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**Title**: *Thaumasiovibrio occultus* gen. nov., sp. nov. and *Thaumasiovibrio subtropicus* sp. nov. within the family *Vibrionaceae*, isolated from coral reef seawater off Ishigaki Island, Japan

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**Abstract**

Two phylogenetically distinct *Vibrionaceae* strains C4II189$^T$ and C4V358$^T$ isolated from the reef seawater off Ishigaki Island, Japan, in 2014 were studied based on advanced genome based taxonomy. All aspects of phylogenetic (16S rRNA phylogeny, MLSA), phenotypic and genetic (ANI, DDH, AAI, and no. of core genes) cohesions between the two *Thaumasiovibrio* spp. were high enough to propose them as a new genus in the *Vibrionaceae*. Here, we propose an eighth genus *Thaumasiovibrio* gen. nov. with two new species of *T. occultus* sp. nov. strain C4II189$^T$ (=DSM 101554$^T$=JCM 31629$^T$) (type species) and *T. subtropicus* sp. nov. strain C4V358$^T$ (=DSM 101555$^T$=JCM 31630$^T$), in the family *Vibrionaceae*. *Thaumasiovibrio* species are phylogenetically distinct from the other *Vibrionaceae* species based on *pyrH* gene sequences. The combination of catalase negative, sensitive to the vibriostatic agent O/129, and green colony on TCBS for the phylogenetically affiliated strains are the current diagnostic features for the tentative identification of this genus.

**Keywords:** *Vibrionaceae*, *Thaumasiovibrio*, new genus, genomic taxonomy, coral reef, seawater

*Footnote: Nucleotide sequence accession number*

The genome data for C4II189$^T$ and C4V358$^T$ were deposited in DDBJ/EMBL/GenBank under the accession numbers BDGP01000001-BDGP01000230, and BDGQ01000001-BDGQ01000271, respectively. The 16S rRNA gene sequences of C4II189$^T$ and C4V358$^T$ were deposited in GenBank under KX713152, and KX713153.

*Abbreviations*: ANI (average nucleotide identity); AAI (average amino acid identity); MLSA (multilocus sequence analyses); DDH (DNA-DNA hybridization); thiosulphate-citrate-bile-sucrose (TCBS)

Bacterial systematics has become a dynamic scientific area integrating classical experimental observations of morphological, biochemical, physiological and genetic features, and state-of-art technologies involving molecular phylogeny and genomics [2,8-9,13-19,21-22]. Two recent landmarks which could strongly affect bacterial systematics are 1) maturation of species threshold boundary based on genome sequence comparison [9,13-14,19,21], and 2) a proposal of rational taxonomic boundaries for high taxa of bacteria and archaea based on the 16S rRNA gene sequences [14,22]. Due to these efforts, we have met not only well-accepted criteria combinations for species delineation with 95-96% ANI of shared genes and 98.7% of 16S rRNA gene sequence identity but also 94.5%, 86.5%, 82.0%, 78.5%, and 75.0% threshold for high taxa of genus, family, order, class, and phylum based on nearly complete 16S rRNA gene sequences [14,22]. Several efforts to find better definite standards of genome cohesions in higher taxonomic ranks have also been made in addition to ANI and the 16S rRNA phylogeny. Genome wide AAI [9,15] could be a better candidate, e.g. 66-86% of genome wide AAI value observed in the transient zone for genus-family boundaries [9].

The family '*Vibrionaceae*' is one of the first bacterial families where advanced microbial genome taxonomy has been applied [1,4-5,7,16-19]. *Vibrionaceae* species are also defined as a group of strains that share >95% MLSA identity and super-tree analysis, >96% AAI, ≤ 10 genome signature dissimilarity [5,17,19]. Currently, the family *Vibrionaceae* contains seven genera consisting of over 160 species [5]. The 8-genes MLSA succeeded in delineating 23 clades, a *Salinivibrio-Grimontia-Enterovibrio* super-clade and four orphan clades [1,17], some of which correspond to "genus" such as the *Fischeri* clade to be the genus *Aliivibrio* [20]. Today the family *Vibrionaceae* has also become an effective taxon for testing thresholds of higher taxa such as the genus boundary testing with multiples species.

In the extensive assessments of vibrio diversity correlated with the coral health in Ishigaki, Japan, two *Vibrionaceae* strains of a quite unique lineage based on *pyrH* gene sequences were isolated [3]. In this study, we tested these strains to fulfill the monophyly, genetic and phenotypic coherences in *Vibrionaceae*, and finally we propose *Thaumasiovibrio* gen. nov. for these strains. Here, phylogenetic, genetic, and phenotypic features of two new species, *Thaumasiovibrio occultus* sp. nov. and *T. subtropicus* sp. nov. are provided.

*Thaumasiovibrio* strains C4II189$^T$ and C4V358$^T$ isolated using TCBS medium (Nissui Pharmacy, Tokyo, Japan) from coral reef seawater off Ishigaki Island, Okinawa, Japan were used [3]. The seawater samples were taken from Osaki (24°25.4178' N 124°04.4892' E, 3 m depth) and Iriomote (24°23.208' N 123°55.681' E, 6 m depth) sites by SCUBA diving. These strains were stored at -80°C in 20% glycerol-supplemented ZoBell broth.

