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## **Avrilella dinanensis gen. nov., sp. nov., a novel bacterium of the family Flavobacteriaceae isolated from human blood**

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1 ***Avrilella dinanensis* gen. nov., sp. nov., a novel bacterium of the family *Flavobacteriaceae***  
2 **isolated from human blood**

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4 Running title: *A. dinanensis* from human blood

5  
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32 **Abstract**

33 Polyphasic taxonomic analysis was performed on a novel bacterium, designated UR159<sup>T</sup>, isolated in  
34 2016 from human blood of a septic patient hospitalized in France. Preliminary 16S rRNA gene  
35 sequence-based phylogenetic analysis indicated that strain UR159<sup>T</sup> belonged to the family  
36 *Flavobacteriaceae*, forming a distinct phyletic line distantly related (<94% sequence similarity) to  
37 known species of the family. Further phenotypic, chemotaxonomic and genomic analyses were  
38 performed. Cells were non-motile, oxidase-negative, catalase-positive Gram-negative rod. It was  
39 strictly aerobic yielding yellow-pigmented colonies, and was metabolically rather inert. Major fatty  
40 acids were iso-branched fatty acids, predominantly iso-C<sub>15:0</sub> (55.5%) and iso-C<sub>17:1</sub>ω<sub>9c</sub> (8.8%). Whole  
41 genome sequencing revealed a 2.3-Mbp genome encoding a total of 2,262 putative genes with a  
42 genomic DNA G+C content at 37.6 mol%. The average nucleotide identity (ANI) and in silico DNA-DNA  
43 hybridization (isDDH) values between strain UR159<sup>T</sup> and the most closely related members of the  
44 *Flavobacteriaceae* were <75% and <39%, respectively, much below the established cut-offs for ANI  
45 (<95-96%) and isDDH (<70%) for species and genus delineation. Average Amino Acid Identity (AAI)  
46 percentages were also estimated and were lower than 65% (cut-off proposed for genus delineation  
47 for uncultivated prokaryotes) in all cases, except for *F. marinum* that was just at the limit (65.1%).  
48 Based on these findings, we propose it as a new genus and species, *Avrilella dinanensis* gen. nov., sp.  
49 nov. (type strain UR159<sup>T</sup> = CIP 111616<sup>T</sup> = DSM 105483<sup>T</sup>).

## 50 **Introduction**

51 The *Flavobacteriaceae* family, belonging to the phylum Bacteroidetes, was described in 1992  
52 [1]. Since this date, it has much evolved with many new taxa allocated and currently 153  
53 genera with a validly published and correct name are part of the *Flavobacteriaceae* family  
54 (www.bacterio.net). Members of this family are Gram-negative non-spore-forming rods  
55 widely described from diverse environmental habitats, especially in the marine environment  
56 [2]. Many species are pathogenic to animals, especially flavobacterial infections in fish [3].  
57 Even though very rarely isolated in humans, some species are considered as opportunistic  
58 pathogens, especially in immunocompromised patients and those with severe underlying  
59 comorbidities (e.g., cancer, diabetes mellitus and chronic hematological, hepatic or kidney  
60 disorders) [4]. Besides *Capnocytophaga* spp., the most frequently found species isolated  
61 from clinical materials belong to the following genera: *Bergeyella* (*B. zoohelcum*),  
62 *Chryseobacterium* (*C. indologenes*), *Elizabethkingia* (*E. meningoseptica*), *Myroides* (*M.*  
63 *odoratus*, *M. odoratimimus*), *Sphingobacterium* (*S. multivorum*, *S. spitivorum*) and *Weeksella*  
64 (*W. virosa*) [5]. Most case reports report these organisms from blood, urine, wounds and  
65 respiratory secretions [4,5].

66 In the present study, we characterized a new isolate (designated UR159<sup>T</sup>) and determined its  
67 taxonomic position using a detailed phenotypic and genotypic analysis (polyphasic approach)  
68 [6-8]. Our data suggest that this strain was not related to any previous taxon and thus, we  
69 propose it as a new genus and species, *Avrilella dinanensis* gen. nov., sp. nov., within the  
70 *Flavobacteriaceae* family.

## 71 *Patient*

72 An unknown Gram-negative rod was isolated in 2016 from two different aerobic blood  
73 culture bottles in a 93-year-old female patient hospitalized in the hospital of Dinan (Brittany,

74 France). The patient suffered from a sepsis (with a temperature at 39°C) after a red blood  
75 cell transfusion for chronic anemia (Hb <8 g/dl) in the context of a myelodysplastic  
76 syndrome. Although the association between infection and transfusion was not clearly  
77 proven, the patient was empirically treated by amoxicillin-clavulanate (1 g tid, 10 days) and  
78 became afebrile after 2 days of antimicrobial therapy.

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79 **Materials and methods**

80 *Strain isolation*

81 Strain UR159<sup>T</sup> was recovered from blood cultures (Bactec™ Plus/aerobic bottles; Becton  
82 Dickinson) of the patient. Positive bottles were used for Gram staining and inoculation onto  
83 blood and chocolate agar plates incubated at 35°C aerobically and in a 5% CO<sub>2</sub> atmosphere,  
84 respectively. Strain UR159<sup>T</sup> was routinely cultivated aerobically at 35°C on blood agar (BA;  
85 ThermoFisher) and preserved for long term at -80°C as a suspension in brain-heart infusion  
86 (BHI; BD Difco™) supplemented with 20% glycerol (v/v).

87 *Cell morphology and growth conditions*

88 Cell morphology was observed using both light microscopy and transmission electron  
89 microscopy (TEM). For light microscopy, Gram staining was performed using cells grown on  
90 BA at 35°C for 24 hours. For TEM, bacterial cells from an exponential culture were collected  
91 after an overnight incubation, transferred to Eppendorf tubes and washed three times in  
92 cacodilate buffer (0.15 M, pH 7.4). Fractions of bacterial suspension were fixed at 4 °C for 60  
93 min with 2% glutaraldehyde in 0.15 M cacodilate buffer to be washed three times in the  
94 same buffer. Cells were post-fixed in 1% OsO<sub>4</sub> for 60 min at 4 °C, rinsed in cacodilate buffer  
95 and embedded in 2% OmniPur® agarose, low melting (Calbiochem, Merck). After the  
96 dehydration series in acetone, the cells samples were embedded in conventional Epon (EMS,  
97 1420) and then polymerized at 60 °C for 48 h. Resins blocks were sectioned into 80-nm  
98 sections using ultramicrotome LEICA UC7 (Leica, Wetzlar, Germany). These sections were  
99 mounted on copper grids and stained. Grids were observed using a JEM-1400 Electron  
100 Microscope (JEOL Ltd, Tokyo, Japan) at an accelerating voltage of 120 kV and equipped with  
101 an Orious® SC1000 camera (Gatan-Ametek, Pleasanton, USA). The cell size measurements

102 were carried out by TEM on two batches of culture and on 50 different bacterial cells for  
103 each culture.

104 Morphology, size and pigmentation of colonies were observed under optimal growth  
105 conditions on BA after 24 hours of incubation at 35°C under ambient air. Ability to grow  
106 under aerobic, microaerophilic and anaerobic conditions was assessed after 24-hour, 48-  
107 hour and 5-day incubation at 35°C while CO<sub>2</sub> requirement was tested in an incubator  
108 supplied with 5% CO<sub>2</sub>. Note that microaerophilic and anaerobic conditions were obtained  
109 using the Anoxomat Anaerobic Cultivation System (Mart Microbiology, Drachten, The  
110 Netherlands). The growth ability was examined on blood agar plates incubated at 15, 30, 37  
111 and 42°C. Gliding motility was tested as previously described [6].

#### 112 *Biochemical and antimicrobial susceptibility testing*

113 Identification was attempted by using the MALDI-TOF mass spectrometry (Microflex™ LT/SH  
114 60 Hz; Bruker Daltonics, Bremen, Germany) according to manufacturer's instructions for  
115 routine. Oxidase activity was determined with the MASTDISCS® ID Oxidase Discs (Mast  
116 Diagnostics) and catalase was detected using a 3% (v/v) aqueous H<sub>2</sub>O<sub>2</sub> solution. Enzyme and  
117 carbon use profiles were generated with the API 20E and Vitek2 GN card microtest systems  
118 (bioMérieux) according to the manufacturer's instructions.

