



HAL
open science

Zobellia roscoffensis sp. nov. and Zobellia nedashkovskayae sp. nov., two flavobacteria from the epiphytic microbiota of the brown alga *Ascophyllum nodosum*, and emended description of the genus *Zobellia*

Tristan Barbeyron, Manon Thiébaud, Nolwen Le Duff, Marjolaine Martin, Erwan Corre, Gwenn Tanguy, Micheline Vandebol, François Thomas

► **To cite this version:**

Tristan Barbeyron, Manon Thiébaud, Nolwen Le Duff, Marjolaine Martin, Erwan Corre, et al.. *Zobellia roscoffensis* sp. nov. and *Zobellia nedashkovskayae* sp. nov., two flavobacteria from the epiphytic microbiota of the brown alga *Ascophyllum nodosum*, and emended description of the genus *Zobellia*. *International Journal of Systematic and Evolutionary Microbiology*, 2021, 71 (8), pp.004913. 10.1099/ijsem.0.004913 . hal-03357314

HAL Id: hal-03357314

<https://hal.science/hal-03357314>

Submitted on 30 Sep 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 ***Zobellia roscoffensis* sp. nov. and *Zobellia nedashkovskayae* sp. nov., two flavobacteria**
2 **from the epiphytic microbiota of the brown alga *Ascophyllum nodosum*, and emended**
3 **description of the genus *Zobellia***

4 Tristan Barbeyron^{1,*}, Manon Thiébaud¹, Nolwen Le Duff¹, Marjolaine Martin², Erwan Corre³,
5 Gwenn Tanguy⁴, Micheline Vandenberg², François Thomas^{1,*}

6
7 **Running Title:** *Zobellia roscoffensis* sp. nov., *Zobellia nedashkovskayae* sp. nov.

8
9 **Author affiliations:**

10 ¹CNRS, Sorbonne Université, Integrative Biology of Marine Models (LBI2M),
11 Station Biologique de Roscoff, 29680 Roscoff, Brittany, France; ²University of Liège,
12 Gembloux Agro-Bio Tech, Microbiology and Genomic Laboratory, TERRA building, avenue
13 de la Faculté 2b, 5030 Gembloux, Belgium; ³CNRS, Sorbonne Université, FR2424, ABiMS,
14 Station Biologique de Roscoff, 29680, Roscoff, Brittany France; ⁴CNRS, Sorbonne
15 Université, FR2424, Genomer, Station Biologique de Roscoff, 29680 Roscoff, Brittany
16 France

17 ***Correspondence:** Tristan Barbeyron, barboun@sb-roscoff.fr; François Thomas,
18 fthomas@sb-roscoff.fr

19 **Keywords:** *Zobellia roscoffensis*; *Zobellia nedashkovskayae*; *Flavobacteriaceae*;
20 polysaccharide; macroalgal microbiome

21 **Abbreviations:** ANI, average nucleotide identity; dDDH, digital DNA–DNA hybridization;
22 GGDC, Genome-Genome Distance Calculator; ML, maximum-likelihood; MP, maximum-
23 parsimony; NJ, neighbour-joining.

24 **Subject category:** New Taxa: *Bacteroidetes*

25

26 The Genbank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences are as
27 follows: MW114830 for strain Asnod1-F08^T, MW114831 for strain Asnod2-B02-B,
28 MW114832 for strain Asnod2-B07-B^T, MW114833 for strain Asnod3-E08-A.

29 The Genbank/ENA/DDBJ accession numbers for the genome sequences are as follows:
30 JADDXT000000000 for strain Asnod1-F08^T, JADDXS000000000 for strain Asnod2-B02-B,
31 JADDXR000000000 for strain Asnod2-B07-B^T, JADDXQ000000000 for strain Asnod3-E08-
32 A.

33 Three supplementary figures and three supplementary tables are available in the online
34 version of this article.

35

36 **Abstract**

37 Four marine bacterial strains were isolated from the thallus of the brown alga *Ascophyllum*
38 *nodosum* collected in Roscoff, France. Cells were Gram-stain-negative, strictly aerobic, non-
39 flagellated, gliding, rod-shaped and grew optimally at 25-30 °C, at pH 7-8 and with 2-4 %
40 NaCl. Phylogenetic analyses of their 16S rRNA gene sequences showed that the bacteria were
41 affiliated to the genus *Zobellia* (family *Flavobacteriaceae*, Phylum *Bacteroidetes*). The four
42 strains exhibited 97.8 - 100% 16S rRNA gene sequence similarity values among themselves,
43 97.9 - 99.1 % with the type strains of *Zobellia amurskyensis* KMM 3526^T and *Z. laminariae*
44 KMM 3676^T, and less than 99% with other species of the genus *Zobellia*. The DNA G+C
45 content of the four strains ranged from 36.7 to 38.1 mol%. Average Nucleotide Identity (ANI)
46 and digital DNA-DNA hybridization (Genome-to-Genome Distance Calculator, GGDC)
47 methods between the new strains and other members of the genus *Zobellia* showed values of
48 76.4 - 88.9 %, and below 38.5 %, respectively. Phenotypic, phylogenetic and genomic
49 analyses show that the four strains are distinct from species of the genus *Zobellia* with validly
50 published names. They represent two novel species of the genus *Zobellia*, for which the
51 names *Zobellia roscoffensis* sp. nov. and *Zobellia nedashkovskayae* sp. nov. are proposed
52 with Asnod1-F08^T (RCC 6906^T = KMM 6823^T = CIP 111902^T) and Asnod2-B07-B^T (RCC
53 6908^T = KMM 6825^T = CIP 111904^T), respectively, as the type strains.

54

55 The genus *Zobellia* was proposed by Barbeyron et al. [1] and contains Gram-negative,
56 aerobic, gliding, bacteria that produce flexirubin-type pigments. At the time of writing, the
57 genus *Zobellia* comprises 5 validly named species, *Zobellia galactanivorans*, *Zobellia*
58 *uliginosa* [1], *Zobellia amurskyensis*, *Zobellia laminariae* and *Zobellia russellii* [2], all
59 isolated from marine environments. *Z. galactanivorans*, a species isolated from the red alga
60 *Delesseria sanguinea* (Hudson) J. V. Lamouroux, 1813, was chosen as the type species.

61 During a previous study of the surface microbiota of the brown alga *Ascophyllum nodosum*
62 (Linnaeus) Le Jolis 1863, 324 bacterial strains were isolated [3]. The taxonomic position of
63 four isolates was investigated in the present study using a polyphasic approach, including
64 some genomic data deduced from their draft genome, whole-genome comparison using the
65 average nucleotide identity (ANI) and dDDH (digital DNA–DNA hybridization) methods,
66 phenotypic and physiological analyses. Based on these results, we report the description of
67 two novel species of the genus *Zobellia*, for which the names *Zobellia roscoffensis* sp. nov.
68 and *Zobellia nedashkovskayae* sp. nov. are proposed.

69 The isolates investigated in this study were obtained by sampling healthy *A. nodosum* plants
70 collected in the English Channel from the foreshore (48°43'36.07" N, -3°59'22.96" W) in
71 Roscoff (Brittany, France) at the end of March 2014 [3]. Strains Asnod1-F08^T, Asnod2-B02-
72 B, Asnod2-B07-B^T and Asnod3-E08-A were isolated by swabbing algal surfaces with sterile
73 cotton tips and then inoculating plates of marine agar 2216 (Difco).

74 For comparison, *Zobellia amurskyensis* KMM 3526^T = CIP 108562^T and *Z. laminariae* KMM
75 3676^T = CIP 108563^T [2] were purchased from the Collection de l'Institut Pasteur (CIP;
76 France) and used as related type strains. Except for the temperature, pH and NaCl ranges of
77 growth, *Z. amurskyensis* KMM 3526^T and *Z. laminariae* KMM 3676^T were studied in parallel
78 with the four new strains for all phenotypic tests and for quinone, fatty acid and polar lipid
79 analyses. All strains were routinely cultivated on ZoBell medium 2216E [4], either liquid or

80 solidified with 1.5 % (w/v) agar. Pure cultures were stored at -80 °C in the culture medium
81 containing 20 % (v/v) glycerol. All experiments were performed in triplicate. Growth was
82 evaluated in ZoBell agar plates at 4, 9, 11, 12, 13, 15, 17, 18, 20, 22, 24, 30, 37, 40, 42, 45
83 and 48 °C. The optimal pH value for growth was determined at 25 °C in ZoBell broth with pH
84 values adjusted by using 100 mM of the following buffers: MES for pH 5.0; Bis Tris for pH
85 5.5, 6.0, 6.5, 7.0 and 7.5; Tris for pH 8.0, 8.5 and 9.0; CHES for pH 9.5 and CAPS for pH
86 10.0, 10.5 and 11.0. The effect of NaCl on growth was determined at 25 °C and at pH 8 in
87 ZoBell broth prepared with distilled water containing 0, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0,
88 10, 12, 15, 17, 20 and 25 % NaCl. To test the influence of other salts on growth, the same
89 NaCl range was used in ZoBell broth prepared with artificial seawater without NaCl but
90 containing 6.3 g MgSO₄ l⁻¹, 4.2 g MgCl₂ l⁻¹, and 0.7 g KCl l⁻¹.

91 Cell morphology and gliding motility were investigated on wet mounts of an exponential
92 phase ZoBell broth culture at 25 °C, by using phase-contrast microscopy on an Olympus
93 BX60 instrument (Olympus, Tokyo, Japan). The Ryu non-staining KOH method [5] was used
94 to determine the Gram type. Production of flexirubin was assessed by flooding a 4 d plate
95 culture with 20% (w/v) potassium hydroxide followed by the observation of changes in
96 colony colour from yellow to red or brown [6]. Colony iridescence was assayed on 3 d plate
97 cultures on R2A-agar (Difco) using a STEMI 2000-C stereomicroscope (Zeiss) equipped with
98 a KC 15 000 LCP light source and a 455170 polarizing analyzer.

