INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY

TAXONOMIC DESCRIPTION

Lucena *et al.*, *Int. J. Syst. Evol. Microbiol.* 2022;72:005498

DOI 10.1099/ijsem.0.005498

MICROBIOLOGY SOCIETY

Parvicella tangerina gen. nov., sp. nov. (*Parvicellaceae* fam. nov., *Flavobacteriales*), first cultured representative of the marine clade UBA10066, and *Lysobacter luteus* sp. nov., from activated sludge of a seawater-processing wastewater treatment plant

Teresa Lucena[1], Olga Sánchez[2], Isabel Sanz-Saez[3], Silvia G. Acinas[3], Laura Garrido[2], Jordi Mas[2], M. Carmen Macián[1], María A. Ruvira[1], David R. Arahal[4] and María J. Pujalte[4,*]

Abstract

Two strains isolated from a sample of activated sludge that was obtained from a seawater-based wastewater treatment plant on the southeastern Mediterranean coast of Spain have been characterized to achieve their taxonomic classification, since preliminary data suggested they could represent novel taxa. Given the uniqueness of this habitat, as this sort of plants are rare in the world and this one used seawater to process an influent containing intermediate products from amoxicillin synthesis, we also explored their ecology and the annotations of their genomic sequences. Analysis of their 16S rRNA gene sequences revealed that one of them, which was orange-pigmented, was distantly related to *Vicingus serpentipes* (family *Vicingaceae*) and to other representatives of neighbouring families in the order *Flavobacteriales* (class *Flavobacteriia*) by 88–89% similarities; while the other strain, which was yellow-pigmented, was a putative new species of *Lysobacter* (family *Xanthomonadaceae*, order *Xanthomonadales*, class *Gammaproteobacteria*) with *Lysobacter arseniciresistens* as closest relative (97.3% 16S rRNA sequence similarity to its type strain). Following a polyphasic taxonomic approach, including a genome-based phylogenetic analysis and a thorough phenotypic characterization, we propose the following novel taxa: *Parvicella tangerina* gen. nov., sp. nov. (whose type strain is AS29M-1^T=CECT 30217^T=LMG 32344^T), *Parvicellaceae* fam. nov. (whose type genus is *Parvicella*), and *Lysobacter luteus* sp. nov. (whose type strain is AS29M^T=CECT 30171^T=LMG 32343^T).

The order *Flavobacteriales* (class *Flavobacteriia*, phylum *Bacteroidota*), comprises seven families: *Blattabacteriaceae*, *Crocinitomicaceae*, *Cryomorphaceae*, *Flavobacteriaceae*, *Ichthyobacteriaceae*, *Schleiferiaceae* and *Weeksellaceae* (https://lpsn.dsmz.de/order/flavobacteriales) [1]. This list has been recently expanded with the recognition of three additional new families out of previous representatives of *Cryomorphaceae*: *Luteibaculaceae*, *Salibacteraceae* and *Vicingaceae* [2, 3]. Internal diversity of this order displayed on phylogenomic trees that include metagenome-assembled genomes (MAGs) suggest that several additional family-level new taxa may be pending of recognition because they lack cultivated representatives [2, 4].

An orange-pigmented bacterial isolate was obtained from a rare environment, a seawater-processing wastewater treatment plant, on the Mediterranean coast of Spain [5, 6]. It was revealed as distantly related to *Vicingus serpentipes* and to other representatives of neighbouring families by 88–89% similarities in their 16S rRNA gene sequences, within the order *Flavobacteriales*. The low level of taxonomic relatedness suggested by these figures prompted us to proceed to characterize the isolate. During the preliminary steps taken to deposit the isolate in the Spanish Type Culture Collection (CECT), a second isolate was recovered from the same

Author affiliations: [1]Colección Española de Cultivos Tipo (CECT), Universitat de València, Valencia, Spain; [2]Departament de Genètica i Microbiologia, Facultat de Biociències, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain; [3]Departament de Biologia Marina i Oceanografia, Institut de Ciències del Mar, ICM-CSIC, Barcelona, Spain; [4]Departamento de Microbiología y Ecología, Universitat de València, Valencia, Spain.
***Correspondence:** María J. Pujalte, maria.j.pujalte@uv.es
Keywords: *Flavobacteriales*; *Lysobacter*; *Parvicella*; saline activated sludge; taxogenomics; UBA10066.
Abbreviations: AAI, average amino acid identity; ANI, average nucleotide identity; DDH, DNA–DNA hybridization; DPG, diphosphatidyl glycerol; GGDC, Genome to-Genome-Distance Calculator; GSI, gene support index; GTDB, Genome Taxonomy Database; LBA, Luria–Bertani agar; MA, marine agar; MB, marine broth; MK, menaquinone; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; R2A, Reasoner's 2A; RAST, Rapid Annotation using Subsystem Technology; UBCG, up-to-date bacterial core gene.
The 16S rRNA gene sequence and draft genome accession numbers for *Lysobacter luteus* CECT 30171^T and *Parvicella tangerina* CECT 30217^T are MZ047093/OU015430 and MZ047088/OU015584, respectively.
Eight supplementary figures and two supplementary tables are available with the online version of this article.

glycerol-supplemented cryotubes containing the *Flavobacteriales* bacterium, a yellow-pigmented isolate, later revealed as a putative new species of *Lysobacter* after analysis of its 16S rRNA gene sequence. *Lysobacter* belongs to the family *Xanthomonadaceae* (order *Xanthomonadales*, class *Gammaproteobacteria*) and is prominent due to the ability of some of its species to lyse a variety of microorganisms [7]. Currently, the genus contains 66 validly named species (https://lpsn.dsmz.de/genus/lysobacter) [1].

Both isolates, deposited as CECT 30217^T (as *Vicingus* sp.) and CECT 30171^T (as *Lysobacter* sp.), have been submitted to a phenotypic (including chemotaxonomic), genomic and phylogenetic characterization in order to resolve their taxonomic position and further explore their genomes and ecological relationships.

ISOLATION AND MAINTENANCE

A sample of activated sludge was obtained from a seawater-based wastewater treatment plant, located in Almeria, on the southeastern Mediterranean coast of Spain (latitude 37.256, longitude −1.763) on November, 2008. Very few plants of this type are running in the world, and this one in particular used seawater to process an influent containing intermediate products from amoxicillin synthesis. The average salinity of water in the Mediterranean Sea in that area is 36 mg l^{-1} and ionic concentrations in the influent are given in [5]. The usage of seawater is justified due to the severe scarcity of fresh water sources. The sludge residence time was 10–14 days. The mean influent flow of the plant was 300 m^3 h^{-1} and had a treatment volume of 32000 m^3. Nitrogen and chemical oxygen demand sludge loads were about 150–170 kg h^{-1} and 900–1000 kg h^{-1} respectively, with total removal rates above 80 and 90%, respectively [5, 6]. The activated sludge sample was cultivated on marine agar (MA; Difco), at room temperature and a culture, supposedly axenic, designated AS29M, was obtained and maintained in 25% glycerol at −80 °C. A partial 16S rRNA gene sequence revealed that strain AS29M was located among the *Flavobacteriales* (phylum *Bacteroidota*) distantly related to *Vicingus serpentipes*.

A subculture was sent to the CECT for deposition: as the recovered strain was being routinely processed, a partial 16S rRNA sequence, obtained as an authenticity check point, did not correspond to the alleged *Flavobacteriales* but to a *Lysobacter* species. Subsequent work allowed the separation of two different bacteria from the subculture, one corresponding to the original *Flavobacteriales*, and another identified as a *Lysobacter* species. Both strains were quite difficult to resolve through subculture and the *Flavobacteriales* was especially difficult to obtain as isolated colonies due to the aggregative character of the cells, its preference for mass growth, instead of individual colonies, and the clumping of the biomass in liquid medium. Finally, the two strains were separated and deposited as pure cultures with numbers CECT 30171^T (*Lysobacter* sp.) and CECT 30217^T (*Flavobacteriales*). They have been also deposited in LMG as LMG 32343^T and LMG 32344^T, respectively.

16S rRNA GENE SEQUENCING AND PHYLOGENY

A partial 16S rRNA sequence of each strain was obtained by using already described methods [8] and compared through BLAST and EzBioCloud searches with available sequences in databases, for tentative taxonomic adscription. For the phylogenetic tree building, the complete 16S rRNA sequences, extracted from genomes (see below) were used, after confirming the partial ones matched them perfectly, thus serving as authentication control check [9].

In the case of strain CECT 30217^T, top similarities obtained using EzBioCloud tool [10] were relatively low (less than 90% to other *Bacteroidota*), being the type of *Vicingus serpentipes* the most similar, with 89.2% identity, followed by *Lishizhenia tianjinensis* (88.5%) *Wandonia haliotis* (88.4%) *Fluviicola hefeinensis* (88.4%) and *Salibacter halophilus* (88.2%). Moreover, none of the 16S rRNA gene sequences (including non-type material, uncultured, phylotypes, etc.) in BLAST or EzBioCloud databases gave more than a 95% sequence similarity to CECT 30217^T, a clear indication of the rareness of this organism.

In turn, strain CECT 30171^T was most similar to several *Lysobacter* species, with the type of *L. arseniciresistens* as the closest one (97.3% 16S rRNA sequence similarity), followed by *L. helvus* (96.2%) and *L. capsici* (96.1%). Similarity to non-type strains and sequences from uncultured bacteria was also explored and results are given under the ecology heading.

Maximum-likelihood and maximum-parsimony trees were inferred for each set of 16S rRNA gene sequences under the GTR+GAMMA model by the Genome to-Genome-Distance Calculator (GGDC) web server [11] available at http://ggdc.dsmz.de/ using the DSMZ phylogenomics pipeline [12] adapted to single genes.

Strain CECT 30217^T was located amid the different *Flavobacteriales* families (Fig. 1), marginally related to *Schleiferiaceae*, *Cryomorphaceae*, *Salibacteraceae* and *Luteibaculaceae* representatives, in a position that suggested that the strain does not belong to any of these families. The low level of bootstrap also suggests that the relationships shown at this node are not heavily supported. In fact, the phylogenomic trees shown later reveals a closer link to *Crocinitomicaceae* than to the above-mentioned families.

With regard to the other isolate, Fig. 2 shows the phylogenetic position of strain CECT 30171^T within the genus *Lysobacter*, with *L. arseniciresistans* as closest neighbour (with high bootstrap support) in a larger clade also containing *L. concretionis* and *L. spongicola* among others.

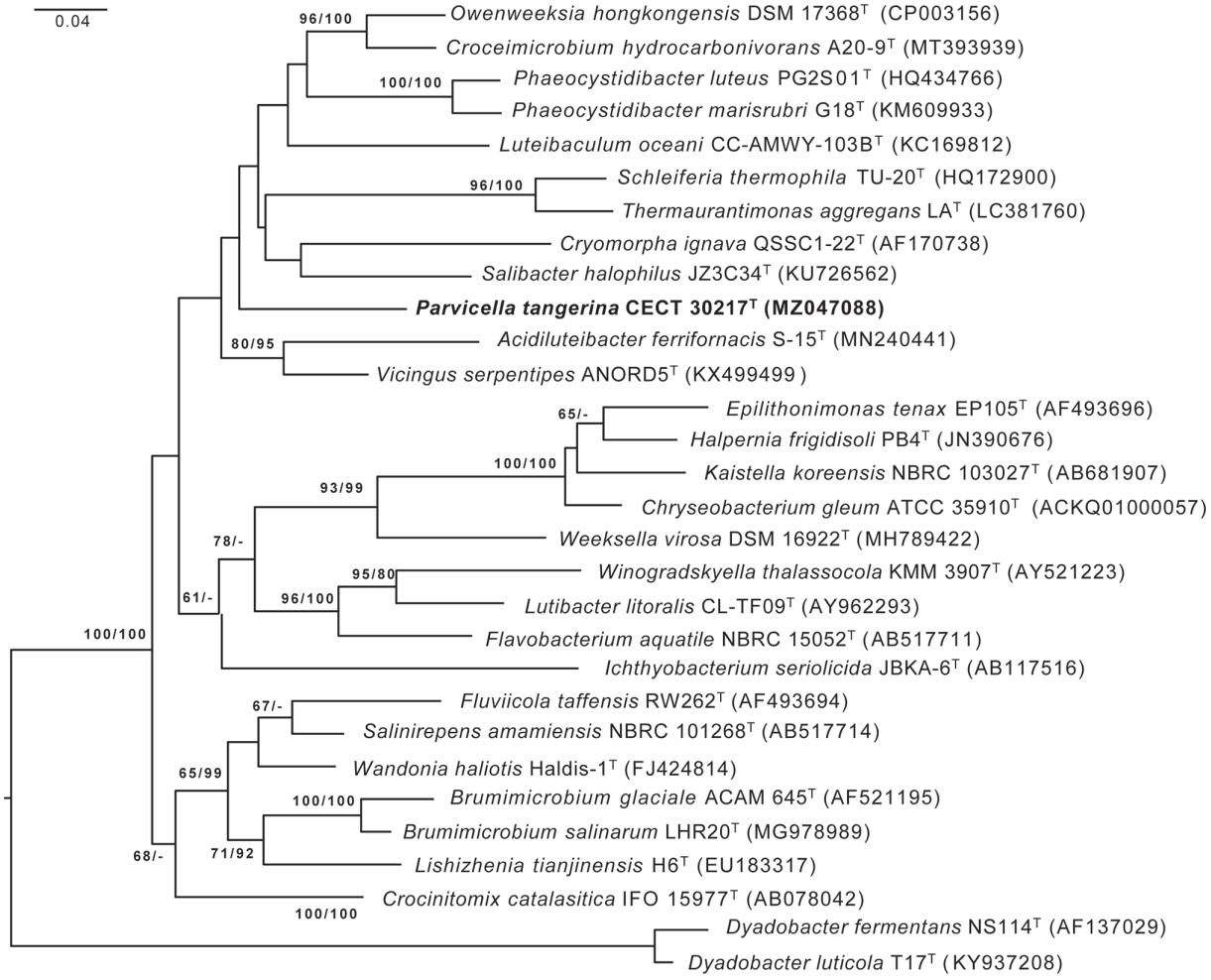

Fig. 1. Maximum-likelihood tree inferred from 16S rRNA gene sequences under the GTR+GAMMA model and rooted by midpoint-rooting including *Parvicella tangerina* gen. nov., sp. nov. CECT 30217^T and representatives of the order *Flavobacteriales*. The branches are scaled in terms of the expected number of substitutions per site. Phylogenies were inferred by the GGDC web server [11] available at http://ggdc.dsmz.de/ using the DSMZ phylogenomics pipeline [12] adapted to single genes. The numbers above the branches are support values when larger than 60% from maximum-likelihood (left) and maximum-parsimony (right) bootstrapping.

