Veronia nyctiphanis gen. nov., sp. nov., isolated from the stomach of the euphausiid *Nyctiphanes simplex* (Hansen, 1911) in the Gulf of California, and reclassification of *Enterovibrio pacificus* as *Veronia pacifica* comb. nov.

Bruno Gomez-Gil,[1]* Adrián González-Castillo,[2] Mario J. Aguilar-Méndez,[3,7] Alejandro López-Cortés,[3] Jaime Gómez-Gutiérrez,[4] Ana Roque[5], Elke Lang[6], and Julissa Enciso-Ibarra[1]

Author affiliations: [1]CIAD, A.C. Mazatlán Unit for Aquaculture and Environmental Management, A.P. 711, Mazatlán, Sinaloa, 82000, Mexico; [2]Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México (Unidad Académica Mazatlán), 82000 Mazatlán, México; [3]Laboratorio de Geomicrobiología y Biotecnología, Centro de Investigaciones Biológicas del Noroeste (CIBNOR), La Paz, BCS, 23096, Mexico; [4]Instituto Politécnico, Nacional, Centro Interdisciplinario de Ciencias Marinas, Av. IPN s/n, Col. Palo de Santa Rita 23096, La Paz, BCS, Mexico; [5]Institut de Recerca i Tecnologia Agroalimentaries, Centre d'Aquicultura, Sant Carles de la Rapita, Spain; [6]Leibniz Institute DSMZ - Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Inhoffenstr. 7B, 38124 Braunschweig Germany; [7]**Present address:** Instituto Politécnico Nacional (IPN), Unidad Profesional Interdisciplinaria de Ingeniería Campus Guanajuato (UPIIG), Silao de la Victoria, Guanajuato, 36275, Mexico.

***** Corresponding author. bruno@ciad.mx, +52 (669) 989 8700, fax +52 (669) 989 8701

Abstract

The bacterial strain 42Xb2^T was isolated from a female adult krill *Nyctiphanes simplex* infected with the apostome parasitoid ciliate *Pseudocollinia brintoni* in January 2007 in the Gulf of California. The strain has the morphological, phenotypic, and molecular characteristics of the bacteria of the family *Vibrionaceae*. The 16S rRNA gene sequence has a similarity of 97.7% with *Enterovibrio pacificus* SW014^T and 96.1% similarity with *Enterovibrio norvegicus* LMG 19839^T. A phylogenomic and a multilocus sequence analyses placed this strain close to the genera *Enterovibrio, Grimontia*, and *Salinivibrio*, but clearly forming a separate branch from these bacterial genera. Genomic analyses presented further support this result. A novel genus *Veronia* gen. nov. and a species *Veronia nyctiphanis* sp. nov. is here described with CAIM 600^T (=DSM 24592^T =CECT 7578^T) as the type strain. Morphological, physiological and genetic evidence presented here support the unification of *Enterovibrio pacificus* and *Veronia nyctiphanis* in the new genus *Veronia*. *Enterovibrio pacificus* is reclassified as *Veronia pacifica*. *V. pacifica* is assigned as the type species of the new genus *Veronia.*

Keywords: Phylogenomic, Euphausiid, *Enterovibrio, Veronia.*

Genome Sequencing Data:

The GenBank/EMBL/DDBJ accession numbers for the genome sequence of *Veronia nyctiphanis* CAIM 600^T is PEIB01 and of *Enterovibrio pacificus* CAIM 1920^T is LYBM01. The 16S rRNA gene sequence of *V. nyctiphanis* CAIM 600^T is JX129353.

Introduction

The family *Vibrionaceae* is a ubiquitous family of aquatic, *Gammaproteobacteria* that inhabit marine and estuarine ecosystems. The family embrace 11 validly named genera and 139 species divided into nine nominal genera (www.bacterio.net) and "*Corallibacterium"* currently without a standing in nomenclature [1]. *Enterovibrio, Grimontia* and *Salinivibrio* are three closely related genera that have been classified as a superclade [2], the common ancestor to these three genera might have occurred between 580 and 620 million years go [3]. The genus *Enterovibrio* was described in 2002 and currently includes five nominal species: *E. norvegicus* [4], *E. coralii* [5], *E. nigricans* [6], *E. calviensis*, originally described as *Vibrio calviensis* [7] but later reclassified [6], and *E. pacificus* [8] described in 2016. *Vibrio hollisae* was described originally as a species of the *Vibrio* genus [9], but latter reclassified as a new genus, *Grimontia* [10]. Three other species of *Grimontia* have been later described [11–13].

The krill *Nyctiphanes simplex* (Order Euphausiacea) is distributed mostly at neritic regions of the southern California Current System, Gulf of California and the northern region of the Humboldt Current System. *Nyctiphanes simplex* is the most abundant krill species in the Gulf of California and frequently forms dense aggregations and swarms that promote a diverse parasitic assemblage ranging from innocuous epibiotic phytoplankton to parasitoid apostome ciliates [14]. The apostome ciliates of the genus *Pseudocollinia* infect several krill species in the entire Northeast Pacific and may have a regulatory population effect on krill [15]. *Nyctiphanes simplex* is infected with the apostome ciliate *Pseudocollinia brintoni* and infection starts and develops rapidly in the krill's hemocoel invading the rest of the body where infection progresses until host death up three days after infection. This parasitoid ciliate is associated with a complex bacterial assemblage originating from the krill stomach [15]. The strain described in the present study was isolated from a bacterial assemblage obtained from the hemocoel of an adult female *N. simplex* host infected with *P. brintoni*.

