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

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

Running title: *Algoriphagus sanaruensis* sp. nov.

Authors: Yoshiaki Maejima[1†], Takao Iino[2†], Yusuke Muraguchi[1], Moriya Ohkuma[2], Kazuhide Kimbara[1], Masaki Shintani[1,2,3,4*]

Author affiliations: [1]Department of Engineering, Graduate School of Integrated Science and Technology, Shizuoka University, 3-5-1 Johoku, Naka-ku, Hamamatsu, 432-8561, Shizuoka, Japan, [2]Japan Collection of Microorganisms, RIKEN BioResource Research Center, 3-1-1 Koyadai, Tsukuba, Ibaraki, 305-0074, Japan, [3]Department of Bioscience, Graduate School of Science and Technology, Shizuoka University, 3-5-1 Johoku, Naka-ku, Hamamatsu, 432-8561, Shizuoka, Japan, [4]Green Energy Research Division, Research Institute of Green Science and Technology, Shizuoka University, 3-5-1 Johoku, Naka-ku, Hamamatsu, 432-8561, Shizuoka, Japan.

Contents List Category: New Taxa – *Bacteroidetes*

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

Keywords: *Cyclobacteriaceae*; ‘filterable’ bacteria; brackish lake

[†]These authors contributed equally to this work.

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

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

maximum-likelihood; MP, maximum-parsimony; NJ, neighbour-joining; OGRI, overall genome related index.

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

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

Strain M8-2^T was routinely cultivated on Marine agar 2216 (MA) (Difco, BD Bioscience) for 6 days at 30ºC. Cells of strain M8-2^T was aerobic, non-spore-forming, non-motile and curved rod-shaped. Gram staining was negative by using the Gram-staining kit (FUJIFILM Wako Pure Chemical Corp.). Colonies of strain M8-2^T are circular, convex and coral with 1.5-2.0 mm diameter on MA plate after 5-6 days of cultivation. Catalase reaction was tested by placing drops of 3% (v/v) H_2O_2 solution directly on the cells cultivated on MA and observing gas evolution. Oxidase reaction was tested by using a Cytochrome Oxidase Test Strip (Nissui). Catalase and oxidase reaction were positive. To confirm that

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

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

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

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

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

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

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

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

Description of *Algoriphagus sanaruensis* sp. nov.

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

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

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

Author contributions

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

Funding information

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

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

Disclosure statement

No potential conflict of interest was reported by the authors.

REFERENCES

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

17. **Maejima Y, Kushimoto K, Muraguchi Y, Fukuda K, Miura T et al.** *Proteobacteria* and *Bacteroidetes* are major phyla of filterable bacteria passing through 0.22 mum pore size membrane filter, in Lake Sanaru, Hamamatsu, Japan. *Biosci Biotechnol Biochem* 2018:1-4.
18. **Komagata K, Suzuki K**. Lipid and cell-wall analysis in bacterial systematics. *Method Microbiol* 1987;19:161-207.
19. **Lechevalier MP, Debievre C, Lechevalier H**. Chemotaxonomy of aerobic *Actinomycetes* - phospholipid composition. *Biochem Syst Ecol* 1977;5:249-260.
20. **Minnikin DE, Odonnell AG, Goodfellow M, Alderson G, Athalye M et al.** An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Meth* 1984;2:233-241.
21. **Sasser M**. Identification of bacteria by gas chromatography of cellular fatty acids, MIDI Technical Note101. Newark,DE: MIDI Inc.1990.
22. **Muraguchi Y, Kushimoto K, Ohtsubo Y, Suzuki T, Dohra H et al.** Complete genome sequence of *Algoriphagus* sp. strain M8-2, isolated from a brackish Lake. *Genome announcements* 2016;4: pii:e00347-16.
23. **Tamura K, Nei M**. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol Biol Evol* 1993;10:512-526.
24. **Kimura M**. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 1980;16:111-120.
25. **Rzhetsky A, Nei M**. Statistical properties of the ordinary least-squares, generalized least-squares, and minimum-evolution methods of phylogenetic inference. *J Mol Evol* 1992;35:367-375.
26. **Thompson JD, Higgins DG, Gibson TJ**. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* 1994;22:4673-4680.
27. **Kumar S, Stecher G, Tamura K**. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol* 2016;33:1870-1874.
28. **Park S, Park JM, Lee KC, Yoon JH**. *Algoriphagus boseongensis* sp. nov., a member of the family *Cyclobacteriaceae* isolated from a tidal flat. *Antonie Van Leeuwenhoek* 2014;105:523-531.
29. **Rau JE, Blotevogel KH, Fischer U**. *Algoriphagus aquaeductus* sp. nov., isolated from a freshwater pipe. *Int J Syst Evol Microbiol* 2012;62:675-682.
30. **Kumar PA, Bhumika V, Ritika C, Bhaskar YV, Priyashanth P et al.** *Algoriphagus shivajiensis* sp. nov., isolated from Cochin back water, India. *Syst Appl Microbiol* 2013;36:106-111.
31. **Yi H, Chun J**. *Hongiella mannitolivorans* gen. nov., sp. nov., *Hongiella halophila* sp. nov. and *Hongiella ornithinivorans* sp. nov., isolated from tidal flat sediment. *Int J Syst Evol Microbiol* 2004;54:157-162.
32. **Nedashkovskaya OI, Kim SB, Kwon KK, Shin DS, Luo X et al.** Proposal of *Algoriphagus vanfongensis* sp. nov., transfer of members of the genera *Hongiella* Yi and Chun 2004 emend. Nedashkovskaya et al. 2004 and

