

Algoriphagus sanaruensis sp. nov., a member of the family Cyclobacteriaceae, isolated from a brackish lake in Hamamatsu, Japan

メタデータ	言語: English 出版者: Microbiology Society 公開日: 2019-07-11 キーワード: 'filterable' bacteria, Cyclobacteriaceae, brackish lake, 465 作成者: Maejima, Yoshiaki, Iino, Takao, Muraguchi, Yusuke, Ohkuma, Moriya, Kimbara, Kazuhide, Shintani, Masaki メールアドレス: 所属:
URL	http://hdl.handle.net/10297/00026716

1 ***Algoriphagus sanaruensis* sp. nov., a member of the family**
2 ***Cyclobacteriaceae*, isolated from a brackish lake in Hamamatsu, Japan**

3 **Running title:** *Algoriphagus sanaruensis* sp. nov.

4 **Authors:** Yoshiaki Maejima^{1†}, Takao Iino^{2†}, Yusuke Muraguchi¹, Moriya
5 Ohkuma², Kazuhide Kimbara¹, Masaki Shintani^{1,2,3,4*}

6 **Author affiliations:** ¹Department of Engineering, Graduate School of
7 Integrated Science and Technology, Shizuoka University, 3-5-1 Johoku, Naka-
8 ku, Hamamatsu, 432-8561, Shizuoka, Japan, ²Japan Collection of
9 Microorganisms, RIKEN BioResource Research Center, 3-1-1 Koyadai,
10 Tsukuba, Ibaraki, 305-0074, Japan, ³Department of Bioscience, Graduate
11 School of Science and Technology, Shizuoka University, 3-5-1 Johoku, Naka-
12 ku, Hamamatsu, 432-8561, Shizuoka, Japan, ⁴Green Energy Research
13 Division, Research Institute of Green Science and Technology, Shizuoka
14 University, 3-5-1 Johoku, Naka-ku, Hamamatsu, 432-8561, Shizuoka, Japan.

15 **Contents List Category:** New Taxa – *Bacteroidetes*

16 ***Correspondence:** Masaki Shintani, shintani.masaki@shizuoka.ac.jp

17 **Keywords:** *Cyclobacteriaceae*; 'filterable' bacteria; brackish lake

18 †These authors contributed equally to this work.

19 **Foot Notes:** The GenBank/DDBJ/EMBL accession numbers for the partial
20 sequence of 16S rRNA and complete genome sequence of *Algoriphagus*
21 *sanaruensis* M8-2^T are LC349734 and CP012836, respectively.

22 **Abbreviation:** ANI, average nucleotide identity; dDDH, digital DNA–DNA
23 hybridization; DDH, DNA–DNA hybridization; ME, minimum-evolution; ML,

- 24 maximum-likelihood; MP, maximum-parsimony; NJ, neighbour-joining; OGRl,
- 25 overall genome related index.

26 Strain M8-2^T, which was isolated from the brackish lake water (Lake Sanaru) in
27 Japan, was characterized for representation of novel species in the genus
28 *Algoriphagus*. Cells of strain M8-2^T were aerobic, Gram-stain-negative, and
29 curved rod shaped (0.2-0.5 µm in width, 0.7-1.9 µm in length). Strain M8-2^T
30 grew optimally at 30°C, pH 6.5-7.5 and in the presence of 0.5-1.0% (w/v) NaCl.
31 MK-7 was a sole isoprenoid quinone for strain M8-2^T. The major polar lipids
32 were phosphatidylethanolamine, an unidentified phospholipid, and an
33 unidentified polar lipid. The predominant cellular fatty acids were iso-C_{15:0} and
34 anteiso-C_{15:0}. Phylogenetic analysis based on 16S rRNA gene sequence
35 showed that strain M8-2^T belonged to the genus *Algoriphagus* and closely
36 related to *Algoriphagus aquatilis* A8-7^T, *Algoriphagus boseongensis* BS-R1^T,
37 *Algoriphagus aquaeductus* T4^T, *Algoriphagus olei* CC-Hsuan-617^T,
38 *Algoriphagus shivajiensis* NIO-S3^T and *Algoriphagus mannitolivorans* DSM
39 15301^T with sequence similarity of 96.6-97.4%. Results of average nucleotide
40 identities (<75%) and digital DNA–DNA hybridization (<19%) showed that M8-2^T
41 was discriminative from its phylogenetic relatives. Based on the acid production,
42 the predominant cellular fatty acid composition, the DNA G+C content and
43 phylogenetic position, a novel species in the genus *Algoriphagus*, for which the
44 name *Algoriphagus sanaruensis* sp. nov. is proposed for strain M8-2^T (=JCM
45 31446^T =LMG 29969^T).

46 The genus *Algoriphagus*, a member of the family *Cyclobacteriaceae*, the order
47 *Cytophagales* of the phylum *Bacteroidetes*, was first proposed with
48 *Algoriphagus ratkowskyi* as a single type species [1]. At the time of writing, the
49 genus *Algoriphagus* was composed of 39 species as validly published names
50 [2] (<http://www.bacterio.net/algoriphagus.html>). In 2017, six species were newly
51 proposed among genus *Algoriphagus* [3-8]. These species were isolated from
52 various environment such as a estuary sediment [3], freshwater lake [9],
53 seawater [10], tidal flat [11], soil [12, 13], marine sediment [4],
54 hexachlorocyclohexane-contaminated dumpsite [14], marine solar saltern [15]
55 and mangrove sediment [16]. In our previous study, strain M8-2 was isolated as
56 one of the 141 'filterable' bacteria, which passed through a 0.22 µm pore size
57 filter, from Lake Sanaru, a brackish lake in Hamamatsu, Shizuoka, Japan
58 (E137°41'15", N34°42'30") [17]. In this study, we characterized strain M8-2 as a
59 representative of a novel species of the genus *Algoriphagus*.

60 Strain M8-2^T was routinely cultivated on Marine agar 2216 (MA) (Difco, BD
61 Bioscience) for 6 days at 30°C. Cells of strain M8-2^T was aerobic, non-spore-
62 forming, non-motile and curved rod-shaped. Gram staining was negative by
63 using the Gram-staining kit (FUJIFILM Wako Pure Chemical Corp.). Colonies of
64 strain M8-2^T are circular, convex and coral with 1.5-2.0 mm diameter on MA
65 plate after 5-6 days of cultivation. Catalase reaction was tested by placing drops
66 of 3% (v/v) H₂O₂ solution directly on the cells cultivated on MA and observing
67 gas evolution. Oxidase reaction was tested by using a Cytochrome Oxidase
68 Test Strip (Nissui). Catalase and oxidase reaction were positive. To confirm that

69 strain M8-2^T was smaller than the pore size of the filter, the colonies of the
70 strain on MA plate were stained with 2% (w/v) ammonium molybdate on
71 electron microscopy grids. Then, they were observed with transmission electron
72 microscopy (TEM: JEM-2000FX-II) at 160 kV. The cells of M8-2^T showed
73 curved rod-shaped and 0.2-0.5 µm wide and 0.7-1.9 µm long (Fig. 1). No
74 flagellation was observed with TEM (Fig. 1).

75 Temperature ranges (4°C and 10-50°C at 5°C intervals) and pH ranges (3.5-
76 10.5, in increments of pH 0.5 units by the addition of HCl or NaOH) for growth of
77 strain M8-2^T were tested in Marine Broth 2216 (Difco, BD Bioscience). The pH
78 of each medium was adjusted after autoclaving them. The pH was also majored
79 when the strain M8-2^T start to grow in the media to confirm whether it was
80 maintained at that time. The longest incubation time was 4 months. Its growth
81 temperature ranged from 15 to 40°C, with an optimum of 30°C and the pH
82 range was 6.0-9.0, with an optimum pH range being 6.5-7.5. Salinity ranges for
83 growth were tested using marine broth omitted NaCl, whose compositions were:
84 5.0 g/L polypeptone, 1.0 g/L yeast extract, 0.1 g/L ferric citrate, 5.9 g/L
85 magnesium chloride, 3.2 g/L magnesium sulfate, 1.8 g/L calcium chloride, 0.55
86 g/L potassium chloride, 0.16 g/L sodium bicarbonate, 0.08 g/L potassium
87 bromide. The 0, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 or 10.0% (w/v) NaCl
88 was added the above broth, and strain M8-2^T grew in the broth with 5.0% (w/v)
89 or less. The optimum salinity for its growth was 0.5-1.0% (w/v) NaCl.

