Rhabdonatronobacter Sediminivivens gen. nov., sp. nov. Isolated from the Sediment of Hutong Qagan Soda Lake

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Abstract

A novel Gram-stain negative bacterium, designated IM2376^T, was isolated from the sediment of Hutong Qagan Lake in Ordos, Inner Mongolia Autonomous Region of China. The strain IM2376^T had the highest similarity with *Roseinatronobacter thiooxidans* DSM 13087^T (96.18%) and *Rhodobaca bogoriensis* LBB1^T (96.18%) of the family *Rhodobacteraceae* according to 16S rRNA gene sequence comparison. Genomic relatedness analyses showed that strain IM2376^T was clearly distinguished from other species in the family *Rhodobacteraceae*, with average nucleotide identities, amino acid identities and in silico DNA-DNA hybridization values not more than 74.1%, 68.5% and 20.2%. The fatty acid was mainly composed of C18:1ω7c (64.86%), iso-C16:0 (16.33%) and C16:1ω7c/C16:1ω6c (6.02%). The major polar lipid was diphosphatidyl glycerol, phosphatidylglycerol and phosphatidylcholine. The predominant ubiquinone was Q-10 (94.9%) and Q-11 (5.1%). The DNA G + C was 66 mol%. Based on all these results, strain IM2376^T was considered to be a novel species of a new genus in the family *Rhodobacteraceae*, for which the name *Rhabdonatronobacter sediminivivens* gen. nov., sp. nov. is proposed. The type strain is IM2376^T (= CGMCC 1.17852^T).

Introduction

The family *Rhodobacteraceae* was firstly established by Garrity et al. (2005). Up to date, approximate two hundred genera were validly published and correctly named (https://lpsn.dsmz.de/family/rhodobacteraceae). *Rhodobacteraceae* are aquatic bacteria that are commonly found in marine environments (Pujalte et al. 2014). Some species in this family could synthesize bioplastic materials polyhydroxyalkanoates (PHAs), or commonly polyhydroxybutyrate (PHB) as carbon reserve material (Boldareva et al. 2007; Hwang and Cho 2008; Li et al. 2017; Pujalte et al. 2014). Soda lake was characteristic of high salinity and pH, which was caused by the accumulation of lots of sodium (bi)carbonate by evaporation (Jones and Grant 2000). Because soda lake was rich in hydrochloride and bicarbonate, but poor of phosphorus, this environment was conducive to the accumulation of PHA or PHB (Chen et al. 2019). Sulfide-quinone oxidoreductase (*Sqr*) or cytochrome subunit of sulfide dehydrogenase (*fccA)/* sulfide dehydrogenase [flavocytochrome c] flavoprotein chain (*fccB*) gene were often found in this family which could be used for the treatment of sulfur-containing wastewater or waste gas (Yu et al. 2011). Isolation and identification of strains of this family from soda lakes would help enriching the microbial resources and providing new resources for industrial development.

Materials And Methods

Isolation and culture conditions

Strain IM2376^T was isolated from the sediment of Hutong Qagan Lake in Ordos, Inner Mongolia Autonomous Region of the People's Republic of China. The longitude and latitude of the sample sites

were 108°58'3" E, 39°14'10" N, and the altitude was 1270 m. The isolation medium was LN with 1.5% agar with the following matters (per liter): 15 g NaCl, 4 g Na_2CO_3, 6 g $NaHCO_3$, 2 g KCl, 0.5 g yeast extract, 0.2 g NH_4Cl, 0.25 g fish peptone, 0.38 g sodium formate, 0.25 g sodium acetate, 0.25 g sodium pyruvate, 2 g $MgSO_4 \cdot 7H_2O$, 0.05 g KH_2PO_4, 0.08 g $CaCl_2$, 0.0046 g $FeSO_4$, 1 ml trace metal solution (SL-6) and 3 ml vitamins solution. The composition of SL-6 solution was (per 100 ml): 0.1 g $ZnSO_4 \cdot 7H_2O$, 0.03 g $MnCl_2 \cdot 4H_2O$, 0.3 g H_3BO_3, 0.2 g $CoCl_2 \cdot 6H_2O$, 0.01 g $CuCl_2 \cdot 2H_2O$, 0.02 g $NiCl_2 \cdot 6H_2O$, 0.03 g $Na_2MoO_4 \cdot H_2O$. SL-6 solution was adjusted final pH to 3 - 4 with HCl to prevent precipitation of metal salts and stored at 4 °C. The composition of vitamins solution was (per liter): 13.0 mg 4-aminobenzoate, 3.0 mg d-(+)-biotin, 33.0 mg nicotinic acid, 17.0 mg hemicalcium D-(+)-pantothenate, 50.0 mg pyridoxamine hydrochloride, 33.0 mg thiamine chloride hydrochloride, 17.0 mg cyanocobalamin, 10.0 mg D, L-6,8-thioctic acid, 10 mg riboflavin and 4.0 mg folic acid.

