Vibrio variabilis sp. nov. and *Vibrio maritimus* sp. nov., isolated from *Palythoa caribaeorum*

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Two novel vibrio isolates (R-40492^T and R-40493^T) originating from the zoanthid *Palythoa caribaeorum* in Brazil in 2005 were taxonomically characterized by means of a polyphasic approach comprising multilocus sequence analysis (MLSA), DNA–DNA hybridization (DDH), ΔT_m analysis and phenotypic characterization. Phylogenetic analysis based on 16S rRNA gene sequences showed that R-40492^T and R-40493^T fell within the genus *Vibrio* and were most closely related to each other with 99 % similarity; similarities of these two novel isolates towards *Vibrio neptunius* LMG 20536^T, *Vibrio coralliilyticus* LMG 20984^T, *Vibrio nigripulchritudo* LMG 3896^T, *Vibrio sinaloensis* LMG 25238^T and *Vibrio brasiliensis* LMG 20546^T varied between 97.1 and 98.5 %. DDH experiments showed that the two isolates had less than 15 % relatedness to the phylogenetically most closely related *Vibrio* species. R-40492^T and R-40493^T had 55–57 % relatedness to each other. The ΔT_m between R-40492^T and R-40493^T was 6.12 °C. In addition, MLSA of concatenated sequences (16S rRNA, *ftsZ*, *gyrB*, *recA*, *rpoA*, *topA*, *pyrH* and *mreB*; 6035 bp in length) showed that the two novel isolates formed a separate branch with less than 92 % concatenated gene sequence similarity towards known species of vibrios. Two novel species are proposed to accommodate these novel isolates, namely *Vibrio variabilis* sp. nov. (type strain, R-40492^T=LMG 25438^T=CAIM 1454^T) and *Vibrio maritimus* sp. nov. (type strain, R-40493^T=LMG 25439^T=CAIM 1455^T).

Zoanthids of the genus *Palythoa* are widespread around the world, most notably on rocky shores and coral reefs. *Palythoa caribaeorum* may cover up to 50 % of the total area of rocky shores in some locations on the coast of Brazil (Oigman-Pszczol *et al.*, 2004). This organism is well known for its ability to produce a very potent nonproteinaceous toxin (palytoxin) and copious amounts of mucus. These features may allow it to occupy areas of periodic desiccation and intense sunlight stress. *Palythoa caribaeorum* is sympatric with several coral species (*Mussismilia* species) on the Brazilian coast, sharing the same habitat with the main Brazilian reef builders. Vibrios are widespread in the aquatic environment and are often found associated with various organisms ranging from plankton to animals (Thompson *et al.*, 2004). In the last few years, particularly due to the application of genomic techniques, the taxonomy of *Vibrio* has improved and many novel species have been described (Chimetto *et al.*, 2011; Sheu *et al.*, 2011; Wang *et al.*, 2011). There are now over 84 formally described species in the genus (http://www.vibriobiology.net/). Some *Vibrio* species have been described that cause severe infections in humans

Abbreviations: DDH, DNA–DNA hybridization; MLSA, multilocus sequence analysis.

The GenBank/EMBL/DDBJ accession numbers for the sequences of *Vibrio variabilis* sp. nov. R-40492^T and *Vibrio maritimus* sp. nov. R-40493^T are GU929924 and GU929925 (16S rRNA gene sequences), GU929926 and GU929927 (*ftsZ* gene sequences), GU929928 and GU929929 (*gyrB* gene sequences), GU929934 and GU929935 (*recA* gene sequences), GU929936 and GU929937 (*rpoA* gene sequences), GU929938 and GU929939 (*topA* gene sequences), GU929932 and GU929933 (*pyrH* gene sequences), and GU929930 and GU929931 (*mreB* gene sequences), respectively.

Three supplementary tables and eight supplementary figures are available with the online version of this paper.

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and in many marine organisms (i.e. fish, crustaceans, molluscs and coral), but vibrios are also considered to be part of the normal microbiota of marine invertebrates and fish (Thompson *et al.*, 2004; Gomez-Gil *et al.*, 2007; Bourne *et al.*, 2009). *Vibrio neptunius*, *Vibrio brasiliensis* and *Vibrio sinaloensis* have been found in association with healthy marine organisms (Thompson *et al.*, 2003; Gomez-Gil *et al.*, 2008), whereas *Vibrio coralliilyticus* has been described as one of the main coral pathogens around the world (Ben-Haim *et al.*, 2003; Sussman *et al.*, 2008).