Materials and Methods

During an oceanographic cruise (11–30 January, 2007), samples of krill were collected with zooplankton nets and analysed for the bacterial diversity associated with them. From one *N. simplex* female infected with the parasitoid ciliate *P. brintoni* [15], several bacterial strains were isolated from the stomach and incubated on Marine Agar 2216 (Difco) at 20°C and 28°C (Table S1). This zooplankton sample was collected on the 18th January, 2007 at the continental shelf off the state of Sonora, in the Gulf of

California (29° 38.22’ N, 112° 36.5’ W) at the first 15 m layer. The strain 42Xb2^T was deposited at the Collection of Important Aquatic Microorganisms (CAIM, www.ciad.mx/caim) as CAIM 600^T, at the Colección Española de Cultivos Tipo (CECT, www.cect.org) as CECT 7578^T and at the Leibniz-Institute Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, www.dsmz.de) as DSM 24592^T.

The phenotypic characterization was done according standard methods [16] and with the API 20E and API 50 CH strips (Bio-Mérieux, France). The pre-cultures were grown on TSA plus 2.0% NaCl, and a suspension in sterile saline solution (2.5% NaCl) was used for the procedure as described by the manufacturer for the API systems, except that the inoculum medium (API 50 CHE) for the API 50 CH system was adjusted to 2.5% NaCl and the test strips were not covered with mineral oil. Additional tests included the determination of temperature and salinity growth ranges, diverse biochemical responses, and the use of 25 substrates as sole carbon and energy sources [16].

Cells of strain CAIM 600^T were grown on Marine Agar 2216 for fatty acid analyses. After incubation for two days at 20 and 28 °C, the total cell fatty acid methyl esters were obtained using standard protocols [17] and separated using gas chromatography (Agilent Technologies, model 6890N). Peaks were automatically integrated and fatty acid names and relative concentrations (%) were estimated using the Microbial Identification standard software package MIDI, Sherlock version 6.1, libraries TSBA 40 and 6.

The 16S rRNA was amplified with universal primers (27F. AGA GTT TGA TCM TGG CTC AG, 1492R. TAC GGY TAC CTT GTT ACG ACT T) to obtain an almost complete sequence (>1450 bp). Amplification program was one cycle at 94° C for 2 min, 35 cycles at 94 °C for 1 min, 56 °C for 1 min, 72 °C for 1 min; and one final cycle at 72 °C for 5 min. Sequencing and PCR purification was done at Macrogen Inc. (Korea). The isolate was identified using the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net) [18] on the basis of 16S rRNA sequence data. Evolutionary analyses were conducted in MEGA7 [19].

The draft genome of CAIM 600^T was sequenced with the Ion Torrent PGM platform as described earlier [20] with minor modifications as stated elsewhere [21]. Assembly of these reads with MIX [22] produced 127 contigs (> 200 pb, N50 =127,144 bp) for a 5.29 Mb genome at a sequencing depth of 52 X. GenBank accession numbers for the genome sequences used in the present study are listed in Table S2.

Results and Discussion

The strain 42Xb2^T showed close relationship with species of the genus *Enterovibrio* of the family *Vibrionaceae* on the basis of 16S rRNA gene sequence. This strain grew as beige colonies on Marine Agar 2216 (Difco), translucid, creamy, with a continuous border. It did not grow on TCBS agar, but did grow in TSA plus 2.0% NaCl. Several phenotypic features, mainly gelatine hydrolysis, growth at 6% NaCl, and utilization of D-turanose, differentiated these strains from the other *Enterovibrio* species (Table 1).

Major fatty acids that accounted for > 1% of the total fatty acids were feature 3 including $C_{16:1}$ ω6c and $C_{16:1}$ ω7c (41.8%), $C_{16:0}$ (20.6%), $C_{18:1}$ ω7c (15.0%), $C_{12:0}$ 3-OH (6.3%), $C_{16:1}$ ω9c (3.2%), $C_{14:0}$ (2.3%), feature 2 including $C_{12:0}$-aldehyde, $C_{14:0}$ 3-OH and iso-$C_{16:1}$ I (1.9%), $C_{17:1}$ ω8c (1.8%), and $C_{18:1}$ ω9c (1.3). This fatty acid pattern had similarities with the patterns detected in other species of the genus *Enterovibrio* [4, 6] (Table S1). Characteristic for strain CAIM 600^T was the higher portion of $C_{12:0}$ 3-OH (6.3%) than those reported to in other *Enterovibrio* strains (1–5%). The fatty acids $C_{12:0}$, $C_{18:0}$ and iso-$C_{16:0}$ reported for other members of the genus *Enterovibrio* [6] were present in strain CAIM 600^T in percentages < 0.5%.

The 16S rRNA sequence tree placed this bacterial isolate in the *Enterovibrio* genus closely related to *E. pacificus* (97.7% similarity) and *E. norvegicus* (96.1%) (Fig. 1). These values are close to the 97% threshold originally proposed [23] to delimit a new species but below the now accepted values of 98.6% [24]. Genomic analyses were performed to have a higher resolution power for this description.

