

# *Aliikangiella coralliicola* sp. nov., a bacterium isolated from coral *Porites lutea*, and proposal of *Pleioneaceae* fam. nov. to accommodate *Pleionea* and *Aliikangiella*

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## Abstract

A novel Gram-stain-negative, non-endospore-forming, motile, and aerobic bacterial strain, M105<sup>T</sup>, was isolated from coral *Porites lutea*, and was subjected to a polyphasic taxonomic study. Global alignment based on 16S rRNA gene sequences indicated that M105<sup>T</sup> shares the highest sequence identity of 94.5% with *Aliikangiella marina* GYP-15<sup>T</sup>. The average nucleotide identity (ANI) and average amino acid identity (AAI) between M105<sup>T</sup> and *A. marina* GYP-15<sup>T</sup> was 69.8 and 71.6%, respectively. On the basis of the results of phenotypic, chemotaxonomic, phylogenetic, phylogenomic, and comparative genomic analyses, it is concluded that M105<sup>T</sup> should represent a novel species in the genus *Aliikangiella*, for which the name *Aliikangiella coralliicola* sp. nov. is proposed. The type strain is M105<sup>T</sup> (=MCCC 1K03773<sup>T</sup>=KCTC 72442<sup>T</sup>). Furthermore, the family *Kangiellaceae* was classified into two families on the basis of phylogenetic, phylogenomic, polar lipid profile and motility variations. The novel family *Pleioneaceae* fam. nov. is proposed to accommodate the genera *Aliikangiella* and *Pleionea*.

The family *Kangiellaceae* was established to accommodate some species of the family *Alcanivoracaceae* in the order *Oceanospirillales*, on the basis of phylogenetic, chemotaxonomic and physiological characteristics, which included the genera *Kangiella*, *Pleionea* and *Aliikangiella* [1]. Cells from the members of the genus *Kangiella* are usually described as non-motile, rod shaped and genome reduced [2–11]. Species from the genera *Pleionea* [12, 13] and *Aliikangiella* [1] have also been described as non-motile rods. However, both genome analysis and microscopic examination indicated that cells of *Pleionea* and *Aliikangiella* are motile by means of a flagellum. So the exact taxonomy of the genera *Pleionea* and *Aliikangiella* needs further study.

Strain M105<sup>T</sup> was isolated from the hermatypic coral *Porites lutea* [sampled from Weizhou Island (21°03'42" N, 109°08'35" E), PR China] by spread dilutions of coral tissue and skeleton homogenate on one-tenth strength marine agar 2216 (BD) plates [14]. Inoculated plates were incubated at 25°C for about 2 weeks, following which a pale-yellow colony

(designated strain M105<sup>T</sup>) was picked and purified by streaking on one-tenth strength marine agar 2216. M105<sup>T</sup> was stored at –70°C in 25% (v/v) glycerol. This strain has also been deposited in the Marine Culture Collection of China and the Korean Collection for Type Cultures under the accession numbers MCCC 1K03773<sup>T</sup> and KCTC 72442<sup>T</sup>, respectively.

The reference strain *Aliikangiella marina* GYP-15<sup>T</sup> (=MCCC 1K01163<sup>T</sup>) was obtained from the Marine Culture Collection of China. *Pleionea mediterranea* DSM 25350<sup>T</sup> was purchased from the German Collection of Microorganisms and Cell Cultures, and *Kangiella koreensis* KCTC 12182<sup>T</sup> was obtained from the Korean Collection for Type Cultures. The novel isolate and the reference strains grew well on marine agar 2216 (BD).

The 16S rRNA gene sequence was obtained by polymerase chain reaction (PCR) amplification with the universal primers 27F and 1492R [15]. Alignment of 16S rRNA gene sequences was performed using the SINA software package [16] in the SILVA rRNA database. Phylogenetic trees were reconstructed

