INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY

TAXONOMIC DESCRIPTION

Khan *et al.*, *Int. J. Syst. Evol. Microbiol.* 2020;70:2239–2246

DOI 10.1099/ijsem.0.003976

MICROBIOLOGY SOCIETY

Aestuariirhabdus litorea gen. nov., sp. nov., isolated from a sea tidal flat and proposal of *Aestuariirhabdaceae* fam. nov.

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Abstract

A Gram-negative, moderately halophilic and facultatively aerobic bacterium, designated strain GTF13^T, was isolated from a sea tidal flat. Cells were curved rods and motile by a single polar flagellum showing catalase and oxidase activities. Growth was observed at 20–37 °C, pH 5.0–8.5 and 1.0–6.0% (w/v) NaCl. Strain GTF13^T contained $C_{16:0}$ summed feature 3 (comprising $C_{16:1}\,\omega6c/C_{16:1}\,\omega7c$), summed feature 8 (comprising $C_{18:1}\,\omega6c/C_{18:1}\,\omega7c$) and $C_{12:0}$ 3-OH as major fatty acids and ubiquinone-9 and ubiquinone-8 as major quinones. Phosphatidylethanolamine and two unidentified phospholipids were detected as major polar lipids. The G+C content of the genomic DNA was 59.8 mol%. Strain GTF13^T was most closely related to *Simiduia agarivorans* SA1^T, *Endozoicomonas montiporae* CL-33^T and *Pseudomonas segetis* FR1439^T, belonging to different families or orders of the class *Gammaproteobacteria*, with less than 92.0% 16S rRNA gene sequence similarities. Phylogenetic analyses based on 16S rRNA gene sequences showed that strain GTF13^T formed a phylogenetic lineage with the family *Litoricolaceae*, but the genome-based phylogenomic tree showed that strain GTF13^T formed a distinct phylogenetic lineage within the order *Oceanospirillales*. The very low 16S rRNA gene sequence similarities and distinct phylogenetic relationships, together with distinct phenotypic and chemotaxonomic properties, served to differentiate strain GTF13^T from phylogenetically closely related families. Here, strain GTF13^T is proposed as a novel genus and species, for which the name *Aestuariirhabdus litorea* gen. nov., sp. nov. is proposed, within a new family *Aestuariirhabdaceae* fam. nov. of the order *Oceanospirillales*. The type strain is GTF13^T (=KACC 19788^T=JCM 32043^T).

The order *Oceanospirillales* belonging to the class *Gammaproteobacteria*, proposed by Garrity *et al.* [1], currently includes ten validly published families, *Oleiphilaceae* [2], *Saccharospirillaceae* [3], *Oceanospirillaceae* [4], *Alcanivoraceae* [5], *Hahellaceae* [6], *Litoricolaceae* [7], *Halomonadaceae* [8], *Kangiellaceae* [9], *Endozoicomonadaceae* [10] and *Balneatrichaceae* [11], which were classified largely based on 16S rRNA gene sequence phylogeny. The order encompasses a diverse range of Gram-negative bacteria that are generally halophilic or halotolerant, rod-shaped, motile by means of a polar flagellum (except for the members of the families *Litoricolaceae* and *Kangiellaceae*) and chemoheterotrophic. Most members of the order *Oceanospirillales* are aerobic, but some members were characterized as facultatively aerobic and/or anaerobic [4, 7]. The genomic DNA G+C contents vary from 40 to 66 mol% and ubiquinone (Q)-8 and/or Q-9 are identified as the major respiratory quinone(s) in most members of the order *Oceanospirillales*, although some members (e.g. *Bacterioplanoides pacificum*) within the family *Oceanospirillaceae* contain Q-10 as the major respiratory quinone [12]. In the study, a presumably novel strain that could not be assigned to a defined family of the order *Oceanospirillales* was isolated and characterized taxonomically using a polyphasic approach.

Strain GTF13^T was isolated from a sea tidal flat sample collected at Gangjin Bay (34° 27′ 51.7″ N 126°45′ 59.4″ E) in the Republic of Korea by the following procedure described previously [13]. In brief, a tidal flat sediment sample was serially diluted in artificial seawater (20 g l^{-1} NaCl, 2.9 g l^{-1} $MgSO_4$, 4.53 g l^{-1} $MgCl_2{\cdot}6H_2O$, 0.64 g l^{-1} KCl and 1.75 g l^{-1} $CaCl_2{\cdot}2H_2O$), spread on marine agar (MA; BD) and incubated

