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TAXONOMIC DESCRIPTION
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Mesonia oceanica sp. nov., isolated from oceans during the *Tara* oceans expedition, with a preference for mesopelagic waters

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Abstract

Strain ISS653^T, isolated from Atlantic seawater, is a yellow pigmented, non-motile, Gram-reaction-negative rod-shaped bacterium, strictly aerobic and chemoorganotrophic, slightly halophilic (1–15% NaCl) and mesophilic (4–37 °C), oxidase- and catalase-positive and proteolytic. Its major cellular fatty acids are iso-$C_{15:0}$, iso-$C_{15:0}$ 2-OH, and iso-$C_{17:0}$ 3-OH; the major identified phospholipid is phosphatidylethanolamine and the major respiratory quinone is MK6. Genome size is 4.28 Mbp and DNA G+C content is 34.9 mol%. 16S rRNA gene sequence similarity places the strain among members of the family *Flavobacteriaceae,* with the type strains of *Mesonia phycicola* (93.2%), *Salegentibacter mishustinae* (93.1%) and *Mesonia mobilis* (92.9%) as closest relatives. Average amino acid identity (AAI) and average nucleotide identity (ANI) indices show highest values with *M. mobilis* (81% AAI; 78.9% ANI), *M. phycicola* (76% AAI; 76.3% ANI), *Mesonia maritima* (72% AAI, 74.9% ANI), *Mesonia hippocampi* (64% AAI, 70.8% ANI) and *Mesonia algae* (68% AAI; 72.2% ANI). Phylogenomic analysis using the Up-to-date-Bacterial Core Gene set (UBCG) merges strain ISS653^T in a clade with species of the genus *Mesonia*. We conclude that strain ISS653^T represents a novel species of the genus *Mesonia* for which we propose the name *Mesonia oceanica* sp. nov., and strain ISS653^T (=CECT 9532^T=LMG 31236^T) as the type strain. A second strain of the species, ISS1889 (=CECT 30008) was isolated from Pacific Ocean seawater. Data obtained throughout the *Tara* oceans expedition indicate that the species is more abundant in the mesopelagic dark ocean than in the photic layer and it is more frequent in the South Pacific, Indian and North Atlantic oceans.

The genus *Mesonia* belongs to the family *Flavobacteriaceae* [1], the bacterial family which includes the largest number of genera to date, 153 according to LPSN [2]. Marine members of the family *Flavobacteriaceae* play fundamental roles as complex organic matter degraders and in the nutrient turnover in oceans, where many of them are found in association with marine phytoplankton and algal live or detritic material [1, 3] and some display proteorodopsin-based photoheterotrophy [3]. The genus *Mesonia* was established in 2003 to accommodate a group of algal-associated marine bacteria that were different from members of the genus *Salegentibacter* and other halophilic flavobacteria [4]. It currently contains eight species with validly published names, all of them isolated from marine environments and organisms: *Mesonia algae*, the type species [4], *M. mobilis* [5], *M. phycicola* [6], *M. ostreae* [7], *M. aquimarina* [8], *M. hippocampi* [9], *M. sediminis* [10] and *M. maritima* [11]. The genus is one of the many included in a recent phylogenomic study of the phylum *Bacteroidetes* [12] and has not been affected by the reclassifications therein proposed.

From the *Tara* oceans expedition (2009–2013) [13] we have isolated in culture a large collection of marina bacteria from different oceanographic regions and depths [14]. Based on such isolation effort, here we present the description of a novel species in this family, based on the phenotypic, genomic and phylogenetic study of the strains ISS653^T and ISS1889.

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Abbreviations: AAI, average amino acid identity; ANI, average nucleotide identity; blast, basic local alignment search tool; DDH, DNA–DNA hybridization; GGDC, genome-to-genome-distance calculator; GSI, gene support index; LTP, the all-species living tree project; PE, phosphatidylethanolamine; RAST, Rapid Annotation using Subsystem Technology; UBCG, Up-to-date-Bacterial Core Gene.
The accession numbers of the *Mesonia oceanica* ISS653^T 16S rRNA gene sequence and draft genome are MH732189 and CABVMM01, respectively.
The accession number of the *Mesonia oceanica* ISS1889 16S rRNA gene sequence is MN836382.
Two supplementary figures and three supplementary tables are available in the online version of this article.

ISOLATION

Strain ISS653^T was isolated in September 2012 from surface seawater at the North Atlantic Ocean (36°10′10.2″N 29°01′13.8″W). Sampling strategy and methodology have been described previously [13]. Briefly, the isolate was obtained by plating 100 µl of undiluted and 10× diluted seawater (pre-filtered by 200 µm and 20 µm meshes to remove large plankton) in Marine Agar 2216 plates (BD Diagnostics). Plates were incubated at room temperature (approximately 20 °C) in the dark until no more colonies appeared (10–30 days). Colonies that grew were streaked on agar plates in duplicate to ensure their purity and avoid contamination. The isolates were stored in the broth medium used with glycerol (25% v/v) in cryovials at −80 °C. The culture was regrown on Marine Agar 2216 (BD Diagnostics) at room temperature for 3 days. The strain has been maintained by lyophilisation at the Spanish Type Culture Collection (CECT) as CECT 9532^T (=LMG 31236^T) where part of the characterization was also conducted. A second strain of the species, ISS1889 (=CECT 30008), was isolated during the same expedition from mesopelagic seawater (at 475.6 m depth) of the Eastern Pacific Ocean (5°15′36.0″S 85°10′04.1″W). A study, including phenotypic, genomic and phylogenetic characterization of the strains was undertaken in order to define their taxonomic position.

16S RNA PHYLOGENY

A partial (1328 bp) 16S rRNA gene sequence of ISS653^T was obtained after DNA extraction as reported previously [15] and deposited under the accession number MH732189. A BLAST search for the closely related taxa, based on this sequence, related the strain to the family *Flavobacteriaceae* and revealed species of the genera *Salengentibacter*, *Mesonia*, *Zunongwangia* and *Gramella* as its closest neighbours, with sequence similarities always lower than 93%. These values indicated that the strain represented a novel taxon. At a different stage, the nearly complete 16S rRNA gene sequence of ISS1889 (1425 bp) was obtained through already reported methods [15] and deposited under the accession number MN836382. It was found to be identical to that of ISS653^T, indicating that both strains could represent the same species.

The sequence (1530 bp) obtained from the genome draft of ISS653^T, described under the next heading, was subsequently used for determining similarity values with the type strains of closely related species [16] and also to reconstruct phylogenetic trees [17, 18] using ARB. Closest neighbours based on 16S rRNA sequence similarity, as determined by the Identifier tool of EzBiocloud [16] were: *Mesonia phycicola* (93.2%), *Salegentibacter mishustinae* (93.1%), *Mesonia mobilis* (92.9%), *Mesonia maritima* (92.9%), *Salegentibacter salarius* (92.9%) and *Mesonia aquimarina* (92.6%). A phylogenetic tree based on 16S rRNA gene sequences is shown in Fig. 1. It places strains ISS653^T and ISS1889 in the clade formed by species of the genus *Mesonia*, as a sister clade. The low 16S rRNA gene similarities found and the position of the branch in the tree support the taxonomic novelty of the strains at the species level and even indicates that they might represent a novel genus in the family. However, genus boundaries are not defined as clearly as species on genomic and phylogenetic grounds and a careful evaluation of all genomic and phenotypic evidence is required for definition of a genus. We thus, explored some other indexes and phylogenetic approaches to decide whether strains ISS653^T and ISS1889 should be classified as representing a novel species of the genus *Mesonia* or as a novel genus in the family *Flavobacteriaceae*.