The similarity of the 16S rRNA gene has been used to circumscribe different genera within the family *Vibrionaceae*, the threshold proposed is 95–96% [25]. A slightly lower threshold of 94.5% was proposed as a strong indicator to delimit a genus for bacteria [26]. The results obtained in the present study agree with those limits, the 16S rRNA sequence similarity of strain CAIM 600^T with members of the closest genus *Enterovibrio* is between 94.7% and 93.9% and lower for all other genera (Table 2). *Enterovibrio pacificus* strain SW014 also showed similarity values that place it as a member of this potential new genus: 95.0–93.6% with other species of *Enterovibrio* (Table 2). The topology of the 16S rRNA tree places *Veronia* more closely related to *Enterovibrio* and *Grimontia* than to the other genera of the *Vibrionaceae* (Fig. 1).

The whole genome sequencing of strain CAIM 600^T produced 718,724 sequences (average sequence length of 188.79 bp). The 16S rRNA gene sequence obtained by Sanger sequencing (JX129353) and the

sequence derived from the WGS (PEIB01) shared a 99.8% similarity, differing in five nucleotides (ambiguities found in the sequences). Further genome purity was evaluated with GenomePeek [27]; 16S rRNA, *recA*, *rpoB*, and *groEL* genes showed only one CD-hit (100%), therefore representing a pure genome. Analysis with CheckM v1.0.7 [28] showed a completeness of 97.0%, a contamination of 0.99%, and strain heterogeneity of 0% compared to 899 genomes in the database, none of which are *Enterovibrio* or *Grimontia*.

A phylogenomic study, as suggested by Chun *et al*. [29], but with 139 single copy genes [30] was performed with Anvi'o v5.1 [31] with the genomes of the type strains of *Enterovibrio, Grimontia, Salinivibrio* and *Veronia*; *Vibrio cholerae* was used as an out-group. The maximum likelihood dendrogram (Fig. 2) clearly shows that *V. nyctiphanis* CAIM 600^T and *E. pacificus* CAIM 1920^T form a monophyletic cluster, for which the closest relatives are *Enterovibrio* and *Grimontia*. It is worth noting that *Enterovibrio* species do not form a monophyletic group but are divided in two, one with *E. corallii* and *E. nigricans*, and another with *E. norvegicus* and *E. calviensis* (Fig. 2).

Multilocus sequence analyses (MLSA) were done with the 16S rRNA, *ftsZ*, *gapA*, *gyrB, mreB, pyrH, recA, rpoA,* and *topA* complete genes using protocols previously described [2]. The dendrogram of MLSA also clearly placed the strains of *V. nyctiphanis* CAIM 600^T and *E. pacificus* CAIM 1920^T as a branch separate from the rest of the *Enterovibrio*, *Grimontia*, and *Salinivibrio* species forming a monophyletic grouping independent of these three genera (Fig. 3).

The DNA G+C content for strain CAIM 600^T obtained from the draft genome sequence was 46.0%. This value is similar to that obtained for *E. pacificus,* 45.4 mol%, but differs from the values obtained for the genera *Enterovibrio* (47 – 48 mol%), *Grimontia* (48 – 49 mol%) and *Salinivibrio* (49 mol%) (Table S3).

The Average Nucleotide Identity (ANI) values [32] among the different genera closest to *Veronia* gen. nov. differed considerably based on the values presented. The new genus showed the highest ANI values with the *Grimontia* species (70.6 – 74.0% ANIb) and the lowest with the *Enterovibrio* species (70.2 – 70.8% ANIb) (Table 3).

DNA-DNA *in silico* hybridization [33] of the new genus similarly showed low values between species of the genera *Grimontia* (21.4 – 22.7%, Table 3) and *Enterovibrio* (22.0 – 23.4%). These results further support the ANI values that delimit the species found.

The Percentage of Conserved Proteins (POCP) was calculated for the genome sequences of the genera closely related to *Veronia* [34] with CMG Biotools [35]. The 50% threshold proposed by [34] to delimit prokaryotic genera based on the POCP permitted us to clearly differentiate *Veronia* as a novel genus because it had POCP values of 27.0% – 35.1% with *Enterovibrio* genomes (Fig. S1) and 29.4% – 34.3% with *Grimontia*. The 50% threshold proposed might be too high for the genera of the family *Vibrionaceae* since species of the same genus can share as low as 48.0% of the conserved proteins, as is the case of *Enterovibrio nigricans* and *Enterovibrio norvegicus*. As reported by Qin [34], using the threshold proposed would be a suitable genomic parameter to delimit the prokaryotic genus boundary, due AAI has presented an extensive overlap in its values, causing misleading results. The type strains of *Veronia nyctiphanis* and *E. pacificus* had 49.9% POCP similarity (3,210 protein coding genes out of a total combined of 6,634, Fig. S1). *Enterovibrio* and *Grimontia* genomes share POCP values between 42.6% and 66.5%, and therefore they cannot be delimited with this methodology.

In silico phenotyping with Traitar ver. 1.1.2 [36] showed different traits for some of the genera related to *Veronia* (Fig. S2). Members of *Salinivibrio* could be differentiated from all other genera by the presence of Voges-Proskauer genes and absence of beta-galactosidase and urea hydrolysis genes (Fig. S2), *Veronia* was negative for D-mannitol and pyrrolidonyl-beta-naphthylamide whereas *Enterovibrio* and *Grimontia*, and most of the *Salinivibrio* genomes analysed were positive. No traits were found to differentiate between *Enterovibrio* and *Grimontia*. Discrepancies were observed between the *in silico* and *in vivo* phenotyping methodologies; test showing different results were D-xylose, D-mannose, D-cellobiose, D-maltose, D-trehalose, D-sucrose, ONPG, lysine decarboxylase, ornithine decarboxylase, indol, and Voges-Proskauer.