The sediment sample was diluted and coated on the surface of the LN agar plates and then incubated in the incubator at 37 °C until the colonies were observed (about two weeks). Then the colonies were inoculated to a new LN agar plate at least three times with the dilution separation method to obtain the pure culture. The isolated strain IM2376^T was stored in 20% (v/v) glycerol with 10% NaCl at -80 °C.

Phylogenetic and phylogenomic analysis

The genomic DNA of strain IM2376^T was prepared according to the method described by Marmur (1961) and Xu et al. (2007). The genome was sequenced using the Illumina Novaseq 6000 platforms by the BioMarker technologies, PR China. The genome sequences were assembled using SPAdes 3.13.0 with default parameters (Nurk et al. 2013). The draft genome was annotated by the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (https://www.ncbi.nlm.nih.gov/genome/annotation_prok/). The 16S rRNA gene sequence similarity of the strain IM2376^T was conducted through the BLAST searching tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi) (Johnson et al. 2008) and the EzBioCloud (Yoon et al. 2017a). The 120 conserved single-copy genes were obtained according to the Genome Taxonomy Database (GTDB) approach (Chaumeil et al. 2019; Parks et al. 2020; Parks et al. 2018). Multiple sequence alignments were performed by using the CLUSTAL W program (Larkin et al. 2007). Phylogenetic tree constructed based on 16S rRNA gene and the 120 conserved single-copy genes in MEGA X software (Sudhir et al. 2018) with the maximum-likelihood (ML) (Felsenstein 1981), neighbor-joining (NJ) (Saitou 1987) and the unweighted pair group method with arithmetic mean (UPGMA) (Sneath and Sokal 1973) methods with bootstrap values based on 1000 replications (Felsenstein 1985). The G + C content was calculated from the whole genome sequence. The OrthoANI algorithm was used to calculate the Average Nucleotide Identity (ANI) value (Yoon et al. 2017b). The genome sequence data was uploaded to the Type (Strain) Genome Server (TYGS), a free bioinformatic platform available under https://tygs.dsmz.de, for the genome-to-genome distance analysis as the *in silico* DNA-DNA hybridization (*is*DDH) (Meier-Kolthoff and Gker 2019). The amino acid identity (AAI) was calculated using the AAI calculator (http://enve-omics.ce.gatech.edu/aai/index) (Rodriguez-R and Konstantinidis 2014).

Physiological characteristics

The LN medium with various NaCl concentrations (0, 0.17, 0.34, 0.51, 0.68, 0.86, 1.03, 1.20, 1.36, 1.54, 1.71, 1.88, 2.05 M) was conducted to test the (optimum) salinity for growth. The pH range for growth (5 - 10.5, at interval of 0.5 pH unit) was also conducted in LN with the addition of various buffer as followed: MES (pH 5.0 - 6.7), PIPES (pH 6.5 - 7.0), Tricine (pH 7.4 - 8.8), CHES (pH 9.0 - 10.1), CAPS (pH 9.7 - 11.1). The temperature range for growth was determined by incubating at 4, 15, 20, 25, 35, 37, 42, 50 ℃. The strain was cultivated in LN at 37 °C for 2 days to examine cell morphology by scanning electron microscopic (SU8010, Hitachi) and motility by light microscopy (BX51, Olympus).