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

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)	0.2-0.5×0.7-1.9	0.2-0.5×0.7-6.0	0.4-0.5×1.1-1.7
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	-	-
N-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	-	-

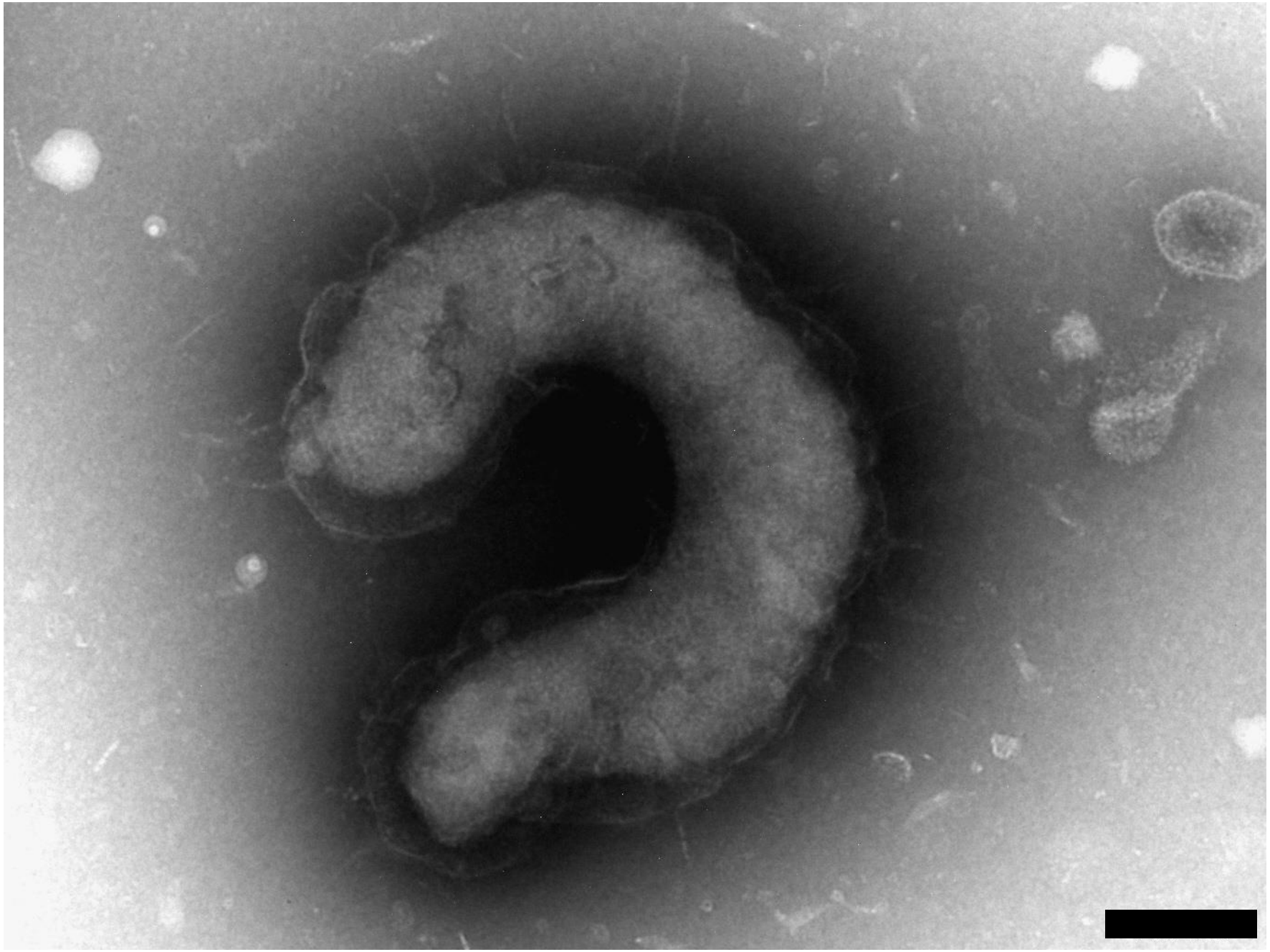
FIGURE LEGENDS

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

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

Figure 3. Neighbour-joining tree 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 (neighbour-joining method/maximum-likelihood method/minimum-evolution method). The tree was reconstructed using MEGA software. *Cytophaga hutchinsonii* ATCC 33406^T was used as the outgroup. Bar shows 0.020 substitutions per nucleotide position.

