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Description of Palleronia rufa sp. nov., a biofilm-forming and AHL-producing Rhodobacteraceae, reclassification of Hwanghaeicola aestuarii as Palleronia aestuarii comb. nov., Maribius pontilimi as Palleronia pontilimi comb. nov., Maribius salinus as Palleronia salina comb. nov., Maribius pelagius as Palleronia pelagia comb. nov. and emended description of the genus Palleronia

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Description of *Palleronia rufa* sp. nov., a biofilm-forming and AHL-producing *Rhodobacteraceae*, reclassification of *Hwanghaeicola aestuarii* as *Palleronia aestuarii* comb. nov., *Maribius pontilimi* as *Palleronia pontilimi* comb. nov., *Maribius salinus* as *Palleronia salina* comb. nov., *Maribius pelagius* as *Palleronia pelagia* comb. nov. and emended description of the genus *Palleronia*.

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Abstract

Strain MOLA 401^T was isolated from marine waters in the southwest lagoon of New Caledonia and was shown previously to produce an unusual diversity of quorum sensing signaling molecules. This strain was Gram-negative, formed non-motile cocci and colonies were caramel. Optimum growth conditions were 30°C, pH 8 and 3 % NaCl (w/v). Based on 16S rRNA gene sequence analysis, this strain was found to be closely related to *Pseudomaribius aestuariivivens* NBRC 113039^T (96.9 % of similarity), *Maribius pontilimi* DSM 104950^T (96.4 % of similarity) and *Palleronia marisminoris* LMG 22959^T (96.3 % of similarity), belonging to the *Roseobacter* group within the family *Rhodobacteraceae*. As its closest relatives, strain MOLA 401^T is able to form a biofilm on polystyrene, supporting the view of *Roseobacter* group strains as prolific surface colonizers. An in-depth genomic study allowed us to affiliate strain MOLA 401^T as a new species of genus *Palleronia* and to reaffiliate some of its closest relatives in this genus. Consequently, we describe strain MOLA 401^T (DSM 106827 = CIP 111607^T = BBCC 401^T) for which we propose the name *Palleronia rufa* sp. nov. We also propose to emend the description of the genus *Palleronia* and to reclassify *Maribius* and *Hwanghaeicola species* as *Palleronia* species.

Keywords: *Rhodobacteraceae*, *Roseobacter* group, *Palleronia*, *Maribius*, quorum sensing, biofilm

45 **Introduction**

46 Most of the *Rhodobacteraceae* are of marine origin and require sodium ion or combined salt for
47 growth [44,52]. The family *Rhodobacteraceae* includes at least 100 genera and among them, the
48 *Roseobacter* group encompasses more than 70 genera. This family as been proposed by Garrity *et al.*
49 [21]. It was registered in the validation list no. 107 [32], but was declared illegitimate because it
50 contained the genus *Hyphomonas*, which was the type of the family *Hyphomonadaceae* Lee *et al.* [30].
51 However, even if Lee *et al.* modified the classification by placing some species in separate families
52 and the *Rhodobacteraceae* in the order *Caulobacterales*, they did not change the family name [30].
53 Strains belonging to this group share more than 89 % identity of 16S rRNA gene sequence [9,44]. The
54 use of culture-independent approaches provided a basis for recognizing the *Roseobacter* group as one
55 of the most abundant in marine environments, that represents up to 20 % of coastal and 15 % of
56 mixed-layer ocean bacterioplankton communities [9,10]. Wagner-Döbler and Biebl [57] highlighted
57 the importance of this group in cycles of carbon, sulphur and nitrogen in the ocean. The *Roseobacter*
58 lineage is physiologically heterogeneous. Members of this group exhibit: aerobic anoxygenic
59 photosynthesis, degradation of algal sulphur compounds, carbon monoxide oxidation, degradation of
60 aromatic compounds (e.g. vanillate, benzoate, coumarate), reduction of trace metals, symbiotic and
61 pathogenic relationships, production of bioactive secondary metabolites and quorum sensing (QS), and
62 association with biotic or abiotic surfaces [8,9,16,20,24,50,52]. However, few of these traits are
63 representative of the entire group. The genome plasticity of *Roseobacter* could explain their wide
64 range of physiological features as well as their wide variety of habitats [9,57].
65 QS is used by bacteria to act as coordinated communities. It has been observed to be linked to the
66 production of virulence factors and secondary metabolites, motility, colonization of particulate matter
67 and biofilm formation [5,6,15,22,37,51]. QS appears a common trait in the *Roseobacter* lineage.
68 Homologs of the N-acyl-homoserine lactone (AHL) synthase gene (*luxI)* were present in more than
69 80 % of roseobacterial genomes available at the time of the study [14,60]. As AHLs are small secreted
70 diffusing signal molecules implicated in QS in Gram-negative bacteria, this study suggests that a
71 majority of *Roseobacter* strains possess AHL based QS systems. However, only few characterized
72 *Roseobacter* isolates have been shown to produce AHL. *Roseobacter* are often reported as prolific

colonizers of inert and living surface in marine environment, and the contribution of QS in these processes is of growing interest.

The present study was undertaken to describe strain MOLA 401^T that was shown to produce a remarkable range of AHLs [18]. The first phylogenetic analyses of strain MOLA 401^T, based on 16S rRNA gene sequence, resulted in its affiliation to the genus *Maribius,* which belongs to the *Roseobacter* group in the family *Rhodobacteraceae* [18]. Three *Maribius* species have already been described: *Maribius salinus*, *Maribius pelagius* and *Maribius pontilimi* [12,31]. However, 16S rRNA gene sequences lack the resolution for a proper phylogenetic reconstruction inside the *Roseobacter* group [9,33,52,59]. Moreover, several strains were described simultaneously, representing a taxonomic challenge to organize this group without redundancy [44]. This is not without consequences, because incorrect species name assignments may cause confusion and mistakes when suggesting evolutionary inference, or in interpreting ecological data concerning this group [33]. We combined classical and genomic markers to define the taxonomic position of strain MOLA 401^T and to clarify that of its closest relatives. In addition, the ability of these bacteria to form biofilms and to produce AHLs, two relevant traits of the *Roseobacter* group, was demonstrated.

Materials and methods

Strains. Strain MOLA 401^T was obtained from MOLA culture collection (WDCM911, Sorbonne Univeristé, France). This strain was isolated on marine agar medium (MA) from marine waters in the southwest lagoon of New Caledonia [13,18]. The type strains used for comparative purposes were obtained from international collections: *Palleronia abyssalis* LMG 27977^T and *Palleronia marisminoris* LMG 22959^T are from Belgian co-ordinated collections of micro-organisms (BCCM), *Hwanghaeicola aestuarii* DSM 22009^T, *Maribius salinus* DSM 26892^T and *Maribius pelagius* DSM 26893^T are from Deutsche Sammlung von Mikroorganismen und Zelkulturen (DSMZ), and *Palleronia soli* NBRC 110740^T is from NITE Biological Resource Center (NBRC).

Physiological and biochemical tests. Cell morphology, motility and size were determined by light microscopy and transmission electron microscopy (TEM). For TEM observation, cells were mounted on Formvar-coated copper grids and negatively stained with 2 % (w/v) uranyl acetate.

101 The temperature range of strain MOLA 401^T was examined at 4, 10, 15, 20, 25, 30, 37, 45 and 50°C in
102 Marine Broth 2216 Difco (MB). The pH range was examined at 25°C in MB by adding MES
103 (4.90 g.L^{-1}), PIPES (7.5 g.L^{-1}), MOPS (5.23 g.L^{-1}), HEPES (5.96 g.L^{-1}), tricine (4.48 g.L^{-1}), bicine
104 (4.08 g.L^{-1}) over a pH range from 5.5 to 9, with 0.5 increments. The NaCl tolerance for growth was
105 determined at 25°C from 0 to 100 g.L^{-1} NaCl, with 10 g.L^{-1} increments, in reconstituted MB
106 (according to the composition indicated by Difco, except for NaCl). Growth was estimated by
107 measuring the turbidity at 600 nm. Growth ranges and optima data missing for the other strains were
108 also determined under the same conditions.
109 Cellobiose, D-arabinose, D-fructose, D-glucose, D-mannose, D-raffinose, D-sorbitol, D-sucrose, D-
110 trehalose, L-rhamnose, L-xylose, melezitose, myo-inositol, xylitol, glycogen (5 g.L^{-1}), starch (1 g.L^{-1}),
111 acetate, gluconate, malate, pyruvate, succinate, ethanol, glycerol, L-proline, peptone, tryptone, ethyl
112 palmitate, lecithin, tricaprylin, cholesterol, triolein, octanol, 1-phenyldecane, pristane,
113 heptamethylnonane, methyl laurate, hexadecane, octane and decane were tested as sole carbon sources
114 in minimal MB (MBmin) with a concentration of 20 mM (unless otherwise specified). The MBmin
115 contained (L^{-1} distilled water): 0.05 g yeast extract, 19.45 g NaCl, 12.6 g $MgCl_2.6H_2O$, 6.65 g
116 $MgSO_4.7H_2O$, 2.35 g $CaCl_2.2H_2O$, 0.55 g KCl, 0.16 g $NaHCO_3$, 0.08 g KBr, 0.053 g $SrCl_2.6H_2O$,
117 0.022 g H_3BO_3, 0.0094 g $Na_2SiO_3.9H_2O$, 0.0024 g NaF, 0.015 g $Na_2HPO_4.7H_2O$, 0.5 g NH_4NO_3, 6 g
118 Trizma base, 1 mL trace element solution. The trace element solution contained (L^{-1} distilled water):
119 15 mg $CuCl_2.2H_2O$, 46 mg $NiCl_2.6H_2O$, 25 mg $Na_2MoO_4.2H_2O$, 70 mg $ZnCl_2$, 100 mg $MnCl_2.4H_2O$,
120 120 mg $CoCl_2.6H_2O$, 4 g $FeCl_3$, 2 g EDTA, 6.5 mL HCl 25 %. The medium was adjusted to pH 8.
121 (bioMérieux). Catalase activity was detected by formation of bubbles by resuspending cells in H_2O_2
122 (30 %). Oxidase activity was tested on a colony with Chemicals Oxidase Reagent kit (VWR
123 chemicals, Belgium). Gelatinase activity was examined in MBmin supplemented with 20 g.L^{-1} gelatin.
124 Strains were cultivated at 30°C. Tubes were then cooled at 4°C during 4 h. Gelatinase activity was
125 considered positive when gelatin liquefaction was observed. Caseinase activity was tested in MBmin
126 supplemented with 10 g.L^{-1} casein. The test is positive if a halo is observed around colonies.
127 Supplementary metabolic and enzymatic activities were assessed using API ZYM and API 20NE
128 systems.