GENOME FEATURES

A draft genome sequence of ISS653^T was obtained through whole genome sequencing at Centre Nacional d'Anàlisi Genòmica (CNAG) (https://www.cnag.crg.eu/) following procedures described previously [15]. The libraries were sequenced on a HiSeq 2500 (HiSeq Rapid SBS Kit V2, Illumina) in paired-end mode 2×251+8+8 bp. Primary data analysis, image analysis, base calling and quality scoring of the run were processed using the manufacturer's software Real Time Analysis (RTA 1.18.66.3), followed by generation FASTQ sequence files. The reads were analysed for quality control using FASTQC, a common quality control tool developed by Babraham Bioinformatics to check raw sequencing data. After filtering, the remaining reads were assembled using SPAdes 3.9.0 software [19]. A plot, coverage versus length of the contigs, was performed to help in the choice of the parameters for contigs filtering. After the filtration of contigs (minimal length 500 bp and coverage 10–50×kmer), evaluation of the final assembly against a reference genome was done with the software QUAST v4.3 [20]. The bioinformatic tool CheckM v1.0.7 [21] was used to assess the genome quality prior to annotation using Prokka v1.12 [22] and RAST v2.0 (Rapid Annotation using Subsystem Technology) [23]. 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 have been observed in this study [24].

The available genomes of type strains of genera of the family *Flavobacteriaceae* closely related to the novel taxon were retrieved from public databases. Table 1 shows their accession numbers and main characteristics.

The draft genome of ISS653^T has an estimated size of 4.28 Mbp. It is composed of 72 contigs with a N50 value of 251300 nucleotides and final assembly coverage of 452×. CheckM results for contamination and completeness were 0.53 and 99.6%, respectively. The assembly contains 4030 protein coding sequences and 45 RNA genes. Only one ribosomal RNA operon is detected and its 16S rRNA gene sequence is complete and 100% coincident with the partial sequence previously amplified (Sanger). The DNA G+C molar content is 34.9%. This genome is the largest one of all available genomes of members of the genus *Mesonia*, in fact, is about 1.0 Mbp larger than the one of *M. phycicola* (which had the maximum genome size recorded until now).

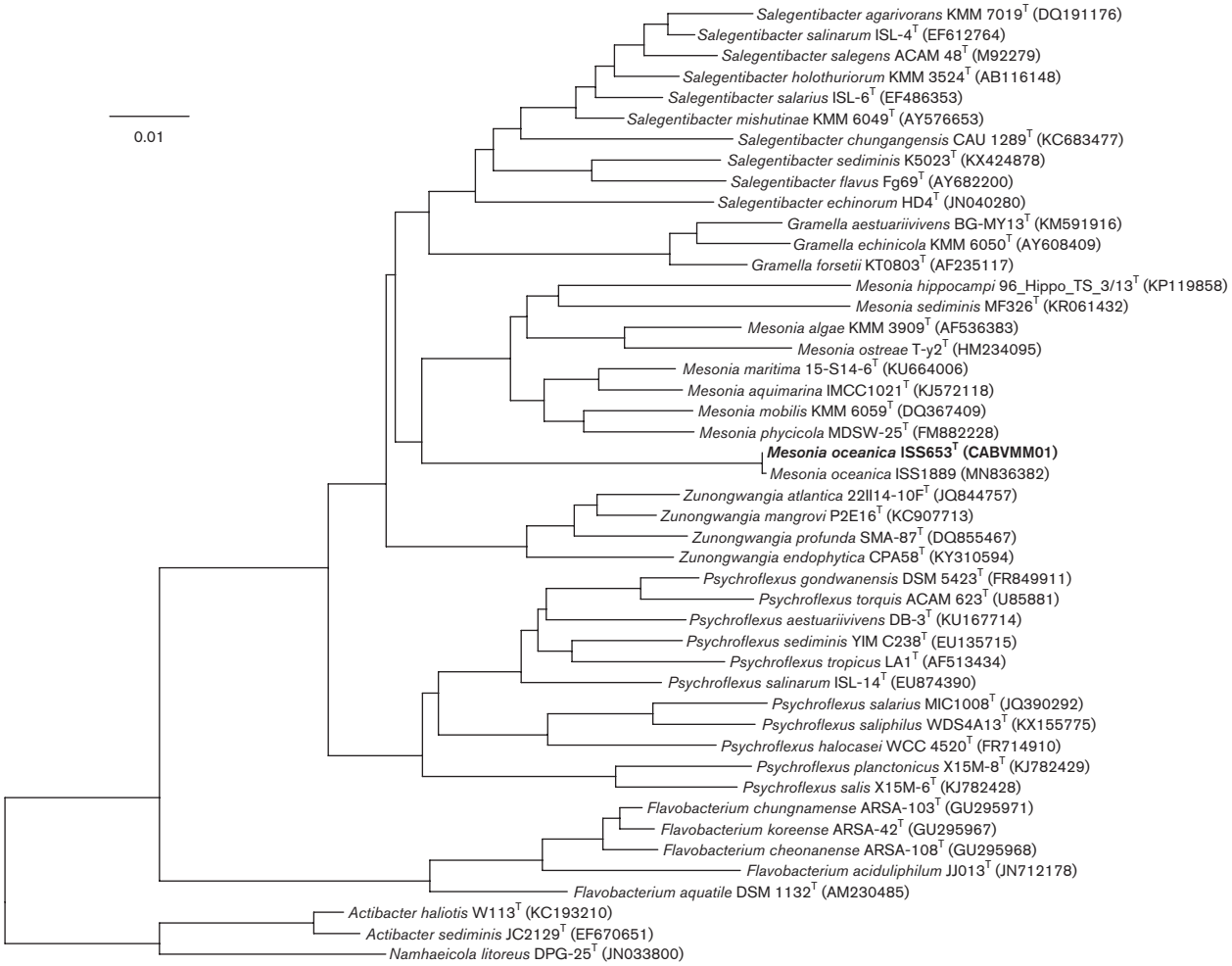

Fig. 1. Phylogenetic reconstruction based on the 16S rRNA gene using the neighbor-joining method. Sequence accession numbers are given in parentheses. Bar, number of substitutions per position.

The presence of four copies of a CTnDOT-like transposon [25] is predicted from the four *Tra* regions (containing *TraJ*, *K*, *M*, *N*, *I* and *G* genes) identified as part of this Bacteroidetes-specific transposon. It is also present in the genomes of *M. maritima* (two copies) and *M. phycicola* (one copy).

Further exploration of the annotated genome of ISS653^T allowed the prediction of some potential abilities of the strain, such as multiple degradative polysaccharide capabilities, a trait characteristic of several taxa in the family. Among them, carbohydrate active enzymes (CAZymes) predicted from the genome of ISS653^T account for pectate lyase (EC 4.2.2.2., two copies), polygalacturonase (EC 3.2.1.15), xylan β-1-4 xylosidase (EC 3.2.1.37), endo 1-4 β-xylanase, pectin estearase (EC 3.1.1.11), a pectin degradation protein (KdgF), rhamnogalacturonate acetyl estearase, endoglucanase, β-glucosidase (five copies), mucin desulfating sulfatase (two copies), phytase, glucan 1-4 α-glucosidase (two copies), a polysaccharide deacetylase, glycogen synthase, laminarinase, β-1-4 glucanase, hyaluronan synthase, oligogalacturonate lyase and neopullullanase (Table S2, available in the online version of this article)

Apart from these, it is also interesting to note the presence of genes coding for phosphatidylserine decarboxylase (in agreement with the presence of PE as major identified polar lipid) and also for cardiolipin synthase (although no DPG was detected among the identified polar lipids). A large CRISPR region and several Cas proteins are also encoded, as well as a type III restriction-modification system, and various gliding-motility-associated ABC transporter permeases. Enzymes involved in carotene metabolism, such as phytoene synthase, phytoene dehydrogenase, lycopene β-cyclase and β-carotene hydroxylase are also found. No rhodopsin-related enzymes are predicted.