16S rRNA gene amplification, sequencing and analyses were performed according to a standard protocol previously described [1,3] using the full dataset included 16S rRNA gene sequences of 157 known *Vibrionaceae* species and *Escherichia coli* K-12 (see supplemental materials for materials and methods in details). The molecular phylogenetic analysis of two novel strains C4II189$^T$ and C4V358$^T$ revealed a tight clustering with each other apart from any known species and genera of *Vibrionaceae* (Fig. 1). The 16S rRNA gene similarity between them was 95.7%, well below the threshold (98.7%) for species delineation [7,13-14,22]. *Vibrionaceae* species which gave the highest similarity were *P. panuliri* (KC990827) (96.9%) followed by *P. aquimaris* (NR_114269) (96.4%), and *P. jeanii* (GU065210) (96.2%) for C4II189$^T$, and that for C4V358$^T$ was C4II189$^T$ (95.6%) followed by *P. jeanii* (95.4%), and *P. damselae* subsp. *damselae* (AB032015) (95.2%). The phylogenetic position of the two strains seems to branch at

the deeper position of the cluster consisting of three genera: *Enterovibrio*, *Grimontia*, *Photobacterium*, and *Salinivibrio* separated from the clusters of genera *Aliivibrio*, *Paraphotobacterium*, *Vibrio* and the *Candidatus* "Photodesmus" (Fig. 1).

The 16S rRNA gene phylogeny was unlikely to be sufficient to demonstrate monophyly of the family *Vibrionaceae*. We performed the 8-genes MLSA to clarify whether *Thaumasiovibrio* strains C4II189$^T$ and C4V358$^T$ form a clade in the same manner previously described [16-17]. The MLSA showed these two strains formed a distinct clade separated from not only all other known clades but also to any genera in the family *Vibrionaceae* (Figs. 2 and S1). A significant branching of the *Thaumasiovibrio* clade from the *Enterovibrio-Grimontia-Salinivibrio* super-clade [17] was also observed. The average amino acid identity of 8-genes between the C4II189$^T$ and C4V358$^T$ was 95.5%. The value is within the range of the two *Thaumasiovibrio* spp. forming the clade (>92%) [17].

As genomic ANI of 95% corresponds to DDH values of 70% [6], two genome-based tools, ANI and *in silico* DDH, are currently common indexes in bacterial taxonomy [11-13]. We used all available genome sequences of type strains of representative species of *Enterovibrio*, *Grimontia*, *Salinivibrio*, and *Photobacterium* (25 species in total). ANI values (%) were calculated using C4II189$^T$ and C4V358$^T$ genomes against genomes of type strains of each genera listed in Table 1 using the orthANI program [10]. The *in silico* DDH values were calculated using the same data set for ANI calculation with the Genome-to-Genome Distance calculator (GGDC 2.1) [12]. The ANI values of the two *Thaumasiovibrio* species against the representative species of different genera of *Vibrionaceae* family (Table 1) showed insufficient cut-off levels (>95%) for species delineation [6,13-14]. Similarly, the *in silico* DDH values of the *Thaumasiovibrio* species against

above mentioned 25 species were sufficiently lower than the delineation threshold (>70%) to propose new bacterial species (Table 1) [13]. These findings strongly suggests that C4II189$^T$ and C4V358$^T$ are new species in *Vibrionaceae*. The determined DNA G+C content based on draft genomes (*in silico*) of these *Thaumasiovibrio* strains were 49.4% and 47.2% for C4II189$^T$ and C4V358$^T$, respectively. *Thaumasiovibrio* spp. could be defined as a high G+C clade in *Vibrionaceae* accompanied by the *Enterovibrio-Grimontia* and *Salinivibrio* clades (Table 2). The estimated genome sizes of *Thaumasiovibrio* spp. based on the draft genome sequencing were 4.5 Mb to 5.4 Mb. These two genetic features could also differentiate from that of the *Paraphotobacterim* species (30.7% G+C content and 2.5 Mb genome). The AAI values could provide a better measurement of evolutionary relatedness [9]. Species belonging to the *Rosenbergii* clade of *Photobacterium* showed slightly higher AAIs against the *Thaumasiovibrio* spp. compared to the other species (Table 1). The relatedness to the *Rosenbergii* clade species was also observed in the MLSA network (Fig. 2). So we further determined the experimental DDH against type strains belonging to the *Rosenbergii* clade species (e.g. *P. rosenbergii*, *P. lutimaris*) of which genome sequences are not currently available in public databases, and the results indicated that C4II189$^T$ and C4V358$^T$ were separate species different from any known related species in *Vibrionaceae* (Table S1) [12].