GENOME FEATURES AND PHYLOGENOMICS

Genomic DNA was isolated using a Jena Bioscience kit (Diffractia) following the standard protocol recommended by the manufacturer. The integrity of the extracted DNA was checked by visualization in a 2.0% (w/v) agarose gel electrophoresis. Its purity and quantity were checked by measuring the absorbance at 260 and 280 nm with a spectrophotometer (Nanodrop 2000c, Thermo Scientific) and calculating the ratio A260:A280. Genome sequencing of the two strains was carried out at the Central Support Service for Experimental Research (SCSIE) of the University of Valencia (Valencia, Spain). Genome sequencing was achieved using Sequel PacBio RS II technology (SMRT Link version 7.0) and assembled with the Hierarchical Genome Assembly Process (HGAP4) *de novo* assembly analysis application.

The bioinformatic tool CheckM version 1.1.3 [13] was used to assess the genome quality prior to annotation using Prokka version 1.14.6 [14] and Rapid Annotation using Subsystem Technology (RAST) version 2.0 [15]. The process of quality assessment of reads, read-processing, assembly and annotation with Prokka was carried out in Linux OS, other tools were accessed online. The minimal standards for the quality of genome sequences and how they can be applied for taxonomic purposes [9] have been observed in this study.

Similarity between genomes was established using *in silico* DNA–DNA hybridization (isDDH) with the GGDC 2.1 [11], average nucleotide identity (ANI) [16] with JSpecies software (http://jspecies.ribohost.com/jspecieswS/) and average amino acid identity (AAI) [17] with AAI matrix tools (http://enve-omics.ce.gatech.edu/g-matrix/). Phylogenomic analyses were performed with the

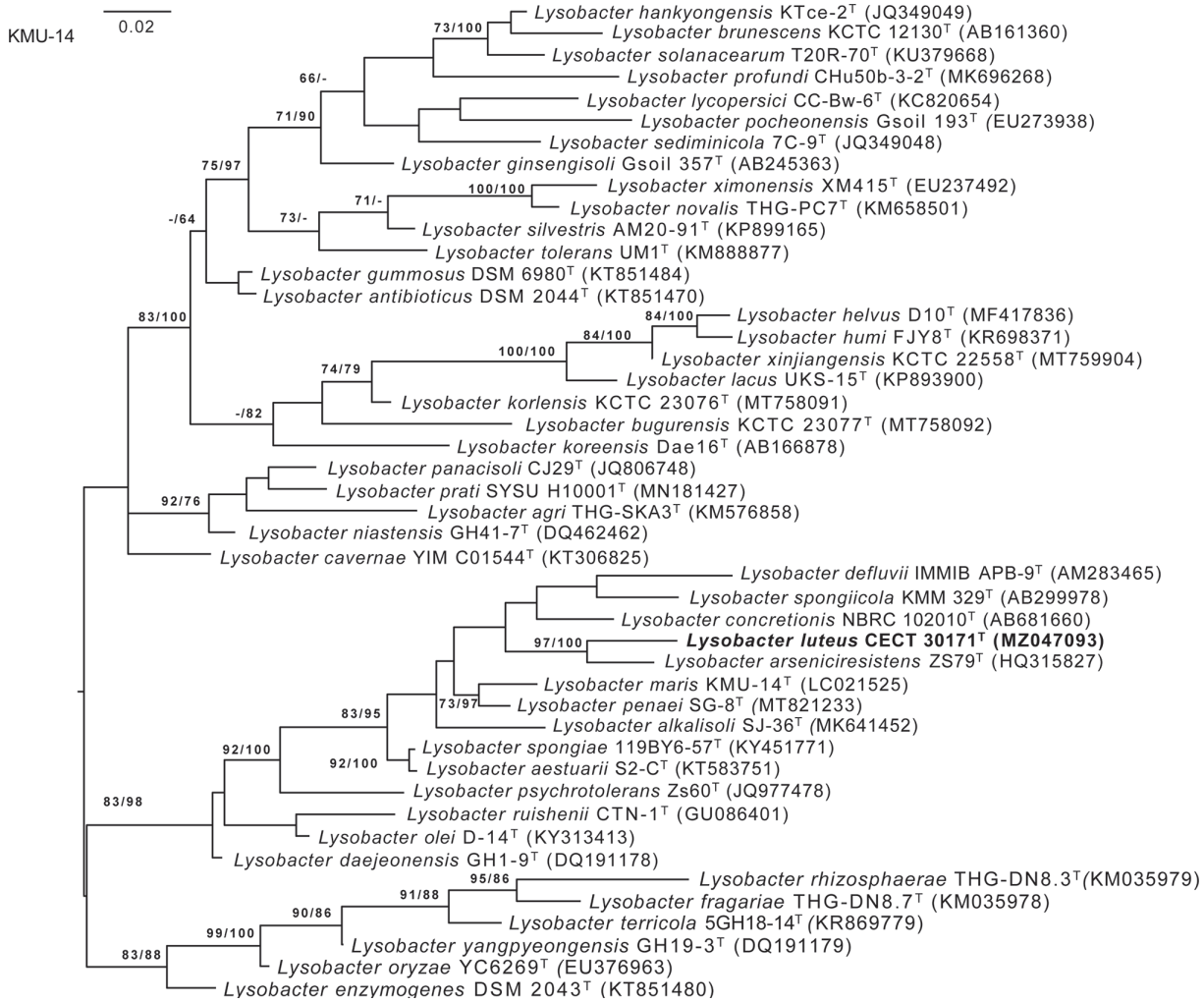

Fig. 2. Maximum-likelihood tree inferred from 16S rRNA gene sequences under the GTR+GAMMA model and rooted by midpoint-rooting including *Lysobacter luteus* sp. nov. CECT 30171^T and representatives of the genus *Lysobacter*. The branches are scaled in terms of the expected number of substitutions per site. Phylogenies were inferred by the GGDC web server [11] available at http://ggdc.dsmz.de/ using the DSMZ phylogenomics pipeline [12] adapted to single genes. The numbers above the branches are support values when larger than 60% from maximum-likelihood (left) and maximum-parsimony (right) bootstrapping.

UBCG (up-to-date bacterial core gene) [18]. This software tool is available for download at EzBioCloud [10] and employs a set of 92 bacterial core genes that are single-copy and commonly present in all bacterial genomes.

General characteristics of the two genomic sequences here determined plus those corresponding to type strains of those taxa used for comparative purposes (obtained from public repositories) are shown in Tables 1 and 2. Further details are discussed in the next subsections.

Strain CECT 30217^T (*Flavobacteriales*)

The genome of strain CECT 30217^T is 3.98 Mb in size (in one chromosome) and includes 3604 coding sequences and 37 RNA genes (with a single rRNA operon). About 63% of the proteins coded are hypothetical, according to RAST annotation. The G+C content is 38.8 mol%.

As could be anticipated by the low 16S rRNA similarities, the ANIb values obtained when comparing the genome of strain CECT 30217^T to other *Flavobacteriales* are also very low as none of them reached 67% (data not shown). Likewise, *is*DDH determinations were below 32% in all comparisons. Thus, the overall genomic indices were well below the commonly accepted threshold for species classification (95~96 and 70%, respectively) [9] confirming that strain CECT 30217^T has sufficient taxonomic novelty at least to the species level.

Table 1. Characteristics of the genomes of strain CECT 30217^T (in bold) and other type strains of *Flavobacteriales* used as reference organisms

	Size (Mb)	Contigs	G+C content (%)	Coverage (×)	N50 (Kpb)	Proteins	rRNA	tRNA	Accession numbers
***Parvicella tangerina* CECT 30217^T**	**3.98**	**1**	**38.8**	**230**	3980026	**3553**	**2**	**36**	**GCA_907165195 (OU015584)**
Lishizhenia tianjinensis CGMCC 1.7005^T	3.57	13	37.4	360	407984	3076	6	40	GCF_900116425 (FPAS01)
Brumimicrobium glaciale IC156^T	4.27	12	33.8	157	627632	3416	7	35	GCF_004152935 (SETE01)
Fluviicola taffensis DSM 16823^T	4.63	1	36.5	30	4633577	3998	6	43	GCF_000194605 (CP002542)
Crocinitomix catalasitica ATCC 23190^T	4.62	77	34.1	unknown	128440	3951	4	42	GCF_000621625 (JHXV01)
Salibacter halophilus KCTC 52047^T	3.12	32	40.3	180	298027	2686	3	35	GCF_008806325 (WACR01)
Cryomorpha ignava QSSC1-22^T	5.0	149	40.3	222	82601	4029	3	35	GCF_010686655 (JAAGVY01)
Luteibaculum oceani JCM 18817^T	2.92	42	39.9	220	302936	2397	5	35	GCF_007995015 (VORB01)
Vicingus serpentipes NCIMB 15042^T	2.92	34	31.0	220	541731	2534	6	34	GCF_007993035 (VOOS01)
Acidiluteibacter ferrifornacis S-15^T	3.52	23	35.6	550	374027	2997	7	32	GCF_009909615 (WWNE01)
Schleiferia thermophila DSM 21410^T	2.60	29	42.9	566	517830	2174	7	36	GCF_003337435 (QPJS01)
Thermaurantimonas aggregans LA^T	2.67	57	42.6	unknown	110953	2254	7	38	GCF_003851885 (BHZE01)
Phaeocystidibacter luteus LMG 25704^T	3.21	27	46.5	1500	200223	2815	3	33	GCF_008933115 (WBVO01)
Croceimicrobium hydrocarbonivorans A20-9^T	4.04	1	43.2	504	4035598	3568	6	36	GCF_014524565 (CP060139)
Owenweeksia hongkongensis DSM 17368^T	4.00	1	40.2	30	4000057	3441	6	37	GCF_000236705 (CP003156)
Weeksella virosa DSM 16922^T	2.27	1	35.9	30	2272954	2061	15	59	GCF_000189415 (CP002455)
Chryseobacterium gleum ATCC 35910^T	5.57	7	36.8	31	3504888	4994	5	41	GCF_000143785 (ACKQ02)
Epilithonimonas tenax DSM 16811^T	3.63	109	37.6	unknown	83168	3306	5	38	GCF_000428485 (AUAA01)
Flavobacterium aquatile LMG 4008^T	3.49	7	32.2	161	642039	3125	4	38	GCF_000757385 (JRHH01)
Winogradskyella thalassocola DSM 15363^T	4.57	34	33.3	316	333368	3883	7	42	GCF_900099995 (FNCZ01)
Ichthyobacterium seriolicida JBKA6^T	1.92	1	32.8	30	1921407	1390	3	32	GCF_002369955 (AP014564)

Table 2. Characteristics of the genomes of strain CECT 30171^T (in bold) and the *Lysobacter* type strains analysed