Figure_1



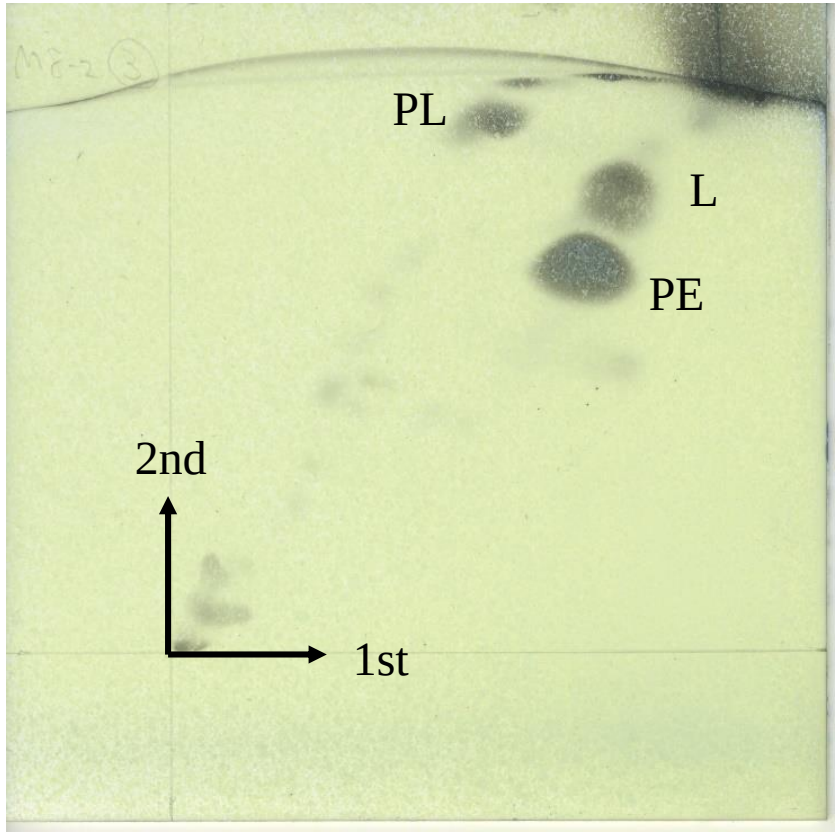
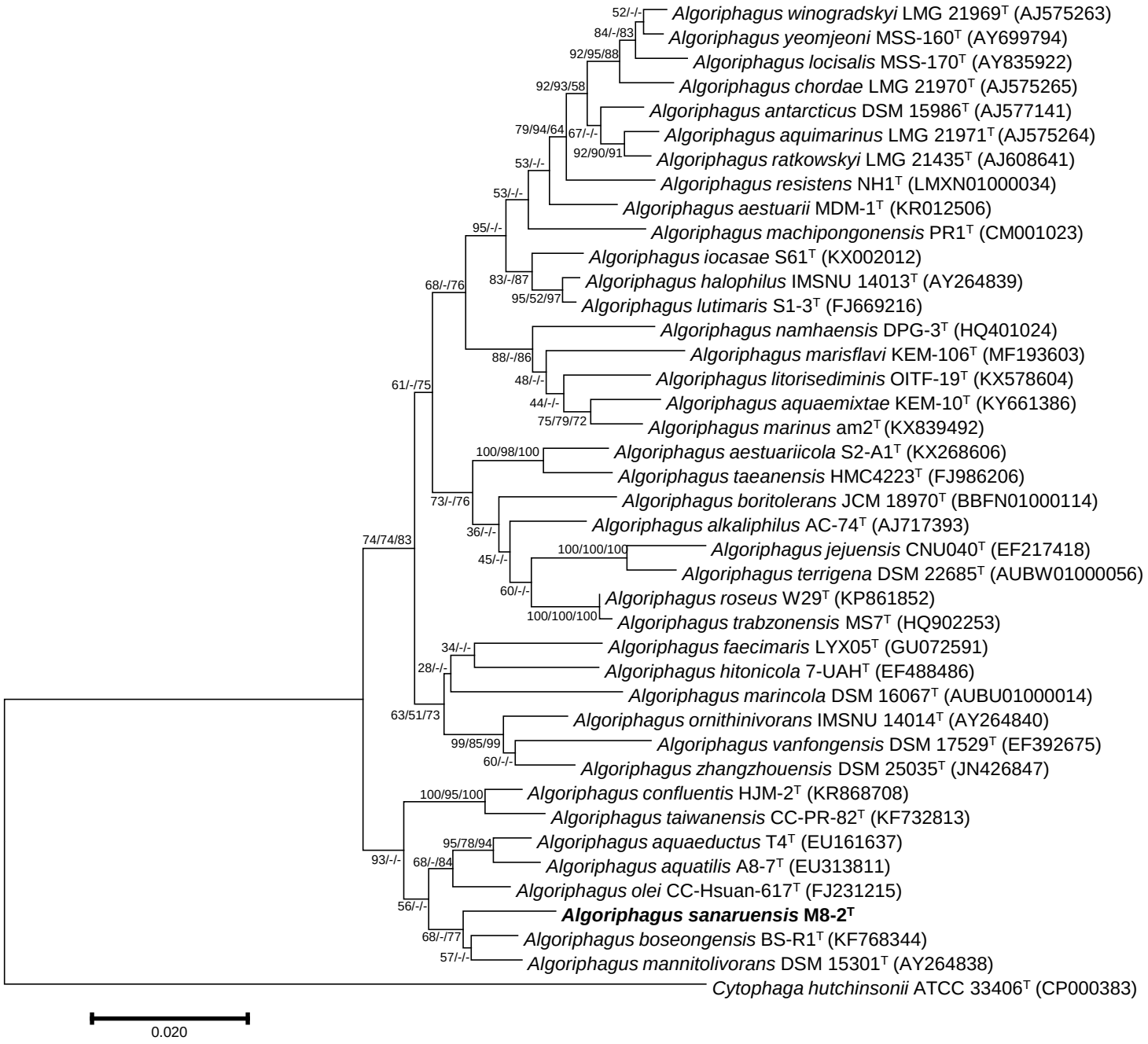