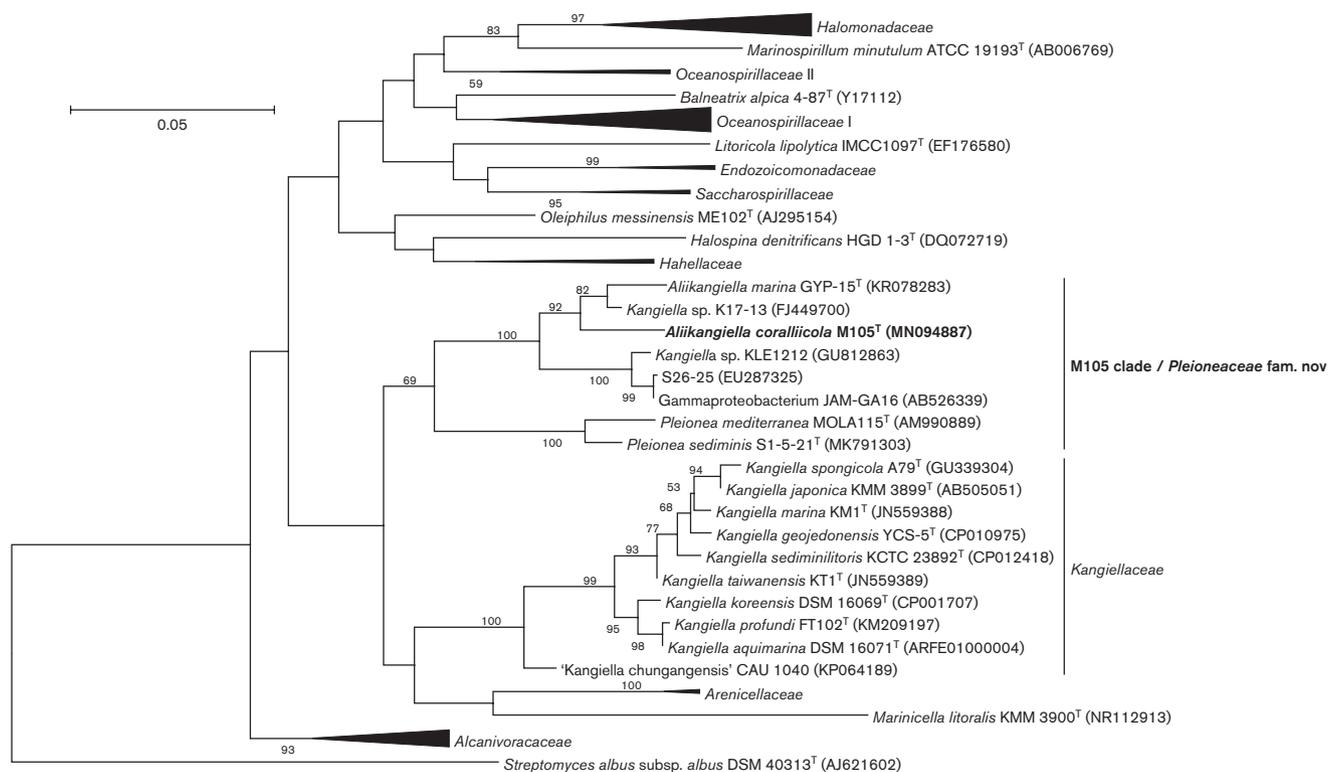
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**Keywords:** *Aliikangiella coralliicola*; *Pleioneaceae*; *Kangiellaceae*.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence and the whole-genome sequence of strain M105<sup>T</sup> are MN094887 and VIKS000000000, respectively. The GenBank/EMBL/DDBJ accession number for the whole-genome sequence of strain *Aliikangiella marina* GYP-15<sup>T</sup> is VIKR000000000.

Eight supplementary figures and three supplementary tables are available with the online version of this article.



**Fig. 1.** Maximum likelihood phylogenetic tree (expanded) based on the 16S rRNA gene sequences of M105<sup>T</sup> and related taxa. The sequence from *Streptomyces albus subsp. albus* was used as an outgroup. Numbers at nodes indicate percentages of 1000 bootstrap resamplings; only values above 50% are shown. Bar, 0.05 substitutions per nucleotide position.