129 Strain MOLA 401^T and its closest relatives genomes were examined to detect genes linked to quorum
130 sensing (*luxI, luxR*), photosynthetic capacity (*pufM, bchG, bchP*), carbon monoxide oxidation (*coxL*),
131 sulfur oxidation (*soxB, soxY*) and nitrogen metabolism (*napA, nasA, narB, narG, norB, nirS*). This
132 detection was conducted by sequence homology searches using the BLAST algorithm. The targeted
133 gene sequences were selected in *Rhodobacteraceae* genomes in which these genes were correctly
134 annotated, based on an examination of both INTERPRO and IMG databases [24, 36].
135 For fatty acids, polar lipids and quinones determination, all strains were cultured in MB. They were
136 harvested during their exponential phase and lyophilized. Analyses were performed by the
137 Identification Service of the DSMZ. FAMEs were prepared from 40 mg cells, as described by Miller
138 [38] and Kuykendall *et al.* [28], and identified using the Sherlock Microbial Identification System
139 (MIDI, Microbial ID, Newark, DE 19 711 USA). Polar lipids were extracted using the method of
140 Bligh and Dyer [4], separated by two dimensional silica gel TLC, and analyzed according to the
141 procedures described by Tindall *et al.* [55,56]. Respiratory lipoquinones (RLQ) were extracted using
142 the method of Tindall *et al.* [55,56], and followed by phase separation into hexane. RLQ were
143 separated by TLC on silica gel, UV absorbing bands corresponding to the different quinone classes
144 were removed from the plate and further analysed by HPLC (detection at 269 nm).
145 **Public genome dataset.** Representative genomes of *Rhodobacteraceae* were downloaded from
146 GenBank and Roseobase (http://www.roseobase.org/), as available in June 2018. In order to root
147 phylogenetic trees, we also downloaded two outgroups from GenBank (*Thiothrix nivea* DSM 5205^T
148 and *Methylococcus capsulatus* ATCC 19069^T). Genomes that seemed incomplete (≤ 3 Mb and L90
149 value above 115 (defined as the smallest number of contigs whose length sum accounts for 90 % of
150 the genome)) were removed. The accession numbers of the 66 genomes are given in Table S1. The
151 main features of MOLA 401^T genome was described by Doberva *et al*. [17]
152 **Orthologous genes.** The 16S rRNA sequences were obtained using the original annotation of
153 genomes. Genomic annotation of strain MOLA 401^T was used to identify sequences of housekeeping
154 genes (*gyrB*, *recA*, *rpoC* and *rpoD*). Annotation of these genes was checked using BLASTP [2] and
155 the non-redundant protein database of NCBI. The proteome of strain MOLA 401^T was then used as a
156 pivot to identify the whole orthologous genes with the 65 genomes (bi-directional best hits, BLASTP

157 (identity ≥ 40 %, coverage ≥ 50 %, E-value ≤ 10^{-5})). The core genome was defined as the intersection
158 of the lists of orthologs between the pivot and each genome.
159 **Measures of similarity between genomes.** For each pair of genomes, we computed three measures of
160 similarity, one based on gene repertoires (POCP) and two others based on the sequence similarity
161 between two genomes (AAI and ANI). The percentage of conserved proteins (POCP) between two
162 genomes was calculated as previously described [45]: [(C1+C2)/(T1+T2)] x 100 %, where C1/C2 and
163 T1/T2 represent the number of orthologous proteins and the total number of proteins in the two
164 genomes being compared, respectively. In particular, the orthologous proteins between a pair of
165 genomes were determined as described above using BLASTP and bi-directional best hits. Pairwise
166 average amino acid identities (AAI) were assessed using BLASTP identity values obtained to estimate
167 POCP. Pairwise average nucleotide identities (ANI) were calculated using the pyani Python3 module
168 (https://github.com/widdowquinn/pyani), with default parameters for ANIb [47].
169 **Alignment and phylogenetic analyses.** Multiple alignments of orthologous genes were performed
170 using Muscle v3.8.31 [19]. Poorly aligned and highly variable regions of alignments were
171 automatically removed using GBlocks 0.91b [11]. In order to compute a reliable phylogenomic tree,
172 kdetrees [58] was used to remove genes that had different evolutionary histories from the majority. In
173 particular, outliers were identified after a phylogenetic reconstruction of each core gene using IQ-
174 TREE v1.3.8 [41] and the LG+I+G4+F model. Then we analyzed how the concatenated alignment of
175 core genes and alignments of housekeeping genes (16S, *gyrB*, *recA*, *rpoC* and *rpoD*) fitted different
176 models of protein or DNA evolution using IQ-TREE v1.3.8. The best parameters were identified
177 based on the Bayesian information criterion (BIC). Lastly, maximum likelihood trees were computed
178 with IQ-TREE v1.3.8 using the appropriate model, and validated via an ultrafast bootstrap procedure
179 of 1000 replicates [39].
180 **Statistical analyses.** Statistical analyses were done using R version 3.3.1 [46]. The correlation
181 between measures of similarity between genomes and phylogenetic trees was assessed using patristic
182 distances (R package {stats}, cophenetic) and Mantel test (R package {vegan}, mantel) [34].
183 **Biofilm growth and quantification.** Biofilm formation was performed on 24 well polystyrene plates
184 (Evergreen Scientific). Biofilm cultures were initiated with 1 mL per well of cells in exponential phase

185 suspended in MBmin at an OD_{600} of 0.1. Plates were incubated for 8 days at 25°C at 90 rpm. Then the
186 culture medium containing planktonic cells was gently removed, and wells were washed with 1 ml
187 desionized water. The biomass bound to the well bottom was quantified by crystal violet staining [40].
188 **Detection of AHL with biosensors.** AHLs production was detected with *Escherichia. coli* MT102
189 (pJBA132) and *Pseudomonas putida* F117 (pRK-C12; Kmr; *ppuI*::*npt*) biosensors [3,48]. Strains were
190 grown in 200 mL MB at 25°C and 200 rpm. Every 2 hours until the stationary phase, $OD_{600\ nm}$ was
191 measured, 1 mL was collected, centrifuged and the supernatant was stored at -20°C. Biosensor tests
192 were performed as described by Doberva *et al.* [15]. After 5 and 24 h, fluorescence was determined
193 with a Synergy HTX multi-mode reader (Bio Tek) at an excitation wavelength of 485 nm and a
194 detection wavelength of 535 nm. Commercial AHLs, C6-AHL and oxo-C10-AHL were used as a
195 positive control for short and long acyl chain AHL respectively. Negative controls were performed
196 with sterile MB. Fluorescence induction was calculated by subtracting the specific fluorescence of the
197 tested sample (gfp535 nm/$OD_{600\ nm}$) by the specific fluorescence of the negative control. Then, the
198 results were presented as a percentage of the positive control, that means: (fluorescence induction x
199 100 / specific fluorescence of the positive control).
200
201 **Results**
202 Strain MOLA 401^T was isolated on MA from a seawater sample collected in the southwest lagoon of
203 New Caledonia (France) (22°21.23′ S, 166°23.43′ E), at 4 m depth, between oligotrophic waters near
204 the coral barrier and mesotrophic waters subjected to terrestrial inputs and effluents from the city of
205 Nouméa [13].
206 BLAST analysis of the 16S rRNA gene sequence revealed that strain MOLA 401^T belonged to
207 the *Rhodobacteraceae* and was closely related to *Pseudomaribius aestuariivivens* NBRC 113039^T
208 (96.9 % of similarity), *Maribius pontilimi* DSM 104950^T (96.4 % of similarity) and *Palleronia*
209 *marisminoris* LMG 22959^T (96.3 % of similarity) (Table S2, Fig. 1).
210 After one week at 30°C on MA, colonies of strain MOLA 401^T were slightly convex, smooth, circular
211 (1-2 mm in diameter), non-swarming and caramel colored. In light microscopy, cells appeared coccoid
212 and non-motile. Electron microscopy of negatively stained cells confirmed this morphology (Fig. S1).