PL
L
PE
2nd
1st

Figure_3


52/-/-
Algoriphagus winogradskyi LMG 21969T (AJ575263)
84/-/83
Algoriphagus yeomjeoni MSS-160T (AY699794)
92/95/88
Algoriphagus locisalis MSS-170T (AY835922)
92/93/58
Algoriphagus chordae LMG 21970T (AJ575265)
Algoriphagus antarcticus DSM 15986T (AJ577141)
79/94/64
67/-/-
Algoriphagus aquimarinus LMG 21971T (AJ575264)
92/90/91
Algoriphagus ratkowskyi LMG 21435T (AJ608641)
53/-/-
Algoriphagus resistens NH1T (LMXN01000034)
53/-/-
Algoriphagus aestuarii MDM-1T (KR012506)
95/-/-
Algoriphagus machipongonensis PR1T (CM001023)
Algoriphagus iocasae S61T (KX002012)
83/-/87
Algoriphagus halophilus IMSNU 14013T (AY264839)
68/-/76
95/52/97
Algoriphagus lutimaris S1-3T (FJ669216)
Algoriphagus namhaensis DPG-3T (HQ401024)
88/-/86
Algoriphagus marisflavi KEM-106T (MF193603)
48/-/-
Algoriphagus litorisediminis OITF-19T (KX578604)
61/-/75
44/-/-
Algoriphagus aquaemixtae KEM-10T (KY661386)
75/79/72
Algoriphagus marinus am2T (KX839492)
100/98/100
Algoriphagus aestuariicola S2-A1T (KX268606)
Algoriphagus taeanensis HMC4223T (FJ986206)
73/-/76
Algoriphagus boritolerans JCM 18970T (BBFN01000114)
36/-/-
Algoriphagus alkaliphilus AC-74T (AJ717393)
74/74/83
100/100/100
45/-/-
Algoriphagus jejuensis CNU040T (EF217418)
Algoriphagus terrigena DSM 22685T (AUBW01000056)
60/-/-
Algoriphagus roseus W29T (KP861852)
100/100/100
Algoriphagus trabzonensis MS7T (HQ902253)
34/-/-
Algoriphagus faecimaris LYX05T (GU072591)
28/-/-
Algoriphagus hitonicola 7-UAHT (EF488486)
Algoriphagus marincola DSM 16067T (AUBU01000014)
63/51/73
Algoriphagus ornithinivorans IMSNU 14014T (AY264840)
99/85/99
Algoriphagus vanfongensis DSM 17529T (EF392675)
60/-/-
Algoriphagus zhangzhouensis DSM 25035T (JN426847)
100/95/100
Algoriphagus confluentis HJM-2T (KR868708)
Algoriphagus taiwanensis CC-PR-82T (KF732813)
95/78/94
Algoriphagus aquaeductus T4T (EU161637)
93/-/-
68/-/84
Algoriphagus aquatilis A8-7T (EU313811)
Algoriphagus olei CC-Hsuan-617T (FJ231215)
56/-/-
Algoriphagus sanaruensis M8-2T
68/-/77
Algoriphagus boseongensis BS-R1T (KF768344)
57/-/-
Algoriphagus mannitolivorans DSM 15301T (AY264838)
Cytophaga hutchinsonii ATCC 33406T (CP000383)
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**