119 The antimicrobial susceptibility profile of the isolate was assessed by determining minimum  
120 inhibitory concentrations (MICs) using a commercial assay (Sensititre Gram-negative MIC  
121 plate, Thermo Scientific) according to manufacturer's instructions. The following antibiotics  
122 were tested: amoxicillin-clavulanate, piperacillin-tazobactam, cefotaxime, ceftazidime,  
123 cefepime, imipenem, meropenem, gentamicin, amikacin, ciprofloxacin, cotrimoxazole,  
124 fosfomycin, tigecycline and colistin.

#### 125 *Chemotaxonomic analysis*



126 The fatty acid methyl ester (FAME) analysis was performed at the Belgian Coordinated  
127 Collections of Microorganisms/Laboratory for Microbiology of the Faculty of Sciences of the  
128 Ghent University (BCCM/LMG), Gent, Belgium. In brief, cells were grown for 24 hours at 35°C  
129 under aerobic conditions on LMG medium 304. Inoculation and harvesting of the cells, and  
130 the extraction and analysis conformed to the recommendations of the commercial MIDI  
131 microbial identification system (Microbial IDentification Inc.). The whole-cell fatty acid  
132 composition was determined by gas chromatography on an Agilent Technologies 6890N gas  
133 chromatograph. The peak naming table MIDI TSBA 5.0 was used.

#### 134 *DNA extraction and 16S rRNA phylogenetic analysis*

135 DNA was isolated from UR159<sup>T</sup> using the using the Quick-DNA fungal/bacterial miniprep kit  
136 (Zymo Research, Irvine, CA) according to the manufacturer's recommendations. The 16S  
137 rRNA gene of UR159<sup>T</sup> was amplified by PCR and sequenced using the universal primers 27F  
138 and 1541R, corresponding to base positions 8-27 and 1541-1525 of the 16S rRNA gene of  
139 *Escherichia coli*, respectively [9]. Both LeBibi-QBPP and NCBI nucleotide collection (nr/nt)  
140 databases were used to identify the nearest neighbor taxa with validly published names  
141 [10,11]. A sequence alignment using 16S rRNA gene sequences was constructed with the  
142 MUSCLE software [12]. All sequences with less than 95% coverage were eliminated, resulting  
143 in a final dataset of 139 sequences (consensus sequence = 1,255 bp). The phylogenetic tree  
144 was constructed using MEGA X version 10.7.1 with neighbor-joining (NJ) and maximum-  
145 likelihood (ML) methods [13]. Branch positions were statically tested with a bootstrap of  
146 1,000 replicates.

#### 147 *Genome sequencing, assembly and annotation*

148 After mechanical DNA shearing (Covaris® ultrasonicator), the DNA libraries were prepared  
149 using the NEBNext® Ultra™ DNA Library Prep Kit for Illumina® (New England Biolabs) and

150 sequenced as paired-end reads (2 x 300 bp) using the Illumina MiSeq platform and MiSeq  
151 reagent kit v3.

152 The Illumina reads were trimmed using Trimmomatic v0.32 [14], quality filtered with the  
153 Fastx-toolkit ([http://hannonlab.cshl.edu/fastx\\_toolkit/](http://hannonlab.cshl.edu/fastx_toolkit/)) and assembled using SPAdes v3.14.0  
154 that includes plasmidSPAdes [15,16]. Note that contigs <1,000 bp were discarded from the  
155 assembly and chromosomal contigs were then scaffolded using the SIS [17] and GapFiller  
156 v1.10 [18] softwares with *Flavobacterium marinum* CGMCC 1.10825<sup>T</sup> (GenBank accession  
157 number NZ\_FNXE00000000.1) as the reference genome sequence.

158 Genome annotation was performed by the NCBI Prokaryotic Genome Annotation Pipeline  
159 (PGAP) ([www.ncbi.nlm.nih.gov/genome/annotation\\_prok/](http://www.ncbi.nlm.nih.gov/genome/annotation_prok/)). For mobile genetic elements,  
160 prophages were predicted using PHAST [19] completed by the Actinobacteriophage  
161 database (<https://phagesdb.org>) and plasmids were searched using the online plasmid  
162 search tool (<https://plasmid.med.harvard.edu/PLASMID/>). Functional analysis was  
163 performed using RPSBLAST program on COG database implemented in WebMGA  
164 (<http://weizhongli-lab.org/webMGA>).

#### 165 *Comparative genomics and phylogenomic analysis*

166 To confirm the status as a new taxon of UR159<sup>T</sup>, Average Nucleotide Identity (ANI), in silico  
167 DNA-DNA hybridization (isDDH), Average Amino Acid Identity (AAI) values were determined.  
168 The calculation of the ANI between strain UR159<sup>T</sup> and 343 genomes of *Flavobacteriaceae*-  
169 related species was performed using BLASTN and Pyani v0.2.10 module  
170 (<https://pypi.org/project/pyani/>) [20], following the algorithm described by Goris *et al.* [21].  
171 According to Pyani and 16S rRNA gene similarities, 25 available *Flavobacteriaceae* genomes  
172 were selected and downloaded from GenBank for comparative genomic analysis. The  
173 protein coding sequences were predicted for all genomes by using Prokka v1.14.5 [22]. The

174 estimation of AAI was determined using the tool AAI calculator ([http://enve-](http://enve-omics.ce.gatech.edu/)  
175 [omics.ce.gatech.edu/](http://enve-omics.ce.gatech.edu/)) [23]. The isDDH values were calculated with the same data set for ANI  
176 calculation using the Genome-to-Genome Distance calculator (GGDC 2.1;  
177 <https://ggdc.dsmz.de/>) with the formula 2. Pangenomic analysis of the 26 genomes was  
178 conducted using PIRATE software [24]. To calculate phylogenomic relations between strain  
179 U159<sup>T</sup> and phylogenetic neighbours, a core-genome tree was constructed as follows. All  
180 predicted protein-coding genes annotated from each available genome were compared  
181 using the all-versus-all BLAST search [25]. Only proteins for which coding sequences with  
182 >50% amino acid identity and >70% sequence coverage were considered as orthologous. The  
183 nucleotide sequences of core gene set were aligned using MUSCLE software and MEGA6  
184 software [26] was used for the phylogenomic tree reconstruction using the neighbour-  
185 joining method with Jukes-Cantor correction.

#### 186 *Sequence accession numbers*

187 The 16S rRNA sequence generated was submitted to GenBank with the accession number  
188 MF278923. The draft genome sequence of UR159<sup>T</sup> was deposited in NCBI under the  
189 accession number NZ\_NIPO000000000.

190

## 191 **Results and discussion**

192 Strain UR159<sup>T</sup> was not identified by the MALDI-TOF mass spectrometry using the Bruker  
193 database (version 2.3) but the profile was reproducible (Figure S1). The almost-complete  
194 sequence of the 16S rRNA gene was obtained (1,516 bp) and used for sequence comparison  
195 and phylogenetic analysis. Strain UR159<sup>T</sup> exhibited the highest level of nucleotide pairwise  
196 similarity (93.1%) with *Flavobacterium marinum* SW105<sup>T</sup> [27]. Sequence similarity was lower  
197 with the next closest relatives (*Flavobacterium* spp., *Myroides* spp.) with percentages of 16S  
198 rRNA gene sequence similarity in all cases lower than 92.9%. The ML and NJ phylogenetic  
199 trees were almost identical and both confirmed the affiliation of UR159<sup>T</sup> to the  
200 *Flavobacteriaceae* (Figure 1). The sequence similarity between the 16S rRNA gene of UR159<sup>T</sup>  
201 and the closest phylogenetic neighbors, which was distinctly below the predetermined cut-  
202 off levels in order to classify bacterial isolates as novel taxa at the genus (<95%) and (<97-  
203 98.65%) species levels [7,8,28,29], suggested that the strain was a potentially undescribed  
204 microorganism requiring further characterization.