In a survey on the diversity of heterotrophic bacteria associated with *Palythoa caribaeorum* in São Paulo (Brazil), two novel *Vibrio* strains, R-40492^T (=LMG 25438^T) and R-40493^T (=LMG 25439^T), were obtained from mucus of apparently healthy *Palythoa caribaeorum*, located at two sites [Preta beach (23° 49′ 10″ S 45° 24′ 37″ W) and Portinho beach (23° 50′ 25″ S 45° 24′ 22″ W)] (Chimetto *et al.*, 2008, 2009). They were isolated on thiosulfate-citrate-bile salt-sucrose (TCBS; Oxoid) agar after 48 h incubation at 28 °C. The aim of the present study was to perform a detailed taxonomic characterization of the two novel strains based on a polyphasic approach.

Sequences of 16S rRNA, cell division protein (*ftsZ*), DNA gyrase B subunit (*gyrB*), recombination repair protein (*recA*), RNA polymerase alpha subunit gene (*rpoA*), topoisomerase I (*topA*), uridylate kinase (*pyrH*) and actin-like cytoskeleton protein (*mreB*) genes were obtained as described previously (Sawabe *et al.*, 2007; Thompson *et al.*, 2001a, 2007). Briefly, PCR products were purified with the enzyme Exosap according to the instructions of the manufacturer (GE Health Care). Subsequently, 5 µl purified PCR product was mixed with 4 µl ET Terminator Mix (GE Health Care), 0.6 µl sequencing primers (20 µmol l^{-1}) and 0.4 µl MilliQ water. The thermal program consisted of 30 cycles of 20 s at 95 °C, 15 s at 50 °C and 1 min at 60 °C. Purification of the sequencing products was done by adding 1 µl ammonium acetate (7.5 mol l^{-1}) and 27.5 µl absolute ethanol to each product, incubating in the dark for 30 min and subsequent centrifugation at 20 800 ***g*** for 75 min at 4 °C. After this, the supernatant was removed and 100 µl 70 % ethanol was added. A final centrifugation step was performed at 3700 r.p.m. for 45 min at 4 °C. Separation of the DNA fragments was performed using a MegaBace 1000 system (GE Health Care). Voltage and time of injection were 3 kV and 80 s, respectively. Running was performed at 9 kV for 100 min at 44 °C. Raw sequence data were transferred to ChromasPro version 1.34 (Technelysium, Tewantin, Australia) where consensus sequences were determined. Sequences were aligned using CLUSTAL W. Pairwise similarities were calculated with the software BioNumerics 4.61 (Applied Maths, Belgium), using an open gap penalty of 100 % and a unit gap penalty of 0 %, and with the software Jalview 2.4.0.b2 (Waterhouse *et al.*, 2009). Similarity matrices and phylogenetic trees were constructed using the software MEGA version 4.0 (Tamura *et al.*, 2007) and BioNumerics 4.61 (Applied Maths, Belgium). Trees were drawn using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony methods (Eck & Dayhoff, 1966). The robustness of each topology was checked by 1000 bootstrap replications (Felsenstein, 1985). The gene sequence data obtained in this study are also available through the website TAXVIBRIO (http://www.taxvibrio.lncc.br/). The GenBank accession numbers for *ftsZ*, *gyrB*, *recA*, *rpoA*, *topA*, *pyrH* and *mreB* gene sequences used in this study are listed in Supplementary Table S1 (available in IJSEM Online). DNA–DNA hybridization (DDH) experiments were performed using Ezaki's microplate method as described previously in detail (Ezaki *et al.*, 1989; Willems *et al.*, 2001). The hybridization temperature was 40 °C in the presence of 50 % formamide. Reciprocal reactions (e.g. A × B and B × A) were performed for every DNA pair and their variation was within the limits of this method (Goris *et al.*, 1998). The degree of DNA–DNA relatedness was also estimated using a fluorimetric method with SYBR Green I, by measuring the divergence between the thermal denaturation midpoint of homoduplex and heteroduplex DNA (ΔT_m) as described by Moreira *et al.* (2011). The DDH experiments were performed four times. DNA G+C contents were determined by HPLC as described previously (Mesbah *et al.*, 1989). Analysis of fatty acid methyl esters was carried out as described by Huys *et al.* (1994). For fatty acid analysis, cells were grown on tryptone soy agar (TSA; Difco) supplemented with 1.5 % NaCl for 24 h at 28 °C. Catalase activity was determined by adding young cells to a drop of 3 % H_2O_2 solution and observing O_2 production. Oxidase activity was tested using 1 % *N*,*N*,*N*′,*N*′-tetramethyl *p*-phenylenediamine (Kovacs, 1956). Phenotypic characterization was performed using the API ZYM, API 20E (both bioMérieux) and Biolog GN2 metabolic fingerprinting kits as described previously (Thompson *et al.*, 2001b, 2002). Type strains of closely related *Vibrio* species (i.e. *V. neptunius* LMG 20536^T, *V. coralliilyticus* LMG 20984^T, *Vibrio nigripulchritudo* LMG 3896^T, *V. sinaloensis* LMG 25238^T and *V. brasiliensis* LMG 20546^T) were included in these analyses. The temperature (0–42 °C) and pH (4–13) ranges for growth were determined by incubating the isolates on TSA supplemented with 2 % (w/v) NaCl. Growth at different NaCl concentrations (0–14 %, w/v) and pH were determined on TSA incubated for 72 h at 28 °C. For 0 % NaCl concentration, TSA was prepared manually without the addition of salt. Acid production from sucrose was tested by incubating the isolates on TCBS agar. Traditional phenotypic tests (i.e. glucose fermentation, gas formation, hydrolysis of Tween 80, citrate utilization, activities of tryptophan deaminase, urease and lysine decarboxylase, and indole and H_2S production) were also performed as described previously (Macián *et al.*, 2001).