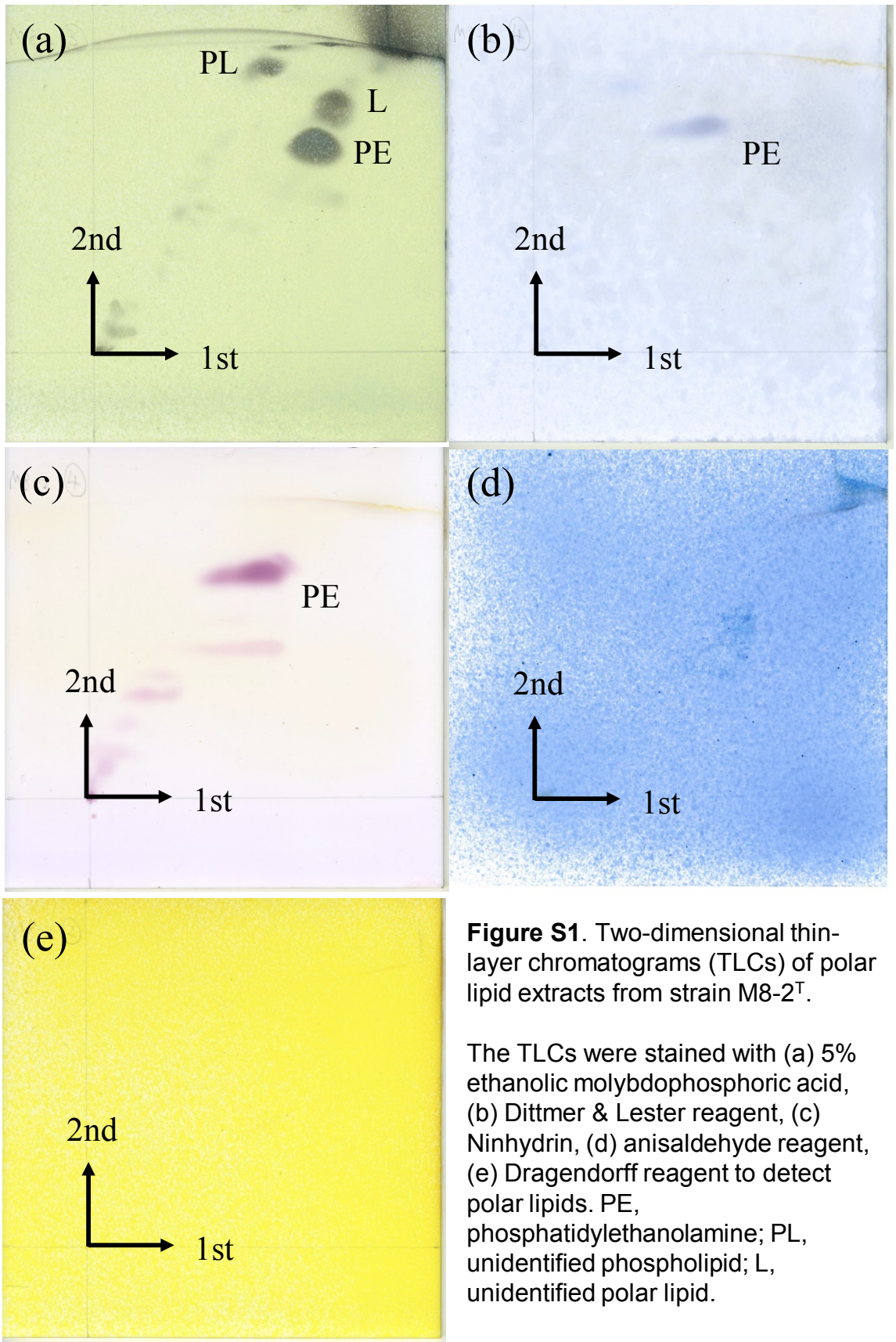
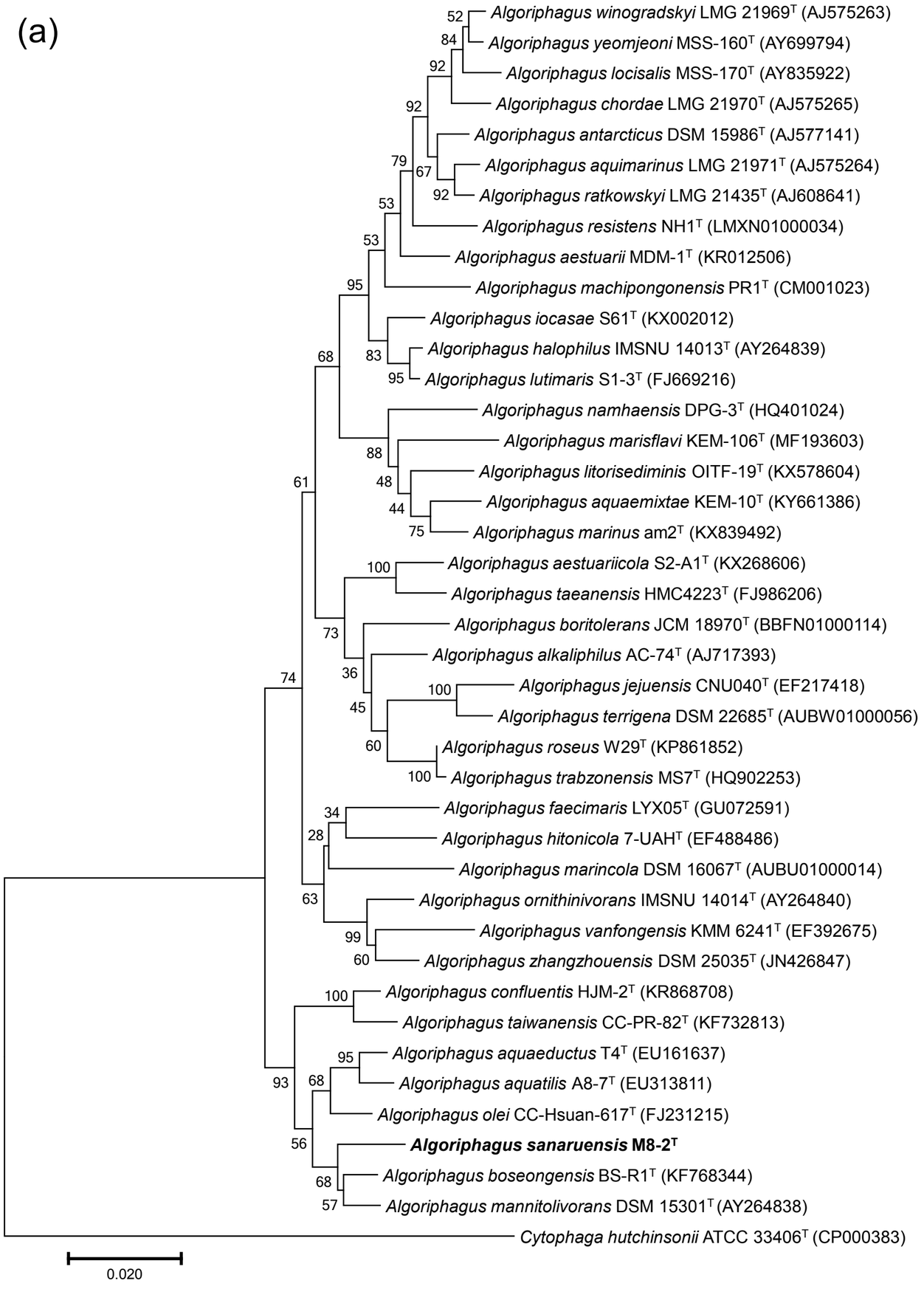


