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## **Eudoraea adriatica gen. nov., sp. nov., a novel marine bacterium of the family Flavobacteriaceae**

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1 *Eudoraea adriatica* gen. nov., sp. nov.,  
2 a novel marine bacterium of the family *Flavobacteriaceae*

3  
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15  
16 Running title: *Eudoraea adriatica* gen. nov. sp. nov.

17  
18 Category: New taxa, *Bacteroidetes*

19  
20 Footnote: The GenBank/EMBL/DDBJ accession number for the 16S rDNA sequence of *Eudoraea*  
21 *adriatica* AS/06/20a<sup>T</sup> is AM745437.

22 A scanning electron micrograph of cells of strain AS06/20a<sup>T</sup> (Fig. S1) and graphs showing  
23 the effects of temperature, pH and salinity on the maximum growth rate ( $\mu_{max}$ ) of the novel  
24 isolate (Fig. S2) are available in IJSEM online.

25  
26 **A novel aerobic, non-motile, Gram-negative bacterium designated AS06/20a<sup>T</sup>, was isolated from**  
27 **coastal waters of the Adriatic Sea and subjected to a polyphasic taxonomic analysis. Cells were rod-**  
28 **shaped and formed non-pigmented punctiform colonies on agar plates. The novel isolate grew**  
29 **heterotrophically on a variety of carbon compounds, including organic acids, carbohydrates, polyols,**

30 amino acids and complex organic substrates. Cells grew at temperature between 15-33°C (optimum  
31 30°C), from pH 6.5 to 8.5 (optimum 7.5-8.0) and between 2 and 6% (w/v) NaCl (optimum 2%). The  
32 dominant fatty acids (> 5%) detected in strain AS06/20a<sup>T</sup> were iso-C<sub>15:1</sub> G, iso-C<sub>15:0</sub>, iso-C<sub>15:0</sub> 3-OH,  
33 iso-C<sub>17:1</sub> ω9c and iso-C<sub>17:0</sub> 3-OH. The major respiratory quinone was MK-6. The G+C content of the  
34 genomic DNA was 38.9 mol%.

35 Phylogenetic analyses based on 16S rRNA gene sequences showed that strain AS06/20a<sup>T</sup> fell within  
36 the family *Flavobacteriaceae*, in the phylum *Bacteroidetes*. Comparative 16S rRNA gene sequence  
37 analysis indicated that strain AS06/20a<sup>T</sup> was most closely related to the genera *Zeaxanthinibacter* and  
38 *Robiginitalea*, sharing 92 to 93% 16S rDNA sequence similarity with representatives of these genera.  
39 It is proposed, from the polyphasic evidence, that the strain should be placed into a novel genus,  
40 *Eudoraea* gen. nov., with *Eudoraea adriatica* sp. nov. as the type species. The type strain is AS06/20a<sup>T</sup>  
41 (= DSM 19308<sup>T</sup> = CIP 109577<sup>T</sup> = OOB 358<sup>T</sup>).

42

43 Bacteria belonging to the phylum *Bacteroidetes* are widely distributed in nature and have been found in  
44 almost every habitat of the biosphere, the lithosphere and the hydrosphere (Bernardet *et al.*, 2002;  
45 Kirchman, 2002). They seem particularly common and abundant in marine surface waters, as indicated by  
46 fluorescence *in situ* hybridization analyses (Glöckner, 1999; Cottrell & Kirchman, 2000a; Simon *et al.*,  
47 2001; Alonso & Pernthaler, 2005). Cultured isolates of *Bacteroidetes* are all heterotrophs and especially  
48 efficient in degrading complex biomacromolecules such as protein, chitin, pectin, agar, starch and cellulose.  
49 In marine ecosystems, the heterotrophic prokaryotes are the main consumers of dissolved organic material  
50 (DOM) and represent a very dynamic compartment in global biogeochemical cycles. Uptake of DOM is the  
51 first step in the microbial loop that ultimately mineralizes over half of the organic matter formed by  
52 photosynthetic bacteria and algae (Cole *et al.*, 1988). Although the importance of DOM uptake is well  
53 recognized, the relative contributions of the different phylogenetic groups to DOM uptake remain to be  
54 elucidated. Because of their abundance and heterotrophic metabolism, *Bacteroidetes* are thought to occupy a  
55 special place in the carbon cycling of oceans. They are obviously involved in the degradation and uptake of  
56 DOM, and likely to contribute to the degradation of the high molecular weight (HMW; > 1 kDa) fraction of  
57 the DOM (Kirchman, 2002). Indeed, the HMW fraction is composed of biopolymers; hydrolysis of these

