APPLIED AND ENVIRONMENTAL MICROBIOLOGY, July 2007, p. 4279–4285 Vol. 73, No. 13
0099-2240/07/$08.00+0 doi:10.1128/AEM.00020-07

Multilocus Sequence Analysis Reveals that *Vibrio harveyi* and *V. campbellii* Are Distinct Species▽†

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Received 4 January 2007/Accepted 26 April 2007

Identification and classification of *Vibrio* species have relied upon band pattern methods (e.g., amplified fragment length polymorphism) and DNA-DNA hybridization. However, data generated by these methods cannot be used to build an online electronic taxonomy. In order to overcome these limitations, we developed the first standard multilocus sequence scheme focused on the ubiquitous and pathogenic *Vibrio harveyi* species group (i.e., *V. harveyi*, *V. campbellii*, *V. rotiferianus*, and a new as yet unnamed species). We examined a collection of 104 isolates from different geographical regions and hosts using segments of seven housekeeping genes. These two species formed separated clusters on the basis of *topA*, *pyrH*, *ftsZ*, and *mreB* gene sequences. The phylogenetic picture obtained by the other three loci, i.e., *gyrB*, *recA*, and *gapA*, was more complex though. *V. campbellii* appeared nested within *V. harveyi* in the *recA* trees, whereas *V. harveyi* formed a tight nested cluster within *V. campbellii* by *gapA*. The *gyrB* gene had no taxonomic resolution and grouped the two species together. The fuzziness observed in these three genes seems not be related to recombination but to low divergence due to the accumulation of only a few substitutions. In spite of this, the concatenated sequences provided evidence that the two species form two separated clusters. These clusters did not arise by recombination but by accumulation of point mutations. *V. harveyi* and *V. campbellii* isolates can be readily identified through the open database resource developed in this study (http://www.taxvibrio.lncc.br/). We argue that the species should be defined by evolutionary criteria. Strains of the same species will share at least 95% concatenated sequence similarity using the seven loci, and, most importantly, cospecific strains will form cohesive readily recognizable phylogenetic clades.

The species *Vibrio harveyi* and *V. campbellii* are widespread in the marine environment and among the main species responsible for disease in many wild and reared aquatic organisms, most notably peneid shrimp, several fish species, and mollusks (2). Luminous vibrios related to *V. harveyi* have been implicated principally in disease outbreaks in shrimp larviculture facilities and in grow-out ponds worldwide. More recently *V. harveyi* has been associated with infections in corals (20). Indeed recent studies have shown that vibrios, including the *V. harveyi* group, are abundant in the mucus of corals and may cause infections during periods of environmental imbalances (15).

In the past, the identification of *V. harveyi* and related species isolated from the marine environment has been imprecise and represents hard work as it involves performing many biochemical and/or physiological tests (14). Presumptive *V. harveyi* isolates grow on thiosulfate-citrate-bile salts-sucrose agar are motile, ferment glucose, and are oxidase positive and sensitive to the vibriostatic agent 0/129 at 150 μg. They are arginine dihydrolase negative and lysine and ornithine decarboxylase positive. Most presumptive *V. harveyi* isolates are luminescent, utilize D-gluconate, L-glutamate, D-glucuronate, heptanoate, D-galactose, and sucrose, and grow at 40°C. These isolates do not utilize L-histidine and L-arabinose (1). Notably a number of diagnostic laboratories still rely on the presumptive identification of the *V. harveyi* species group by means of phenotypic tests (1). Because *V. campbellii* and *V. harveyi* share nearly 100% 16S rRNA gene sequence similarity and around 70% DNA-DNA hybridization (DDH) similarity (14), discriminating these species remains a hard task for taxonomy. Also, infections by phages may contribute to changing phenotypes in *V. harveyi* (19).

