Marivita hallyeonensis sp. nov., isolated from seawater, reclassification of *Gaetbulicola byunsanensis* as *Marivita byunsanensis* comb. nov. and emended description of the genus *Marivita* Hwang *et al.* 2009

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A Gram-stain-negative, non-motile, non-spore-forming, aerobic, rod-shaped bacterial strain, designated DPG-28^T, was isolated from seawater on the southern coast of Korea. Strain DPG-28^T grew optimally at 30 °C and in the presence of 2 % (w/v) NaCl. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain DPG-28^T formed a coherent cluster with members of the genera *Marivita* and *Gaetbulicola*, with which it exhibited sequence similarity values of 97.8–98.5 %. The DNA G+C content of strain DPG-28^T was 65.1 mol%. The predominant ubiquinone of strain DPG-28^T was ubiquinone-10 (Q-10), consistent with data for the genera *Marivita* and *Gaetbulicola*. The cellular fatty acid profiles of strain DPG-28^T and the type strains of *Marivita cryptomonadis*, *Marivita litorea* and *Gaetbulicola byunsanensis* were essentially similar in that the common predominant fatty acid was C_{18:1}ω7c. Major polar lipids found in strain DPG-28^T and the type strains of *M. cryptomonadis*, *M. litorea* and *G. byunsanensis* were phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine and an unidentified aminolipid. From these data, it is proposed that *Gaetbulicola byunsanensis* be reclassified as a member of the genus *Marivita*, for which the name *Marivita byunsanensis* comb. nov. is proposed, with the type strain SMK-114^T (=CCUG 57612^T =KCTC 22632^T), and that strain DPG-28^T be classified in the genus *Marivita*. Differential phenotypic properties and genetic distinctiveness of strain DPG-28^T demonstrated that this strain is distinguishable from *M. cryptomonadis*, *M. litorea* and *G. byunsanensis*. On the basis of the data presented, strain DPG-28^T is considered to represent a novel species of the genus *Marivita*, for which the name *Marivita hallyeonensis* sp. nov. is proposed. The type strain is DPG-28^T (=KCTC 23421^T =CCUG 60522^T). An emended description of the genus *Marivita* is also provided.

During the screening of polysaccharide-degrading microorganisms from a seaweed farm in the South Sea, Korea, many bacterial strains were isolated and characterized. One of these isolates, designated DPG-28^T, is the subject of this work. Comparative 16S rRNA gene sequence analysis showed that this novel strain is phylogenetically related most closely to members of the genera *Marivita* and *Gaetbulicola* of the *Alphaproteobacteria*. The genus *Marivita* was erected by Hwang *et al.* (2009) and, at the time of

writing, comprises two recognized species, *Marivita cryptomonadis* and *Marivita litorea*. The genus *Gaetbulicola* was erected by Yoon *et al.* (2010) with the description of a single species, *Gaetbulicola byunsanensis*. The aim of the present work was to determine the exact taxonomic position of strain DPG-28^T by using a polyphasic characterization and to elucidate the taxonomic relationship between the genera *Gaetbulicola* and *Marivita*.

Strain DPG-28^T was isolated by the standard dilution plating technique on marine agar 2216 (MA; Difco) at 25 °C. *M. cryptomonadis* KCCM 90070^T and *M. litorea* KCCM 90071^T were obtained from the Korean Culture Center of

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain DPG-28^T is JF260872.

A supplementary figure is available with the online version of this paper.