58 macromolecules requires specific extracellular enzymes which are produced by numerous known  
59 *Bacteroidetes* members. Using a method combining microautoradiography and hybridization of fluorescent  
60 rRNA-targeted oligonucleotide probes to whole cells from natural marine assemblages (MICRO-FISH),  
61 Cottrell and Kirchman (2000b) demonstrated that *Bacteroidetes* represented, for the considered habitats, a  
62 large fraction of the community efficient in degrading chitin, *N*-acetylglucosamine and protein, part of the  
63 HMW fraction of the DOM. All these data together suggest strongly that, in marine environments,  
64 *Bacteroidetes* are key players in the degradation of the high molecular weight fraction of the DOM, which  
65 has been demonstrated to be more bioreactive and more bioavailable than the low molecular weight fraction  
66 (Amon & Benner, 1996). As relevant work on cultured representatives from marine origin is lacking, there is  
67 a great interest in isolating and describing phenotypic and genotypic characteristics of marine *Bacteroidetes*  
68 known only by their 16S rDNA sequence. For example, there are notably very few sequence data on  
69 enzymes catalyzing biopolymer hydrolysis (Kirchman, 2002). The isolation of strains is still the easiest way  
70 to access to their physiological and genomic capital. This is probably the reason why the number of strains  
71 of *Bacteroidetes* from marine ecosystems described the last five years has increased exponentially.

72

73 In this study, a novel marine bacterium belonging to the phylum *Bacteroidetes* is described. Based on the  
74 results of a polyphasic taxonomic analysis, the strain AS06/20a<sup>T</sup> represents a novel species and genus,  
75 *Eudoraea adriatica* gen. nov., sp. nov.

76

77 In April 2006, marine surface waters were collected on the coast of the Adriatic Sea, Italy (44.690°N,  
78 12.520°E). One subsample collected by 10 m depth was spread on a marine agar 2216 (MA; Difco) plate,  
79 and then incubated at 25°C. After 2 weeks, a small non-pigmented colony was picked and purified by  
80 repeated streaking on MA plates. It was referenced as strain AS06/20a<sup>T</sup>. Stock cultures were stored at  
81 –80°C in marine broth 2216 (MB; Difco) supplemented with 5% (v/v) DMSO or 35% (v/v) glycerol, until  
82 characterization.

83

84 The almost complete 16S rRNA gene (1393 bp) of the strain was double-strand sequenced from one single  
85 colony, as described elsewhere (Agogué *et al.*, 2005). This sequence was compared to those in available