90 Physiological and biochemical characterizations were conducted using API
91 ZYM and API 50 CH (bioMérieux). These characteristics of M8-2^T are shown in
92 Table 1 and the species description.

93 The major isoprenoid quinone of strain M8-2^T was determined by the HPLC
94 method described by Komagata & Suzuki [18]. In brief, the quinone was
95 extracted from the lyophilized cells by chloroform:methanol (2:1). Then, filtered
96 and concentrated sample was purified by TLC. The resultant sample was
97 subjected to HPLC [LC-20AD and SPD-M20A (SHIMADZU)] with COSMOSIL
98 5C18 column. The detection was performed with 270 nm UV. The polar lipids
99 pattern of the strain was determined by using two-dimensional TLC and
100 spraying with 5% ethanolic molybdophosphoric acid, ninhydrin, Dittmer & Lester
101 reagent, anisaldehyde reagent and Dragendoff's reagent, as described
102 previously [19, 20]. The Sherlock Microbial Identification System (MIDI) version
103 6 (Microbial ID; Agilent Technologies) was used for identifying and quantifying
104 the cellular fatty acids of strain M8-2^T based on the previous method [21]. Strain
105 M8-2^T contained menaquinone-7 (MK-7) as a sole isoprenoid quinone. The
106 polar lipids of strain M8-2^T mainly comprised phosphatidylethanolamine, an
107 unidentified phospholipid, and an unidentified polar lipid (Figs. 2 and S1). The
108 percentages of detected fatty acids are shown in Table 2. The predominant
109 cellular fatty acids of strain M8-2^T were iso-C_{15:0} (36.7%) and anteiso-C_{15:0}
110 (11.5%). The DNA G+C content of strain M8-2^T was 41.4 mol% based on the
111 complete nucleotide sequence, which was already published [22]. The genome
112 size of M8-2^T has a single 3,882,610-bp chromosome with 3,377 coding
113 sequences, nine sets of rRNA genes, and 40 tRNA genes [22].

114 Phylogenetic analyses were performed by the neighbour-joining method [23]
115 (Kimura 2-parameter method [24]), the maximum likelihood method (Tamura-

116 Nei model [23]), the minimum-evolution method [25] (Kimura 2-parameter
117 method [24]), and the maximum-parsimony method with ClustalW [26] in MEGA
118 7.0 [27]. According to the complete sequence of strain M8-2^T
119 (GenBank/DDBJ/EMBL accession no. CP012836, [22]) it has three identical
120 sequences of 16S rRNAs at 1798630-1800155 nt, 3812980-3811455 nt, and
121 3882483-3880958 nt. Based on the comparisons of 16S rRNA gene sequences,
122 strain M8-2^T belonged to the genus *Algoriphagus* (Figs. 3 and S2). Strain M8-2^T
123 located near *Algoriphagus aquatilis* A8-7^T [9], *Algoriphagus boseongensis* BS-
124 R1^T [28], *Algoriphagus aquaeductus* T4^T [29], *Algoriphagus olei* CC-Hsuan-617^T
125 [12], *Algoriphagus shivajiensis* NIO-S3^T [30] and *Algoriphagus mannitolivorans*
126 DSM 15301^T (=JC2050^T) [31, 32], whose nucleotide sequence similarities of
127 16S rRNA gene were 97.4%, 96.9%, 96.8%, 96.8%, 96.6% and 96.6%,
128 respectively {the reference data were obtained from EzBioCloud server
129 (<https://www.ezbiocloud.net/identify>) [33]}. These similarities were lower than
130 the cut-off value recommended for species differentiation (98.7-99.0 % [34]).
131 Then, overall genome related index (OGRI) was calculated according to a
132 recent proposal by Chun and colleagues [35]. Available whole genomic data of
133 closely related strains T4^T (accession no. QKTX01), DSM15301^T (accession
134 no. AUBV01) and several other strains *A. zhangzhouensis* DSM 25035^T
135 (FRXN01), *A. vanfongensis* DSM 17529^T (AUBX01), *A. faecimaris* DSM 23095^T
136 (FNAC01), *A. terrigena* DSM 22685^T (AUBW01), *A. marinus* am2^T (MSPQ01),
137 *A. halophilus* DSM 15292^T (FSRC01), and *A. resistens* NH1^T (LMXN01) were
138 used to calculate the OGRI. The average nucleotide identity (ANI) was
139 calculated by using OAT software [36] and *in silico* DNA-DNA hybridization

140 (DDH) analyses were performed by using Genome-to-Genome Distance
141 Calculator 2.1 [37] (<http://ggdc.dsmz.de/ggdc.php>). The ANI values of strain M8-
142 2^T with them were less than 75%, 73.28% with strain DSM 15301^T and 74.81%
143 with strain T4^T (Table S2). The results of DDH showed less than 20%, 17.2%
144 with strain DSM 15301^T and 18.3% with strain T4^T (Table S2). These results
145 clearly indicated that strain M8-2^T could be assigned as different species of
146 them.

147 Morphological, biochemical and physiological characteristics of strain M8-2^T,
148 along with those of members of the phylogenetically related taxa, are
149 summarized in Table 1. In strain M8-2^T, acid production was weakly confirmed
150 from xylose and *N*-acetyl-D-glucosamine. Furthermore, strain M8-2^T differed in
151 the composition of major cellular fatty acids from the related bacteria as shown
152 in Table 2; e.g. the ratio of anteiso-C_{15:0} of strain M8-2^T was highest than those
153 of related species. The DNA G+C content of strain M8-2^T was lower than those
154 of members of the genus *Algoriphagus* (Table 1). Therefore, strain M8-2^T
155 should be classified as the representative of a new species within the genus
156 *Algoriphagus*. We proposed that strain M8-2^T represent a novel species, for
157 which, the name *Algoriphagus sanaruensis* sp. nov. is proposed.

158 **Description of *Algoriphagus sanaruensis* sp. nov.**

159 *Algoriphagus sanaruensis* (sa.na.ru.en´sis. N.L. masc.adj. *sanaruensis* of or
160 belonging to Lake Sanaru, Hamamatsu, Japan, referring to the isolation of the
161 type strain).

162 Aerobic bacterium. Cells are curved rod shaped (0.2-0.5 μm in width and 0.7-
163 1.9 μm in length). Catalase-positive and oxidase positive. Colonies are circular,
164 convex and coral on MA after 5 days of cultivation. The temperature for growth
165 is 15-40°C with an optimum growth at 30°C. Grows at pH 6.0-9.0 with an
166 optimum around pH 6.5-7.5. Growth occurs 5.0% (w/v) NaCl or less. In assays
167 with API 50 CH, the acid was produced (positive) for D-galactose, D-glucose, D-
168 fructose, D-mannose, amygdalin, arbutin, esculin ferric citrate, salicin, D-
169 cellobiose, D-maltose, D-lactose, D-melibiose, D-sucrose, D-trehalose, D-
170 melezitose, D-raffinose, starch and gentiobiose, weakly positive for D-xylose, L-
171 xylose, methyl- β -D-xylopyranoside, L-sorbose, L-rhamnose, methyl- α -D-
172 mannopyranoside, methyl- α -D-glucopyranoside, *N*-acetylglucosamine, inulin,
173 glycogen, D-turanose and D-lxyose. In assays with API ZYM MicroPlates,
174 positive for alkaline phosphatase, leucine arylamidase, valine arylamidase,
175 trypsin, α -chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase,
176 α -galactosidase, β -galactosidase, α -glucosidase, β -glucosidase and *N*-acetyl- β -
177 glucosaminidase, weakly positive for esterase (C4), esterase lipase (C8), and
178 cystine arylamidase. The major menaquinone is MK-7. The main polar lipids
179 were phosphatidylethanolamine, an unidentified phospholipid, and an
180 unidentified polar lipid. The predominant cellular fatty acids were iso-C_{15:0} and
181 anteiso-C_{15:0}.