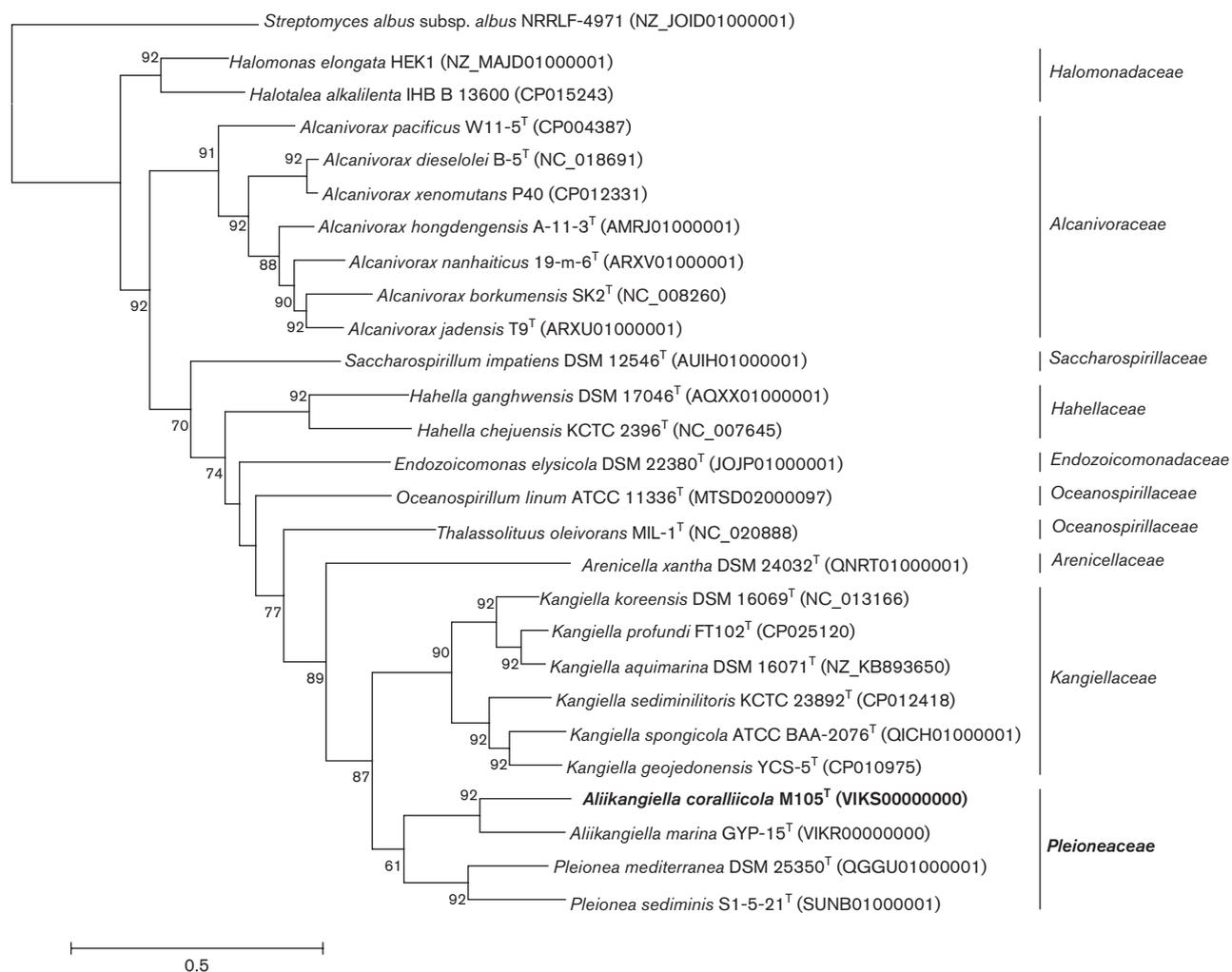
using the maximum likelihood [17], neighbor-joining [18], and maximum parsimony [19] methods using MEGA software v7.0 [20]. Phylogenetic distance matrices were estimated by the Kimura two-parameter model [21]. Topology of the phylogenetic tree was evaluated using Felsenstein's bootstrap resampling method [22] with 1000 replicates.

About 1410 bp of 16S rRNA gene sequence was obtained and deposited in GenBank under the accession number MN094887. Global alignment based on the 16S rRNA gene sequences in the EzBioCloud database (<https://www.ezbiocloud.net/>) indicated that M105<sup>T</sup> shares 94.5% sequence similarity with *A. marina* GYP-15<sup>T</sup>, 89.8–90.6% sequence similarities with members of the genus *Kangiella* and 88.9–91.5% sequence similarities with members of the genus *Pleionea*. This indicated that M105<sup>T</sup> may represent a novel species of the genus *Aliikangiella* [23]. The results of phylogenetic analysis based on the maximum likelihood algorithm indicated that M105<sup>T</sup> forms a distinct branch in a large clade composed of members of the genera *Aliikangiella* and *Pleionea* (M105 clade) (Figs 1 and S1). Meanwhile, the members of the genera *Kangiella*, *Arenicella* and *Marinicella* formed another large clade (Figs 1 and S1). Trees reconstructed using the neighbor-joining and maximum parsimony algorithms indicated that M105<sup>T</sup> and members of the genera *Aliikangiella*, *Pleionea* and *Kangiella* form a large clade, where M105<sup>T</sup> and members of the genera *Aliikangiella* and *Pleionea* form one branch, and

members of the genus *Kangiella* form another branch (Figs S2 and S3).