The following matters were used as sole carbon and energy source (2 g/l for sugars, alcohols and organic acids while 1 g/l for amino acids) in LN medium with 0.1g/l yeast extract and the fish peptone, sodium formate, sodium acetate, sodium pyruvate were omitted: galactose, starch, trehalose, mannitol, D-xylose, D-maltose, sucrose, D-glucose, D-mannose, L-rhamnose, lactose, arabinose, cellobiose, fructose, sorbose, glycerol, sorbitol, acetate, malate, pyruvate, DL-lactate, succinate, fumarate, citrate, ornithine, arginine, glutamate, glycine, histidine, cysteine, isoleucine, valine, lysine and aspartate. Biochemical tests were performed in LN (containing 0.01 yeast extract) according to methods described by Xu et al. (2007) and Mata et al. (2002), including the activity of oxidase, catalase and urease, the reduction of nitrate and nitrite, the production of H_2S and indole, and the O-nitrophenyl-β-D-galactopyranoside (OPNG) and vogesproskauer (VP) test. The antibiotic sensitivity test was conducted on LN agar plate with the following antibiotic discs (ug per disc unless otherwise noted): Nitrofurantoin (300), Erythromycin (15), Bacitracin B (10 Units), Rifampicin (5), Ciprofloxacin (5), Novobiocin (5), Neomycin (30), Norfloxacin (10), Cefoxitin (30), Tetracycline (30), Tobramycin (10), Amoxycillin (10), Cefotaxime (30), Vancomycin (30), Chloramphenicol (30), Penicillin G (10 Units), Streptomycin (10).

Chemotaxonomy characteristics

The strain was cultivated in LN for two days, and then the fatty acid extraction and GC-MS determination was performed by following the previous method (Kuykendall et al. 1988). The frozen dry cells (about 200 mg) were used to extract isoprenoid quinones with chloroform/methanol (2:1, by vol.), and then the extracted isoprenoid quinones was measured by reversed-phase HPLC (Wu et al. 2009). The polar lipids analysis followed the method described by Kamekura and Kates (1988) using one- and two-dimensional TLC.

Results And Discussion

Morphology, physiology and biochemical analysis

The colonies of strain IM2376^T on LN agar plate under 37 ℃ for 48h were pink, circle, convex and with smooth edge, and the diameter was 1.0 - 2.0 mm. The cells were Gram-stain negative, and were short rod or oval (0.5 - 1.06 μm × 1 - 2.27 μm) (Fig. S1, Table 1), non-motile and no flagellum. The NaCl concentrations, pH values and temperature ranges for growth were 0 - 2.05 M (optimum: 0.34 - 0.68 M), 5.5 - 10.5 (optimum: 7.0 - 8.0) and 4 - 42℃ (optimum: 37 ℃). The strain IM2376^T was sensitive to

Nitrofurantoin, Erythromycin, Bacitracin B, Rifampicin, Ciprofloxacin, Novobiocin, Neomycin, Norfloxacin, Cefoxitin, Tetracycline, Tobramycin, Amoxycillin, Cefotaxime, Vancomycin, while resistant to Chloramphenicol, Penicillin G, Streptomycin. The other features of strain IM2376^T could be found in the species description. The detail of feature comparison between strain IM2376^T and its close species were listed in Table 1.

Chemotaxonomic characterization

The major fatty acid was C18:1ω7c (64.86%), iso-C16:0 (16.33%) and C16:1ω7c/C16:1ω6c (6.02%), and the details of the fatty acid categories were listed in Table S1. The major polar lipids were diphosphatidyl glycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine and one unknown amino phospholipid. Phospholipid were also detected, but no glycolipid was found (Fig. S2). The quinones were Q-10 (94.9%) and Q-11 (5.1%).

Phylogenetic and phylogenomic analysis

The 16S rRNA gene (1503bp) and whole-genome sequence of strain IM2376^T were obtained. The genome size was 4.063 M bps. The G + C content was 66 mol% which was higher than that of the reference species (59 - 62 mol%, Table 1). The strain IM2376^T had one 16S rRNA gene, one 23S rRNA gene, three 5S rRNA genes, forth-one tRNAs and three ncRNAs. Three clustered regularly interspaced short palindromic repeat sequences (CRISPRs) arrays were predicted. A total of 3671 coding sequences (CDS) were predicted.