	Size (Mb)	Contigs	G+C content (%)	Coverage (×)	N50 (Kpb)	Proteins	rRNA	tRNA	Accession numbers
***L. luteus* CECT 30171^T**	**2.77**	**1**	**69.1**	**387**	2768593	**2513**	**3**	**52**	**GCF_907164845 (OU015430)**
L. aestuarii JCM 31130^T	3.49	156	69.4	330	41908	3096	3	48	GCF_006546775 (VICE01)
L. alkalisoli SJ-36^T	3.86	1	66.6	73	3857091	3265	6	49	GCF_006547045 (CP041242)
L. arseniciresistens ZS79^T	3.09	98	69.6	272	101761	2827	3	45	GCF_000768335 (AVPT01)
L. concretionis Ko07^T	3.03	26	67.2	308	386139	2603	3	49	GCF_000768345 (AVPS01)
L. defluvii DSM 18482^T	2.72	12	70.3	unknown	309919	2504	7	46	GCF_000423325 (AUHT01)
L. enzymogenes ATCC 29487^T	6.26	23	69.3	208	630088	4835	6	56	GCF_900106525 (FNOG01)
L. lacus UKS-15^T	2.86	301	68.0	30	16358	2709	3	44	GCF_008274655 (VTRV01)
L. lycopersici CC-Bw-6^T	2.60	1	66.9	unknown	2596073	2498	3	46	GCF_007556775 (CP041742)
L. panacisoli JCM 19212^T	3.88	2	67.5	300	3731398	3546	6	52	GCF_009765165 (VLNU01)
L. psychrotolerans ZS60^T	3.91	19	67.7	200	534630	3289	3	51	GCF_003719825 (RIBS01)
L. profundi CHu50b-3-2^T	2.71	21	66.8	200	720016	2486	3	45	GCF_009792795 (VKHQ01)
L. spongiae 119BY6-57^T	3.64	54	69.4	400	172446	3238	5	48	GCF_014145325 (JACHTF01)
L. tolerans UM1^T	2.54	20	61.6	441	640892	2389	3	45	GCF_900155935 (FTLW01)
L. xinjiangensis KCTC 22558^T	3.04	10	69.6	156	1741615	2851	6	48	GCF_014651995 (BMXY01)

In order to ascertain a possible separate genus status, suggested by the low 16S rRNA gene similarities, AAI values were also investigated. A definite threshold value of AAI for genus delimitation has not been universally stablished, but values higher than, at least, 60% are often encountered. After determination of AAI values of strain CECT 30217^T to a group of representatives of all the families included in the *Flavobacteriales* (except for *Blattabacteriaceae*), *Vicingus serpentipes* yielded 51% AAI and all other comparisons resulted in less than 50% (Table 3), including *Acidiluteibacter ferrifornacis*, which is the second representative of the family *Vicingaceae*. These low values point to a separate status at the genus level for strain CECT 30217^T and suggested that it might even constitute a new family within the order *Flavobacteriales*. In fact, a close examination of data in Table 3 revealed that species in the same family display AAI figures of 50% or higher (see coloured triangles) with only one exception (the pair *Crocinitomix catalassitica–Fluviicola taffensis*). On the other hand, AAI inter-family values above 50% are not observed except for two instances were 51% is reached (in both cases involving *Vicingus serpentipes*). These data point to a boundary of circa 50% AAI as a tentative level for definition of families in this context.

To further explore the family-level position of strain CECT 30217^T, we also determined AAI indexes of its genome to five MAGs, selected as representatives of family-level clades entirely composed of MAGs, flanking the family *Crocinitomicaceae* in the recent work of Bowman [2]: these comprise the clades UBA10066 (represented by SP19 and SP82, assemblies GCA_002723085.1 and GCA_002716065.1, respectively), UBA2798 (GCA_002351955.1), PHOS-HE28 (UBA3364, GCA_002360495.1) and UA16 (UBA1531, GCA_002323795.1). The assemblies were selected among those with at least a 90% completeness. The AAI values obtained were lower than 50% for UBA2798, UBA3364 and UBA1531, but attained 52 and 54% for SP19 and SP82, the two MAGs representative of clade UBA10066, suggesting a family-level relationship to strain CECT 30217^T. Thus, it might be considered the first cultivated strain of this clade and, consequently, type genus of a newly defined family in *Flavobacteriales*.

Two phylogenomic trees based on UBCGs were obtained by using amino acid and nucleotide sequences, and they are displayed as Fig. 3 and Fig. S1 (available in the online version of this article), respectively. In both, families are clearly defined, including the most recently described ones, and both trees show strain CECT 30217^T as a distant relative to *Crocinotomicaceae*, without a closer link to *Vicingaceae*, whose representatives form a clade not directly related to *Crocinitomicaceae* either. A moderate gene support index (GSI) value is obtained for the node relating strain CECT 30217^T to *Crocinitomicaceae* (73 for the amino acids tree and 57 for nucleotide tree, where the maximum possible value is 92).

Addition of MAGs from clades UBA10066, UBA2798, PHOS-HE28 and UA16 to the UBGC analysis resulted in the trees seen in Fig. 4 (amino acid-based) and Fig. S2 (nucleotide-based), that show a robust branching between strain CECT 30217^T and both representatives of UBA10066 clade. All three lineages merge as a well-defined clade with a high GSI (87), comparable to the ones on the nodes defining family *Crocinitomicaceae* (83) and *Vicingaceae* (92).

Combining the information of the phylogenomic trees with that of AAI indices, it is clear that strain CECT 30217^T bears enough distance from all other families in the order *Flavobacteriales* as to be considered as the type genus of a new family. This new family would encompass the *Flavobacteriales* clade UBA10066, until now represented only by uncultivated bacteria whose MAGs have been recovered from seawater oceanic samples.

The genome of strain CECT 30217^T was annotated with Prokka, RAST and also with the recently published Bakta [19]: all three annotations resulted in a high percentage of hypothetical protein genes, a common observation when examining genomes of 'uncommon' lineages. Thus, RAST annotation found a 63% of hypothetical protein-coding genes while Bakta annotation resulted in 56%. Several interesting features could be predicted from genome and others are confirmed by using the annotations. Firstly, we tried to detect the *Bacteroidota* hallmark genes postulated by Munoz *et al.* [20], a task hampered by the various synonymous labels used for some of the genes and also by the differences in annotations obtained from different tools. Gliding motility associated genes were found as follows in RAST annotated genome: *gldJ* (five copies), *gldB* and the components of the ABC-type transporter, *gldA*, *gldF* and *gldG*; genes for the type IX secretion system outer membrane protein channel, *porV*, and the cell surface protein, *sprA*, were detected in Bakta annotation. However, other components of T9SS seem to be absent. The gene for the flagellar motor protein A (MotA) was also detected.

SusC/D, involved in TonB-dependent uptake of polysaccharide/oligosaccharide, was not found under this label but 12 copies of a *tonB*-dependent receptor, plus three copies of a *tonB*-dependent receptor plug are present.

The Na^+ pumping NADH:quinone oxidorreductase cluster ABCDEF, which is present in *Bacteroidota* bacteria isolated from seawater and other sodium-rich habitats, was consistently annotated by all tools.

A cluster of several genes involved in carotene synthesis was also detected, containing genes for a β-carotene hydroxylase, a phytoene desaturase (neurosporene/lycopene-producing, EC 1.3.99.27, *crtD*), a phytoene synthase (15 cis phytoene synthase EC 2.5.1.32, *crtB*) and a phytoene dehydrogenase (zeta-carotene forming phytoene desaturase, EC 1.3.99.29, *carA2*). The genes were grouped in a gene cluster also containing genes apparently non related to carotenoid synthesis and the cluster was preceded by an MerR family transcriptional regulator (annotated as HTH-type transcriptional repressor CarH /YcgE in Prokka and Bakta annotations). No gene involved on flexirubin synthesis was identified and no proteorhodopsin-encoding gene was annotated in the genome of strain CECT 30217^T.

Table 3. AAI values between strain CECT 30217^T (in bold) and type strains of representative species of the nearest families

AAI (%)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1 ***Parvicella tangerina*** **CECT 30217^T**	*																				
2 *Lishizhenia tianjinensis* CGMCC 1.7005^T	**49**	*																			
3 *Brumimicrobium glaciale* IC156^T	**48**	56	*																		
4 *Fluviicola taffensis* DSM 16823^T	**47**	53	51	*																	
5 *Crocinitomix catalasitica* ATCC 23190^T	**48**	51	50	48	*																
6 *Salibacter halophilus* KCTC 52047^T	**48**	48	47	47	47	*															
7 *Cryomorpha ignava* QSSC1-22^T	**47**	47	48	46	47	48	*														
8 *Luteibaculum oceani* JCM 18817^T	**48**	49	47	47	47	49	48	*													
9 *Vicingus serpentipes* NCIMB 15042^T	**51**	50	49	49	50	51	50	49	*												
10 *Acidiluteibacter ferrifornacis* S-15^T	**48**	49	48	47	48	50	49	49	54	*											
11 *Schleiferia thermophila* DSM 21410^T	**46**	45	45	45	45	47	46	46	47	46	*										
12 *Thermaurantimonas aggregans* LA^T	**46**	46	45	45	45	47	46	46	47	46	72	*									
13 *Phaeocystidibacter luteus* LMG 25704^T	**47**	47	46	45	45	48	47	47	49	48	50	50	*								
14 *Croceimicrobium hydrocarbonivorans* A20-9^T	**46**	46	45	44	45	48	46	47	48	48	50	50	51	*							
15 *Owenweeksia hongkongensis* DSM 17368^T	**47**	47	47	46	47	49	48	48	50	49	50	50	51	56	*						
16 *Weeksella virosa* DSM 16922^T	**45**	45	45	45	45	45	45	45	47	46	46	46	47	46	47	*					
17 *Chryseobacterium gleum* ATCC 35910^T	**43**	44	44	45	43	44	43	44	45	44	45	45	44	44	45	51	*				
18 *Epilithonimonas tenax* DSM 16811^T	**44**	44	44	45	44	44	44	44	46	45	45	45	45	44	46	51	68	*			
19 *Flavobacterium aquatile* LMG 4008^T	**46**	46	46	47	46	47	46	46	49	47	47	47	47	47	48	50	49	49	*		
20 *Winogradskyella thalassocola* DSM 15363^T	**46**	47	47	45	47	47	47	46	49	48	47	47	47	47	49	49	46	47	56	*	
21 *Ichthyobacterium seriolicida* JBKA6^T	**45**	46	46	45	46	47	46	46	48	47	47	47	47	47	48	47	46	49	49	49	*
Fam. *Crocinitomicaceae*																					
Fam. *Vicingaceae*																					
Fam. *Schleiferiaceae*																					
Fam. *Weeksellaceae*																					
Fam. *Flavobacteriaceae*																					

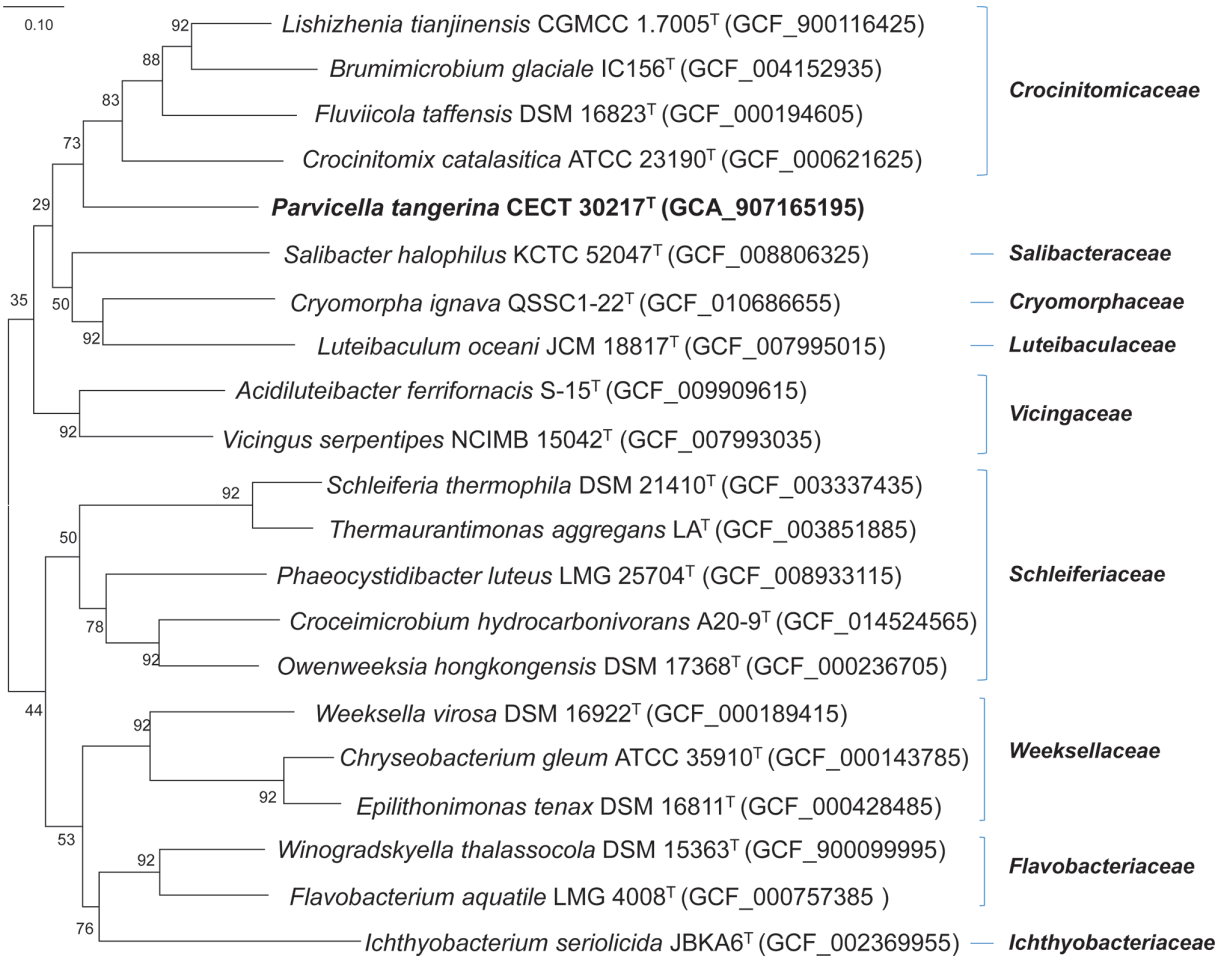

Fig. 3. Phylogenetic tree generated with the UBCG [18] by using amino acid sequences including *Parvicella tangerina* gen. nov., sp. nov. CECT 30217^T and representatives of the order *Flavobacteriales*. The numbers at the nodes indicate the gene support index (maximal value is 92). Genome accession numbers are indicated in parentheses. Bar, 0.05 substitutions per position. Genomes of strain *Dyadobacter luticola* T7^T (GCF_005860805) and *Dyadobacter fermentans* DSM 18053^T (GCF_000023125) were used as outgroup.