182 The type strain is M8-2^T (=JCM 31446^T =LMG 29969^T), which was isolated from
183 a brackish lake (Lake Sanaru) in Hamamatsu, Japan. The DNA G+C content of
184 the type strain is 41.4 mol% (Genome sequence)

185 **Author contributions**

186 MS conceived, designed, and supervised the study. YM, TI, MY, and MS
187 performed the experiments and analyzed the data. YM, TI, MO, KK, and MS
188 wrote, reviewed, and edited the manuscript. All authors read and approved the
189 final manuscript.

190 **Funding information**

191 This study was supported by Institute for Fermentation, Osaka (IFO), Japan.

192 **ORCID** Masaki Shintani: ORCID 0000-0002-6505-9850

193 **Disclosure statement**

194 No potential conflict of interest was reported by the authors.

195

196 **REFERENCES**

- 197 1. **Bowman JP, Nichols CM, Gibson JA.** *Algoriphagus ratkowskyi* gen.
 198 nov., sp. nov., *Brumimicrobium glaciale* gen. nov., sp. nov., *Cryomorpha ignava*
 199 gen. nov., sp. nov. and *Crocinitomix catalasitica* gen. nov., sp. nov., novel
 200 flavobacteria isolated from various polar habitats. *Int J Syst Evol Microbiol*
 201 2003;53:1343-1355.
- 202 2. **Parte AC.** LPSN--list of prokaryotic names with standing in
 203 nomenclature. *Nucleic Acids Res* 2014;42:D613-616.
- 204 3. **Jia X, Jia B, Kim KH, Jeon CO.** *Algoriphagus aestuariicola* sp. nov.,
 205 isolated from estuary sediment. *Int J Syst Evol Microbiol* 2017;67:914-919.
- 206 4. **Han JR, Zhao JX, Wang ZJ, Chen GJ, Du ZJ.** *Algoriphagus resistens*
 207 sp. nov., isolated from marine sediment. *Int J Syst Evol Microbiol* 2017;67:1275-
 208 1280.
- 209 5. **Park S, Park JM, Yoon JH.** *Algoriphagus marisflavi* sp. nov., isolated
 210 from water of an estuary environment. *Int J Syst Evol Microbiol* 2017;67:4168-
 211 4174.
- 212 6. **Han JR, Geng QL, Wang FQ, Du ZJ, Chen GJ.** *Algoriphagus marinus*
 213 sp. nov., isolated from marine sediment and emended description of the genus
 214 *Algoriphagus*. *Int J Syst Evol Microbiol* 2017;67:2412-2417.
- 215 7. **Sun QL, Sun L.** Description of *Algoriphagus iocasae* sp. nov., isolated
 216 from deep-sea sediment. *Int J Syst Evol Microbiol* 2017;67:243-249.
- 217 8. **Park S, Jung YT, Park JM, Yoon JH.** *Algoriphagus aquaemixtae* sp.
 218 nov., isolated from water in an estuary environment. *Int J Syst Evol Microbiol*
 219 2017;67:3231-3236.
- 220 9. **Liu Y, Li H, Jiang JT, Liu YH, Song XF et al.** *Algoriphagus aquatilis* sp.
 221 nov., isolated from a freshwater lake. *Int J Syst Evol Microbiol* 2009;59:1759-
 222 1763.
- 223 10. **Lee DH, Kahng HY, Lee SB.** *Algoriphagus jejuensis* sp. nov., isolated
 224 from seawater. *Int J Syst Evol Microbiol* 2012;62:409-413.
- 225 11. **Park S, Ha MJ, Yoon SY, Jung YT, Yoon JH.** *Algoriphagus*
 226 *litorisediminis* sp. nov., isolated from a tidal flat. *Int J Syst Evol Microbiol*
 227 2016;66:5437-5443.
- 228 12. **Young CC, Lin SY, Arun AB, Shen FT, Chen WM et al.** *Algoriphagus*
 229 *olei* sp. nov., isolated from oil-contaminated soil. *Int J Syst Evol Microbiol*
 230 2009;59:2909-2915.
- 231 13. **Yoon JH, Lee MH, Kang SJ, Oh TK.** *Algoriphagus terrigena* sp. nov.,
 232 isolated from soil. *Int J Syst Evol Microbiol* 2006;56:777-780.
- 233 14. **Kohli P, Nayyar N, Sharma A, Singh AK, Lal R.** *Algoriphagus roseus*
 234 sp. nov., isolated from a hexachlorocyclohexane-contaminated dumpsite. *Int J*
 235 *Syst Evol Microbiol* 2016;66:3558-3565.
- 236 15. **Yoon JH, Kang SJ, Jung SY, Lee CH, Oh TK.** *Algoriphagus yeomjeoni*
 237 sp. nov., isolated from a marine solar saltern in the Yellow Sea, Korea. *Int J Syst*
 238 *Evol Microbiol* 2005;55(Pt 2):865-870.
- 239 16. **Yang C, Li Y, Guo Q, Lai Q, Zheng T et al.** *Algoriphagus*
 240 *zhangzhouensis* sp. nov., isolated from mangrove sediment. *Int J Syst Evol*
 241 *Microbiol* 2013;63:1621-1626.

- 242 17. **Maejima Y, Kushimoto K, Muraguchi Y, Fukuda K, Miura T et al.**
243 *Proteobacteria* and *Bacteroidetes* are major phyla of filterable bacteria passing
244 through 0.22 µm pore size membrane filter, in Lake Sanaru, Hamamatsu,
245 Japan. *Biosci Biotechnol Biochem* 2018;1-4.
- 246 18. **Komagata K, Suzuki K.** Lipid and cell-wall analysis in bacterial
247 systematics. *Method Microbiol* 1987;19:161-207.
- 248 19. **Lechevalier MP, Debievre C, Lechevalier H.** Chemotaxonomy of
249 aerobic *Actinomycetes* - phospholipid composition. *Biochem Syst Ecol*
250 1977;5:249-260.
- 251 20. **Minnikin DE, Odonnell AG, Goodfellow M, Alderson G, Athalye M et**
252 **al.** An integrated procedure for the extraction of bacterial isoprenoid quinones
253 and polar lipids. *J Microbiol Meth* 1984;2:233-241.
- 254 21. **Sasser M.** Identification of bacteria by gas chromatography of cellular
255 fatty acids, MIDI Technical Note 101. Newark, DE: MIDI Inc. 1990.
- 256 22. **Muraguchi Y, Kushimoto K, Ohtsubo Y, Suzuki T, Dohra H et al.**
257 Complete genome sequence of *Algoriphagus* sp. strain M8-2, isolated from a
258 brackish Lake. *Genome announcements* 2016;4: pii:e00347-16.
- 259 23. **Tamura K, Nei M.** Estimation of the number of nucleotide substitutions
260 in the control region of mitochondrial DNA in humans and chimpanzees. *Mol*
261 *Biol Evol* 1993;10:512-526.
- 262 24. **Kimura M.** A simple method for estimating evolutionary rates of base
263 substitutions through comparative studies of nucleotide sequences. *J Mol Evol*
264 1980;16:111-120.
- 265 25. **Rzhetsky A, Nei M.** Statistical properties of the ordinary least-squares,
266 generalized least-squares, and minimum-evolution methods of phylogenetic
267 inference. *J Mol Evol* 1992;35:367-375.
- 268 26. **Thompson JD, Higgins DG, Gibson TJ.** CLUSTAL W: improving the
269 sensitivity of progressive multiple sequence alignment through sequence
270 weighting, position-specific gap penalties and weight matrix choice. *Nucleic*
271 *Acids Res* 1994;22:4673-4680.
- 272 27. **Kumar S, Stecher G, Tamura K.** MEGA7: Molecular evolutionary
273 genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol* 2016;33:1870-
274 1874.
- 275 28. **Park S, Park JM, Lee KC, Yoon JH.** *Algoriphagus boseongensis* sp.
276 nov., a member of the family *Cyclobacteriaceae* isolated from a tidal flat.
277 *Antonie Van Leeuwenhoek* 2014;105:523-531.
- 278 29. **Rau JE, Blotevogel KH, Fischer U.** *Algoriphagus aquaeductus* sp.
279 nov., isolated from a freshwater pipe. *Int J Syst Evol Microbiol* 2012;62:675-682.
- 280 30. **Kumar PA, Bhumika V, Ritika C, Bhaskar YV, Priyashanth P et al.**
281 *Algoriphagus shivajiensis* sp. nov., isolated from Cochin back water, India. *Syst*
282 *Appl Microbiol* 2013;36:106-111.
- 283 31. **Yi H, Chun J.** *Hongiella mannitolivorans* gen. nov., sp. nov., *Hongiella*
284 *halophila* sp. nov. and *Hongiella ornithinivorans* sp. nov., isolated from tidal flat
285 sediment. *Int J Syst Evol Microbiol* 2004;54:157-162.
- 286 32. **Nedashkovskaya OI, Kim SB, Kwon KK, Shin DS, Luo X et al.**
287 Proposal of *Algoriphagus vanfongensis* sp. nov., transfer of members of the
288 genera *Hongiella* Yi and Chun 2004 emend. Nedashkovskaya et al. 2004 and

