Bruguiierivorax albus gen. nov. sp. nov. Isolated from Mangrove Sediment and Proposal of Bruguiierivoracaceae fam. nov

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Abstract
A novel Gram-negative, motile, aerobic rod-shaped bacterium designated BGMRC 2031T was isolated from mangrove sediment collected from Guangxi Province, China. Optimal growth occurred at 28 °C and pH 7.0–8.0 in the presence of 1% (w/v) NaCl. Alignment based on 16S rRNA gene sequences indicated that strain BGMRC 2031T is most closely related to Sodalis praecaptivus HS1T (95.6%, sequence similarity), followed by Biostraticola tofi DSM 19580T (95.5%), Sodalis glossinidius DSM 16929T (95.4%), and Brenneria goodwinii FRB141T (94.9%) sequence similarity. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain BGMRC 2031T formed a distinct branch in a robust cluster and revealed that strain BGMRC 2031T, genera Biostraticola and Sodalis, formed a novel family-level clade in the order Enterobacterales. The novel strain showed an average nucleotide similarity of 74.7%, 74.2%, and 73.1% for S. praecaptivus HS1T, S. glossinidius DSM 16929T, and B. tofi DSM 19580T, respectively. The genomes of the BGMRC 2031T shared the presence of a riboflavin synthesis gene cluster. The menaquinones of strain BGMRC 2031T were MK-8 and Q-8, which were similar to those of genus Biostraticola. The major fatty acids (> 10%) were C16:0 (19.9%), summed feature 2 (iso-C16:1 and/or C14:0 3-OH, 18.10%), summed feature 3 (C16:1 ω7c and/or C16:1 ω6c, 15.3%), C12:0 (13.9%), C17:0 cyclo (11.4%), and C14:0 (10.4%). The main polar lipids were phosphatidyl methylethanolamine, phosphatidyl glycerol, diphosphatidyl glycerol, phosphatidyl inositol, one unidentified phospholipid, and one unknown polar lipid. The G+C content of strain BGMRC 2031T was 55.4%. Strain BGMRC 2031T could extend the mean lifespan and maximum lifespan of Caenorhabditis elegans by 4.5% and 12.5%, respectively. Overall, the results of this study indicate that BGMRC 2031T is a novel species in a new genus, for which the name Bruguiierivorax albus gen. nov. sp. nov. is proposed, and the type of strain is designated as BGMRC 2031T (= NBRC 111907T = KCTC 52119T). In addition, a novel family, Bruguiierivoracaceae fam. nov., is proposed to accommodate the genera Bruguiierivorax, Biostraticola, and Sodalis.

Introduction
The order Enterobacterales is a large group within the class Gammaproteobacteria which was revised in 2016 by Adeolu et al. [1]. This order is characterized by its non-spore-forming, rod-shaped bacteria, as well as its Gram-negative and facultatively anaerobic characteristics [1]. At the time of its revision, the Enterobacterales comprised seven families according to the EzTaxon database, Enterobacteriaceae, Erwiniaeae, Pectobacteriaceae, Yersiniaeae, Hafniaceae, Morganellaceae, and Budviciaceae [1].

The genera Biostraticola and Sodalis were first proposed by Verbarg et al. [2] and Dale et al. [3], respectively. In 2016, Biostraticola and Sodalis were affiliated to two families, Enterobacteriaceae and Pectobacteriaceae, respectively, based on their genome phylogeny and taxonomy [1]. At the time of writing, the genus Biostraticola...
contained a single species, *B. tofi*, while *Sodalis* contained two validly named species (*S. praecaptivus* [4] and *S. glossinidius* [3]) and two candidatus species (Candidatus *S. melophagi* [5] and Candidatus *S. baculum* [6]). Two Candidatus strains were live in symbiosis with various groups of insects and, respectively, symbiose relationships with *Melophagus ovinus* and Hemipteran insects. To date, the majority of *Sodalis* members have been found in several insect groups, and *S. praecaptivus* has been found in human hand wounds [4, 6]. The strain *S. lignotolerans* 159R was isolated from an anaerobic lignin degrading consortium.