Microorganisms (KCCM), Seoul, Korea. *G. byunsanensis* SMK-114^T was obtained from our previous study (Yoon *et al.*, 2010). The morphological, physiological and biochemical characteristics of strain DPG-28^T were investigated by using routine cultivation on MA at 30 °C. Cell morphology was examined by using light microscopy (DP70; Olympus) and transmission electron microscopy (CM-20; Philips). Flagellation was determined by using transmission electron microscopy on cells from an exponentially growing culture. For this, cells were negatively stained with 1% (w/v) phosphotungstic acid and the grids were examined after being air-dried. The Gram reaction was determined by using the bioMérieux Gram stain kit according to the manufacturer's instructions. The presence of poly- β -hydroxybutyrate granules was investigated by epifluorescence microscopy (DP70; Olympus) after staining with Nile blue A as described by Ostle & Holt (1982). Growth in the absence of NaCl and in the presence of 0.5, 1.0, 2.0 and 3.0% (w/v) NaCl was investigated by using trypticase soy broth prepared according to the formula of the Difco medium except that NaCl was omitted and 0.45% (w/v) MgCl₂·6H₂O was added. Growth with 2.0–10.0% (w/v) NaCl (in increments of 1.0%) was investigated in marine broth 2216 (MB; Difco). Growth at 4, 10, 20, 25, 28, 30, 35, 37, 40 and 45 °C was measured on MA. The pH range for growth was determined in MB adjusted to pH 4.5–9.5 (increments of 0.5 pH units) by the addition of HCl or Na₂CO₃. The pH was verified after autoclaving. Growth under anaerobic conditions was determined after incubation in a Forma anaerobic chamber on MA and on MA supplemented with potassium nitrate (0.1%, w/v), both of which had been prepared anaerobically under a nitrogen atmosphere. Catalase and oxidase activities were determined as described by Cowan & Steel (1965). Hydrolysis of casein, starch, hypoxanthine, tyrosine and xanthine was tested on MA, by using the substrate concentrations described by Cowan & Steel (1965). Hydrolysis of aesculin, gelatin, Tweens 20, 40, 60 and 80 and urea and reduction of nitrate were investigated as described by Lányi (1987) with the modification that artificial seawater was used for preparation of media. The artificial seawater contained (per litre distilled water) 23.6 g NaCl, 0.64 g KCl, 4.53 g MgCl₂·6H₂O, 5.94 g MgSO₄·7H₂O and 1.3 g CaCl₂·2H₂O (Bruns *et al.*, 2001). Utilization of substrates as sole carbon and energy sources was tested as described by Baumann & Baumann (1981), by using supplementation with 2% (v/v) Hutner's mineral base (Cohen-Bazire *et al.*, 1957) and 1% (v/v) vitamin solution (Staley, 1968). Susceptibility to antibiotics was tested on MA plates using discs containing the following antibiotics: polymyxin B (100 U), streptomycin (50 µg), penicillin G (20 U), chloramphenicol (100 µg), ampicillin (10 µg), cephalothin (30 µg), gentamicin (30 µg), novobiocin (5 µg), tetracycline (30 µg), kanamycin (30 µg), lincomycin (15 µg), oleandomycin (15 µg), neomycin (30 µg) and carbenicillin (100 µg). Enzyme activities were determined by using the API ZYM system (bioMérieux).

Cell biomass of strain DPG-28^T for DNA extraction and for analyses of isoprenoid quinones and polar lipids was obtained

Table 1. Differential phenotypic characteristics between strain DPG-28^T and the type strains of *M. cryptomonadis*, *M. litorea* and *G. byunsanensis*

Strains: 1, DPG-28^T; 2, *M. cryptomonadis* KCCM 90070^T; 3, *M. litorea* KCCM 90071^T; 4, *G. byunsanensis* SMK-114^T. Data for reference strains are from Hwang *et al.* (2009) and Yoon *et al.* (2010) unless otherwise indicated. All strains are Gram-stain-negative and aerobic. All strains are positive for catalase and oxidase, activity* of esterase (C4) and esterase lipase (C8) and susceptibility* to ampicillin, carbenicillin, cephalothin, chloramphenicol, gentamicin, kanamycin and novobiocin. All strains are negative for hydrolysis of starch, utilization* of sucrose, trehalose, benzoate, formate, salicin and L-glutamate, activity* of lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, N-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase and susceptibility* to lincomycin. w, Weakly positive reaction.