289 Chimaereicella Tiago et al. 2006 to the genus *Algoriphagus*, and emended
290 description of the genus *Algoriphagus* Bowman et al. 2003 emend.
291 Nedashkovskaya et al. 2004. *Int J Syst Evol Microbiol* 2007;57:1988-1994.
292 33. **Yoon SH, Ha SM, Kwon S, Lim J, Kim Y et al.** Introducing
293 EzBioCloud: a taxonomically united database of 16S rRNA gene sequences
294 and whole-genome assemblies. *Int J Syst Evol Microbiol* 2017;67:1613-1617.
295 34. **Stackebrandt E, Ebers J.** Taxonomic parameters revisited: tarnished
296 gold standards. *Microbiology Today* 2006;33:152-155.
297 35. **Chun J, Oren A, Ventosa A, Christensen H, Arahal DR et al.**
298 Proposed minimal standards for the use of genome data for the taxonomy of
299 prokaryotes. *Int J Syst Evol Microbiol* 2018;68:461-466.
300 36. **Lee I, Ouk Kim Y, Park SC, Chun J.** OrthoANI: An improved algorithm
301 and software for calculating average nucleotide identity. *Int J Syst Evol*
302 *Microbiol* 2016;66:1100-1103.
303 37. **Meier-Kolthoff JP, Auch AF, Klenk HP, Goker M.** Genome sequence-
304 based species delimitation with confidence intervals and improved distance
305 functions. *BMC bioinformatics* 2013;14:60.

306

307
308
309
310
311

Table 1. Differential characteristics of strain M8-2^T and related species.

Strains: 1, *Algoriphagus sanaruensis* sp. nov. M8-2^T; 2, *A. boseongensis* BS-R1^T [28]; 3, *A. mannitolivorans* DSM 15301^T [31, 32].

+, positive; w, weakly positive; -, negative; n.d., no data available. All strains are positive for oxidase and catalase. All have MK-7 as predominant respiratory quinone. Data for reference strains are from Park et al., 2014 [28], Yi and Chun 2004 [31], and Nedashkovskaya et al., 2007 [32].

Characteristics	1	2	3
Cell size (µm)	0.2-0.5x0.7-1.9	0.2-0.5x0.7-6.0	0.4-0.5x1.1-1.7
Pigmentation	Coral	Strong orange	Opaque and orange
Temperature for growth (°C)			
Optimum	30	30	35-40
Range	15-40	15-40	10-40
pH for growth			
Optimum	6.5-7.5	7.0-8.0	7.0
Range	6.0-9.0	6.0-8.0	6-11.0
NaCl requirement for growth (% w/v)			
Optimum	0.5-1.0	2.0	1.0
Range	0-5.0	1.0-4.0	0-7.0
Acid production from			
D-Fructose	+	-	-
D-Glucose	+	+	-
Lactose	+	-	-
Maltose	+	+	-
D-Mannose	+	-	-

Melibiose	+	-	-
L-Rhamnose	w	-	-
Xylose	w	-	-
<i>N</i> -Acetyl-D-glucosamine	w	n.d.	-
Enzyme activity (API ZYM)			
Alkaline phosphatase	+	-	+
Esterase (C4)	w	+	-
Esterase lipase (C8)	w	+	-
Cystine arylamidase	w	+	-
α -Galactosidase	+	-	+
α -Glucosidase	+	+	-
β -Glucosidase	+	-	-
DNA G+C (mol%)	41.4	42.3	42.0

312

313

Table 2. Cellular fatty acid composition of strain M8-2^T and the type strains of phylogenetically related species^a.

Strains: 1, *Algoriphagus sanaruensis* sp. nov. M8-2^T; 2, *A. boseongensis* BS-R1^T [28]; 3, *A. mannitolivorans* DSM 15301^T[31].

^a‘tr’ and ‘-’ indicates trace (<1%) and not detected/not reported, respectively, and fatty acids with more than 10% are in bold.

*Summed features consist of one or more fatty acids that cannot be separated by the method used. Summed feature 3 comprised iso-C_{15:0} 2-OH, C_{16:1} ω7c and/or C_{16:1} ω6c; summed feature 4 comprised anteiso-C_{17:1} B and/or iso-C_{17:1} I; summed feature 9 comprised iso-C_{17:1} ω9c and/or C_{16:0} 10-methyl. ECL, Equivalent chain-length; –, not detected/not reported. Data for reference strains are from Park et al., 2014 [28], and Yi and Chun 2004 [31].

	1	2	3
iso-C _{11:0}	-	tr	-
anteiso-C _{11:0}	2.6	3.1	1.7
C _{14:0}	-	tr	-
iso-C _{14:0}	5.4	1.3	1.2
C _{15:0}	2.9	-	1.7
iso-C _{15:0}	36.7	30.6	28.7
anteiso-C _{15:0}	11.5	6.8	4.6
iso-C _{15:0} 3-OH	4.2	4.8	3.6
iso-C _{15:1} G	-	-	1.0
C _{15:1} ω6c	1.6	tr	2.5
C _{16:0}	-	tr	-
C _{16:0} 3-OH	1.3	tr	tr
iso-C _{16:0}	6.5	5.5	3.7
iso-C _{16:0} 3-OH	7.3	4.5	4.0
iso-C _{16:1} H	2.0	1.7	2.8
C _{16:1} ω5c	1.3	1.6	1.3
iso-C _{17:0}	-	tr	-
C _{17:0} 2-OH	-	1.3	tr
iso-C _{17:0} 3-OH	5.2	11.1	7.9
C _{17:1} ω6c	1.7	1.2	3.1
C _{17:1} ω8c	-	tr	-
iso-C _{17:1} ω9c	-	7.9	5.6
Summed features*			
Summed features 3	6.6	11.0	13.7
Summed features 4	1.7	2.0	4.8
Summed features 9	1.4	-	-