The present polyphasic taxonomic study, which involved phenotypic, genomic, phylogenomic, and phylogenetic analyses support the establishment of a novel genus *Veronia* gen. nov. and a novel species, *V. nyctiphanis* sp. nov., with CAIM 600^{T} as the type strain. Information also supports the unification of *E. pacificus* and *V. nyctiphanis* into one genus, i.e. to reclassify *E. pacificus* as *Veronia pacifica* comb. nov. In order to avoid any future confusion and respect temporal taxonomic priority, the older species, *V. pacifica* is here assigned as the type species of the genus *Veronia.*

Description of *Veronia* gen. nov.

Ve.ron'i.a. N.L. fem. n. *Veronia* named after M. Michel Véron, French microbiologist who described the *Vibrionaceae* family.

Gram-negative rod-shaped to coccoid bacterium, facultative anaerobic, motile, chemoorganotrophic. Unable to grow in TCBS medium, in Marine Agar does not produce pigments. Oxidase positive, arginine dehydrolase negative, lysine and ornithine decarboxylase negative. DNA G+C content is 45.4 – 46.0 mol%. The most abundant fatty acids are $C_{16:1}$ ω6c and ω7c, $C_{18:1}$ ω7c, $C_{16:0}$ and $C_{12:0}$ 3-OH. Slightly halophilic, growth in 2.5% and 3% NaCl but not at 0 and 8% NaCl. Mesophilic, growth occurs between 10 and 30°C. The range of estimated genome sizes based on draft genome sequencing is between 5.18 Mb and 5.29 Mb. Type species is *Veronia pacifica* comb. nov.

Description of *Veronia pacifica* comb. nov. (Basonym *Enterovibrio pacificus* LIU *ET AL*. 2016)

Veronia pacifica (pa.ci'fi.ca. L. fem. adj. *pacifica*, peaceful, referring to the Pacific Ocean from where the type strain was isolated).

The description of *V. pacifica* is as the one was given by Liu *et al*. (2016) for the basonym *E. pacificus* [8] with the following amendments: The DNA G+C content of the type strain is 45.4 mol%, its genome size is 5.29 Mbp. The type strain SW014^T (=KCTC 42425^T =MCCC 1K00 500^T =CAIM 1920^T) was isolated from surface seawater of the South Pacific Gyre. The 16S rRNA accession number of the type strain is KP216206 and the genome assembly code is LYMB01.

Description of *Veronia nyctiphanis* sp. nov.

V. nyctiphanis (nyc.ti.pha'nis. N.L. gen. n. *nyctiphanis *of the krill genus *Nyctiphanes*).

Motile, distal flagellated, Gram negative rod-shaped to coccoid bacterium, 0.7 μm width and 1.0 μm in length; positive for oxidase and catalase. No growth in TCBS medium was observed. Sensitive to the vibriostatic agent 0/129 at 10 and 150 μg. Accumulation of polyhydroxybutyrate (PHB) was not observed. Positive reactions were observed for citrate, D-glucose, gelatinase, nitrate reduction, and Voges-Proskauer in the API 20E strip. Positive fermentation of D-glucose and weak fermentation of D-

turanose, fumarate, glycerol, and N-acetylglucosamine was observed with the API 50 CH test strip. Negatives for API 20E and API 50 CH are listed in Table S4.

Negative utilization of 2-ketoglutarate, acetate, citrate, D,L-lactate, D-galacturonate, D-gluconate, D-glucosamine, D-glucuronate, L-alanine, L-aspartate, L-glutamate, L-glutamine, L-histidine, L-leucine, L-lysine, L-ornithine, L-threonine, malate, p-hydroxybenzoate, propionate, putrescine, pyruvate, succinate, γ-aminobutyrate was observed in corresponding media.

The main (> 10%) fatty acids present are $C_{16:1}$ ω 6c and ω7c, $C_{18:1}$ ω 7c, $C_{16:0}$ and $C_{12:0}$ 3-OH. The DNA G+C content of the type strain is 46.0 mol%, its genome size is 5.18 Mbp.

The type strain is *V. nyctiphanis* CAIM 600^T (=DSM 24592^T =CECT 7578^T) isolated from a female krill *Nyctiphanes simplex*.

The 16S rRNA accession number of the type strain is JX129353 and the genome assembly code is PEIB01.

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Conflict of interest

The authors of this paper deny any financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

Author's contribution statement

All authors contributed to the study conception and design. Bruno Gomez-Gil, Adrián González-Castillo and Julissa Enciso-Ibarra performed material preparation, data collection and analysis. Conceptualization was performed by Mario J. Aguilar-Méndez; Visualization was performed by Alejandro López-Cortés; Data Curation was performed by Jaime Gómez-Gutiérrez; Methodology and Validation were performed by Ana Roque and Elke Lang. Bruno Gomez-Gil wrote the first draft of the manuscript and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Table 1 Phenotypic differences between *Veronia nyctiphanis* sp. nov., *Veronia pacifica* comb. nov. and the species of *Enterovibrio* and *Grimontia.*

1, *Veronia nyctiphanis* sp. nov. CAIM 600^T; **2**, *Veronia pacifica* SW014^T[8]; **3**, *Grimontia celer* CECT 9029^T[12]; **4**, *Grimontia hollisae* ATCC 33564^T[9]; **5**, *Grimontia indica* AK-16^T[13]; **6**, *Grimontia marina* IMCC5001^T[11]; **7**, *Enterovibrio calviensis* CAIM 595^T[6-7] and present study; **8**, *Enterovibrio coralii* CAIM 912^T[5] and present study; **9**, *Enterovibrio nigricans* CAIM 661^T [6] and present study; and **10**, *Enterovibrio norvegicus* CAIM 430^T[4] and present study.