The differentiating characteristics of these novel bacteria, in particular compared to the *Rosenbergii* clade species, were observed in pigmentation, growth temperature range, growth NaCl range, oxidase, catalase, acetoin production, indole production, amylase, gelatinase, nitrate reduction, utilization of 39 carbon sources (Tables 2 and S2). Among 88 phenotypic traits tested, 85% were similar between the two *Thaumasiovibrio* species. The similarity was likely to be high enough to propose a phenotypic cohesion of two *Thaumasiovibrio* species comparing those

similarities against *Photobacterium*, *Enterovibrio*, *Grimontia*, and *Salinivibrio* (below 72%). Increasing number of *Vibrionaceae* species rapidly reduces effective diagnostic features of the genus definition, and even eight major features reported previously cannot be reliably used to delineate the genera of *Vibrionaceae* today (Table 2). Negative catalase activity of isolates showing green colony on TCBS seemed to be the only common feature of the two *Thaumasiovibrio* species currently differentiating the other genera (Tables 2 and S3). The low resistance to active oxygen species may have caused the delay in the discovery of the *Thaumasiovibrio* species until now. These *Thaumasiovibrio* possessed typical fatty acid biosynthesis (Fab) gene sets closely similar to those of *E. coli* which produces $C_{16:0}$, $\Delta$9-$C_{16:1}$ and $\Delta$11-$C_{18:1}$ as majors [11].

The phylogenetic and phenotypic cohesions of two *Thaumasiovibrio* species could support the proposal for the eighth genus in the family *Vibrionaceae*. In fact, the inter-genus similarities based on 16S rRNA gene sequences between *Thaumasiovibrio* and the other genera were below 94.7%, which fulfilled a recently reported taxonomic thresholds (minimum sequence identity 94.8%, threshold sequence identify 94.5%) for "genus" delineation of *Bacteria* and *Archaea* [14,22] (Table 3). In addition to this, the genome wide AAI [9] between two *Thaumasiovibrio* spp. is 68.9%, of which value was >2.9% higher than the other type strains of the representative genera (Table 1). As the lowest intra-genus and the highest inter-genus AAIs were 66.6% and 65.1%, the genus threshold based on AAI for *Vibrionaceae* could be found around 65-66% (Fig. S2). The number of core genes defined as >90% amino acid identity and alignment between two *Thaumasiovibrio* spp. was 328, higher than those (51-120) of the representative strains of the related genera of *Enterovibrio*, *Grimontia*, *Photobacterium*, and *Salinivibrio* (Fig. 2), which

could also support the two *Thaumasiovibiro* species as being genetically more coherent than the other genera.

In conclusion, we propose *Thaumasiovibrio occultus* gen. nov. sp. and *T. subtropicus* sp. nov. as unusual rare vibrios in the marine tropical environments in Ishigaki, Japan. Unique phylogenetic features of these proposed novel bacteria, disclosed by this study would provide new insights not only for '*Vibrionaceae*' taxonomy and ecophysiology but also for reef system-*Vibrionaceae* interactions.

**Description of *Thaumasiovibrio* gen. nov. (Thau.ma.si.o.vi'bri.o. Gr. adj. thaumasios, unusual, unique; N.L. masc. n. vibrio, a motile bacterium; N.L. masc. n. *Thaumasiovibrio*, an unusual bacterium)**

Bacteria are Gram-negative, facultatively anaerobic, motile rod. Sodium ions are essential for growth. No endospores are formed. The single polar flagellation is observed when the bacterium is cultivated on solidified and/or liquid media. No swarming is observed. Green colonies are observed on TCBS. The bacterium is grown in 1% to 3% NaCl broth. Negative for lysine decarboxylase, arginine dihydrolase and ornithine decarboxylase. Catalase, acetoin and indole productions are negative. Susceptible to the vibriostatic agent O/129 (150 µg/disc). Isolated from the seawater off Ishigaki coral reef. DNA G+C content is 47.2-49.4%. The range of estimated genome sizes based on the draft genome sequencing is 4.5 Mb to 5.4 Mb. Type species is *Thaumasiovibrio occultus* DSM 101554$^{T}$= JCM 31629$^{T}$=C4II189$^{T}$. Taxonumber of Digital Protologue is GA00017.

**Descriptions of *Thaumasiovibrio occultus* sp. nov. (oc.cul'tus. L. adj. occultus, hidden; referring to resistant to the discovery of the species in the diverse active coral reef ecosystem)**

Cells are 1.0-1.2 μm long and 0.7-0.8 μm width when the bacterium is grown on ZoBell 2216E agar. Colonies on ZoBell 2216E agar are beige, circular, smooth and convex grown in 2 mm diameter after 3 days' culture. Mesophilic and neutrophilic chemo-organotroph which grow at 15-30°C. No bioluminescence is observed, and does not required organic growth factors. Oxidase positive. Produces acid from glucose without gas production, hydrolyses tween 80, gelatin, DNA; assimilates D-mannose, D-fructose, fumarate, N-acetyl-D-glucosamine, citrate, glycerol, DL-malate, acetate, D-glucosamine, pyruvate, L-glutamate, glycerate, DL-lactate, L-alanine, and L-asparagine. No pigmentation is observed; does not hydrolyse starch, alginate, agar, and κ-carrageenan; does not reduce nitrate. Does not assimilate D-galactose, sucrose, maltose, melibiose, lactose, D-gluconate, succinate, aconitate, meso-erythritol, D-mannitol, γ-aminobutyrate, D-sorbitol, α-ketoglutarate, xylose, trehalose, glucronate, δ-aminovalate, cellobiose, L-proline, putrescine, propionate, amygdalin, D-galacturonate, D-raffinose, rhamnose, D-ribose, salicine, L-arginine, L-citrulline, glycine, histidine, L-ornithine, and L-serine. The DNA G+C content is 49.4%. The estimated genome size is 4.5 Mb. Type strain is *Thaumasiovibrio occultus* DSM 101554$^{T}$= JCM 31629$^{T}$=C4II189$^{T}$. The Taxonumber of Digital Protologue is TA00038.