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Keywords: *Aestuariirhabdus litorea*; Aestuariirhabdaceae; new family; sea tidal flat.
Abbreviations: MA, marine agar; MB, marine broth; ML, maximum-likelihood; MP, maximum-parsimony; NJ, neighbour-joining; Q, ubiquinone; RDP, Ribosomal Database Project.
The GenBank accession numbers for the 16S rRNA gene and genome sequences of strain GTF13^T and the genome sequence of *Litoricola lipolytica* IMCC1097^T are MH742310, QWEZ00000000 and CP045871, respectively.
Three supplementary figures and one supplementary table are available with the online version of this article.

at 25 °C for 5 days under an aerobic condition. The 16S rRNA genes of colonies grown on MA were PCR-amplified using the universal primers F1 (5′-AGAGTTTGATCMTGGCTCAG-3′) and R13 (5′-TACGGYTACCTTGTTACGACTT-3′) and double-digested with *Hae*III and *Hha*I as described previously [14]. Representative PCR products showing discrete fragment patterns were partially sequenced using the universal primer 340F (5′-CCTACGGGAGGCAGCAG-3′). The resulting 16S rRNA gene sequences were compared with those of all type strains of validly published species using the Nucleotide Similarity Search program in the EzBioCloud server (www.ezbiocloud.net/identify) [15]. A putative novel strain belonging to the order *Oceanospirillales*, designated strain GTF13^T, was selected for the further taxonomic characterization. Strain GTF13^T was routinely cultured aerobically on MA at 30 °C for 3 days. For a long-term storage, strain GTF13^T was preserved at −80 °C in marine broth (MB) containing a final concentration of 15% (v/v) glycerol. *Endozoicomonas montiporae* LMG 24815^T, *Oleiphilus messinensis* LMG 20357^T, *Marinospirillum minutulum* KACC 15214^T and *Zooshikella ganghwensis* KACC 14329^T were purchased from their corresponding collection centres and *Litoricola lipolytica* IMCC1097^T was obtained from Professor JC Cho at Inha University (Republic of Korea) as a gift to use them as reference strains for the comparisons of phenotypic properties and fatty acid compositions.

The amplified PCR product of strain GTF13^T was further sequenced using the universal primers 518R (5′-ATTACCGCGGCTGCTGG-3′) and 805F (5′-GATTAGATACCCTGGTAGTC-3′) [14] at Macrogen (Republic of Korea) to get its almost-complete 16S rRNA gene sequence. The sequences obtained by 340F, 518R and 805F primers were assembled together and an almost-complete 16S rRNA gene sequence (1466 nucleotides) of strain GTF13^T was generated. Phylogenetic analysis of strain GTF13^T through multiple alignment and generation of phylogenetic trees of 16S rRNA gene sequences using the ARB package based on the ARB database [16] was carried out to infer its phylogenetic position. The 16S rRNA gene sequence similarity values between strain GTF13^T and closely related type strains were calculated using the EzTaxon-e server (www.ezbiocloud.net) [15]. The 16S rRNA gene sequences of strain GTF13^T and its closely related type strains were aligned using the fast secondary-structure-aware infernal aligner available in the Ribosomal Database Project (RDP) [17] and their phylogenetic trees were reconstructed by using the MEGA7 software [18]. The Kimura two-parameter model, the nearest-neighbour-interchange heuristic search method and the complete deletion option with bootstrap values (1000 replications) were used for the reconstruction of neighbour-joining (NJ), maximum-parsimony (MP) and maximum-likelihood (ML) trees, respectively. An additional taxonomic analysis of strain GTF13^T was also performed against RDP 16S rRNA training set 16 using the RDP Naïve Bayesian rRNA Classifier (version 2.11; http://rdp.cme.msu.edu/classifier/) [17] to infer its phylogenetic position.

For the whole genome sequencing of strain GTF13^T and *L. lipolytica* IMCC1097^T, their genomic DNA was extracted using a Wizard Genomic DNA purification kit (Promega) according to the protocol described by the manufacturer and the phenol–chloroform extraction and ethanol precipitation method, respectively [19], and sequenced with 151 bp paired-end reads by an Illumina Hiseq X instrument at Macrogen. The sequences of strain GTF13^T were *de novo* assembled by using SOAPdenovo2 [20]. The genomic DNA of *L. lipolytica* IMCC1097^T was additionally sequenced using an Oxford Nanopore MinION sequencer (Nanopore) and *de novo* assembled by using Unicycler (version 0.4.7) [21]. The sequences of *L. lipolytica* IMCC1097^T derived from the Illumina sequencing were used for error corrections by mapping them on the assembled genome derived from the MinION sequencing.