### 205 *Phenotypic properties*

206 Strain UR159<sup>T</sup> grew optimally on BA after 24-h incubation at 35°C under ambient air, yielding  
207 ~1-3-mm, yellow-pigmented, circular, convex, smooth colonies (Figure S2). No growth was  
208 observed when incubated anaerobically and it was demonstrated UR159<sup>T</sup> was strictly  
209 aerobic (Figure S2). It failed to grow on Drigalski, cetrinide and Hektoen agar (data not  
210 shown). It was a non-motile, oxidase-negative, catalase-positive Gram-negative rod (Figure  
211 2A) able to grow between 15°C and 37°C. TEM confirmed that cells of UR159<sup>T</sup> were rod-  
212 shaped bacteria measuring an average size of 0.35 ± 0.02 µm wide and 1.12 ± 0.16 µm long  
213 (Figure 2B). Biochemically, it was metabolically rather inert since almost all reactions were  
214 negative (especially indole production, Voges-Proskauer, nitrate reduction, H<sub>2</sub>S production

215 and urease tests) except of the production of gelatinase, and presented some differences  
216 with species of closely-related genera (Table 1) [27,30-33]. Furthermore, strain UR159<sup>T</sup>  
217 appeared susceptible to amoxicillin-clavulanate (MIC <2/2 mg/L), ciprofloxacin (MIC = 0.25  
218 mg/L), cotrimoxazole (MIC = 2/38 mg/L) and tigecycline (MIC = 0.5 Mg/L) whereas MICs  
219 were higher for piperacillin-tazobactam (MIC = 8 mg/L), cefotaxime (MIC >4 mg/L),  
220 ceftazidime (MIC = 128 mg/L), cefepime (MIC = 32 mg/L), imipenem (MIC = 4 mg/L),  
221 meropenem (MIC = 8 mg/L), gentamicin (MIC >4 mg/L), amikacin (MIC >16 mg/L),  
222 fosfomycin (MIC >64 mg/L) and colistin (MIC >8 mg/L).

223 The cellular fatty acids of strain UR159<sup>T</sup> mainly comprised iso-branched fatty acids,  
224 predominantly iso-C<sub>15:0</sub> (55.5%), iso-C<sub>17:1</sub>ω<sub>9c</sub> (8.8%) iso-C<sub>17:0</sub> 3-OH and summed feature 3  
225 (comprising C<sub>16:1</sub>ω<sub>9c</sub> and/or iso-C<sub>15:0</sub> 2-OH) (Table 2). Strain UR159<sup>T</sup> and *F. marinum* SW105<sup>T</sup>  
226 shared similar fatty acid profiles, with minor differences in the respective proportions of the  
227 components (Table 2) [27].

### 228 *Genomic features*

229 The draft genome size of UR159<sup>T</sup> was 2,331,799 bp long (3 scaffolds with a final coverage of  
230 124×) (Table 3 and Figure S3), which is smaller than those of most other members of the  
231 *Flavobacteriaceae* family (Table 4). Based on whole genome sequence, the genomic DNA  
232 G+C content was determined at 37.6 mol%, which is consistent with those of the type strains  
233 of closely related genera (31.4-44.2 mol%) (Tables 3 and 4). The genome encoded a total of  
234 2,262 putative genes, including 2,176 protein-coding genes and 61 RNAs (50 tRNAs, 7 rRNAs  
235 and 4 ncRNAs) and 25 pseudogenes (Table 3). A total of 1,645 genes (75.5%) were assigned  
236 to a COG functional category with 1406 genes (64.5% of the total genes) with known  
237 putative function, and 773 genes were annotated as hypothetical proteins (35.5%) (Table  
238 S1). Whereas no extrachromosomal element was found, a 6.5-kb prophage-like element was

239 found (Figure S3), showing sequence homology to the phage RAP44 of *Riemerella*  
240 *anatipestifer* [34].

#### 241 *Comparative genomic analysis*

242 Phylogenomic analysis revealed a lack of relatives at the species or genus level, with ANI  
243 values between strain UR159<sup>T</sup> and the most closely related members of the  
244 *Flavobacteriaceae* between 69.7 and 74.0% (alignment coverage from 7.0% to 29.0%) while  
245 those of isDDH ranged from 17.9 to 38.7% (Table 4 and Figures S4). These calculated  
246 similarity values were below the proposed and generally accepted species boundaries for  
247 ANI and isDDH that are 95-96% and 70%, respectively [8,35]. These results indicate that  
248 strain UR159<sup>T</sup> constitutes a novel taxon at least at the species level, not related at this level  
249 to any of the closest phylogenetic neighbors, which can be visualized with the core-genome  
250 tree (Figure 3). Additionally, species that share less than 80% ANI are too divergent to be  
251 compared only based on this parameter, and AAI, which provides a more robust resolution,  
252 should be used instead. Thus, AAI percentages were also estimated with a threshold AAI  
253 value of 65%, which has been proposed for genus delineation for uncultivated prokaryotes  
254 [29]. AAI values for strain UR159<sup>T</sup> and the most closely related species were in all cases  
255 lower than 65% (Table 4), except for *F. marinum* that is just at the limit (65.1%) (Table 4 and  
256 Figure S5). By pangenomic analysis, only 472 orthologous genes (20.8%) were determined to  
257 be encoded in all genomes whereas 514 genes (22.7%) were predicted to be unique to strain  
258 UR159<sup>T</sup> compared to other genomes tested. The complete genome of UR159<sup>T</sup> shared  
259 between 761 (35.5%) to 1403 (65.4%) common gene families with *Weeksella virosa* and *F.*  
260 *marinum* genomes, respectively (Table 4).

261 In conclusion, taking into account all the findings from phenotypic, chemotaxonomic,  
262 phylogenetic, and genomic analysis, strain UR159<sup>T</sup> cannot be allocated to any genus and

263 species previously described. Hence, this strain represents a novel taxon within the  
264 *Flavobacteriaceae* family, for which the name *Avrilella dinanensis* gen. nov., sp. nov. is  
265 proposed. The descriptions of the *Avrilella* gen. nov. and *Avrilella dinanensis* sp. nov. are  
266 given in Table 5.