99 Oxidase activity was assayed using small pieces of 3MM paper (Whatman) soaked in the
100 reagent N,N,N',N'-tetramethyl-p-phenylenediamine dihydrochloride (bioMérieux). Catalase
101 activity was assayed by mixing one colony from a ZoBell agar plate with a drop of hydrogen
102 peroxide (3 %, v/v). Amylase activity was assayed on 0.2 % (w/v) soluble starch ZoBell agar
103 plates. DNase activity was detected on DNA agar (Difco) prepared with seawater. Amylase
104 and DNase activities were revealed by flooding the plates with Lugol's solution or 1 M HCl,

105 respectively. The degradation of Tween compounds (1 %, v/v) was assayed in ZoBell agar
106 according to Smibert & Krieg [7]. Agarase, κ -carrageenase and ι -carrageenase activities were
107 tested by inoculating ZoBell media solidified with (per litre): 15 g agar (Sigma-Aldrich, ref.
108 A7002), 10 g κ -carrageenan (X-6913; Danisco) or 20 g ι -carrageenan (X-6905; Danisco)
109 respectively. ZoBell media solidified with alginate was made using with the calcium
110 carbonate / gluconolactone method [8]. Strains were considered positive when colonies
111 liquefied or produced craters in the solidified substrate. Moreover, agarase activity was
112 revealed by flooding the plates with Lugol's solution. Additional phenotypic characterizations
113 were performed using API 20 E, API 20 NE, API 50CH and API ZYM strips according to the
114 manufacturer's instructions (bioMérieux) except that API AUX medium and API 50 CHB/E
115 medium were adjusted to 2.5 % NaCl. All strips were inoculated with cell suspensions in
116 artificial seawater and incubated at 25 °C for 48 h. The ability to use carbohydrates as sole
117 carbon and energy sources was also tested in marine minimal medium [9] containing 2 g l⁻¹ of
118 the following sugars (all from Sigma-Aldrich unless otherwise stated): D-glucose, D-
119 galactose, D-fructose, L-fucose, D-mannose, L-arabinose, L-rhamnose, D-xylose, lactose,
120 sucrose, maltose, D-raffinose, D-mannitol, glucomannan (Megazyme), galactan (arabic gum),
121 galactomannan from carob seeds (Megazyme), arabinan (Megazyme), arabinoxylane
122 (Megazyme), xylan, xyloglucan (Megazyme), amylopectine (Merck), pectin (from apple),
123 lichenin (Megazyme), laminarin (Goëmar), agar, porphyrin (extracted from *Porphyra* sp.), κ -
124 carrageenin (Danisco), ι -carrageenin (Danisco), λ -carrageenin (Danisco), acid alginic
125 (Danisco), fucoidin from *Ascophyllum nodosum* (kindly provided by Algues et Mer) and
126 ulvan (kindly provided by Pr. Bruno Moerschbacher, University of Münster, Germany).

127 Sensitivity to antibiotics was tested by the disc-diffusion method on ZoBell agar plates and
128 using antibiotic discs (Bio-Rad) containing (μ g per disc, unless otherwise stated): penicillin G
129 (10 IU), ampicillin (10), carbenicillin (100), oxacillin (5), streptomycin (500), kanamycin

130 (30), chloramphenicol (30), tetracycline (30), lincomycin (15), bacitracin (130), rifampicin
131 (30), vancomycin (30), trimethoprim/sulfamethoxazole (1.25/23.75), colistin (50),
132 gentamicin (15), neomycin (30), nalidixic acid (30), polymixin (50), erythromycin (15). The
133 effects of the antibiotics on cell growth were assessed after 48 h of incubation at 25 °C, and
134 susceptibility was scored based on the diameter of the clear zone around the disc.

135 Analysis of respiratory quinones [10, 11], fatty acids [12, 13] and polar lipids [14, 15] were
136 performed on freeze-dried cultures of strains Asnod1-F08^T, Asnod2-B02-B, Asnod2-B07-B^T,
137 Asnod3-E08-A and of *Z. amurskyensis* KMM 3526^T and *Z. laminariae* KMM 3676^T grown at
138 room temperature in ZoBell medium 2216E, by Susanne Verbarq and Dr. Brian Tindall from
139 the DSMZ Identification Service, Braunschweig, Germany.

140 Genomic DNA was extracted from strains Asnod1-F08^T, Asnod2-B02-B, Asnod2-B07-B^T and
141 Asnod3-E08-A using the DNeasy UltraClean Microbial kit (QIAGEN). Sequencing libraries
142 were prepared using the Nextera XT DNA kit (Illumina) and sequenced using Illumina MiSeq
143 v3 PE300, resulting in 5,312,862 quality-filtered reads for Asnod1-F08^T, 5,534,396 for
144 Asnod2-B02-B, 4,493,736 for Asnod2-B07-B^T and 2,037,244 for Asnod3-E08-A (Table S1).
145 Reads were assembled using SPAdes v3.11 [16] and scaffolds were built using MeDuSa v1.6
146 [17] using the closed genome of *Z. galactanivorans* Dsij^T as backbone. In silico gap filling
147 was performed using GapCloser v1.12 [18]. The final assemblies consist of 2 scaffolds (6
148 contigs) for Asnod1-F08^T, 6 scaffolds (11 contigs) for Asnod2-B02-B, 3 scaffolds (5 contigs)
149 for Asnod2-B07-B^T and 9 scaffolds (13 contigs) for Asnod3-E08-A. Completeness was
150 checked with CheckM v1.0.11 [19] by searching for 571 lineage-specific marker genes, and
151 was estimated at 100% for the four strains (Table S1).

152 The 16S rRNA gene sequence was amplified by PCR using pure genomic DNA as template
153 and the bacteria-specific primer pair, 8F [20] and 1492R [21]. PCR reactions were typically
154 prepared in a volume of 25 µl containing 10-100 ng template, 0.4 µM each specific primer,

155 250 μ M each dNTP, 0.1 mg BSA, 1X GoTaq buffer (Promega) and 1.25 U GoTaq DNA
156 polymerase (Promega). PCR amplification was performed as previously described [22]. PCR
157 products were purified using the Exostar kit according to the manufacturer's protocol (GE
158 Healthcare) and sequenced using BigDye Terminator V3.1 (Applied Biosystems) and an ABI
159 PRISM 3130xl Genetic Analyzer automated sequencer (Applied Biosystems/Hitachi).
160 Chargaff's coefficient (G+C content) of the genomic DNA of strains Asnod1-F08^T, Asnod2-
161 B02-B, Asnod2-B07-B^T and Asnod3-E08-A was deduced from the draft genome sequence
162 and expressed as the molar percentage of guanine + cytosine. The nucleotide sequences of the
163 16S rRNA gene deduced from the draft genomes were compared to those obtained by direct
164 sequencing of PCR-amplified products, showing high identity for each strain (99.85% for
165 Asnod1-F08^T, 99.71% for Asnod2-B02-B, 99.86% for Asnod2-B07-B^T and 99.93% for
166 Asnod3-E08-A). Genome-extracted 16S rRNA sequences for the four new strains were
167 aligned with sequences of the 16S rRNA genes from all valid species of the genus *Zobellia*
168 and from some species of the closest genera using the software MAFFT version 7 with the L-
169 INS-I strategy [23]. The alignment was then manually refined and phylogenetic analyses,
170 using the neighbour-joining (NJ) [24], maximum-parsimony (MP) [25] and maximum-
171 likelihood (ML) [26] methods, were performed using the MEGA 6 package [27]. The
172 different phylogenetic trees were built from a multiple alignment of 41 sequences and 1435
173 positions. For the NJ algorithm, the evolutionary model Kimura Two Parameters [28] was
174 used. The ML tree was calculated using the evolutionary model Kimura Two Parameters with
175 a discrete Gamma distribution to model evolutionary rate differences among sites (4
176 categories). This substitution model was selected through submission of the alignment to the
177 online server IQ-TREE (<http://iqtree.cibiv.univie.ac.at/>) [29]. The MP tree was obtained using
178 the Subtree-Pruning-Regrafting algorithm [30]. Bootstrap analysis (1000 replicates) was
179 performed to provide confidence estimates for the phylogenetic tree topologies [31]. A

180 phylogenomic tree was constructed with FastTree MP based on a concatenated alignment of
181 2573 translated protein-coding genes from the core genome of *Zobellia* species (50% amino
182 acid identity, 80% coverage) using custom scripts. Pairwise comparisons of 16S rRNA gene
183 sequences were made by using the database EzBioCloud
184 (<https://www.ezbiocloud.net/identify>) [32] and the FASTA software [33]. Genomic
185 relatedness was investigated by comparing the new isolates genome sequence with those of
186 the type strains of other *Zobellia* species using ANI
187 (<http://jspecies.ribohost.com/jspeciesws/#analyse>) [34–36] and dDDH via the online server
188 Genome to Genome Distance Calculator 2.1 (GGDC; <http://ggdc.dsmz.de/distcalc2.php>) [37].
189 The results from GGDC analysis were obtained from the alignment method Blast+ and the
190 formula 2 (sum of all identities found in HSPs / by overall HSP length; HSP: High-scoring
191 segment pairs) for incomplete genome sequences [38, 39]. Exploration of carbohydrate active
192 enzyme-coding genes in the genomes of the strains Asnod1-F08^T, Asnod2-B02-B, Asnod2-
193 B07-B^T, Asnod3-E08-A and comparison with *Z. amurskyensis* KMM 3526^T and *Z.*
194 *laminariae* KMM 3676^T [40] was carried out via the online server Microscope from the
195 French National Sequencing Center (<http://www.genoscope.cns.fr/agc/microscope/mage>) [41]
196 and the CAZy database (www.cazy.org) [42].

197 Phylogenetic analyses of 16S rRNA genes of species from a subset of the family
198 *Flavobacteriaceae* showed that strains Asnod1-F08^T, Asnod2-B02-B, Asnod2-B07-B^T and
199 Asnod3-E08-A belong to the genus *Zobellia* (Figs 1 and S1). The 16S rRNA genes from
200 Asnod1-F08^T and Asnod2-B02-B were included in a clade containing the type strains of *Z.*
201 *amurskyensis* KMM 3526^T and *Z. laminariae* KMM 3676^T while the 16S rRNA genes from
202 Asnod2-B07-B^T and Asnod3-E08-A were distantly related to type strains of *Z.*
203 *galactanivorans* Dsij^T and *Z. uliginosa* 553^T (Figs 1 and S1). The best pairwise comparison
204 scores with 16S rRNA gene from the strains Asnod1-F08^T and Asnod2-B02-B (1525 bp for

205 both), were obtained with *Zobellia galactanivorans* Dsij^T and *Z. uliginosa* 553^T (99.0 %;
206 Table S2, available in the online version of this article) and with *Z. galactanivorans* Dsij^T and
207 *Z. uliginosa* 553^T (98.8 %; Table S2), respectively. From the 16S rRNA sequence of Asnod2-
208 B07-B^T and Asnod3-E08-A (1523 for both), the best scores were obtained with *Z. laminariae*
209 KMM 3676^T (99.1%; Table S2) for both strains. The 16S rRNA gene sequence similarities
210 between the four new strains and other *Zobellia* species were in the range of 97.4 % with *Z.*
211 *russellii* KMM 3677^T and 98.5 % with *Z. amurskyensis* KMM 3526^T (Table S2). The draft
212 genomes of strains Asnod1-F08^T, Asnod2-B02-B, Asnod2-B07-B^T and Asnod3-E08-A were
213 sequenced. The genomic sizes were between 4,941,018 (Asnod2-B07-B^T) and 5,025,849
214 nucleotides (Asnod3-E08-A) and the Chargaff's coefficients (G+C content) were between
215 36.7 % for Asnod3-E08-A and 37.7 % for Asnod2-B02-B. Analysis of a phylogenomic tree
216 based on 2573 genes from the core genome of sequenced *Zobellia* strains showed that
217 Asnod2-B07-B^T and Asnod3-E08-A formed a clade with *Z. laminariae* KMM 3676^T, while
218 Asnod1-F08^T and Asnod2-B02-B were distant to all other strains (Fig S2). ANI and GGDC
219 methods (formula 2) for the new isolates, when compared with the valid species of *Zobellia*,
220 were less than 89 % and less than 38.5 % respectively (88.9 % and 38.3 % with *Z. laminariae*
221 KMM 3676^T; Table S3). As the normally accepted thresholds of species delineation for ANI
222 and GGDC are 95 % and 70%, respectively [34, 36, 43, 44], these values suggest that strains
223 Asnod1-F08^T, Asnod2-B02-B, Asnod2-B07-B^T and Asnod3-E08-A do not belong to any of
224 the valid species of the genus *Zobellia*. In addition, the genomes of the new isolates showed
225 the highest ANI and GGDC percentages with those of *Z. laminariae* KMM 3676^T and *Z.*
226 *amurskyensis* KMM3526^T, suggesting that these two species were closest to the new isolates
227 (Table S3). The strains *Z. laminariae* KMM 3676^T and *Z. amurskyensis* KMM3526^T were
228 therefore studied in parallel with the new isolates. Finally, the ANI and GGDC percentages
229 between Asnod1-F08^T and Asnod2-B02-B (97.5 and 81.6% respectively) showed that these

230 two strains represent a new species, for which the name *Zobellia roscoffensis* sp. nov. is
231 proposed (Table S3). Similarly, the ANI and GGDC percentages between Asnod2-B07-B^T
232 and Asnod3-E08 (97.6 and 82.1%, respectively) showed that these two strains represent a
233 new species, for which the name *Zobellia nedashkovskayae* sp. nov. is proposed.