Examples are abundant in the literature illustrating the difficulties of correctly identifying strains of *V. campbellii*, *V. harveyi*, and *V. rotiferianus* and the related species *V. alginolyticus*, *V. natriegens*, and *V. parahaemolyticus*. These studies point out several instances of ambiguous identifications, possibly due to the limitations of the identification methods. An alternative explanation for the difficulty of identifying these strains is the plasticity of the vibrio genomes with hybridization events in the marine environment, leading to soft species boundaries (5). In a most detailed study using molecular fingerprinting methods and DDH, we showed that presumptive *V. harveyi* isolates, identified by phenotypic features, belonged in fact to the species *V. campbellii* (8).

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† Supplemental material for this article may be found at http://aem.asm.org/.

▽ Published ahead of print on 4 May 2007.

Molecular fingerprinting methods (e.g., amplified fragment length polymorphism and repetitive extragenic palindrome-PCR [rep-PCR]) and DDH are very reliable for species identification, but they are restricted to a few reference laboratories and, most importantly, are not readily available through the Internet to many end users of taxonomy. Thus, researchers still lack a standard taxonomic scheme for the identification and classification of the *Vibrio harveyi* species group. An alternative to these shortcomings is the development of an electronic online taxonomy based on segments (ca. 500 nucleotides [nt]) of housekeeping genes (17).

Here we report on the first standard multilocus sequence analysis (MLSA) scheme for the *V. harveyi* species group based on sequences of genes encoding recombination repair protein (*recA*), urydilate kinase (*pyrH*), glyceraldehyde 3-phosphate dehydrogenase (*gapA*), an actin-like cytoskeleton protein (*mreB*), a cell division protein (*ftsZ*), DNA gyrase B subunit (*gyrB*), and topoisomerase I (*topA*). This standard multilocus scheme has indeed a high resolution for resolving closely related vibrio species. We show that the widespread marine bacterium *V. harveyi* and its sister species, *V. campbellii*, form separated gene clusters and that the overall clusters are not fuzzy. We suggest an alternative species definition based on gene sequence similarity. In addition, we developed an online identification system and related strain database that will allow the identification of isolates through the Internet.

MATERIALS AND METHODS

Representative strains included in this study are deposited in the BCCM/LMG Bacteria Collection (http://bccm.belspo.be/about/lmg.php) and in the Collection of Aquatic Important Microorganisms (www.ciad.mx/caim), CIAD Mazatlan (Table 1). Isolates originated from a variety of sources, places, and dates, providing a broad representation of the distribution of *Vibrio harveyi* and *V. campbellii* (Table 1). Reference and type strains were selected on the basis of previous polyphasic taxonomic analyses using rep-PCR, amplified fragment length polymorphism, and DDH data in order to represent the overall known genomic diversity of *Vibrio harveyi* and *V. campbellii*. MLSA was performed essentially as described previously by Thompson et al. (18). Genomic bacterial DNA was prepared using the Promega Wizard DNA purification kit. Approximately 100 ng of DNA was used as a PCR template for amplification of the seven genetic loci: *recA*, *pyrH*, *gapA*, *mreB*, *ftsZ*, *gyrB*, and *topA*. PCR primers are listed elsewhere (T. Sawabe, K. Kita-Tsukamoto, and F. L. Thompson, submitted for publication). Overall, the primers yielded specific amplicons of 600 to 1,200 bp at 50°C. In a few cases the annealing temperature was adjusted to provide a specific amplification. The PCR products were analyzed on a 1.5% agarose gel with a molecular weight standard for quantification of the PCR yield. The PCR products which produced a single band on agarose gels were purified using Promega gel and PCR purification kits. DNA sequencing was done by Shimadzu Biotech sequencing service (Kyoto, Japan) using a RISA384 sequencer and a Megabase 4000 system (Amersham Biosciences).

The sequences were aligned using ClustalX, and phylogenetic trees were constructed by the neighbor-joining (16) and maximum-parsimony methods (MEGA version 3.0; close neighbor interchange). The robustness of each topology was checked by 500 bootstrap replications. Trees were drawn by using MEGA version 3.0 (11).