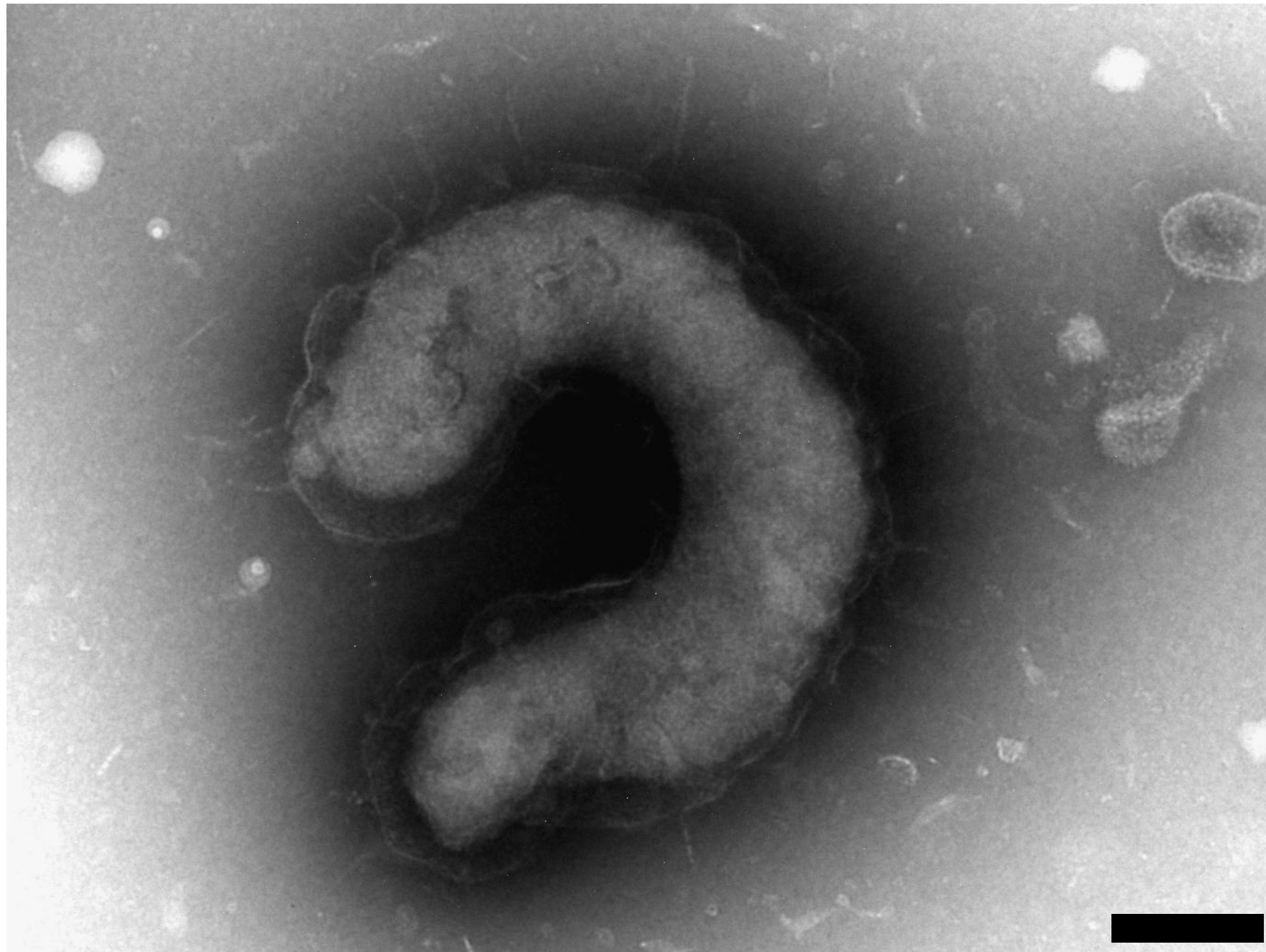
329 **FIGURE LEGENDS**

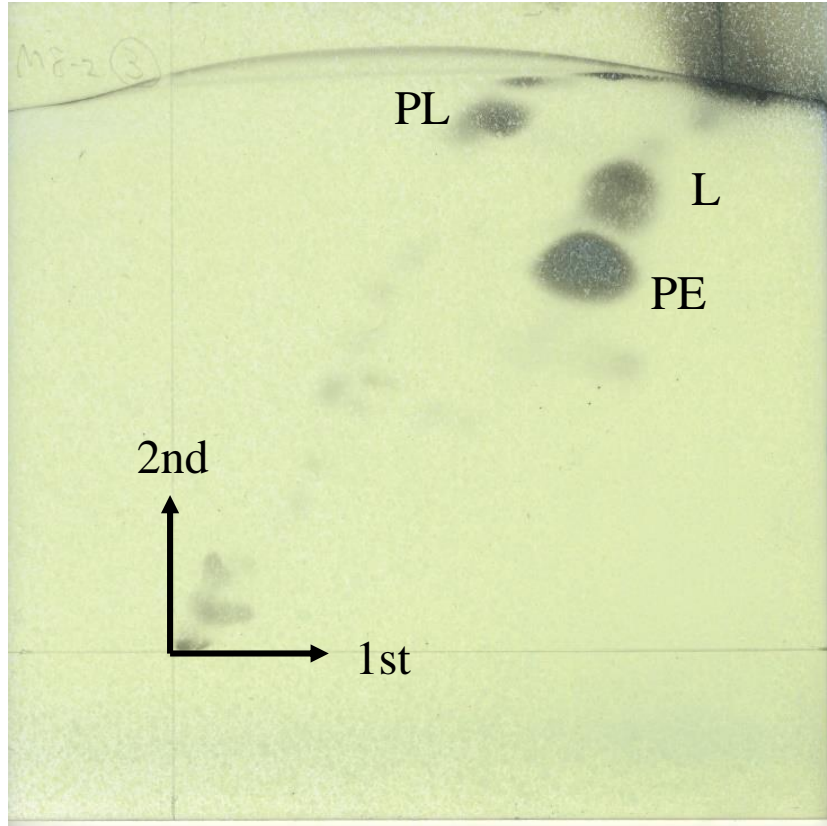
330 **Figure 1.** Morphological characterization of strain M8-2^T observed by
331 transmission electron microscopy after 5 days of incubation on the above MB
332 agar at 30°C. A bar in the figure indicates 0.2 µm.

333 **Figure 2.** A two-dimensional thin-layer chromatogram of polar lipid extracts
334 from strain M8-2^T, stained with 5% ethanolic molybdophosphoric acid to detect
335 total lipids. PE, phosphatidylethanolamine; PL, unidentified phospholipid; L,
336 unidentified polar lipid.

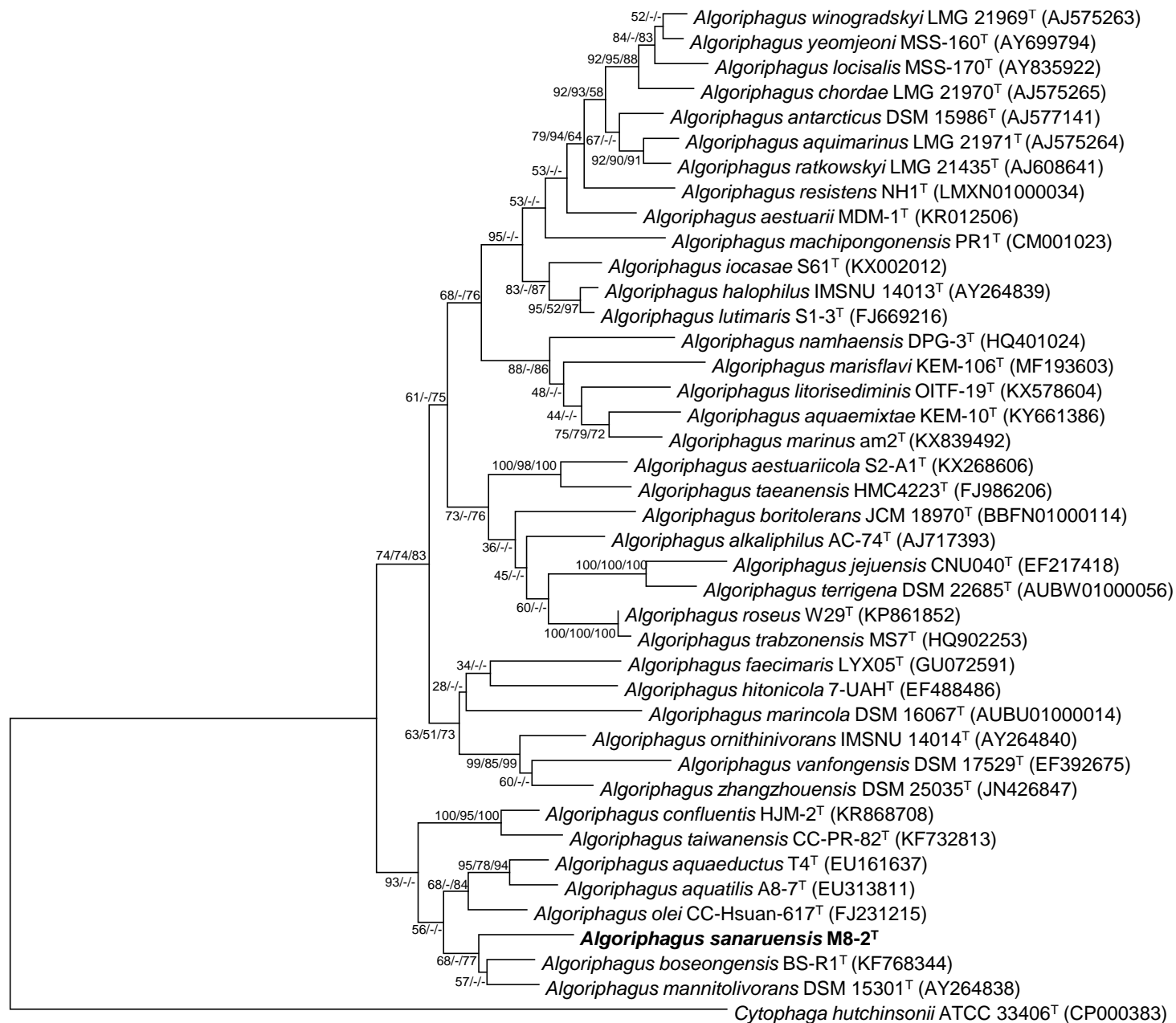
337 **Figure 3.** Neighbour-joining tree of partial 16S rRNA gene sequences of strain
338 M8-2^T with representative members of selected species belonging to the genus
339 *Algoriphagus*. Bootstrap values (1000 replications) are shown as percentages
340 at nodes (neighbour-joining method/maximum-likelihood method/minimum-
341 evolution method). The tree was reconstructed using MEGA software.
342 *Cytophaga hutchinsonii* ATCC 33406^T was used as the outgroup. Bar shows
343 0.020 substitutions per nucleotide position.