Phylogenetic analysis based on 16S rRNA gene sequences of R-40492^T (1521 bp) and R-40493^T (1504 bp) classified the novel strains in the genus *Vibrio* (Fig. 1). The two novel strains were most closely related to each other with 99 % similarity, and to *V. neptunius* LMG 20536^T, *V. coralliilyticus* LMG 20984^T, *V. nigripulchritudo* LMG 3896^T, *V.*

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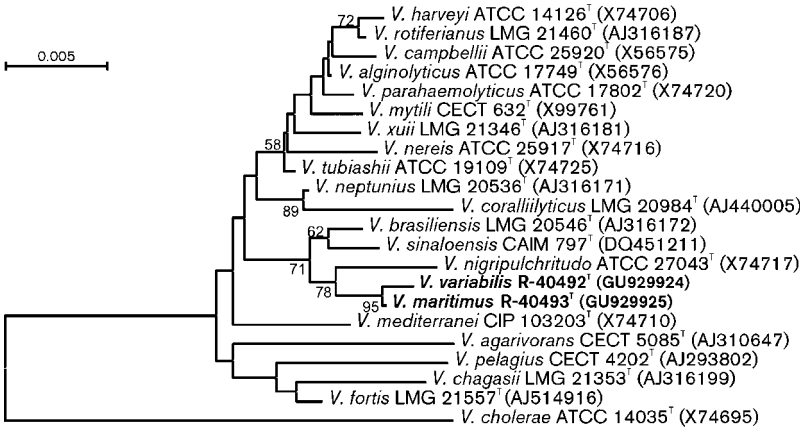

Fig. 1. Neighbour-joining phylogenetic tree showing the phylogenetic position of *V. variabilis* sp. nov. and *V. maritimus* sp. nov. based on 16S rRNA gene sequences. Evolutionary distances were computed using the Jukes–Cantor method. All positions containing alignment gaps and missing data were eliminated only in pairwise sequence comparisons (pairwise deletion option). Phylogenetic analyses were conducted in MEGA4. Bootstrap values (>50 %) based on 1000 repetitions are shown. *Vibrio cholerae* ATCC 14035^T was used as outgroup. Bar, 0.5 % estimated sequence divergence. Fragments of 1521 bp and 1504 bp were obtained for *V. variabilis* and *V. maritimus*, respectively.

sinaloensis LMG 25238^T and *V. brasiliensis* LMG 20546^T with similarities of 97.1–98.5 % (Table 1).