86 databases by use of the BLAST program (Altschul *et al.*, 1990) and then aligned to its nearest neighbours  
87 using the CLUSTALX program (Thompson *et al.*, 1997). Alignments were refined manually using the  
88 SEAVIEW program (Galtier *et al.*, 1996). Phylogenetic trees were constructed by the PHYLIP (PHYlogeny  
89 Inference Package) version 3.63 software (<http://evolution.genetics.washington.edu/phylip/getme.html>) on  
90 the basis of evolutionary distance (neighbour-joining method with Jukes and Cantor corrections) (Saitou and  
91 Nei, 1987) and maximum likelihood (Felsenstein, 1981). The robustness of the inferred topologies was  
92 assessed by bootstrap analyses based on 1000 bootstrap resamplings for the neighbour-joining and 100  
93 replications for the maximum likelihood method (Felsenstein, 1985). The 16S rRNA gene-based analysis  
94 located the strain AS06/20a<sup>T</sup> within the phylum *Bacteroidetes*. The novel isolate was affiliated to the family  
95 *Flavobacteriaceae*, one of the main phyletic lines of the phylum *Bacteroidetes* (Reichenbach, 1989;  
96 Bernardet *et al.*, 1996; Bernardet *et al.*, 2002) (Fig. 1). The results of different phylogenetic reconstructions  
97 performed with different treeing algorithms were in accordance with each other. The novel isolate formed a  
98 distinct lineage together with *Zeaxanthinibacter enoshimensis* TD-ZE<sub>3</sub><sup>T</sup> and *Robiginitalea biformata* HTCC  
99 2501<sup>T</sup>, but this lineage did not cluster robustly with any of the recognized genera in the family. Within this  
100 lineage, the novel isolate showed only a distant relatedness to its nearest phylogenetic neighbours, the most  
101 closely genus being *Zeaxanthinibacter* (93% 16S rDNA sequence similarity), followed by *Robiginitalea*  
102 (92%). These low 16S rDNA sequence similarities between the strain AS06/20a<sup>T</sup> and the genera described  
103 so far suggest that the novel isolate represents a new genus within the family *Flavobacteriaceae*.

104 The DNA G+C content was determined, by the Identification Service of the DSMZ (Deutsche Sammlung  
105 von Mikroorganismen und Zellkulturen GmbH, Braunschweig,  
106 Germany), by HPLC analysis of deoxyribonucleosides as described by Mesbah *et al.* (1989). The G+C  
107 content of strain 26III/A02/215<sup>T</sup> was 38.9 mol%.

108

109 Colonies on MA were non-pigmented and cream in colour. They were circular, punctiform, opaque, with an  
110 entire edge and possessed a smooth surface. After 1 week incubation, colonies were less than 1 mm in  
111 diameter. Morphological characteristics of the cells were determined by light microscopy (Olympus AX70)  
112 and by transmission electron microscopy (Hitachi H-7500) after negative staining with uranyl acetate  
113 (Raguénès *et al.*, 1997). Gram-staining was determined using standard procedures. Gliding motility was

114 determined using the hanging drop method on cells grown at 30°C in low nutrient medium (0.1% (w/v)  
115 marine broth solidified with 1% agar), as described in Bernardet *et al.* (2002). In summary, cells of strain  
116 AS06/20a<sup>T</sup> were Gram-negative, non-motile, straight rods of 1.48-2.99 µm in length (mean 2.22 ± 0.54 µm,  
117 n=8) and 0.61-0.78 µm in width (mean 0.70 ± 0.07 µm, n=8) in the mid-exponential phase of growth (see  
118 Supplementary Fig. S1 in IJSEM online). Gliding motility was not observed under the conditions tested.  
119 Cells swell into balloon-like shapes during the late stationary growth phase.

120

121 In order to analyse respiratory quinones and polar lipids, strain AS06/20a<sup>T</sup> was grown for 5 days on MB  
122 medium at 30°C, and checked for purity. Analyses of isoprenoid quinones and polar lipids were carried out  
123 by the Identification Service (Dr. Brian Tindall, DSMZ culture collection, Braunschweig, Germany). As  
124 described for the other members of the family *Flavobacteriaceae*, the main respiratory quinone was  
125 menaquinone 6 (MK-6; 83%) (Bernardet *et al.*, 2002); Menaquinone 7 was also detected (MK-7; 17%).  
126 Phosphatidylethanolamine was the only major phospholipid identified in strain AS06/20a<sup>T</sup>. Three other  
127 major unidentified polar lipids, two containing amino groups, were also present.