213 Cells were 0.77 +/- 0.07 µm in diameter. The main characteristics are presented Table 1. Strain
214 MOLA 401^T was able to grow between 4 and 37 °C with an optimum at 30 °C. Growth was observed
215 in a pH range between 6.5 and 8.5, with an optimum at 8.0. Strain MOLA 401^T could grow without
216 NaCl in the medium, but growth was observed until 90 g.L^{-1} NaCl with an optimum at 30 g.L^{-1}. No
217 growth was observed on MBmin with yeast extract at 0.05 g.L^{-1}, confirming that yeast extract at this
218 concentration could not sustain growth. Strain MOLA 401^T could develop with D-glucose, D-
219 mannose, D-raffinose, D-sucrose, L-rhamnose, acetate, gluconate, pyruvate, D-sorbitol, glycerol, myo-
220 inositol and succinate as carbon sources. A weak growth was observed with melezitose, malate and
221 ethanol. Cellobiose, D-arabinose, D-fructose, D-trehalose, D-fructose, L-xylose, xylitol, glycogen,
222 starch, peptone and tryptone did not support growth. A comparison of substrate profiles between strain
223 MOLA 401^T and its closest relatives is shown in Table 2. Strain MOLA 401^T was positive for alkaline
224 phosphatase, esterase (C4), esterase lipase (C8), valine arylamidase, cystine arylamidase, leucine
225 arylamidase, acid phosphatase and naphtol-AS-BI-phosphohydrolase. All the other tests were
226 negative, including nitrate reduction and urease activity. The comparison of enzymatic profile of strain
227 MOLA 401^T with those of its closest relatives is shown in Table 2. Tests for catalase and oxidase
228 activities were negative.
229 The presence of genes involved in particular pathways in strain MOLA 401^T and its closest relatives
230 was investigated (Table 3). Carbon monoxide oxidation gene *coxL* as well as genes involved in
231 sulphur and nitrogen metabolism (i.e. *soxB*, *soxY*, *narB*, *nirS*) were detected in strain MOLA 401^T,
232 *Palleronia* and *Hwanghaeicola* species genomes. Autoinducers synthetase and receptor (*luxI* and
233 *luxR*) were also present in all strains. Strain MOLA 401^T owns the photosynthetic reaction center M
234 subunit gene (*pufM*), as well as *Maribius salinus* DSM 26892^T, *Maribius pelagius* DSM 26893^T and
235 *Hwanghaeicola aestuarii* DSM 22009^T.
236 The main cellular fatty acids of strain MOLA 401^T were $C_{18:1}$ω7c (63.14 %), $C_{18:0}$ (12.49 %), $C_{19:0}$
237 cyclo ω8c (8.97 %) (Table 4). Polar lipid profile of strain MOLA 401^T showed five classes of lipids:
238 aminolipid, glycolipid, phosphatidylglycerol, diphosphatidylglycerol and phosphatidylcholine. The
239 only respiratory quinone detected was ubiquinone-10.

240 The genome sequence of strain MOLA 401^T has been compared to 65 genome sequences of
241 *Rhodobacteraceae* strains (Table S1). The highest ANI percentages were obtained for *Palleronia*
242 *marisminoris* LMG 22959^T (76.98 %), *Maribius salinus* DSM 26892^T (76.37 %), *Maribius pelagius*
243 DSM 26893^T (76.08 %) and *Hwanghaeicola aestuarii* DSM 22009^T (75.77 %). *Ponticoccus*
244 *marisrubri* JCM 19520^T (74.88 %) was the next one. AAI analysis led to quite similar results (Table
245 S3): *Palleronia marisminoris* LMG 22959^T (73.40 %), *Hwanghaeicola aestuarii* DSM 22009^T
246 (71.35 %), *Maribius salinus* DSM 26892^T (71.06 %) and *Maribius pelagius* DSM 26893^T (70.98 %).
247 The next was *Roseivivax isoporae* LMG 25204^T (66.74 %). These strains were also those which had
248 the highest POCP compared to strain MOLA 401^T (Table S4): *Maribius salinus* DSM 26892^T
249 (62.77 %), *Maribius pelagius* DSM 26893^T (61.95 %), *Palleronia marisminoris* LMG 22959^T
250 (57.88 %), and *Hwanghaeicola aestuarii* DSM 22009^T (54.68 %), the next one being *Roseivivax*
251 *halodurans* JCM 10272^T (53.64 %).
252 Other phylogenetic markers have been tested: *gyrB*, *recA*, *rpoC* and *rpoD*. Phylogenetic
253 reconstructions obtained with *gyrB*, *rpoC* and *rpoD* and a concatenate of the 3 sequences were
254 consistent. Strain MOLA 401^T was close to *Palleronia marisminoris* LMG 22959^T, in a robust cluster
255 (99 to 100 % bootstrap values) with *Palleronia marisminoris* LMG 22959^T, *Maribius salinus*
256 DSM 26892^T, *Maribius pelagius* DSM 26893^T and *Hwanghaeicola aestuarii* DSM 22009^T (Fig. 2, 3
257 and S2). The use of *recA* sequences gave a totally different distribution, with a less robust structure
258 where strain MOLA 401^T was still close to *Palleronia marisminoris* LMG 22959^T (Fig. S3). Another
259 phylogenetic reconstruction, based on 585 core genes, has been done. It showed a similar clustering of
260 the same strains to that mentioned above, and the same positioning of strain MOLA 401^T (Fig. 4). The
261 correlation between AAI, ANI, POCP and identity percentages between 16S RNA, *gyrB, rpoC*, *rpoD*
262 and *recA* genes was estimated by calculating Pearson correlation coefficients (Table S5). The best
263 correlation was between AAI and identity percentages of core genes (0.907). With Pearson
264 coefficients between 0.799 and 0.825, core genes and housekeeping genes appeared also strongly
265 correlated. In contrast, ANI showed poor correlations with identity percentages of *recA* and 16S rRNA
266 genes. POCP did not correlate with any other metrics, except with AAI for which the coefficient was
267 0.765.

268 To further position strain MOLA 401^T within the *Rhodobacteraceae*, AAI values were plotted against
269 POCP values obtained by comparing strain MOLA 401^T with the others *Rhodobacteraceae* genomes
270 (Fig. 5). On this graph, genomes were spread in two clusters, one that contained most of the genomes
271 and the other grouping *M. salinus* DSM 26892^T, *M. pelagius* DSM 26893^T, *P. marisminoris*
272 LMG 22959^T and *H. aestuarii* DSM 22009^T. The same analysis repeated with *M. salinus*
273 DSM 26892^T, *M. pelagius* DSM 26893^T, *P. marisminoris* LMG 22959^T or *H. aestuarii* DSM 22009^T
274 as reference genome again clustered the same four genomes (Fig. S4).
275 Strain MOLA 401^T has been recognized for its ability to produce a wide range of AHLs. We therefore
276 investigated the production of long and short chain AHLs in the related types strains. Both types of
277 AHLs were detected in culture supernatants of the three *Palleronia*, only long chain AHLs in cultures
278 of strain MOLA 401^T. *Maribius salinus* DSM 26892^T, *Maribius pelagius* DSM 26893^T and
279 *Hwanghaeicola aestuarii* DSM 22009^T strains produced low amount of AHL. COG3916,
280 corresponding to the AHL synthase LuxI, was found in strain MOLA 401^T as well as in *Palleronia*
281 *soli* NBRC 110740^T, *Palleronia abyssalis* LMG 27977^T, *Palleronia marisminoris* LMG 22959^T,
282 *Maribius salinus* DSM 26892^T, *Maribius pelagius* DSM 26893^T and *Hwanghaeicola aestuarii*
283 DSM 22009^T genomes (Table 5).
284 Strains belonging to the *Roseobacter* group were frequently observed to be surface-associated. The
285 ability of strain MOLA 401^T, *Palleronia* and *Maribius* strains to form a biofilm was assessed. All
286 these strains were able to develop a biofilm on polystyrene in MB after 8 days of culture and were
287 among the best biofilm formers when compared to a set of 29 marine strains (Fig. 6). It is worth noting
288 that all the strains that form the most biofilm belong to the *Roseobacter* group.
289

290 **Discussion**

291 The characterization of strain MOLA 401^T carried out in this study supports that strain MOLA 401^T
292 represents a new species. Strain MOLA 401^T exhibits identity percentages ranging from 96.9 to
293 96.3 % with its closest relatives *Pseudomaribius aestuariivivens* NBRC 113039^T, *Maribius pontilimi*
294 DSM 104950^T and *Palleronia marisminoris* LMG 22959^T, which are below the usual boundary of
295 97 % used for species delineation. Strain MOLA 401^T presents substrates and enzymatic profiles that

296 are different from its relatives (Table 1 and 2). Strain MOLA 401^T has the same major fatty acids as
297 *Palleronia*, *Maribius*, *Pseudomaribius* and *Hwanghaeicola* type species, but exhibits differences
298 within minor fatty acids (Table 3). ANI values between genome of strain MOLA 401^T and available
299 genomes of the four closest relatives were below 77 %, much less than the 94 % boundary that
300 corresponds to 70 % of DNA-DNA hybridization used to separate species [54]. Physiological,
301 metabolic, morphological, and biochemical characteristics as well as genomic data support the
302 delineation of strain MOLA 401^T as a new species, but do not provide a basis for its affiliation to a
303 genus. Moreover, the current partition of all its closest relatives at the genus level needs to be clarified.
304 The three closest relatives of strain MOLA 401^T, based on 16S rRNA gene sequence, belonged to
305 three different genera. On the sole basis of this sequence, strain MOLA 401^T would be affiliated to the
306 genus *Pseudomaribius* (Table S2). However, it has been argued that 16S rRNA gene sequence was not
307 a pertinent marker for the *Rhodobacteraceae* as it is for many other bacterial families [52,59]. In fact,
308 many genera in the family *Rhodobacteraceae* have been validated in spite of more than 96 % 16S
309 rRNA gene sequence identity between the type species and the closest relatives. Such cases would not
310 have been accepted in the majority of bacterial families, unless providing supplementary data
311 discriminating at genus rank. To determine which genus strain MOLA 401^T belonged, physiological,
312 metabolic, morphological, and biochemical characteristics have been compared to those of the ten
313 closest type species (Table 1) [1,12,25,26,29,31,37,42,43]. Strain MOLA 401^T had the same major
314 fatty acids as *Palleronia*, *Maribius*, *Pseudomaribius* and *Hwanghaeicola* type species. However, the
315 interpretation of the fatty acid profiles should be taken with caution because they are not always
316 reproducible in the *Roseobacter* group [59]. For instance, variations in the amount of the fatty acids
317 $C_{17:0}$, $C_{10:0}$ 3OH, $C_{12:0}$ 3OH and $C_{19:0}$ cyclo ω8c between *Palleronia* species, or between *Maribius*
318 species, were greater than those between species of different genera. This made it difficult to use fatty
319 acid profiles to support the discrimination at genus rank. Moreover, differences in phenotypical
320 characteristics and enzymatic and substrate profiles of these strains were not sufficient to separate
321 them into distinct genera.
322 The sequence alignments of *gyrB*, *rpoC* and *rpoD* showed that strain MOLA 401^T was close to
323 *Palleronia marisminoris* LMG 22959^T and both belonged to a cluster including *Hwanghaeicola*