The 16S rRNA gene sequences similarity analysis showed that strain IM2376^T was closely related to *Roseinatronobacter thiooxidans* DSM 13087^T (96.18%), *Rhodobaca bogoriensis* LBB1^T (96.18%) and followed by *Pararhodobacter aggregans* D1-19^T (96.12%). The OrthoANI and *is*DDH values between strain IM2376^T and reference strains were 73.51 - 74.06% and 19.4 - 20.9%, respectively (Table 2), which was lower than the threshold values of the species boundary (ANI 95-96% and *is*DDH 70%) (Goris et al. 2007; Meier-Kolthoff et al. 2013; Richter and Rosselló-Móra 2009). Besides, the AAI value (66.51 - 69.25%) was among 60-80% (the genus-level boundary) (Luo et al. 2014). The ML trees based on 16S rRNA sequences and 120 conserved single-copy genes from the whole genome sequences showed that strain IM2376^T formed a distinct clade (bootstrap value > 75%, Fig. 1a, b), separated from *Roseibaca ekhonensis* EL-50^T (Labrenz et al. 2009), *Rhodobaca bogoriensis* LBB1^T (Milford et al. 2000), *Rhodobaca barguzinensis* VKM B-2406^T (Boldareva et al. 2008), *Roseinatronobacter thiooxidans* DSM 13087^T (Sorokin et al. 2000) and *Roseinatronobacter monicus* ROS 35^T (Boldareva et al. 2007). The stability of the ML trees was further supported by NL and UPGM trees (bootstrap value > 85%, Fig. S3a, b, and Fig. S4a, b). The phenotypic, chemotaxonomic, and phylogenetic properties suggested that strain IM2376^T represented a novel species of a new genus within the family *Rhodobacteraceae*, for which the name *Rhabdonatronobacter sediminivivens* gen. nov., sp. nov. was proposed.

Description of *Rhabdonatronobacter* gen. nov.

Rhabdonatronobacter (*Rhab.do.nat.ro.no.bac'ter*. M. L. adj. *rhabdos*, rod; M. L. N. natron, soda; M. L. masc. n. bacter, short rod; M. L. n. *Rhabdonatronobacter* short rod from soda lake)

Cells are Gram-stain negative, non-motile, short rod or oval. The major respiratory quinone is Q-10. The major fatty acid was C18:1ω7c and iso-C16:0. The major polar lipid was diphosphatidyl glycerol (DPG), phosphatidylglycerol (PG), and phosphatidylcholine (PC). According to 16S rRNA sequences similarity analysis, the genus is belonged to the family *Rhodobacteraceae*, order *Rhodobacterales*, class *Alphaproteobacteria*. The type species is *Rhabdonatronobacter sediminivivens*.

Description of *Rhabdonatronobacter sediminivivens* sp. nov.

Rhabdonatronobacter sediminivivens (se.di.mi.ni.vi'vens L. n. *sedimen -inis* sediment; L. part. *vivens* living; N.L. part. adj. *sediminivivens living in the sediment*)

The cells were short rod or oval (0.50-1.06 μm × 1.00-2.27 μm), non-motile, no flagellum. The cells were Gram-stain negative. Colonies on optimum agar plate were pink, circle, convex, with smooth edge, and the diameter were 1.0 - 2.0 mm. The optimum condition for growth is occurred at 0.34 - 0.68 M (range of 0 - 2.05 M) NaCl, 37℃ (range of 4 - 42 ℃), pH 7.0 - 8.0 (range of 5.5 - 10.5). The oxidase and catalase activity were positive. The hydrolase of gelatin, Tween 80, and casein was positive. Starch hydrolase was negative. Nitrate and nitrite reduction were positive. H_2S was produced while indole was not. No urease activity. The OPNG and VP test was positive. The following compounds are utilized as the sole carbon and energy source: galactose, trehalose, mannitol, D-xylose, D-maltose, sucrose, D-glucose, D-mannose, L-rhamnose, arabinose, cellobiose, glycerol, acetate, malate, pyruvate, DL-lactate, succinate, histidine, cysteine, isoleucine, aspartate, while the following compounds were not used: starch, lactose, fructose, sorbose, sorbitol, fumarate, citrate, ornithine, arginine, glutamate, glycine, valine, lysine, aspartate. The major fatty acid was composed of C18:1ω7c (64.86%), iso-C16:0 (16.33%) and C16:1ω7c/C16:1ω6c (6.02%). The major quinone was ubiquinone Q-10 (94.9%) and Q-11 (5.1%). The major polar lipids were diphosphatidyl glycerol, phosphatidylglycerol (PG), Phosphatidylethanolamine, phosphatidylcholine and one unknown amino phospholipid. Phospholipid were also detected, but no glycolipid was found.