Genome sequencing was performed by Beijing Novogene Bioinformatics Technology (Beijing, China) under its standard procedure. Genomic DNA was extracted using the sodium dodecyl sulphate (SDS) method [24]. Harvested DNA was detected by agarose gel electrophoresis and quantified with a Qubit 2.0 Fluorometer (Thermo Fisher Scientific). The whole genomes of M105<sup>T</sup> and *A. marina* GYP-15<sup>T</sup> were sequenced using a NovaSeq PE150 platform (Illumina). The Illumina PCR adapter reads and low-quality reads were filtered during quality control using Readfq v10. All good-quality paired reads were assembled using the SOAPdenovo [25, 26], SPAdes [27], and ABySS [28] methods and integrated into a number of scaffolds by CISA [29]. Genes were predicted using GeneMarkS programme [30]. Genome information was extracted according to the method of Chun *et al.* [31]. The average nucleotide identity (ANI) was calculated using the ChunLab's online calculator [32]. Average amino acid identity (AAI) was calculated using the online AAI-profiler [33]. The phylogenomic tree was reconstructed using the up-to-date bacterial core gene set (UBCG v.3) [34].

Genome sequences for *Kangiella koreensis* KCTC 12182<sup>T</sup> (CP001707), *Kangiella profunda* FT102<sup>T</sup> (CP025120), *Kangiella sediminilitoris* KCTC 23892<sup>T</sup> (CP012418),



**Fig. 2.** Phylogenomic tree of strain M105<sup>T</sup> and related species based on 92 core genes using maximum likelihood algorithm. *Streptomyces albus* subsp. *albus* was used as an outgroup.

*Kangiella geojedonensis* YCS-5<sup>T</sup> (CP010975), *Kangiella spongicola* A79<sup>T</sup> (QICH00000000), *Kangiella aquimarina* DSM 16071<sup>T</sup> (ARFE00000000), *Pleionea mediterranea* DSM 25350<sup>T</sup> (QGGU00000000) and *Pleionea sediminis* S1-5-21<sup>T</sup> (SUNB01000001) were obtained from GenBank. If any gene sequence was unavailable, GeneMarkS was used to obtain the coding information. The Clusters of Orthologous Groups (COGs) annotation was performed by WebMGA [35]. Genomic alignment between the sample and reference genomes was performed using the OrthoVenn2 online pipeline (<https://orthovenn2.bioinfotoolkits.net/home>) [36]. Shared gene (protein) IDs special for members of the genera *Aliikangiella* and *Pleionea*, and the novel isolate were obtained from the OrthoVenn2 output. The protein sequence was extracted using faSomeRecords ([http://hgdownload.cse.ucsc.edu/admin/exe/linux.x86\\_64/](http://hgdownload.cse.ucsc.edu/admin/exe/linux.x86_64/)) from the original predicted .pep file. Function of the shared proteins was annotated in the KEGG database (<https://www.genome.jp/tools/kaas/>) using the partial genome module.

Genome sequencing depth for strain M105<sup>T</sup> was 175× and the N50 was 563692 bp. A total of 22 scaffolds were obtained, and the genome size was predicted to be 7.04 Mb with a genomic DNA G+C content of 41.5 mol%. The full length 16S rRNA gene was 1527 bp, which was identical to the Sanger sequencing result (accession number MN094887). The genome sequence was deposited in GenBank under the accession number VIKS00000000. Genome sequencing depth for *A. marina* GYP-15<sup>T</sup> was 211× and the N50 was 1289802 bp. A total of eight scaffolds were obtained, and the genome size was predicted to be 5.34 Mb with a genomic DNA G+C content of 41.9 mol%. The full length 16S rRNA gene was 1524 bp in length, which was identical to the Sanger sequencing result (KR078283). The genome sequence was deposited in GenBank under the accession number VIKR00000000. The ANI and AAI between M105<sup>T</sup> and *A. marina* MCCC 1K01163<sup>T</sup> was 69.8 and 71.6%, respectively, which indicated that the two strains represented different species [31, 37, 38]. Phylogenomic analysis based on 92 core genes indicated that

M105<sup>T</sup> forms a distinct branch in the M105 clade, separate from the members of the genus *Kangiella* (Fig. 2). This topology is consistent with the 16S rRNA sequence-based phylogenetic results (Figs 1 and S1–S3).