Tests	1	2	3	4	5	6	7	8	9	10
Growth on TCBS	-	-	+	-	ND	-	+	+	+	+
Pigment production	-	+	-	-	ND	ND	-	-	+	-
Indol production	-	-	+	+	+	-	-	+	-	+
β-galactosidase (ONPG)	-	+	+	-	+	+	+	+	-	+
Gelatinase	+	+	+	-	+	+	-	-	-	-
Citrate	-	-	+	-	ND		-	+	-	-
Growth at										
6% NaCl	-	+	+	+	+	+	+	+	+	+
4ºC	w	-	-	ND	-	-	w	-	-	+

Utilization of										
D-fructose	-	-	+	ND	ND	+	+	+	+	+*
D-mannitol	-	+	+	-	-		+	(+)	(-)	+
D-mannose	-	-	+	+	ND	+	+	+	+	+*
D-melibiose	-	-	+	-	ND	-	-	+	-	-
D-turanose	w	+	ND	ND	ND	+	-	-*	-	-

+ positive, - negative, (+) most strains positive, (-) most strains negative, v variable results between the strains, w positive but weak result, ND not determined,

*discrepancies exist between the literature and the test done in this study.

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Table 2 16S rRNA sequence similarity values (%) within species of *Veronia* gen. nov. and with validly named species of genera of the family *Vibrionaceae*, including the not-yet validated name. Analysis include nominal species plus the not yet validated *"Corallibacterium"*.

Genera and references	No. species	*Veronia nyctiphanis* CAIM 600^T		*Veronia pacifica* SW014^T	
		Max.	Min.	Max.	Min.
Aliivibrio [37]	6	91.3	88.6	91.1	88.1
Catenococcus [38]	1	89.7		90.0	
"Corallibacterium" [1]	1	87.5		87.1	
Echinimonas [39]	1	87.6		87.9	
Enterovibrio [4]	4	94.7	93.9	95.0	93.6
Grimontia [10]	4	93.4	93.2	94.2	93.6
Paraphotobacterium [40]	1	93.1		93.1	
Photobacterium [41]	36	95.1	89.0	94.4	89.3
Salinivibrio [42]	9	91.6	90.1	91.7	89.2
Thaumasiovibrio [43]	2	93.6	91.9	93.5	92.5
Vibrio [44]	131	93.8	86.7	93.6	86.8

Table 3 Results of Average Nucleotide Identity (ANIb) and digital DNA-DNA hybridization (GGDC) calculations (with OAT v0.93.1, formula 2) between traits of the genera more closely related to *Veronia* gen. nov. obtained from *Grimontia*, and *Enterovibrio.*

1, *Veronia nyctiphanis* gen. nov. sp. nov. CAIM 600^T (PEIB01); **2**, *G. celer* CECT 9029^T (FIZX01); **3**, *G. hollisae* CIP 101886^T (ADAQ01); **4**, *G. indica** AK16 (ANFM01); **5**, *G. marina* CECT 8713^T (FIZY01); **6**, *V. pacifica* CAIM 1920^T (LYBM01); **7**, *E. calviensis* DSM 14347^T (JHZA01); **8**, *E. coralii* CAIM 912^T (LNTY01); **9**, *E. nigricans* CAIM 661^T (FUXU01); **10**, *E. norvegicus* DSM 15893^T (FOWE01). Strain names are reported here as originally published. *species without standing in nomenclature.

		OrthoANIb									
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
	1		71.1	71.3	71.1	71.1	74.0	70.6	70.8	70.6	70.6
	2	22.2		79.1	88.8	87.4	70.8	74.6	75.4	74.6	74.0
	3	21.7	19.4		79.2	78.9	71.1	74.6	75.2	74.5	74.1
G	**4**	22.5	10.9	19.2		88.5	70.8	74.6	75.6	74.7	74.2
G	**5**	21.7	12.0	19.2	10.8		70.6	74.5	75.4	74.7	74.0
D	**6**	21.6	22.6	21.4	22.7	22.1		70.3	70.6	70.3	70.2
C	**7**	23.0	22.1	22.2	22.1	22.1	22.8		74.4	74.3	76.8
	8	22.0	21.0	21.3	20.8	20.8	22.4	21.9		79.8	74.3
	9	22.5	21.7	22.0	21.7	21.6	22.7	22.0	19.2		74.2
	10	23.4	21.9	22.5	21.6	21.9	23.0	19.2	21.6	21.7	

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Fig. 1 16S rRNA evolutionary relationships of type strains of the family *Vibrionaceae* as inferred using the Neighbour-Joining method (bootstrap test 1000 replicates, percentage values above 60% are denoted). The evolutionary distances were computed using the Jukes-Cantor method and are in the units of the number of base substitutions per site (scale). All positions containing gaps and missing data were eliminated. There were a total of 1500 positions in the final dataset. *Escherichia coli* was used as out-group. *species without standing in nomenclature.