Trees based on partial sequences of the housekeeping genes *ftsZ* (525 bp), *gyrB* (743 bp), *recA* (556 bp), *rpoA* (790 bp), *topA* (553 bp), *pyrH* (531 bp) and *mreB* (830 bp) confirmed the phylogenetic position of the two isolates in the genus *Vibrio* and revealed they constitute a separate branch (Supplementary Figs S1–S8, available in IJSEM Online). Similarities between R-40492^T and R-40493^T were 94.5 % (for ftsZ), 95.4 % (for *gyrB*), 95.7 % (for *recA*), 98.5 % (for *rpoA*), 97.8 % (for *topA*), 96.7 % (for *pyrH*) and 95.6 % (for *mreB*), which confirms their close relationship to each other. Although the similarities obtained for some housekeeping genes (i.e. *pyrH*) between R-40492^T and R-40493^T are above the limiting value (>94–95 %) for species differentiation suggested by Thompson *et al.* (2005, 2009), data from multilocus sequence analysis (MLSA) clearly split these two novel strains in two phylogenetic branches (Fig. 2). Gene sequence similarities among R-40492^T, R-40493^T and the type strains of the phylogenetically most closely related species (i.e. *V. neptunius*, *V. coralliilyticus*, *V. nigripulchritudo* and *V. brasiliensis*), for which these housekeeping gene sequences were available in GenBank/EMBL or the website TAXVIBRIO (http://www.taxvibrio.lncc.br/), were less than 92 % (Supplementary Table S2 available in IJSEM Online). A neighbour-joining tree based on concatenated gene sequences of 16S rRNA, *ftsZ*, *gyrB*, *recA*, *rpoA*, *topA*, *pyrH* and *mreB* (6035 bp in length) confirmed the separate phylogenetic position of the novel strains R-40492^T and R-40493^T in the genus *Vibrio*, with high bootstrap support values in both neighbour-joining and maximum-parsimony methods (Fig. 2).

DDH experiments were performed with strains R-40492^T, R-40493^T and the type strains of the closest phylogenetic neighbours (Table 1). Results showed that the novel strains R-40492^T and R-40493^T had 55–57 % mutual DNA–DNA relatedness. This value is below the threshold for species delineation, considering the deviation of the methodology which is known to be around 7 %. DNA–DNA relatedness towards other species was 15 % (Table 1). DNA–DNA relatedness estimates using a ΔT_m method confirmed that strains R-40492^T and R-40493^T should be classified as different species. The ΔT_m value between R-40492^T and R-40493^T was 6.12 °C (Fig. 3), which is significantly above

Table 1. DNA–DNA hybridization data and 16S rRNA gene sequence similarities of *V. variabilis* sp. nov., *V. maritimus* sp. nov. and related *Vibrio* species

Values are means. Standard deviations are given in parentheses.

Strain	16S rRNA similarity (%)		DNA–DNA hybridization values (%)						
	1	2	1	2	3	4	5	6	7
1. *V. variabilis* sp. nov. R-40492^T	100	–	100	57 (8)	10 (1)	11 (3)	3 (1)	9 (2)	5 (1)
2. *V. maritimus* sp. nov. R-40493^T	99.0	100	55 (7)	100	13 (2)	11 (2)	2 (1)	7 (1)	8 (2)
3. *V. neptunius* LMG 20536^T	98.5	97.1	8 (3)	9 (3)	100	33 (6)	4 (2)	8 (4)	14 (4)
4. *V. coralliilyticus* LMG 20984^T	98.2	97.3	9 (2)	11 (3)	48 (7)	100	3 (2)	11 (5)	12 (4)
5. *V. nigripulchritudo* LMG 3896^T	98.2	98.5	11 (2)	12 (2)	13 (8)	21 (6)	100	8 (0)	8 (4)
6. *V. sinaloensis* LMG 25238^T	98.2	98.3	11 (1)	9 (2)	17 (5)	15 (6)	7 (1)	100	10 (3)
7. *V. brasiliensis* LMG 20546^T	98.1	98.4	15 (1)	13 (2)	24 (6)	22 (7)	6 (2)	14 (4)	100