Recombination in each gene was analyzed by SplitsTree version 4 (9); the phi statistical test (3); and the suite of tests including MaxChi2, SIscan, Bootscan, and Sawyers that are implemented in Recombination Detection Program version 2.0 (13). The GC content, index of association (Ia), and ratio of mean synonymous substitutions per synonymous site/mean nonsynonymous substitutions per nonsynonymous site (*ds*/*dn*) were calculated using the software package START, which was obtained from http://pubmlst.org/software/analysis/start/ (10). The Ia was also evaluated by the online tool Lian3.5 (http://pubmlst.org/perl/mlstanalyse/mlstanalyse.pl?site=pubmlst&page=lian&referer=pubmlst.org).

Nucleotide sequence accession numbers. The gene sequences determined in this study are deposited in the GenBank under the accession no. EF596024 to EF596735. The sequence data are also available at our website, http://www.taxvibrio.lncc.br/.

RESULTS

The genetic loci chosen in this study are typical housekeeping genes, as is evident from the very low *dn*/*ds* values (Table 2). Little gene sequence variation was observed in *ftsZ*, *gapA*, and *pyrH*. The *topA*, *pyrH*, *ftsZ*, and *mreB* trees provided clear-cut discrimination of the species *V. harveyi* and *V. campbellii*, with strains falling into quite tight clusters (see Fig. S1 in the supplementary material). These clusters shared mutual gene sequence similarities of 94 to 96% (except for *topA*, which was 90%), while strains within each species cluster had less than 1.5% gene sequence variation, with most of the substitutions located in the third position of each codon. The *topA* gene has, thus, the highest resolution, followed by *mreB*, *ftsZ*, and *pyrH*, for both species and strain differentiation. For instance, *V. harveyi* LMG 19643, LMG 19714, and 823WBZ7 were identical in all loci except in *topA*, where 823WBZ7 accumulated three silent point mutations.

On the other hand, the *recA*, *gyrB*, and *gapA* trees revealed a somewhat more complex grouping. *Vibrio campbellii* formed a cluster nested within *V. harveyi* on the basis of *recA* gene sequences, with strains sharing at least 94% similarity, whereas *V. harveyi* appeared in a tight nested cluster (99.5% sequence similarity) within *V. campbellii* by *gapA*. *V. campbellii* was more heterogeneous in the *gapA* gene, with strains grouping as low as 97.7% gene sequence similarity. These two species seem to be indistinguishable by *gyrB* sequences though. Among these three genes, *recA* has the highest resolution for differentiating isolates at the strain and species levels. Overall, *V. harveyi* strains seemed more homogeneous in all seven loci examined than *V. campbellii* strains, indicating some sort of unknown environmental selection more effectively acting on *V. harveyi*. For instance, R-623 and R-626, both isolated from cnidarians in Brazil, were nearly identical to the Japan strain 823DZ5 and to the Ecuador strain R264, respectively. On the other hand, every *V. campbellii* strain produced a new sequence type, defined by the number of alleles found in each locus (our unpublished data). The gene with the lowest discriminatory power was *gyrB*, for which *V. campbellii* CAIM 1173, CAIM 757, R300, CAIM 249, CAIM 134, CAIM 1073, CAIM 401, CAIM 372, CAIM 109, CAIM 115, and R633 had up to 99.5% sequence similarity with the type strain of *V. harveyi* and several reference strains. Indeed *Vibrio harveyi* and *V. campbellii* isolates were rather mixed on the basis of *gyrB*.