The similarity between genomes was assessed using several indexes useful for species and genus delineation. Average amino acid identity (AAI) was calculated with the online server ANI/AAI-Matrix [26]. DNA–DNA hybridization

Table 1. Genomes used in the study and their main characteristics

Strain	Accession no.	Size (Mbp)	DNA G+C content (mol%)	Protein-encoding genes	RNA-encoding genes
Mesonia oceanica ISS653^T	GCF_902499555 (CABVMM01)	4.28	34.9	3854	45
Mesonia algae DSM 15361^T	GCF_003253545 (QKYV01)	3.09	33.1	2859	70
Mesonia mobilis DSM 19841^T	GCF_000423405 (AUHX01)	3.21	35.2	2875	46
Mesonia phycicola DSM 21425^T	GCF_900141885 (FQYY01)	3.23	31.4	2911	47
Mesonia maritima DSM 102814^T	jgi.1227497*	3.14	33.7	2946	67
Mesonia hippocampi DSM 29568^T	jgi.1220074	2.59	34.5	2378	45
Salegentibacter salegens ACAM 48^T	GCF_900142975 (LT670848.1)	4.01	37.2	3415	58
Gramella echinicola DSM 19838^T	GCF_000423065 (AUHG01)	3.51	36.9	3112	50
Zunongwangia profunda SM-A87^T	GCF_000023465 (CP001650.1)	5.13	36.2	4270	60

*These sequence data were produced by the US Department of Energy Joint Genome Institute (http://www.jgi.doe.gov/) in collaboration with the user community and are part of The One Thousand Microbial Genomes Phase 4 Project (KMG-4) by M. Göker.

(DDH) was estimated in silico with the Genome-to-Genome Distance Calculator (GGDC 2.1), using the BLAST method and recommended formula 2 [27]; Average nucleotide identities, according to BLAST (ANIb) were determined in JSpeciesWS [28]. AAI and ANIb values among referenced genomes are presented in Table 2. ANI values confirm, as expected from 16S rRNA gene data, that ISS653^T does not belong to any of the compared species, since the figures obtained are always lower than 95%. *In silico* DDH values for ISS653^T genome against *M. algae*, *M. hippocampi*, *M. maritima*, *M. mobilis* and *M. phycicola* type strain genomes are 18.0–22.4%, confirming that the strain does not represent any of the mentioned species. AAI values to other species of the genus *Mesonia* are mostly over 70%, but they range from 64 to 81%, with 68% to the type species of the genus, *M. algae*. Finally, phylogenomic relationships among ISS653^T, members of the genus *Mesonia* and other representatives of the family *Flavobacteriaceae* were explored with UBCG, based on the analysis of 92 universal bacterial core gene sequences [29]. The resulting trees, based on amino acid and nucleotide sequences are shown in Fig. 2 and Fig. S1, respectively. In both trees, ISS653^T forms a branch deeply embedded within the *Mesonia* clade; this branch shows the highest possible gene support index (GSI) value on the immediate nodes relating ISS653^T to *M. mobilis* and *M. phycicola*.

ECOLOGY

Additionally, we explored the biogeographic distribution of ISS653^T across oceans and depths by comparing the amplicon sequencing of the V4–V5 region of the 16S rRNA gene (16S iTAGs, primers 515F-Y and 926R [30]; sequenced using Illumina MiSeq platform (iTAGs) datasets from *Tara* oceans [31]. We have been able to compare at 100% similarity the 16S rRNA gene sequences of ISS653^T and ISS1889 with zOTUs (zero-radius OTUS, i.e. Operational Taxonomic Units defined at 100% sequence similarity) denoted from high-throughput sequencing of the 16S rRNA of surface and mesopelagic

Table 2. Average amino acid identity (AAI, yellow cells) and average nucleotide identity (ANIb, blue cells) indexes among genomes of type strains of species of the family *Flavobacteriaceae* related to ISS653^T. Values relating Mesonia *oceanica* with all other species are shown in bold type

		1	2	3	4	5	6	7	8	9
1	*Mesonia oceanica* ISS653^T		**78.9**	**76.3**	**74.9**	**70.8**	**72.2**	**70.0**	**68.8**	**72.3**
2	*Mesonia mobilis* DSM 19841^T	**81**		78.4	74.4	70.9	72.4	69.7	68.6	70.7
3	*Mesonia phycicola* DSM 21425^T	**76**	80		73.4	71.5	73.5	69.6	68.9	70.0
4	*Mesonia maritima* DSM 102814^T	**72**	72	70		71.3	72.9	70.4	69.0	70.9
5	*Mesonia hippocampi* DSM 29568^T	**64**	65	65	65		71.0	69.1	68.2	68.9
6	*Mesonia algae* DSM 15361^T	**68**	70	70	70	65		69.5	68.8	69.6
7	*Salegentibacter salegens* ACAM 48^T	**63**	64	63	64	61	63		71.1	71.3
8	*Gramella echinicola* DSM 19838^T	**62**	63	63	63	61	63	70		70.2
9	*Zunongwangia profunda* SM-A87^T	**65**	64	63	64	60	62	68	67	

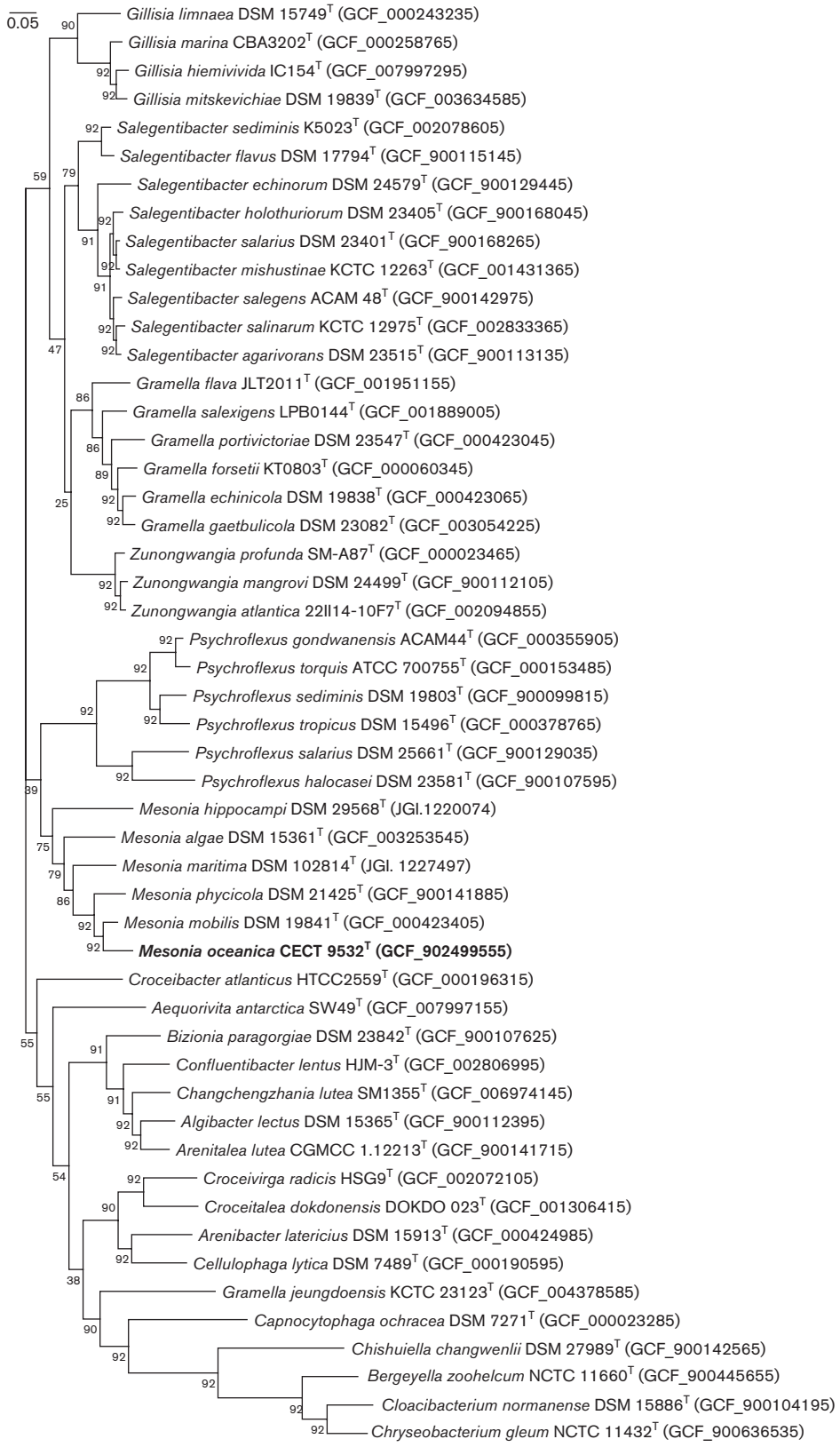

Fig. 2. Phylogenetic tree generated with UBCG [29] by using amino acid sequences. The numbers at the nodes indicate the gene support index (GSI, maximal value is 92). Genome accession numbers are indicated in parentheses. Bar, 0.05 substitutions per position.