Fig. 2 Maximum likelihood tree (Jones-Taylor-Thorton model [45]) constructed with the amino acid sequences of 130 single-copy genes [30] of type strains of the *Vibrionaceae*. In parenthesis, NCBI assembly accession code. Scale, substitutions per site.
Amino acid sequences were obtained with Anvi'o v5.1 [31], aligned with Muscle [46], ML tree constructed with FastTree v2.1.7 [47], and rendered with FigTree v1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/). *species without standing in nomenclature.

Fig. 3. Split tree confidence (95 %) network with bootstrap support (1000 replications) based on the concatenated complete genes (*ftsZ*, *gapA*, *gyrB*, *mreB*, *pyrH*, *recA*, *rpoA*, *topA*, and the 16S rRNA gene) of representative species of the family *Vibrionaceae*. Scale, base substitutions per site. Tree characteristics: NeighborNet, uncorrected-P and equal angle. Concatenated sequences were aligned with MAFFT v7.215 [48], uninformative nucleotides were removed with Gblocks v0.91 [49], the tree was constructed with SplitTree4 v4.14.6 [50]. *species without standing in nomenclature.

Figure 1

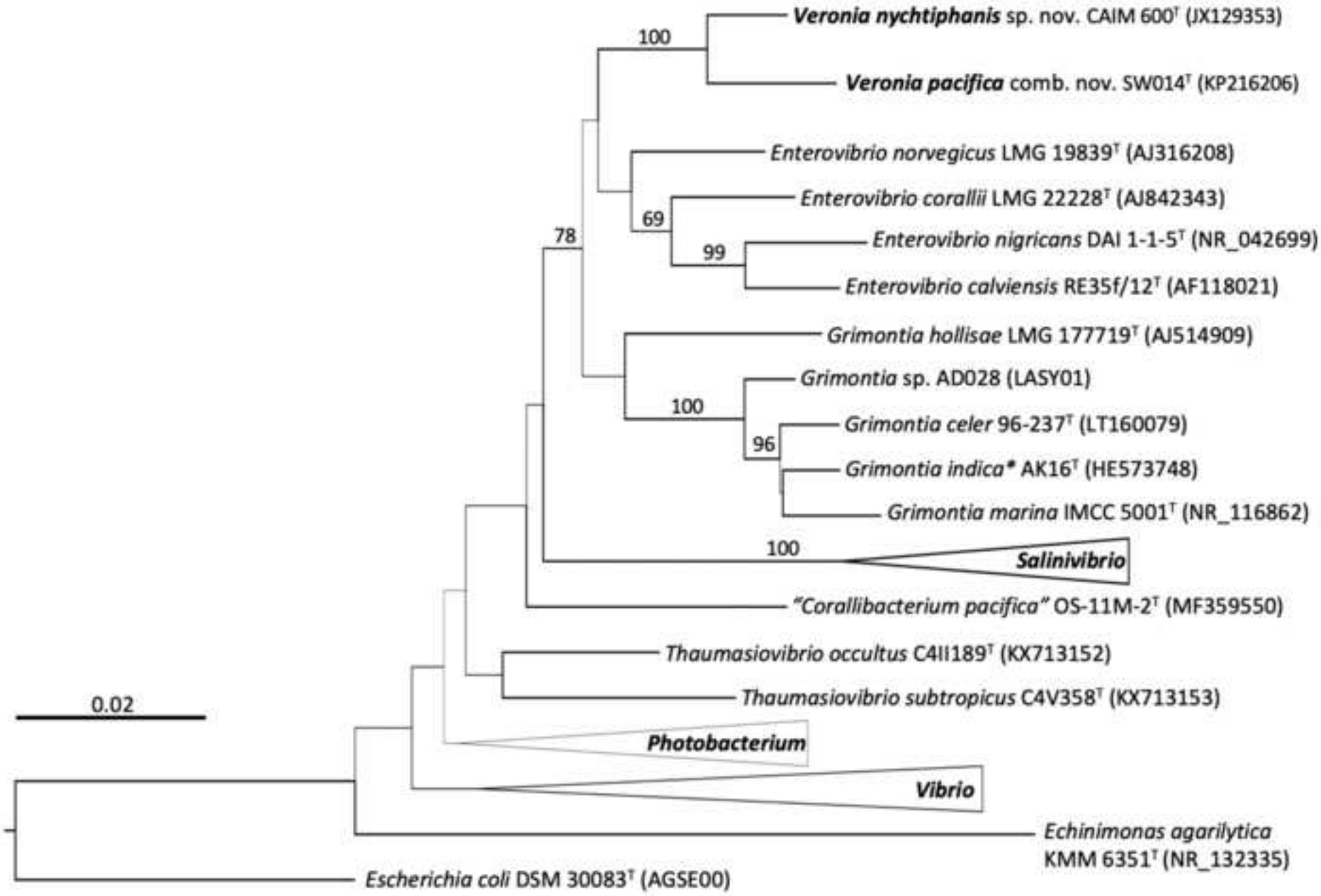

Veronia nychtiphanis sp. nov. CAIM 600T (JX129353)
Veronia pacifica comb. nov. SW014T (KP216206)
Enterovibrio norvegicus LMG 19839T (AJ316208)
Enterovibrio corallii LMG 22228T (AJ842343)
Enterovibrio nigricans DAI 1-1-5T (NR_042699)
Enterovibrio calviensis RE35f/12T (AF118021)
Grimontia hollisae LMG 177719T (AJ514909)
Grimontia sp. AD028 (LASY01)
Grimontia celer 96-237T (LT160079)
Grimontia indica* AK16T (HE573748)
Grimontia marina IMCC 5001T (NR_116862)
Salinivibrio
"Corallibacterium pacifica" OS-11M-2T (MF359550)
Thaumasiovibrio occultus C4II189T (KX713152)
Thaumasiovibrio subtropicus C4V358T (KX713153)
Photobacterium
Vibrio
Echinimonas agarilytica KMM 6351T (NR_132335)
Escherichia coli DSM 30083T (AGSE00)
100
69
78
99
100
96
100
0.02