267

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270

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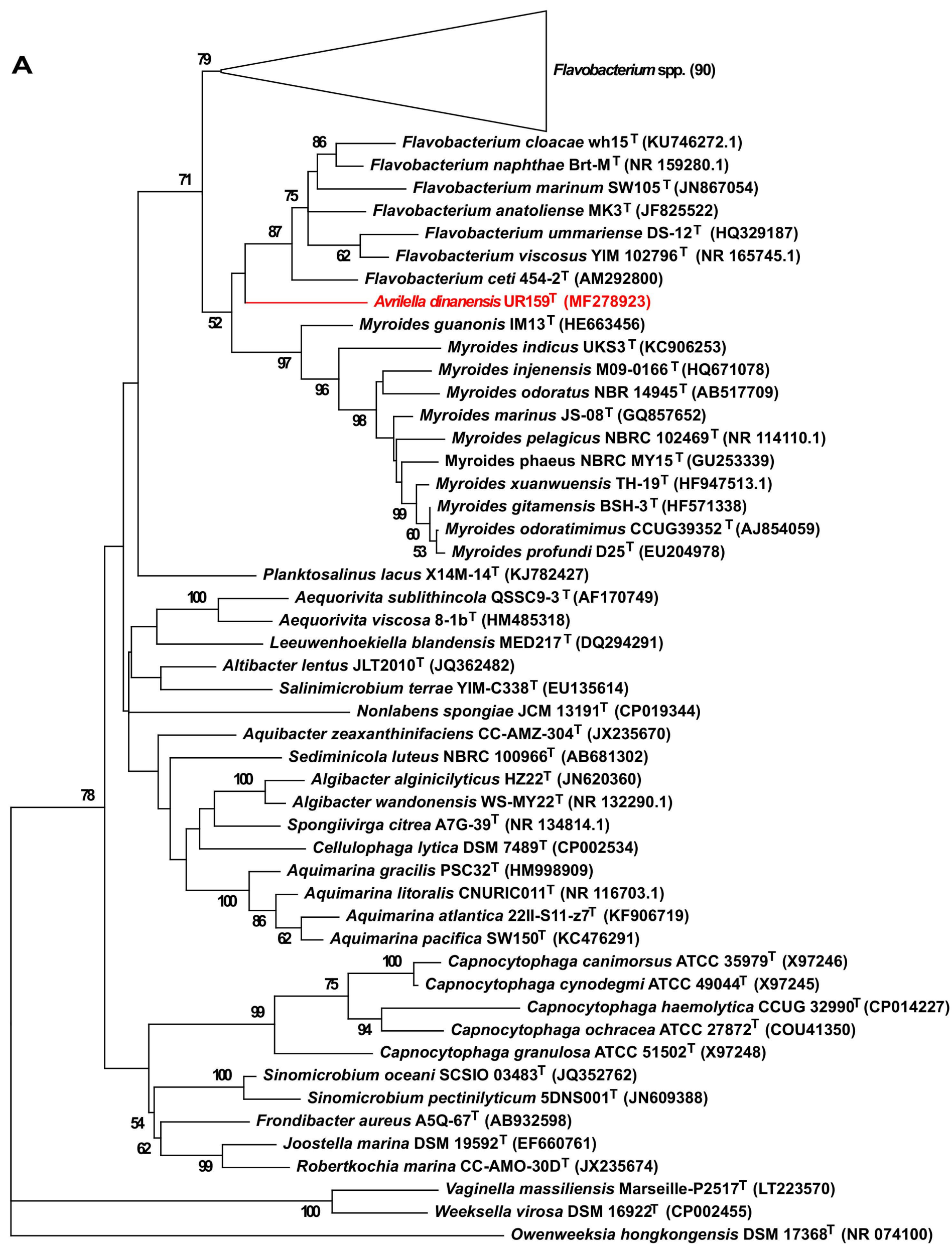
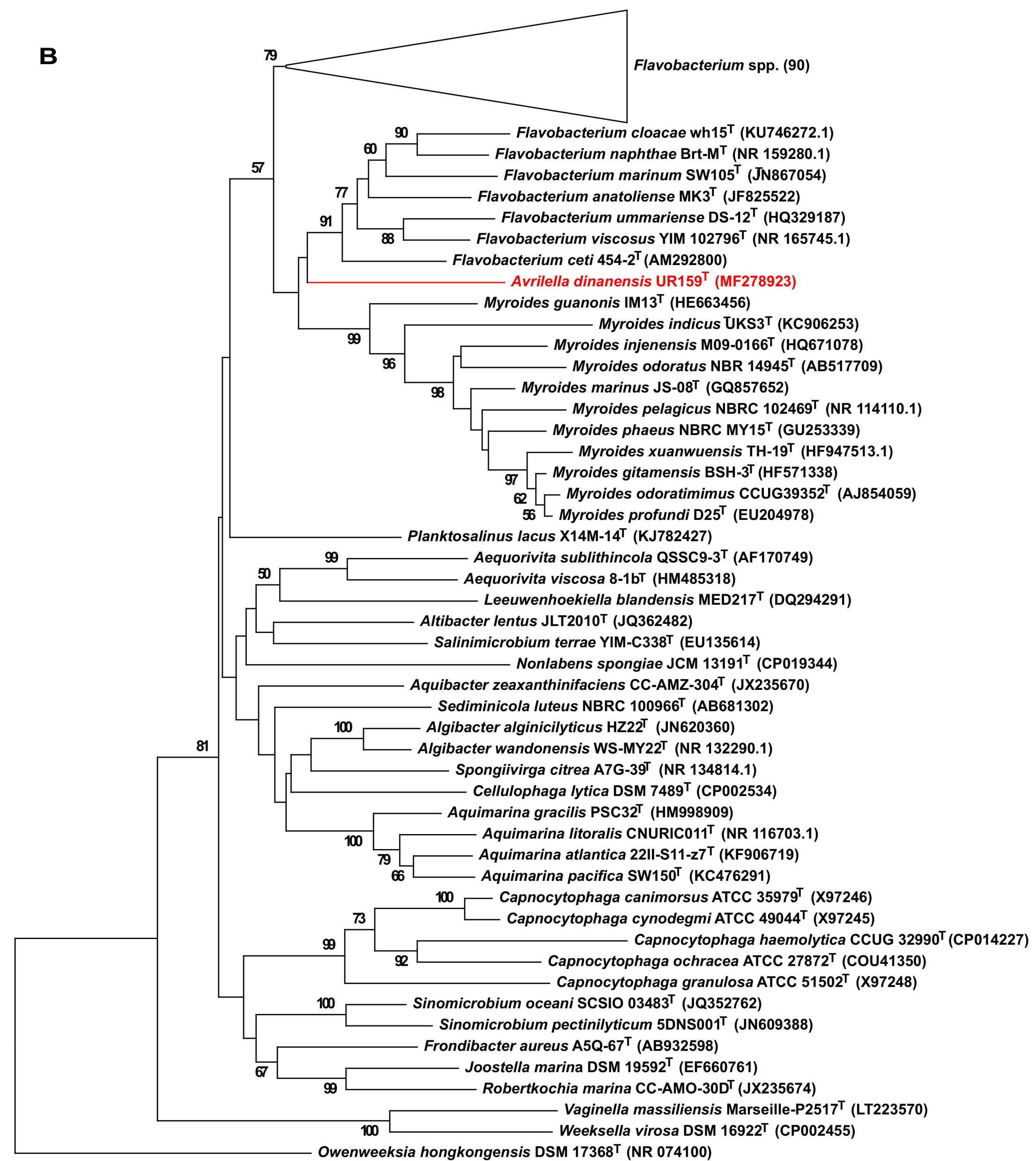
351

352 **Legends of the figures**

353 **Figure 1.** Maximum-likelihood (A) and neighbor-joining (B) trees based on the alignment of  
354 1,255 bp of 16S rRNA gene sequences, showing the phylogenetic position of strain U159<sup>T</sup>  
355 among the *Flavobacteriaceae* family. Bootstrap values higher than 50% are indicated at  
356 branch points. The strain *Owenweeksia hongkongensis* DSM 17368<sup>T</sup> was used as an  
357 outgroup. Sequence accession numbers are shown in parentheses. Scale bars: 0.1 or 0.02  
358 substitutions per nucleotide position.

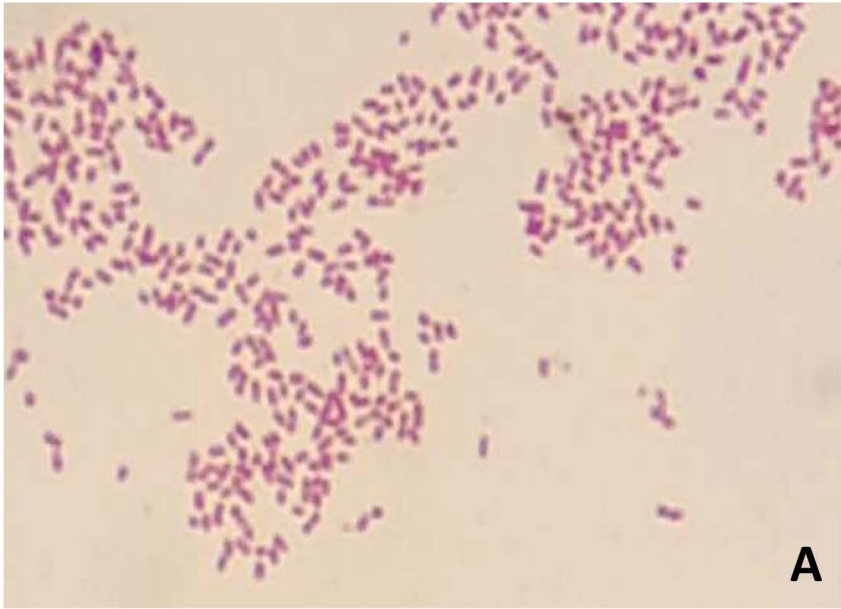
359 **Figure 2.** (A) Gram staining image (at × 1000 magnification) and (B) transmission electron  
360 microscopy (scale bar = 0.5 μm) of *Avrillella dinanensis* UR159<sup>T</sup>.