V. harveyi and *V. campbellii* formed separated phylogenetic groups when a tree was constructed using concatenated sequences (Fig. 1). This consensus tree also suggests that *V. harveyi* and *V. campbellii* form monophyletic groups, possibly with *V. harveyi* evolving from *V. campbellii*. This finding supports the notion that vibrios form species and that species are not fuzzy in nature. The analysis of concatenated sequences also revealed some interesting groups of *Vibrio harveyi*. For instance, the strains 823DZ1, 823DZ3,

TABLE 1. Strain list

Strain(s) (synonym[s])	Isolation		Host/origin
	Place	Date	
V. harveyi			
Australia			
1977 (CRC D53), 1978 (CRC D52)	Tasmania	1994	Atlantic salmon (*Salmo salar*)
1979 (99/0805-1-1), 1847 (99/0425-4)	Tasmania	1999	Abalone (*Haliotis* sp.)
1000 (02/1034)	South Australia	2002	Abalone (*Haliotis* sp.)
0436 (93/0793-1)	Tasmania	1993	Abalone (*Haliotis* sp.)
0802 (00/0163-T5b)	Tasmania	2000	Abalone (*Haliotis* sp.)
Brazil			
R-623	Praia Portinho, São Sebastião	2006	Mucus of coral (*Palithoa caribaeorum*)
R-626	Praia Preta, São Sebastião	2006	Mucus of coral (*Mussismilia hispida*)
Ecuador			
R259, R264	ND[a]	ND	Shrimp (*Penaeus* sp.)
Japan			
LMG 19643, LMG 19714	Shizuoka	1995	Diseased horse mackerel (*Trachurus japonicus*)
AS11, AS59, AS71, AS93, AS131, S20, S30, S35, S92, 913SDZ20, 818ODDZ2, 823DZ6	Kumaishi	2002	Internal organs of dead abalone (*Haliotis discus hannai*)
99WT11	Kumaishi	2003	Seawater
913BZ62	Kumaishi	2004	Seawater tank of abalone (*Haliotis discus hannai*)
720WT44	Kumaishi	2005	Seawater before outbreak
89SF200-3	Kumaishi	2005	Sand-filtrated seawater before outbreak
823BAZ5	Kumaishi	2005	Seawater tank of abalone (*Haliotis discus hannai*)
823WBZ7	Kumaishi	2005	Seawater
818ODEZ12	Okushiri	2005	Gill of abalone (*Haliotis discus hannai*)
823TEZ2, 823TEZ1, 823TDZ13	Taisei	2005	Gill of moribund abalone (*Haliotis discus hannai*)
913SDZ12, 913SDZ14, 913SDZ16, 913SDZ18	Kumaishi	2004	Internal organs of dead abalone (*Haliotis discus hannai*)
818ODDZ6, 818ODDZ8, 818ODDZ10, 818ODDZ14	Okushiri	2005	Internal organs of abalone (*Haliotis discus hannai*)
823DZ1, 823DZ2, 823DZ3, 823DZ5	Kumaishi	2005	Internal organs of abalone (*Haliotis discus hannai*)
Mexico			
CAIM 1	Mazatlan, Sinaloa	1998	Seawater from shrimp (*Litopenaeus stylirostris*) broodstock tank
CAIM 2	Mazatlan, Sinaloa	1998	Diseased shrimp nauplii (*Litopenaeus stylirostris*)
CAIM 79	Gulf of Santa Clara, Sonora	1999	Shrimp (*Litopenaeus stylirostris*) hatching system
CAIM 107	Rosario, Sinaloa	1999	Diseased shrimp (*Penaeus* sp.) hemolymph
CAIM 973	Mazatlan, Sinaloa	2004	Snapper (*Lutjanus guttatus*) kidney
CAIM 1075	Huatabampo, Sonora	2003	Oyster (*Crassostrea gigas*)
CAIM 1266	Mazatlan, Sinaloa	2004	Snapper (*Lutjanus guttatus*) liver
CAIM 1159, CAIM 1173, CAIM 1333	Mazatlan, Sinaloa	2004	Snapper (*Lutjanus guttatus*) spleen
CAIM 1508, CAIM 1511	Mazatlan, Sinaloa	2005	Diseased puffer fish (*Spheroides annulatus*), external lesion
CAIM 1510	Mazatlan, Sinaloa	2005	Diseased puffer fish (*Spheroides annulatus*), anus
CAIM 1614	San Quintin Bay, Baja California	2004	Oyster (*Crassostrea gigas*)
CAIM 1761	Mazatlan, Sinaloa	2005	Puffer fish (*Spheroides annulatus*), kidney
CAIM 1766	Mazatlan Aquarium, Sinaloa	2005	Sea horse (*Hipocampus ingens*), liver
New Zealand			
0772 (99/1052), 1976 (99/0736-4)	ND	1999	Rock lobster (*Jasus verreauxi*)
Spain			
R825, R826, R827, R828, R829, R830, R831	Mediterranean coast	2005	Internal organs or ulcers of cultured gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchis labrax*)