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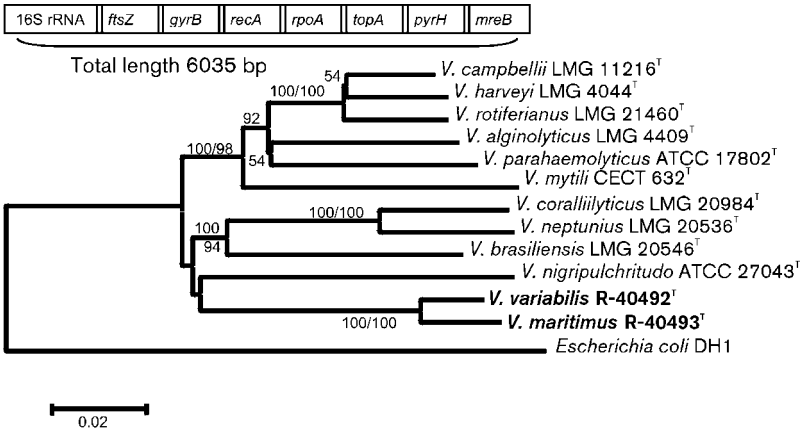

Fig. 2. Neighbour-joining phylogenetic tree showing the phylogenetic position of *V. variabilis* sp. nov. and *V. maritimus* sp. nov. based on concatenated 16S rRNA, *ftsZ*, *gyrB*, *recA*, *rpoA*, *topA*, *pyrH* and *mreB* gene sequences (6035 bp). Evolutionary distances were computed using the Jukes–Cantor method. Phylogenetic analyses were conducted in MEGA4. Bootstrap values (>50 %) based on 1000 repetitions are shown. Numbers at nodes denote bootstrap values derived from the neighbour-joining and maximum-parsimony methods, respectively; single values were obtained by neighbour-joining analysis. *Escherichia coli* DH1 was used as outgroup. Bar, 2 % estimated sequence divergence.

the 5 °C cut-off level recommended for species delineation (Wayne *et al.*, 1987; Rosselló-Mora & Amann, 2001). The regression curve of the DDH versus ΔT_m values determined by Moreira *et al.* (2011) indicates that the ΔT_m expected for DDH between R-40492^T and R-40493^T should be 6.42 °C, indicating a good correlation between the data. DDH data clearly provide evidence that the two novel strains should be allocated to two novel *Vibrio* species.

The two novel strains shared the main phenotypic and chemotaxonomic features of the genus *Vibrio* (Baumann & Schubert, 1984). They were Gram-negative, motile, oxidase- and catalase-positive, and showed prolific growth on TCBS agar with production of acid from sucrose. The major fatty acids were summed feature 3 (comprising iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$), $C_{18:1}\omega7c$ and $C_{16:0}$, which corresponded to 64 % (R-40492^T) and 70 % (R-40493^T) of the total fatty acids (Supplementary Table S3 available in IJSEM Online). Prolific growth occurred in TSA medium containing 2.0 % (w/v) NaCl at 28 °C; no growth was observed without NaCl.

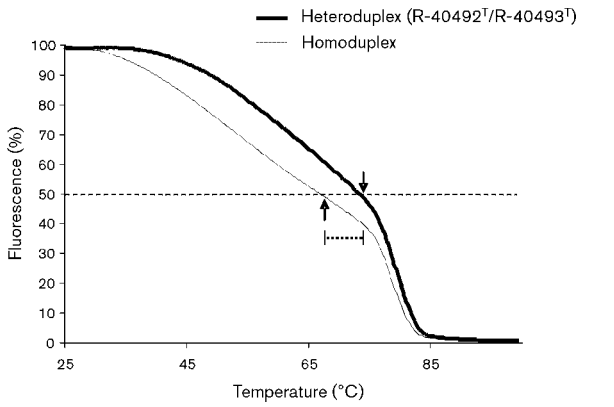

Fig. 3. Melting curves of homoduplex and heteroduplex (R-40492^T/R-40493^T) DNA. The ΔT_m is: mean 6.12 °C ± standard error of 0.26; n=8). The dashed horizontal line delimits a reduction of 50 % in the fluorescence, reflecting a 50 % denaturation of the double-stranded DNAs.