The *de novo* assembly of the Illumina genome sequencing data of strain GTF13^T resulted in the draft genome of seven contigs with an N50 value of 2189 kb and average genome coverage of 604×. The genome size of strain GTF13^T had a total length of approximately 3663 kb with an average G+C content of 59.8 mol% and contained 57 tRNA genes for 20 amino acids. A total of 3417 genes were predicted and among them, 3342 protein-coding genes were identified. On the other hand, *de novo* assembly of the MinION sequencing data of *L. lipolytica* IMCC1097^T produced a complete genome with an average genome coverage of 656.7× and the complete genome was error-corrected using the Illumina genome sequencing data of *L. lipolytica* IMCC1097^T with an average genome coverage of 1751.7×. The genome of *L. lipolytica* IMCC1097^T had a circular chromosome of approximately 2,346 kb with an average G+C content of 58.8 mol%, and 2395 total genes, 2332 protein coding sequences, three rRNA gene operons (16S, 23S and 5S) and 40 tRNA genes encoding 20 amino acids were predicted. The genome sequences of strain GTF13^T and *L. lipolytica* IMCC1097^T have been deposited into GenBank with the accession numbers QWEZ00000000 and CP045871, respectively. The 16S rRNA gene sequences of strain GTF13^T and *L. lipolytica* IMCC1097^T obtained by the above PCR approach were identical to those in their genomes. The up-to-date bacterial core gene (UBCG) pipeline (www.ezbiocloud.net/tools/ubcg) [22] was used to extract 92 housekeeping core genes from the genomes of strain GTF13^T and related taxa for a genome-based phylogenomic analysis. An ML tree with bootstrap values (100 replications) based on the concatenated 92 housekeeping core genes was reconstructed by using the MEGA7 software.

Because 16S rRNA gene sequence similarities of strain GTF13^T and validly reported type strains were very low, first its phylogenetic inference using the ARB package based on the ARB database was performed, which suggested that strain GTF13^T may belong to the order *Oceanospirillales* of the class *Gammaproteobacteria*. The comparative analysis of 16S rRNA gene sequences between strain GTF13^T and all type strains showed that the strain was most closely related to *Simiduia agarivorans* SA1^T (92.0%), *Endozoicomonas montiporae* CL-33^T (91.9%), *Pseudomonas segetis* FR1439^T (91.8%), *Mangrovitalea sediminis* M11-4^T (91.8%), *Zooshikella ganghwensis* JC2044^T (91.7%) and *Alkalimarinus sediminis* FA028^T (91.7%), all of which belong to different