128 The determination of the whole-cell fatty acid composition was performed on cultures grown at 30°C for  
129 72h on marine agar 2216. This analysis was carried out at the DSMZ according to the standard protocol of  
130 the Microbial Identification System (MIDI Inc., Del. USA, 2001). Extracts were analysed using a Hewlett  
131 Packard model HP6890A gas chromatograph equipped with a flame-ionization detector as described by  
132 Kämpfer & Kroppenstedt (1996). Results are summarized in Table 1. The dominant fatty acids in strain  
133 AS06/20a<sup>T</sup> were iso-C<sub>15:1</sub> G, iso-C<sub>15:0</sub>, iso-C<sub>15:0</sub> 3-OH, iso-C<sub>17:1</sub>ω9c, iso-C<sub>17:0</sub> 3-OH and the fatty acids summed  
134 in feature 3.

135

136 Unless stated otherwise, physiological characterization was carried out aerobically in marine broth medium  
137 (MB 2216; Difco), in triplicate, and incubation were done in the dark and under agitation. Growth was  
138 routinely monitored by measuring the increase in optical density at 600 nm using a spectrophotometer. Cell  
139 numbers were determined by flow cytometry in order to calculate calibration curves 'Cell numbers =  
140 f(OD<sub>600</sub>)'. Growth rates were calculated using linear regression analysis of four to nine points along the  
141 logarithmic portions of the resulting growth curves. Growth temperature was tested over the range 9-44°C

142 (i.e. 9, 15, 20, 25, 30, 33, 37, 44°C). The temperature range for growth was 15-33°C, with optimum growth  
143 rate at 30°C (see Supplementary Fig. S2a in IJSEM online). No growth was observed at 9 and 37°C. The pH  
144 range for growth was tested at 30°C in MB medium, buffered and adjusted to the required pH as described  
145 elsewhere (Alain *et al.*, 2002). The pH range for growth was rather narrow (Fig. S2b); Growth was observed  
146 from pH 6.5 to pH 8.5, the optimum being around pH 7.5-8.0. Salt tolerance was tested at 30°C in MB  
147 medium prepared with various concentrations of NaCl (0.02, 0.5, 1, 2, 3, 4, 5, 6, 7 and 9% w/v). The isolate  
148 required NaCl for growth (Fig. S2c). This is congruent with the fact that this strain was isolated from  
149 brackish waters of the North Adriatic Sea. Growth was observed at salt concentrations ranging from 2 to 6%  
150 (w/v) NaCl, the optimum salinity being around 2%. No growth was observed at 1 and 7% (w/v) NaCl.

151

152 Strain AS06/20a<sup>T</sup> was found to be aerobic. Conventional phenotypic tests including those for oxidase,  
153 catalase, tween esterase and nitrate reductase were performed according to standard methods (Smibert &  
154 Krieg, 1994). The results are given in Table 1. Biochemical tests were performed at 30°C using api®ZYM  
155 (bioMérieux) and Biolog GN2 microplates (Oxoid). These tests were inoculated with cells grown on MA  
156 plates, swabbed from the surface of the agar plates and then suspended in ASW ½ (diluted artificial  
157 seawater) to the density specified by the manufacturer. Supplementary biochemical tests were also  
158 performed using api®20NE strips (bioMérieux), following the manufacturer's instructions. The data  
159 obtained are given in Table 1. Testing for oxidation of carbon sources with Biolog GN2 plates indicated that  
160 the strain was able to oxidize a range of sugars, organic acids and amino acids. To confirm these results and  
161 to test for the capability of the strain to catabolize different substrates as sole carbon and energy source, with  
162 oxygen as a terminal electron acceptor, the strain was grown aerobically, in the dark, on a mineral medium  
163 supplemented with one substrate. The defined medium had the following composition (l<sup>-1</sup>): phosphate  
164 buffer, 30 mM; NaCl 20 g, MgCl<sub>2</sub>·6H<sub>2</sub>O 3 g, CaCl<sub>2</sub>·2H<sub>2</sub>O 1.0 g, NH<sub>4</sub>Cl 0.3 g, KCl 0.5 g, Na<sub>2</sub>SO<sub>4</sub> 3 g, NaNO<sub>3</sub>  
165 1 g; trace element solution, 1 ml; selenite-tungstate solution, 1 ml; vitamin solution, 1 ml. The strain was  
166 found to grow chemoorganoheterotrophically on a variety of carbon compounds, including pentoses,  
167 hexoses, polyols, organic acids, amino acids and complex organic substrates (Table 1).