234 The strains Asnod1-F08^T, Asnod2-B02-B, Asnod2-B07-B^T and Asnod3-E08-A cultivated on
235 ZoBell medium 2216E showed yellow-orange colonies of 1 mm in diameter strongly attached
236 to agar after three days at 25 °C. The flexirubin-type pigments were present for the four
237 strains. A spreading aspect of colonies was observed only for the strains Asnod1-F08^T and
238 Asnod2-B02-B. A weak iridescence was observed only for Asnod1-F08^T and Asnod2-B02-B.
239 The cells were Gram stain negative. Under the microscope, they appeared as rods attached to
240 the glass of the slide or coverslip and showed gliding mobility.

241 The four strains Asnod1-F08^T, Asnod2-B02-B, Asnod2-B07-B^T and Asnod3-E08-A used the
242 non-gelling agaroid porphyrin, as sole carbon source (Table 1). Surprisingly, agar, although
243 hydrolysed by the strains Asnod1-F08^T, Asnod2-B02-B and *Z. amurskyensis* KMM 3526^T, is
244 not used as sole carbon source by any of the strains including *Z. amurskyensis* KMM 3526^T
245 and *Z. laminariae* KMM 3676^T (Table 1). This result might be explained by the presence of
246 the gene *agaC* in the genomes of Asnod1-F08^T, Asnod2-B02-B and *Z. amurskyensis* KMM
247 3526^T only. An outer-membrane AgaC ortholog was recently characterized in *Z.*
248 *galactanivorans* Dsij^T (Genbank accession CAZ98402), and shown to be an agarase active on
249 complex agars [45]. The studied strains might miss one or several gene(s) for utilization of
250 agar as sole carbon source, such as *agaA* (CAZ98338 in *Z. galactanivorans* Dsij^T) that is
251 absent from their genomes. None of the tested carrageenins were hydrolysed or used as
252 carbon and energy source (Table 1). This observation is consistent with the absence of genes
253 *cgkA* and *cgiA* encoding a κ- and ι-carrageenase, respectively, in the six studied genomes
254 whereas the genome of the carrageenolytic *Z. galactanivorans* Dsij^T contains one *cgkA*

255 (CAZ94309) and three *cgiA* genes (CAZ98400, CAZ96312, CAZ96032) [46]. Interestingly, all
256 strains analysed in this study feature the complete operon responsible for the assimilation of
257 3,6-anhydro-D-galactose [47], a monosaccharide component of carrageenins. Although all
258 *Zobellia* strains in this study possess between 4 to 8 genes encoding alginate lyases, alginic
259 acid was degraded and used as the sole carbon source by Asnod2-B07-B^T, Asnod3-E08-A and
260 *Z. amurskyensis* KMM 3526^T only (Table 1). Furthermore, the strains Asnod2-B07-B^T and
261 Asnod3-E08-A liquefied the alginate while *Z. amurskyensis* KMM 3526^T only formed a
262 crater. This difference in behaviour could be due to the absence of the gene *alyA1*, encoding a
263 secreted endo-alginate lyase in *Z. galactanivorans* Dsij^T (CAZ95239) [48], in the genome of
264 *Z. amurskyensis* KMM 3526^T, while *alyA1* is present in the genomes of strains Asnod2-B07-
265 B^T and Asnod3-E08-A. The strains Asnod1-F08^T and Asnod2-B02-B did not degrade or utilize
266 alginic acid as unique carbon source (Table 1). This might be due to the absence of a gene
267 encoding the alginate-specific SusD-like lipoprotein (CAZ96770 in *Z. galactanivorans* Dsij^T),
268 involved in the binding and internalisation of oligo-alginates [49]. Finally, it remains
269 unexplained why *Z. laminariae* KMM 3676^T does not degrade alginic acid, since no
270 difference in the alginate lyase gene content could be observed compared to *Z. amurskyensis*
271 KMM 3526^T. Starch was hydrolysed and used as sole carbon source by *Z. amurskyensis*
272 KMM 3526^T, the only strain that showed a hydrolysis area on soluble starch ZoBell agar
273 plates (Table 1). This result is consistent with the presence of the *susA* gene encoding an outer
274 membrane α -amylase in its genome, while it is absent from the strains Asnod1-F08^T, Asnod2-
275 B02-B, Asnod2-B07-B^T, Asnod3-E08-A and *Z. laminariae* KMM 3676^T. Although all strains
276 possess *malsS*, encoding an α -amylase of the cytoplasmic membrane, *susA* might be necessary
277 to use starch as unique carbon source. The other physiological features of Asnod1-F08^T,
278 Asnod2-B02-B, Asnod2-B07-B^T, Asnod3-E08-A compared with *Z. amurskyensis* KMM
279 3526^T and *Z. laminariae* KMM 3676^T are listed in Table 1.

280 The six strains were resistant to penicillin, oxacillin, polymyxin B, bacitracin, colistin,
281 gentamicin, neomycin, kanamycin, trimethoprim/sulfamethoxazole and nalidixic acid, and
282 were sensitive to rifampicin. Asnod2-B07-B^T, Asnod3-E08-A, *Z. amurskyensis* KMM 3526^T
283 and *Z. laminariae* KMM 3676^T were sensitive to lincosamycin and carbenicillin, while Asnod1-
284 F08^T and Asnod2-B02-B were resistant to these antibiotics.

285 The major fatty acids (>10 % of the total fatty acids) of strains Asnod1-F08^T, Asnod2-B02-B,
286 Asnod2-B07-B^T and Asnod3-E08-A were iso-C_{15:0} (>20 %), iso-C_{17:0} 3-OH (>18 %), iso-C_{15:1}
287 G (>10 %) and those contained in summed feature 3 (>10 %; C_{16:1} ω7c and/or iso-C_{15:0} 2OH)
288 (Table 2). These fatty acid profiles were similar to those of *Z. amurskyensis* KMM 3526^T and
289 *Z. laminariae* KMM 3676^T (Table 2) and confirmed previous results [2]. From all strains, the
290 respiratory quinone was menaquinone-6 (MK-6). The major polar lipids of all strains were
291 unidentified lipids and aminolipids and phosphatidylethanolamine (Fig. S2). *Z. amurskyensis*
292 KMM 3526^T and *Z. laminariae* KMM 3676^T were characterized by the presence of two
293 phosphatidylethanolamine spots. In conclusion, phenotypic characterizations and
294 phylogenetic analysis using 16S rRNA gene sequences together with whole-genome pairwise
295 comparisons show that strains Asnod1-F08^T, Asnod2-B02-B on one hand, and Asnod2-B07-
296 B^T and Asnod3-E08-A on the other hand represent two novel species in the genus *Zobellia*,
297 for which the names *Zobellia roscoffensis* sp. nov. and *Zobellia nedashkovskayae* sp. nov. are
298 proposed, respectively.

299 **EMENDED DESCRIPTION OF THE GENUS *ZOBELLIA* BARBEYRON *ET AL.* 2001**

300 The description of the genus is as given by Barbeyron et al. [1] with the following modified
301 features. The hydrolysis of galactans from red seaweeds such as agar, κ-carrageenan and ι-
302 carrageenan and of alginic acid from brown seaweeds depends on the species. The G + C
303 content of the genomic DNA ranges from 36.7 to 43 mol%.

304 **DESCRIPTION OF *ZOBELLIA ROSCOFFENSIS* SP. NOV.**

305 *Zobellia roscoffensis* (ros.coff.en'sis. N.L. fem. adj. *roscoffensis* referring to Roscoff in
306 Brittany, France, where the type strain was isolated).

307 Cells are Gram-stain-negative, aerobic, chemoorganotrophic, heterotrophic, and rod-shaped,
308 approximately 0.5 μm in diameter and 2.0-4.0 μm in length; a few cells greater than 4 μm in
309 length may occur. Flagella are absent. Prosthecae and buds are not produced. Colonies on
310 ZoBell agar are orange-coloured, convex, circular and mucoid in consistency and 2.0–3.0 mm
311 in diameter strongly attached to agar after incubation for 3 days at 25 °C. A weak iridescence
312 is visible on R2A agar after 3 days at 18°C. Growth in ZoBell broth occurs from 4 to 40 °C
313 (optimum, 25 °C), at pH 5.5 to 8.5 (optimum pH 7.5) and in the presence of 2 to 6 % NaCl
314 (optimum, 2 %). In presence of magnesium and KCl, the growth also occurs with 0 and 1 %
315 NaCl. Positive for the gliding motility and the flexirubin-type pigment production. Nitrate is
316 reduced. β -galactosidase, oxidase- and catalase-positive. DNA, aesculin, Tween 20, 60 (for
317 the type strain only) and 80 and agar are hydrolysed, but Tween 40, gelatin (for the type strain
318 only), starch, κ -carrageenan, ι -carrageenan and alginic acid are not. D-glucose, D-galactose,
319 D-fructose, rhamnose, N-acetyl-glucosamine, L-fucose (weakly for the type strain), raffinose
320 sucrose, D-maltose, lactose, L-arabinose, mannose, xylose, mannitol, xylan and porphyrin are
321 utilized as carbon and energy sources, but caprate, adipate, citrate, gluconate, malate,
322 phenylacetate, glucomannan, galactomannan, arabinan, arabinoxylan, xyloglucan, laminarin,
323 amylopectine, agar, κ -, ι - and λ -carrageenan, ulvan, fucoidin (*Ascophyllum nodosum*), alginic
324 acid and pectin (apple) are not. Acid is produced from D-glucose, D-galactose, D-fructose,
325 mannose, D-arabinose, rhamnose, L-fucose, D-tagatose, D-Lyxose methyl-D-
326 mannopyranoside, methyl-D-glucopyranoside, N-acetyl-glucosamine, amygdaline, arbutine,
327 salicine, D-cellobiose, D-lactose, D-melibiose, D-sucrose, D-trehalose, D-Turanose and D-
328 maltose, but not from L-arabinose, D-ribose, D-xylose, L-sorbose, D-melezitose, L-xylose, D-