***Thaumasiovibrio subtropicus* sp. nov. (sub.tro'pi.cus. N.L. adj. subtropicus, subtropical, referring to the isolation site of the Ishigaki coral reef)**

Cells are 1.9-2.0 μm long and 0.9-1.0 μm width when the bacterium is grown on ZoBell 2216E

agar. Colonies on ZoBell 2216E agar are beige, circular, smooth and convex grown in 3 mm diameter after 3 days culture. Mesophilic and neutrophilic chemo-organotroph which grow at 15-37°C. No bioluminescence is observed, and does not required organic growth factors. Produces soluble brown pigments; acid from glucose without gas production, hydrolyses tween 80, gelatin, DNA; reduces nitrate; assimilates D-mannose, D-fructose, N-acetyl-D-glucosamine, fumarate, citrate, D-mannitol, D-sorbitol, DL-malate, glucronate, acetate, D-glucosamine, pyruvate, cellobiose, L-proline, L-glutamate, propionate, glycerate, DL-lactate, L-alanine, L-asparagine, histidine, and L-serine. Does not hydrolyse starch, alginate, agar, and κ-carrageenan; capable to reduce nitrate. Oxidase negative. Does not assimilate D-galactose, sucrose, maltose, melibiose, lactose, D-gluconate, succinate, aconitate, meso-erythritol, glycerol, γ-aminobutyrate, α-ketoglutarate, xylose, trehalose, δ-aminovalate, putrescine, amygdalin, D-galacturonate, D-raffinose, rhamnose, D-ribose, salicine, L-arginine, L-citrulline, glycine, and L-ornithine. The DNA G+C content is 47.2%. The estimated genome size is 5.4 Mb. Type strain is *Thaumasiovibrio subtropicus* DSM 101555$^{T}$= JCM 31630$^{T}$=C4V358$^{T}$. The Taxonumber of Digital Protologue is TA00043.

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**Figure legends**

**Fig. 1.** Rooted phylogenetic tree on the basis of 16S rRNA gene sequences. This figure combines the results of three analyses, neighbor-joining (NJ), maximum-parsimony (MP) and maximum-likelihood (ML). The topology shown was obtained by NJ, and the percentage values are the results of a bootstrap analysis using 1000 replications. Branches also obtained in the ML and MP analyses are only indicated with bootstrap value.

**Fig. 2.** Concatenated split network tree based on eight protein coding gene loci of the *Thaumasiovibrio* species and the related taxa. The *gapA*, *gyrB*, *ftsZ*, *mreB*, *pyrH*, *recA*, *rpoA*, and *topA* gene sequences of the related taxa were concatenated including the representative of novel genus *Thaumasiovibrio* species (C4II189$^{T}$ and C4V358$^{T}$). Phylogenetic tree was generated using the SplitsTree4program (see supplemental materials). Two novel species of this study formed a

cluster not associated with any known clade in the family *Vibrionaceae* proposed previously. The clade names are shown in bold Italic.

**Fig. 3.** Relationship of core genes amongst representative strains of five genera; *Thaumasiovibrio, Enterovibrio, Grimontia*, *Photobacterium*, and *Salinivibrio*. Circos was used for drawing the linkage plot of the core genes showing >90% amino acid identity and alignment amongst representative strains of genera *Enterovibrio*, *Grimontia*, *Photobacterium*, and *Salinivibrio*.

**Supporting information legends**

**Table S1**. Experimental DND-DNA similarity data of the two *Thaumasiovibrio* species against the representative *Vibrionaceae* species.

**Table S2.** Phenotypic characteristics for distinguishing *Thaumasiovibrio occulutus* gen. nov., sp. nov., *Thaumasiovibrio subtropicus* sp. nov., and their related species. Taxa are indicated as: (1) *T. occultus* C4II189$^T$, (2) *T. subtropicus* C4V358$^T$, (3) *P. sanctipaulii* CAIM 1892$^T$, (4) *P. rosenbergii* CAIM 911$^T$, (5) *P. lutimaris* CAIM 1851$^T$, (6) *P. marinum* CAIM 1887$^T$, (7) *P. ganghwense* CAIM 512T, (8) *G. hollisae* LMG 17718$^T$, (9) *E. norvegicus* LMG 18839$^T$, (10) *S. costicola* subsp. *costicola* LMG 11651$^T$.