168 Antibiotic sensitivity tests were performed by using susceptibility discs (Biorad) or filter-paper discs  
169 impregnated with different antibiotics. Discs were placed on MA plates spread with a culture of the isolate

170 and were then incubated at 30°C for one week. Susceptibility was scored as positive at zone diameters above  
171 10 mm. The results are summarized in Table 1.

172

173 The phenotypic and genotypic characteristics of the novel isolate generally met the minimal standards for  
174 the family *Flavobacteriaceae* (Bernardet *et al.*, 2002). Nevertheless, strain AS06/20a<sup>T</sup> differed from other  
175 *Flavobacteriaceae* genera, with validly published names, in terms of a number of phylogenetic, genotypic,  
176 chemotaxonomic, morphological and physiological features (Table 2). In brief, in addition to the  
177 phylogenetic distance, the novel taxon can be distinguished from its closest relatives by some of its  
178 phenotypic features, including its absence of colonial pigmentation, the narrow NaCl and pH ranges for  
179 growth and its restricted degrading-capabilities regarding to the tested macromolecules. In addition to these  
180 phenotypic differences, the novel isolate is unambiguously distinct from its two closest neighbours by its  
181 genomic DNA G+C content which is more than 5% lower than those of *Zeaxanthinibacter enoshimensis* and  
182 *Robiginitalea biformata*. In conclusion, in view of all the above-mentioned distinctive features, we propose  
183 that the isolate AS06/20a<sup>T</sup> should be assigned as the type strain of a novel genus and species, for which the  
184 name *Eudoraea adriatica* gen. nov., sp. nov. is proposed.

185

#### 186 **Description of *Eudoraea* gen. nov.**

187 *Eudoraea* (N. L. fem. n. *Eudora* was a sea goddess of the Greek mythology; N. L. fem. n. *Eudoraea*, named after  
188 *Eudora*).

189 Cells are Gram-negative, non-spore forming, non-motile, non-gliding rods. Pigments are not produced. Aerobic and  
190 chemoorganoheterotrophic. Catalase- and oxidase- positive. Mesophilic. Neutrophilic. Requires NaCl for growth. The  
191 predominant quinone is MK-6. Polar lipids include phosphatidylethanolamine. Major fatty acids are iso-C<sub>15:1</sub> G, iso-  
192 C<sub>15:0</sub>, iso-C<sub>15:0</sub> 3-OH, iso-C<sub>17:1</sub> ω<sup>9</sup>c, iso-C<sub>17:0</sub> 3-OH and summed feature 3. The G+C content of the DNA is close to 39  
193 mol%. Phylogenetically, the genus *Eudoraea* belongs to the phylum *Bacteroidetes*, family *Flavobacteriaceae*, showing  
194 a distant relatedness to the marine genera *Zeaxanthinibacter* and *Robiginitalea*. The type species is *Eudoraea adriatica*.

195

#### 196 **Description of *Eudoraea adriatica* sp. nov.**

197 *Eudoraea adriatica* (a.dri.a'ti'ca. L. fem. adj. *adriatica*, of the Adriatic Sea, where the type strain was isolated). In  
198 addition to the characters described for the genus, the following properties apply. Colonies on MA medium are



199 punctiform, cream in colour, opaque and smooth. Optimal growth occurs at 30°C, with a growth range from 15 to 33°C.  
200 The pH and NaCl ranges are 6.5-8.5 (optimum 7.5-8.0) and 2-6% (w/v) (optimum, 2% w/v NaCl), respectively.  
201 Aesculin is hydrolysed but agar, cellulose, gelatin, starch and urea are not.  $\beta$ -galactosidase activity is positive. Glucose  
202 is not fermented. Nitrate is not reduced. A variety of carbon compounds are used as sole carbon sources, including  
203 pentoses, hexoses, polyols, organic acids, amino acids and complex substrates (Table 1). The G+C content of the type  
204 strain AS06/20a<sup>T</sup> is 38.9 mol%.