The ratio of COG annotated Open Reading Frames (ORFs) to COG categories indicated that the genomes of the M105 clade (1.66–2.03) are more redundant than those of members of the genus *Kangiella* (1.13–1.33) (Fig. S4). The OrthoVenn2 alignment result indicated that functional genes from ten species of the genera *Aliikangiella*, *Pleionea* and *Kangiella* and the novel isolate form about 5030 clusters in total, in which 1230 clusters are shared by the ten species. A pair-wise heatmap based on the OrthoVenn2 alignment indicated that M105<sup>T</sup> is much more closely related to *A. marina* GYP-15<sup>T</sup>, *P. mediterranea* DSM 25350<sup>T</sup>, and *P. sediminis* S1-5-21<sup>T</sup> than species of the genus *Kangiella* (Fig. S5). Approximately 436 clusters were unique to species of the M105 clade after the OrthoVenn2 alignment. While a few of these 436 clusters were also observed in *K. koreensis* KCTC 12182<sup>T</sup> (JGI database). These annotation differences might be attributed to isoenzymes or use of different cutoffs for protein cluster analysis. After a manual check, it was observed that the M105 clade-specific clusters are mainly annotated to the two-component system, bacterial chemotaxis, flagella assembly and some amino acid metabolism pathways (Table S1, available in the online version of this article). A total of 189 clusters were also identified to be specific to the members of the genus *Kangiella*, however, these clusters did not annotate to any complete KEGG pathway.

Cellular morphology was observed using optical microscopy (BX53, Olympus) and transmission electron microscopy (TEM; H-7650 TEM System; Hitachi) after incubation for 2–3 days in marine broth (BD) at 25 °C. Cell mobility was tested using the hanging drop technique [39]. The Gram staining was performed as described by Gerhardt *et al.* [40]. Catalase activity was determined by observing bubble production in a 3% (v/v) hydrogen peroxide solution, and oxidase activity was determined using oxidase test strips (Huankai). Endospore forming ability was examined as described by Dong and Cai [41]. Anaerobic growth was tested in marine broth (BD) at 25 °C for 3 weeks in a 96-well plate (each cell was sealed with mineral oil, similar to API 20NE test). NaCl requirement and tolerance were tested at 25 °C for 7 days in R2A liquid medium (Haibo) with NaCl concentrations ranging from 0 to 16% (w/v), specifically 0, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14 and 16% (w/v). Growth at different pH levels was tested in R2A liquid medium at 25 °C for 7 days. The pH was amended using different buffers: pH 5–8 with 0.1 M KH<sub>2</sub>PO<sub>4</sub>/K<sub>2</sub>HPO<sub>4</sub>, pH 8.5–10 with 0.1 M NaHCO<sub>3</sub>/Na<sub>2</sub>CO<sub>3</sub> and pH 10.5–11 with 0.1 M Na<sub>2</sub>CO<sub>3</sub>/NaOH. Optimal growth temperature was determined after 7–30 days growth on marine agar 2216 (BD) plates at temperatures of 5, 10, 15, 20, 25, 30, 33, 37, 40 and 45 °C. Metabolic ability was tested using API 20NE strips, API ZYM strips and Biolog Gen III microplates according to the manufacturers' instructions. Anaerobic fermentation was determined using the API 50CH strips according to the manufacturer's protocol using R2A liquid medium as the inoculum medium. Hydrogen sulfide

(H<sub>2</sub>S) production was tested by following standard procedures compiled by Tindall *et al.* [42].

Cells of M105<sup>T</sup> were Gram-stain-negative, non-spore-forming, aerobic rods, motile by means of a single polar flagellum (Fig. S6). Both *A. marina* GYP-15<sup>T</sup> (Fig. S6) and *P. mediterranea* DSM 25350<sup>T</sup> (checked by photomicroscopy, data not shown) were rechecked as motile by means of flagella. Though *P. sediminis* S1-5-21<sup>T</sup> has been reported to be non-motile [12], genome annotation results indicated the existence of most of the flagellum assembly coding genes (Fig. S7). All members of the genus *Kangiella* have been reported to be non-motile [2–9], which was consistent with the genome analysis result that there were no flagellum coding genes detected. Hence, members of the family *Kangiellaceae* could be divided into two groups on the basis of motility. Both M105<sup>T</sup> and *A. marina* MCCC 1K01163<sup>T</sup> could hydrolyze starch, while members of the genera *Pleionea* [12, 13] and *Kangiella* [2–9] could not. This starch hydrolysis feature might be niche related. Both M105<sup>T</sup> and *A. marina* MCCC 1K01163<sup>T</sup> were isolated from algae-related environments (*Symbiodinium* sp. or *Picochlorum* sp.). While members of the genera *Pleionea* and *Kangiella* were isolated from seawater or sediment [2–9, 12, 13], genome analysis indicated the starch hydrolysis encoding genes are incomplete or absent (Table S1). Other characteristics of M105<sup>T</sup> are listed in Table 1 and the species description.