Continued on following page

TABLE 1—*Continued*

Strain(s) (synonym[s])	Isolation		Host/origin
	Place	Date	
United States			
LMG 4044T (CAIM 513T)	Massachusetts	1936	Dead plankton (*Talorchestia* sp.); type strain of species
LMG 7890 (CAIM 517)	Baltimore	1982	Dead brown shark (*Carcharhiunus plumbeus*)
V. campbellii			
Brazil			
R-603	Praia Grande, São Sebastião	2006	Mucus of coral (*Mussismilia hispida*)
R-644	Praia Portinho, São Sebastião	2006	Mucus of coral (*Mussismilia hispida*)
China			
CAIM 392 (STD3-1002)	Feng Cheng	ND	Diseased protozoea stage 3 shrimp (*Penaeus chinensis*) larvae
Mexico			
CAIM 4	Bay of Santa Barbara, Sonora	1998	Seawater
CAIM 109	Matatipac shrimp farm, Nayarit	1999	Shrimp (*Litopenaeus* sp.) hemolymph
CAIM 115	Santa Rosalia shrimp farm	1999	Shrimp (*Litopenaeus* sp.) hemolymph
CAIM 134	La Brecha shrimp farm, Guasave, Sinaloa	2000	Diseased shrimp (*Litopenaeus* sp.) hemolymph
CAIM 149, CAIM 150, CAIM 155	Costa Azul shrimp farm	1995	Diseased shrimp (*Litopenaeus* sp.) hemolymph
CAIM 198	Clementina shrimp farm, Sinaloa	1999	Shrimp (*Litopenaeus* sp.) hepatopancreas
CAIM 249	Costa Azul shrimp farm	1995	Diseased shrimp (*Litopenaeus* sp.) hepatopancreas
CAIM 757	Oyster culture, La Cruz, Sinaloa	2003	Oyster (*Crassostrea gigas*)
CAIM 1283, CAIM 1500	Mazatlan, Sinaloa	2004	Snapper (*Lutjanus guttatus*) liver
CAIM 1558	Oyster culture, La Cruz, Sinaloa	2004	Oyster (*Crassostrea gigas*)
CAIM 1074	Oyster culture, Huatabampo, Sonora	2003	Oyster (*Crassostrea gigas*)
Philippines			
CAIM 9	Negros Island	ND	Juvenile shrimp (*Penaeus monodon*) hepatopancreas
CAIM 372	Negros Island	ND	Lymphoid organ of a diseased shrimp (*Penaeus monodon*) juvenile
CAIM 401	Iloilo	ND	Seawater
Spain			
R300, R376	Oyster culture, Bay of Alfacs, Catalonia	2005	Oyster (*Crassostrea gigas*)
R633	Oyster culture, Bay of Fangar, Catalonia	2005	Oyster (*Crassostrea gigas*)
United States			
LMG 11216T (CAIM 519T)	Hawaii	1971	Seawater; type strain of species
Thailand			
CAIM 3	ND	ND	Diseased shrimp (*Penaeus monodon*)
V. rotiferianus			
Belgium			
LMG 21460T (CAIM 577T)	Artemia Reference Center	1999	Rotifer (*Brachionus plicantilis*) flowthrough system; type strain of species
Brazil			
R-601, R-646	Praia Portinho, São Sebastião	2006	Mucus of coral (*Mussismilia hispida*)
Mexico			
CAIM 994*	Mazatlan, Sinaloa	2004	Snapper (*Lutjanus guttatus*) kidney
1975 (99/0736-1a)	ND	1999	Snapper (*Pagrus auratus*)