205 The type strain, AS06/20a<sup>T</sup> (DSM 19308<sup>T</sup>, CIP 109577<sup>T</sup>, OOB 358<sup>T</sup>), was isolated from coastal waters of the Adriatic  
206 Sea, Italy (44.690°N, 12.520°E).

207

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219

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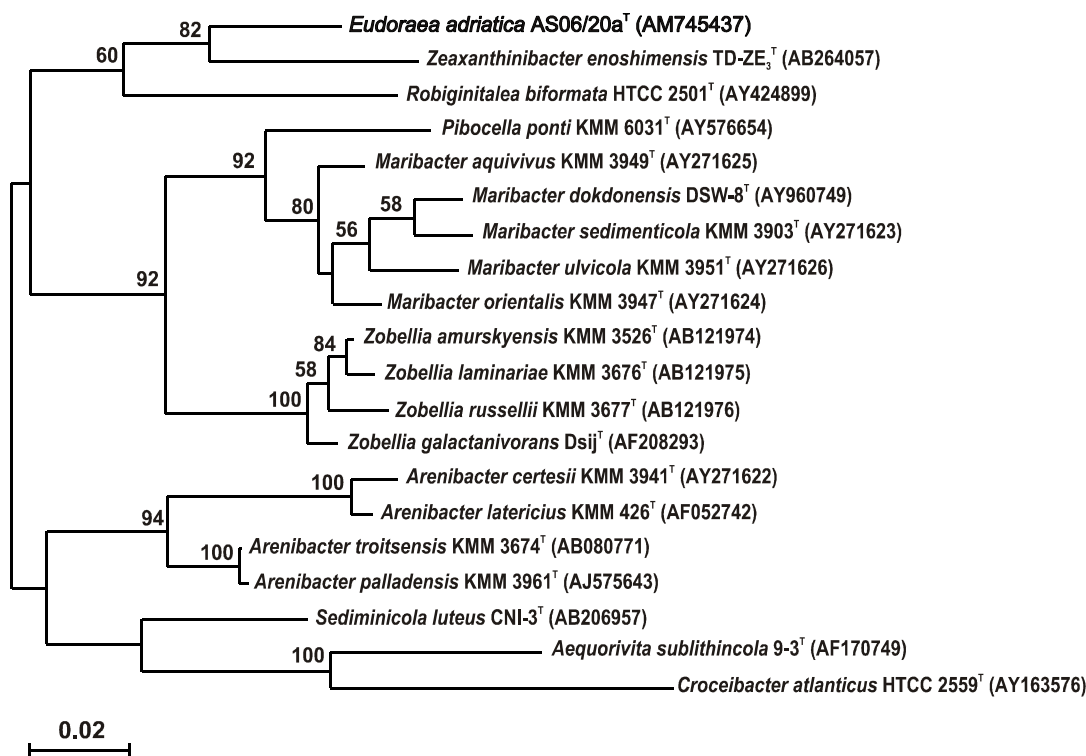
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289 **Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences of strain AS06/20a<sup>T</sup> and representative**  
 290 **members of related genera within the family *Flavobacteriaceae*.** Sequence data of reference strains were  
 291 obtained from the GenBank/EMBL and/or RDP databases. Accession numbers are indicated in parentheses.  
 292 The topology shown corresponds to an unrooted tree obtained by the maximum likelihood algorithm,  
 293 established using the PHYLIP package. Bootstrap values (from 100 replicates) are indicated at the branch  
 294 nodes. The positioning of the novel isolate was confirmed by the neighbour-joining method. The scale bar  
 295 indicates 2.0 nt substitutions per 100 nt.