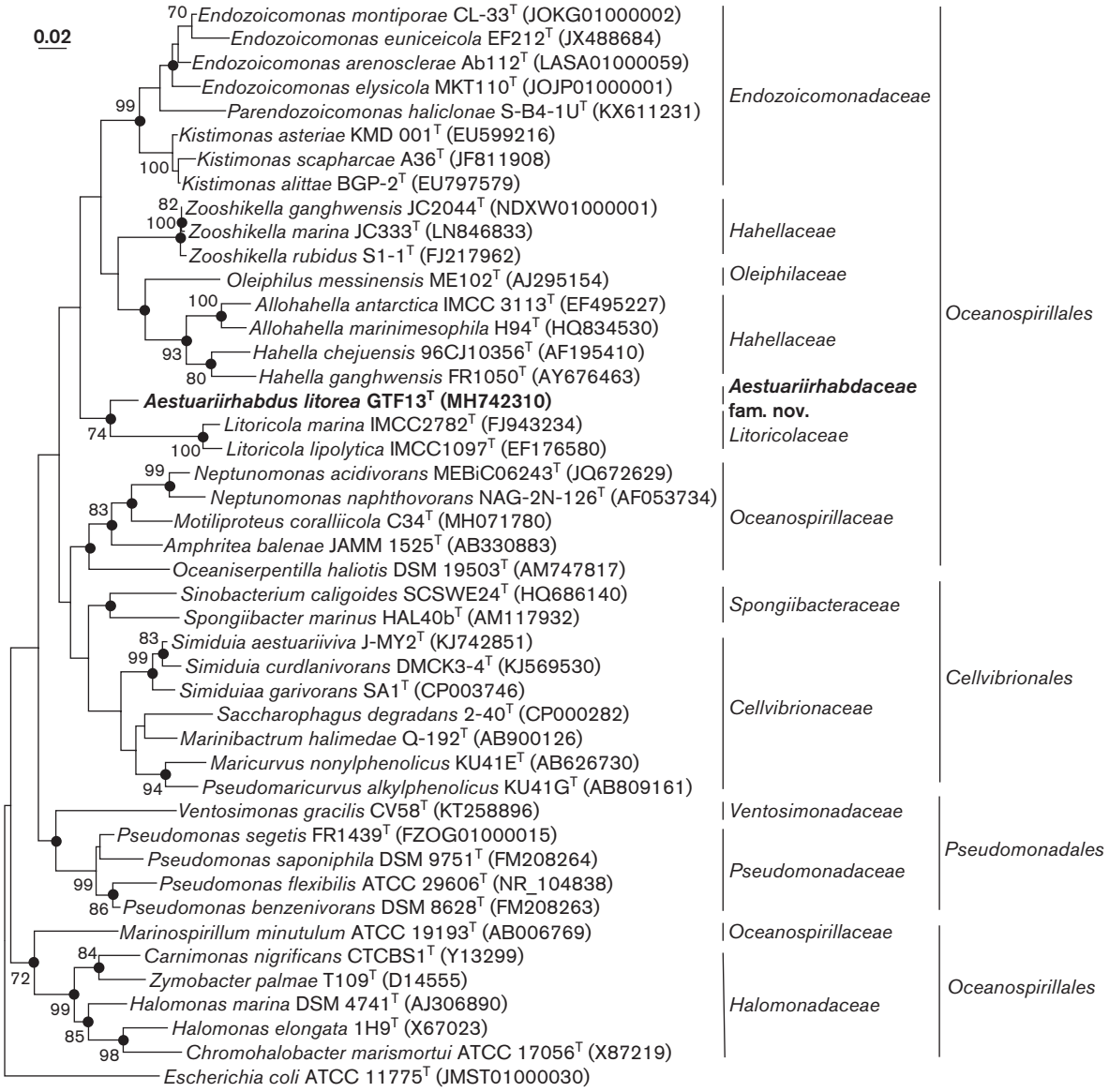

Fig. 1. A maximum-likelihood phylogenetic tree showing the phylogenetic relationships between strain GTF13^T and representatives of the class *Gammaproteobacteria*, based on 16S rRNA gene sequences. Filled circles (●) on the nodes indicate that the relationships were also recovered by the neighbour-joining and maximum-parsimony algorithms. Family and order names in the *Gammaproteobacteria* are indicated at right sides. Bootstrap values are shown on nodes as percentages of 1000 replicates for values over 70%. *Escherichia coli* ATCC 11775^T (JMST01000030) was used as an outgroup. Bar, 0.02 changes per nucleotide position.

families or orders in the class *Gammaproteobacteria*, probably suggesting that the strain cannot be assigned to any of the defined families of the order *Oceanospirillales*. The phylogenetic analyses using the ML, NJ and MP algorithms based on the 16S rRNA gene sequences showed that strain GTF13^T formed a phyletic lineage with the members of the genus *Litoricola* of the family *Litoricolaceae* within the order *Oceanospirillales* (Fig. 1, Fig. S1, available in the online version of this article). However, strain GTF13^T were distantly related to the members of the genus *Litoricola* in the phylogenetic trees and their 16S rRNA gene sequence similarities were very low (less than 91.1%). The taxonomic analysis of strain GTF13^T using the RDP classifier tool showed that strain GTF13^T was classified as an unclassified family of the order *Oceanospirillales* even at low confidence threshold (60%). In addition, the genome-based phylogenomic tree using 92 orthologous housekeeping core genes clearly showed that strain GTF13^T formed a distinct phylogenetic lineage from other members, especially the genus *Litoricola* of the family *Litoricolaceae*, within the order *Oceanospirillales* (Fig. 2), suggesting that strain GTF13^T represents a new family member of the order *Oceanospirillales*. The very low sequence similarities (<92%) and the distant relationships between strain GTF13^T and other