In our study of microbial biodiversity in medicinal mangrove plants, strain BGMRC 2031T was isolated from a *Bruguiera gymnorrhiza* rhizosphere soil sample. Comparative 16S rRNA gene sequence analysis showed that strain BGMRC 2031T was closely related to species in the genera *Biostraticola* (95.5%) and *Sodalis* (95.4–95.6%). However, strain BGMRC 2031T could not be assigned to any species of the genera *Biostraticola* or *Sodalis* because of its low sequence similarity with the two type strains (≤ 95.6%). Therefore, the present study is conducted to report the taxonomic characterization of the new isolate, BGMRC 2031T.

**Materials and Methods**

**Bacterial Strain and Culture Conditions**

Strain BGMRC 2031T was isolated from sediment of *Bruguiera gymnorrhiza* roots collected from Guangxi Province, China (21° 55′ N, 108° 50′ E). Samples were immediately stored in sterile plastic bags at 4 °C, then transported to the laboratory within 12 h. Soil (2 g) was added to 20 mL of sterilized seawater, then shaken at 37 °C for 1 h. Next, 1 mL of the suspension was transferred to 9 mL sterilized seawater, then shaken at 37 °C for 1 h. Next, 1 mL of the suspension was transferred to 9 mL sterilized seawater and serially diluted to 10⁻², 10⁻³, 10⁻⁴, and 10⁻⁵. The serial dilutions of the samples (200 μL) were subsequently plated onto R2A medium (0.5 g yeast extract powder, 0.5 g peptone, 0.5 g casein hydrolysate, 0.5 g glucose, 0.5 g starch, 0.3 g K₂HPO₄, 0.024 g MgSO₄, 0.3 g sodium pyruvate, 15.0 g agar, 1 L seawater, pH 7.2), then incubated at 28 °C for 2 days. Single colony was selected and purified on modified Yeast Malt Extract (ISP2) (2.0 g yeast extract, 0.5 g malt extract, 2.0 g D- (+)-glucose anhydrous, 15.0 g agar powder and 1 L seawater) at 28 °C. Strain BGMRC 2031T was isolated using the preceding method, then preserved in 20% (v/v) glycerol suspensions at -80 °C. The reference strain, *B. tofi* DSM 19580T, was obtained from the Leibniz Institut DSMZ-German Collection of Microorganisms and Cell Cultures GmbH.

**Morphological and Physiological Characteristics**

Morphological and physiological characteristics were observed on modified ISP2 medium unless stated. Growth and colony morphology were observed after 2 days incubation at 28 °C. Cell morphology was observed using a scanning electron microscope (FEI Quanta 250 Environmental Scanning Electron Microscope), and the flagellum of the strain was observed via transmission electron microscopy (Hitachi Transmission Electron Microscope HT7700) after growth on ISP2 at 28 °C for 2 days. Cell motility determination was conducted by observing the development of turbidity in a tube using ISP2 semisolid medium containing 0.4% agar [7]. Gram staining was determined on ISP2 plates following the protocols described by Gerhardt et al. [8]. Oxidase activity was examined using 1% (w/v) *N, N', N'-tetramethyl-p-phenylenediamine* reagent. Catalase activity was assessed using 3% (w/v) H₂O₂ solution [9]. Growth at various concentrations of NaCl (0%–15%, w/v, with an interval of 1.0%), was tested on ISP2 agar (Difco) at 28 °C. The temperature range was determined by incubating cells in ISP2 medium broth at 4 °C, 10 °C, 15 °C, 20 °C, 25 °C, 28 °C, 37 °C, 40 °C, and 45 °C for 2 weeks. The pH range for growth (pH 4.0–12.0 at intervals of 1 pH unit) was tested in ISP2 broth at 28 °C using the buffer system developed by Xu et al. [10]. Cultural characteristics were determined by observing growth of the strain at 28 °C for 2 weeks on ISP2, ISP3, ISP4, ISP5, and ISP7 agar plates, lysogeny broth (LB agar), R2A agar, and tryptic soy agar. ISCC-NBS color charts [11] were used to assess colony colors. Biochemical tests, including H₂S production, hydrolysis of cellulose, gelatin, starch, Tweens 20, 40, and 80, were performed using the methods described by Tindall [12]. Coagulation and peptonisation of milk were evaluated as described by Gonzalez [13]. Carbohydrate metabolism was determined using API ZYM and API 20E strips (BioMérieux, Marcyl’Etoile, France) according to the manufacturer’s instructions. Anaerobic fermentation was evaluated using the API 50CH system (BioMérieux). The incubation temperature for all API kits was 28 °C, and results were observed after 48 h.