Figure S1. Two-dimensional thin-layer chromatograms (TLCs) of polar lipid extracts from strain M8-2^{T}.

The TLCs were stained with (a) 5% ethanolic molybdophosphoric acid, (b) Dittmer & Lester reagent, (c) Ninhydrin, (d) anisaldehyde reagent, (e) Dragendorff reagent to detect polar lipids. PE, phosphatidylethanolamine; PL, unidentified phospholipid; L, unidentified polar lipid.

(a)



(b)

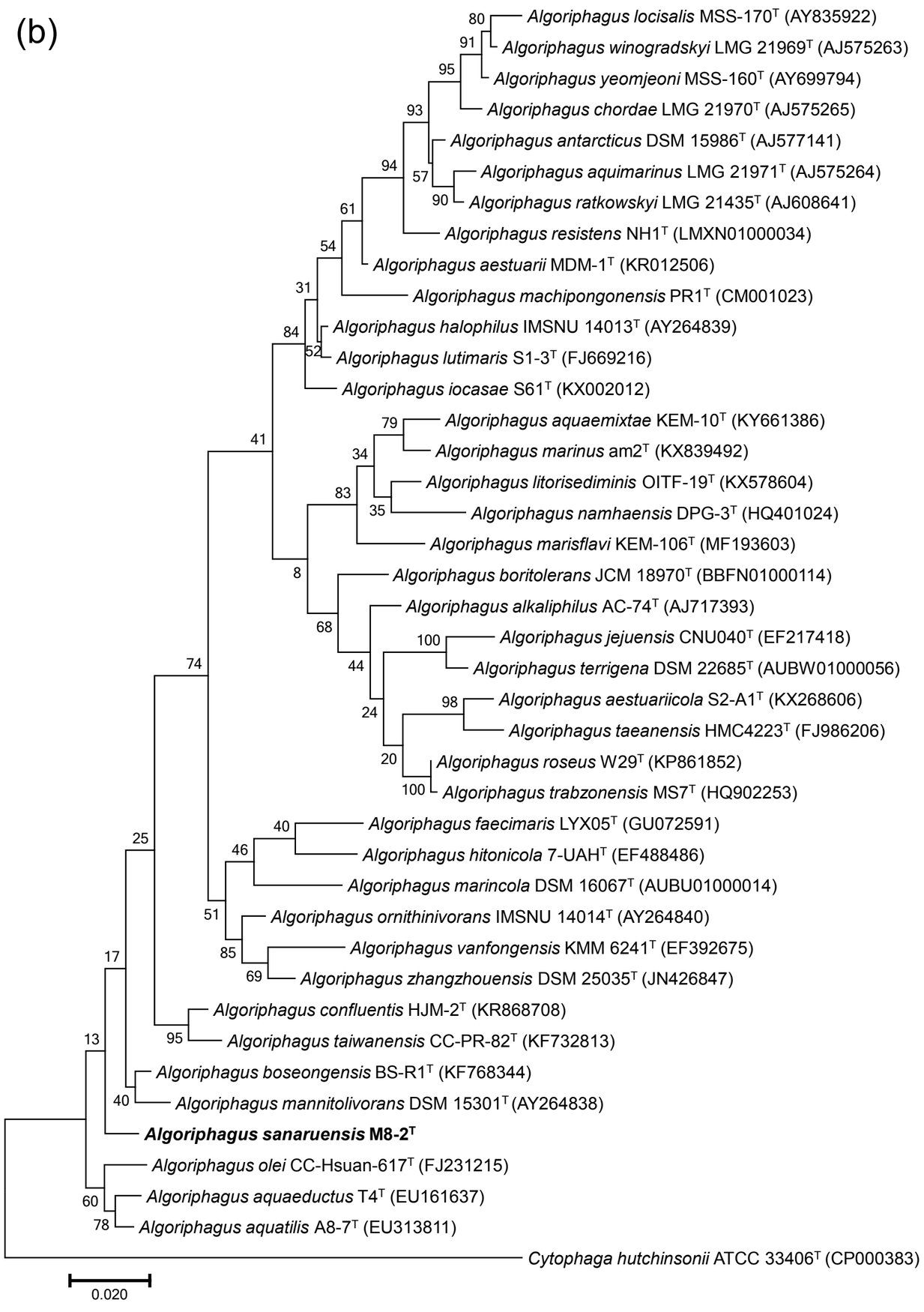
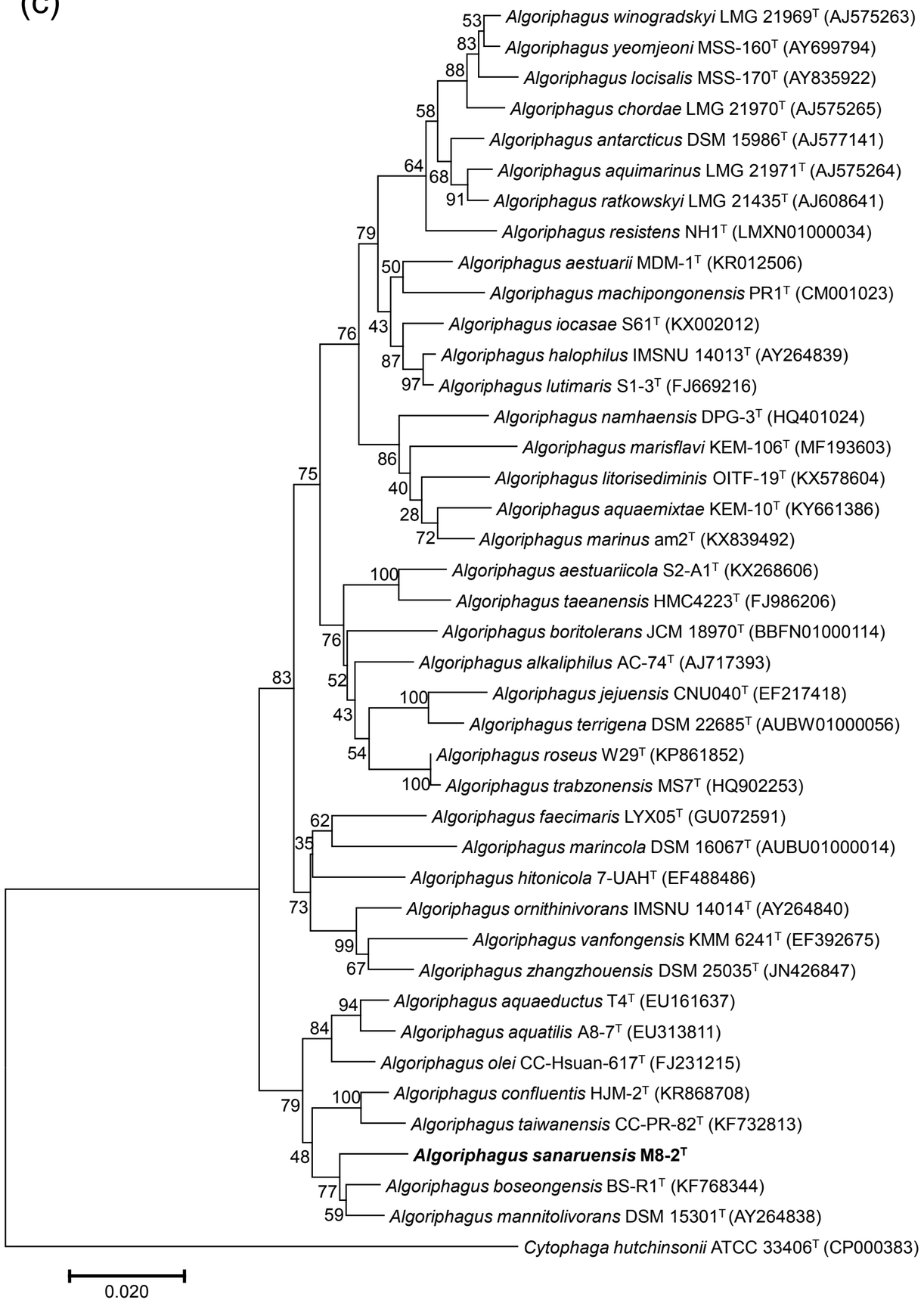