329 fucose, methyl-D-xylopyranoside, glycerol, inositol, D-mannitol, D-sorbitol, adonitol, L- and
330 D-arabitol, dulcitol, erythritol, xylitol, gentiobiose, raffinose, inulin, starch, glycogen,
331 gluconate, 2-ketogluconate and 5-ketogluconate. Negative for indole and H₂S production and
332 for arginine dihydrolase, tryptophan deaminase, urease, lysine decarboxylase, ornithine
333 decarboxylase activities. In the API ZYM system, activities from acid phosphatase, alkaline
334 phosphatase, esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine
335 arylamidase, trypsin, α -glucosidase, β -glucosidase and naphthol-AS-BI-phosphohydrolase are
336 present, but activities from lipase (C14), α -chymotrypsin, β -glucuronidase and α -fucosidase
337 are absent. The only lipoquinone detected is MK-6. The major fatty acids (>10 % of the total
338 fatty acids) are iso-C_{15:0}, iso-C_{17:0} 3-OH, iso-C_{15:1} G and summed feature 3 (C_{16:1} ω 7c and/or
339 iso-C_{15:0} 2OH). The major polar lipids are phosphatidylethanolamine and unknown
340 aminolipids. The DNA G+C content is 37.6-37.7 mol%.

341 The type strain Asnod1-F08^T (RCC 6906^T = KMM 6823^T = CIP 111902^T) and the strain
342 Asnod2-B02-B (RCC 6907 = KMM 6824 = CIP 111903) were isolated from *Ascophyllum*
343 *nodosum* surface microbiota. The Genbank/EMBL/DDBJ accession number for the 16S
344 rRNA gene sequence of strains Asnod1-F08^T and Asnod2-B02-B are MW114830 and
345 MW114831, respectively. The Genbank/ENA/DDBJ accession number for the genome
346 sequence of strains Asnod1-F08^T and Asnod2-B02-B are JADDXT000000000 and
347 JADDXS000000000 respectively.

348 **DESCRIPTION OF ZOBELLIA NEDASHKOVSKAYAE SP. NOV.**

349 *Zobellia nedashkovskayae* (ne.dash.kovs'ka.yae N.L. gen. n. *nedashkovskayae* in honour of
350 Olga Nedashkovskaya for her great contribution to the study of marine *Bacteroidetes*).

351 Cells are Gram-stain-negative, aerobic, chemoorganotrophic, heterotrophic, and rod-shaped,
352 approximately 0.5 μ m in diameter and 2.0-4.0 μ m in length; a few cells greater than 4 μ m in
353 length may occur. Flagella are absent. Prosthecae and buds are not produced. Colonies on

354 ZoBell agar are orange yellow-coloured, convex, circular and mucoid in consistency and 2.0–
355 3.0 mm in diameter strongly attached to agar after incubation for 3 days at 25 °C. Growth in
356 ZoBell broth occurs from 4 to 40 °C (optimum, 25-30 °C), at pH 5.5 to 8.0 (optimum pH 7.5)
357 and in the presence of 3 to 6 % NaCl (optimum, 2 %). In presence of magnesium and KCl, the
358 growth also occurs with 1 % NaCl. Positive for the gliding motility and the flexirubin-type
359 pigment production. Nitrate is reduced. β -galactosidase, oxidase- and catalase-positive. DNA,
360 aesculin, Tween 20, 40, 60 and 80 (for the type strain only) and alginic acid are hydrolysed,
361 but gelatin, starch, agar, κ -carrageenan and ι -carrageenan are not. D-glucose, D-galactose, D-
362 fructose, rhamnose, raffinose sucrose, D-maltose, lactose, L-arabinose, mannose, xylose,
363 mannitol, laminarin, porphyrin, fucoidin (*Ascophyllum nodosum*) and alginic acid are utilized
364 as carbon and energy sources, but L-fucose, N-acetyl-glucosamine, caprate, adipate, citrate,
365 gluconate, malate, phenylacetate, glucomannan, galactomannan, arabinan, arabinoxylan,
366 xyloglucan, xylan, amylopectine, agar, κ -, ι - and λ -carrageenan, ulvan and pectin (apple) are
367 not. Acid is produced from D-glucose, D-galactose, D-fructose, mannose, L-arabinose,
368 rhamnose, D-xylose, D-Lyxose, methyl-D-mannopyranoside, methyl-D-glucopyranoside, N-
369 acetyl-glucosamine, D-mannitol, amygdaline, arbutine, salicine, D-cellobiose, D-lactose, D-
370 sucrose, D-trehalose, D-Turanose and D-maltose, but not from D-arabinose, D-ribose, L-
371 sorbose, D-melezitose, L-xylose, D-fucose, L-fucose, D-tagatose, methyl-D-xylopyranoside,
372 glycerol, inositol, D-sorbitol, adonitol, L- and D-arabitol, dulcitol, erythritol, xylitol, D-
373 melibiose, gentiobiose, raffinose, inulin, starch, glycogen, gluconate, 2-ketogluconate and 5-
374 ketogluconate. Negative for indole and H₂S production and for arginine dihydrolase,
375 tryptophan deaminase, urease, lysine decarboxylase, ornithine decarboxylase activities. In the
376 API ZYM system, activities from acid phosphatase, alkaline phosphatase, esterase lipase
377 (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin,
378 α -glucosidase, β -glucosidase and naphthol-AS-BI-phosphohydrolase are present, but

379 activities from lipase (C14), β -glucuronidase, α -galactosidase, N-acetyl- β -glucosaminidase
380 and α -fucosidase activities are absent. The only lipoquinone detected is MK-6. The major
381 fatty acids (>10 % of the total fatty acids) are iso-C_{15:0}, iso-C_{17:0} 3-OH, iso-C_{15:1} G and
382 summed feature 3 (C_{16:1} ω 7c and/or iso-C_{15:0} 2OH). The major polar lipids are
383 phosphatidylethanolamine and unknown aminolipids. The DNA G+C content is 36.7-36.8
384 mol%.

385 The type strain Asnod2-B07-B^T (RCC 6908^T = KMM 6825^T = CIP 111904^T) and the strain
386 Asnod3-E08-A (RCC 6909 = KMM 6826 = CIP 111905) were isolated from *Ascophyllum*
387 *nodosum* surface microbiota. The Genbank/EMBL/DDBJ accession number for the 16S
388 rRNA gene sequence of strains Asnod2-B07-B^T and Asnod3-E08-A are MW114832 and
389 MW114833, respectively. The Genbank/ENA/DDBJ accession number for the genome
390 sequence of strains Asnod2-B07-B^T and Asnod3-E08-A are is JADDXR000000000 and
391 JADDXQ000000000 respectively.

392

393 **Acknowledgements**

394 The LABGeM (CEA/Genoscope & CNRS UMR8030), the France Génomique and French
395 Bioinformatics Institute national infrastructures (funded as part of Investissement d'Avenir
396 program managed by Agence Nationale pour la Recherche, contracts ANR-10-INBS-09 and
397 ANR-11-INBS-0013) are acknowledged for support within the MicroScope annotation
398 platform. We are grateful to the Institut Français de Bioinformatique (ANR-11-INBS-
399 0013) and the Roscoff Bioinformatics platform ABiMS (<http://abims.sb-roscoff.fr>) for
400 providing help for computing and storage resources. This work has benefited from the
401 facilities of the Genomer platform, which is part of the Biogenouest core facility network. The
402 authors are grateful to Dr. Gurvan Michel for initial discussions on the project, to Dr.

403 Bernhard Schink for his advice on Latin grammar and to Dr. Sébastien Bridel for his help
404 with the construction of the phylogenomic tree.

405

406 **Funding information**

407 This work was supported by the French ANR investment expenditure program IDEALG
408 (grant agreement ANR-10- BTBR-04) and the French ANR project ALGAVOR (grant
409 agreement ANR-18-CE02-0001-01).

410

411 **Conflicts of interest**

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

413

414 **References**

- 415 1. **Barbeyron T, L'Haridon S, Corre E, Kloareg B, Potin P.** *Zobellia galactanovorans*
416 gen. nov., sp. nov., a marine species of *Flavobacteriaceae* isolated from a red alga, and
417 classification of [*Cytophaga*] *uliginosa* (ZoBell and Upham 1944) Reichenbach 1989
418 as *Zobellia uliginosa* gen. nov., comb. nov. *Int J Syst Evol Microbiol* 2001;51:985–97.
- 419 2. **Nedashkovskaya OI, Suzuki M, Vancanneyt M, Cleenwerck I, Lysenko AM, et al.**
420 *Zobellia amurskyensis* sp. nov., *Zobellia laminariae* sp. nov. and *Zobellia russellii* sp.
421 nov., novel marine bacteria of the family *Flavobacteriaceae*. *Int J Syst Evol Microbiol*
422 2004;54:1643–8.
- 423 3. **Martin M, Barbeyron T, Martin R, Portetelle D, Michel G, et al.** The Cultivable
424 Surface Microbiota of the Brown Alga *Ascophyllum nodosum* is Enriched in
425 Macroalgal-Polysaccharide-Degrading Bacteria. *Front Microbiol* 2015;6:1–14.

- 426 4. **Zobell C.** Studies on marine bacteria I The cultural requirements of heterotrophic
427 aerobes. *J Mar Res* 1941;4:42–75.
- 428 5. **Powers EM.** Efficacy of the Ryu nonstaining KOH technique for rapidly determining
429 gram reactions of food-borne and waterborne bacteria and yeasts. *Appl Environ*
430 *Microbiol* 1995;61:3756–3758.
- 431 6. **Reichenbach H, Kleinig H, Achenbach H.** The pigments of *Flexibacter elegans*:
432 Novel and chemosystematically useful compounds. *Arch Microbiol* 1974;101:131–144.
- 433 7. **Smibert R, Krieg N.** General characterization. In: Gerhardt P, Murray R, Costilow R,
434 Nester E, Wood W, et al. (editors). *Manual of Methods for General Bacteriology*.
435 Washington, DC., USA: American Society for Microbiology; 1981. pp. 409–443.
- 436 8. **Draget KI, Ostgaard K, Smidsrød O.** Alginate-based solid media for plant tissue
437 culture. *Appl Microbiol Biotechnol* 1989;31:79–83.
- 438 9. **Thomas F, Barbeyron T, Michel G.** Evaluation of reference genes for real-time
439 quantitative PCR in the marine flavobacterium *Zobellia galactanivorans*. *J Microbiol*
440 *Methods* 2011;84:61–6.
- 441 10. **Tindall BJ.** A comparative study of the lipid composition of *Halobacterium*
442 *saccharovorum* from various sources. *Syst Appl Microbiol* 1990;13:128–130.
- 443 11. **Tindall B.** Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol Lett*
444 1990;66:199–202.
- 445 12. **Miller L.** A single derivatization method for bacterial fatty acid methyl esters
446 including hydroxy acids. *J Clin Microbiol* 1982;16:584–586.