Aerotolerance proteins Bat (*Bacteroides* aerotolerance) A, B and C are encoded in the genome, but catalase/peroxidase genes are not detected, in agreement with the findings of the phenotypic characterization (see below).

Regarding extracellular hydrolytic capabilities, a chitinase gene was present, and also many different genes encoding peptidases.

The HigA and HigB toxin-antitoxin system was present as well. Also, a restriction modification (R-M) system type I and a CRISPR region containing Cas1, Cas 2 and Cas 9 genes plus CRISPR repeats with 17 spacers, were annotated.

Because of the origin of the wastewater processed in the plant, we examined the genome of strain CECT 30217^T using CARD to search for antibiotic resistance [21]. A total of 121 resistance related genes were tagged, including diverse β-lactamases (most of them, class C) as well as several RND efflux systems were located along the genome, plus an arsenate reductase and a mercuric reductase operon (*merR*, *merT* and mercuric ion reductase). Mobile genetic elements were not found in the RAST annotated genome, except for one transposase close to a tetracycline resistance element mobilization regulatory protein, RteC. In contrast, Prokka annotated genome showed 12 transposases distributed along the genome sequence, but only one beta-lactamase related gene. Plasmids were apparently absent, as the whole genome was resolved in a single contig.

Strain CECT 30171^T (*Lysobacter*)

The genome of strain CECT 30171^T is 2.77 Mb in size (in one chromosome) and contains 2549 coding sequences (40% hypothetical, according to RAST annotation) and 53 RNAs (this includes two rRNA operons). The G+C content is 69.1 mol%, a value within the range described for the genus.

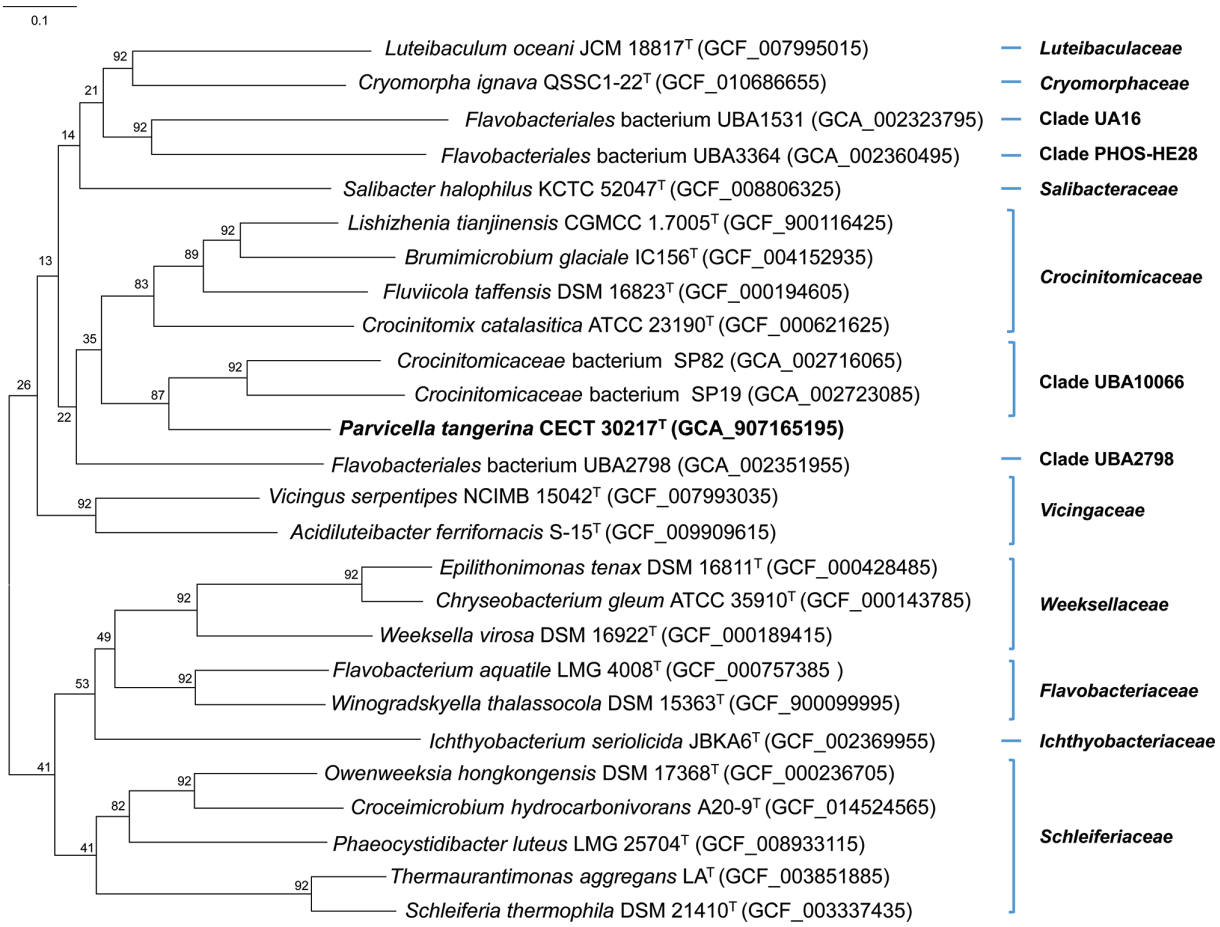

Fig. 4. Phylogenetic tree generated with the UBCG [18] by using amino acid sequences including *Parvicella tangerina* gen. nov., sp. nov. CECT 30217^T and representatives of the order *Flavobacteriales* and clades UBA10066, UBA2798, UA16 and PHOS-HE28. The numbers at the nodes indicate the gene support index (maximal value is 92). Genome accession numbers are indicated in parentheses. Bar, 0.05 substitutions per position. Genomes of strain *Dyadobacter luticola* T7^T (GCF_005860805) and *Dyadobacter fermentans* DSM 18053^T (GCF_000023125) were used as outgroups.

ANIb and *is*DDH values were obtained for strain CECT 30171^T and the close species in the genus (Table 4) using the same tools as before. The highest ANI and *is*DDH values (85.3 and 28.3%), were obtained with *L. arseniciresistans*, in agreement with the findings with 16S rRNA gene comparisons and tree. These values are well below the boundary for species definition and confirm that this strain is different at genomic level from all other species of *Lysobacter*.

Phylogenomic trees were also obtained with nucleotide and amino acid sequences from the genome of strain CECT 30171^T and relatives by using UBCG [18]. Fig. 5 shows the amino acid-based tree: as it can be observed, strain CECT 30171^T merges with *L. arseniciresistans* as closest species, with a moderate GSI value (75). The nucleotide-based tree is shown as Fig. S3.

The annotated genome of strain CECT 30171^T contains several genes involved in secretion systems: T2SS (six genes), T4SS, encoding type IV pilus (21 genes) and T7SS (two genes), as well as three proteins involved in twitching motility (PilH, PilG and PilT). These features are interesting in relation to the polar filaments observed in scanning microphotographs (see Fig. 6). It also contains a large amount of flagellar motility and chemotactic-related genes, in accordance with the swimming motility shown by the strain; flagellar motility is a trait excluded from the genus definition, but present in a few *Lysobacter* species (*L. arseniciresistans*, *L. spongicola*) and in strain CECT 30171^T.

Other remarkable features of the annotation are a large number of protein-degradation genes, the presence of alkane synthesis genes (*oleABCD*), a phospholipase/hemolisin, two restriction–modification systems, type I and type III (but no CRISPR region), and a large number of DNA repair genes (52). A Cu-nitrite reductase and a quinol-dependent nitric oxide reductase genes are present. Polyphosphate kinase and exopolyphosphatase genes are also present, suggesting the strain may be able to store polyphosphate. Several β-lactamases, RND efflux systems, ABC type antimicrobial peptide transport systems and genes related with arsenic resistance were also detected in the RAST annotated genome.

Table 4. Overall genome relatedness indexes ANIb and *is*DDH between strain CECT 30171^T (in bold) to the nearest *Lysobacter* species. ANIb figures are median values among reciprocal ANIb

	***is*DDH\ANIb**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
1	***Lysobacter luteus* CECT 30171^T**	*	**76.9**	**77.0**	**85.3**	**77.8**	**77.8**	**78.0**	**75.0**	**75.2**	**77.2**	**77.8**	**74.8**	**76.9**	**72.0**	**75.9**
2	*L. aestuarii* JCM 31130^T	**21.3**	*	79.0	78.0	76.1	76.1	77.2	74.7	75.1	76.8	76.7	74.7	91.5	72.2	75.7
3	*L. alkalisoli* SJ-36^T	**21.9**	22.9	*	78.1	76.1	76.1	77.1	74.5	75.4	77.0	76.7	74.6	79.0	72.3	75.6
4	*L. arseniciresistens* ZS79^T	**29.3**	21.6	22.4	*	78.9	78.9	78.9	75.9	76.2	78.2	78.6	75.6	78.0	72.5	77.1
5	*L. concretionis* Ko07^T	**22.5**	21.6	22.1	23.5	*	77.5	77.5	74.0	74.4	75.7	76.0	75.0	76.5	72.1	75.1
6	*L. defluvii* DSM 18482^T	**21.0**	21.1	21.3	22.0	21.4	*	76.5	74.0	74.4	75.7	76.0	73.8	76.0	71.5	75.1
7	*L. enzymogenes* ATCC 29487^T	**23.0**	22.6	22,8	23.2	22.7	21.9	*	75.8	76.6	78.9	76.0	76.0	76.9	73.1	77.0
8	*L. lacus* UKS-15^T	**19.9**	20.5	21.0	20.2	20.6	20.2	21.7	*	74.3	75.5	75.4	73.7	74.6	71.3	80.9
9	*L. lycopersici* CC-Bw-6^T	**20.3**	20.8	21.2	20.8	20.9	20.1	22.0	20.0	*	76.4	75.5	75.2	75.2	73.1	75.3
10	*L. panacisoli* JCM 19212^T	**21.1**	21.3	21.8	21.7	21.5	20.4	24.1	20.7	21.0	*	78.1	75.4	76.8	72.6	76.6
11	*L. psychrotolerans* ZS60^T	**22.3**	21.9	22.0	22.9	22.4	21.2	23.1	20.9	21.2	22.6	*		74.7	71.9	76.4
12	*L. profundi* CHu50b-3-2^T	**19.8**	20.5	29.9	19.9	20.4	19.5	21.6	19.7	21.1	20.0	20.7	*	76.6	72.4	74.4
13	*L. spongiae* 119BY6-57^T	**21.1**	46.3	22.7	21.6	21.5	21.0	22.5	20.4	20.8	21.0	21.8	20.5	*	72.4	75.6
14	*L. tolerans* UM1^T	**18.9**	20.8	20.5	19.4	19.7	18.4	21.0	19.0	19.5	20.2	20.3	19.2	21.1	*	72.0
15	*L. xinjiangensis* KCTC 22558^T	**19.8**	20.4	21.2	20.8	20.7	19.8	22.3	23.9	20.1	20.8	21.4	19.7	20.5	18.9	*