**Chemotaxonomic Characterization**

Cell biomass for the chemotaxonomic characterization was obtained from ISP2 medium after incubation at 28 °C for 3 days. Polar lipids were extracted as described by Kamekura [14] and identified by two-dimensional thin-layer chromatography (TLC) on silica gel 60 GF₂₅₄ plates (Merck KGaA, Darmstadt Germany) that had been sprayed with ethanolic molybdophosphoric acid, molybdenum
blue, and ninhydrin after two-dimensional TLC [15]. Respiratory quinones were extracted and analyzed using reverse-phase HPLC [16, 17]. Cellular fatty acid composition was analyzed by gas chromatography (G6890N; Agilent Technologies, Savage, MD, USA) and identified using the Sherlock Microbial Identification System (version 6.0) according to the manufacturer’s instructions and as previously described [18].

**Phylogenetic Analyses**

PCR amplification of strain BGMRC 2031T with the universal primers 27F and 1492R and subsequent 16S rRNA gene sequencing [19] were conducted as described by Li et al.[20]. The purified DNA product was cloned into the pEASY-T1 vector and transformed into *Escherichia coli* DH5α using the pEASY-T1 cloning kit. The 16S rRNA gene sequence was compared with that of recognized species using EzBioCloud (http://www.ezbiocloud.net) [21]. Multiple alignments of the sequence data were conducted using CLUSTAL X 1.83 [22]. Phylogenetic analyses were conducted based on the neighbor-joining [23], maximum-likelihood [24], and maximum-parsimony [25] algorithms using the MEGA software (version 7.0) [26]. Kimura’s two-parameter model was used to calculate evolutionary distance matrices of the neighbor-joining method [27]. The topology of the phylogenetic tree was evaluated by bootstrap analysis with 1000 replicates [28].

**Genomic Characterization**

To further distinguish strain BGMRC 2031T from its closely related species, whole-genome sequencing was conducted by BGI (Wuhan, China) using the Illumina Hiseq 4000 system (Illumina, San Diego, CA, USA) according to the manufacturer’s suggested protocols. The draft genome was assembled using SOAP de novo version 2.04, and the short oligonucleotides of the obtained results were further optimized using SOAP aligner 2.21 [29, 30]. The obtained genome sequences were annotated by using the NCBI Prokaryotic Genome Annotation Pipeline and deposited at DDBJ/ENA/GenBank. Genomes were annotated using the Rapid Annotation Subsystems Technology (RAST) servers [31]. Genomic information of *B. tofii* DSM 19580T (SMCR00000000), *S. praeceptivus* H5T (CP006569.1), *S. glossinidius* DSM 16929T (GCA_000010085.1), and Candidatus *S. baculum* HBA(LT897836) was downloaded from GenBank and was used to evaluate genomic relatedness with strain BGMRC 2031T. The average nucleotide identity (ANI) was calculated using the ANI calculator tool from EzBioCloud [32]. The estimated genome sequence-based digital DNA-DNA hybridization values were calculated using formula 2 from the online Genome-to-Genome Calculator (http://ggdc.dsmz.de/ggcdc.php) as described by Meier-Kolthoff et al. [33].