Respiratory lipoquinones were extracted as described elsewhere [43] and analysed using reverse-phase high-performance liquid chromatography (HPLC) as described by Komagata and Suzuki [44]. Polar lipids were extracted as described by Kamekura [45] and identified using two-dimensional thin-layer chromatography (TLC) [46]. Cellular biomass was collected from marine agar 2216 plates in late growth phase (incubated at 25 °C) to analyse cellular fatty acid content. Cellular fatty acid composition was determined using the Sherlock Microbial Identification System (MIDI) according to the manufacturer's instructions. Fatty acids were analysed by gas chromatography (GC) (G6890N; Agilent) and identified using the Microbial Identification Software (Sherlock v6.0).

The only respiratory quinone detected in strain M105<sup>T</sup> was ubiquinone 8 (Q-8), which was consistent with those of *A. marina* GYP-15<sup>T</sup> [1] and members of the genera *Pleionea* [12, 13] and *Kangiella* [2–9]. The major polar lipids present in strain M105<sup>T</sup> were phosphatidylethanolamine (PE), phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), one unidentified ninhydrin-positive phospholipid (NPL) and one unidentified ninhydrin-positive lipid (NL) (Fig. S8). Generally, M105<sup>T</sup>, *A. marina* GYP-15<sup>T</sup>, *P. mediterranea* DSM 25350<sup>T</sup> and *K. koreensis* KCTC 12182<sup>T</sup> shared similar major polar lipids, such as PE, PG, DPG and NL (Fig. S8). While phosphatidylmonomethylethanolamine (PME) was a common feature of species of the genus *Kangiella* (Fig. S8) [2, 3, 6, 7, 9]. The unidentified polar lipid L2 from *A. marina* GYP-15<sup>T</sup> could be readily used to distinguish M105<sup>T</sup> from *A. marina* GYP-15<sup>T</sup> (Fig. S8). No NPL was

**Table 1.** Characteristics differentiate strain M105<sup>T</sup> from related type strainsStrains: 1, M105<sup>T</sup>; 2, *Aliikangiella marina* GYP-15<sup>T</sup>; 3, *Pleionea mediterranea* DSM 25350<sup>T</sup>. +, Positive; -, negative; w, weakly positive.

Characteristics	1	2	3
Habitat	<i>Porites lutea</i>	Marine microalga‡	Coastal seawater§
Cell size (µm)	0.2–0.4×1.3–3.6	0.2–0.4×2.6–4.3‡	0.3×2.2§
Colony colour	Pale-yellow	Pale-yellow‡	Pale-pink§
Temperature range (°C)	15–40 (25–30)	15–37(30)	15–37(20–30)
NaCl tolerance (% w/v)	0–10 (1–3)	2–8 (2–3)	2–7 (2–3)
pH range	7–8	8–9	7–9
Hydrolysis of starch	+	+	–
Hydrolysis of Tween 80	–	–	+
Hydrolysis of Tween 40	–	+	+
<b>API 20NE</b>			
Nitrate reduction	+	w	–
Arginine dihydrolase	+	+	–
Urease	+	+	–
Beta-glucosidase	+	–	–
Gelatin hydrolysis	+	+	+
Beta-galactosidase	+	–	–
ANI to strain M105 <sup>T</sup>	100%	69.8%	67.8%
AAI to strain M105 <sup>T</sup>	100%	71.6%	63.9%
DNA G+C content mol%*	41.5	41.9	42.5
Major polar lipids†	PE, PG, DPG, NL	PE, PG, DPG, NL, Ls	PE, PG, DPG, NL
Major fatty acids (>5%)	C <sub>16:0</sub> , C <sub>18:0</sub> , iso-C <sub>15:0</sub> , iso-C <sub>16:0</sub> , C <sub>16:0</sub> -10-methyl	iso-C <sub>15:0</sub> , C <sub>16:0</sub> -10-methyl, C <sub>16:0</sub> , C <sub>18:0</sub> , iso-C <sub>16:0</sub>	C <sub>16:0</sub> , C <sub>18:0</sub> , C <sub>16:0</sub> -10-methyl, iso-C <sub>15:0</sub> , iso-C <sub>16:0</sub>

\*Data from genome sequences VIKS000000000, VIKR000000000 &amp; QGGU01000000, respectively

†PE, phosphatidylethanolamine; PG, phosphatidylglycerol; DPG, diphosphatidylglycerol; NL, unidentified ninhydrin-positive lipid; L, unidentified lipid.