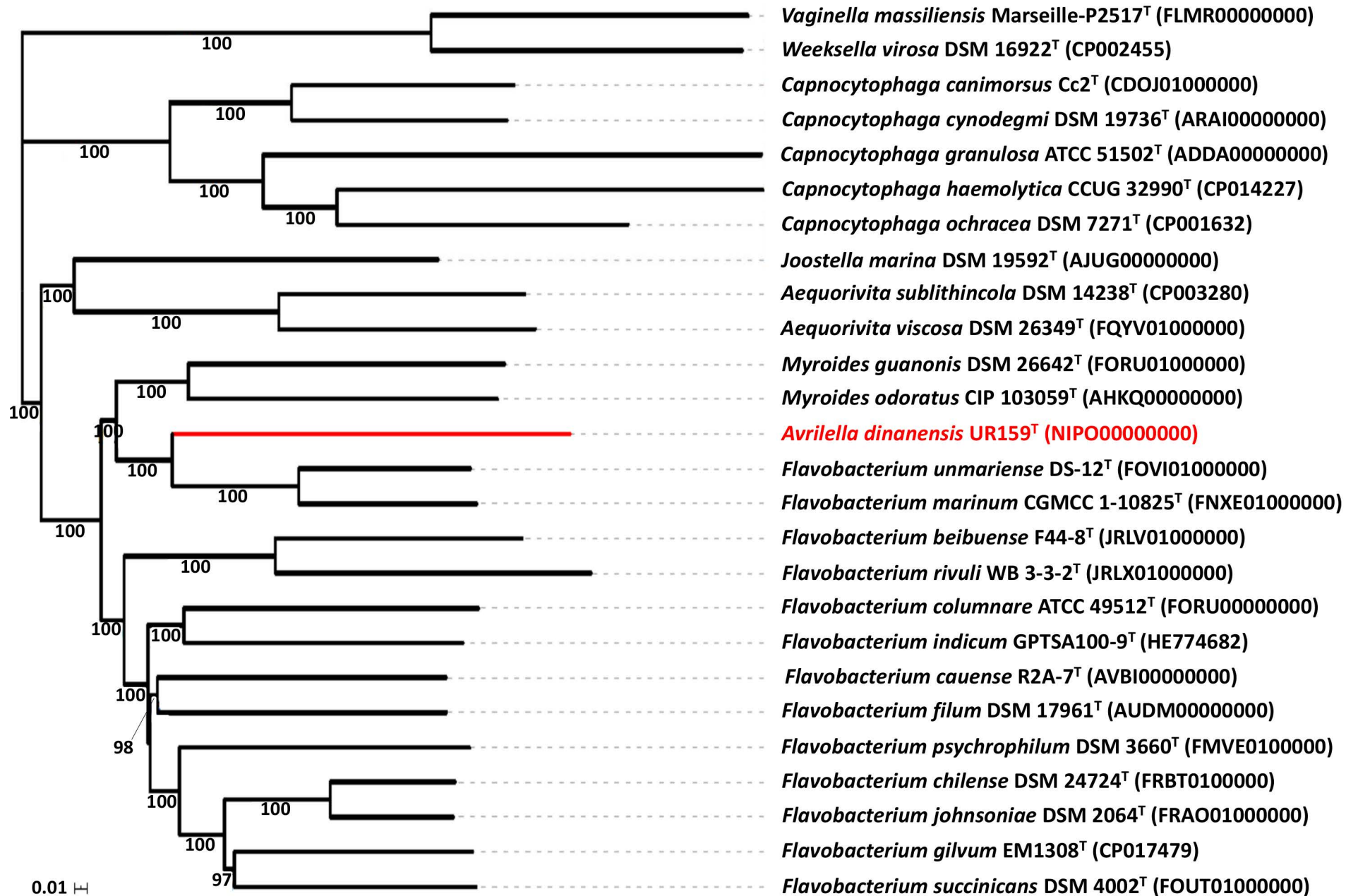
361 **Figure 3.** Neighbour-joining core gene phylogenetic tree including genomes of 26 members  
362 *Flavobacteriaceae* family. Bootstrap values higher than 60% are indicated at indicated at  
363 branch points. Scale bar: 0.01 substitution per nucleotide position.

**A****B**

0.10

0.020





**Table 1.** Differential characteristics of strain UR159<sup>T</sup> and type strains of closely related genera [20,23-26].

Characteristic	<i>Avrilella</i> <i>dinanensis</i> UR159 <sup>T</sup>	<i>Flavobacterium</i> <i>marinum</i> SW105 <sup>T</sup>	<i>Flavobacterium</i> <i>unmariense</i> DS-12 <sup>T</sup>	<i>Myroides</i> <i>guanonis</i> IM13 <sup>T</sup>
Colony pigmentation	Yellow	Yellow	Yellow	Yellow
Growth at:				
15°C	+	+	+	+
30°C	+	+	+	+
37°C	+	+	+	+
42°C	-	+	-	-
Gliding motility	-	-	-	-
Respiratory metabolism	Strictly aerobic	Strictly aerobic	Strictly aerobic	Strictly aerobic
Oxidase	+	+	+	+
Catalase	+	+	+	+
<b>Substrates utilized:</b>				
D-glucose	-	-	-	-
D-mannose	-	+	+	-
Maltose	-	+	+	-
L-arabinose	-	+	-	-
D-Mannitol	-	+	-	-
D-Sorbitol	-	-	-	-
<b>Enzyme activity :</b>				
Arginine dihydrolase	-	-	-	-
Lysine decarboxylase	-	-	nd	-
Ornithine decarboxylase	-	-	nd	-
Gelatinase	+	-	-	+
Urease	-	-	+	+
α-galactosidase	-	-	-	nd
β-galactosidase	-	-	-	-
β-glucosidase	-	-	-	nd
Citrate utilization	-	w	-	+
Indole production	-	-	-	-
Acetoin production (Voges-Proskauer reaction)	-	nd	nd	-
Nitrate reduction	-	-	-	-
H <sub>2</sub> S production	-	-	nd	-

-, negative; nd: not determined; +, positive; w, weakly positive.

**Table 2.** Cellular fatty acid compositions of *Avrilella dinanensis* UR159<sup>T</sup> gen. nov. sp. nov. and *Flavobacterium marinum* SW105<sup>T</sup> [20]

<b>Fatty acid (%)</b>	<b><i>A. dinanensis</i> UR159<sup>T</sup></b>	<b><i>F. marinum</i> SW105<sup>T</sup></b>
iso-C <sub>14:0</sub>	-	-
iso-C <sub>15:0</sub>	55.5	52.7
C <sub>16:0</sub>	2.2	-
iso-C <sub>15:0</sub> 3-OH	3.2	-
iso-C <sub>16:0</sub>	-	1.1
iso-C <sub>17:1</sub> ω <sup>9</sup> c	8.8	13.1
iso-C <sub>15:0</sub> 3-OH	-	2.5
iso-C <sub>16:0</sub> 3-OH	1.4	-
C <sub>18:1</sub> ω <sup>9</sup> c	1.5	-
iso-C <sub>17:0</sub> 3-OH	4.0	11.4
Unknown 13.565	8.9	-
Unknown 16.582	2.5	-
Summed feature 3	4.9	15.1
Summed feature 4	1.7	-

The fatty acids amounting to <1% of the total fatty acids are not shown. Summed feature 3 comprises (C<sub>16:1</sub> ω<sup>7</sup>c and/or iso-C<sub>15:0</sub> 2-OH) and summed feature 4 comprises (iso-C<sub>17:1</sub> I and/or anteiso-C<sub>17:1</sub> B). -, not detected or Trace (<1% of total).



**Table 3.** Genomic characteristics of *Avrilella dinanensis* UR159<sup>T</sup> gen. nov. sp. nov.

<b>Characteristic</b>	<b><i>A. dinanensis</i> UR159<sup>T</sup></b>
Genbank accession no.	NZ_NIPO00000000
Genome size	2,331,799
No. scaffolds (sizes)	3 (2,273,643 bp; 5,646 bp; 52,510 bp)
G+C content (%)	37.6
Total genes	2,262
Protein-coding genes	2,176
No. hypothetical proteins	1,183
rRNAs	7
tRNAs	50
ncRNAs	4
Pseudogenes	25
Extrachromosomal elements	0

**Table 4.** Relatedness of the sequenced genome of *Avrilella dinanensis* UR159<sup>T</sup> gen. nov. sp. nov. to whole genome sequences of types strains of closely related genera based on genome size, G+C content, ANI, in silico DDH, AAI and orthologous proteins.