**Effects on Lifespan of *Caenorhabditis elegans***

The antiaging activities of crude extract of strain BGMRC 2031T were investigated as previously described [34]. Briefly, the strain was fermented in ISP2 liquid medium at 28 °C and 180 rpm for 7 days. The fermentation liquor was then extracted with ethyl acetate, after which it was concentrated and desiccated to yield crude extract [35]. Wild-type *C. elegans* strains (N2) were purchased from the Caenorhabditis Genetic Center (CGC) at the University of Minnesota (Minneapolis, MN, USA). Synchronized worms can eliminate variation in results due to age differences [34]; therefore, adult worms were seeded with *E. coli* OP50 on nematode growth medium (NGM) plates and incubated for about 2 days at 20 °C. Next, M9 buffer (0.3% KH2PO4, 0.6% Na2HPO4, 0.5% NaCl, 1 mM MgSO4) was poured onto the plate and gently swirled it to dislodge the worms. Alkaline hypochlorite (20%) was subsequently used to completely lyse the adult worms, after which synchronized eggs were collected. The synchronized eggs were grown in M9 buffer overnight at 20 °C, then put on NGM plates at the L4 stage. Synchronized L4 larvae were subsequently used to analyze the life span of worms at 20 °C. Forty L4 stage larvae were randomly transferred onto fresh NGM plates seeded with dead *E. coli* OP50 (day 0 of lifespan), then treated with 100 μL, 0.1% (v/v) DMSO (blank control) or 500 μg·mL⁻¹ BGMRC 2031T crude extract. The BGMRC 2031T crude extracts were dissolved in dimethyl sulfoxide, and the final concentration of DMSO was less than 0.1%. During the lifespan experiments, media were exchanged every 2 days, and survival of the animals was measured daily based on touch-provoked movement. All lifespan experiments were repeated at least two independent times.

**Results and Discussion**

**Morphological and Physiological Characteristics**

Colonies of strain BGMRC 2031T were round, flat, and white with diameters of 0.5–1.0 mm after cultivation for 2 days on ISP2 at 28 °C. Cells of BGMRC 2031T were Gram-negative and motile. Cells of BGMRC 2031T were Gram-negative and motile. Scanning electron microscopy showed that the cells were short rods of about 0.4–0.6 x 1.0–1.6 μm (Fig. S3). No growth was observed under anaerobic conditions. Strain BGMRC 2031T growth occurred at 15 °C–37 °C (optimum, 28 °C) and pH 5.0–9.0 (optimum, pH 7.0–8.0) in the presence of 0%–6% (v/v)
Bruguierivorax albus gen. nov. sp. nov. Isolated from Mangrove Sediment and Proposal of…

NaCl (optimum, 0–1%) (Table 1). Growth occurred on ISP2, LB, and R2A agar plates, but not ISP3, ISP4, ISP5, ISP7, or trypticase soy yeast agar plates. The strain was positive for catalase activities and negative for oxidase. Milk coagulation and peptonisation were positive, and hydrolysis of gelatin, nitrate reduction, cellulose, starch, and Tween 20, 40, and 80 were negative. The differences in the physiological and biochemical characteristics of strain BGMRC 2031T and its closest related type strains are listed in Table 1 and Tables S1 and S2. Strain BGMRC 2031T and the other related species were motile and catalase positive; however, strain S. glossinidius DSM 19580T was non-motile and catalase negative. Strain BGMRC 2031T was VP, valine arylamidase, cystine arylamidase, trypsin, 2-ketogluconate, and 5-ketogluconate positive, as well as positive for milk coagulation, peptonisation and fermentation of d-mannose, d-adonitol, d-glucose, dulcitol, d-sorbitol, L-fucose, d-arabinitol, and L-arabinitol. However, the strain was negative for esterase (C4) and esterase lipase (C8), as well as fermentation of d-cellobiose. These characteristics enable strain BGMRC 2031T to be clearly distinguished from its closest phylogenetic relatives.