- 447 13. **Kuykendall L, Roy M, O'Neill J, Devine T.** Fatty acids, antibiotic resistance, and
448 deoxyribonucleic acid homology groups of *Bradyrhizobium japonicum*. *Int J Syst*
449 *Bacteriol* 1988;38:358–361.
- 450 14. **Bligh E, Dyer W.** A rapid method of total lipid extraction and purification. *Can J*
451 *Biochem Physiol* 1959;37:911–917.
- 452 15. **Tindall B, Sikorski J, Smibert R, Kreig N.** Phenotypic characterization and the
453 principles of comparative systematics. In: Reddy C, Beveridge T, Breznak J, Marzluf
454 G, Schmidt T, et al. (editors). *Methods for General and Molecular Microbiology*.
455 Washington, DC., USA: ASM Press; 2007. pp. 330–393.
- 456 16. **Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, et al.** SPAdes: A New
457 Genome Assembly Algorithm and Its Applications to Single-Cell Sequencing. *J*
458 *Comput Biol* 2012;19:455–477.
- 459 17. **Bosi E, Donati B, Galardini M, Brunetti S, Sagot MF, et al.** MeDuSa: A multi-draft
460 based scaffold. *Bioinformatics* 2015;31:2443–2451.
- 461 18. **Luo R, Liu B, Xie Y, Li Z, Huang W, et al.** SOAPdenovo2: An empirically improved
462 memory-efficient short-read de novo assembler. *Gigascience* 2012;1:1–6.
- 463 19. **Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW.** CheckM:
464 Assessing the quality of microbial genomes recovered from isolates, single cells, and
465 metagenomes. *Genome Res* 2015;25:1043–1055.
- 466 20. **Hicks R, Amann R, Stahl D.** Dual staining of natural bacterioplankton with 4',6-
467 diamidino-2-phenylindole and fluorescent oligonucleotide probes targeting kingdom-
468 level 16S rRNA sequences. *Appl Environ Microbiol* 1992;58:2158–2163.

- 469 21. **Kane M, Poulsen L, Stahl D.** Monitoring the enrichment and isolation of sulfate-
470 reducing bacteria by using oligonucleotide hybridization probes designed from
471 environmentally derived 16S rRNA sequences. *Appl Environ Microbiol* 1993;59:682–
472 686.
- 473 22. **Barbeyron T, Carpentier F, L’Haridon S, Schüler M, Michel G, et al.** Description
474 of *Maribacter forsetii* sp. nov., a marine *Flavobacteriaceae* isolated from North Sea
475 water, and emended description of the genus *Maribacter*. *Int J Syst Evol Microbiol*
476 2008;58:790–797.
- 477 23. **Katoh K, Rozewicki J, Yamada KD.** MAFFT online service: multiple sequence
478 alignment, interactive sequence choice and visualization. *Brief Bioinform* 2017;1–7.
- 479 24. **Saitou N, Nei M.** The neighbor-joining method: a new method for reconstructing
480 phylogenetic trees. *Mol Biol Evol* 1987;4:406–425.
- 481 25. **Fitch WM.** Toward defining the course of evolution: Minimum change for a specific
482 tree topology. *Syst Biol* 1971;20:406–416.
- 483 26. **Felsenstein J.** Evolutionary trees from DNA sequences: A maximum likelihood
484 approach. *J Mol Evol* 1981;17:368–376.
- 485 27. **Tamura K, Stecher G, Peterson D, Filipski A, Kumar S.** MEGA6: Molecular
486 evolutionary genetics analysis version 6.0. *Mol Biol Evol* 2013;30:2725–2729.
- 487 28. **Kimura M.** A simple method for estimating evolutionary rates of base substitutions
488 through comparative studies of nucleotide sequences. *J Mol Evol* 1980;16:111–120.
- 489 29. **Trifinopoulos J, Nguyen LT, von Haeseler A, Minh BQ.** W-IQ-TREE: a fast online
490 phylogenetic tool for maximum likelihood analysis. *Nucleic Acids Res* 2016;44:W232–

- 491 W235.
- 492 30. **Nei M, Kumar S.** *Molecular Evolution and Phylogenetics*. New York: Oxford
493 University Press.; 2000.
- 494 31. **Felsenstein J.** Confidence limits on phylogenies: an approach using the bootstrap.
495 *Evolution (N Y)* 1985;39:783–791.
- 496 32. **Yoon SH, Ha SM, Kwon S, Lim J, Kim Y, et al.** Introducing EzBioCloud: A
497 taxonomically united database of 16S rRNA gene sequences and whole-genome
498 assemblies. *Int J Syst Evol Microbiol* 2017;67:1613–1617.
- 499 33. **Pearson W, Lipman D.** Improved tools for biological sequence comparison. *Proc Natl*
500 *Acad Sci* 1988;85:2444–2448.
- 501 34. **Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Vandamme P, et al.**
502 DNA-DNA hybridization values and their relationship to whole-genome sequence
503 similarities. *Int J Syst Evol Microbiol* 2007;57:81–91.
- 504 35. **Figueras MJ, Beaz-Hidalgo R, Hossain MJ, Liles MR.** Taxonomic affiliation of new
505 genomes should be verified using average nucleotide identity and multilocus
506 phylogenetic analysis. *Genome Announc* 2014;2:2–3.
- 507 36. **Chun J, Oren A, Ventosa A, Christensen H, Arahal DR, et al.** Proposed minimal
508 standards for the use of genome data for the taxonomy of prokaryotes. *Int J Syst Evol*
509 *Microbiol* 2018;68:461–466.
- 510 37. **Auch AF, von Jan M, Klenk HP, Göker M.** Digital DNA-DNA hybridization for
511 microbial species delineation by means of genome-to-genome sequence comparison.
512 *Stand Genomic Sci* 2010;2:117–134.

- 513 38. **Meier-Kolthoff JP, Auch AF, Klenk HP, Göker M.** Genome sequence-based species
514 delimitation with confidence intervals and improved distance functions. *BMC*
515 *Bioinformatics*;14. Epub ahead of print 2013. DOI: 10.1186/1471-2105-14-60.
- 516 39. **Meier-Kolthoff JP, Göker M, Spröer C, Klenk HP.** When should a DDH experiment
517 be mandatory in microbial taxonomy? *Arch Microbiol* 2013;195:413–418.
- 518 40. **Chernysheva N, Bystritskaya E, Stenkova A, Golovkin I, Nedashkovskaya O, et al.**
519 Comparative genomics and CAZyme genome repertoires of marine *Zobellia*
520 *amurskyensis* KMM 3526T and *Zobellia laminariae* KMM 3676T. *Mar Drugs*;17.
521 Epub ahead of print 2019. DOI: 10.3390/md17120661.
- 522 41. **Vallenet D, Belda E, Calteau A, Cruveiller S, Engelen S, et al.** MicroScope - An
523 integrated microbial resource for the curation and comparative analysis of genomic and
524 metabolic data. *Nucleic Acids Res* 2013;41:636–647.
- 525 42. **Lombard V, Golaconda Ramulu H, Drula E, Coutinho PM, Henrissat B.** The
526 carbohydrate-active enzymes database (CAZy) in 2013. *Nucleic Acids Res*
527 2014;42:490–495.
- 528 43. **Richter M, Rosselló-Móra R.** Shifting the genomic gold standard for the prokaryotic
529 species definition. *Proc Natl Acad Sci U S A* 2009;106:19126–19131.
- 530 44. **Auch AF, Klenk HP, Göker M.** Standard operating procedure for calculating
531 genome-to-genome distances based on high-scoring segment pairs. *Stand Genomic Sci*
532 2010;2:142–148.
- 533 45. **Naretto A, Fanuel M, Ropartz D, Rogniaux H, Larocque R, et al.** The agar-specific
534 hydrolase ZgAgaC from the marine bacterium *Zobellia galactanivorans* defines a new

- 535 GH16 protein subfamily. *J Biol Chem* 2019;294:6923–6939.
- 536 46. **Barbeyron T, Thomas F, Barbe V, Teeling H, Schenowitz C, et al.** Habitat and
537 taxon as driving forces of carbohydrate catabolism in marine heterotrophic bacteria:
538 Example of the model algae-associated bacterium *Zobellia galactanivorans* Dsij^T.
539 *Environ Microbiol* 2016;18:4610–4627.
- 540 47. **Ficko-Blean E, Préchoux A, Thomas F, Rochat T, Larocque R, et al.** Carrageenan
541 catabolism is encoded by a complex regulon in marine heterotrophic bacteria. *Nat*
542 *Commun*;8. Epub ahead of print 2017. DOI: 10.1038/s41467-017-01832-6.
- 543 48. **Thomas F, Lundqvist LCE, Jam M, Jeudy A, Barbeyron T, et al.** Comparative
544 characterization of two marine alginate lyases from *Zobellia galactanivorans* reveals
545 distinct modes of action and exquisite adaptation to their natural substrate. *J Biol Chem*
546 2013;288:23021–37.
- 547 49. **Thomas F, Barbeyron T, Tonon T, Génicot S, Czjzek M, et al.** Characterization of
548 the first alginolytic operons in a marine bacterium: from their emergence in marine
549 *Flavobacteriia* to their independent transfers to marine *Proteobacteria* and human gut
550 *Bacteroides*. *Environ Microbiol* 2012;14:2379–94.
- 551

Table 1. Phenotypic characteristics of strains Asnod1-F08^T, Asnd2-B02-B, Asnod2-B07-B^T, Asnod3-E08-A and of two *Zobellia* species used as related type strains.

1, Strain Asnod1-F08^T (*Zobellia roscoffensis* sp. nov.); 2, Asnd2-B02-B (*Zobellia roscoffensis* sp. nov.); 3, Asnod2-B07-B^T (*Zobellia nedashkovskayae* sp. nov.); 4, Asnod3-E08-A (*Zobellia nedashkovskayae* sp. nov.); 5, *Zobellia amurskyensis* KMM 3526^T; 6, *Zobellia laminariae* KMM 3676^T. Cells of all strains share the following characteristics: Gram-negative, aerobic, heterotroph, chemorganotroph with respiratory metabolism, gliding motility, do not form endospores, do not accumulate poly- β -hydroxybutyrate as an intracellular reserve product; require Na⁺ ion or seawater for growth. All strains are positive for flexirubin production and nitrate reduction; for the utilization as sole carbon source of D-glucose, D-galactose, D-fructose, rhamnose, sucrose, D-maltose, lactose, L-arabinose, mannose, xylose, mannitol, and porphyrin; for the hydrolysis of DNA, aesculin and Tween 20; for acid and alkaline phosphatase, esterase lipase (C8), leucine, valine and cystine arylamidases, trypsin, α - and β -glucosidase, β -galactosidase (PNPG test), oxidase and catalase activities; for the acid production from D-glucose, D-galactose, D-fructose, mannose, rhamnose, D-xylose and D-maltose. All strains are negative for indole production; for utilization of caprate, adipate, citrate, gluconate, malate, phenylacetate, glucomannan, galactomannan, arabinan, xyloglucan, agar, κ -, ι - and λ -carrageenan and pectin; for the hydrolysis of κ - and ι -carrageenan; for urease, Lipase (C14) and α -fucosidase activities; for the acid production from L-sorbose, D-melezitose, L-xylose, methyl-D-xylopyranoside, adonitol, L-arabitol, dulcitol, erythritol, xylitol, gentiobiose, raffinose, inulin, starch, glycogen, gluconate, 2-ketogluconate and 5-ketogluconate. +, Positive; -, negative; W, weakly positive; NA, not available.