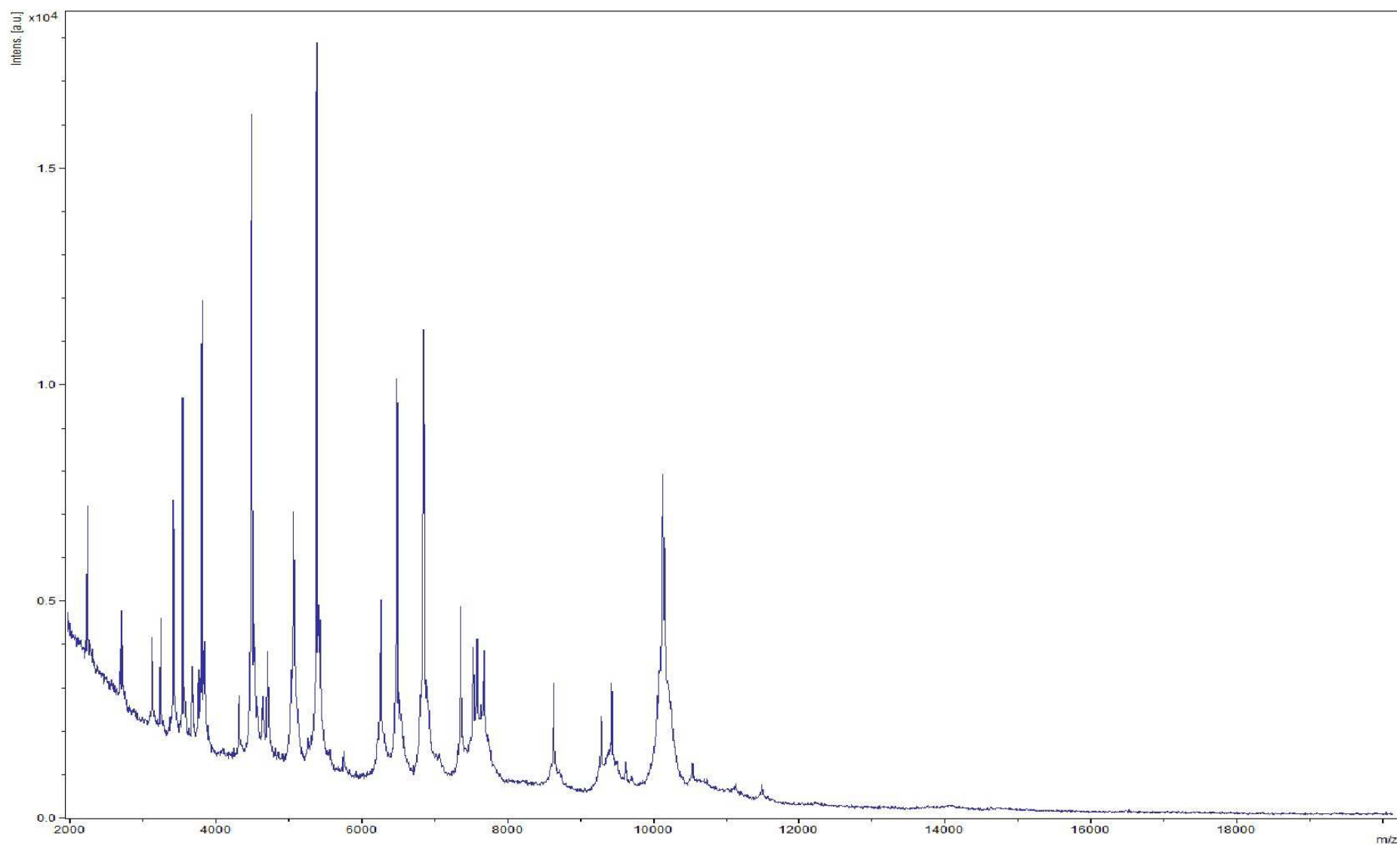
Strain	Genbank accession no.	Size	%						
			G+C	Mean ANI identity	Mean ANI coverage	In silico DDH	Mean AAI identity	Mean AAI coverage	Orthologous proteins
<i>Avrilella dinanensis</i> UR159 <sup>T</sup>	NZ_NIPO00000000.1	2,331,799	37.7	100.0	100.0	100.0	100.0	100.0	100.0
<i>Aequorivita sublithicola</i> DSM 14238 <sup>T</sup>	CP003280.1	3,520,671	36.2	70.7	11.2	23.5	54.5	68.0	47.2
<i>Aequorivita viscosa</i> DSM 26349 <sup>T</sup>	FQYV01000000.1	3,526,954	36.5	70.5	10.5	38.7	54.6	69.0	48.1
<i>Capnocytophaga canimorsus</i> Cc2 <sup>T</sup>	CDOJ01000000.1	2,504,412	36.1	71.4	10.7	20.5	54.4	58.1	39.6
<i>Capnocytophaga cynodegmi</i> DSM 19736 <sup>T</sup>	ARAI00000000.1	2,657,104	34.4	71.5	10.2	21.5	54.5	57.9	39.7
<i>Capnocytophaga granulosa</i> ATCC 51502 <sup>T</sup>	ADDA00000000.1	2,746,278	41.4	69.7	7.3	19.7	52.2	57.5	37.2
<i>Capnocytophaga haemolytica</i> CCUG 32990 <sup>T</sup>	CP014227.1	2,688,484	44.2	70.1	7.0	27.9	53.4	54.7	37.3
<i>Capnocytophaga ochracea</i> DSM 7271 <sup>T</sup>	CP001632.1	2,612,925	39.6	70.8	8.6	25.9	53.5	56.1	37.4
<i>Flavobacterium beibuense</i> F44-8 <sup>T</sup>	JRLV01000000.1	3,800,758	37.7	71.2	14.8	19.2	58.3	73.5	54.1
<i>Flavobacterium cauense</i> R2A-7 <sup>T</sup>	AVBI00000000.1	3,110,902	38.2	72.4	18.4	17.9	60.2	71.1	54.9
<i>Flavobacterium chilense</i> DSM 24724 <sup>T</sup>	FRBT0100000.1	6,113,833	33.7	72.0	13.4	20.3	57.4	76.2	55.5
<i>Flavobacterium columnare</i> ATCC 49512 <sup>T</sup>	FORU00000000.1	3,162,432	31.5	72.0	15.7	21.8	58.8	65.6	51.0
<i>Flavobacterium filum</i> DSM 17961 <sup>T</sup>	AUDM00000000.1	3,192,093	33.8	72.6	16.4	20.2	59.3	66.6	50.6
<i>Flavobacterium gilvum</i> EM1308 <sup>T</sup>	CP017479.1	4,402,594	35.2	72.5	13.3	21.3	57.7	69.0	50.7
<i>Flavobacterium indicum</i> GPTSA100-9 <sup>T</sup>	HE774682.1	2,993,089	31.4	72.2	16.8	18.9	58.1	68.4	51.5
<i>Flavobacterium johnsoniae</i> DSM 2064 <sup>T</sup>	FRAO01000000.1	6,052,484	34.0	71.9	13.2	19.9	57.6	76.7	55.9
<i>Flavobacterium marinum</i> CGMCC 1-10825 <sup>T</sup>	FNXE01000000.1	2,977,689	35.4	74.0	29.0	22.5	65.1	80.1	65.4
<i>Flavobacterium psychrophilum</i> DSM 3660 <sup>T</sup>	FMVE0100000.1	2,626,144	32.3	71.7	16.5	19.0	59.0	64.7	50.6
<i>Flavobacterium rivuli</i> WB 3-3-2 <sup>T</sup>	JRLX01000000.1	4,479,972	39.6	70.7	12.1	18.2	57.3	73.6	54.0
<i>Flavobacterium succinicans</i> DSM 4002 <sup>T</sup>	FOUT01000000.1	3,664,904	35.5	71.8	14.0	18.8	57.5	68.8	51.0
<i>Flavobacterium ummariense</i> DS-12 <sup>T</sup>	FOVI01000000.1	3,493,887	34.7	73.1	25.9	19.8	63.8	80.0	64.3
<i>Joostella marina</i> DSM 19592 <sup>T</sup>	AJUG00000000.1	4,508,243	33.6	71.1	10.8	19.6	55.2	68.0	46.9
<i>Myroides guanonis</i> DSM 26542 <sup>T</sup>	FORU01000000.1	3,140,132	33.3	71.3	19.6	18.1	60.5	73.5	58.2
<i>Myroides odoratus</i> CIP 103059T	AHKQ00000000.1	4,227,641	35.5	71.8	16.4	19.9	58.8	75.6	56.8
<i>Vaginella massiliensis</i> Marseille-P2517 <sup>T</sup>	FLMR00000000.1	2,434,476	38.2	72.0	9.6	22.5	51.7	61.9	36.9
<i>Weeksella virosa</i> DSM 16922 <sup>T</sup>	CP002455.1	2,272,954	35.9	71.9	9.8	26.4	51.1	63.9	35.5