‡Date from Wang et al. [1].

§Date from Fagervold et al. [12].

detected from *P. mediterranea* DSM 25350<sup>T</sup> in this study, which has been detected previously [12]. So M105<sup>T</sup> and *P. mediterranea* DSM 25350<sup>T</sup> might have the same polar lipids. The major cellular fatty acids detected in M105<sup>T</sup> were C<sub>16:0</sub>, C<sub>18:0</sub>, iso-C<sub>15:0</sub>, iso-C<sub>16:0</sub> and C<sub>16:0</sub>-10-methyl (Table S2). The fatty acid profile from M105<sup>T</sup> was similar to that of *A. marina* GYP-15<sup>T</sup>, *P. mediterranea* DSM 25350<sup>T</sup> and *K. koreensis* KCTC 12182<sup>T</sup>, while the exact percentage was quite different. C<sub>16:0</sub> was the most abundant fatty acid in M105<sup>T</sup>, *P. mediterranea* DSM 25350<sup>T</sup> and *K. koreensis* KCTC 12182<sup>T</sup>, while iso-C<sub>15:0</sub> was the major fatty acid in *A. marina* GYP-15<sup>T</sup> (Table S2).

The 16S rRNA gene sequence similarity (max. 94.5%), ANI (69.8%) and AAI (max. 71.6%, Table S3) indicated that M105<sup>T</sup> represents a novel species (Table 1). The AAI

to *A. marina* GYP-15<sup>T</sup> was near the threshold for assigning species to the same genus (65–72%), so M105<sup>T</sup> should be assigned to the genus *Aliikangiella* [47]. Therefore, M105<sup>T</sup> is suggested to represent a novel species of the genus *Aliikangiella*.

Both 16S rRNA gene sequence phylogenetic results (Figs 1, S2 and S3) and phylogenomic results (Fig. 2) indicated there are two lineages in the family *Kangiellaceae* Wang et al. 2015, namely the strain M105 clade (strain M105<sup>T</sup>, *A. marina* GYP-15<sup>T</sup>, *P. mediterranea* DSM 25350<sup>T</sup> and *P. sediminis* S1-5-21<sup>T</sup>) and the genus *Kangiella*. This division was also supported by genome redundant (ORFs/COGs), cell motility and polar lipid differences (Table 2). The small genome of members of the genus *Kangiella* was partially due to low genome redundancy [11]. The members of the

**Table 2.** Characteristics differentiate the *Pleioneaceae* to related families

+, positive, -, negative, ND, unknown.

Characteristics	<i>Pleioneaceae</i> fam. nov		<i>Kangiellaceae</i>	<i>Arenicellaceae</i>	
	<i>Aliikangiella</i>	<i>Pleionea</i> *	<i>Kangiella</i> †	<i>Perspicuibacter</i> ‡	<i>Arenicella</i> §
Genome size	5.34–7.04	4.04–5.19	2.5–2.85	ND	4.44
Genome ORFs/COGs	1.75–2.03	1.66–1.9	1.13–1.33	ND	1.79
Motility	+	+	-	-	-
Flagella assembly genes	+	+	-	ND	-
Chemotaxis genes	+	+	-	ND	-
AAI to strain M105 <sup>T</sup>	0.716–1	0.634–0.639	0.631–0.646	ND	0.575
DNA G+C content (mol%)	41.5–41.9	40.1–42.5	43.7–46.3	41.7	46.3–47.3
Polar lipids	PE, PG, DPG, NL, PL	PE, PG, DPG, NL, PL	PE, PG, DPG, PME	PE, PG	PE, PG, DPG, NPL

\*Data from this study and Luo et al. [13].

†Data from this study and the literature (references [2–9]).

‡Data from Teramoto et al. [48].