324 *aestuarii* DSM 22009^T, *Maribius salinus* DSM 26892^T and *Maribius pelagius* DSM 26893^T. Bootstrap
325 values of 99-100 % indicated a strong robustness and showed these genes were pertinent markers in
326 the *Roseobacter* group, as stated by Wirth and Whitman [59] for *rpoC*. Phylogeny based on the 585
327 core genes was in strong accordance with phylogeny based on *gyrB*, *rpoC* and *rpoD* genes. In contrast,
328 *recA* gave inconsistent tree topology and therefore should not be used within the *Rhodobacteraceae*.
329 AAI percentages between genome of strain MOLA 401^T and available genomes of the four closest
330 relatives (*Palleronia marisminoris* LMG 22959^T, *Hwanghaeicola aestuarii* DSM 22009^T, *Maribius*
331 *salinus* DSM 26892^T and *Maribius pelagius* DSM 26893^T) were between 71.06 % and 73.40 %, which
332 fell within the range of 60 %-80 %, suggesting that these strains may belong to the same genus
333 [27,49]. In addition, all these strains shared AAI values above 70 % (Table S3), suggesting that they
334 could be grouped within the same genus. A genus boundary of 50 % POCP has been suggested [45].
335 Percentages between strain MOLA 401^T and its closest relatives were higher than 54 %, those with the
336 *Maribius* species being the highest (Table S4). These values could support the affiliation of strain
337 MOLA 401^T to the genus *Maribius*, but percentages between *Palleronia marisminoris* LMG 22959^T,
338 *Hwanghaeicola aestuarii* DSM 22009^T, *Maribius salinus* DSM 26892^T and *Maribius pelagius*
339 DSM 26893^T suggested that all these strains belonged to the same genus, consistently with the
340 conclusion drawn from AAI values (Table S4). Limit values of AAI and POCP for genus delineation
341 have been questioned [59]. We confirmed that 50 % POCP alone could not be applied to
342 *Rhodobacteraceae* for genus-level circumscription because nearly half of the percentages we
343 calculated were greater than this value. Similarly, the limits given by AAI for belonging to a same
344 genus, ranging from 60 to 80 %, could not be the considered alone because all values we obtained fell
345 in this range. But the combination of these two metrics provided a clear discrimination (Fig. 5 and Fig.
346 S4). Moreover, all the combinations sharing POCP > 50 % and AAI > 70 % corresponded to the
347 genomes of strain MOLA 401^T, *M. salinus* DSM 26892^T, *M. pelagius* DSM 26893^T, *P. marisminoris*
348 LMG 22959^T and *H. aestuarii* DSM 22009^T only. These are the 5 strains that group in a robust
349 monophyletic cluster when using sequences of *gyrB*, *rpoC*, *rpoD*, separately or in concatenate, or the
350 core-genome. This consistency shows the interest and the usefulness of combining these
351 phylogenomic analyses. In our study, the limit of AAI > 0.80 suggested recently [59] would have been

352 too restrictive by maintaining each strain in its genus and leading to propose strain MOLA 401^T as the
353 type species of a new genus without any significant discriminating phenotypic trait and leaving
354 besides phylogenomic metrics and a phylogenetic position in a robust cluster encompassing three
355 genera containing only one fully sequenced genome each. Like the fundamental of polyphasic
356 taxonomy, it is still important to achieve a consistency by combining various analyses including
357 phylogenomic ones.
358 An unexpected observation concerns *Maribius salinus* DSM 26892^T and *Maribius pelagius*
359 DSM 26893^T. Their genomic, metabolic, chemotaxonomic and phenotypic differences are very minor
360 and, depending on the study, some of them are not always observed. With ANI, AAI and POCP values
361 of 96.12, 97.46 and 86.80 % respectively, these 2 strains should belong to the same species. This is
362 also supported by the 16S rRNA gene sequence identity percentage of 99.86 %. *M. Pelagius* could be
363 a synonym of *M. marinus*.
364
365 **Surface colonization**
366 Because strain MOLA 401^T was firstly studied for its remarkable AHLs produced diversity [18], an
367 experiment to detect AHL production was conducted on the 6 closest relatives of strain MOLA 401^T.
368 As expected, strain MOLA 401^T was positive for long chain AHLs production. The three *Palleronia*
369 strains were positive for both long and short chain AHLs production, and the two *Maribius* strains and
370 *H. aestuarii* DSM 22009^T exhibited a limited ability for AHL production under our conditions. The
371 presence of the COG3916 coding for AHL synthase in all the genomes studied is consistent to
372 consider QS as a common trait of the *Roseobacter* group, even if the two *Maribius* strains and *H.*
373 *aestuarii* DSM 22009^T tested did not show high AHL production. The low AHL detection for these 3
374 strains could be due to environmental factors such as carbon source, temperature, pH, and others that
375 could disturb the AHL production [7].
376 Our results show that strain MOLA 401^T and its relatives were strong biofilm formers and possess
377 AHL based QS systems. Surface colonization and QS systems have been often linked, and especially
378 in the *Roseobacter* group, which has been identified as ubiquitous colonizers of marine surfaces [16].

By offering a structured environment where transport of solutes is diffusion limited, biofilms represent a habitat where communication by QS system appears best suited.

Conclusion

Our QS and biofilm results strengthen the observation previously made about the *Roseobacter* group, and place them as biofilm specialists increasing their common QS potential in the marine environment.

On the basis of all the data presented, strain MOLA 401^T should be placed in the genus *Palleronia*. We propose the inclusion of *Maribius* species as well as *H. aestuarii* within the genus *Palleronia* (which has been described first).

Several genome sequences are not available yet, preventing genomic analyses. Therefore, and even if taxonomic positions of *M. pontilimi* DSM 104950^T, *P. soli* NBRC 110740^T and *P. abyssalis* LMG 27977^T could be considered now as uncertain, their affiliations should be maintained in their respective genera. Consequently, they also have to be renamed to be in accordance with the proposed new delineation. In the case of *M. pontilimi* DSM 104950^T, its 16S rRNA gene sequence similarity with *M. salinus* DSM 26892^T and *Maribius pelagius* DSM 26893^T is 97.63 %. Now, Whirth and Whitman [59] have observed that, among the *Rhodobacteraceae*, a 16S rRNA gene sequence similarity above 97 % was a strong predictor of an AAI greater than 0.80, in accordance with a maintenance in the same genus. The case of *Pseudomaribius aestuariivivens* NBRC 113039^T is different, as this type strain is the only one of its genus. Thus even if it is likely that this species would be grouped with the others, until new data is available, it should be maintained as the type strain of this separate genus.

A clear delineation of species and genera in the family *Rhodobacteraceae* is not easy. Genomic metrics and phylogenetic markers provide data that help to clarify an uncertain positioning after having observed the usual parameters required in a polyphasic taxonomy approach. Recent references regarding the *Roseobacter* group and *Rhodobacteraceae* emphasize the importance of genome sequences and taxonomy to understand the evolutionary ecology and phylogenomics of these bacteria [33,52]. Here we suggest a reorganization based on phylogenetic markers and genomic metrics, used

407 in combination with all the other usual parameters, that could be applied to other cases concerning this
408 family in order to achieve a more robust and usable taxonomic structure.
409
410 **Emended description of the genus *Palleronia* Martínez-Checa *et al.* 2005** [37]**.**
411 The genus was already described [37] and emended by Albuquerque *et al.* [1] and Kim *et al.* [25].
412 Oxidase and catalase tests are positive or negative. The principal fatty acid is $C_{18:1}\omega c7$ and $C_{19:0}$ cyclo
413 ω8c, $C_{16:0}$ and $C_{18:0}$ are among the major ones. The type species of the genus is *P. marisminoris*.
414
415 **Reclassification of *M. pelagius* as *Palleronia pelagia* comb. nov.**
416 *Palleronia pelagia* (pe.la'gia L. fem adj. *pelagia* of or belonging to the sea)
417 Basonym: *Maribius pelagius* Choi *et al.* 2007. Description is as that given by Choi *et al.* [12], with the
418 following modification: G+C content is 66.9 % on the basis of the genome sequence.
419 The type strain is *Palleronia pelagia* DSM 26892^T (KCCM 42336^T = JCM 14009^T).
420
421
422 **Reclassification of *Maribius salinus* as *Palleronia salina* comb. nov.**
423 *Palleronia salina* (sa.li'na. N.L. fem. adj. *salina* salted, salty).
424 Basonym: *Maribius salinus* Choi *et al.* 2007. Description is as that given by Choi *et al.* [12], with the
425 following modifications: D-fructose and D-raffinose do not support growth and G+C content is 67.7 %
426 on the basis of the genome sequence.
427 The type strain is *Palleronia salina* DSM 26892^T (KCCM 42113^T = JCM 13037^T).
428
429 **Reclassification of *Maribius pontilimi* as *Palleronia pontilimi* comb. nov.**
430 *Palleronia pontilimi* (pon.ti.li'mi. L. masc. n. *pontus* sea; L. masc. n. *limus* mud; N.L. gen. n. *pontilimi*
431 of mud of the sea, where the type strain was isolated).
432 Basonym: *Maribius pontilimi* Lee 2018. Description is as that given by Lee [31].
433 The type strain is *Palleronia pontilimi* DSM 104950^T (KCTC 52957^T = DSM 104950^T).
434

435 **Reclassification of *Hwanghaeicola aestuarii* as *Palleronia aestuarii* comb. nov.**

436 *Palleronia aestuarii* (aes.tu.a'ri.i L. gen. n. *aestuarii* of the tidal flat, from where the organism was

437 first isolated).