**Table 5.** Protologue for *Avrilella dinanensis*.

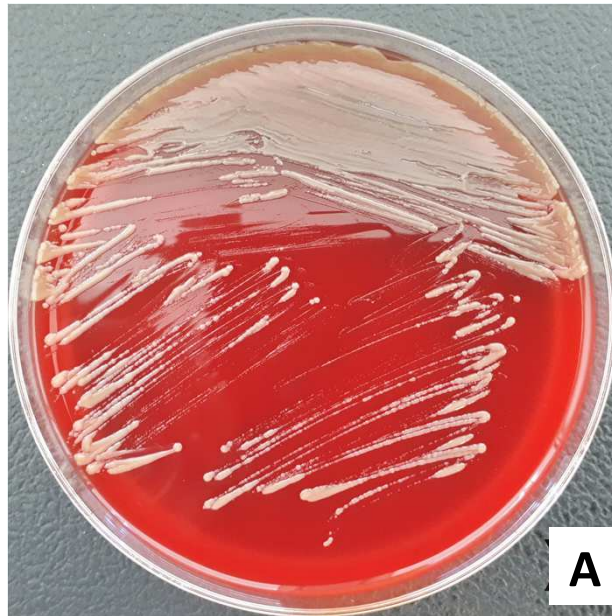
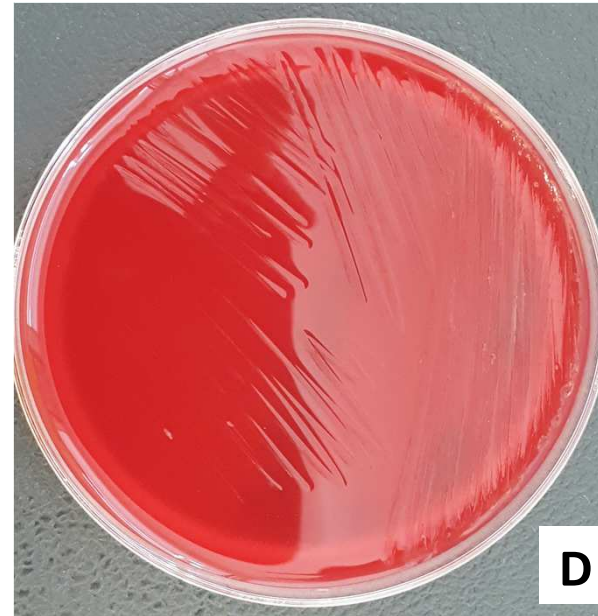
Genus name	<i>Avrilella</i>	
Species name		<i>Avrilella dinanensis</i>
Genus status	gen. nov.	
Genus etymology	[A.vril.el'la. N.L. dim. suff. -ella. <i>Avrilella</i> , named after the French bacteriologist and writer Jean-Loup Avril, Rennes, France]	
Type species of the genus	<i>Avrilella dinanensis</i>	
Specific epithet	-	<i>dinanensis</i>
Species status	sp. nov.	
Species etymology	<i>Avrilella dinanensis</i> (di.nan.en'sis. N.L. fem. adj. <i>dinanensis</i> , of Dinan, after the name of the city in Brittany where the strain was originally isolated)	
Description of the new taxon and diagnostic traits	Gram-negative, aerobic rods that grow as yellow-pigmented, circular, convex, smooth colonies. Strains are positive for catalase and oxidase, and metabolically rather inert except for a gelatinase activity. Major fatty acids are iso-branched fatty acids, predominantly iso-C15:0, iso-C17:1 <i>ω</i> 9c and iso-C17:0 3-OH. The genus is affiliated to the family 'Flavobacteriaceae', order 'Flavobacteriales', class 'Flavobacteriia'.	Non-motile, oxidase-negative, catalase-positive Gram-negative rod able to grow between 15°C and 37°C. Strictly aerobic growing optimally on blood agar after 24-h incubation at 35°C under ambient air, yielding ~1-3-mm, yellow-pigmented, circular, convex, smooth colonies. Metabolically rather inert, except the production of gelatinase. Susceptibility to amoxicillin-clavulanate (MIC, 0.5 mg/L), ceftazidime (MIC, 1.5 mg/L), ertapenem (MIC, 1.5 mg/L) imipenem (MIC, 2 mg/L), meropenem (MIC, 2 mg/L), ciprofloxacin (MIC, 0.19 mg/L), cotrimoxazole (MIC 0.094 mg/L) and tigecycline (MIC, 0.5 Mg/L) but resistant to cefotaxime (MIC >32 mg/L), ceftazidime (MIC >32 mg/L), cefepime (MIC, 16 mg/L), aminoglycosides (MICs >16 mg/L), fosfomycin (MIC >64 mg/L) and colistin (MIC >8 mg/L).
Country of origin	France	
Region of origin	Brittany	
Date of isolation	01/09/2016	
Source of isolation	Human blood	
Sampling date	01/09/2016	
Latitude	48° 27' 0" N	
Longitude	2° 2' 49.2" W	
Altitude (meters above sea level)	76 m	
16S rRNA gene accession number	MF278923	
Genome accession number	NZ_NIPO00000000	
Genome size (bp)	2,331,799	
GC mol%	37.6	
Number of strains in the study	1	
Information related to the Nagoya protocol	Not applicable	
Designation of the type strain	UR159 <sup>T</sup>	
Strain collection numbers	CIP 111616 <sup>T</sup> = DSM 105483 <sup>T</sup>	

**Table S1.** Proportions of genes associated with general COG functional categories.

<b>Code</b>	<b>% of total genes</b>	<b>% of unique genes</b>	<b>Description</b>
J	9,54	0,84	Translation, ribosomal structure and biogenesis
K	5,48	10,56	Transcription
L	5,92	7,69	Replication, recombination and repair
D	1,25	0,76	Cell cycle control, cell division, chromosome partitioning
V	2,23	1,75	Defense mechanisms
T	3,42	0,00	Signal transduction mechanisms
M	9,40	12,97	Cell wall/membrane/envelope biogenesis
N	0,42	0,61	Cell motility
U	2,42	0,90	Intracellular trafficking, secretion, and vesicular transport
O	5,26	6,59	Posttranslational modification, protein turnover, chaperones
C	6,24	1,23	Energy production and conversion
G	3,18	2,86	Carbohydrate transport and metabolism
E	8,13	5,06	Amino acid transport and metabolism
F	3,49	1,01	Nucleotide transport and metabolism
H	5,30	4,05	Coenzyme transport and metabolism
I	5,29	1,67	Lipid transport and metabolism
P	6,38	7,85	Inorganic ion transport and metabolism
Q	2,13	1,68	Secondary metabolites biosynthesis, transport and catabolism
R	14,52	21,00	General function prediction only
S	8,29	15,16	Function unknown