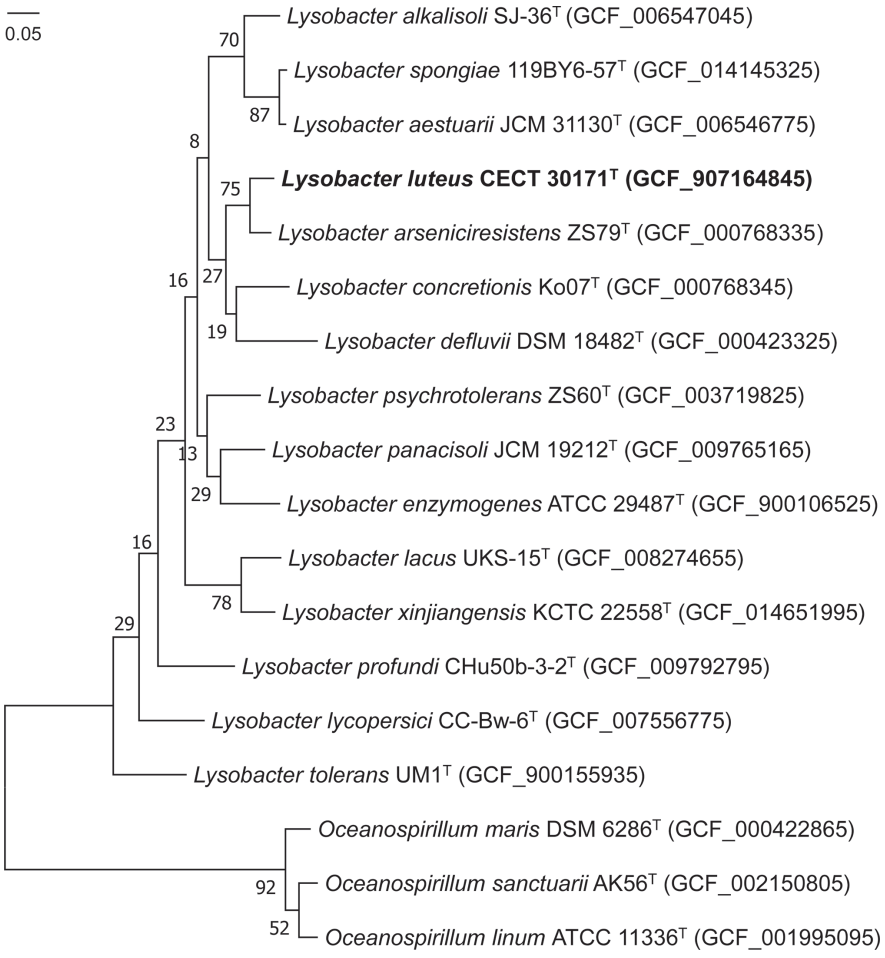

Fig. 5. Phylogenetic tree generated with the UBCG [18] by using amino acid sequences including *Lysobacter luteus* sp. nov. CECT 30171^T and representatives of the genus *Lysobacter*. The numbers at the nodes indicate the gene support index (maximal value is 92). Genome accession numbers are indicated in parentheses. Bar, 0.05 substitutions per position.

PHENOTYPIC CHARACTERIZATION

Strains were routinely cultivated at 26 °C aerobically, on MA prepared with marine broth (MB; Difco) plus an added, autoclaved double strength 1.2% w/v solution of agar. This was done to avoid the deleterious effect described by Tanaka *et al.* [22]. Marine Reasoner's 2A (R2A) medium (R2A with 2% sea salts, Oxoid), also prepared with a double-strength agar solution autoclaved separately, was also assayed but resulted in lower biomass recovery for both strains. Pigment production, colony morphology and gliding on solid medium were determined on strains grown on MA plates at 26 °C for 2–5 days. Flexirubin-type pigments were determined according to Bernardet *et al.* [23].

Cell morphology and motility were observed on wet mounts by phase-contrast microscopy. Cell size was determined by using scanning electron microscopy (SEM) at the SCSIE (University of Valencia). For these observations, cells were filtered with a 47 mm polycarbonate membrane filter of 0.2 µm pore size (Millipore) with a peristaltic pump. Filters were fixed in glutaraldehyde with final concentration 3% at room temperature and washed three times with 0.1 M phosphate buffer. A series of sequential ethanol dehydrations were performed for 10min each (50, 70, 95 and 100%) and post-fixed with 2% osmium before drying samples under CO_2 using a critical point drier apparatus (CPD030, Baltec). Samples were examined in a Hitachi S4800 field emission scanning microscope with field emission gun and a resolution of 1.4 nm to 1 kV. Pictures were stored digitally and processed using the software Quantax 400.

The temperature range for growth was determined on MA incubated at 5 and 15 °C for weeks, and at 26, 30, 37, 40 and 45 °C for 48 h. The pH range was determined in MB adjusted to pH 5.0–9.5, at 0.5 pH unit intervals, incubated at 26 °C for 1 week. Salinity range and NaCl requirement were determined for strain CECT 30171^T in liquid medium containing tryptone (5 g l^{-1}) and yeast extract (1 g l^{-1}) and supplemented with various amounts of sea salts (Oxoid): 0, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0,

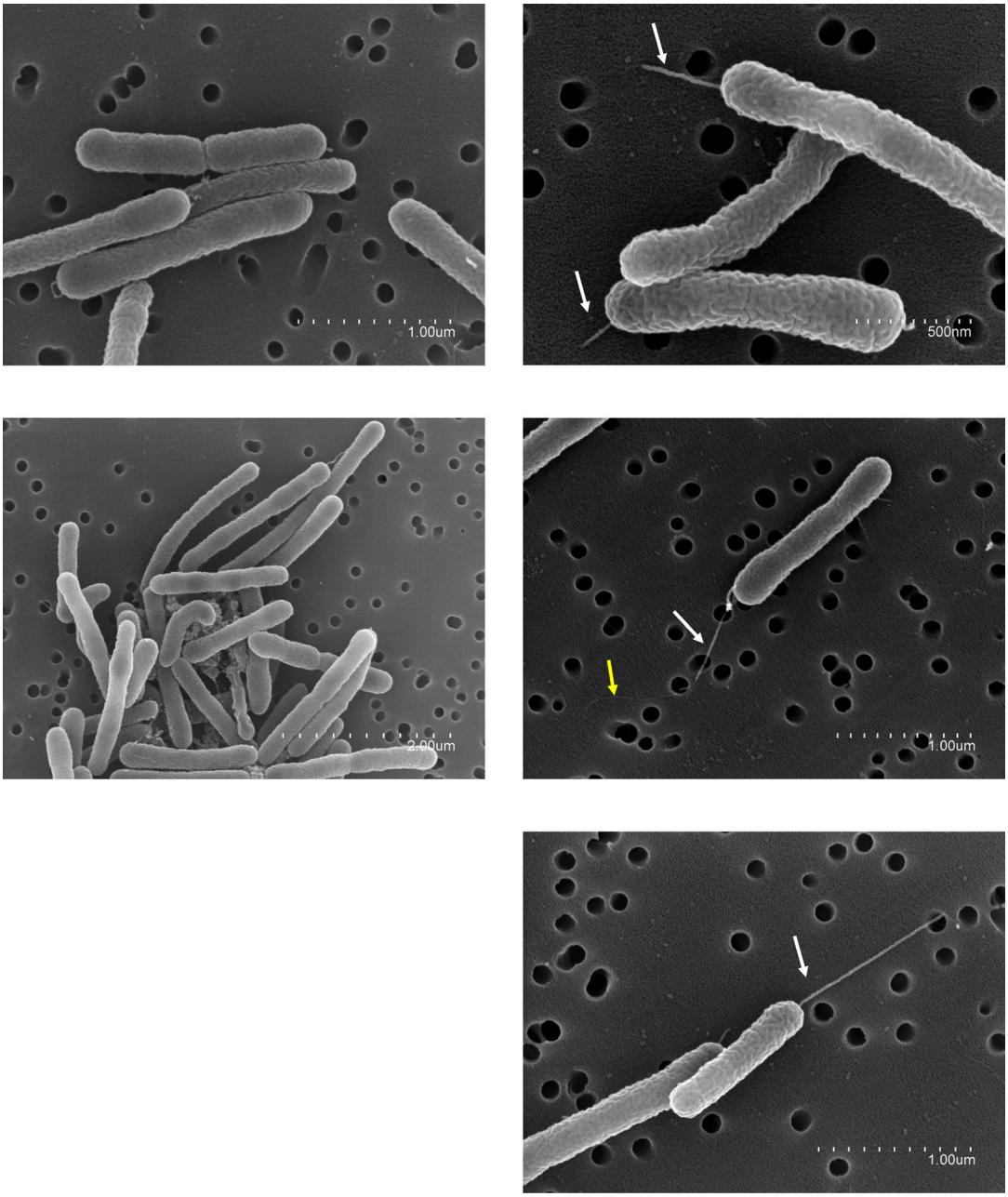

Fig. 6. Cells of strain CECT 30171^T observed under SEM. Cell morphology: binary fission is observed. Polar straight filaments (white arrows), flagellum (yellow arrow).

12.0 and 15.0% and with only 1% NaCl in a separate test (to determine if NaCl alone was able to sustain growth). Strain CECT 30217^T was tested on MA plates with the following salinities: 0.5, 1.0, 2.0% salinity (obtained by diluting MB, and supplementing with tryptone and yeast extract up to 5 and 1 g l^{-1}, respectively) and MB plus NaCl up to 4, 5, 6, 7, 8, 9, 10, 12 and 15% total salts. Catalase and oxidase, plus casein, starch, DNA and Tween 80 hydrolysis were determined according to Lucena *et al.* [24]. Anaerobic growth in denitrification medium [25] was also assayed. Other tests were recorded from API galleries (API 20NE, API ZYM and API 50CH, bioMérieux). Marine cations supplement was added (1/10) to diluents and AUX medium used to obtain cell suspensions and inoculate these galleries, which were incubated for 6 h (API ZYM) and 48 h (API 20NE and API 50CH) at 26 °C.

Fatty acid methyl esters were extracted from biomass grown for 48 h on MA at 26 °C and prepared according to standard protocols as described for the MIDI Microbial Identification System [26]. Cellular fatty acid content was analysed by gas chromatography with an Agilent 6850 chromatographic unit, with the MIDI Microbial Identification System using the TSBA6 method [27] and

identified using the Microbial Identification Sherlock software package. Analysis of respiratory quinones and polar lipids were carried out by the Identification Service, Leibniz-Institut DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany.

Strain CECT 30217^T (*Flavobacteriales*)

Strain CECT 30217^T was able to grow on MA but it was difficult to obtain individual colonies by regular streaking and viability of the cultures dropped quite rapidly. Growth was easier if the inoculum was administrated as drops of grown liquid media or cell suspensions on the agar surface without any streaking or spreading. This resulted in more biomass yield and extended viability. When resolved, colonies were round, elevated, shiny, with a regular margin and a mucous appearance. Pigment was orange to deep orange, more pronounced in old cultures (Fig. S4). The pigment did not react to the KOH test for flexirubin-type pigments. No gliding motility was observed. Following growth in liquid medium (MB) formed surface masses that converged to form a pellicle and adhered to the tube wall. Theses sticky masses were very difficult to disperse. Thus, uniformly turbid suspension of cells was not achieved. Photographs of the growth behaviour of the strain are displayed in Fig. S4. R2A medium supplemented with 2% sea salts did not support good growth of this strain, even when drop-inoculated. Since the amount of salts is within the physiological range of this organism (see below), it means that R2A, a meagre medium, does not meet its nutritional requirements showing a preference for richer ones such as MA.

Cells were observed as wet mounts under phase-contrast microscopy, appearing as small, non-motile coccobacilli. SEM observations revealed small cocci, coccobacilli and short bacilli (0.3–0.5×0.5–1.2 µm) aggregates, joined together by a network of abundant fibrous extracellular material, closely resembling *Vicingus serpentipes* SEM micrographs [28]. Selected images are shown in Fig. 7.

Cells of strain CECT 30217^T were Gram-staining and Gram-reaction negative, although clumping of cells hinder an easy observation of the KOH test.

Temperature, pH and salinity ranges were determined with the following results: the strain grew from 15–40°C but not at 5 °C (optimum 26–30 °C), at pH 6.0–8.5, but not at pH 5.5 or less or pH 9.0 or more. Salinities sustaining growth spanned from 1 to 7% (optimum, 3–4%), no growth was obtained at 0.5% or less or 8% or more. The strain did not grow with only NaCl, indicating a requirement for combined sea salts. Thus, the strain is classified as mesophilic, neutrophilic and slightly halophilic with complex ionic requirements.

The strain behaved as a strict aerobe: anaerobic, microaerophilic and capnophilic growth were tested on MA incubated in a Genbag Anaer (EZ Anaerobe), a *Campylobacter* Gaspak EZ campylopouch system and in a CO_2 incubator (5% CO_2 at 37 °C), respectively. No growth was obtained in any of these conditions after 4 days of incubation. Growth in agar-sealed denitrification medium was also negative. The oxidase test was positive, but catalase was negative. Nitrate was not reduced to nitrite or N_2 and glucose was not fermented. Hydrolysis of gelatin, casein and DNA were positive, Tween 80 was also hydrolysed but starch hydrolysis was negative. Prominent activities in the API ZYM galleries were alkaline and acidic phosphatases and leucine and valine arylamidases. All other activities, including carbohydrate-related enzymes, were negative. None of the carbohydrates included in the API 50CH gallery were acidified aerobic or anaerobically. Enzymatic tests on API 20NE were also negative except for gelatine hydrolysis, while assimilation tests were slightly positive except for the citrate and caprate microtubes.