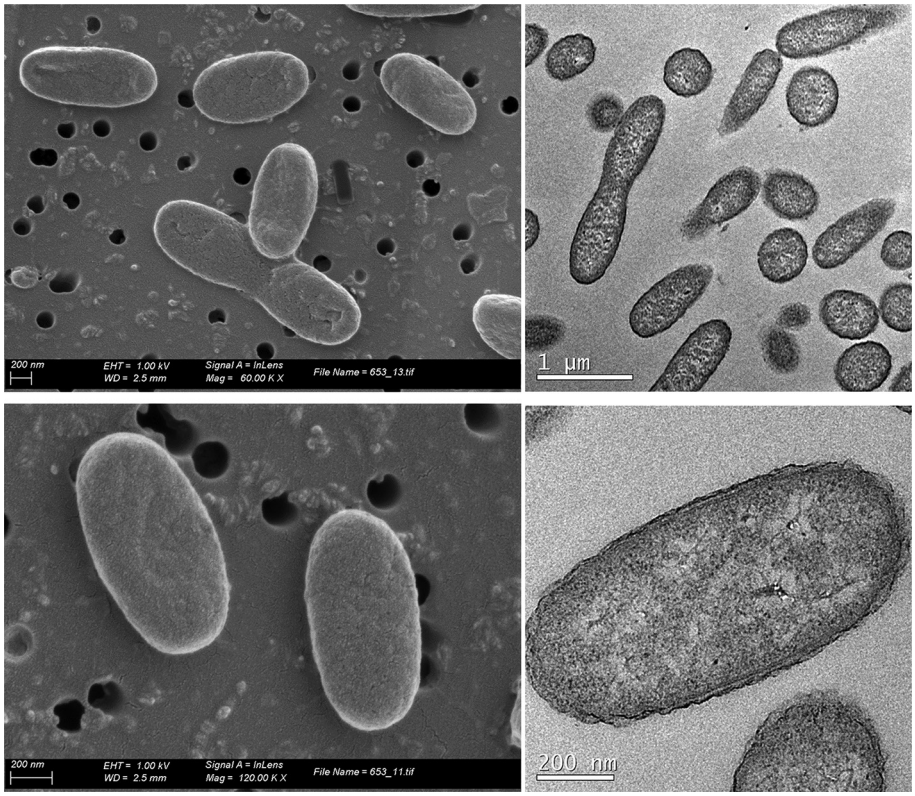

Fig. 3. SEM and TEM images of cells of ISS653^T. Samples were gold sputter coated in order to visualize them with SEM Zeiss MERLIN Fe. Visualizations were done at the Microscopy Service of the Universitat Autònoma de Barcelona (http://sct.uab.cat/microscopia/en/content/inici).

samples (Fig. 3). The two strains were identical to one zOTU that, based on rank abundance analysis (Fig. 4), belongs to the rare biosphere in the surface layer (0.0045% of the total reads) but to the mid-abundant biosphere (0.31%) in the mesopelagic. In addition, if we look the average of reads that were 100% identical to the mentioned strains per oceanographic region, the higher abundances were found in the mesopelagic samples from the South Pacific Ocean (Fig. 4) where ISS1889 was isolated. Interestingly, higher abundances were found in the mesopelagic layer, indicating probably a higher preference of these strains for aphotic layers.

PHYSIOLOGY AND CHEMOTAXONOMY

Phenotypic characterization included morphological, cultural, biochemical, physiological and nutritional screening and was performed by methods described previously [32]. *Mesonia algae* CECT 9441^T, *Salegentibacter salegens* CECT 9443^T, *Gramella echinicola* CECT 9439^T and *Zunongwangia profunda* CECT 9445^T were characterized in parallel for comparative purposes.

Flexirubin type pigmentation was tested according to the methods of Bernardet *et al.* (2002) [33]. In addition, anaylses of cellulose degradation, nitrate reduction acid production from carbohydrates in API 50CH/E, API ZYM and API 20NE profile were performed as described previously [15]. Transmission electron microscopy (TEM) and Scanning Electron Microscopy (SEM) observations of the morphology and ultrastructure also followed previously described procedures [15].

ISS653^T and ISS1889 were Gram-reaction-negative, rod-shaped and non-motile, strictly aerobic and chemoorganoheterotrophic, oxidase- and catalase-positive. They were unable to grow anaerobically, either by carbohydrate fermentation or through nitrate reduction. The strains grew well on Marine Agar and Marine Broth 2216 (BD Diagnostics). Colonies on Marine Agar were yellow, round, with regular borders, with no indication of swarming or gliding. Temperature range for growth was 4–37 °C (30 °C for ISS1889), with optimum at 26–30 °C. No growth was obtained at 40 °C. pH range was 5 to 8, with optimum at pH 6–8, very weak growth at pH 9 and 10 and no growth at pH 4 and below. The strains grew at total salinities of 1–15%, optimally at 2–4%, they did not grow at 0.5% or less nor 18% or higher salinities. The growth was strictly Na^+-dependent. They are, thus, mesophilic, neutrophilic and slightly halophilic. They were able to hydrolyse gelatin and casein but the hydrolyses of Tween 80 (negative for ISS1889) and DNA were weak. Alginate, starch, cellulose and agar were not hydrolysed. Good growth was obtained on Baumann's basal medium with yeast extract (positive

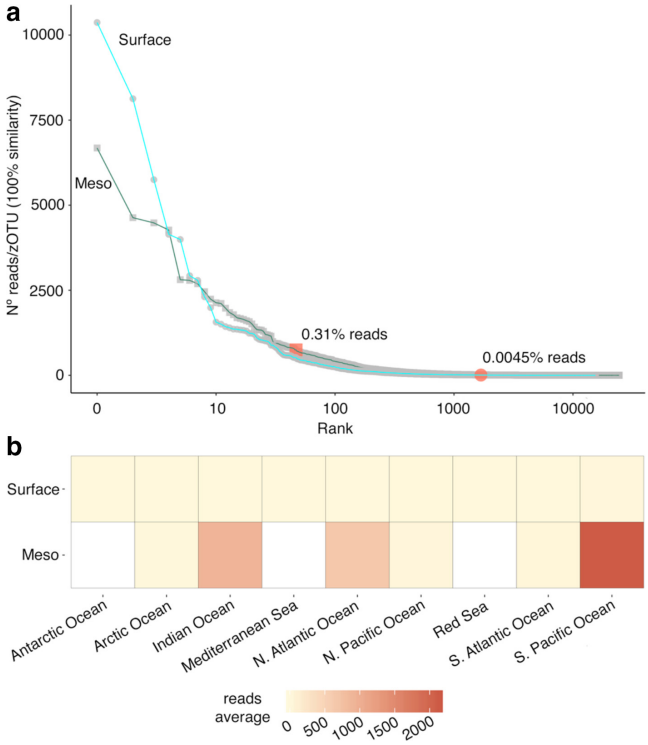

Fig. 4. Distribution of strains ISS653^T and ISS1889. (a) Rank abundance based on the number of reads per zOTU denoted from the 16S rRNA sequences. Color indicates the layer were the zOTUs come from: light-blue, surface; turquoise, mesopelagic. Orange circle and square indicate the position in the rank of the zOTU 100% identical to the two strains. (b) Heatmap indicating the average of reads 100% identical to the isolates per oceanographic region in surface and mesopelagic (Meso) samples. Lighter colors indicate lower numbers of reads, while strong colors indicate higher number of reads.