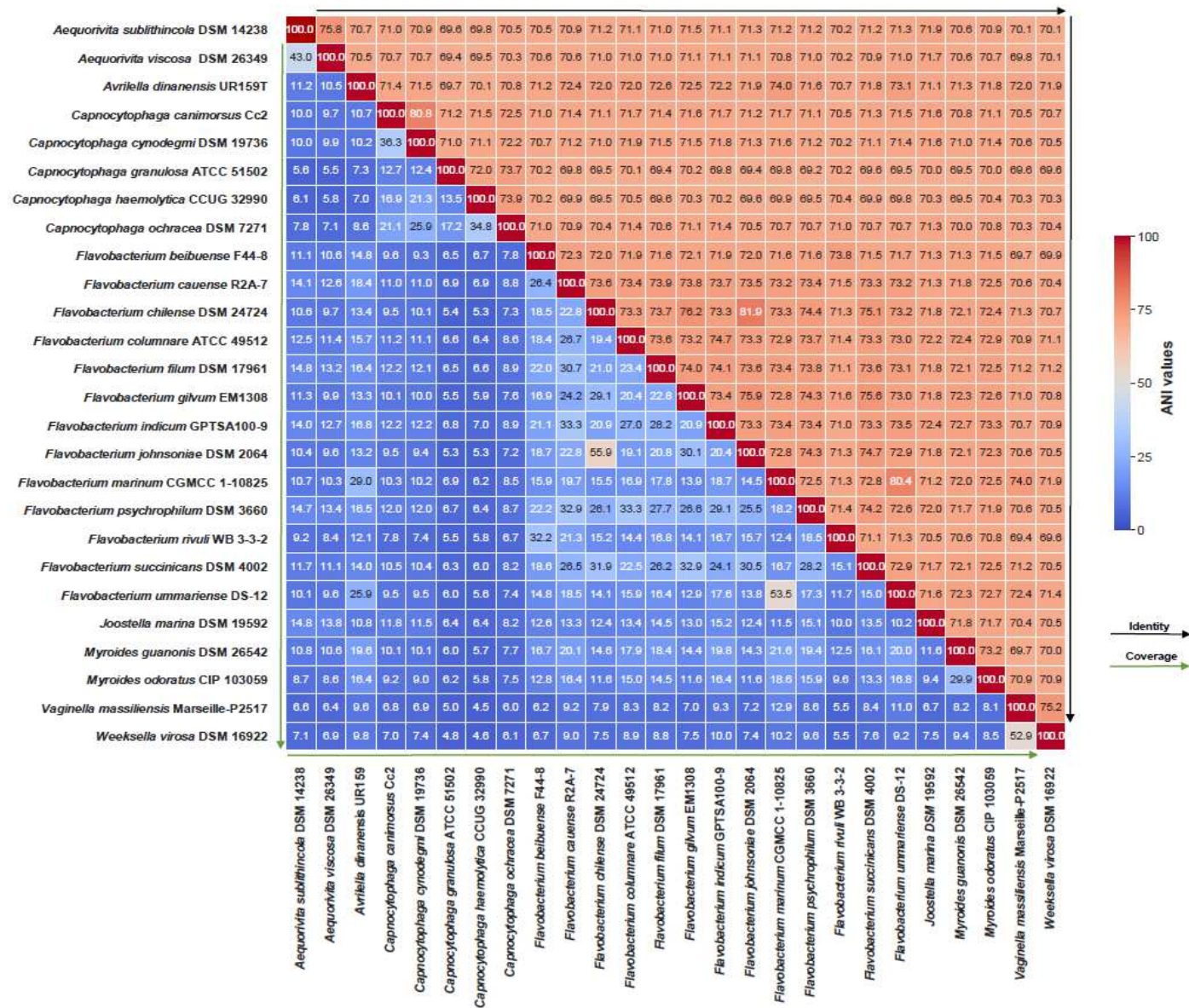
**Figure S1.** Mass spectrum from *Avriella dinanensis* UR159<sup>T</sup> obtained with the Microflex MALDI-TOF instrument (Bruker Daltonics).

**A****B****C****D**

**Figure S2.** Growth of strain UR159<sup>T</sup> on blood agar after 24-h incubation at 35°C under (A) ambient air, 5% CO<sub>2</sub> atmosphere (B), microaerophily (C), and anaerobiosis (D).

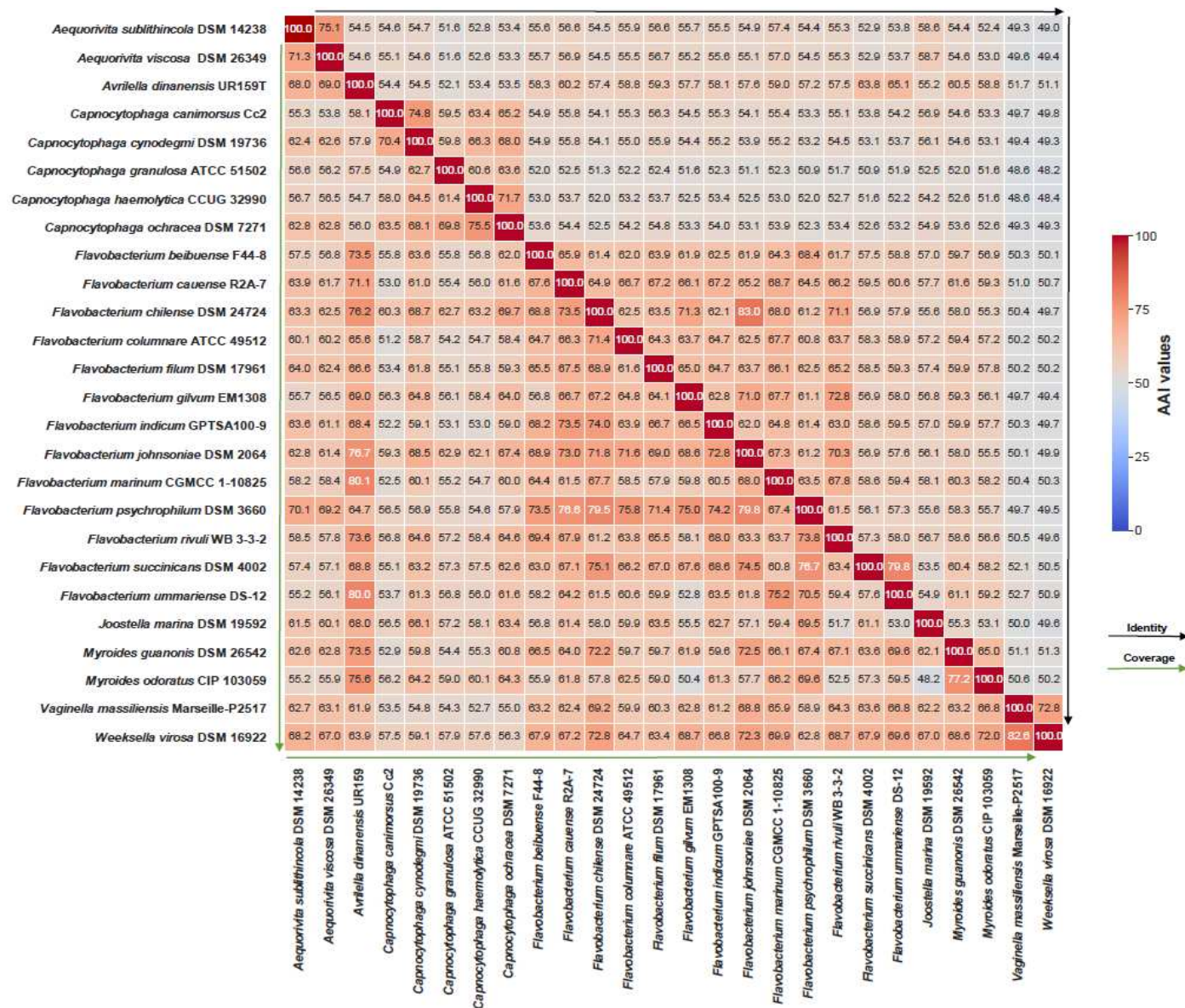


**Figure S3.** Graphical circular map of UR159<sup>T</sup> genome. Seven rings, outwards: (1) nucleotide positions, (2) G+C %mol content, (3) G+C skew, (4) Assembly coverage, (5) scaffolds, (6) RNA sequences, and (7) annotated genes (orange) versus unknown proteins (blue)



**Figure S4.** Average nucleotide identity (ANI) and average nucleotide coverage (ANC) percentages between strain UR159<sup>T</sup> and the most closely related members of the *Flavobacteriaceae* family. ANI (black arrows) and ANC (green arrows) are represented by heat maps, where similarity percentages are represented by the color key histograms on the right panel.





**Figure S5.** Average amino acid identity (AAI) and average amino acid coverage (AAC) percentages between strain UR159<sup>T</sup> and the most closely related members of the *Flavobacteriaceae* family. AAI (black arrows) and AAC (green arrows) are represented by heat maps, where similarity percentages are represented by the color key histograms on the right panel.