438 Basonym: *Hwanghaeicola aestuarii* Kim *et al.* 2010. Description is as that given by Kim *et al.* [26].

439 The type strain is *Palleronia aestuarii* DSM 22009^T (KACC 13705^T = DSM 22009^T).

440

441 **Description of *Palleronia rufa* sp. nov.**

442 *Palleronia rufa* (ru'fa. L. fem. adj. *rufa*, being rufous, roux).

443 In addition to the traits reported for the genus description, colonies on MA are caramel coloured,

444 slightly domed, smooth, circular (1-2 mm in diameter) and non-swarming. Cells are cocci of 0.7 to

445 0.8 µm in diameter. Growth occurs within the temperature range of 4 to 37°C (optimum 30°C), at pH

446 values between 6.5 and 8.5 (optimum 8), and with 0 to 9 % (w/v) NaCl (optimum 3 %). Gelatin is

447 hydrolysed. Catalase negative and positive for oxidase. Growth occurs on D-glucose, D-mannose, D-

448 raffinose, D-sucrose, L-rhamnose, acetate, gluconate, pyruvate, D-sorbitol, glycerol, myo-inositol and

449 succinate as carbon sources. Weak growth with melezitose, malate and ethanol. No growth with

450 cellobiose, D-arabinose, D-fructose, D-trehalose, D-fructose, L-xylose, xylitol, glycogen, starch,

451 peptone and tryptone. The major cellular fatty acids are $C_{18:1}\omega7c$, $C_{18:0}$, C_{11}-Me-$C_{18:1}\omega7c$ and $C_{19:0}$

452 cyclo ω8c. The only ubiquinone is Q-10. The DNA G+C content of the strain is 67.6 %. The type

453 strain is MOLA 401^T (DSM 106827 = CIP 111607^T = BBCC 401^T), isolated from a water sample

454 collected from marine waters in the southwest lagoon of New Caledonia [13,18]. The taxonumber is

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456

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1 **Table 1. Characteristics comparing strain MOLA 401^T with its closest relatives.** 1: strain MOLA 401^T; 2:
2 *Palleronia soli* NBRC 110740^T; 3: *Palleronia abyssalis* LMG 27977^T; 4: *Palleronia marisminoris*
3 LMG 22959^T; 5: *Maribius salinus* DSM 26892^T; 6: *Maribius pelagius* DSM 26893^T; 7: *Maribius pontilimi*
4 DSM 104950^T; 8: *Pseudomaribius aestuariivivens* NBRC 113039^T; 9: *Hwanghaeicola aestuarii* DSM 22009^T;
5 10: *Profundibacterium mesophilum* JCM 17872^T; 11: *Boseongicola aestuarii* CECT 8489^T. *Data from this
6 study.
7

	1	2	3	4	5	6	7	8	9	10	11
G+C content (%)	67.6	64.3	64.7	64.2	67.7	66.9	66.7	68.7	61	64	58.7
motility	-	-	-	-	-	-	-	-	+	-	-
colony color	caramel	cream	red	pale pink	cream	cream	cream	greyish yellow	pale pink	colorless	pale yellow
shape	coccus	short rod	coccus/ rod	short rod	rod	rod	rod	ovoid/ rod	coccoid	coccoid	coccus/ rod
size (µm)	0.77	0.5-0.8/ 1.2-2.6	0.8-1	0.75-1/ 2-2.5	0.7-1.4/ 1.0-4.5	0.4-0.8/ 1.3-2.8	0.8-1.2/ 1.2-2.3	0.3-1.6/ 0.5-10.0	0.8-1.2	5.0-8.0	0.5-2/ 0.7-10.0
oxidase	-	-	+	-	+*	+*	+	+	+	+	+
catalase	-	+	+	+*	-*	-*	+	+	+	+	+
temperature											
growth range (°C)	15-37	20-40	15-37	20-37	10-35	10-40	10-30	10-37	15-35	15-25	10-30
optimum (°C)	30	37	30	30*	30-35	30-35	30	25-30	25-30	20-25	25
pH growth range	6.5-8.5	4.5-11	6.0-8.0	5.0-10	7-9	6-9	6-10	6-8	6-8	6-8.5	6.5-8
optimum	8	7	6.5-7	7*	8*	7-8*	7	7-8	6.5-7.5	7-8	7
NaCl tolerance for growth (%)	0-9	1-11	0-13	0.5-15	1-10	2-15	0.5-5	0.5-5	1.5-6	0.5-20	0.5-5
optimum (%)	3	3	2-5	5	1.5*	2.5*	1	2	2-3	2-6	2
ubiquinone	Q-10	Q-10	Q-10	Q-10	Q-10	Q-10	Q-10	Q-10	Q-10	Q-10	Q-10

8

9

10

11 **Table 2. Comparison of substrate utilization and enzyme activity profiles of strain MOLA 401^T and its**
12 **closest relatives.** 1: strain MOLA 401^T; 2: *Palleronia soli* NBRC 110740^T; 3: *Palleronia marisminoris*
13 LMG 22959^T; 4: *Palleronia abyssalis* LMG 27977^T; 5: *Maribius salinus* DSM 26892^T; 6: *Maribius pelagius*
14 DSM 26893^T; 7: *Maribius pontilimi* DSM 104950^T; 8: *Hwanghaeicola aestuarii* DS 22009^T; 9:
15 *Pseudomaribius aestuariivivens* NBRC 113039^T. +, positive; -, negative; w, weak. All strains are positive for
16 esterase (C4), esterase lipase (C8) and leucine arylamidase. All strains are negative for trypsin, β-galactosidase,
17 N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase.

18

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Substrate:									
cellobiose	-	-	-	+	+	-	-	+	+
D-fructose	-	-	-	+	w	w	+	w	+
D-glucose	+	-	+	+	-	+	+	+	+
D-mannose	+	-	-	+	-	-	+	w	+
D-raffinose	+	-	w	-	-	-	+	-	+
D-sucrose	+	+	-	-	+	+	-	-	+
D-trehalose	-	-	-	w	-	-	+	+	+
L-rhamnose	+	-	-	+	-	-	+	w	-
melezitose	w	-	-	w	-	-	+	-	+
D-sorbitol	+	-	-	-	+	-	-	+	+
myo-inositol	+	-	+	-	+	-	-	-	+
Activity:									
alkaline Phosphatase	+	+	+	w	-	-	-	+	+
lipase (C14)	-	-	-	-	-	-	-	w	-
valine arylamidase	+	-	-	-	-	-	w	w	-
cystine arylaminidase	+	w	w	-	-	-	-	w	-
α-chymotrypsin	-	-	-	-	-	-	-	w	-
acid phosphatase	+	+	+	w	-	-	-	w	+
naphthol-AS-BI-phosphohydrolase	+	-	+	+	-	-	+	w	w
α-galactosidase	-	-	-	-	-	-	+	-	-
β-glucuronidase	-	-	-	-	-	-	-	w	-
α-glucosidase	-	-	+	+	-	-	-	-	-
β-glucosidase	-	-	-	-	-	-	+	-	+

19

20

21

22 **Table 3. Metabolic genes searched in MOLA 401^{T} and its closest relatives genomes.** All genomes contain

23 *luxI*, *luxR*, *nirS*, *narB* and *coxL*. All genomes lack *norB*. u: uncertain.

	pufM	*bchG*	*bchP*	*soxB*	*soxY*	*narG*	*nasA*	*napA*
MOLA 401^{T}	+	+	+	+	+	-	+	-
M. salinus	+	+	+	u	-	-	+	-
M. pelagius	+	+	+	+	+	-	+	-
P. marisminoris	-	-	-	u	+	-	+	-
P. abyssalis	+	-	-	-	-	u	u	+
H. aestuarii	+	+	+	+	+	-	+	-

24 25 26 27 28 29 30 31

32

33

34 **Table 4. Fatty acids composition of strain MOLA 401^T and its closest relatives.** Percentage ranges are

35 based on data from references [1,12,24,25,28,29,34,39,40] and this study. 1: strain MOLA 401^T; 2: *Palleronia*

36 *soli* NBRC 110740^T; 3: *Palleronia abyssalis* LMG 27977^T; 4: *Palleronia marisminoris* LMG 22959^T; 5:

37 *Maribius salinus* DSM 26892^T; 6: *Maribius pelagius* DSM 26893^T; 7: *Maribius pontilimi* DSM 104950^T; 8:

38 *Pseudomaribius aestuariivivens* NBRC 113039^T; 9: *Hwanghaeicola aestuarii* DSM 22009^T; 10:

39 *Profundibacterium mesophilum* JCM 17872^T; 11: *Boseongicola aestuarii* CECT 8489^T. -, not detected. tr, trace

40 (< 0.5 %).