control for the carbon source screening) but it was negative with all the substrates used (D-ribose, L-arabinose, D-xylose, D-glucose, D-fructose, D-galactose, trehalose, D-mannose, L-rhamnose, maltose, cellobiose, lactose, sucrose, melibiose, amygdalin, salicin, *N*-acetyl-D-glucosamine, D-gluconate, D-glucuronate, D-galacturonate, D-saccharate, D-glycerol, D-mannitol, D-sorbitol, *myo*-inositol, acetate, pyruvate, propionate, butyrate, citrate, *t*-aconitate, 2 ketoglutarate, succinate, fumarate, malate, lactate, 3-hydroxybutyrate, glycine, L-leucine, L-alanine, L-glutamate, L-serine, L-arginine, L-tyrosine, L-treonine, L-aspartate, L-citrulline, L-ornithine, L-histidine, L-lysine, L-sarcosine and putrescine) indicating that ISS653^T may have a growth factor requirement. ISS1889 was not tested for carbon source utilization in this medium. On the assimilation tests of API 20NE, both strains grew well with malate and, less conspicuously, on glucose, mannose, maltose, arabinose, mannitol, *N*-acetyl D-glucosamine, gluconate and adipate, but not on caprate or citrate; growth on phenyacetate was positive only for ISS653^T. Both strains were positive for PNPG test (β-galactosidase activity) and aesculin and gelatin hydrolysis, but negative for indole, arginine dihydrolase and urease. On API ZYM tests both strains were positive for alkaline and acid phosphatases, leucine and valine arylamidases and α- and β-glucosidases and were negative for other tests. Acid production from carbohydrates on API50CH/E strips was very scarce, with positive reaction only for aesculin hydrolysis and a slight acidification in D-glucose, D-mannose, amygdalin, salicin, cellobiose and maltose wells after 48 h incubation. ISS1889 was almost identical except for the acid production from arbutin and 2-ketoglutarate (weak). They also showed slight differences in minor fatty acids (Table S1).

Fig. 3 displays SEM and TEM images of ISS653^T cells: they are regular straight rods with rounded ends, 0.5–0.6×0.9–1.5 µm in size, appearing singly or in pairs and showing the profile typical for a Gram-reaction-negative bacterium in TEM images. No appendages or internal structures were seen. Additional images are included in Fig. S2.

Fatty acid methyl esters were extracted from ISS653^T and ISS1889 biomass grown in Marine Agar at 26 °C after 72 h incubation. Extracts were prepared according to standard protocols as described for the MIDI Microbial Identification System [34] at the CECT. Cellular fatty acid content was analysed by gas chromatography with a 6850 chromatographic unit (Agilent), with the MIDI Microbial Identification System using the TSBA6 method [35] and identified using the Microbial Identification Sherlock software package. Table S1 shows the cellular fatty acids detected in the two strains, that include iso-$C_{15:0}$, summed feature 3 and iso-$C_{17:0}$ 3-OH as major components, followed by iso-$C_{15:1}$G, summed feature 9 ($C_{16:0}$ 10-methyl and/or iso-$C_{17:1}$ ω9*c*) and iso-$C_{15:0}$ 3-OH. An issue has been reported [36] about the peak names for summed feature 3 in flavobacteria that makes us believe it might correspond to iso-$C_{15:0}$ 2-OH, and so it is reported as such in the species description. The fatty acid profile of the strains closely resembles those of other species of the genus *Mesonia* and all the fatty acids present at percentages higher than 5% are found in similar amounts in the six species of the genus reported previously [11].

Analysis of respiratory quinones and major polar lipids of ISS653^T were carried out by the Identification Service and Dr. Brian Tindall, DSMZ, Braunschweig. Germany. Detailed methods for the analyses have been reported previously [15]. MK6 was identified as the major quinone and phosphatidylethanolamine (PE) was the only identified polar lipid, among others detected (three unidentified lipids, two glycolipids and three aminolipids, Fig. S3). Both MK6 and PE are typical chemotaxonomic features of members of the genus *Mesonia*, present in all species so far described.

In summary, phylogenetic, genomic and phenotypic distinctiveness of the strains are indications of them representing, at least, a novel species, a novelty confirmed by 16S rRNA gene sequence similarity (less than 94%) and ANI values (less than 95%) exhibited between ISS653^T and the rest of the species of the genus *Mesonia* and the neighboring taxa. In fact, the strains initially appeared to represent a novel genus in the family due to the low 16S rRNA gene similarity and to the topology of the 16S rRNA gene-based tree (Fig. 1). However,

when considering genomic indexes, such as AAI, and the UBCG phylogenomic analysis, the relationship to their neighbors seems closer than anticipated from the results of the 16S rRNA analysis: instead of representing a marginal and distant branch, ISS653^T merges in the core of the genus *Mesonia*. Its AAI values to other species of the genus *Mesonia* fall in the lower range of AAIs among congeneric species of the family *Flavobacteriaceae,* such as, for example, species of the genus *Psychroflexus* (62–91% intragenus AAIs, data not shown). AAI has been used to define genus boundaries in the family, and particularly with the genus *Chryseobacterium* and related genera, in a recent paper [37]. These authors propose a cutoff value of 76% AAI for assignment of a novel species to an existing genus and consider that all type strain of congeneric species should present at least a 74% AAI to each other. By following this proposal, the novel species characterized in our study would constitute a novel genus, different from *Mesonia*, and this genus would encompass *M. mobilis* and *M. phycicola*. The species *M. hippocampi* and *M. maritima* should, in turn, be classified in two novel, different genera, while *M. algae* would be the only representative of the genus *Mesonia sensu stricto*. However, such major rearrangements should wait until genome sequences for the type strains of all species of the genus *Mesonia* are available. For the time being, we consider the best option is to describe the novel species as a member of the genus *Mesonia*, provided that the genus does not become polyphyletic with this inclusion and that the novel species fits well with the genus description: the presence of iso-$C_{15:0}$ as major fatty acid, a genomic DNA G+C content of 34.9%, PE as the major identified polar lipid, MK-6 as the major respiratory quinone, the pigmentation type, the aerobic chemoorganotrophic metabolism, a positive response for catalase, oxidase and alkaline phosphatase and the strict requirement of sodium for growth are features that qualify the strains as members of the genus *Mesonia*. On the other hand, several traits shown in Table 3 allow them to be differentiated phenotypically from any other species of the genus *Mesonia*.

Table 3. Differential characteristics between ISS653^T and ISS1889 and their closest phylogenomic relatives. Taxa: 1, Mesonia *oceanica* ISS653^T and ISS1889; 2, *M. algae* CECT 9441^T; 3, *M. mobilis* KMM 6059^T [5]; 4, *M. phycicola* MDSW-25^T [6]; 5, *M. ostreae* T-y2^T [7]; 6, *M. aquimarina* IMCC1021^T [8]; 7, *M. hippocampi* 96_Hippo_TS_3/13^T [9]; 8, *M. sediminis* MF326^T [10]; 9, *M. maritima* 15-S14-6^T [11]; 10, *Salegentibacter salegens* CECT 9443^T; 11, *Gramella echinicola* CECT 9439^T; 12, *Zunongwangia profunda* CECT 9445^T. All data are from this study unless indicated. +, Positive; –, negative; w, weakly positive; ND, not determined; Y, yellow; Wh, white; Or, orange; G, gliding; Fl, flagellated. All strains were Gram-reaction-negative, aerobic, chemoorganotrophic bacteria, positive for oxidase, catalase, alkaline and acid phosphatases and leucine arylamidase; and negative for cellulose hydrolysis, α-galactosidase, *β*-glucuronidase, α-mannosidase and α-fucosidase