[a] ND, not determined.

913SDZ12, AS11, AS59, AS71, AS93, AS131, S20, S30, and S92 represent a persistent clone, observed from 2002 until 2005, associated with mass mortalities of abalones in Japan. We also disclosed a widespread clone present in Spain and Japan, comprising 720WT44, 913SDZ12, 913SDZ14, 913SDZ18, R826, and R827. Another interesting aspect of the genetic diversity of vibrios relates to the fact that often in environmental surveys some strains appear in intermediate (taxonomic) positions and thus may be hard to identify on the basis of single genes. For instance, strain CAIM 994

TABLE 2. Summary of gene features[a]

Gene	Length (nt)	*dn*/*ds*	% GC	Nucleotide diversity/site (π)	No. of alleles	Recombination (*P*)
ftsZ	445	0.010	47.7 ± 0.6	0.027	22	0.03
topA	583	0.023	48.7 ± 0.1	0.058	49	0.009
pyrH	360	0.000	48.5 ± 0.3	0.027	22	0.04
mreB	507	0.000	49.1 ± 0.4	0.033	44	0.6
recA	498	0.002	46.2 ± 0.3	0.028	56	0.08
gyrB	596	0.000	46.7 ± 0.6	0.04	41	0.001
gapA	607	0.045	46.4 ± 0.2	0.01	20	0.12

[a] Phi recombination statistics were used (3).

had different species type strains as its closest neighbor; it showed 100% *ftsZ* and 98.4% *gapA* sequence similarity to *V. harveyi* LMG 4044^T; 96.5% *mreB* and 97% *pyrH* sequence similarity to *V. campbellii* LMG 11216^T; and 97% *recA*, 95.5% *gyrB*, and 91% *topA* sequence similarity to *V. rotiferianus* LMG 21460^T. Overall, the groups obtained by gene sequence data revealed a complete agreement with the groups obtained in previous studies using rep-PCR and DDH (7, 8) (see Fig. S2 in the supplemental material; http://www.taxvibrio.lncc.br/).

DISCUSSION

DDH has a number of limitations, but it is clear that this tool has been instrumental to prokaryotic taxonomy so far, in that it established cohesive, stable taxonomic groups, based upon their overall genomic similarity. DDH data have underpinned also the concepts for interpreting environmental microbiological sequence data such as those for the 16S rRNA gene. However prokaryotic taxonomy is in flux in the last years due to the new avenues of research opened up by whole-genome sequencing and the development of multilocus sequence typing (6). Here we found complete agreement between the DDH values and grouping obtained in previous studies (7, 8) and the gene sequence similarity values and grouping observed in the *V. harveyi* species group (Fig. 1). Strains 1000, 0772, and 1976 to 1979 form a clearly separated phylogenetic clade. These strains belong to a new species related to *V. harveyi* (J. Carson et al., unpublished data). These strains form a homogeneous group on the basis of DDH, having around 50% DDH similarity to *V. harveyi*, *V. campbellii*, and *V. rotiferianus*. It is particularly informative and reassuring to confirm that groups based on DDH correlate well with gene sequence data shown here. It is evidence of the power of MLSA as an alternative to DDH for defining the boundaries of species. *V. harveyi* and *V. campbellii* species can be defined as strains sharing around 95% gene sequence similarity in their concatenated loci. This threshold should not be taken strictly though, because it may vary as new isolates are analyzed. Eventually the significant phylogenetic clades, obtained by different tree-building methods, will equate species and will lead to an alternative bacterial species definition based on evolutionary relationships.