The fatty acid composition of the cells was investigated as previously described, on MA cultures (26 °C, 48 h). Major fatty acids accounting for more than 10% of the total were iso-$C_{15:0}$ (56%) and iso-$C_{15:1}$ G (15%). Complete results are reported in Table S1.

The only respiratory quinone detected was menaquinone 7 (MQ-7). Polar lipids present in strain CECT 30217^T comprised one identified component, phosphatidylethanolamine (PE), along with three unidentified lipids, two phospholipids and one aminolipid (Fig. S5).

Strain CECT 30171^T (*Lysobacter*)

Strain CECT 30171^T grew on MA and on Luria–Bertani agar (LBA) supplemented with 1% NaCl forming bright yellow colonies after 72 h incubation. Colonies were round, smooth, translucent, elevated, with regular borders (Fig. S6). In liquid media, MB, it produced uniform turbidity and was readily suspended. It also grew on R2A+2% (w/v) sea salts (the pigment displayed in this medium was less intense and more ochre). The pigment did not react to the KOH test for flexirubin-type pigments [23]. Phase contrast observations of living cells showed swimming-type, very active motility, suggesting the presence of flagella. Gram staining and Gram reaction on 3% KOH were consistently negative.

Cells grown for 5 days on MA at 26 °C were examined by SEM at the SCSIE (University of Valencia). Microscopy images illustrating cell size and morphology of strain CECT 30171^T are shown in Fig. 6. The strain presented rod-shaped cells, 0.2–0.3×1.4–2.0 µm, that divided by binary fission. Some cells had a polar-anchored, straight filament of variable length adherent to substratum/filter. A polar flagellum could also be seen in some cells.

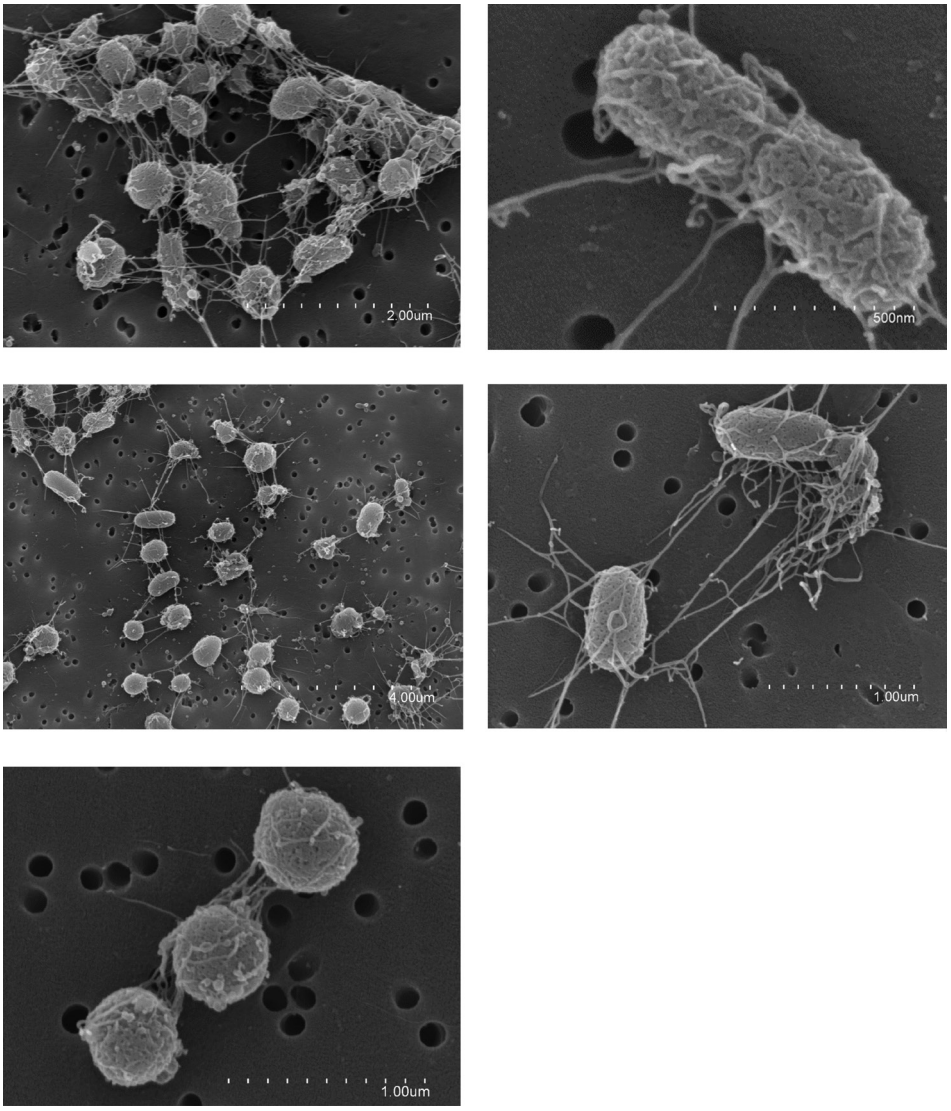

Fig. 7. SEM images of strain CECT 30217^T cells. Coccus, cocobacilli and bacilli are seen within a network of extracellular, fibrous material. Unequal division of cells is suggested in the fourth photomicrograph, where a dividing cell is seen resulting in a bacillus and a coccoid cell (upper right cluster).

Strain CECT 30171^T grew from 4 (after 15 days) to 40 °C, but not at 45 °C. Optimal temperature was 30–37 °C. The strain did not grow at pH 5.0 or lower and pH 9.0 or higher. Growth was obtained at pH 5.5–8.5 and was optimal at pH 7.5–8.0. Salinity range allowing growth, determined with sea salts was 0.5–8.0% (w/v), no growth occurred at 0% salinity or more than 9.0%. Optimal salinity was 1–3%. The strain was able to growth with only NaCl added to the medium (1%). Thus, the strain is considered mesophilic, neutrophilic and slightly halophilic.

Strain CECT 30171^T did not grow in Baumann's denitrification medium and did not ferment glucose. It behaved as a strict aerobe and was oxidase- and catalase-positive. Nitrate reduction to nitrite or molecular nitrogen was negative. The strain was strongly proteolytic, according to its ability to degrade gelatin quickly and the positive response to leucine arylamidase, trypsin and α-chemotrypsin in the API ZYM galleries. Alkaline and acid phosphatases were also strongly positive. Naphthol-AS-BI-phosphohydrolase was positive. A weak response was observed for estearase and estearase lipase, valine arylamidase and α-glucosidase. Other activities, mainly carbohydrate activity enzymes, were negative. Assimilation tests in API 20NE galleries were weakly positive after 48 h, except for caprate and citrate assimilation, which were negative. All other tests, except for gelatine hydrolysis, were also negative. None of the carbohydrates included in API 50CH galleries was acidified, aerobic or anaerobically, by strain CECT 30171^T.

Table 5. Differential characteristics among CECT 30217^T and representatives of closest families in the order *Flavobacteriales*

Strains: 1, *Parvicella tangerina* sp. nov. CECT 30217^T (this study); 2, *Vicingus serpentipes* [28]; 3, *Acidiluteibacter ferrifornacis* [39]; 4, *Crocinitomix* species [40, 41]; 5, *Bruminimicrobium* species [40, 42–44]; 6, *Lishizhenia* species [45, 46]; 7, *Putridiphycobacter roseus* [47]; 8, *Salinirepens amamiensis* [48]; 9, *Cryomorpha ignava* [40]; 10, *Salibacter halophilus* [49]; 11, *Luteibaculum oceani* [50]. +, Positive; –, negative; v, variable among species; ND, not determined; R, rods; CB, cocobacilli; C, cocci; F, filaments; O, orange; Y, yellow; P, pink; MK, menaquinone; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; DPG, diphosphatidylglycerol.

	Parvicellaceae	*Vicingaceae*		*Crocinitomicaceae**					*Cryomorphaceae*	*Salibacteraceae*	*Luteibaculaceae*
Characteristic	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Cell morphology	R, CB, C	CB	CB	R, F	R	R, CB, F	R	R	R	R	R
Gliding motility	–	+	–	+	v	+	–	+	–	–	+
Pigment color	O	Y	Y	Y	O	O	P	Y/O	O	O	Y
Oxidase	+	+	+	–	–	v	+	+	–	–	+
Catalase	–	+	+	+	+	v	+	+	+	+	–
Maximum salinity (%)	7	6	10	6–10	6–13	6.5–7.5	5	6	6	18	5
Growth at 5°C	–	+	–	v	v	+	+	–	+	–	–
Strictly aerobic	+	+	+	+	–	+	+	+	+	–	+
Nitrate reduction	–	ND	ND	–	v	–	–	–	–	–	+
Casein hydrolysis	+	+	ND	–	–	+/–	–	–	–	ND	–
Trypsin (API ZYM)	–	–	–	v	v	+	+	ND	ND	–	+
Proteorhodopsin†	–	+	ND	+	+	+	+	+	+	+	ND
Major quinone	MK7	MK7	MK7	MK7	MK6	MK6	MK7	MK6	ND	MK7	MK6
Polar lipids identified	PE	PE	PE	–	PE (PG)	nd	PE	–	ND	PE, DPG	PE
G+C content (mol%)	39	31	36	35	34–40	34–36	34	36	36	39	44

**Fluviicola* and *Wandonia* can be differentiated from all taxa included in the table by their production of flexirrubins and ability to growth without NaCl.
†According to [2].

Table 6. Characteristics differentiating *Lysobacter luteus* CECT 30171^T from other *Lysobacter* species

Strains: 1, *L. luteus* sp. nov. CECT 30171^T (this study); 2, *L. arseniciresistens* [51]; 3, *L. concretionis* [52]; 4, *L. spongiae* [53]; 5, *L. defluvii* [54]; 6, *L. spongiicola* [55]; 7, *L. aestuarii* [56]; 8, *L. maris* [57]; 9, *L. penaei* [58]; 10, *L. alkalisoli* [59]; 11, *L. enzymogenes* [7]. +, Positive; –, negative; v, variable; ND, not determined; Y, yellow; O, orange; PG, phosphatidylglycerol; PE, phosphatidylethanolamine.

Feature	1	2	3	4	5	6	7	8	9	10	11
Flagellar motility	+	+	–	–	–	+	–	–	–	–	–
Pigment colour	Y	Y	Y	Y/O	Y	Y	Y	O	Y	Y	-/Y
Salinity range	0.5–8.0	0–4	0–2	0–9	0–6	0–6	0–7	ND–5	0–6	0–6	0–1
Maximum growth temperature	40	37	ND	37	37	41	40	40	45	40	40
Nitrate reduction	–	–	–	+	–	–	+	–	+	+	–
Oxidase	+	+	+	+	+	+	+	–	+	+	+
Aesculin hydrolysis	–	–	–	ND	–	–	+	+	+	+	+
β-Galactosidase	–	–	–	–	–	ND	–	+	v	–	+
Polar lipids include PG	+	+	+	+	+	+	+	–	+	+	+
Polar lipids include PE	+	+	+	+	+	+	+	+	+	–	+
G+C content (mol%)	69.1	69.5	63.8	69.9	67.1	69.0	63.8	64.9	68.8	66.6	68.5

The cellular fatty acid profile was determined for cultures obtained after 48 h incubation at 26 °C on MA and LBA. Main fatty acids, accounting for more than 10% of the total, were iso-$C_{15:0}$ (32–36%), sumemd feature 9 (iso-$C_{17:1}$ ω9*c*, 25–30%) and iso-$C_{16:0}$ (14–15%) (Table S2).

The main respiratory quinone was Q8 (99.4%) and Q7 represented a minor component (0.6%) of the system. The polar lipids present were DPG, PG and PE, plus two unidentified glycolipids, three unidentified phospholipids and one lipid (Fig. S7).

ECOLOGY

To explore occurrence of bacteria similar to our isolates and determine the potential distribution of the species, BLAST searches with 16S rRNA gene sequences (default parameters) were examined for hits yielding the highest similarities.

Strain CECT 30217^T appears to be very uncommon. The BLAST search explained above resulted on similarities below 95.4%, which is quite low for such a conserved gene. The top results corresponded to uncultured clones obtained from deep-sea hydrothermal vents on Logatchev field, Atlantic Ocean (95.3%) [29] and a deep sea vent on the East Pacific Rise (95.0%) [30, 31], showing 91–94% similarity. All corresponded to uncultured clones of different marine origins and geographical locations, suggesting that the only known relatives of strain CECT 30217^T in the range of putative genera of the same family are marine bacteria that have never been cultivated.

In addition, the affiliation of the new genus and species to the UBA10066 clade, until now found only by MAGs, suggests again a seawater-related distribution: the two MAGs showing family-level AAI indices and phylogenomic relationships to strain CECT 30217^T were obtained as a part of the *Tara* Oceans expedition dataset [32] and were obtained from seawater from the South Pacific Ocean. It is interesting to note that clade UBA10066 includes clade Agg58, detected in seawater in one of the first molecular surveys of uncultivated marine bacteria in the early 1990s by De Long and coworkers [33], as pointed by Bowman [2].