Phenotypic features such as acetoin production, activities of *N*-acetyl-β-glucosaminidase, β-galactosidase, trypsin, lipase (C14) and cystine arylamidase, fermentation of mannitol, inositol, L-rhamnose and amygdalin, and assimilation of D-glucuronic acid, α-ketoglutaric acid, succinamic acid, glucuronamide, DL-carnitine, glucose 1-phosphate and glucose 6-phosphate can be used to differentiate the two novel species from each other and from phylogenetically related *Vibrio* species (Table 2). Divergent results were observed for indole production, assimilation of Tween 80, and activities of tryptophan deaminase and lysine decarboxylase when traditional methods were compared with phenotypic characterization using the systems described in this study. The phenotypic profiles of each of the two novel strains are based on single isolates. However, five strains (R-77, R-78^T, R-91^T, R-616 and R-619), isolated as described by Chimetto *et al.* (2009), have been reported (*Vibrio* L2 sp. nov.) to be closely related based on 16S rRNA and *pyrH* gene sequences. Unfortunately, all strains apart from R-78^T (=R-40492^T) and R-91^T (=R-40493^T) lost viability. As more strains of these species are isolated and tested, the phenotypic profile may change slightly.

Based on data from polyphasic analysis including MLSA, DDH and phenotypic tests performed in this study, it is proposed that two novel species, *Vibrio variabilis* sp. nov. and *Vibrio maritimus* sp. nov., should be created to accommodate isolates R-40492^T and R-40493^T, respectively.

Description of *Vibrio variabilis* sp. nov.

Vibrio variabilis (va.ri.a′bi.lis. L. masc. adj. *variabilis* changeable, variable, referring to the change of colour of the colonies).

Cells are 0.9 μm wide and 1.5–2.5 μm long, Gram-negative, motile rods. Forms translucent, convex, smooth-rounded colonies with entire margins, 1 mm in size after 1 day

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Table 2. Differential features of *V. variabilis* sp. nov., *V. maritimus* sp. nov. and the most closely related *Vibrio* species

Strains: 1, *V. variabilis* sp. nov. R-40492^T (=LMG 25438^T); 2, *V. maritimus* sp. nov. R-40493^T (=LMG 25439^T); 3, *V. neptunius* LMG 20536^T; 4, *V. coralliilyticus* LMG 20984^T; 5, *V. nigripulchritudo* LMG 3896^T; 6, *V. sinaloensis* LMG 25238^T; 7, *V. brasiliensis* LMG 20546^T. +, Positive; −, negative; W, weak; ND, not determined. All data were obtained in this study except where indicated otherwise.

Characteristic	1	2	3	4	5	6	7
Growth in 8 % (w/v) NaCl	−	W	−	−	−	+	−
Growth at 40 °C	+	−	−	ND	−	+	+
Acetoin production (Voges–Proskauer)	−	−	+	+	−	−	−
Fermentation of:							
Mannitol	+	+	−	+	−	−	+
Inositol	−	+	−	−	−	−	−
L-Rhamnose	+	−	−	−	−	−	−
Amygdalin	+	+	−	−	+	−	+
Melibiose	−	−	−	−	+	−	−
Enzyme activity:							
Lipase (C14)	−	+	+	W	−	−	+
Cystine arylamidase	+	−	−	−	−	−	−
Trypsin	+	−	+	+	−	−	−
N-Acetyl-β-glucosaminidase	+	W	+	−	−	−	−
β-Galactosidase	+	+	−	−	W	−	+
Tryptophan deaminase	−	−	−	+	−	+	−
Utilization of:							
D-Glucuronic acid	−	+	−	−	ND	−	−
Propionic acid	−	−	−	W	ND	−	−
Glucose 1-phosphate	−	+	+	−	ND	−	−
Glucose 6-phosphate	−	+	+	+	ND	−	−
α-Ketoglutaric acid	−	+	+	+	ND	−	−
DNA G+C content (mol%)	46.8	46.3	46*	46.2	46–47†	45.8	45.9*
Fatty acid composition:‡							
$C_{12:0}$	2.3	5.0	2.0	1.8	−	4.5	2.1
iso-$C_{15:0}$ 3-OH	1.3	−	−	1.0	−	−	−
$C_{16:0}$	13.0	16.0	18.4	15.5	23.0	20.0	17.0
$C_{17:0}$	−	−	1.8	2.0	−	−	−
iso-$C_{17:0}$	9.0	3.9	−	2.2	−	−	−
$C_{18:0}$	−	1.4	1.8	1.0	2.4	−	1.3

*Thompson *et al.* (2003).