§Data from Romanenko et al. [49].

genus *Kangiella* lack flagellum assembly and chemotaxis coding genes in their genomes, while almost all members of the M105 clade are motile by means of a flagellum. Additionally, PME was detected in almost all species of the genus *Kangiella* with validly published names, while it was absent from the M105 clade. On the basis of phylogenetic, phylogenomic, flagellum and PME differences (Table 2), the M105 clade is suggested to represent a novel family, for which the name *Pleioneaceae* fam. nov. is proposed, which includes the genera *Aliikangiella* and *Pleionea*. The genus *Kangiella* remains in the family *Kangiellaceae*.

## DESCRIPTION OF ALIIKANGIELLA CORALLICOLA SP. NOV.

*Aliikangiella coralliicola* (co.ral.li.i'co.la. L. neut. n. *corallium* coral; L. suff. *-cola* (from L. masc or fem. n. *incola*), inhabitant; N.L. fem. n. *coralliicola* inhabitant of corals)

Cells are Gram-stain-negative, non-endospore-forming, motile, aerobic rods. Catalase and nitrate reduction are positive, oxidase is negative. Cells are usually 0.2–0.4 µm wide and 1.3–3.6 µm long, and motile by means of a single polar flagellum. Colonies are pale-yellow or colourless and circular on marine agar. Growth occurs at pH 7–8, at 15–40 °C and in the presence of 0–10% (w/v) NaCl, and optimally at pH 7.0–8.0, at 25–30 °C and with 1–3% (w/v) NaCl. Production of H<sub>2</sub>S does not occur. Starch is hydrolysed. Tweens 20, 40 and 60 are not hydrolysed. In the API 20NE tests, arginine dihydrolase, urease, β-glucosidase, gelatin hydrolysis and β-galactosidase are positive. In the API ZYM tests, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase and naphthol-AS-BI phosphohydrolase are

positive. Results for all anaerobic fermentation tests in API 50CH are negative. In the Biolog GenIII microplates, sodium lactate, acetoacetic acid, propionic acid, acetic acid and formic acid are oxidized. The only detected respiratory quinone is Q-8. Major polar lipids are phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, one unidentified ninhydrin-positive lipid and one unidentified ninhydrin-positive phospholipid. Major cellular fatty acids are C<sub>16:0</sub>, C<sub>18:0</sub>, iso-C<sub>15:0</sub>, iso-C<sub>16:0</sub> and C<sub>16:0</sub> 10-methyl.

The type strain, M105<sup>T</sup> (=MCCC 1K03773<sup>T</sup>=KCTC 72442<sup>T</sup>), was isolated from the coral *Porites lutea*. The genomic DNA G+C content is 41.5 mol%. The GenBank/EMBL/DBJ accession numbers for the 16S rRNA gene sequence and the whole genome sequence are MN094887 and VIKS00000000, respectively.

## EMENDATION OF THE DESCRIPTION OF THE GENUS ALIIKANGIELLA WANG ET AL. 2015

The description is identical to that given by Wang et al. [1] with the following emendations. Cells are motile by means of a single polar flagellum. The genomic DNA G+C content is 41.5–41.9 mol%. The major polar lipids are phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol and an unidentified ninhydrin-positive lipid.

## EMENDATION OF THE DESCRIPTION OF THE GENUS PLEINOEA FAGERVOLD ET AL. 2013 AND LUO ET AL. 2019

The description is identical to that given by Fagervold et al. [12] and Luo et al. [13] with the following emendations: Cells are motile by means of a flagellum.

## DESCRIPTION OF PLEIONEACEAE FAM. NOV.

*Pleioneaceae* (Ple.io.ne.a.ce'ae. N.L. fem. n. *Pleionea*, name of a bacterial genus; suff. *-aceae*, ending to denote the name of a family; N.L. fem. n. *Pleioneaceae*, the *Pleionea* family)

This family is a member of the order *Oceanospirillales* and encompasses Gram-stain-negative, motile bacteria retrieved from marine environments. Currently, the family comprises the genera *Aliikangiella* and *Pleionea*. The major respiratory quinone is Q-8. The major polar lipids are phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol. The major fatty acids are C<sub>16:0</sub>, C<sub>18:0</sub>, iso-C<sub>15:0</sub>, iso-C<sub>16:0</sub>, and C<sub>16:0</sub>-10-methyl. The type genus is *Pleionea*.

## EMENDATION OF THE DESCRIPTION OF THE FAMILY KANGIELLACEAE WANG ET AL. 2015

The description is identical to that given by Wang et al. [1] with the following emendations. This family contains solely the genus *Kangiella*.

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### Conflicts of interest

The authors declare that there are no conflicts of interest.

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