Characteristic	1	2	3	4	5	6
Growth conditions:						
Optimum temperature (°C)	25 - 30	25 - 30	25 - 30	25 - 30	23 - 25 [†]	21 - 23 [†]
Temperature range (°C)	4 - 40	4 - 37	4 - 40	4 - 40	4 - 32 [†]	4 - 30 [†]
Optimum pH	7.5	7.5	7.5	7.5	NA	NA
pH range	5.5 - 8.5	5.5 - 8.5	5.5 - 8.0	5.5 - 8.0	NA	NA
NaCl range (%)	2- 6	2 - 6	3 - 6	3 - 4	1 - 6 [†]	1.5 - 6 [†]
Optimum NaCl (%)	2	2	2	2	2 [†]	2 [†]
Iridescence	w	w	-	-	-	-
Enzyme (API 20 NE)						
Arginine dihydrolase	-	-	-	-	+	-
Gelatinase	-	+	-	-	+	+
Hydrolysis of:						
Starch (lugol assay)	-	-	-	-	+	-
Agar (lugol assay)	+	+	-	-	+	-
Alginic acid	-	-	+	+	+	-
Tween 40	-	-	+	+	-	-
Tween 60	+	-	+	+	+	-
Tween 80	+	+	+	-	-	-

Acid production						
Glycerol	-	-	-	-	+	+
D-arabinose	+	+	-	-	+	+
L-arabinose	-	-	+	+	+	+
D-ribose	-	-	-	-	+	+
Inositol	-	-	-	-	+	+
D-mannitol	-	-	+	+	+	+
D-sorbitol	-	-	-	-	+	+
Methyl-D-mannopyranoside	+	+	+	+	+	-
Methyl-D-glucopyranoside	+	+	+	+	+	-
N-acetyl-glucosamine	+	+	+	+	-	-
Amygdaline	+	+	+	+	+	-
Arbutine	+	+	+	+	-	-
Salicine	+	+	+	+	-	-
D-cellobiose	+	+	+	+	w	-
D-lactose	+	+	+	+	+	-
D-melibiose	+	+	-	-	+	+
D-sucrose	+	+	+	+	+	-
D-trehalose	+	+	+	+	+	-
D-turanose	+	+	+	+	+	-
D-lyxose	+	+	+	+	+	-
D-tagatose	+	+	-	-	+	-
D-fucose	-	-	-	-	+	+
L-fucose	+	+	-	-	+	+
D-arabitol	-	-	-	-	+	-
Utilization of						
N-acetyl-glucosamine	+	+	-	-	+	+
L-fucose	w	+	w	w	+	-
Raffinose	+	+	+	-	+	+
Amylopectine	-	-	-	-	+	-
Laminarin	-	-	+	+	+	w
Lichenin	w	w	-	-	w	w
Arabinoxylan	-	-	-	-	+	-
Fuoidin	-	-	+	+	+	w
Xylan	+	+	-	-	+	-
Ulvan	w	w	w	+	+	w
Alginic acid	-	-	+	+	w	w
Enzymes (API ZYM)						
α -Chymotrypsin	-	-	+	+	-	-
Naphtol phosphohydrolase	+	+	w	+	+	+
α -galactosidase	-	+	-	-	+	-

β -glucuronidase	-	-	-	-	-	-
N-acetyl- β -glucosaminidase	-	+	-	-	+	+
α -mannosidase	-	+	-	-	+	-
DNA G+C mol %	37.56	37.7	37.69	36.8	38.1	36.8

38.09

†Data from Nedashkovskaya [2]

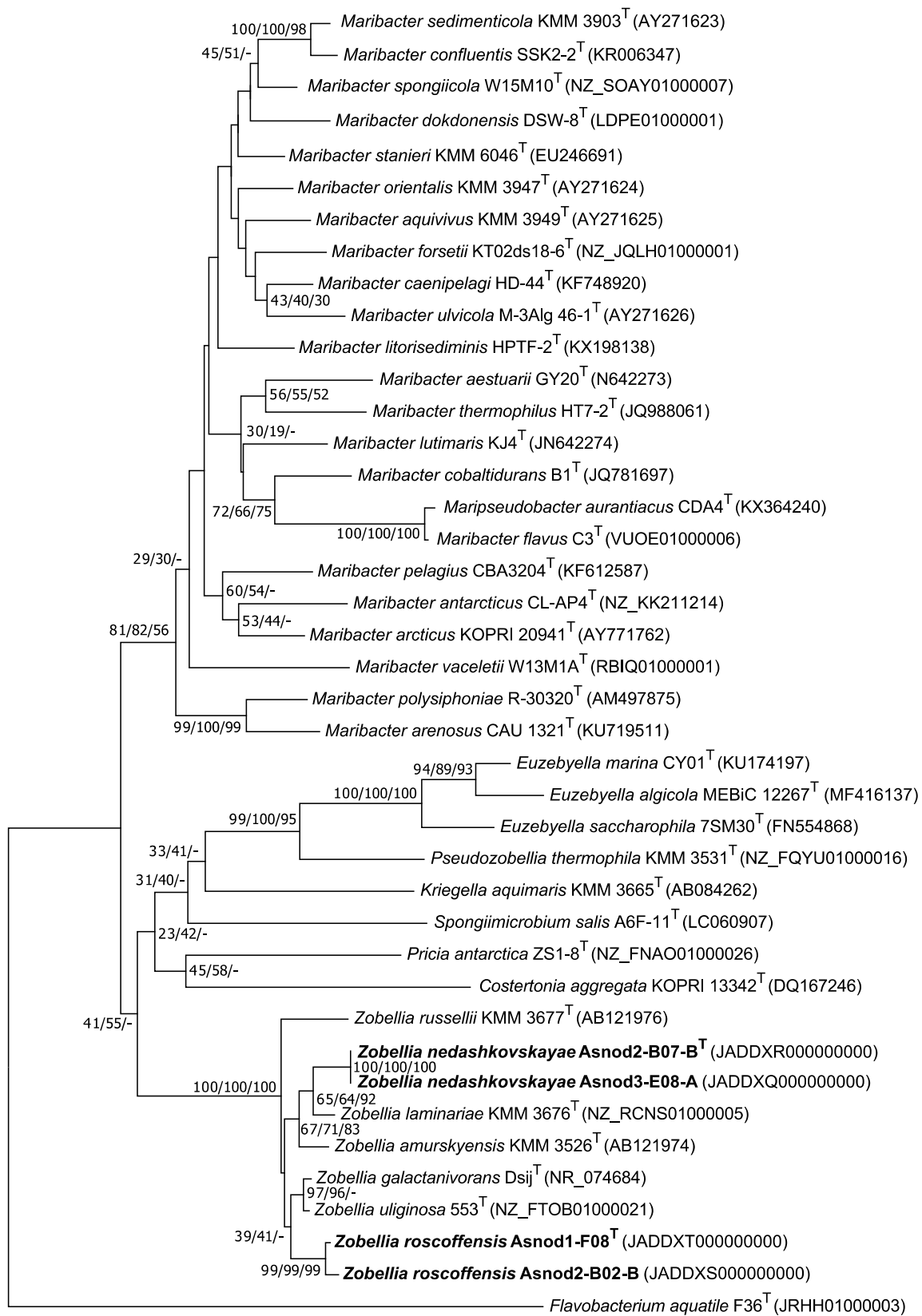
Table 2. Cellular fatty acid composition of *Zobellia* species studied.

Strains: 1, Asnod 1-F08^T (*Zobellia roscoffensis* sp. nov.); 2, Asnod2-B02-B (*Z. roscoffensis* sp. nov.); 3, Asnod2-B07-B^T (*Z. nedashkovskayae* sp. nov.); 4, Asnod3-E08-A^T (*Z. nedashkovskayae* sp. nov.); 5, *Z. amurskyensis* KMM 3526^T; 6, *Z. laminariae* KMM 3676^T. Data are percentages of the total fatty acids. Fatty acids that represented <1.0 % in the six strains were omitted. Fatty acids that represented >10.0 % are indicated in bold. -, Fatty acids not detected or below 1 %.

Fatty acid	1	2	3	4	5	6
Straight-chain						
C _{14:0}	1.0	1.0	-	-	1.5	1.0
C _{15:0}	7.5	8.0	8.6	9.3	6.5	10.3
C _{15:1} <i>ω6c</i>	1.0	1.0	1.7	1.8	1.2	1.1
C _{16:0}	-	1.0	-	-	-	1.0
C _{16:0} 3-OH	1.2	1.0	1.1	1.0	1.6	1.4
C _{17:1} <i>ω6c</i>	-	-	1.0	1.0	-	-
C _{18:1} <i>ω5c</i>	-	-	1.5	1.4	1.0	1.0
Branched chain						
iso-C _{15:0}	26.7	25.2	20.9	22.6	29.5	25.6
iso-C _{15:0} 3-OH	3.2	3.1	3.6	3.4	3.0	2.8
anteiso-C _{15:0}	-	-	1.3	1.4	1.7	1.5
iso-C _{15:1} G	12.9	12.2	10.7	12.3	11.8	9.4
iso-C _{17:0} 3-OH	19.8	20.3	21.2	18.8	15.4	18.7
iso-C _{17:1} <i>ω9c</i>	6.3	7.3	7.9	7.7	4.7	5.6
Summed features*						
3	10.7	10.9	13.8	12.9	14.9	14.2
4	1.0	1.0	1.0	1.0	1.0	-
ECL†						
13.565	2.7	2.8	1.4	1.3	1.6	1.4
16.582	1.2	1.2	1.2	1.2	1.0	1.0

* Summed feature 3 contained C_{16:1} *ω7c* and/or iso-C_{15:0} 2OH; summed feature 4 contained iso-C_{17:1} I and/or anteiso-C_{17:1} B.