Much has been argued in the literature for the role of recombination in species diversification (4). We analyzed several features of our gene sequences in order to evaluate possible cases of horizontal gene transfer (HGT) that would lead to hybrid, intermediate strains, e.g., CAIM 994. The GC content of the genes is within the range observed in vibrios, suggesting no signs of HGT. The GC content alone is not a good predictor of gene transfer, as genes may undergo the process of amelioration (12). For this reason, we also analyzed the linkage disequilibrium within and between species on the basis of allelic profiles. The observed values of variance (2.00 for *V. harveyi* and 1.10 for *V. campbellii*) were always higher than the expected variance (0.96 for *V. harveyi* and 0.52 for *V. campbellii*) if the strains were experiencing free recombination. The phi recombination test (3) did point to recombination though. Recombination might have occurred in several loci, including *gyrB*, *ftsZ*, *topA*, and *pyrH*, with a high statistical significance level according to this test (Table 2), but we should look at these analyses with the greatest caution. The phi recombination test takes polymorphic sites in an alignment with opposing incongruent phylogenetic signals as breakpoints of recombination, but this assumption may lead to false positives, possibly explaining the results found here. The nucleotide substitution patterns observed in this study suggest that recombination is not the main evolutionary force leading to speciation in *V. harveyi* and *V. campbellii*. Although we cannot rule out the effect of recombination in the reassortment of genomic sequences in the *V. harveyi* group, the fuzziness in the *gyrB*, *gapA*, and *recA* trees, our data show, is due to slower molecular clocks, not to recombination. Possibly in these loci there occurred a smaller number of accumulated nucleotide substitutions, leading to a close relationship between species.

The use of MLSA has far-reaching beneficial consequences in environmental microbiology. First, researchers will be able to readily identify their isolates through the Internet using a common tool that is highly reliable taxonomically. Second, this type of portable data will allow for testing new concepts and standards for culture-dependent and -independent studies, particularly concerning the definition and diversification of species. We developed a new online identification scheme that will allow the end users of the taxonomy of vibrios to promptly identify their isolates (http://www.taxvibrio.lncc.br/). *V. harveyi* and *V. campbellii* are very intriguing animal pathogens. Apparently they are both able to cause disease in a wide range of aquatic organisms by complex mechanisms involving quorum sensing, toxin (hemolysin) production, and biofilm formation. The present study shows the usefulness of a simple electronic