41

	1	2	3	4	5	6	7	8	9	10	11
$C_{12:0}$	tr	0.6-1.1	0-0.5	-	0- tr	0- tr	-		1.1-1.3	-	-
$C_{15:0}$	-	-	-	-	0- tr	-	-		-	-	-
$C_{16:0}$	2.1	2.6-5.1	3.1-6.3	1.5-4.3	3.5-4.9	0-2.4	4.7	1.9-2.1	8.31-11.9	3.4	1.5-2.0
$C_{17:0}$	0.6	0.8-7.5	tr-1.5	0-1.7	1.1-2.7	0-0.7	-	4.9-6.4	-	-	-
$C_{18:0}$	12.5	6.1-9.7	2.5-3.1	3.0-4.2	2.7-5.4	2.7-4.6	7.2	3.0-3.4	2.5-2.8	0.5-0.7	5.5-5.6
$C_{19:0}$	-	-	-	-	-	-	-		-	0-0.6	-
$C_{16:1}\omega7c/_{16:1}\omega6c/$ C_{15} iso 2OH	-	0-1.2	0- tr	0- tr	0-0.6	0- tr	-	0.6-0.7	-	1.0-3.6	tr-0.8
$C_{17:1}\omega6c$	-	-	-	0- tr	-	-	-	1.0-1.4	-	-	-
$C_{17:1}\omega7c$	-	0- tr	-	-	-	-	-	-	-	0.9	-
$C_{17:1}\omega8c$	-	0-1.5	0-0.7	-	0- tr	tr	-	1.6-2.0	-	-	-
$C_{18:1}\omega7c$	63.1	62.9-79.1	39.7-80.9	54.8-79.6	32.7-69.7	36.3-79.4	51	67.2-70.7	63.4-82.9	83.3-85.6	73.1-81.1
$C_{18:1}\omega9c$	-	-	-	-	-	-	-	-	-	-	1.4-1.7
$C_{18:3}\omega6c$	-	-	-	-	0-1.7	-	-		-	-	-
$C_{19:1}\omega6c/$ $C_{19:0}$ cyclo ω10c	2.5	0-1.3	-	-	1.7-5.5	1.8-2.8	4	1.1-1.5	-	-	tr-0.7
$C_{20:1}\omega7c$	0.5	0- tr	-	0- tr	0- tr	0-1.0	-	tr	0.7	-	0.9-1.2
$C_{10:0}$ 3OH	1.5	2.2-2.8	1.6-2	2.2-5	1.3-4.3	3.2-5.2	-	2.3-2.5	0-2.7	1.8	2.2-2.7
$C_{11:0}$ 3OH	-	0- tr	0- tr	0- tr	-	-	-	tr-0.6	-	-	-
$C_{12:0}$ 3OH	-	0-2.6	1.9-2.9	-	-	-	-	-	-	1.7	tr
$C_{12:1}$ 3OH	-	-	-	-	-	0- tr	-	-	-	-	-
$C_{14:0}$ 3OH	-	-	-	-	-	-		-	1.0-1.5	-	-
$C_{19:0}$ cyclo ω8c	8.9	0-6.6	5.2-43.5	10.8-31.3	5.8-38.9	3.3-36.5	22.3	3.7-6.8	0.6-9.3	-	-
C_{11}-Me-$C_{18:1}\omega7c$	7.5	2.1-6.3	0-1.6	0-1.4	3.4-8.4	3.4-10.8	6.8	5.4-7.4	2.3-4.5	5.7-7.2	5.3-12.9
C_{10}-Me-$C_{19:0}$	-	-	-	-	0- tr	-	-	-	-	-	-
unknown ECL 11.799	tr	2.8	2.1-2.6	0.8-1	2.3-2.4	2.3-2.8	-	-	1.23-1.5	-	-

42 **Table 5. AHL production by strain MOLA 401^T and relatives.** Results are presented as percentage
43 of the positive control. Number of COG3916, corresponding to AHL synthetase, was reported against
44 the AI-1 production results. 1: strain MOLA 401^T; 2: *Maribius pelagius* DSM 26893^T ; 3: *Maribius*
45 *salinus* DSM 26892^T; 4: *Hwanghaeicola aestuarii* DSM 22009^T; 5: *Palleronia marisminoris*
46 LMG 22959^T; 6: *Palleronia abyssalis* LMG 27977^T; 7: *Palleronia soli* NBRC 110740^T. NA, no
47 genome available.

	1	**2**	**3**	**4**	**5**	**6**	**7**
Maximum AHL with a long fatty acyl chain (as % of the positive control / OD_{600})	53.72±18.97	0.95±0.13	1.93±0.46	3.64±1.43	174.68±41.22	87.58±12.81	135.06±13.38
Maximum AHL with a short fatty acyl chain as % of the positive control / OD_{600})	0.19±0.22	0.16±0.02	1.35±0.53	1.98±0.44	65.33±6.45	15.18±3.07	45.46±1.98
AHL synthetase (COG3916)	2	1	1	2	2	NA	NA

48

49

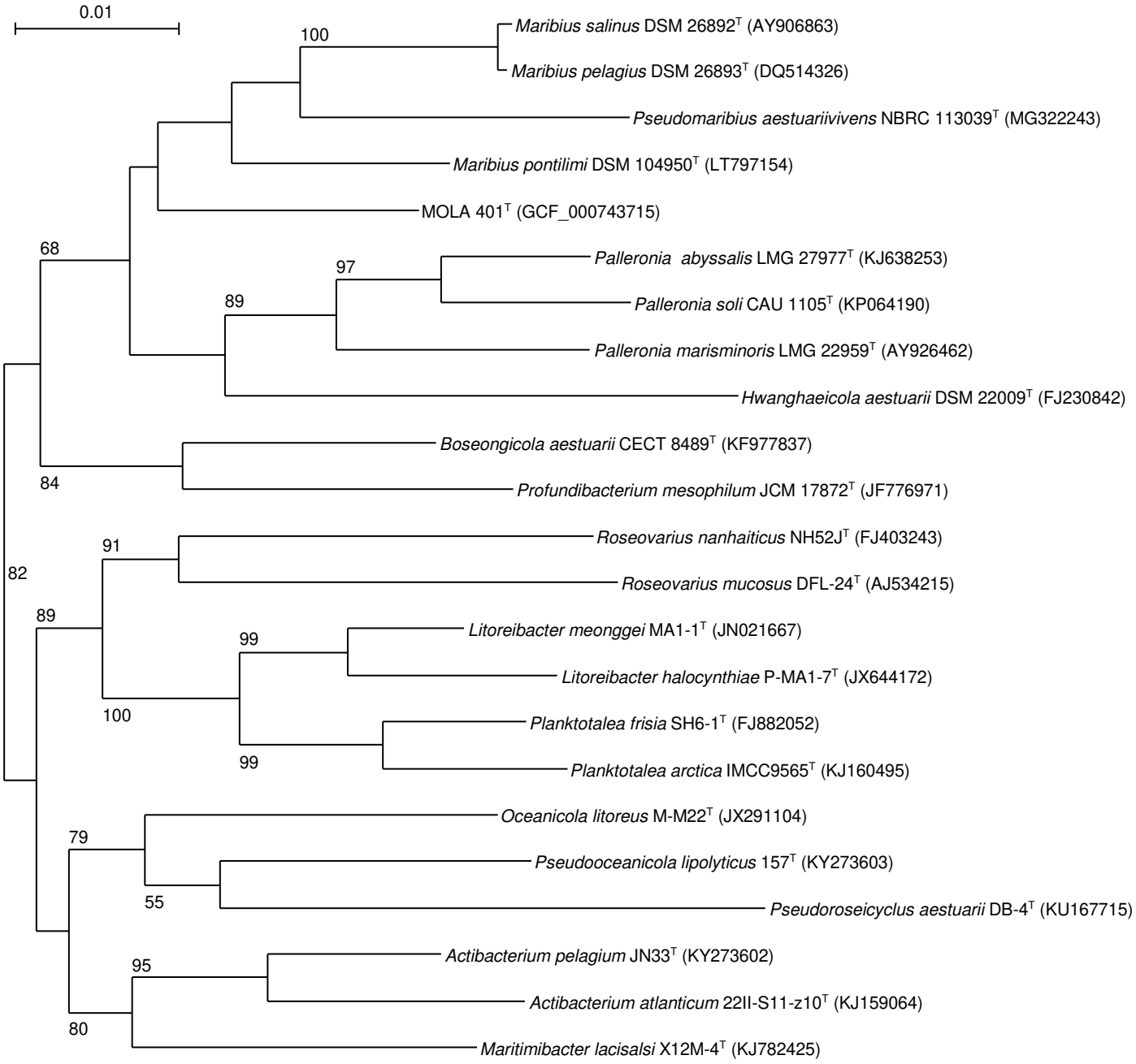

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences showing the position of strain MOLA 401^T and its closest relatives. Bootstrap values (percentages of 1000 replications) greater than 50 % are indicated. Bar: 0.01 substitution per nucleotide position.