**Fig. S1.** A broad concatenated split network tree based on eight protein coding gene loci for *Vibrionaceae* taxa. The *gapA*, *gyrB*, *ftsZ*, *mreB*, *pyrH*, *recA*, *rpoA*, and *topA* gene sequences of 120 taxa were concatenated including the representative of novel genus *Thaumasiovibrio* species (C4II189$^T$ and C4V358$^T$). Phylogenetic tree was generated using the SplitsTree4program (see supplemental materials). Two novel species of this study formed a cluster not associated with any known clade in the family *Vibrionaceae* proposed previously.

**Fig. S2.** Inter-genus AAI between *Thaumasiovibiro* gen. nov. and the other representative genera. Same genus is indicated by the same shape of symbol. AAI (66.6%) between *P. phosphoreum* vs. *P. halotorelans* was the lowest in intra-genus comparison. AAI (65.1%) between *S. costicola* vs. *G. hollisae* was the highest in inter-genus comparison.

Figure

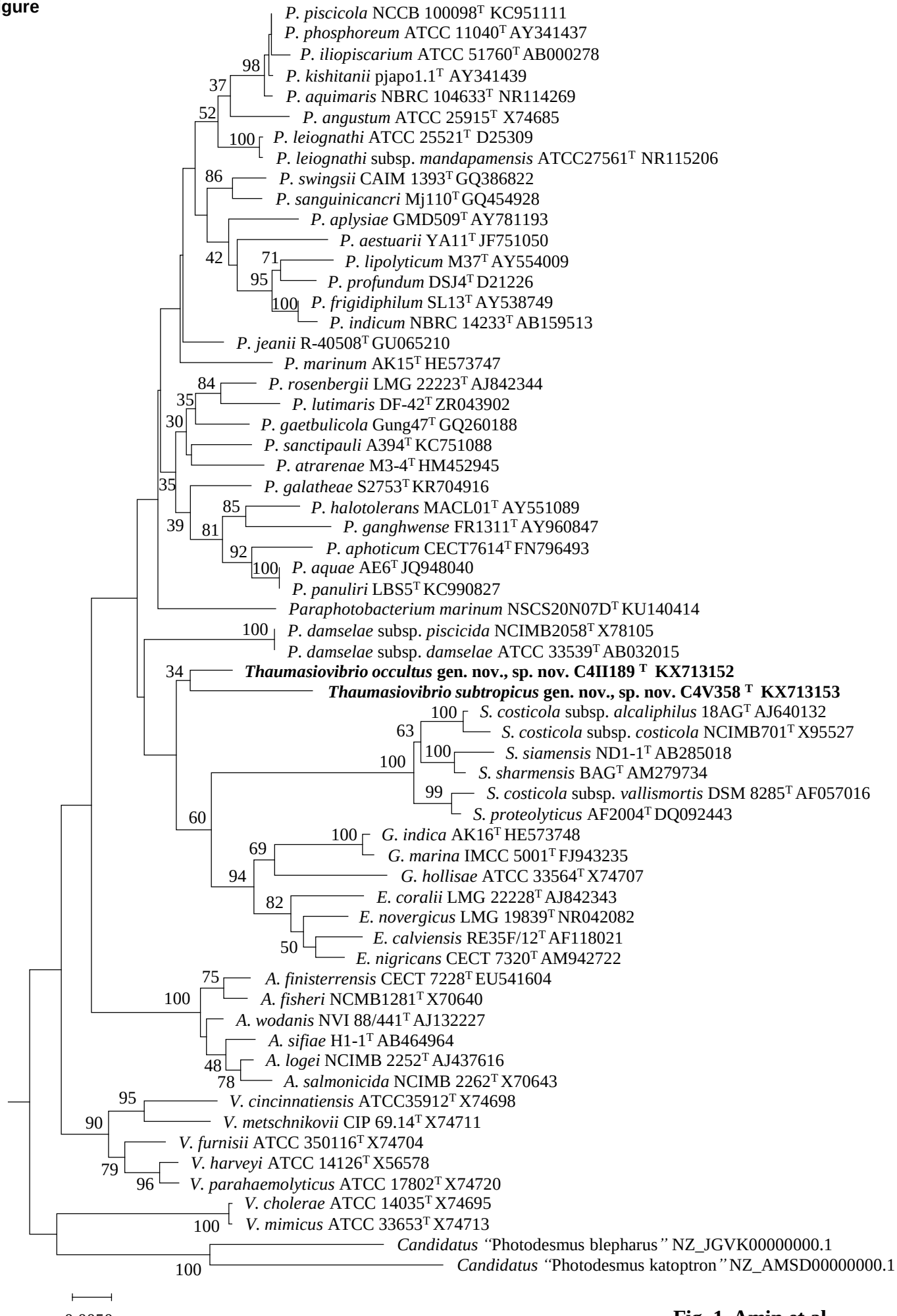



**Fig. 1. Amin et al.**

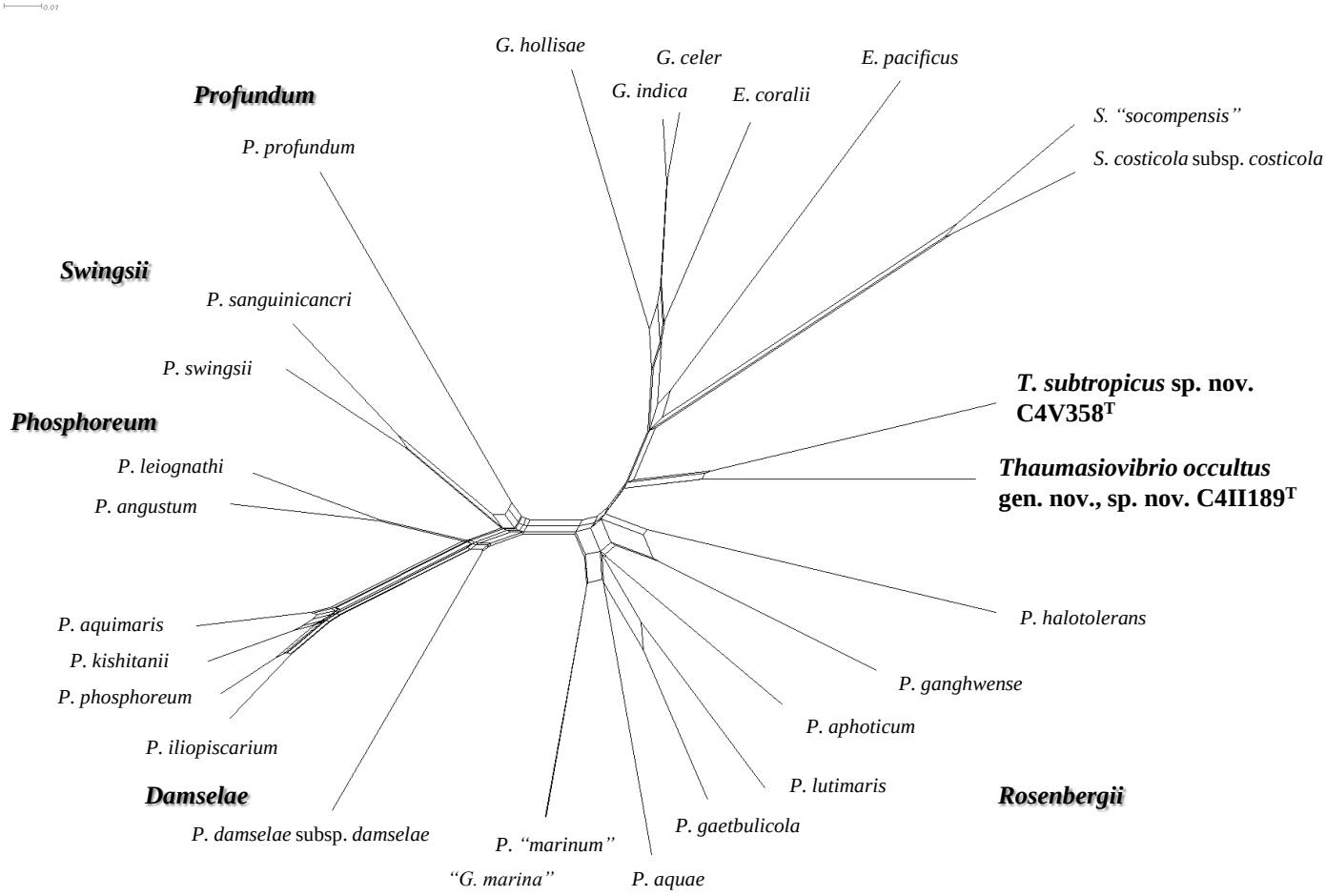



**Fig. 2. Amin et al.**

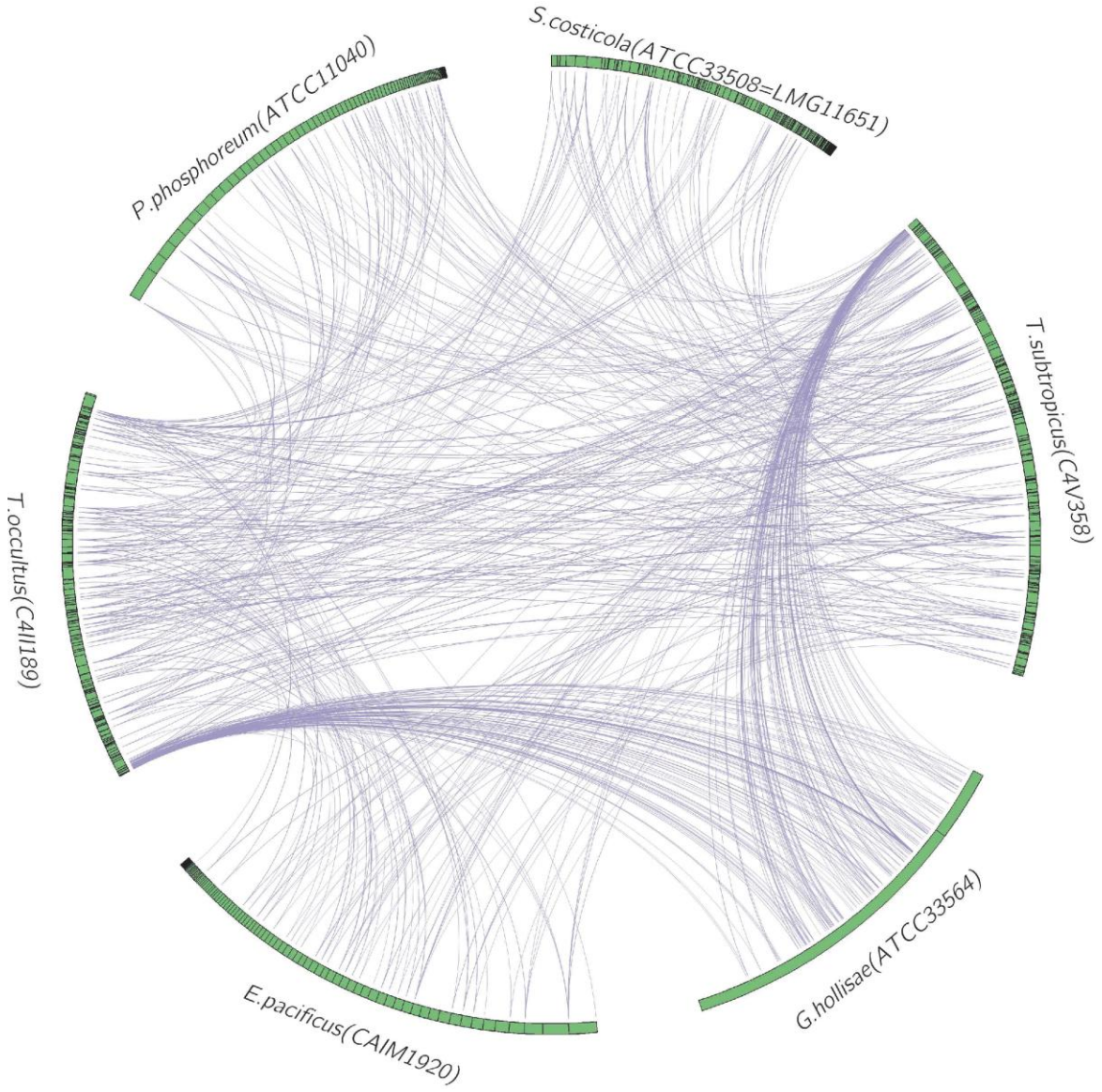



**Fig. 3. Amin et al.**

**Table 1**

ANI and *in silico* DDH values, and AAI between the proposed two novel *Thaumasiovibrio* species and the related species