Figure 2

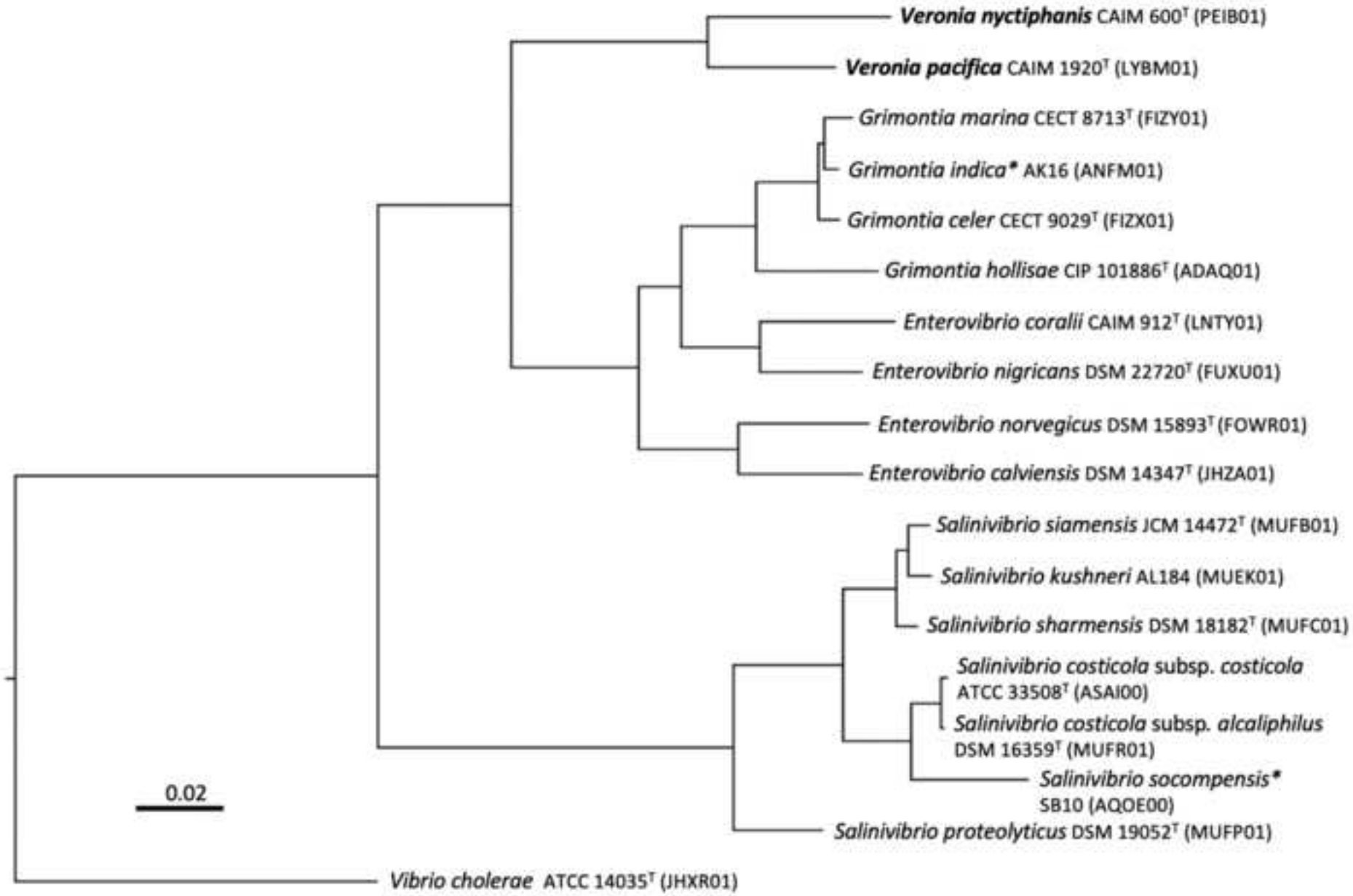
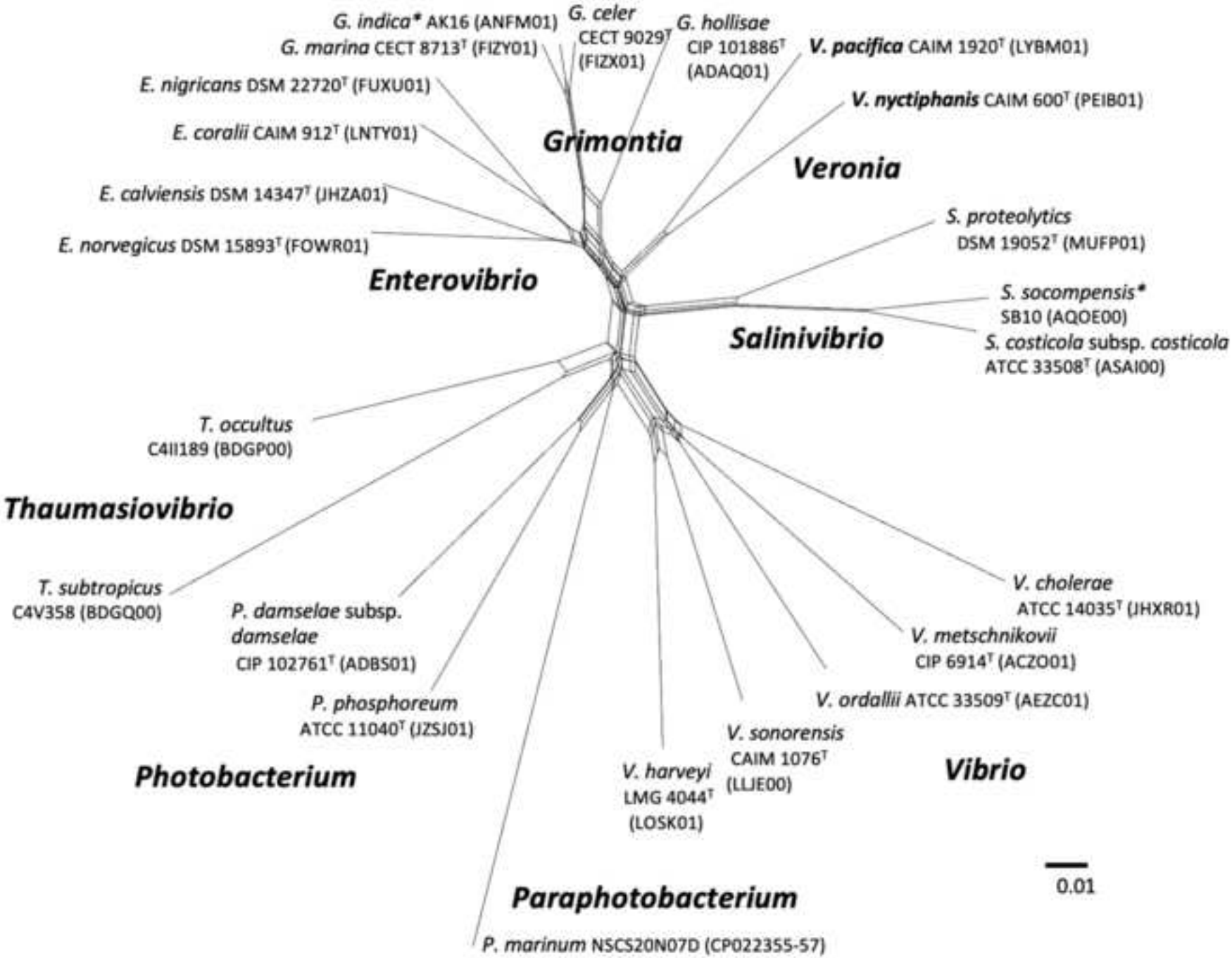