Figure S2. Different phylogenetic trees of partial 16S rRNA gene sequences of strain M8-2^T with representative members of selected species belonging to the genus *Algoriphagus*. Bootstrap values (1000 replications) are shown as percentages at nodes. (a) neighbour-joining (NJ) method, (b) maximum-likelihood (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)

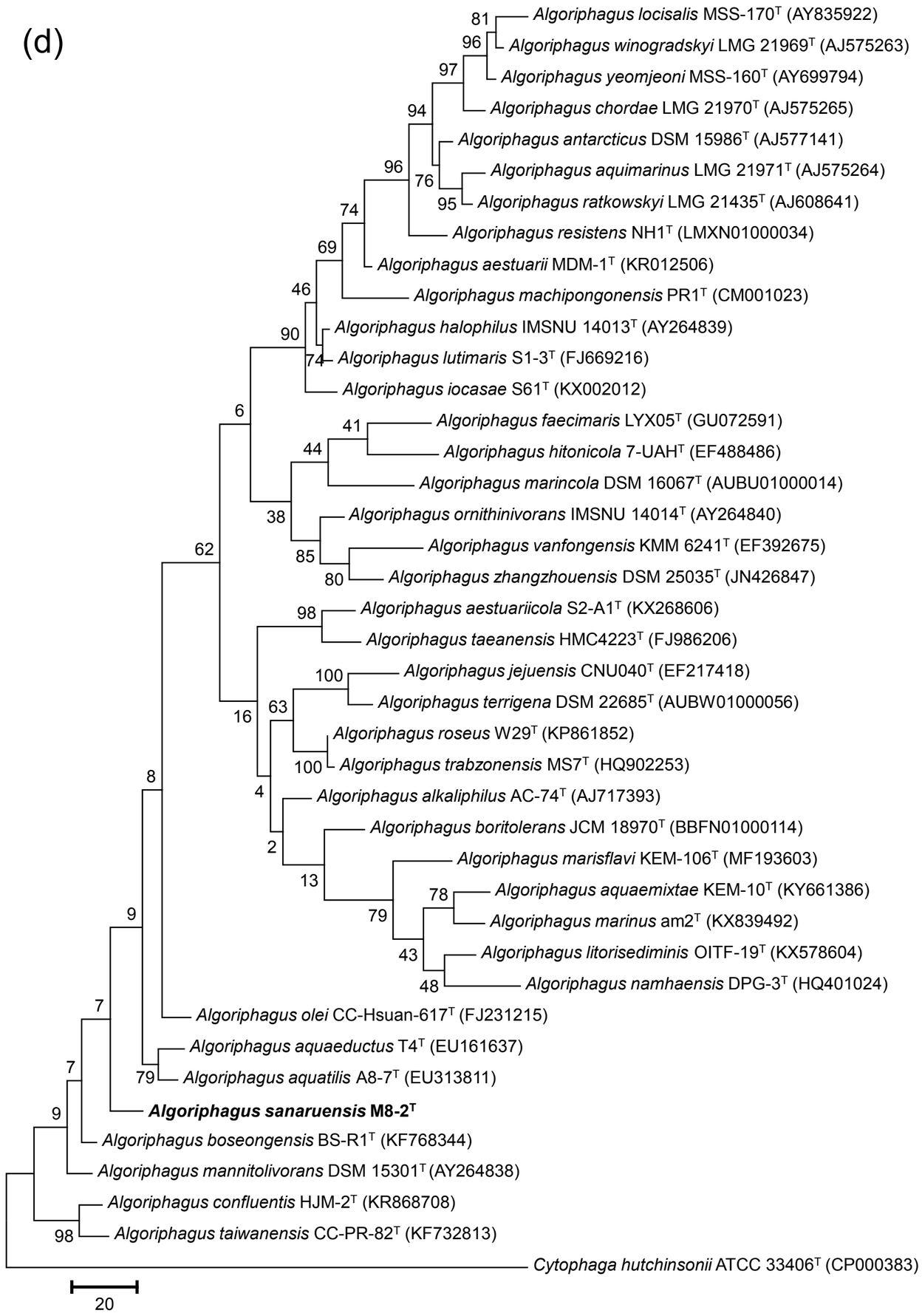


Figure S2. Continued.