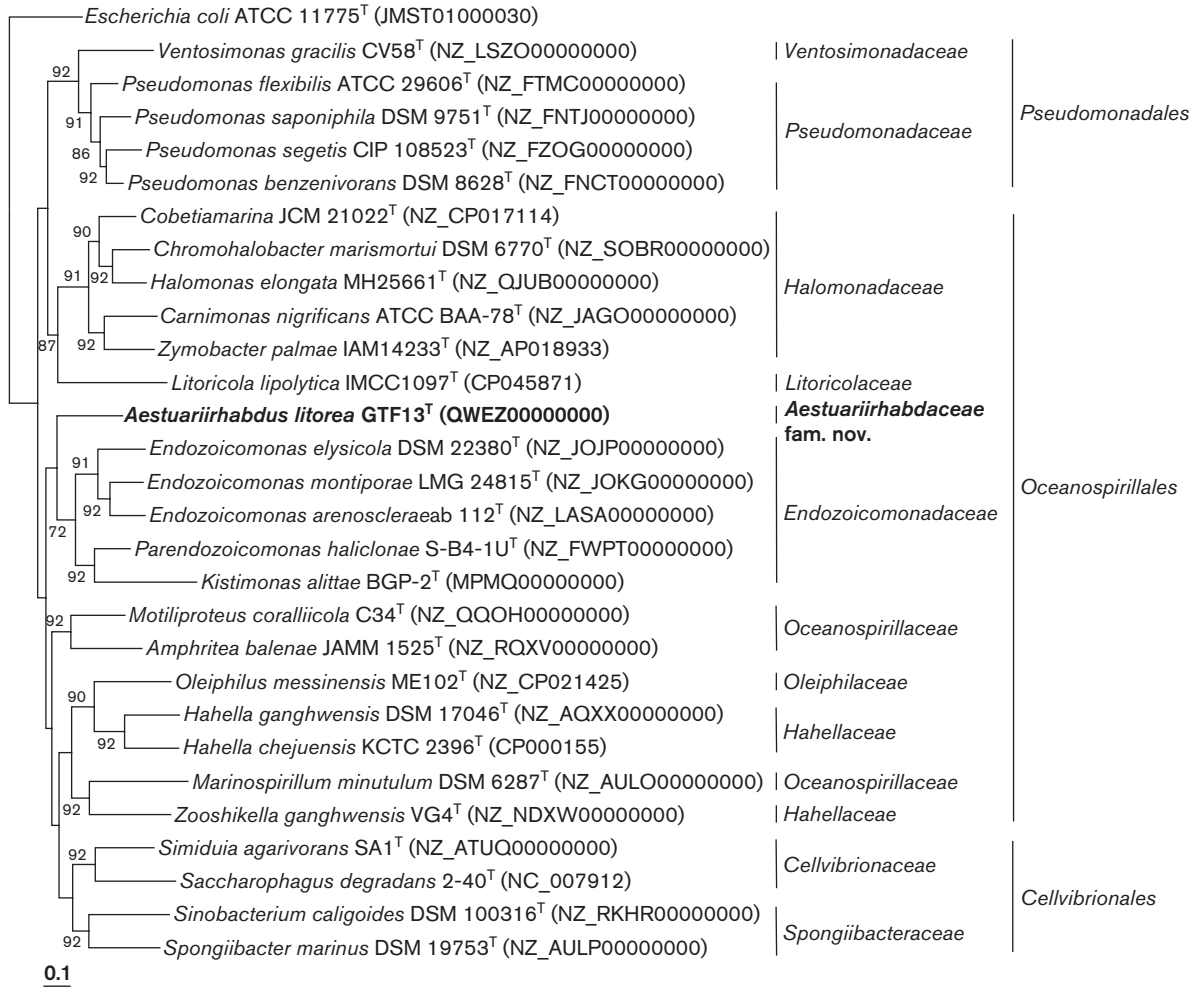

Fig. 2. A phylogenomic tree based on the concatenated 92 housekeeping core genes showing the phylogenetic relationships between strain GTF13^T and closely related taxa. Bootstrap values are shown on nodes as percentages of 1000 replicates for values over 70%. *Escherichia coli* ATCC 11775^T (JMST01000030) was used as an outgroup. Bar, 0.1 changes per nucleotide position.

family members of the order *Oceanospirillales* suggest that strain GTF13^T can represent a novel family of the order *Oceanospirillales*. On the other hand, results of both 16S rRNA gene sequence-based and genome-based phylogenetic analyses showed that members of the order *Oceanospirillales* were split into two lineages, suggesting that they may be separated into two different orders.

The growth of strain GTF13^T was tested at 30 °C for 3 days on various bacteriological agar media: MA, Reasoner's 2A (R2A) agar (BD), tryptic soy agar (BD), nutrient agar (BD) and laboratory-prepared Luria-Bertan (LB) agar. The growth of strain GTF13^T was assessed at different pH values (4.0–10.0 at 0.5 pH unit intervals) and temperatures (4, 15, 20, 25, 30, 37, 40 and 45 °C) in MB and MA, respectively, for 3 days. Sodium citrate, Na_2HPO_4/NaH_2PO_4, and Tris-HCl buffer systems were used to prepare MB media with pH 4.0–5.5, pH 6.0–7.0 and pH 8.0–10.0, respectively [23]. The pH was readjusted if necessary, after autoclaving for 15 min at 121 °C. The tolerance to NaCl of strain GTF13^{T}was evaluated in modified MB media with different NaCl concentrations (0–10%, w/v, at 0.5% intervals), which were prepared in the laboratory according to the BD formula. The following biochemical physiological tests of strain GTF13^T were performed using cells grown on MA for 3 days at 30 °C. Cell morphology and flagella of strain GTF13^T were investigated by transmission electron microscopy (JEM-1010, JEOL) after negative staining and phase-contrast microscopy (Scope.A1, Carl Zeiss after flagellum staining using Ryu staining solution [24]. Gram staining was investigated using the bioMérieux Gram stain kit, according to the protocol suggested by the manufacturer. Catalase and oxidase activities of strain GTF13^T were investigated by the production of oxygen bubbles in aqueous H_2O_2 (3%, v/v) and oxidation of 1% (w/v) tetramethyl-*p*-phenylenediamine, respectively (Merck) [25]. The anaerobic growth of strain GTF13^T was examined on MA at 30 °C for 21 days under an anaerobic condition generated by the BBL GasPak Plus anaerobic system (BD). The following properties of strain GTF13^T