The type strain IM2376^T (= CGMCC 1.17852) was isolated from a soda lake. The DNA G + C content of the type strain is 66 mol%. The GenBank accession number for the 16S rRNA gene sequence is MW750412. The whole genome has been deposited at GenBank under the accession number of JACBXS000000000.

Abbreviations

DPG diphosphatidyl glycerol

PG phosphatidylglycerol

PC phosphatidylcholine

***is*DDH** the *in silico* DNA-DNA hybridization

AAI the amino acid identity

ANI the average nucleotide identity

PHA polyhydroxyalkanoate

PHB polyhydroxybutyrate

sqr sulfide-quinone reductase

fccA cytochrome subunit of sulfide dehydrogenase

fccB sulfide dehydrogenase [flavocytochrome c] flavoprotein chain

GTDB the Genome Taxonomy Database

ML the maximum-likelihood

NJ neighbor-joining

UPGMA the unweighted pair group method with arithmetic mean

TYGS the Type (Strain) Genome Server

OPNG the O-nitrophenyl-β-D-galactopyranoside

VP Vogesproskauer

tmRNAtransfer-messenger-RNA

CDS coding sequences

Declarations

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Conflict of interest

The authors declare that there are no conflicts of interest.

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Tables

Table 1 Characteristics of strain IM2376^T and closely related genera within the family *Rhodobacteraceae*.

Taxa: 1, strain IM2376^T; 2, *Roseibaca ekhonensis* EL-50^T (data from Labrenz et al. (2009)); 3, *Rhodobaca barguzinensis* VKM B-2406^T *(data from Boldareva et al. (2008))*; 4, *Rhodobaca bogoriensis* LBB1^T *(data from Milford et al. (2000))*; 5, *Roseinatronobacter thiooxidans* DSM 13087^T *(data from Sorokin et al. (2000))*; 6, *Roseinatronobacter monicus* ROS 35^T *(data from Boldareva et al. (2007))*.

Characteristics	1*	2	3	4	5	6
Cell size (um)	0.50-1.06 × 1.00-2.27	0.80-1.20 × 1.20-4.00	1.0 × 1.5	0.8-1 × 1-1.5	0.5-0.8 × 0.8-2.2	0.5-0.7 × 1.2-1.7
Cell shape	short rod	rod	short rod	short rod	rod with elongated ends	short rod
NaCl range for growth (M)	0 - 2.05	0-0.68	0.17-1.37	0.17-0.51	0.1-2	0-1.37
Optimum NaCl for growth (M)	0.34 - 0.68	0.43	0.34-0.51	0.17-0.51	0.4-0.6	0.68
Temperature range for growth (°C)	4-42	10-30	10-45	30-43	mesophilic	mesophilic
Optimum temperature for growth (°C)	37	16	23-35	39	30	25-30
pH range for growth	5.5-10.5	5.5-9.5	7.5-9	7.5-10	8.5-10.4	8-10
Optimum pH for growth	7.0-8.0	7.0-9.5	8.2	9	10	8.5-9.5
Utilization as sole carbon and energy source:						
pyruvate	+	+	+	+	+	+
aspartate	+	+	+	+	+	-
succinate	+	+	+	+	+	-
fructose	-	+	+	+	+	+
glutamate	-	+	+	+	+	-
citrate	-	+	-	-	+	+
Major polar lipids**	DPG, PG, PE, PC, APL, L1	DPG, PG, PE, PC,	ND	ND	ND	ND
G+C content (mol%)	66	61	60	59	62	59

Note: * determined in this study. ** DPG, diphosphatidyl glycerol; PG, phosphatidyl glycerol; PE, phosphatidyl ethanolamine; PC, phosphatidyl choline; APL, unidentified aminophospholipid, L1, unidentified lipid. ND: No data. +, positive; -, negative.

Table 2 Overall genome relatedness index (%) of strain IM2376^T and closely related species within the family *Rhodobacteraceae*.