Veronia nyctiphanis CAIM 600T (PEIB01)
Veronia pacifica CAIM 1920T (LYBM01)
Grimontia marina CECT 8713T (FIZY01)
Grimontia indica* AK16 (ANFM01)
Grimontia celer CECT 9029T (FIZX01)
Grimontia hollisae CIP 101886T (ADAQ01)
Enterovibrio coralii CAIM 912T (LNTY01)
Enterovibrio nigricans DSM 22720T (FUXU01)
Enterovibrio norvegicus DSM 15893T (FOWR01)
Enterovibrio calviensis DSM 14347T (JHZA01)
Salinivibrio siamensis JCM 14472T (MUFB01)
Salinivibrio kushneri AL184 (MUEK01)
Salinivibrio sharmensis DSM 18182T (MUFC01)
Salinivibrio costicola subsp. costicola ATCC 33508T (ASAI00)
Salinivibrio costicola subsp. alcaliphilus DSM 16359T (MUFR01)
Salinivibrio socompensis* SB10 (AQOE00)
Salinivibrio proteolyticus DSM 19052T (MUFP01)
0.02
Vibrio cholerae ATCC 14035T (JHXR01)

Figure 3


G. indica* AK16 (ANFM01)
G. marina CECT 8713T (FIZY01)
G. celer CECT 9029T (FIZX01)
G. hollisae CIP 101886T (ADAQ01)
V. pacifica CAIM 1920T (LYBM01)
V. nyctiphanis CAIM 600T (PEIB01)
E. nigricans DSM 22720T (FUXU01)
E. coralii CAIM 912T (LNTY01)
Grimontia
Veronia
E. calviensis DSM 14347T (JHZA01)
E. norvegicus DSM 15893T (FOWR01)
S. proteolytics DSM 19052T (MUFP01)
Enterovibrio
S. socompensis* SB10 (AQOE00)
S. costicola subsp. costicola ATCC 33508T (ASAI00)
Salinivibrio
T. occultus C4II189 (BDGP00)
Thaumasiovibrio
T. subtropicus C4V358 (BDGQ00)
P. damselae subsp. damselae CIP 102761T (ADBS01)
V. cholerae ATCC 14035T (JHXR01)
V. metschnikovii CIP 6914T (ACZO01)
P. phosphoreum ATCC 11040T (JZSJ01)
V. ordallii ATCC 33509T (AEZC01)
V. sonorensis CAIM 1076T (LLJE00)
V. harveyi LMG 4044T (LOSK01)
Photobacterium
Vibrio
0.01
Paraphotobacterium
P. marinum NSCS20N07D (CP022355-57)