†Baumann & Schubert (1984).

‡Fatty acid profiles of known *Vibrio* species (type strain) were obtained in this study under the same conditions. Data are expressed as percentages of total fatty acids. Fatty acids representing <1 % are not shown.

incubation at 28 °C on TSA. Colonies are beige in colour, but may turn black after some time under limited light conditions. Growth occurs between 15 and 40 °C, at NaCl concentrations of 0.5–7.0 % (w/v) and at pH 6–12. No growth is observed in 0 or ⩾8 % NaCl, at temperatures ⩽7 or ⩾42 °C, or at pH ⩾5. Optimum growth occurs at 28–35 °C, in 1.5–5.0 % NaCl and at pH 7–10. Yellow colonies are able to grow on the selective medium TCBS (positive for acid production from sucrose). Positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase, arginine dihydrolase, gelatinase, valine arylamidase, cystine arylamidase, trypsin, *N*-acetyl-β-glucosaminidase, indole production, fermentation of glucose, mannitol, sucrose, amygdalin and rhamnose, nitrate reduction to nitrite, and assimilation of dextrin, glycogen, Tween 40, Tween 80, *N*-acetyl-D-glucosamine, cellobiose, D-fructose, D-galactose, gentiobiose, α-D-glucose, maltose, D-mannitol, D-mannose, melibiose, methyl β-D-glucoside, psicose, raffinose, D-sorbitol, sucrose, trehalose, turanose, methyl pyruvate, acetic acid, DL-lactic acid, succinic acid, alaninamide, L-alanine, L-alanyl glycine, L-asparagine, L-glutamic acid, glycyl-L-aspartic acid, glycyl-L-glutamic acid, L-serine, L-threonine, inosine, uridine, thymidine, glycerol, DL-α-glycerol phosphate and α-hydroxybutyric acid. Negative for α-chymotrypsin, β-glucuronidase, β-glucosidase, α-mannosidase, α-fucosidase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase, urease, lipase (C14), production of H_2S and acetoin (Voges–Proskauer),

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reduction of nitrate to N_2 gas, fermentation of sorbitol, melibiose, arabinose and inositol, and assimilation of α-cyclodextrin, citrate, *N*-acetyl-D-galactosamine, adonitol, L-arabinose, D-arabitol, *i*-erythritol, L-fucose, *myo*-inositol, α-lactose, lactulose, L-rhamnose, xylitol, monomethyl succinate, *cis*-aconitic acid, citric acid, formic acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, itaconic acid, α-ketobutyric acid, α-ketovaleric acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, sebacic acid, bromosuccinic acid, D-alanine, L-aspartic acid, L-histidine, hydroxy-L-proline, L-leucine, L-ornithine, L-phenylalanine, L-proline, L-pyroglutamic acid, D-serine, γ-aminobutyric acid, urocanic acid, phenylethylamine, putrescine, 2-aminoethanol, 2,3-butanediol, D-glucuronic acid, α-ketoglutaric acid, succinamic acid, glucuronamide, DL-carnitine, glucose 1-phosphate and glucose 6-phosphate. The main cellular fatty acids are summed feature 3 (iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$), $C_{18:1}\omega7c$ and $C_{16:0}$. The following fatty acids are present in small amounts: iso-$C_{17:0}$, $C_{14:0}$, iso-$C_{15:0}$, $C_{12:0}$, summed feature 2 ($C_{14:0}$ 3-OH and/or iso-$C_{16:1}$ I, an unidentified fatty acid with an equivalent chain-length of 10.928 and/or $C_{12:0}$ ALDE), iso-$C_{13:0}$, $C_{12:0}$ 3-OH and iso-$C_{15:0}$ 3-OH.

The type strain is R-40492^T (=LMG 25438^T=CAIM 1454^T), isolated from mucus of the zoanthid *Palythoa caribaeorum* in Preta Beach, São Sebastião channel, São Paulo, Brazil. The DNA G+C content of strain R-40492^T is 46.8 mol%.