	1	2	3	4	5	6	7	8	9	10	11	12
Pigment colour	Y	Y	Y	Y	Wh	Y	Or	Y	Y	Y	Y	Y
Flexirubin	–	–	–	–	–	–	+	ND	–	–	–	–
Motility	–	–	+, G	–	–	–	–	–	+, Fl	–	+, G	–
Temperature. range (°C)	4–30	4–34	10–30	10–30	4–31	10–30	4–36	4–42	10–35	4–28	4–37	15–37
NaCl range (% w/v)	1–15	1–15	0.5–15	0.5–12	0–12	0.5–12	1–10	0.5–7	0.5–11	1–12	1–12	0.5–15
Hydrolysis of:												
Aesculin	+	–	+	+	–	–	–	+	–	+	+	+
Casein	+	+	+	–	–	+	ND	ND	+	w	+	–
DNA	w	–	+	+	–	–	ND	ND	+	w	+	+
Tween 80	w	+	+	–	–	+	ND	+	+	–	–	+
Enzymatic activity (API ZYM)												
Valine arylamidase	+	+	+	+	+	+	–	+	+	+*	+†	+‡
Trypsin	–	–	–	–	–	–	+	w	–	–*	w†	+‡
β-Galactosidase	–	–	–	–	–	–	–	–	–	+*	–†	+‡
α-Glucosidase	+	–	+	+	–	–	–	–	–	–*	+†	+‡
β-Glucosidase	+	–	–	–	–	+	–	–	–	ND	w†	+‡
DNA G+C content (mol%)	34.9	33.1	35.2	31.4	42.1§	41.4§	34.5	40.7§	33.7	37.2	36.9	36.2

*, Data from [38]
†, Data from [39]
‡, Data from [40]
§, Determined by HPLC (all other values, from WGS).

In consequence, we propose a novel species of the genus *Mesonia*, with the name *Mesonia oceanica* and strain ISS653^T = CECT 9532^T=LMG 31236^T as the type strain.

DESCRIPTION OF *MESONIA OCEANICA* SP. NOV.

Mesonia oceanica (o.ce.a'ni.ca, N.L. fem. adj. *oceanica*, of or pertaining to the ocean).

Cells are Gram-reaction-negative, rod-shaped, 0.5–0.6 µm×0.9–1.5 µm and non-motile. Strictly aerobic and chemoorganotrophic; positive for catalase and oxidase. Colonies in Marine Agar medium are regular and yellow pigmented. Flexirubin-type pigments are not produced. Mesophilic, neutrophilic and slightly halophilic, with optima at: 26 °C (range: 4–30 °C; 40 °C negative), pH6–8 (range: 5–8; pH 4 negative; pH 9 and 10, weak) and 2–4% total salinity (range: 1–15%; 0.5 and 18% negative). Requires sodium ions for growth. Nitrate is not reduced to nitrite or N_2. Hydrolyses aesculin, casein, gelatin, Tween 80 (weakly) and DNA (weakly), but not alginate, cellulose (as filter paper) or agar. Indole production from tryptophan, arginine dihydrolase and urease are negative. PNPG test (β-galactosidase) is positive in API 20NE. Assimilates malate, as well as glucose, mannose, maltose, arabinose, mannitol, *N*-acetyl D-glucosamine, gluconate and adipate, but not caprate or citrate, on API 20NE. The type strain was unable to grow in minimal medium (Basal Medium) with any of 52 sole carbon and energy sources but grew with yeast extract. The following carbohydrates are metabolised with weak acid production in aerobic API 50CH/E tubes: D-glucose, D-mannose, amygdalin, salicin, cellobiose and maltose. Enzymatic activities displayed on API ZYM are alkaline and acid phosphatases, leucine and valine arylamidases and α- and β-glucosidases. Naphthol-AS-BI-phosphohydrolase, estearase and estearase lipase are weakly positive; lipase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase are negative. Major polar lipids are phosphatidylethanolamine (PE), two unidentified glycolipids, three unidentified aminolipids and three unidentified lipids. Major respiratory quinone is MK6. Major cellular fatty acids include iso-$C_{15:0}$, iso-$C_{15:0}$ 2-OH [although reported as summed feature 3 ($C_{16:1}$ ω7*c*/ω6*c*)] and iso-$C_{17:0}$ 3-OH.

The type strain is ISS653^T (=CECT 9532^T=LMG 31236^T), which was isolated from surface seawater of the Atlantic Ocean during the *Tara* oceans expedition. Strain ISS1889 (=CECT 30008) is an additional strain of the species. The DNA G+C content of the type strain is 34.9 mol% and its genome size is 4.28 Mbp. The GenBank/EMBL/DDBJ accession numbers for the whole genome sequence and 16S rRNA gene sequence of strain ISS653^T are CABVMM01 and MH732189, respectively.

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Author contributions
Conceptualization: I.S.S., S.G.A. and M.J.P. Data curation, formal analysis and investigation: T.L., I.S.S., S.G.A., O.S. and M.J.P. Funding acquisition: S.G.A. and C.P.A. Project administration and resources: D.R.A., S.G.A., R.A. 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.

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Supplementary Material

Mesonia oceanica sp. nov., isolated from oceans during *Tara* Oceans Expedition, with a preference for mesopelagic waters

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Supplementary Table S1. Cellular fatty acid composition of *Mesonia oceanica* strains ISS653^T and ISS1889. tr : less than 1%; -: not detected. Fatty acids amounting to < 1% of the total fatty acids in both strains are not shown. Boldface, percentages above 5%.

	ISS653^T	**ISS1889**
Hydroxylated		
iso-$C_{15:0}$ 3-OH	3.9	**5.1**
$C_{15:0}$ 2-OH	1.1	1.2
iso-$C_{16:0}$ 3-OH	1.9	1.9
Iso-$C_{17:0}$ 3-OH	**9.2**	**14.6**
$C_{17:0}$ 2-OH	1.7	1.8
Saturated		
$C_{12:0}$	1.0	-
iso-$C_{13:0}$	1.0	-
iso-$C_{15:0}$	**24.0**	**26.0**
anteiso-$C_{15:0}$	2.2	4.4
iso-$C_{16:0}$	1.0	tr
$C_{16:0}$	4.5	1.9
$C_{18:0}$	1.9	-
Unsaturated		
iso-$C_{15:1}$ G	**7.9**	**9.0**
$C_{15:1}$ *ω8c*	1.6	-
$C_{15:1}$ *ω6c*	1.8	1.1
$C_{15:1}$ *ω5c*	3.6	-
iso-$C_{16:1}$ H	1.3	1.0
anteiso-$C_{17:1}$ *ω9c*	-	1.1
$C_{17:1}$ *ω6c*	1.7	1.3
$C_{18:1}$ *ω9c*	3.9	tr
Summed features		
SF 3 ($C_{16:1}$ *ω7c*/$C_{16:1}$ *ω6c*)*	**11.2**	**14.5**
SF 4 (iso-$C_{17:1}$ I/anteiso-$C_{17:1}$ B)	1.3	1.3
SF 5 (anteiso-$C_{18:0}$/$C_{18:2}$ *ω6,9c*)	1.0	-
SF 9 ($C_{16:0}$ 10-methyl/iso-$C_{17:1}$ *ω9c*)	**5.2**	**10.5**

