogenies may be attributable as much to the slower rate of evolution, compared with DNA, and also to noise and homoplasy in the 5S rRNA molecule as to clustering procedure.

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