Description of *Vibrio maritimus* sp. nov.

Vibrio maritimus (ma.ri′ti.mus. L. masc. adj. *maritimus* of the sea, marine).

Cells are 1 μm wide and 1.5–4.0 μm long, Gram-negative, motile bacilli. Forms translucent, convex, smooth-rounded colonies with entire margins, 1 mm in size and beige in colour after 1 day incubation at 28 °C on TSA. Growth occurs between 15 and 37 °C, at NaCl concentrations of 0.5–8.0 % (w/v) and at pH 5–12. Weak growth is observed in the presence of 8 % NaCl and at pH 5. No growth is observed in 0 or ⩾9 % NaCl, at temperatures ⩽7 or ⩾40 °C, or at pH ⩾4. Yellow colonies are able to grow on the selective medium TCBS (positive for acid production from sucrose). Optimum growth occurs at 28–37 °C, in 1.5–6.0 % NaCl and at pH 7–10. Positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase, arginine dihydrolase, gelatinase, lipase (C14), indole production, fermentation of glucose, mannitol, sucrose, amygdalin and inositol, nitrate reduction to nitrite, and assimilation of dextrin, glycogen, Tween 40, Tween 80, *N*-acetyl-D-glucosamine, cellobiose, D-fructose, D-galactose, gentiobiose, α-D-glucose, maltose, D-mannitol, D-mannose, melibiose, methyl β-D-glucoside, psicose, raffinose, D-sorbitol, sucrose, trehalose, turanose, methyl pyruvate, acetic acid, DL-lactic acid, succinic acid, alaninamide, L-alanine, L-alanyl-glycine, L-asparagine, L-glutamic acid, glycyl-L-aspartic acid, glycyl-L-glutamic acid, L-serine, L-threonine, inosine, uridine, thymidine, glycerol, DL-α-glycerol phosphate, D-glucuronic acid, α-ketoglutaric acid, succinamic acid, glucuronamide, DL-carnitine, glucose 1-phosphate and glucose 6-phosphate. Negative for α-chymotrypsin, β-glucuronidase, β-glucosidase, α-mannosidase, α-fucosidase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase, urease, cystine arylamidase, trypsin, production of H_2S and acetoin (Voges–Proskauer), reduction of nitrate to N_2 gas, fermentation of sorbitol, melibiose, arabinose and rhamnose, and assimilation of α-cyclodextrin, citrate, *N*-acetyl-D-galactosamine, adonitol, L-arabinose, D-arabitol, *i*-erythritol, L-fucose, *myo*-inositol, α-lactose, lactulose, L-rhamnose, xylitol, monomethyl succinate, *cis*-aconitic acid, citric acid, formic acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, itaconic acid, α-ketobutyric acid, α-ketovaleric acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, sebacic acid, bromosuccinic acid, D-alanine, L-aspartic acid, L-histidine, hydroxy-L-proline, L-leucine, L-ornithine, L-phenylalanine, L-proline, L-pyroglutamic acid, D-serine, γ-aminobutyric acid, urocanic acid, phenylethylamine, putrescine, 2-aminoethanol and 2,3-butanediol. Weak reactions are observed for *N*-acetyl-β-glucosaminidase, valine arylamidase and α-hydroxybutyric acid assimilation. The main cellular fatty acids are summed feature 3 (comprising iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$), $C_{18:1}\omega7c$ and $C_{16:0}$. The following fatty acids are present in small amounts: $C_{14:0}$, $C_{12:0}$, iso-$C_{17:0}$, summed feature 2 ($C_{14:0}$ 3-OH and/or iso-$C_{16:1}$ I, an unidentified fatty acid with an equivalent chain-length of 10.928 and/or $C_{12:0}$ ALDE), $C_{12:0}$ 3-OH, iso-$C_{15:0}$, $C_{18:0}$ and iso-$C_{13:0}$.

The type strain is R-40493^T (=LMG 25439^T=CAIM 1455^T), isolated from mucus of the zoanthid *Palythoa caribaeorum* in Portinho Beach, São Sebastião Channel, São Paulo, Brazil. The DNA G+C content of strain R-40493^T is 46.3 mol%.

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