† ECL, Equivalent chain-length. The identity of the fatty acid is not known



0.01

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences, showing the phylogenetic relationships between strains Asnod1-F08^T, Asnod2-B02-B, Asnod2-B07-B^T, Asnod3-E08-A and related taxa from the family *Flavobacteriaceae*. Numbers at the nodes indicate bootstrap values (in percentage of 1000 replicates) in neighbour-joining, maximum-likelihood and maximum-parsimony analyses respectively, while dashes instead of numbers indicate that the node was not observed in the corresponding analysis. For nodes conserved in at least two trees, all bootstrap values are shown. Nodes without bootstrap value are not conserved in other trees and < 70 %. *Flavobacterium aquatile* F36^T was used as an outgroup. Bar, 0.01 changes per nucleotide position.

Supplementary Table S1: Properties of the draft genomes obtained for the four studied strains.

Strain	Asnod1-F08^T	Asnod2-B02-B	Asnod2-B07-B^T	Asnod3-E08-A
Species	<i>Z. roscoffensis</i>	<i>Z. roscoffensis</i>	<i>Z. nedashkovskayae</i>	<i>Z. nedashkovskayae</i>
Genome accession	JADDXT0000000000	JADDXS0000000000	JADDXR0000000000	JADDXQ0000000000
Draft genome length (b)	4,979,074	5,012,136	4,941,018	5,025,849
Number of contigs	6	11	5	13
Number of scaffolds	2	6	3	9
Estimated completeness	100%	100%	100%	100%
N50	1,935,264	1,275,558	2,631,201	1,255,004
Paired sequencing reads	5,312,862	5,534,396	4,493,736	2,037,244
Total sequencing data (b)	1,259,406,978	1,360,453,660	1,105,016,829	464,679,866
Estimated coverage	253 X	271 X	224 X	92 X
G+C content	37.56%	37.69%	36.81%	36.68%
Number of CDS	4,199	4,205	4,290	4,412

Supplementary Table S2

Pairwise nucleotide comparisons (in percentage of similarity sequence) between *Zobellia* species 16S rRNA gene sequences.

	1										
<i>Zobellia roscoffensis</i> Asnod1-F08 ^T	1	100	2								
<i>Z. roscoffensis</i> Asnod2-B02-B	2	99.7	100	3							
<i>Z. nedashkovskayae</i> Asnod2-B07-B ^T	3	97.8	98.0	100	4						
<i>Z. nedashkovskayae</i> Asnod3-E08-A	4	97.8	98.0	100	100	5					
<i>Z. amurskyensis</i> KMM 3526 ^T	5	98.5	98.3	98.5	98.5	100	6				
<i>Z. laminariae</i> KMM 3676 ^T	6	98.2	97.9	99.1	99.1	99.3	100	7			
<i>Z. galactanivorans</i> Dsjj ^T	7	99.0	98.8	98.1	98.1	99.0	98.5	100	8		
<i>Z. russellii</i> KMM 3677 ^T	8	98.0	97.7	97.4	97.4	98.4	97.9	98.4	100	9	
<i>Z. uliginosa</i> 553 ^T	9	99.0	98.8	97.9	97.9	98.9	98.4	99.8	98.4	100	

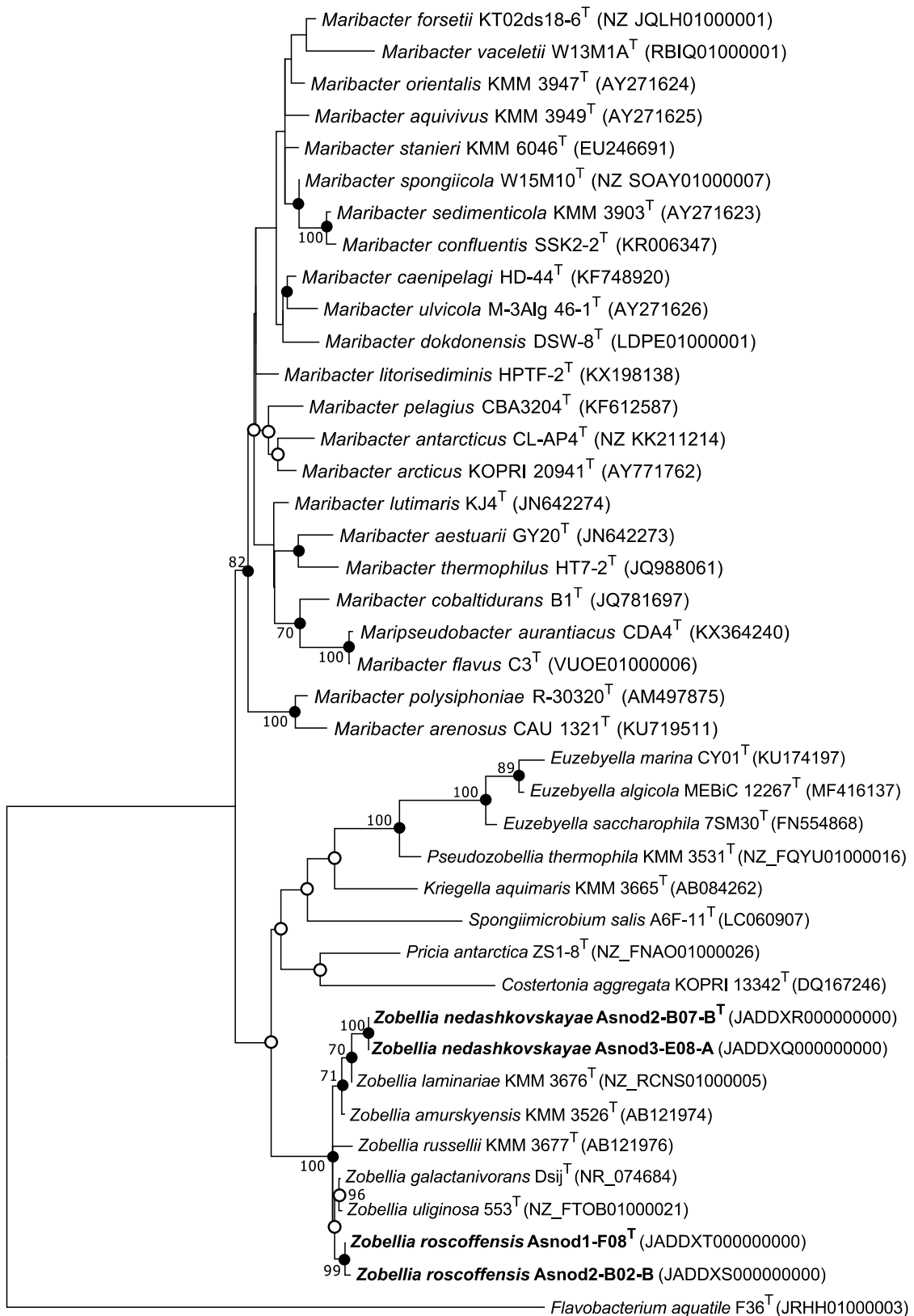
Supplementary Table S3

Whole genome relatedness analysis (in percentage) of strains Asnod1-F08^T, Asnod2-B02-B, Asnod2-B07-B^T, Asnod3-E08-A and *Zobellia* species for which whole genome sequences are available.

Average nucleotide identities (ANI) are shown below the diagonal and genome-to-genome distance calculations (GGDC) are shown above the diagonal. GGDC values were calculated online (<http://ggdc.dsmz.de/distcalc2.php>) and the results from formula 2 were used. ANI values were calculated with an online program (<http://jspecies.ribohost.com/jspeciesws/#analyse>). The GenBank accession numbers for the whole genome sequences are given in the top row.

		GGDC							
		<i>Zobellia</i> <i>roscoffensis</i> Asnod1-F08 ^T	<i>Z.</i> <i>roscoffensis</i> Asnod2-B02-B	<i>Z.</i> <i>nedashkovskayae</i> Asnod2-B07-B ^T	<i>Z.</i> <i>nedashkovskayae</i> Asnod3-E08-A	<i>Z.</i> <i>amurskyensis</i> KMM 3526 ^T	<i>Z.</i> <i>laminariae</i> KMM 3676 ^T	<i>Z.</i> <i>galactanivorans</i> Dsjj ^T	<i>Z.</i> <i>uliginosa</i> 553 ^T
Accession number		JADDXT000000000	JADDXS000000000	JADDXR000000000	JADDXQ000000000	GCA_009725985	GCA_009725995	GCA_000973105	GCA_900156625
ANI	<i>Zobellia roscoffensis</i> Asnod1-F08 ^T		81.60	24.70	24.70	24.00	23.90	20.10	19.80
	<i>Z. roscoffensis</i> Asnod2-B02-B	97.54		24.60	24.60	23.90	23.90	20.90	20.30
	<i>Z. nedashkovskayae</i> Asnod2-B07-B ^T	81.09	81.19		82.10	26.00	38.30	20.60	20.30
	<i>Z. nedashkovskayae</i> Asnod3-E08-A	81.04	81.07	97.62		25.90	38.10	20.70	20.20
	<i>Z. amurskyensis</i> KMM 3526 ^T	80.87	80.76	82.18	82.06		28.60	21.00	21.00
	<i>Z. laminariae</i> KMM 3676 ^T	80.67	80.66	88.86	88.74	83.91		21.00	20.30
	<i>Z. galactanivorans</i> Dsjj ^T	76.51	77.10	76.99	76.91	77.61	77.04		55.90
	<i>Z. uliginosa</i> 553 ^T	76.38	76.80	76.67	76.67	77.49	77.44	93.04	

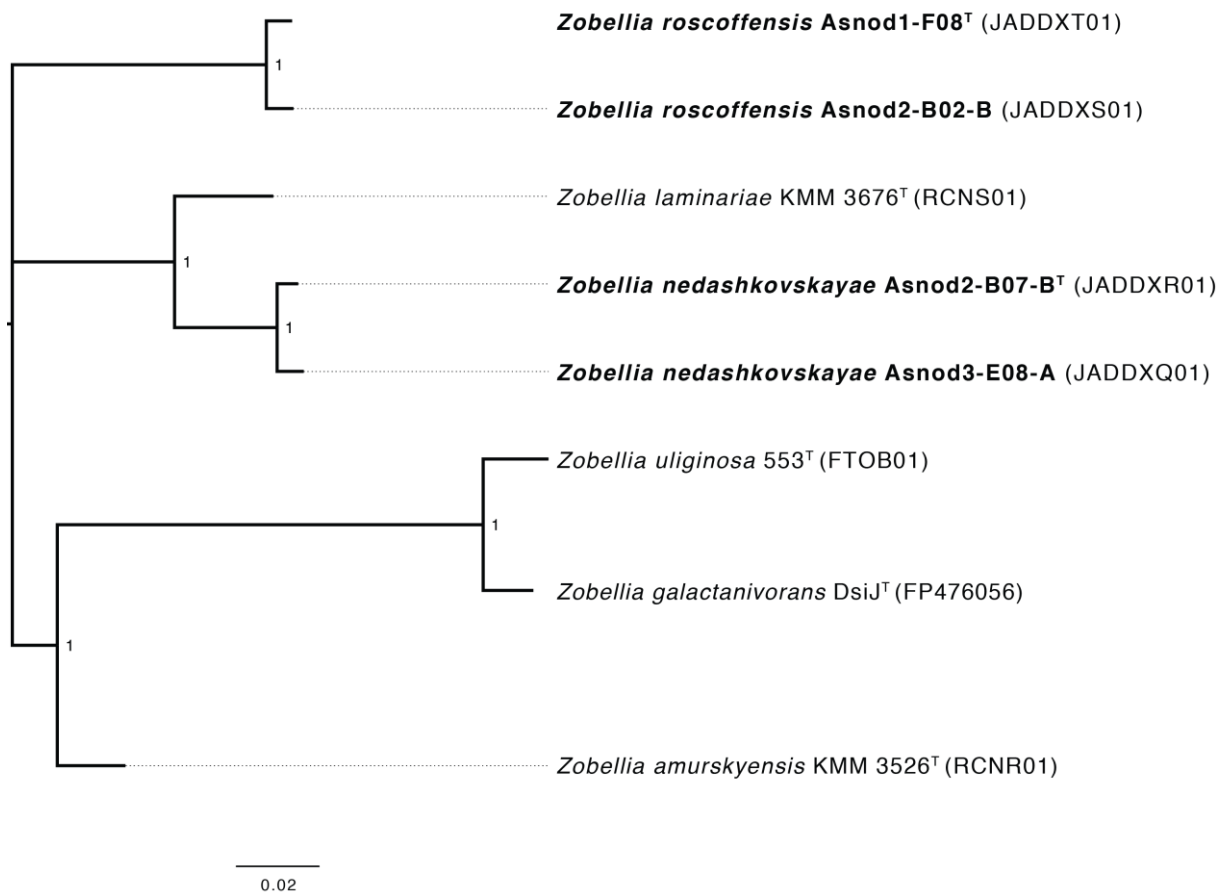
The percentages in red indicate the values above the thresholds of species boundary (95 % for ANI and 70 % for GGDC).