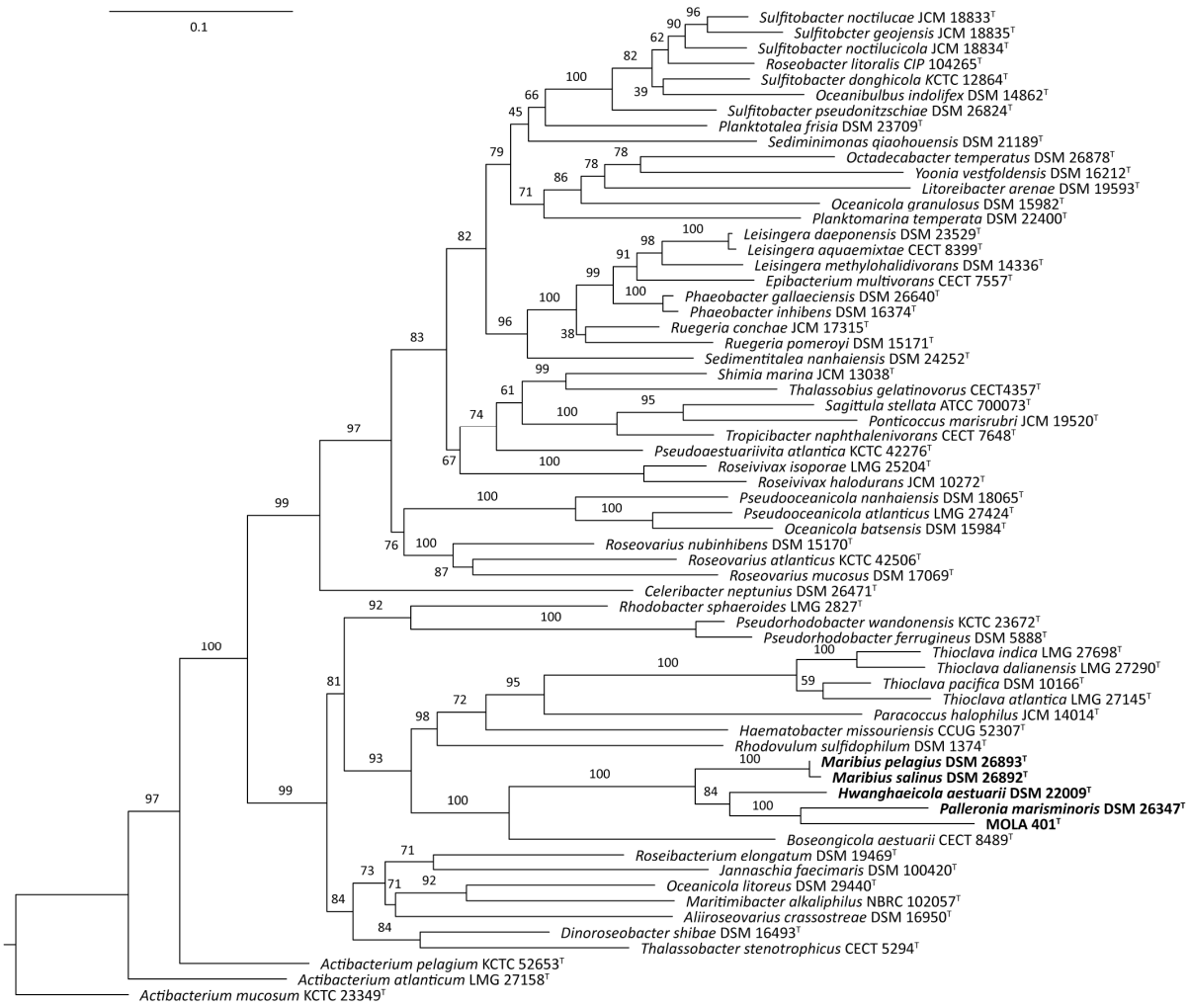

Fig. 2. Phylogenetic tree based on *gyrB* sequences showing the position of strain MOLA 401^{T} and its closest relatives. Bootstrap values are percentages of 1000 replications. Bar: 0.1 substitution per nucleotide position.

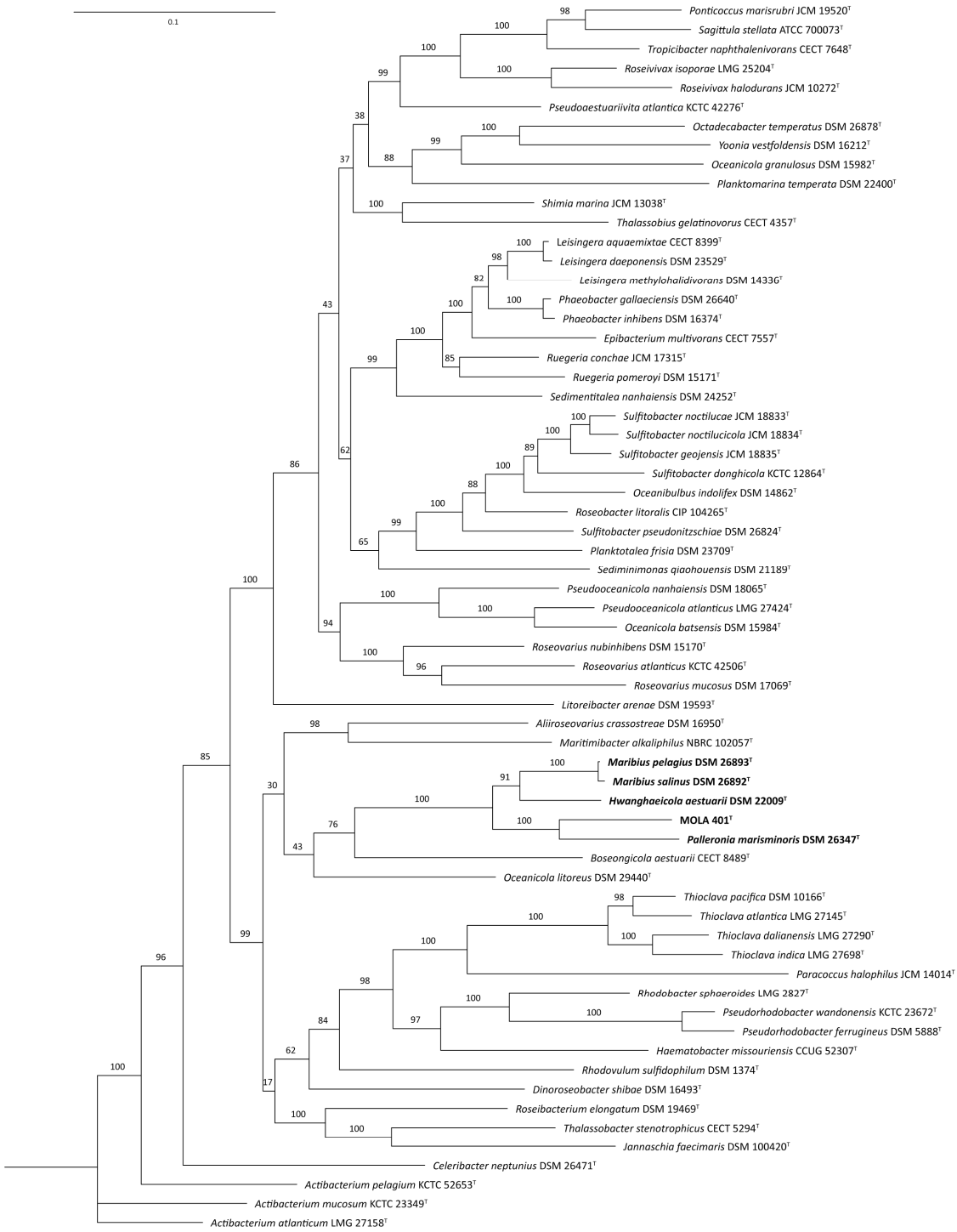

Fig. 3. Phylogenetic tree based on concatenated sequences of *gyrB*, *rpoC* and *rpoD* showing the position of strain MOLA 401^{T} and its closest relatives. Bootstrap values are percentages of 1000 replications. Bar: 0.1 substitution per nucleotide position.