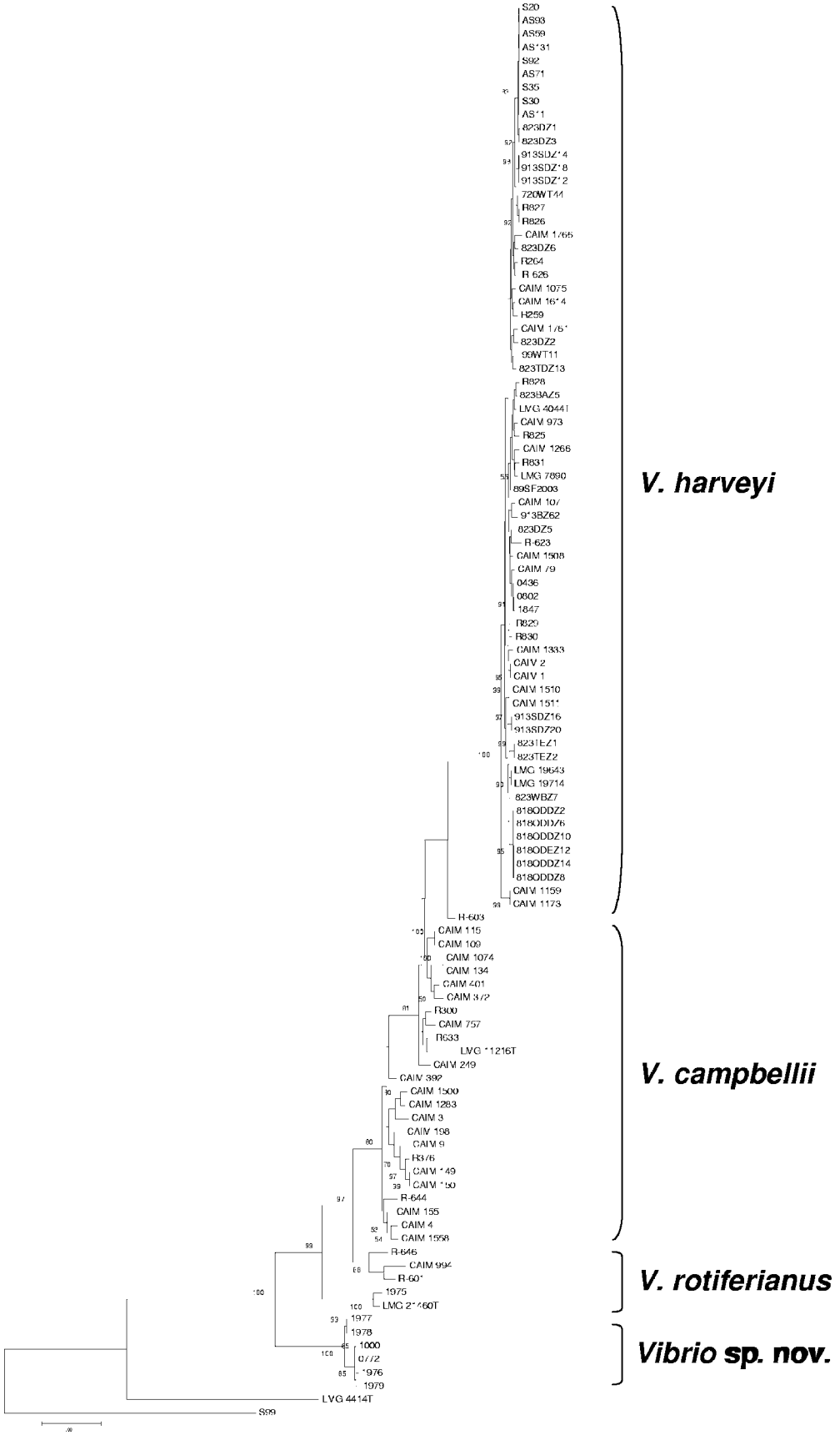

FIG. 1. Consensus maximum-parsimony phylogenetic tree based on the concatenated gene sequences of the seven loci (3,596 nt). Values of bootstrap after 500 repetitions are shown at the nodes. Tree length, 2,762. Scale bar, 100 substitutions. *Vibrio fischeri* LMG 4414T and *Photobacterium profundum* SS9 were used as out-groups.

tool for comparing vibrio strains on the molecular level which will certainly be useful for pinpointing the widespread successful clones associated with animal health worldwide.

ACKNOWLEDGMENTS

F.L.T. acknowledges young researcher grant FAPESP (no. 2004/00814-9). This work was supported by the Japan Society for the Promotion of Science, Institute of Fermentation of Osaka, Goho Life Science Foundation (Japan), and the FOSEMARNAT-2004-01-33 project (Mexico).

We thank Jeremy Carlson and Maria de Jesus Pujalte for providing some strains. We also thank Renato Camara da Silva, Vicente de Araujo Calfo, Yasuhiro Tsuruya, and Youhei Fukui for their excellent technical assistance. We thank Jean Swings for fruitful discussions. We appreciate the comments of the two referees.

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