0.05

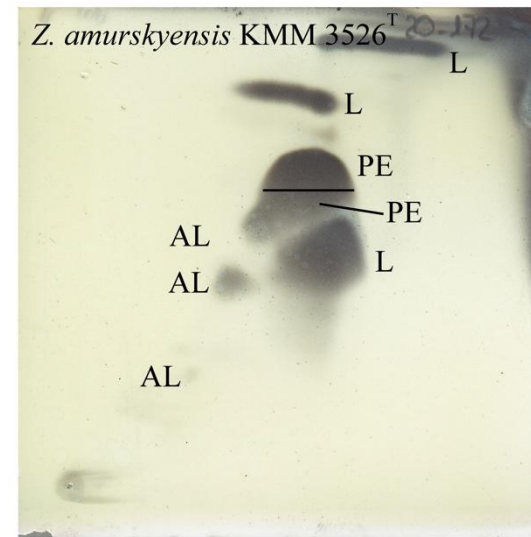
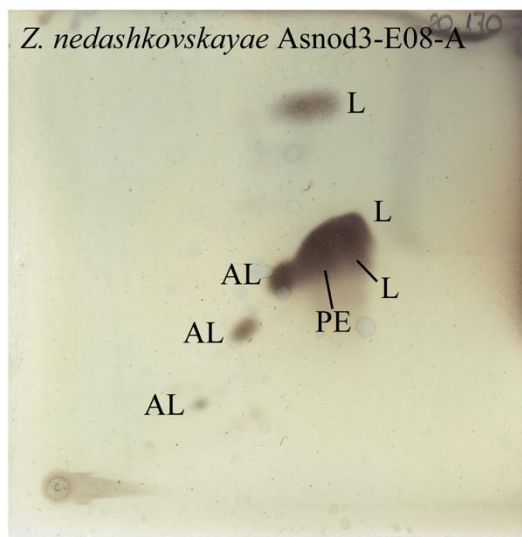
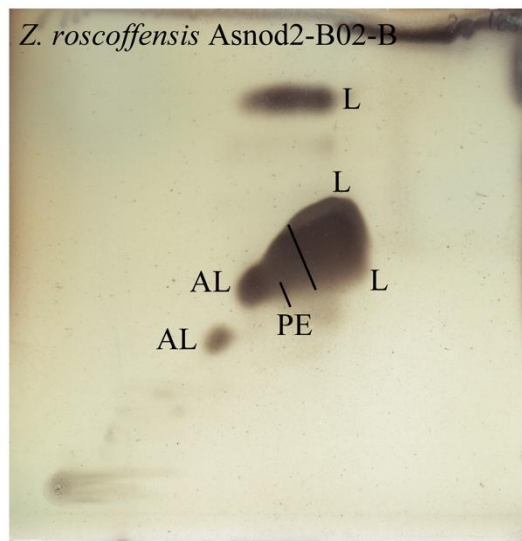
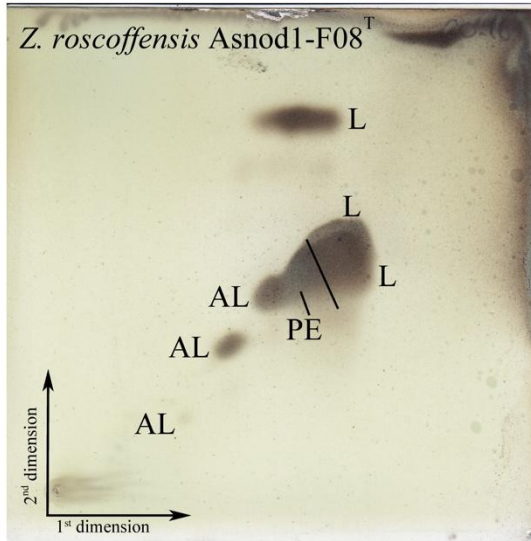
Supplementary Fig. S1

Maximum-likelihood (ML) tree based on 16S rRNA gene sequences, showing the phylogenetic relationships between strains Asnod1-F08^T, Asnod2-B02-B, Asnod2-B07-B^T, Asnod3-E08-A and related taxa from the family *Flavobacteriaceae*. Numbers at nodes are bootstrap values shown as percentages of 1000 replicates; only values >70 % are shown. Filled circles indicate that the corresponding nodes were also recovered in the trees generated with the neighbour-joining (NJ) and maximum-parsimony algorithms, while open circles indicate that the nodes were only recovered in the tree generated with the NJ and ML algorithms. *Flavobacterium aquatile* F36^T was used as an outgroup. Bar, 0.05 changes per nucleotide position.



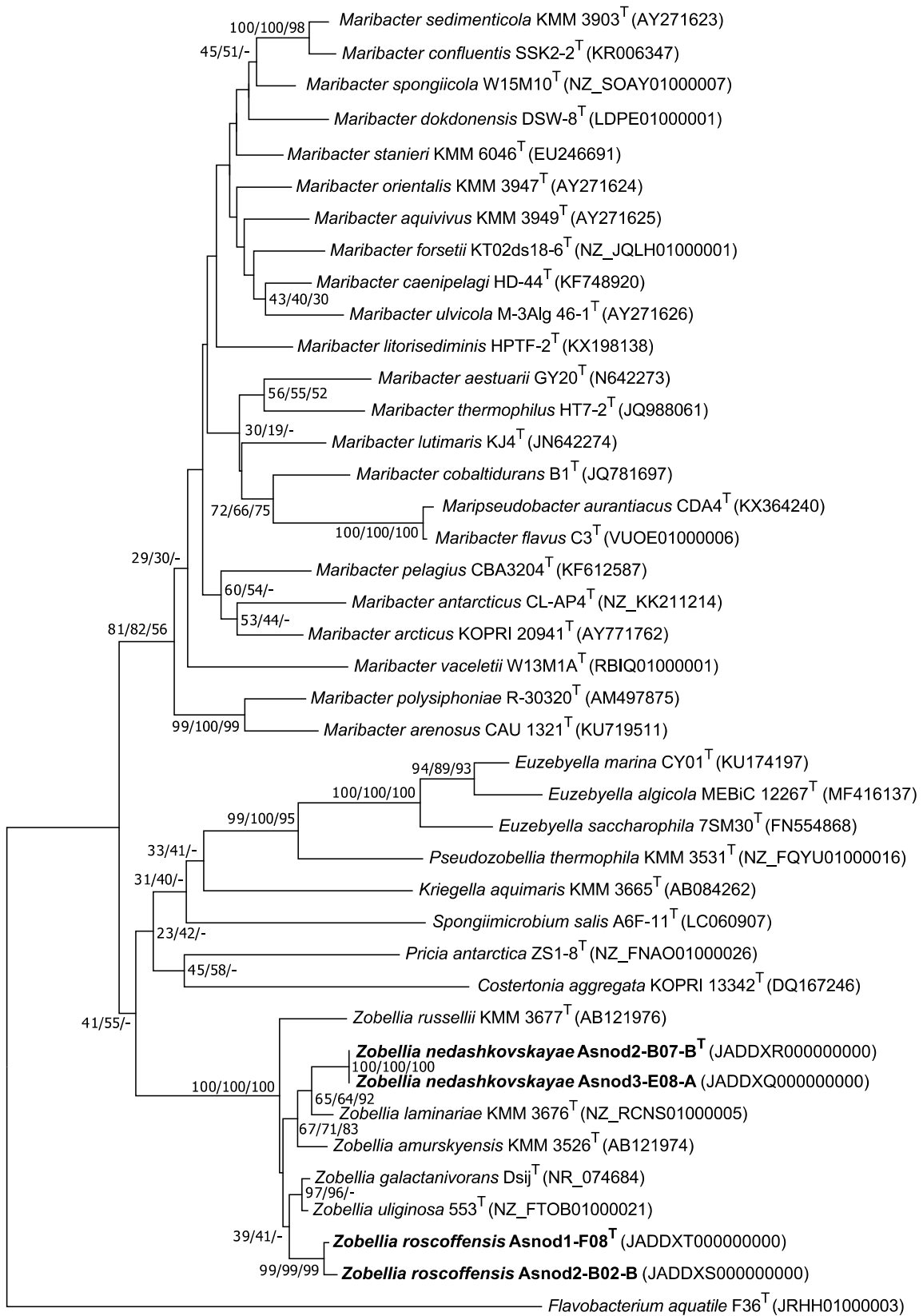
Supplementary Figure S2

Phylogenomic tree inferred with FastTree MP on a concatenated alignment of 2573 translated protein-coding genes of the core genome of *Zobellia* isolates with available complete or draft genomes (genome accessions are given in brackets). Bar: 0.02 change per position.



Supplementary Fig. S3

Two-dimensional thin layer chromatography of the polar lipids of strains Asnod1-F08^T and Asnod2-B02-B (*Zobellia roscoffensis* sp. nov.), Asnod2-B07-B^T and Asnod3-E08-A (*Z. nedashkovskayae* sp. nov.), *Z. amurskyensis* KMM 3526^T and *Zobellia laminariae* KMM 3676^T. The first dimension is developed in chloroform:methanol:water (65:25:4, v/v/v), and the second in chloroform:methanol:acetic acid:water (80:12:15:4, v/v/v/v). AL, aminolipid; L, lipid; PE, phosphatidylethanolamine.



0.01