Figure_1





Figure_3



0.020

Supplemental Materials

***Algoriphagus sanaruensis* sp. nov., a member of the family
Cyclobacteriaceae, isolated from a brackish lake in Hamamatsu, Japan**

Maejima & Iino et al.

Correspondence: shintani.masaki@shizuoka.ac.jp

The Supplementary Materials include:

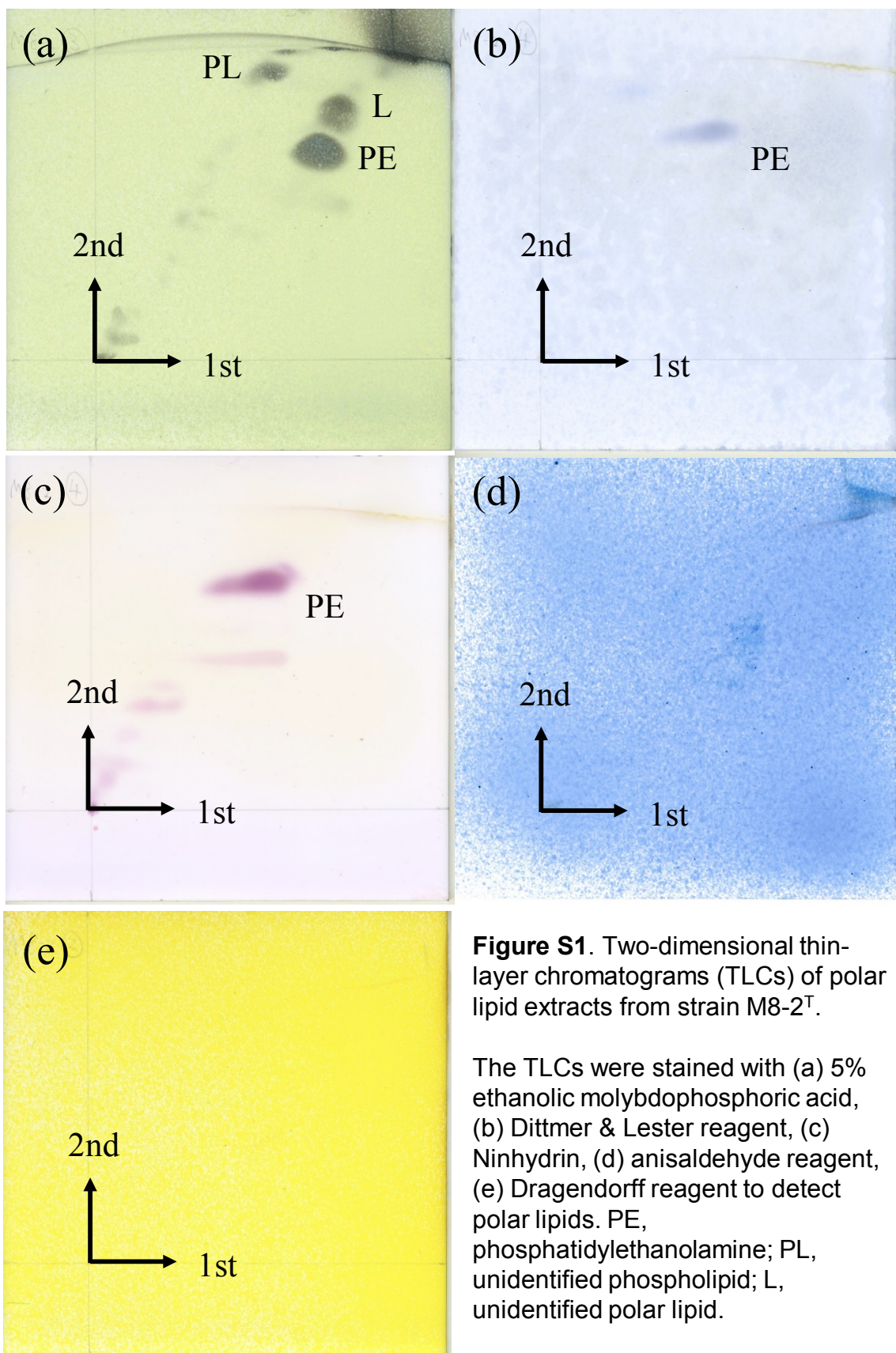
- ✓ Table S1-S2
- ✓ Figures S1-S2

Table S1. OrthoANI values calculated from the OAT software (Lee et al. 2016). Strains: 1, *Algoriphagus sanaruensis* sp. nov. M8-2^T (accession no. CP012836); 2, *Algoriphagus aquaeductus* T4^T (QKTX01); 3, *Algoriphagus mannitolivorans* DSM 15301^T (AUBV01); 4, *Algoriphagus zhangzhouensis* DSM 25035^T (FRXN01); 5, *Algoriphagus vanfongensis* DSM 17529^T (AUBX01); 6, *Algoriphagus faecimaris* DSM 23095^T (FNAC01); 7, *Algoriphagus terrigena* DSM 22685^T (AUBW01); 8, *Algoriphagus marinus* am2^T (MSPQ01); 9, *Algoriphagus halophilus* DSM 15292^T (FSRC01); 10, *Algoriphagus resistens* NH1^T (LMXN01).

	1	2	3	4	5	6	7	8	9	10
1		74.81	73.28	71.18	70.83	70.83	71.79	70.66	70.44	69.91
2			74.00	71.00	71.17	71.18	73.49	70.78	70.32	70.18
3				71.49	71.41	71.30	72.58	70.67	70.60	70.26
4					76.74	72.12	69.97	71.38	71.60	70.97
5						72.09	70.66	70.90	70.96	70.82
6							70.04	70.76	70.51	70.35
7								70.09	69.37	70.53
8									71.61	72.01
9										71.50
10										

Table S2. DDH values calculated by using Genome-to-Genome Distance Calculator 2.1 [33]. Only 'Formula 1' values were shown. Strains: 1, *Algoriphagus sanaruensis* sp. nov. M8-2^T (accession no. CP012836); 2, *Algoriphagus aquaeductus* T4^T (QKTX01); 3, *Algoriphagus mannitolivorans* DSM 15301^T (AUBV01); 4, *Algoriphagus zhangzhouensis* DSM 25035^T (FRXN01); 5, *Algoriphagus vanfongensis* DSM 17529^T (AUBX01); 6, *Algoriphagus faecimaris* DSM 23095^T (FNAC01); 7, *Algoriphagus terrigena* DSM 22685^T (AUBW01); 8, *Algoriphagus marinus* am2^T (MSPQ01); 9, *Algoriphagus halophilus* DSM 15292^T (FSRC01); 10, *Algoriphagus resistens* NH1^T (LMXN01).