Table 1. Cellular fatty acid compositions (%) of strain GTF13^T and closely related taxa of the order *Oceanospirillales*

Taxa: 1, strain GTF13^T; 2, *Litoricola lipolytica* IMCC1097^T; 3, *Endozoicomonas montiporae* LMG 24815^T; 4, *Oleiphilus messinensis* LMG 20375^T; 5, *Marinospirillum minutulum* KACC 15214^T; 6, *Zooshikella ganghwensis* KACC 14329^T. All data were from this study. Data are expressed as percentages of the total fatty acids and fatty acids amounting to less than 1.0% in all strains are not shown. Major components (>5.0%) are highlighted in bold. TR, Trace amount (<1.0%); –, not detected.

Fatty acid	1	2	3	4	5	6
Saturated:						
$C_{10:0}$	TR	TR	–	–	–	3.5
$C_{12:0}$	2.5	3.0	TR	4.9	–	–
$C_{14:0}$	1.6	2.1	**9.9**	**12.7**	3.0	**9.7**
$C_{16:0}$	**18.6**	**12.5**	**16.7**	**26.3**	**17.1**	**21.4**
$C_{17:0}$	1.0	–	–	–	–	–
$C_{18:0}$	TR	2.1	TR	3.8	1.0	TR
Unsaturated:						
$C_{16:1}$ ω9*c*	–	–	–	**8.8**	–	–
$C_{16:1}$ ω11*c*	–	–	–	–	4.0	–
$C_{17:1}$ ω8*c*	2.8	–	–	**12.4**	–	–
$C_{18:1}$ ω9*c*	–	TR	–	**13.8**	TR	–
Hydroxy:						
$C_{10:0}$ 3-OH	–	**12.0**	4.2	–	–	3.1
$C_{12:0}$ 3-OH	**6.9**	4.7	TR	**5.7**	–	4.6
$C_{16:0}$ 3-OH	3.1	–	TR	–	–	–
Branched:						
iso-$C_{15:0}$	–	–	–	–	**10.3**	–
anteiso-$C_{15:0}$	–	–	–	–	**33.1**	–
iso-$C_{15:0}$ 3-OH	TR	–	TR	–	–	**7.2**
iso-$C_{16:0}$	–	–	–	–	1.5	–
iso-$C_{17:0}$	–	–	–	–	4.9	–
anteiso-$C_{17:0}$	–	–	–	–	**9.4**	–
Summed features:*						
2	2.3	–	1.9	3.4	–	–
3	**36.9**	**36.1**	**41.0**	**16.0**	2.8	**36.4**
8	**19.5**	**24.2**	**23.3**	3.0	**5.1**	**7.5**

*Summed features represent groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 2, $C_{12:0}$ aldehyde; summed feature 3, $C_{16:1}$ ω6*c* and/or $C_{16:1}$ ω7*c*; summed feature 8, $C_{18:1}$ ω6*c* and/or $C_{18:1}$ ω7*c*.

and reference strains were investigated at their optimum growth conditions in parallel. Hydrolysis of tyrosine, casein, aesculin, starch, Tween 20 and Tween 80 was tested on MA, as described previously [25, 26]. Additional biochemical features and enzymatic activities of strain GTF13^T and the reference strains were evaluated using the API 20NE and API ZYM system (bioMérieux), according to the instructions of the manufacturer.

Strain GTF13^T grew well on MA and showed a slow growth on R2A agar, but did not grow on LB agar, tryptic soy agar or nutrient agar. Cells of strain GTF13^T were observed to be Gram-stain-negative, oxidase- and catalase-positive, and motile by means of a single polar flagellum and slightly curved long rods (0.5–0.6 µm wide and 2.0–5.0 µm long; Fig. S2). After 21 days of anaerobic incubation, weak growth of strain GTF13^T was observed. Catalase-positive activity, the slightly curved long rod shape and flagellum motility of strain GTF13^T clearly differentiated it from the members of *Litoricolaceae*, the phylogenetically most closely related family of the order *Oceanospirillales*, in which type strains are non-motile and short rods [7, 27] (Table S1).