Characteristic	1	2	3	4
Motility	–	+	+	–
Growth at/with:				
10 °C	+	–	–	+
40 °C	w	–	–	+
10% NaCl	–	+	+	–
Nitrate reduction	+	–	–	+
Hydrolysis of:				
Aesculin	+	+	+	–
Gelatin	–	+	–	–
Tween 80	+	+	+	–
Enzyme activity (API ZYM)*				
Alkaline phosphatase	–	+	+	–
Leucine arylamidase	w	w	+	–
Utilization of:*				
L-Arabinose	–	–	–	w
Cellobiose	–	–	+	w
D-Fructose	–	–	+	w
D-Galactose	–	–	+	w
D-Glucose	–	–	+	w
Maltose	–	+	–	–
D-Mannose	–	–	+	w
D-Xylose	–	–	–	+
Acetate	–	–	+	+
Citrate	–	+	–	–
L-Malate	–	+	–	+
Pyruvate	–	+	+	+
Succinate	–	+	–	+
Antibiotic susceptibility*				
Neomycin	+	–	–	+
Oleandomycin	–	+	+	–
Penicillin G	+	–	–	+
Polymyxin B	–	+	+	–
Streptomycin	+	–	+	+
Tetracycline	+	–	–	–
DNA G + C content (mol%)	65.1	58.6	61.0	60.0

*Data for *M. cryptomonadis* KCCM 90070^T and *M. litorea* KCCM 90071^T are from this study.

from cultures grown for 5 days in MB at 30 °C. Cell biomass of *M. cryptomonadis* KCCM 90070^T, *M. litorea* KCCM 90071^T and *G. byunsanensis* SMK-114^T for analysis of polar lipids was obtained from cultures grown for 5 days in MB at 30 °C. Chromosomal DNA was isolated and purified according to the method described previously (Yoon *et al.*, 1996), with the exception that RNase T1 was used in combination with RNase A to minimize contamination by RNA. The 16S rRNA gene was amplified by PCR using two universal primers as described previously (Yoon *et al.*, 1998). Sequencing of the amplified 16S rRNA gene and phylogenetic analysis were performed as described by Yoon *et al.* (2003). Isoprenoid quinones were extracted according to the method of Komagata & Suzuki (1987) and were analysed using reversed-phase HPLC and a YMC ODS-A (250 × 4.6 mm) column. For analysis of fatty acid methyl esters, cell mass of strain DPG-28^T, *M. cryptomonadis* KCCM 90070^T, *M. litorea* KCCM 90071^T and *G. byunsanensis* SMK-114^T was harvested from MA plates after incubation for 7 days at 30 °C. Fatty acids were saponified, methylated and extracted using the standard protocol of the MIDI system (Sherlock Microbial Identification System, version 4.0). Fatty acids were analysed by GC (Hewlett Packard 6890) and identified by using the TSBA40 database of the Microbial Identification System (Sasser, 1990). Polar lipids were extracted according to the procedures described by Minnikin *et al.* (1984) and identified by two-dimensional TLC followed by spraying with appropriate detection reagents (Minnikin *et al.*, 1984; Komagata & Suzuki, 1987). The position of phosphatidylcholine on thin-layer chromatographs was confirmed by spraying with Dragendorff's reagent (Sigma). The DNA G+C content was determined by the method of Tamaoka & Komagata (1984) with the modification that DNA was hydrolysed and the resultant nucleotides were analysed by reversed-phase HPLC. DNA–DNA hybridization was performed fluorometrically

according to the method of Ezaki *et al.* (1989) by using photobiotin-labelled DNA probes and microdilution wells. Hybridization was performed at 45 °C with five replications for each sample. The highest and lowest values obtained in each sample were excluded, and the means of the remaining three values were quoted as DNA–DNA relatedness values.

Morphological, cultural, physiological and biochemical characteristics of strain DPG-28^T are given in the species description and in Table 1. The almost-complete 16S rRNA gene sequence of strain DPG-28^T determined in this study comprised 1384 nt (approx. 96 % of the *Escherichia coli* 16S rRNA gene sequence). In the neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, strain DPG-28^T formed a coherent cluster with the type strains of *M. cryptomonadis*, *M. litorea* and *G. byunsanensis* (Fig. 1). Strain DPG-28^T shared 98.5, 97.8 and 97.8 % 16S rRNA gene sequence similarity with *M. cryptomonadis* CL-SK44^T, *M. litorea* CL-JM1^T and *G. byunsanensis* SMK-114^T, respectively. 16S rRNA gene sequence similarity among *M. cryptomonadis* CL-SK44^T, *M. litorea* CL-JM1^T and *G. byunsanensis* SMK-114^T was in the range 97.9–99.2 %. The DNA G+C content of strain DPG-28^T was 65.1 mol%.