For strain CECT 30171^T, searches resulted in several hits above 98.7% similarity including two uncultured bacterial clones from a soil sample in the Yellow River delta (99.87%) and from seawater near dolphins in San Diego, USA (99.85%). These findings point to a marine/coastal environment as putative natural habitat of the species. However, some of the sequences from cultivated strains challenge this conclusion. Strains NC344 and NC471 (both 99.93% similarity to strain CECT 30171^T) lack a full record about the source and only the country (China) is mentioned. There is no associated publication, being the depositor's institution the Chinese Academy of Agricultural Sciences. Then, strains ZGLJ7-2 and ZGLJ7-1 (99.71 and 99.57% similarity, respectively) were isolated from a pit mud involved in the production of white wine in Henan, an inner province in China. Strain ZGLJ7-1 was proposed as type strain of '*Lysobacter zhanggongensis*' [34] but the name has not been validated. Interestingly, the submitter's institution of these sequences is the Institute of Oceanology, Chinese Academy of Sciences. Thus, despite having found much closer relatives to strain CECT 30171^T, compared to strain CECT 30217^T, we end up with a less defined ecological niche preference.

Given the possible marine origin of the strains coming from a seawater-processing wastewater treatment plant, and in order to elucidate a little bit more about their ecology and biogeographic distribution, we have used the partial 16S rRNA sequence of strains CECT 30171^T and CECT 30217^T to look for related environmental sequences in the Ocean Barcode Atlas (https://oba.mio.osupytheas.fr/ocean-atlas/OBA_query), which include metagenomic and amplicon 16S rRNA sequences from global *Tara* Oceans and Malaspina circumnavigation expeditions [35, 36]. The results for both strains showed that no sequence in the database presented a percentage of similarity above 97% [37]. Instead, we found some similarities at the genus level with 94.8% in the case of strain CECT 30171^T, specifically sequences taxonomically classified as *Lysobacter* sp. HJHJ-0803 and detected in Malaspina Bathypelagic samples (Fig. S8), and 95.3% for strain CECT 30217^T, in this case sequences affiliated to *Flavobacteriales* and found in surface and Deep Chlorophyll Maximum (DCM) samples from the Arctic Ocean (Fig. S8).

We further explored other metagenomic databases available in the European Nucleotide Archive (ENA, https://www.ebi.ac.uk/ena/browser/home) in order to better describe the ecology of these new strains. Specially, we explored the sequences available in the MIDAS project [38] which aimed to study the micro-organisms present in activated sludge systems. Thanks to their online tool (www.midasfieldguide.org/guide) a BLAST search can be performed between the database and the 16S rRNA sequence of interest. All these analyses gave us also negative results at the species level, confirming that these strains are very rare and found in nature in extremely low abundances. In fact, a previous 454-pyrosequencing analysis [6] of the sample of activated sludge from which both micro-organisms were isolated showed that a sequence similar to strain CECT 30217(98.3% similarity) was in a very low proportion in the sample (0.05%).

DISCUSSION AND TAXONOMIC CONCLUSIONS

The uniqueness of the environment, a wastewater treatment plant that uses seawater instead of freshwater due to the shortage of the later and abundance of the first in its location [5], rendering strains CECT 30171^T and CECT 30217^T sustains the interest of their study, not only because of their taxonomic novelty but also for the biotechnological potentialities they might present. The plant has operated since 1998 and its main influent corresponds to intermediate products from amoxicillin synthesis [5].

Both strains are phenotypically distinct from their closest phylogenetic relatives, as can be seen in Tables 5 and 6, which display differences in the phenotypic profile of the new taxa.

Table 5 displays differential traits allowing discrimination of strain CECT 30217^T from the families *Crocinitomicaceae*, *Vicingaceae*, *Cryomorphaceae*, *Salibacteraceae* and *Luteibaculaceae*. Strain CECT 30217^T could be clearly differentiated by the oxidase and catalase tests and the menaquinone type from all other marine representatives of these families, but there are several additional phenotypic differences. Thus, its position as a new genus and species in the order *Flavobacteriales* is soundly established on the basis of distinctive phenotype and low levels of ANI and AAI indices, combined with its detachment from other family lineages in phylogenomic trees. The suitability of defining a new family to allocate the new genus is based on the distances and branching pattern respect to their neighbours in phylogenomic trees. Other options are less pragmatic. For example, if we would consider that CECT 30217^T should be included in an existing family, this should be *Crocinitomicaceae*, but phenotypically it does not fit with the more common traits of the family (catalase, menaquinone, proteorhodopsin, gliding) and the overall genome similarity measured as AAI index would be lower than the usual intra-family values observed for the order (>50%). After exploring the possibility of the new taxon being representative of any of the potentially new families defined by Bowman [2] and the Genome Taxonomy Database (GTDB) [4], clades UBA10066, UBA2798, UA16 and PHOS-HE28, we conclude that this strain is a member of the UBA10066 clade, according with phylogenomic analysis, and should be considered as the first cultivated representative of this lineage.

In addition, Table 6 differentiated strain CECT 30171^T from neighbouring *Lysobacter* species by a minimum of three traits, giving support to its proposal as a new species. *Lysobacter* already contains 64 validly named species, only a few from marine origin (*L. spongiae*, *L. spongicola*, *L maris*, *L. aestuarii*, *L. penaei*). The new species is related to a group of species that show atypical features: ability to form functional flagella that allow swiming motility (along with *L. arseniciresistens*, *L. mobilis* and *L. spongicola*) and requirement of NaCl for growth, a trait rarely found among *Lysobacter s*pecies (and only in *L. maris*, among those of marine origin).

Thus, we describe strain CECT 30217^T as representing a new genus and species in a new family in the order *Flavobacteriales* [2, 4] and strain CECT 30171^T as representing a new species of *Lysobacter*.

DESCRIPTION OF *PARVICELLA* GEN. NOV.

Parvicella (Par.vi.cel'la. L. masc. adj. *parvus*, small; L. fem. n. *cella*, a storeroom and in biology a cell; N.L. fem. n. *Parvicella*, a small cell).

Gram-negative, non-motile cells, strictly aerobic and chemoorganotrophic. Orange-pigmented due to carotenoid production. Flexirubin or rhodopsin are not produced. Catalase-negative, oxidase-positive, proteolytic and non-fermentative. Does not reduce

nitrates. Main fatty acid is iso-$C_{15:0}$. Menaquinone 7 is the only respiratory quinone. Phosphatidyl ethanolamine is present in the polar lipid profile. The G+C content of the type and only known species is 38.8mol%.

The type species is *Parvicella tangerina*.

DESCRIPTION OF *PARVICELLA TANGERINA* SP. NOV.

Parvicella tangerina (tan.ge.ri'na. N.L. fem. adj. *tangerina*, tangerine-coloured, referring to the colony colour).

In addition to the characteristics of the genus, the species present cells that produce abundant extracellular fibrous material, aggregating cells in masses. Cells are cocci, coccobacilli or short bacilli (0.3–0.5 µm wide and 0.5–1.2 µm long). Grows on MA forming round elevated, shiny colonies with regular borders, orange-pigmented, with the pigment becoming deeper with time. No gliding motility is observed. Growth on liquid media is limited to a superficial pellicle and aggregates adhered to recipient walls, no turbidity is seen in the liquid. Grows from 15–40 °C, but not at 5 or 45 °C. Optimum temperature is 26–30 °C. Salinity range is 1–7% sea salts (optimum 3–4%). No growth without salts or with only NaCl added. The pH range allowing growth is pH 6.0–8.5. No growth in anaerobic, microaerophilic or CO_2-enriched atmosphere. Hydrolyses gelatin, casein, Tween 80 and DNA, but not starch. Negative for nitrate reduction to nitrite or gas, arginine dihydrolase, tryptophanase, urease and aesculin hydrolysis. Enzymatic activities include also alkaline and acidic phosphatases and leucine and valine aryl amidases. Negative for trypsin, naphthol-AS-BI-phosphohydrolase, α- and β-galactosidases, β-glucuronidase, α- and β-glucosidases, *N*-acetyl-β-glucosaminidase, α-manosidase and α-fucosidase. Does not produce acid from carbohydrates, either aerobic or anaerobically. Slight growth is obtained with D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, maltose, gluconate, adipate, malate and phenylacetate but not on citrate or caprate. Main fatty acids are iso-$C_{15:0}$, iso-$C_{15:1}$ G and iso-$C_{17:0}$ 3OH. Polar lipids include PE and one unidentified major lipid plus two phospholipids, two lipids and one aminolipid. The only menaquinone is MK7. The G+C content is 38.8mol%.

The type strain is CECT 30217^T (=AS29M-1^T=LMG 32344^T), which was isolated from activated sludge from a seawater-processing wastewater treatment plant in southeast coast of Spain.

DESCRIPTION OF *PARVICELLACEAE* FAM. NOV.

Parvicellaceae (Par.vi.cel.la.ce'ae. N.L. fem. n. *Parvicella* type genus of the family; *-aceae* ending to denote a family; N.L. fem. pl. n. *Parvicellaceae* the family of the genus *Parvicella*).

Gram-negative, non-spore-forming, non-flagellated, non-gliding bacteria. Metabolism is strictly aerobic and chemoorganotrophic. Sea salts are required for growth. Carotenoid pigments are formed. Fatty acids include iso-$C_{15:0}$. The main respiratory quinone is MK7.

Includes six placeholder genera encompassed in the family-level clade UBA10066, based on GTDB. The G+C content ranges from 30 to 41mol%, based on genome sequences within that clade. Affiliated to the order *Flavobacteriales*.

The type genus is *Parvicella*.

DESCRIPTION OF *LYSOBACTER LUTEUS* SP. NOV.

Lysobacter luteus (lu'te.us. L. masc. adj. *luteus*, yellow, reflecting the colony colour).

Gram reaction and staining negative, rod-shaped cells, 0.2–0.3 µm wide and 1.4–2.0 µm long that divide by binary fission and display swimming motility. One polar flagellum is present. Polar straight filament occurs in most cells that, according with genome content, corresponds to a type IV fimbria. No gliding motility is detected on solid media. Grows on MA as yellow, round, smooth, translucent, elevated colonies with regular borders. The pigment does not react to KOH test for flexirubin. LBA and R2A+2% NaCl also support growth, but in the latter, pigment produced is yellowish ochre. Growth is optimal at 30–37 °C and is positive at 4 and 40 °C (but not at 45 °C). No grow at pH 5.0 or lower and pH 9.0 or higher and is optimal at pH 7.5–8.0. NaCl is required for growth, which occurs from 0.5–8.0% sea salts (but not at 9% or more or without added salts). Optimal salinity is 1–3% sea salts.

Strictly aerobic chemoorganotroph, unable to ferment sugars. Oxidase- and catalase-positive. Does not reduce nitrates and does not produce acid from carbohydrates aerobically. Positive for gelatine hydrolysis, alkaline and acid phosphatases, naphthol-AS-BI-phosphohydrolase, leucine aryl amidase, trypsin and α-chemotrypsin. Weakly positive for valine arylamidase, estearase, estearase lipase and α-glucosidase. Negative for arginine dihydrolase, urease, tryptophanase, lipase, cystine arylamidase, α- and β-galactosidases, β-glucuronidase, β-glucosidase, *N*-acetyl-β glucosaminidase, α-manosidase and α-fucosidase. Grows weakly on D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, maltose, gluconate, adipate, malate and phenylacetate but not on citrate or caprate. Main cellular fatty acids are iso-$C_{15:0}$, iso-$C_{17:1}$ ω9*c* and iso-$C_{16:0}$. The main respiratory quinone is Q8. DPG, PG and PE are the main identified polar lipids present. The G+C content of the genome is 69.1mol%.

The type strain is CECT 30171^T (=AS29M^T=LMG 32343^T), which was isolated from activated sludge from a seawater-processing wastewater treatment plant in southeast coast of Spain.

Funding information
Financial support was received from grant AICO-2020-181 (Generalitat Valenciana) to M.J.P. S.G.A. was supported by Plan Nacional I+D+I 2020 (PID2020-116489RB-I00).

Authors and contributors
Conceptualization: O.S., S.G.A. and M.J.P. Data curation, formal analysis and investigation: T.L., I.S.S., S.G.A., O.S., L.G., J.M., M.C.M., M.A.R., D.R.A and M.J.P. Funding acquisition: SGA and M.J.P. Project administration and resources: D.R.A., O.S. and M.J.P. Supervision and writing original draft: M.J.P. All authors contributed to review and editing.