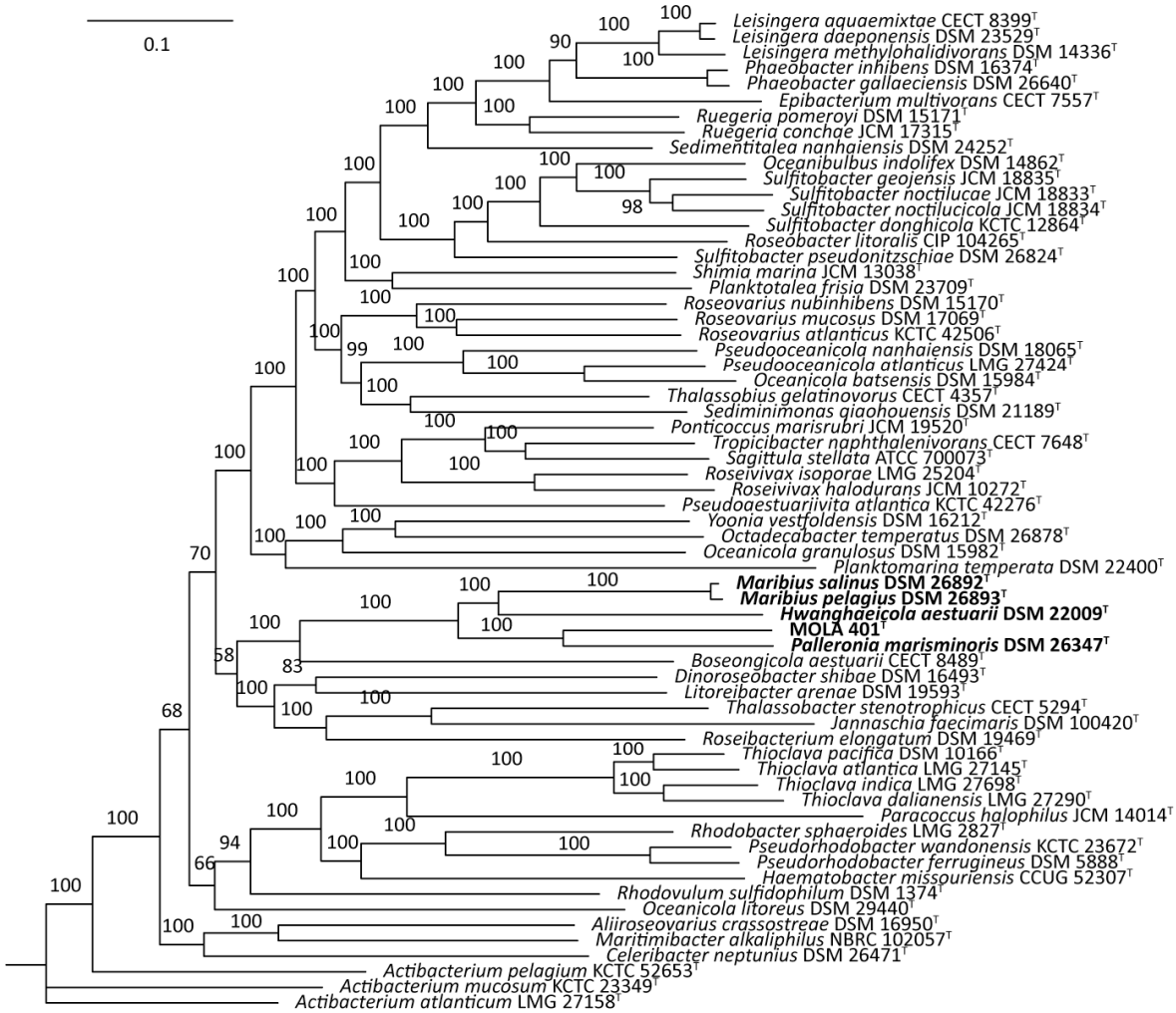

Fig. 4. Phylogenetic tree based on core genes sequences showing the position of strain MOLA 401^{T} and its closest relatives. Bootstrap values are percentages of 1000 replications. Bar: 0.1 substitution per nucleotide position.

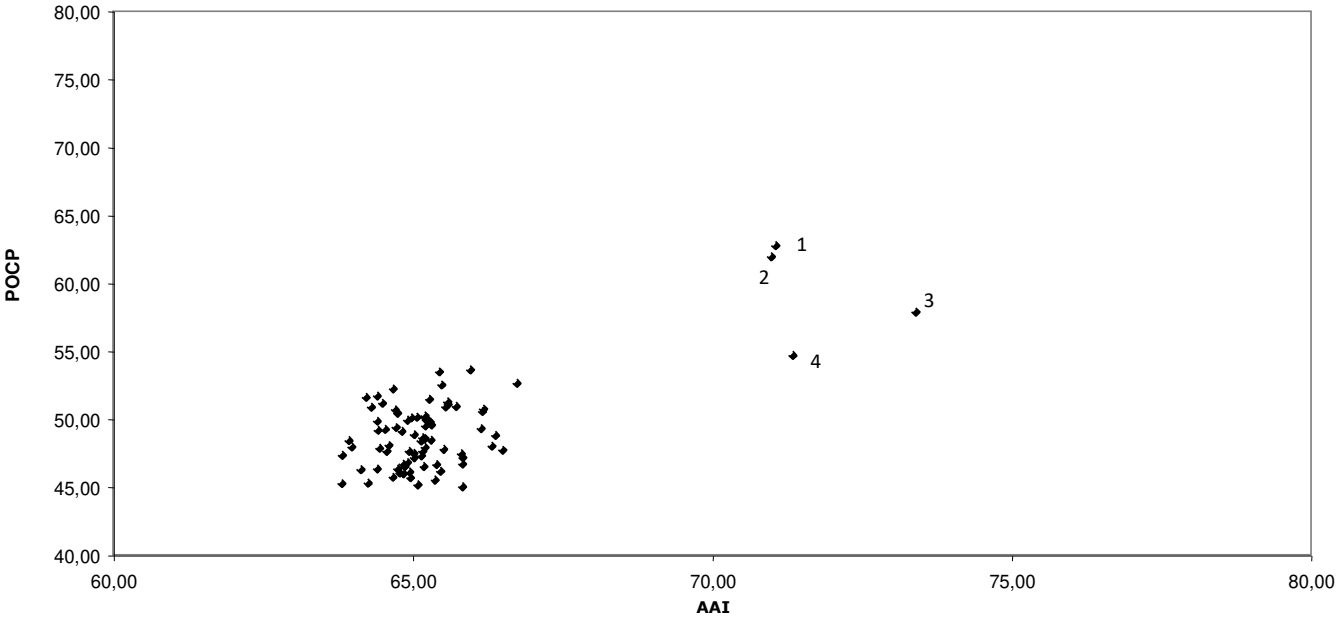

Fig. 5. Distribution of the comparisons of strain MOLA 401^T genome to its closest relatives (> 95 % rRNA gene sequence identity) according to AAI and POCP values. 1, strain MOLA 401^T and *M. salinus* DSM 26892^T; 2, strain MOLA 401^T and *M. pelagius* DSM 26893^T; 3, strain MOLA 401^T and *P. marisminoris* LMG 22959^T; 4, strain MOLA 401^T and *H. aestuarii* DSM 22009^T.

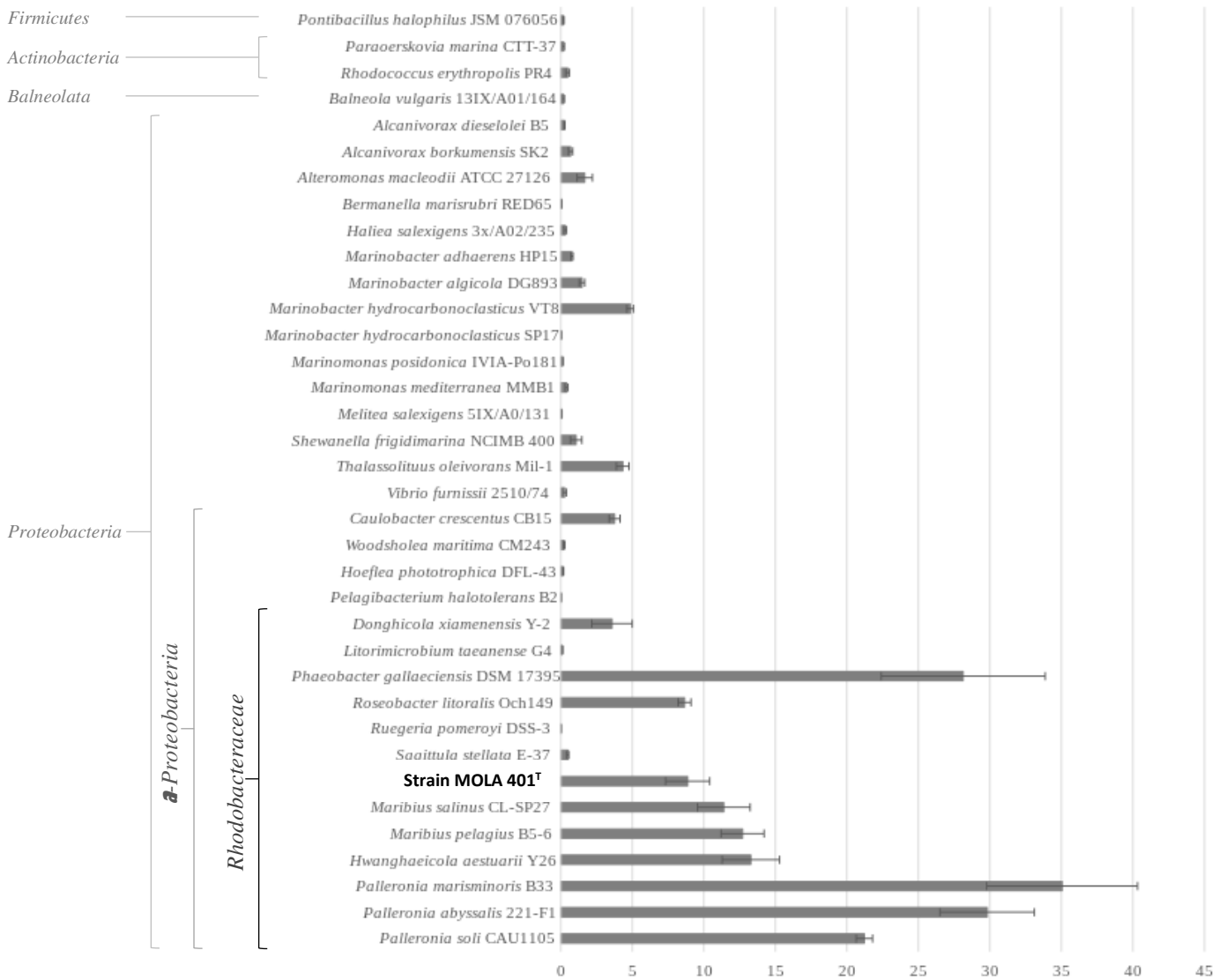

Fig. 6. Amount of biofilm production on polystyrene (Abs $_{595\ nm}$). Biomass bound to the wells bottom was quantified by crystal violet staining.