	1	2	3	4	5	6	7	8	9	10
1		18.3	17.2	14.1	13.8	14.0	14.2	14.0	13.9	13.3



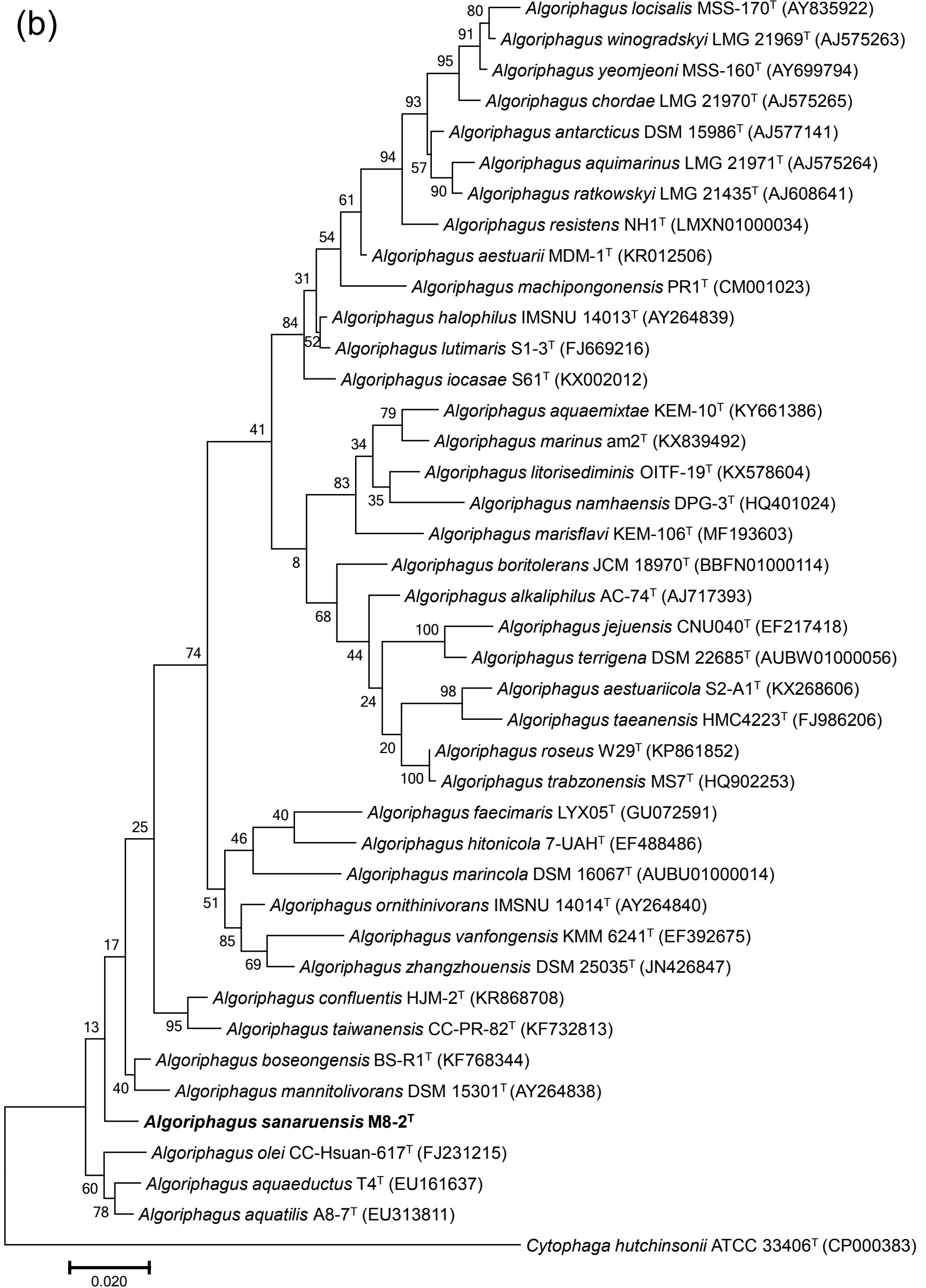
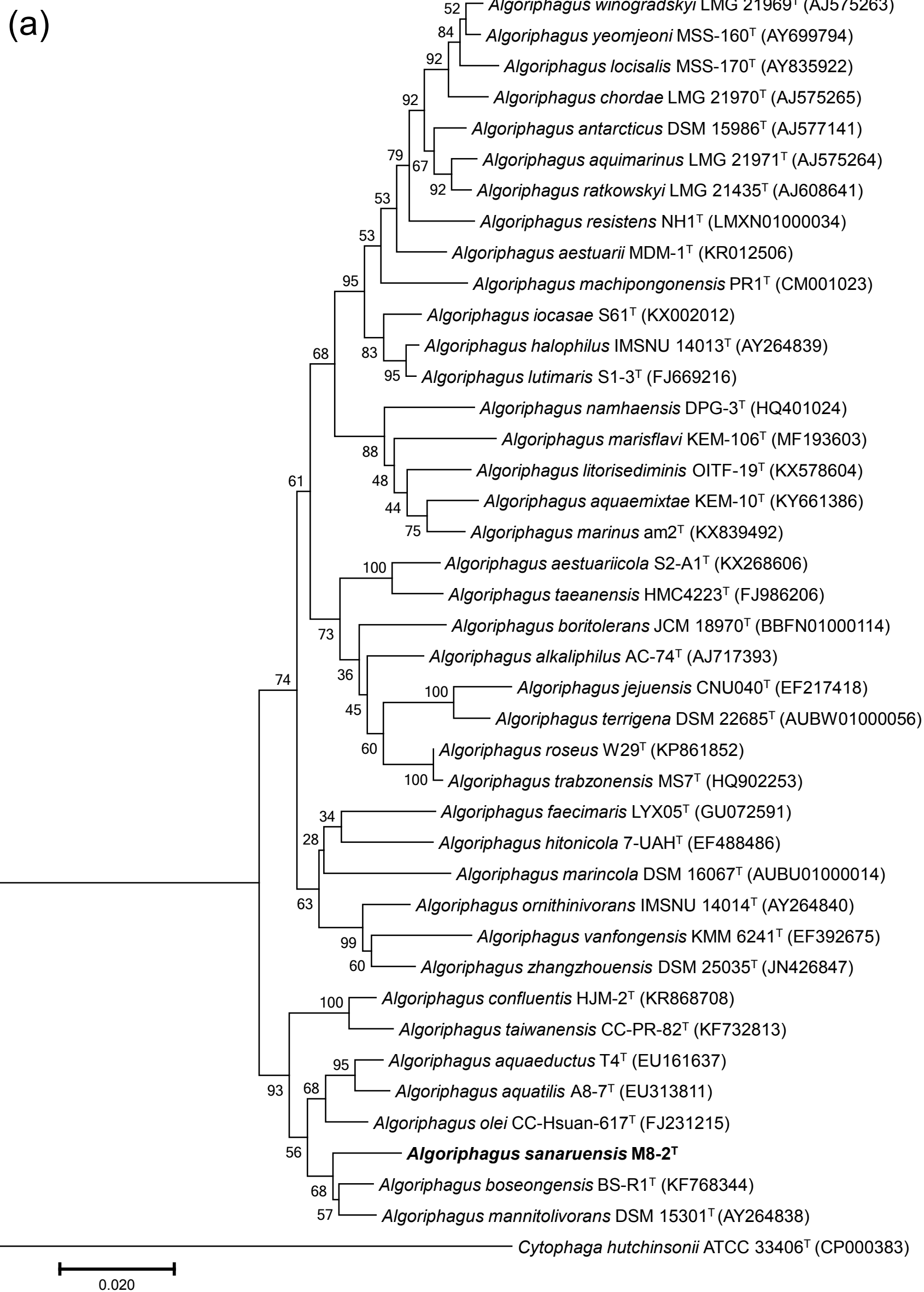
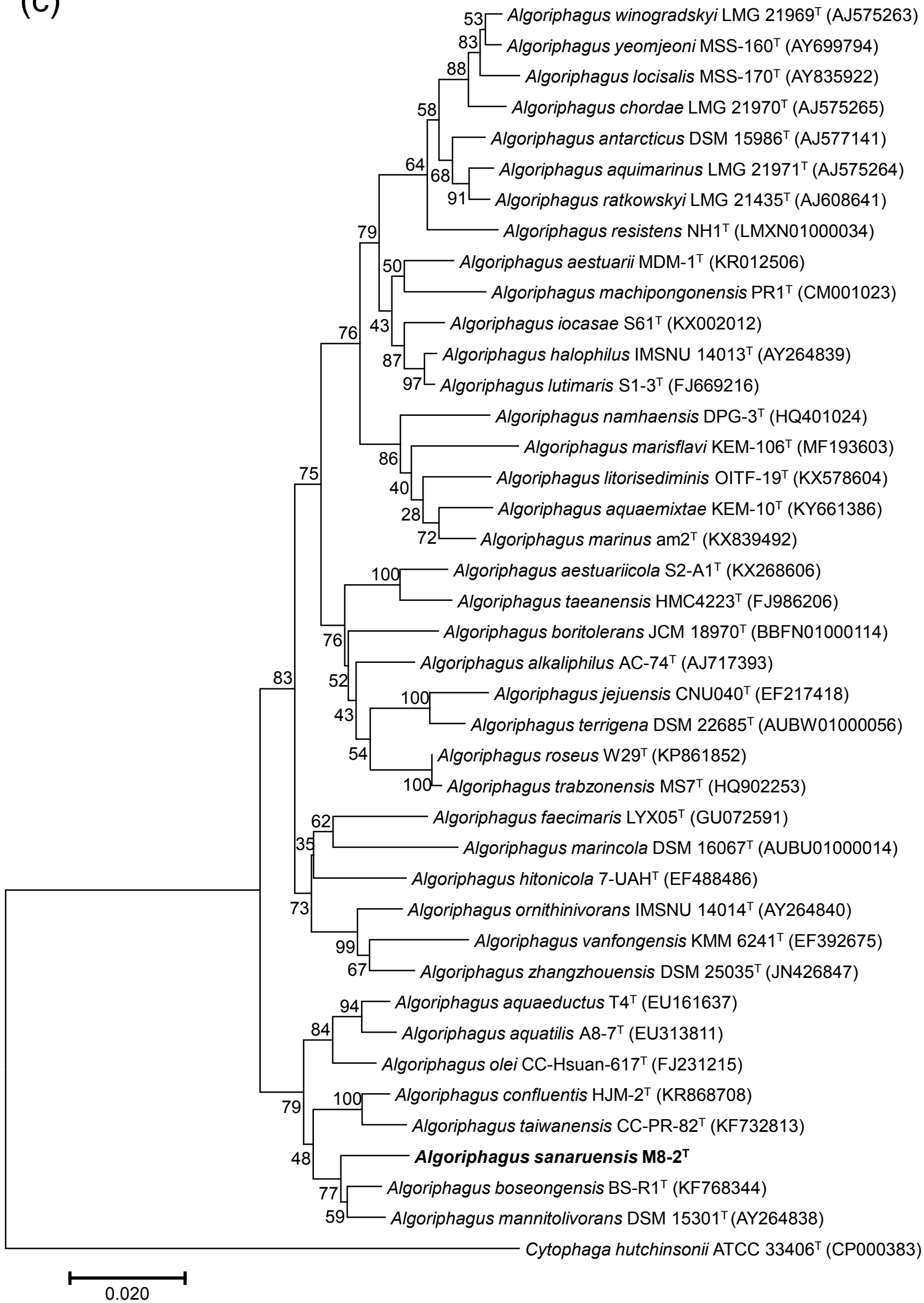