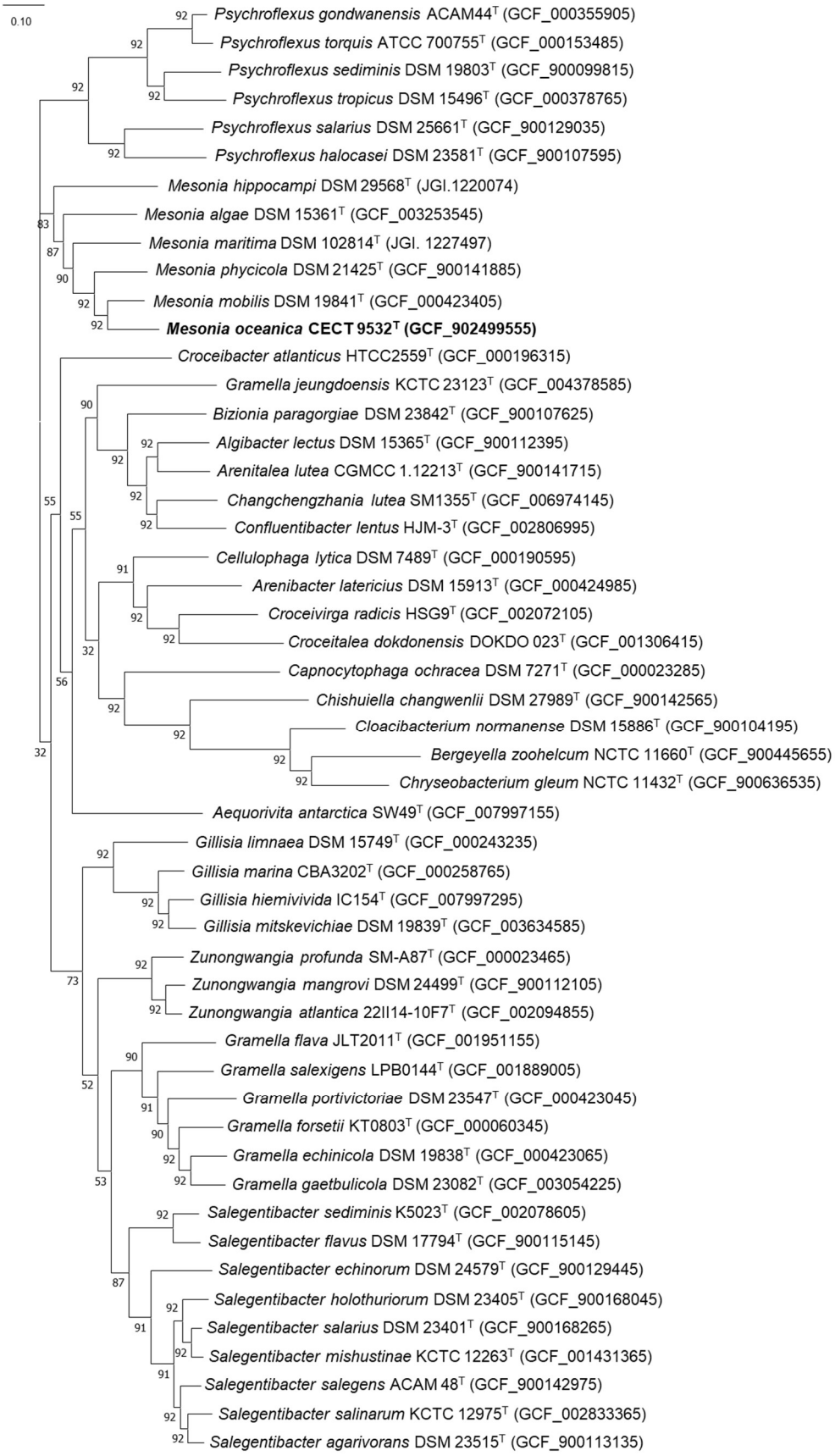
*According to Montero-Calasanz et al. (2014) it corresponds indeed to iso-$C_{15:0}$ 2-OH.

Montero-Calasanz MC, Göker M, Rohde M, Spröer C, Schumann P *et al*. *Chryseobacterium oleae* sp. nov., an efficient plant growth promoting bacterium in the rooting induction of olive tree (*Olea europaea* L.) cuttings and emended descriptions of the genus *Chryseobacterium*, *C. daecheongense*, *C. gambrini*, *C. gleum*, *C. joostei*, *C. jejuense*, *C. luteum*, *C. shigense*, *C. taiwanense*, *C. ureilyticum* and *C. vrystaatense*. *Syst Appl Microbiol* 2014;37:342–350. doi: 10.1016/j.syapm.2014.04.004

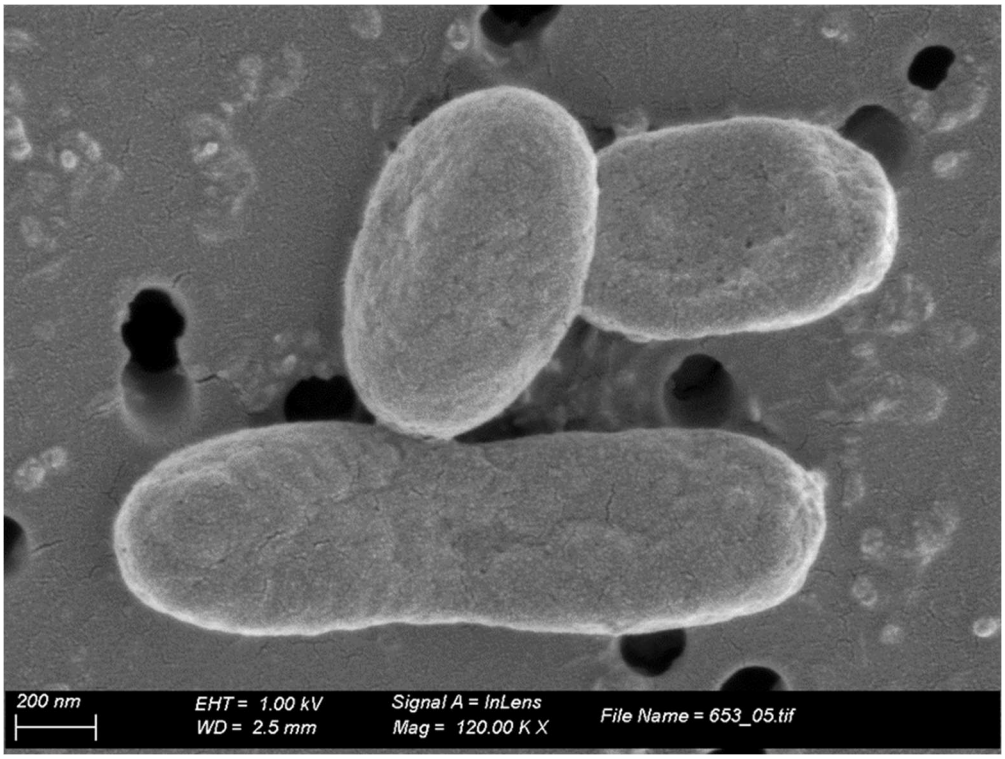
Supplementary Table S2. Some CAZyme activities of ISS653^T predicted from RAST genome annotation