Isoprenoid quinones of strain GTF13^T, *L. lipolytica* IMCC1097^T and *O. messinensis* LMG 20357^T were extracted and analysed using a model LC-20A HPLC system (Shimadzu) equipped with a diode array detector (SPD-M20A, Shimadzu) and a reversed-phase column (250×4.6 mm; Kromasil, Akzo Nobel), as described previously [28]. For the whole-cell fatty acids analysis, strain GTF13^T and five reference strains were cultured in MB under their optimum growth conditions and harvested during their exponential phases (optical density=0.8 at 600 nm). The cellular fatty acids of the harvested cells were saponified, methylated and extracted according to the MIDI standard protocol. The fatty acid methyl esters extracted were examined by gas chromatography (Hewlett Packard 6890) and identified by using the RTSBA6 database of the Microbial Identification System (Sherlock version 6.0B) [29]. The polar lipids of strain GTF13^T, *L. lipolytica* IMCC1097^T, *E. montiporae* LMG 24815^T and *M. minutulum* KACC 15214^T were extracted from cells harvested during their exponential growth phase and analysed by two-dimensional TLC, according to the procedure described by Minnikin *et al.* [30]. The following spray reagents were used to detect different types of polar lipids: 10% ethanolic molybdophosphoric acid (for total polar lipids), ninhydrin (for aminolipids), Dittmer–Lester reagent (for phospholipids) and α-naphthol/sulfuric acid (for glycolipids).

The isoprenoid quinone analysis showed that strain GTF13^T contained Q-9 (80%) and Q-8 (20%) as the major respiratory quinones, while *L. lipolytica* and *O. messinensis* contained Q-8 as the sole respiratory quinone, which supports that they may be phylogenetically different. The quinone profile of strain GTF13^T was also different from those of other phylogenetically closely related taxa, *M. minutulum* and *Z. ganghwensis* (Table S1). $C_{16:0}$, summed feature 3 (comprising $C_{16:1}$ ω6*c* and/or $C_{16:1}$ ω7*c*), summed

Table 2. General features of strain GTF13^T and phylogenetically closely related genera of the order *Oceanospirillales*

Taxa: 1, strain GTF13^T [this study]; 2, *Litoricola* [7, 27]; 3, *Endozoicomonas* [31–38]; 4, *Parendozoicomonas* [10]; 5, *Kistimonas* [39–41]; 6, *Oleiphilus* [2]. +, Positive; –, negative; v, variable (numbers in the parentheses indicate the numbers of positive species/total species reported in each genus).

Characteristic	1	2	3	4	5	6
Cell morphology	Curved rods	Short rods	Long rods	Long rods	Long rods	Thick rods
Flagellum motility	+	–	v (6/9)	+	+	+
Anaerobic growth	+	+	v (4/9)	+	v (2/3)	–
Growth at:						
4 °C	–	–	v (1/9)	–	–	–
40 °C	–	–	–	–	v (1/3)	–
0.5% NaCl	–	–	v (2/9)	+	v (2/3)	+
6% NaCl	+	+	–	–	v (1/3)	+
Catalase activity	+	v (1/2)	+	+	v (1/3)	+
Oxidase activity	+	+	+	+	v (1/3)	+
Major quinone	Q-9	Q-8	Q-9	Q-9	Q-9	Q-8
Major hydroxyl fatty acids	$C_{12:0}$ 3-OH	$C_{10:0}$ 3-OH	$C_{10:0}$ 3-OH	$C_{10:0}$ 3-OH	$C_{10:0}$ 3-OH	$C_{10:0}$ 3-OH, $C_{12:0}$ 3-OH
DNA G+C content (mol%)	59.8	58.8–59.6	47–51	50.1–51.4	47.3–52.5	47.8
Major phospholipids*	PE, PL	PE, PL	PE, PS, PG, PL	PE, PS, PG, DPG, PL	PE, PS, PG, PL, DPG	PE, PG, DME, PL

*PE, phosphatidylethanolamine; PS, phosphatidylserine; DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; DME, phosphatidyl dimethylethylamine; PL, unidentified phospholipid.

feature 8 (comprising $C_{18:1}$ ω6*c* and/or $C_{18:1}$ ω7*c*) and $C_{12:0}$ 3-OH were detected from strain GTF13^T as major cellular fatty acids (>5% of total fatty acids), which were quite different from those of the reference strains in some fatty acids (Table 1). For example, $C_{10:0}$ 3-OH was not detected from strain GTF13^T, but it was detected as one of major fatty acids from *L. lipolytica* IMCC1097^T. In addition, $C_{11:0}$, $C_{17:0}$ and $C_{17:1}$ ω6*c* were detected from strain GTF13^T, but they were not detected from the reference strains. Phosphatidylethanolamine and two unidentified phospholipids as major polar lipids and an unidentified polar lipid as a minor polar lipid were identified from strain GTF13^T, of which profile was different from those of phylogenetically closely related taxa (Fig. S3 and Table S1). In particular, the polar lipid profile of strain GTF13^T was quite different from that of *O. messinensis* having phosphatidylglycerol and phosphatidyl dimethylethylamine.