307 **Table 1. Phenotypic and genotypic characteristics of strain AS06/20a<sup>T</sup>.**

308 Legend: +, positive; –, negative; PE, phosphatidylethanolamine; L, unidentified, polar lipid; AL,  
 309 unidentified aminolipid.

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Characteristic	<i>Eudoraea adriatica</i> strain AS06/20a <sup>T</sup>
<b>Temperature range for growth (°C) [optimum]</b>	15-33 [30]
<b>NaCl range for growth (%) [optimum]</b>	2-6 [2]
<b>pH range for growth [optimum]</b>	6.5-8.5 [7.5-8.0]
<b>Biochemical properties (API 20NE)</b>	
Catalase, oxidase, aesculin hydrolysis, β-galactosidase production	+
Denitrification, indole production, glucose fermentation, arginine dihydrolase, urease, gelatin hydrolysis	–
<b>Enzymatic activities (APIZYM)</b>	
Alkaline phosphatase, esterase (C4), esterase lipase (C8), valine arylamidase, leucine arylamidase, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase	+
Lipase (C14), cystine arylamidase, trypsin, α-galactosidase, α-glucosidase, α-fucosidase	–
<b>Substrate assimilation</b>	
D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetylglucosamine, potassium gluconate, m-inositol, acetic acid, citric acid, pyruvic acid, propionic acid, capric acid, adipic acid, malic acid, phenylacetic acid, L-arginine, yeast extract	+
Formic acid, tween 40, tween 80, D-maltose	–
<b>Degradation of macromolecules</b>	
Aesculin	+
Agar, cellulose (filter paper), gelatin, starch, urea	–
<b>Susceptibility to antibiotics</b>	
Vancomycin, rifampicin, tetracycline, polymyxin B	+
Oxacillin, penicillin, ciprofloxacin	–
<b>Fatty acid composition (%)*</b>	iso-C <sub>15:1</sub> G (15.97%), iso-C <sub>15:0</sub> (29.37%), iso-C <sub>15:0</sub> 3-OH (7.27%), iso-C <sub>17:1</sub> ω9c (8.96%), iso-C <sub>17:0</sub> 3-OH (14.13%) and summed feature 3 § (11.57%).
<b>Polar lipids</b>	PE, L, AL1, AL2
<b>Quinones</b>	MK-6 (83%), MK-7 (17%)
<b>DNA G+C content (mol%)</b>	38.9

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312 §, Summed feature 3 comprises iso-C<sub>15:0</sub> 2-OH and/or C<sub>16:1</sub> ω7c.

313 \* Fatty acids comprising more than 5% of the total content are listed. The following fatty acids were present in lesser  
 314 amounts: iso-C<sub>13:0</sub> (0.48%), C<sub>14:0</sub> (0.17%), anteiso-C<sub>15:0</sub> (0.72%), C<sub>15:1</sub> ω6c (1.06%), iso-C<sub>16:0</sub> (0.25%), C<sub>16:0</sub> (0.67%), C<sub>15:0</sub>  
 315 2-OH (0.30%), C<sub>15:0</sub> 3-OH (1.33%), iso-C<sub>17:0</sub> (0.17%), C<sub>17:1</sub> ω8c (0.48%), C<sub>17:1</sub> ω6c (0.83%), iso-C<sub>16:0</sub> 3-OH (1.38%),  
 316 C<sub>16:0</sub> 3-OH (0.37%), C<sub>18:1</sub> ω5c (0.70%), C<sub>17:0</sub> 3-OH (0.20%) and three unknown fatty acids designated by their equivalent  
 317 chain-length [ECL 11.54 (0.21%), ECL 13.56 (2.26%) and ECL 16.58 (1.15%)].