### Chemotaxonomic Characterization

The major cellular fatty acids of strain BGMRC 2031T (> 10%) were C16:0 (19.9%), summed feature 2 (iso-C16:1 and/or C14:0 3-OH (18.1%)), summed feature 3 (C16:1ω7c and/or C16:1ω6c (15.3%)), C12:0 (13.9%), C17:0 cyclo (11.4%), and C14:0 (10.4%), whereas C17:0 cyclo (21.0%) and C16:0 (20.6%) were the predominant fatty acids of strain B. tofi DSM 19580T (Table S3). S. glossinidius DSM 19629T was different from BGMRC 2031T and B. tofi DSM 19580T in the absence of C19:0 cyclo aβ8c. BGMRC 2031T was different from B. tofi DSM 19580T based on the percentage of C17:0 cyclo and summed feature 2 (iso-C16:1 and/or C14:0 3-OH). The C16:0 was main cellular fatty acid of BGMRC 2031T and other neighboring families (Table 2). The major polar lipids consisted of phosphatidylmethylethanolamine, phosphatidyl glycerol, diphosphatidyl glycerol, phosphatidyl inositol, one unidentified phospholipid and one unknown polar lipid (Fig. S4). The polar lipid profile of BGMRC 2031T was similar to that of B. tofi DSM 19580T, while one unknown polar lipid was detected in BGMRC 2031T. The menaquinones were MK-8 (60.7%) and Q-8 (39.3%), which were similar to those of B. tofi DSM 19580T and neighboring families (Table 2).
Phylogenetic Analyses

The nearly complete 16S rRNA gene sequence of strain BGMRC 2031T (1472 nucleotides) has been deposited in National Center for Biotechnology Information (NCBI GenBank) under accession No. MN059649. Alignment based on the 16S rRNA gene sequence in the EzBioCloud database indicated that strain BGMRC 2031T is a member of the order Enterobacterales and showed the highest 16S rRNA gene sequence similarity to S. praecaptivus HS1T (95.6% sequence similarity), B. tofi DSM 19580T (95.5%), S. glossinidius DSM 16929T (95.4%), Candidatus M. melophagi CZT (95.3%), Candidatus S. baculum HBA (91.5%), and Brennanneria goodwinii FRB141T (94.9%), suggesting that it is a novel species. This suggested that strain BGMRC 2031T represented a novel species. Phylogenetic analysis based on the neighbor-joining algorithm revealed that strain BGMRC 2031T and S. praecaptivus HS1T, S. baculum HBA, and S. lignotolerans 159R were 73.1%, 74.7%, 74.2%, 71.24%, and 77.69%, respectively, which are below the standard ANI criteria for prokaryotic species identity (95–96%) [37]. The DDH estimated values between strain BGMRC 2031T and B. tofi DSM 19580T, S. praecaptivus HS1T, S. glossinidius DSM 16929T, Candidatus S. baculum HBA, and S. lignotolerans 159R were 20.5%, 21.1%, 21.1%, 26.4%, and 26.8%, respectively, which were all much lower than the standard criteria (DDH < 70%) [36]. These findings confirmed that strain BGMRC 2031T represents a novel species.

An overview of some characteristics of the respective gene content of the strain BGMRC 2031T, B. tofi, S. praecaptivus, S. glossinidius, Candidatus S. baculum, and S. lignotolerans 159R was given in Table 4. The genomes of