It is evident that the low 16S rRNA gene sequence similarity (<92), the distant phylogenetic relationships by the genome-based phylogenomic analysis and the difference of morphology, major respiratory quinone, hydroxyl fatty acids and polar lipids and some distinguishable phenotypic features of strain GTF13^T can differentiate strain GTF13^T from closely related genera of the families in the order *Oceanospirillales* (Table 2). Therefore, in this study, it is proposed that strain GTF13^T represents a novel genus and species, for which the name *Aestuariirhabdus litorea* gen. nov., sp. nov. is proposed, within a new family *Aestuariirhabdaceae* fam. nov. of the order *Oceanospirillales*.

DESCRIPTION OF THE GENUS *AESTURIIRHABDUS* GEN. NOV.

Aestuariirhabdus (Aes.tu.a.ri.i.rhab′dus. L. neut. n. *aestuarium* mud flat; Gr. fem. n. *rhabdos* rod; N.L. fem. n. *Aestuariirhabdus* a rod-shaped bacterium from mud flat).

Cells are Gram-stain-negative and motile curved rods. Facultatively aerobic. Q-9 and Q-8 are detected as the major isoprenoid quinones. Phosphatidylethanolamine and two unidentified phospholipids are detected as the major polar lipids. Catalase- and oxidase-positive. Phylogenetically, the genus is a member of the family *Aestuariirhabdaceae* within the order *Oceanospirillales* of the phylum *Proteobacteria*. The type species is *Aestuariirhabdus litorea*.

DESCRIPTION OF *AESTURIIRHABDUS LITOREA* SP. NOV.

Aestuariirhabdus litorea (li.to′re.a. L. fem. adj. *litorea* inhabiting seashore).

In addition to the characteristics given in the genus description above, this species has the following properties. Colonies are circular, convex, opaque, entire, smooth and glistening

with regular margins. Growth occurs at 20–37 °C (optimum, 30 °C), at pH 5.0–8.5 (optimum, pH 5.0) and in the presence of 1.0–6.0% (w/v) NaCl (optimum, 2.0%). Major cellular fatty acids are $C_{16:0,}$ summed feature 3 (comprising $C_{16:1}\,\omega6c$ and/or $C_{16:1}\,\omega7c$), summed feature 8 (comprising $C_{18:1}\,\omega6c$ and/or $C_{18:1}\,\omega7c$) and $C_{12:0}$ 3-OH. Tween 20 hydrolysis, nitrate reduction, arginine dihydrolase, urease, gelatinase, alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase activities and D-mannitol assimilation are positive. Tween 80, tyrosine, casein, starch and aesculin hydrolysis, indole production, glucose fermentation, *β*-galactosidase, cystine arylamidase, trypsin, *α*-chymotrypsin, *α*-galactosidase, *β*-glucuronidase, *β*-glucosidase, *α*-glucosidase, *N*-acetyl-*β*-glucosaminidase, *α*-mannosidase and *α*-fucosidase activities and D-glucose, L-arabinose, D-mannose, *N*-acetyl-glucosamine, maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid assimilation are negative.

The type strain is GTF13^T (=KACC 19788^T = JCM 32043^T), isolated from a sea tidal flat, in the Republic of Korea. The type strain has 59.8 mol% G+C content in the genomic DNA. The GenBank accession numbers of strain GTF13^T for the 16S rRNA gene and genome sequences are MH742310 and QWEZ00000000, respectively.

DESCRIPTION OF *AESTURIIRHABDACEAE* FAM. NOV.

Aestuariirhabdaceae (Aes.tu.a.ri.i.rhab.da.ce'ae. N.L. fem. n. *Aestuariirhabdus* type genus of the family; *-aceae* ending to denote a family; N.L. fem. pl. n. *Aestuariirhabdaceae* the family of the genus *Aestuariirhabdus*).

The family *Aestuariirhabdaceae* is within the order *Oceanospirillales* and encompasses Gram-negative bacteria retrieved from marine environments. Based on results of 16S rRNA gene sequence analysis and its genotypic, chemotaxonomic and phenotypic properties, the family is a member of the order *Oceanospirillales* in the class *Gammaproteobacteria*. Currently, the family comprises the genus *Aestuariirhabdus*. The description is the same as described for the genus *Aestuariirhabdus*. The type genus of the family is *Aestuariirhabdus*.

Funding information
This work was supported by the Korea Environmental Industry and Technology Institute (KEITI) through the Public Technology Program based on Environmental Policy (Project No. 2016000200012) and the Program for Collection of Domestic Biological Resources from the National Institute of Biological Resources (NIBR No. 2019-02-001) of the Ministry of Environment (MOE), Republic of Korea.

Conflicts of interest
The authors declare that there are no conflicts of interest.

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