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331 **Table 2. Characteristics differentiating *Eudoraea* from related genera of the family *Flavobacteriaceae*.**  
 332 Genera: 1, *Eudoraea adriatica* AS06/20a<sup>T</sup> (this study); 2, *Zeaxanthinibacter enoshimensis* TD-ZE<sub>3</sub><sup>T</sup>; 3,  
 333 *Robiginitalea biformata* HTCC 2501<sup>T</sup>; 4, *Pibocella ponti* KMM 6031<sup>T</sup>; 5, *Maribacter sedimenticola* KMM  
 334 3903<sup>T</sup>; 6, *Zobellia galactanivorans* Dsij<sup>T</sup>; 7, *Arenibacter latericius* KMM 426<sup>T</sup>; 8, *Sediminicola luteus* CNI-  
 335 3<sup>T</sup>.

336 Legend: +, positive; −, negative; ND, not determined; Y, yellow; O, orange; DO, dark orange; w, weak. The  
 337 percentage of 16S rDNA sequence similarity is calculated in reference to the 16S rDNA sequence of the  
 338 novel isolate AS06/20a<sup>T</sup>.

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Characteristic	1	2	3	4	5	6	7	8
<b>Oxidase/catalase</b>	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/ w
<b>Pigmentation</b>	−	Y	O	Y	Y	Y	DO	Y
<b>Flexirubin pigments</b>	−	−	−	−	−	+	−	−
<b>Gliding motility</b>	−	+	−	+	+	+	−	−
<b>Growth in/at</b>								
<b>8% NaCl</b>	−	−	+	+	+	−	−	−
<b>7% NaCl</b>	−	+	+	+	−	−	+	+
<b>1% NaCl</b>	−	ND	+	+	+	+	+	+
<b>4°C</b>	−	−	−	+	+	−	−	−
<b>10°C</b>	−	−	+	+	+	−	+	+
<b>42°C</b>	−	−	+	−	−	+	−	−
<b>Hydrolysis of</b>								
<b>Agar</b>	−	−	ND	−	+	+	−	ND
<b>Gelatin</b>	−	+	−	+	+	+	−	+
<b>Starch</b>	−	+	+	+	−	+	−	+
<b>Cellulose</b>	−	−	−	−	−	ND	−	−
<b>Aesculin</b>	+	+	+	ND	ND	+	−	ND
<b>Urease activity</b>	−	−	−	−	−	−	+	−
<b>β-galactosidase</b>	+	+	−	ND	−	+	+	ND
<b>Nitrate reduction</b>	−	−	−	−	−	+	+	+
<b>DNA G+C content (mol%)</b>	38.9	46.5	56.4	35.5	37	43	37-38	38
<b>16S rDNA similarity (%)</b>	100	93	92	90	91	91	91	90

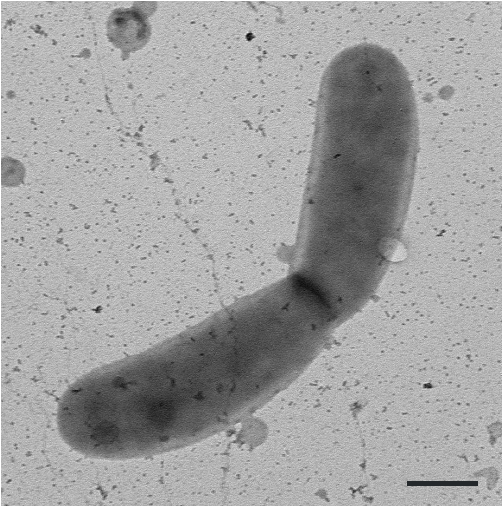
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346 **Fig. S1. Transmission electron micrograph of cells of strain AS06/20a<sup>T</sup> in a state of division.** Cells were  
347 grown at 30°C on marine broth and harvested during the mid-exponential phase of growth. The preparation  
348 was negatively stained with uranyl acetate. Bar, 0.5 μm.

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379 **Fig. S2. Effects of temperature (A), pH (B) and salinity (C) on the maximum growth rate of strain**  
380 **AS06/20a<sup>T</sup>.** Bars indicate confidence intervals.

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