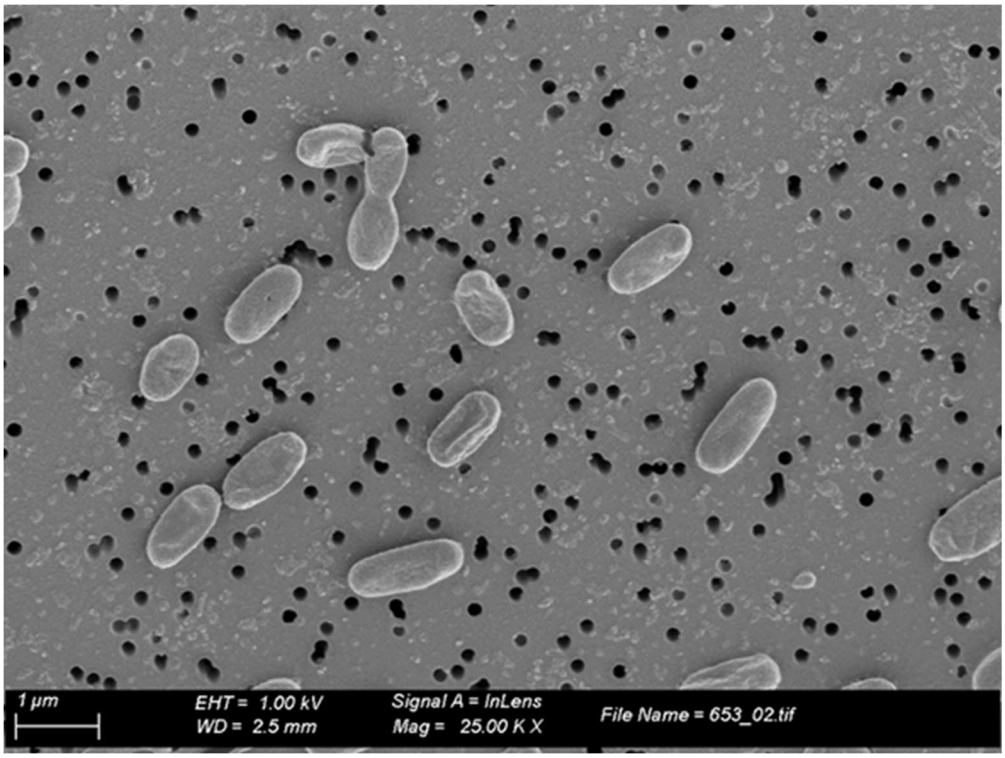
	Position	Node
Alpha-1,2-mannosidase	121	10
Pectate lyase (EC 4.2.2.2)	147	10
Polygalacturonase (EC 3.2.1.15)	148	10
Xylan 1,4-beta-xylosidase (EC 3.2.1.37)	153	10
Pectinesterase (EC 3.1.1.11)	154	10
rhamnogalacturonan acetylesterase	155, 157	10
Pectin degradation protein KdgF	158	10
Endoglucanase	398	12
beta-glucosidase (EC 3.2.1.21)	449, 451, 1490, 2812, 4159	12, 3, 1, 8
Polysaccharide biosynthesis protein	687	13
Glucan 1,4-alpha-glucosidase (EC 3.2.1.3)	1486	1
Beta-glucanase precursor (EC 3.2.1.73)	1987	25
Endo-1,4-beta-xylanase (EC 3.2.1.8)	2151, 4163	2, 8
beta-galactosidase (EC 3.2.1.23)	2155, 3547	2, 6
beta-N-acetylglucosaminidase (EC 3.2.1.52)	2194	2
Polysaccharide deacetylase	2338, 3804	2, 7
Alpha-glucosidase, family 31 of glycosyl hydrolases, COG1501	2602	3
1,4-alpha-glucan (glycogen) branching enzyme, GH-13-type (EC 2.4.1.18)	2603	3
laminarinase	2813	3
Arabinogalactan endo-1,4-beta-galactosidase (EC 3.2.1.89)	2932	4
Glucoamylase (EC 3.2.1.3)	3244	5
Hyaluronan synthase (EC 2.4.1.212)	3262	5
Predicted alpha-L-rhamnosidase	3508	6
Oligogalacturonate lyase (EC 4.2.2.6)	3509	6
Alpha-glucosidase (EC 3.2.1.20)	3552	6
Pectate lyase (EC 4.2.2.2)	3553	6
Cytoplasmic alpha-amylase (EC 3.2.1.1)	4132	8
Glucan 1,4-alpha-glucosidase (EC 3.2.1.3)	4141	8
Neopullulanase (EC 3.2.1.135)	4142	8
Endo-1,4-beta-mannosidase	4160	8
beta-1,4-glucanase (cellulase) (EC 3.2.1.4)	4167	8

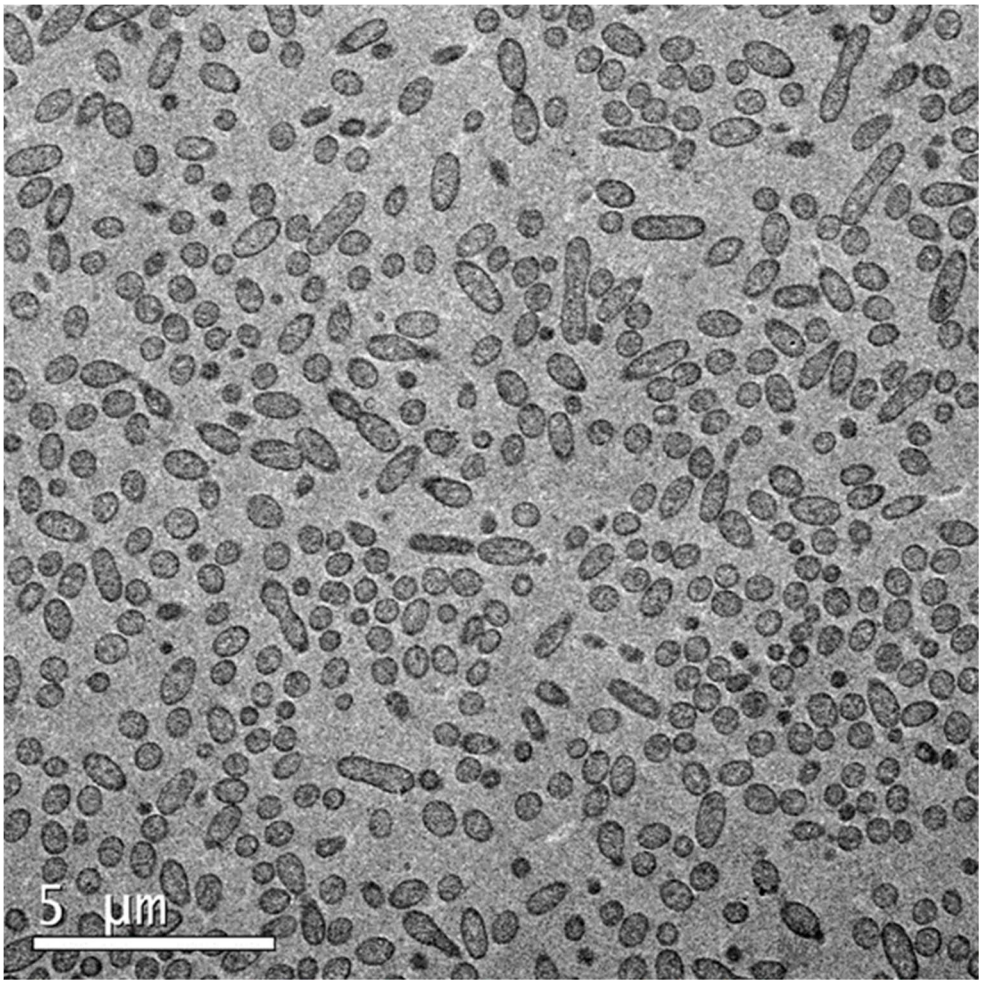
Supplementary Fig. S1. Phylogenetic tree generated with UBCG by using nucleotide sequences. Numbers at the nodes indicate the gene support index (GSI, maximum: 92). Genome accession numbers are indicated in parentheses. Bar, 0.05 substitutions per position.



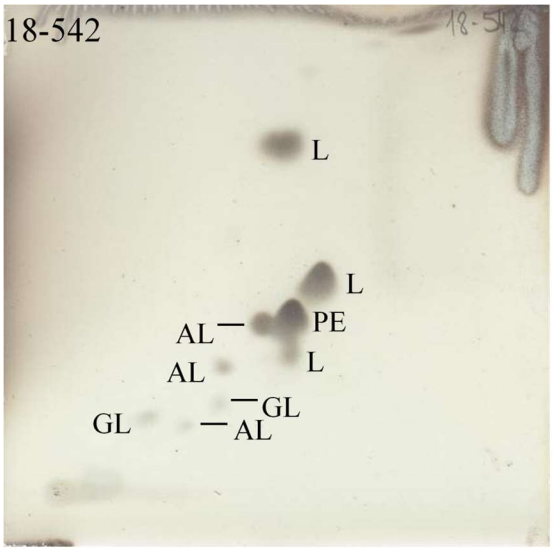
Supplementary Figure S2. SEM and TEM images of strain ISS653^{T} cells.






5 µm

Supplementary Fig. S3. Polar lipid profiles of strain ISS653^{T} separated by two dimensional silica gel thin layer chromatography.



L = Lipid

GL = Glycolipid

AL = Aminolipid

PE = Phosphatidylethanolamine