Figure S2. Different phylogenetic trees of partial 16S rRNA gene sequences of strain M8-2^T with representative members of selected species belonging to the genus *Algoriphagus*. Bootstrap values (1000 replications) are shown as percentages at nodes. (a) neighbour-joining (NJ) method, (b) maximum-likelihood (ML) method, (c) minimum evolution (ME) method, and (d) maximum parsimony (MP) method. The tree was reconstructed using MEGA software. *Cytophaga hutchinsonii* ATCC 33406^T was used as the outgroup.

(c)



(d)

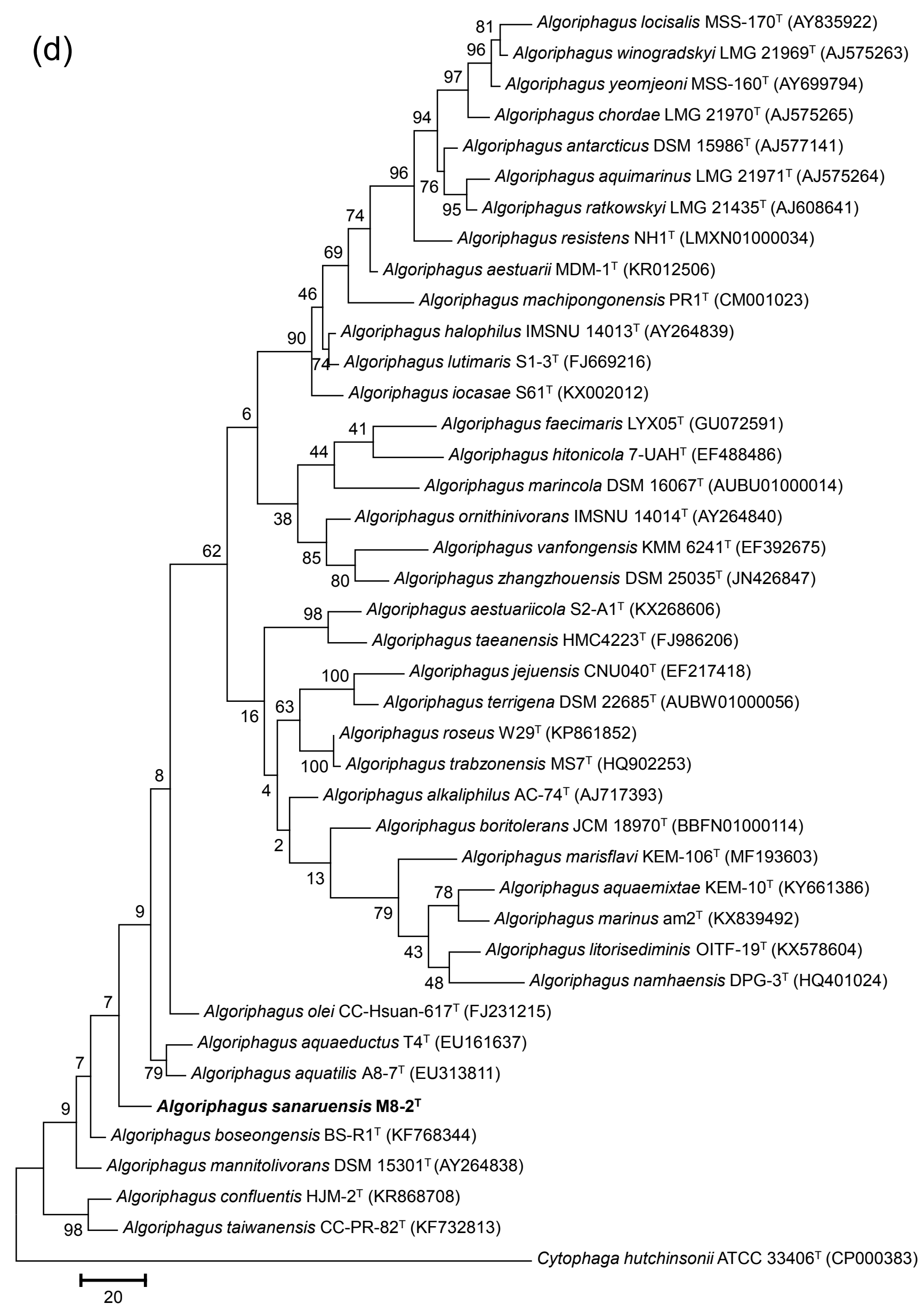


Figure S2. Continued.