The predominant isoprenoid quinone detected in strain DPG-28^T was ubiquinone-10 (Q-10), which is the same as reported for members of the genera *Marivita* and *Gaetbulicola* (Hwang *et al.*, 2009; Yoon *et al.*, 2010). The cellular fatty acid profiles of strain DPG-28^T and the type strains of *M. cryptomonadis*, *M. litorea* and *G. byunsanensis*, which were analysed under identical conditions and with identical methods, are shown in Table 2. The major fatty acids (>10 % of the total) found in strain DPG-28^T were C_{18:1}ω7c and 11-methyl C_{18:1}ω7c (Table 2). The fatty acid profiles of the four strains were essentially similar in that the common predominant component was C_{18:1}ω7c, although

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the positions of strain DPG-28^T, members of the genera *Marivita* and *Gaetbulicola* and some other related taxa. Bootstrap values (expressed as percentages of 1000 replications) >50 % are shown at branch points. Filled circles indicate that the corresponding nodes were also recovered in trees generated with the maximum-likelihood and maximum-parsimony algorithms; open circles indicate that the corresponding nodes were also recovered in the tree generated with the maximum-parsimony algorithm. The sequence of *Stappia stellulata* IAM 12621^T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

Table 2. Cellular fatty acid compositions of strain DPG-28^T and the type strains of *M. cryptomonadis*, *M. litorea* and *G. byunsanensis*

Strains: 1, DPG-28^T; 2, *M. cryptomonadis* KCCM 90070^T; 3, *M. litorea* KCCM 90071^T; 4, *G. byunsanensis* SMK-114^T. All data are from this study. Values are percentages of total fatty acids; fatty acids that represented <0.5 % in all strains were omitted. —, Not detected; ECL, equivalent chain-length.

Fatty acid	1	2	3	4
Straight-chain				
C _{14:0}	—	—	—	2.0
C _{16:0}	2.8	2.5	4.0	3.1
C _{17:0}	3.0	2.8	2.4	—
C _{18:0}	7.7	3.6	6.9	7.5
Unsaturated				
C _{14:1} ω5c	—	—	—	0.7
C _{18:1} ω7c	69.9	71.2	67.9	72.3
C _{18:1} ω9c	1.2	—	1.1	—
C _{20:1} ω7c	—	—	—	1.5
Branched				
iso-C _{15:1}	—	—	—	0.9
Hydroxy				
C _{12:1} 3-OH	3.2	3.1	2.6	—
C _{13:0} 2-OH	—	—	—	1.1
11-Methyl C _{18:1} ω7c	10.5	16.9	14.1	4.9
C _{19:0} cyclo ω8c	—	—	—	2.0
Summed features*				
5	—	—	—	4.0
7	0.9	—	0.4	—
Unknown ECL 18.814	—	—	0.6	—

*Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 5 comprised anteiso-C_{18:0} and/or C_{18:2}ω6,9c; summed feature 7 comprised an unknown fatty acid (ECL 18.846), C_{19:1}ω6c and/or C_{19:0} cyclo ω10c.

there were differences in the proportions of some fatty acids and in the presence or absence of some fatty acids (Table 2). The major polar lipids detected in strain DPG-28^T were phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine and an unidentified aminolipid (see Fig. S1, available in IJSEM Online). The major polar lipids of *M. cryptomonadis* KCCM 90070^T, *M. litorea* KCCM 90071^T and *G. byunsanensis* SMK-114^T, which were analysed in this study, were the same as those of strain DPG-28^T (Fig. S1). The polar lipid profiles of *M. cryptomonadis* KCCM 90070^T and *M. litorea* KCCM 90071^T determined in this study differed from those given by Hwang *et al.* (2009) in that diphosphatidylglycerol and an unidentified lipid were absent. Taking these data into account, it is our view that the genera *Marivita* and *Gaetbulicola* should be integrated into one genus, *Marivita*. Accordingly, we propose that *Gaetbulicola byunsanensis* be reclassified as a member of the genus *Marivita*, as *Marivita*

byunsanensis comb. nov., and that strain DPG-28^T be assigned to the genus *Marivita*. An emended description of the genus *Marivita* is also given.