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

References

1. Parte AC, Sardà Carbasse J, Meier-Kolthoff JP, Reimer LC, Göker M. List of Prokaryotic names with Standing in Nomenclature (LPSN) moves to the DSMZ. *Int J Syst Evol Microbiol* 2020;70:5607–5612.
2. Bowman JP. Out from the shadows - resolution of the taxonomy of the family *Cryomorphaceae*. *Front Microbiol* 2020;11:795.
3. Oren A, Garrity GM. Validation list no.199. list of new names and new combinations previously effectively, but not validly, published. *Int J Syst Evol Microbiol* 2021;71:4773.
4. Parks DH, Chuvochina M, Rinke C, Mussig AJ, Chaumeil P-A, *et al*. GTDB: an ongoing census of bacterial and archaeal diversity through a phylogenetically consistent, rank normalized and complete genome-based taxonomy. *Nucleic Acids Res* 2022;50:D785–D794.
5. Sánchez O, Garrido L, Forn I, Massana R, Maldonado MI, *et al*. Molecular characterization of activated sludge from a seawater-processing wastewater treatment plant. *Microb Biotechnol* 2011;4:628–642.
6. Sánchez O, Ferrera I, González JM, Mas J. Assessing bacterial diversity in a seawater-processing wastewater treatment plant by 454-pyrosequencing of the 16S rRNA and *amoA* genes. *Microb Biotechnol* 2013;6:435–442.
7. Christensen P. *Lysobacter*. In: *In Bergey's Manual of Systematics of Archaea and Bacteria*. John Wiley and Sons, 2015.
8. Arahal DR, Sánchez E, Macián MC, Garay E. Value of recN sequences for species identification and as a phylogenetic marker within the family "*Leuconostocaceae*." *Int Microbiol* 2008;11:33–39.
9. Chun J, Oren A, Ventosa A, Christensen H, Arahal DR, *et al*. Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. *Int J Syst Evol Microbiol* 2018;68:461–466.
10. Yoon S-H, Ha S-M, Kwon S, Lim J, Kim Y, *et al*. Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *Int J Syst Evol Microbiol* 2017;67:1613–1617.
11. Meier-Kolthoff JP, Auch AF, Klenk HP, Göker M. Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics* 2013;14:60.
12. Meier-Kolthoff JP, Hahnke RL, Petersen J, Scheuner C, Michael V, *et al*. Complete genome sequence of DSM 30083(T), the type strain (U5/41(T)) of *Escherichia coli*, and a proposal for delineating subspecies in microbial taxonomy. *Stand Genomic Sci* 2014;9:2.
13. Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Res* 2015;25:1043–1055.
14. Seemann T. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* 2014;30:2068–2069.
15. Overbeek R, Olson R, Pusch GD, Olsen GJ, Davis JJ, *et al*. The SEED and the rapid annotation of microbial genomes using subsystems technology (RAST). *Nucleic Acids Res* 2014;42:D206-14.
16. Richter M, Rosselló-Móra R, Oliver Glöckner F, Peplies J. JSpeciesWS: a web server for prokaryotic species circumscription based on pairwise genome comparison. *Bioinformatics* 2016;32:929–931.
17. Rodriguez-R LM, Konstantinidis KT. The enveomics collection: a toolbox for specialized analyses of microbial genomes and metagenomes. *PeerJ Preprints*. 2016. DOI: 10.7287/peerj.preprints.1900v1.
18. Na S-I, Kim YO, Yoon S-H, Ha S-M, Baek I, *et al*. UBCG: p-to-date bacterial core gene set and pipeline for phylogenomic tree reconstruction. *J Microbiol* 2018;56:280–285.
19. Schwengers O, Jelonek L, Dieckmann MA, Beyvers S, Blom J, *et al*. Bakta: rapid and standardized annotation of bacterial genomes via alignment-free sequence identification. *Microb Genom* 2021;7:000685.
20. Munoz R, Teeling H, Amann R, Rosselló-Móra R. Ancestry and adaptive radiation of *Bacteroidetes* as assessed by comparative genomics. *Syst Appl Microbiol* 2020;43:126065.
21. Alcock BP, Raphenya AR, Lau TTY, Tsang KK, Bouchard M, *et al*. CARD 2020: antibiotic resistome surveillance with the comprehensive antibiotic resistance database. *Nucleic Acids Res* 2020;48:D517–D525.
22. Tanaka T, Kawasaki K, Daimon S, Kitagawa W, Yamamoto K, *et al*. A hidden pitfall in the preparation of agar media undermines microorganism cultivability. *Appl Environ Microbiol* 2014;80:7659–7666.
23. Bernardet JP, Nakagawa Y, Holmes B. Subcommittee on the taxonomy of *Flavobacterium* and cytophaga-like bacteria of the International Committee on Systematics of Prokaryotes. Proposed minimal standards for describing new taxa of the family *Flavobacteriaceae* and emended description of the family. *Int J Syst Evol Microbiol* 2002;52:1049–1070.
24. Lucena T, Sanz-Sáez I, Arahal DR, Acinas SG, Sánchez O, *et al*. *Mesonia oceanica* sp. nov., isolated from oceans during the *Tara* oceans expedition, with a preference for mesopelagic waters. *Int J Syst Evol Microbiol* 2020;70:4329–4338.
25. Baumann P, Baumann L. The marine Gram-negative eubacteria: genera *Photobacterium, Beneckea*, *Alteromonas*, *Pseudomonas*, and *Alcaligenes*. In: Starr MP, Stolp H, Trüper HG, Balows A and SchlegelHG (eds). *The Prokaryotes*. New York: Springer Verlag; 1981. pp. 1352–1394.
26. Sasser M. Technical Note 101: Identification of bacteria by gas chromatography of cellular fatty acids. MIDI. 1990.
27. MIDI. *Sherlock Microbial Identification System Operating Manual*. version 6.1. Newark, DE: MIDI Inc; 2008.
28. Wiese J, Saha M, Wenzel-Storjohann A, Weinberger F, Schmaljohann R, *et al*. *Vicingus serpentipes* gen. nov., sp. nov., a new member of the *Flavobacteriales* from the North Sea. *Int J Syst Evol Microbiol* 2018;68:333–340.
29. Hügler M, Gärtner A, Imhoff JF. Functional genes as markers for sulfur cycling and CO_2 fixation in microbial communities of hydrothermal vents of the Logatchev field. *FEMS Microbiol Ecol* 2010;73:526–537.

30. Sylvan JB, Toner BM, Edwards KJ. Life and death of deep-sea vents: bacterial diversity and ecosystem succession on inactive hydrothermal sulfides. *mBio* 2012;3:e00279-11.
31. Tian F, Yu Y, Chen B, Li H, Yao Y-F, *et al*. Bacterial, archaeal and eukaryotic diversity in arctic sediment as revealed by 16S rRNA and 18S rRNA gene clone libraries analysis. *Polar Biol* 2009;32:93–103.
32. Tully BJ, Graham ED, Heidelberg JF. The reconstruction of 2,631 draft metagenome-assembled genomes from the global oceans. *Sci Data* 2018;5:170203.
33. DeLong EF, Franks DG, Alldredge AL. Phylogenetic diversity of aggregate-attached vs. free-living marine bacterial assemblages. *Limnol Oceanogr* 1993;38:924–934.
34. Zhang XF, Wang HH, Sun XY, Pan CM. *Lysobacter zhanggongensis* sp. nov. isolated from a pit mud. *Curr Microbiol* 2017;74:1389–1393.
35. Alberti A, Poulain J, Engelen S, Labadie K, Romac S, *et al*. Viral to metazoan marine plankton nucleotide sequences from the Tara Oceans expedition. *Sci Data* 2017;4:170093.
36. Duarte CM. Seafaring in the 21st century: the malaspina 2010 circumnavigation expedition. *Limnol Oceanogr Bull* 2015;24:11–14.
37. McIlroy SJ, Saunders AM, Albertsen M, Nierychlo M, McIlroy B, *et al*. MiDAS: the field guide to the microbes of activated sludge. *Database* 2015;2015:bav062.
38. Yarza P, Yilmaz P, Pruesse E, Glöckner FO, Ludwig W, *et al*. Uniting the classification of cultured and uncultured bacteria and archaea using 16S rRNA gene sequences. *Nat Rev Microbiol* 2014;12:635–645.
39. Gui X, Wu S, Huang F, Wang Y, He R, *et al*. *Acidiluteibacter ferrifornacis* gen. nov., sp. nov., a new member of the *Flavobacteriales* from Tielu Harbour, Hainan, PR China. *Int J Syst Evol Microbiol* 2020;70:5812–5817.
40. Bowman JP, Nichols CM, Gibson JAE. *Algoriphagus ratkowskyi* gen. nov., sp. nov., *Brumimicrobium glaciale* gen. nov., sp. nov., *Cryomorpha ignava* gen. nov., sp. nov. and *Crocinitomix catalasitica* gen. nov., sp. nov., novel *Flavobacteria* isolated from various polar habitats. *Int J Syst Evol Microbiol* 2003;53:1343–1355.
41. Shi M-J, Han J-R, Zhang H, Xie Z-H, Du Z-J. *Crocinitomix algicola* sp. nov., isolated from *Gracilaria blodgettii*. *Int J Syst Evol Microbiol* 2017;67:4020–4023.
42. Yang S-H, Seo H-S, Oh H-M, Kim S-J, Lee J-H, *et al*. *Brumimicrobium mesophilum* sp. nov., isolated from a tidal flat sediment, and emended descriptions of the genus *Brumimicrobium* and *Brumimicrobium glaciale*. *Int J Syst Evol Microbiol* 2013;63:1105–1110.
43. Zhang H, Han J-R, Shi M-J, Du Z-J, Chen G-J. *Brumimicrobium aurantiacum* sp. nov., isolated from coastal sediment. *Int J Syst Evol Microbiol* 2017;67:3256–3260.
44. Luo HR, Chen ZY, Fei JJ, Du ZJ. *Brumimicrobium salinarum* sp. nov., isolated from a marine solar saltern. *Int J Syst Evol Microbiol* 2018;68:3447–3451.
45. Lau KWK, Ren J, Wai NLM, Qian P-Y, Wong P-K, *et al*. *Lishizhenia caseinilytica* gen. nov., sp. nov., a marine bacterium of the phylum *Bacteroidetes*. *Int J Syst Evol Microbiol* 2006;56:2317–2322.
46. Chen L-P, Xu H-Y, Fu S-Z, Fan H-X, Zhou Y-G, *et al*. *Lishizhenia tianjinensis* sp. nov., isolated from coastal seawater. *Int J Syst Evol Microbiol* 2009;59:2400–2403.
47. Wang X-J, Xu L, Wang N, Sun H-M, Chen X-L, *et al*. *Putridiphycobacter roseus* gen. nov., sp. nov., isolated from Antarctic rotten seaweed. *Int J Syst Evol Microbiol* 2020;70:648–655.
48. Muramatsu Y, Takahashi M, Kamakura Y, Suzuki KI, Nakagawa Y. *Salinirepens amamiensis* gen. nov., sp. nov., a member of the family *Cryomorphaceae* isolated from seawater, and emended descriptions of the genera *Fluviicola* and *Wandonia*. *Int J Syst Evol Microbiol* 2012;62:2235–2240.
49. Lu D-C, Xia J, Dunlap CA, Rooney AP, Du Z-J. *Salibacter halophilus* gen. nov., sp. nov., isolated from a saltern. *Int J Syst Evol Microbiol* 2017;67:1784–1788.
50. Shahina M, Hameed A, Lin S-Y, Lai W-A, Liu Y-C, *et al*. *Luteibaculum oceani* gen. nov., sp. nov., a carotenoid-producing, lipolytic bacterium isolated from surface seawater, and emended description of the genus *Owenweeksia Lau et al*. 2005. *Int J Syst Evol Microbiol* 2013;63:4765–4770.
51. Luo G, Shi Z, Wang G. *Lysobacter arseniciresistens* sp. nov., an arsenite-resistant bacterium isolated from iron-mined soil. *Int J Syst Evol Microbiol* 2012;62:1659–1665.
52. Bae HS, Im WT, Lee ST. *Lysobacter concretionis* sp. nov., isolated from anaerobic granules in an upflow anaerobic sludge blanket reactor. *Int J Syst Evol Microbiol* 2005;55:1155–1161.
53. Choi H, Im WT, Park JS. *Lysobacter spongiae* sp. nov., isolated from spongin. *J Microbiol* 2018;56:97–103.
54. Yassin AF, Chen W-M, Hupfer H, Siering C, Kroppenstedt RM, *et al*. *Lysobacter defluvii* sp. nov., isolated from municipal solid waste. *Int J Syst Evol Microbiol* 2007;57:1131–1136.
55. Romanenko LA, Uchino M, Tanaka N, Frolova GM, Mikhailov VV. *Lysobacter spongiicola* sp. nov., isolated from a deep-sea sponge. *Int J Syst Evol Microbiol* 2008;58:370–374.
56. Jeong SE, Lee HJ, Jeon CO. *Lysobacter aestuarii* sp. nov., isolated from estuary sediment. *Int J Syst Evol Microbiol* 2016;66:1346–1351.
57. Yoon J. Polyphasic characterization of *Lysobacter maris* sp. nov., a bacterium Isolated from seawater. *Curr Microbiol* 2016;72:282–287.
58. Xu S, Li A, Zhang MX, Yao Q, Zhu H. *Lysobacter penaei* sp. nov., isolated from intestinal content of a pacific white shrimp (*Penaeus vannamei*). *Int J Syst Evol Microbiol* 2019;71.
59. Xu L, Huang X-X, Fan D-L, Sun J-Q. *Lysobacter alkalisoli* sp. nov., a chitin-degrading strain isolated from saline-alkaline soil. *Int J Syst Evol Microbiol* 2020;70:1273–1281.