Strain	*is*DDH	ANI	AAI
Rhodobaca barguzinensis VKM b-2406^T	20.9	73.7291	69.25
Rhodobaca bogoriensis LBB1^T	20.9	73.869	69.22
Roseibaca ekhonensis EL-50^T	19.4	73.5157	66.51
Roseinatronobacter thiooxidans DSM 13087^T	19.6	74.0568	68.65
Roseinatronobacter monicus ROS 35^T	19.8	73.76	68.78

Figures

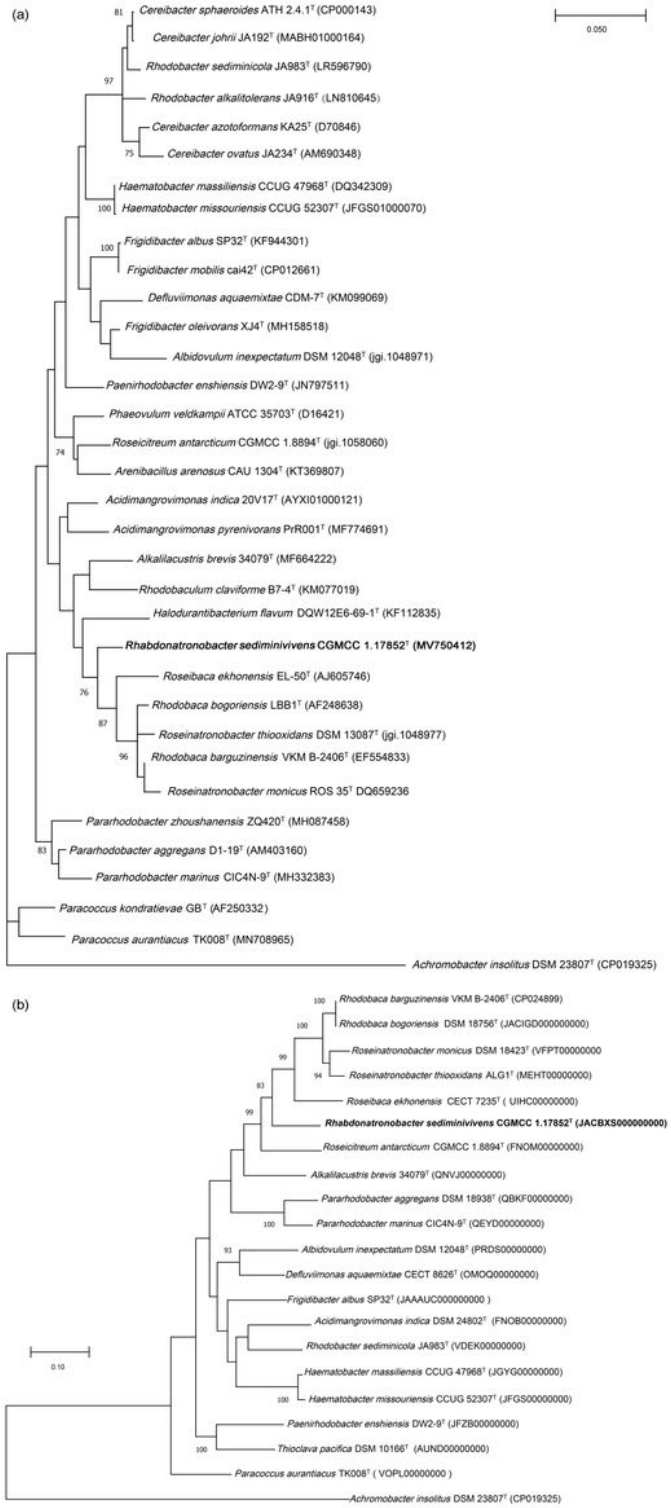

Figure 1

Maximum-likelihood tree reconstructions based on 16S rRNA gene (a) and 120 conserved single-copy proteins (b) sequences, showing the relationship between strain IM2376T and related members with the family Rhodobacteraceae. Bootstrap values (%) are based on 1000 replicates and are shown for branches with more than 70% bootstrap values. Bar, 0.05 (a) and 0.1 (b) substitutions per nucleotide position.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarymaterials.pdf