Strain DPG-28^T was distinguishable from *M. cryptomonadis*, *M. litorea* and *G. byunsanensis* based on differences in several phenotypic characteristics, many of which were determined under the same conditions and with the same methods (Table 1). Strain DPG-28^T exhibited levels of DNA–DNA relatedness of 15, 17 and 11 % to *M. cryptomonadis* KCCM 90070^T, *M. litorea* KCCM 90071^T and *G. byunsanensis* SMK-114^T, respectively. Accordingly, the phylogenetic and genetic distinctiveness and differential phenotypic properties of strain DPG-28^T are sufficient to show that this strain represents a species separate from *M. cryptomonadis*, *M. litorea* and *G. byunsanensis* (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994). On the basis of the phenotypic, chemotaxonomic, phylogenetic and genetic data, strain DPG-28^T is considered to represent a novel species of the genus *Marivita*, for which the name *Marivita hallyeonensis* sp. nov. is proposed.

Description of *Marivita hallyeonensis* sp. nov.

Marivita hallyeonensis (hall.yeo.nen'sis. N.L. fem. adj. *hallyeonensis* pertaining to Hallyeo Marine National Park, the location of Geojedo, the Korean island from where the type strain was isolated).

Cells are Gram-stain-negative, non-spore-forming, non-flagellated rods (0.4–1.3 × 1.0–8.0 µm). Colonies on MA are circular, slightly convex, smooth, glistening, reddish orange and 0.7–1.2 mm in diameter after incubation for 7 days at 30 °C. Cells contain poly-β-hydroxybutyrate granules. Optimal growth occurs at 30 °C; growth occurs at 10 and 40 °C, but not at 4 or 45 °C. Optimal growth occurs between pH 7.0 and 7.5; growth occurs at pH 6.0 but not at pH 5.5. Optimal growth occurs in the presence of 2 % (w/v) NaCl; growth occurs with 0.5–8.0 % (w/v) NaCl. Mg²⁺ ions are required for growth. Growth does not occur under anaerobic conditions on MA or on MA supplemented with nitrate. Catalase- and oxidase-positive. Nitrate is reduced to nitrite. Hydrolyses aesculin and Tweens 40 and 80, but not casein, gelatin, hypoxanthine, xanthine, tyrosine or starch. Does not utilize L-arabinose, cellobiose, D-fructose, D-galactose, D-glucose, maltose, D-mannose, sucrose, trehalose, D-xylose, acetate, benzoate, citrate, formate, L-malate, pyruvate, succinate, salicin or L-glutamate as carbon and energy sources. The predominant ubiquinone is Q-10. The major fatty acids (>10 % of the total) are C_{18:1}ω7c and 11-methyl C_{18:1}ω7c. The major polar lipids are phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine and an unidentified aminolipid. The DNA G+C content of the type strain is 65.1 mol% (HPLC). Other phenotypic properties are given in Table 1.

The type strain, DPG-28^T (=KCTC 23421^T =CCUG 60522^T), was isolated from seawater from a seaweed farm in Geojedo on the South Sea, Korea.

Description of *Marivita byunsanensis* Yoon et al. 2010 comb. nov.

Marivita byunsanensis (byun.sa.nen'sis. N.L. fem. adj. *byunsanensis* of Byunsan, where the type strain was isolated).

Basonym: *Gaetbulicola byunsanensis* Yoon et al. 2010.

The description is the same as that given by Yoon et al. (2010).

Emended description of the genus *Marivita* Hwang et al. 2009

The description of the genus *Marivita* is as given by Hwang et al. (2009) with the following amendments. Cells are rods or pleomorphic. Motile or non-motile. The major fatty acid is C_{18:1}ω7c. Major polar lipids are phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine and an unidentified aminolipid. The DNA G+C content is 58.6–65.1 mol%.

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