| Strains | Accession numbers | ANI (%) | | *in silico* DDH (%) | | AAI (%) | |
|---|---|---|---|---|---|---|---|
| | | C4II189 | C4V358 | C4II189 | C4V358 | C4II189 | C4V358 |
| *Thaumasiovibrio occultus* DSM 101554$^{T}$ (C4II189) | BDGP01000001-BDGP01000230 | - | 71.89 | - | 20.8 | - | 68.9 |
| *T. subtropicus* DSM 101555$^{T}$ (C4V358) | BDGQ01000001-BDGQ01000271 | 71.9 | - | 20.8 | - | 68.9 | - |
| *Enterovibrio calviensis* DSM 14347$^{T}$ | NZ_JHZA00000000 | 69.9 | 69.4 | 21.6 | 21.0 | 61.0 | 60.7 |
| *E. coralii* CAIM 912$^{T}$ | NZ_LNTY00000000 | 70.5 | 69.5 | 20.9 | 21.0 | 52.0 | 51.8 |
| *E. pacificus* CAIM 1920$^{T}$ | NZ_LYBM00000000 | 69.2 | 68.8 | 21.2 | 22.1 | 58.9 | 59.2 |
| *Grimontia hollisae* ATCC 33564$^{T}$ | NZ_CP014056 and NZ_CP014055 | 70.3 | 69.9 | 21.9 | 23.0 | 62.2 | 62.6 |
| *G. celer* CECT 9029$^{T}$ | NZ_FIZX00000000 | 70.4 | 69.5 | 22.2 | 21.3 | 61.0 | 60.7 |
| *G. indica* AK16$^{T}$ | NZ_ANFM00000000 | 70.5 | 69.6 | 22.0 | 21.3 | 58.3 | 58.8 |
| *G. marina* CECT 8713$^{T}$ | NZ_FIZY00000000 | 70.4 | 69.7 | 21.1 | 21.0 | 61.2 | 60.9 |
| *Photobacterium angustum* ATCC 25915$^{T}$ | NZ_JZSO00000000 | 70.3 | 70.0 | 21.6 | 20.9 | 62.3 | 62.6 |
| *P. aphoticum* DSM 25995$^{T}$ | NZ_LDOV00000000 | 71.7 | 70.3 | 22.1 | 21.3 | 64.0 | 63.8 |
| *P. aquae* CGMCC 1.12159$^{T}$ | NZ_LDOT00000000 | 70.7 | 70.0 | 21.9 | 21.2 | 63.4 | 63.4 |
| *P. aquimaris* NCCB 100386$^{T}$ | NZ_LNQZ00000000 | 70.0 | 70.0 | 22.6 | 21.7 | 62.1 | 62.4 |
| *P. damselae* subsp. *damselae* CIP 102761$^{T}$ | NZ_ADBS00000000 | 70.4 | 70.4 | 23.7 | 22.9 | 62.7 | 62.8 |
| *P. gaetbulicola* KCTC 22804$^{T}$ | NZ_CP005973 and NZ_CP005974 | 70.8 | 70.2 | 23.3 | 23.2 | 63.5 | 64.5 |
| *P. galatheae* S2753$^{T}$ | NZ_JMIB00000000 | 70.5 | 69.8 | 21.7 | 22.0 | 61.2 | 61.1 |
| *P. ganghwense* DSM 22954$^{T}$ | NZ_LDOU00000000 | 71.1 | 70.0 | 22.8 | 20.9 | 63.3 | 63.6 |
| *P. halotolerans* DSM 18316$^{T}$ | NZ_AULG00000000 | 70.5 | 69.8 | 20.2 | 20.2 | 62.6 | 62.7 |
| *P. iliopiscarium* ATCC 51760$^{T}$ | NZ_JZSQ00000000 | 69.9 | 69.9 | 21.3 | 20.4 | 61.9 | 62.1 |
| *P. jeanii* R-40508$^{T}$ | NZ_LVHF00000000 | 71.0 | 70.6 | 22.2 | 21.1 | 64.6 | 66.0 |
| *P. kishitanii* ATCC BAA-1194$^{T}$ | NZ_JZSP00000000 | 69.7 | 69.8 | 21.7 | 20.6 | 61.9 | 62.0 |
| *P. leiognathi* ATCC 25521$^{T}$ | NZ_JZSK00000000 | 70.5 | 70.2 | 21.1 | 20.8 | 62.5 | 62.9 |
| *P. phosphoreum* ATCC 11040$^{T}$ | NZ_JZSJ00000000 | 69.9 | 69.9 | 21.6 | 21.2 | 62.0 | 62.3 |
| *P. sanctipauli* A-394$^{T}$ | NZ_JGVO00000000 | 71.0 | 70.4 | 22.1 | 21.7 | 63.1 | 63.6 |
| *P. swingsii* CAIM 1393$^{T}$ | NZ_LELC00000000 | 70.5 | 70.2 | 21.2 | 20.3 | 61.4 | 62.3 |
| *Salinivibrio costicola* subsp. *costicola* LMG 11651$^{T}$ | NZ_AQOF00000000 | 69.2 | 68.9 | 23.0 | 20.8 | 59.6 | 59.8 |
| *S. socompensis* S10B$^{T}$ | NZ_AQOE00000000 | 69.1 | 68.8 | 21.4 | 21.3 | 59.2 | 59.6 |

**Table 2**
Useful features for differentiating *Thaumasiovibrio* gen. nov. in the family *Vibrionaceae*

| Characteristic | *"Thaumasiovibrio"* | *Aliivibrio* | *Enterovibrio* | *Grimontia* | *Photobacterium* | *Salinvivrio* | *Vibrio* | *"Paraphotobacterium"* |
|---|---|---|---|---|---|---|---|---|
| D-mannitol fermentation | v | + | − | v | − | v | v | + |
| Gelatinase acivity | + | v | − | v | v | + | v | NA |
| Voges-Proskauer | − | v | − | − | v | + | v | − |
| Indole production | − | v | v | + | v | − | v | NA |
| Arginine dihydrolase | − | v | + | − | v | + | v | − |
| Ornithine decarboxylase | − | − | − | − | − | − | v | − |
| Susceptibility to O/129 (150 μg) | + | + | v | + | v | + | v | NA |
| Growth at | | | | | | | | |
| 10% NaCl | − | − | − | v | − | + | − | − |
| 40 °C | − | − | − | − | − | + | − | − |
| Catalase | − | + | + | + | v | + | v | NA |
| DNA G + C content (mol%) | 47.2-49.4 | 38-42 | 47.1-49.5 | 48.4-51.0 | 39-53.6 | 49-51 | 38.7-51.3 | 30.7 |

+, positive; -, negative; v, variable; NA, data not available. Data of "Paraphotobacterium" is obtained from Huang et al. [23].

**Table 3**

Inter-genus similarity on the basis of 16S rRNA gene sequence between *Thaumasiovibrio* gen. nov. and the other genera of the family *Vibrionaceae*

| | *Vibrio* | *Entero-vibrio* | *Grimontia* | *Photo-bacterium* | *Aliivibrio* | *Salinivibrio* |
|---|---|---|---|---|---|---|
| *Enterovibrio* | 92.5 | | | | | |
| *Grimontia* | 92.1 | 95.0 | | | | |
| *Photobacterium* | 93.5 | 93.6 | 93.4 | | | |
| *Aliivibrio* | 94.4 | 91.8 | 91.0 | 93.7 | | |
| *Salinivibrio* | 90.8 | 92.6 | 92.3 | 91.7 | 90.2 | |
| *Thaumasiovibrio* | 93.5 | 94.2 | 94.2 | 94.7 | 93.0 | 92.9 |