| Characteristic | Type genus | Catalase/Oxidase | Major cellular fatty acids | Respiratory quinone |
|---------------|------------|-----------------|---------------------------|--------------------|
| 1             | Bruguierivorax | ± | C_{16:0} feature 3 (C_{16:1}ω7c and/or C_{16:1}ω6c)) | MK-8 & Q-8 |
| 2             | Pectobacterium [38] | ± | C_{16:0} summed feature 3 (C_{16:1}ω7c and/or C_{16:1}ω6c) | MK-8 & Q-8 |
| 3             | Erwinia [39] | ± | C_{16:0} summed feature 3 (C_{16:1}ω7c and/or C_{16:1}ω6c) | Q-8 |
| 4             | Escherichia [40–42] | ± | C_{16:0} summed feature 3 (C_{16:1}ω7c and/or C_{16:1}ω6c), C_{17:0} cyclo | Q-8 |
| 5             | Yersinia [43–45] | /nd | C_{16:0} C_{18:1}ω7c, summed feature 3 (C_{16:1}ω7c and/or C_{16:1}ω6c) | MK-8 & Q-8 |
| 6             | Morganella [46, 47] | nd/− | C_{16:0} | nd |
| 7             | Hafnia [48] | ± | C_{16:0} and C_{17:0} cyclo | nd |
| 8             | Budvicia [49] | ± | C_{16:0} summed feature 3 (C_{16:1}ω7c and/or C_{16:1}ω6c) | MK-8 & Q-8 |

Families: 1, Bruguierivoraceae; 2, Pectobacteriaceae; 3, Erwiniaeae; 4, Enterobacteriaceae; 5, Yersiniaceae; 6, Morganellaceae; 7, Hafniaceae; 8, Budviciaceae. +, positive; −, negative; nd, not determined.

Genomic Characterization

The draft genome sequences of B. tofi DSM 19580T, S. praecaptivus HS1T, and S. glossinidius DSM 16929T were obtained from NCBI (Table 3). The genome sequencing depth of strain BGMRC 2031T was 199 ×, and its N50 and L50 values were 147,949 bp and 12, respectively. Compared with the reference strain, the largest genome size was observed for strain BGMRC 2031T (5.66 Mb). The DNA G+C content of strain BGMRC 2031T was determined to be 55.4 mol%, which was higher than that of B. tofi DSM 19580T (53.9%) and S. glossinidius DSM 16929T (54.4%), but lower than that of S. praecaptivus HS1T (57.1%), Candidatus S. baculum HBA, and S. lignotolerans 159R. The ANI values between strain BGMRC 2031T and B. tofi DSM 19580T, S. praecaptivus HS1T, S. glossinidius DSM 16929T, Candidatus S. baculum HBA, and S. lignotolerans 159R were 73.1%, 74.7%, 74.2%, 71.24%, and 77.69%, respectively, which are below the standard ANI criteria for prokaryotic species identity (95–96%) [37]. The DDH estimated values between strain BGMRC 2031T and B. tofi DSM 19580T, S. praecaptivus HS1T, S. glossinidius DSM 16929T, Candidatus S. baculum HBA, and S. lignotolerans 159R were 20.5%, 21.1%, 21.1%, 26.4%, and 26.8%, respectively, which were all much lower than the standard criteria (DDH < 70%) [36]. These findings confirmed that strain BGMRC 2031T represents a novel species.
Bruguierivorax albus gen. nov. sp. nov. Isolated from Mangrove Sediment and Proposal of…

Family Enterobacteriaceae and Erwiniaceae

- Gibbsiella quercinecans FRB 97T (CP014136)
- Serratia marcescens sub sp. marcescens ATCC 13880T (JMPQ01000005)

Family Budviciaceae

- Plesiomonas shigelloides NCTC 10360T (LT575468)
- Nissabacter archeti 2134T (FQXW01000003)
- Ewingella americana ATCC 33852T (JMPJ01000013)
- Rahnella aquatilis CIP 78.65T (CP003244)
- Rouxiella chamberiensis 130333T (JRWU010000013)
- Hafnia paralvei ATCC 29927T (LXET01000073)
- Obesumbacterium proteus DSM 2777T (CP014608)
- Chania multitudinisentens RB-25T (CP007044)
- Izhakiella capsodis N6PO6T (KF436763)
- Edwardsiella tarda NBRC 105688T (BANW01000030)

Family Morganellaceae

- Dickeya chrysanthemi LMG 2804T (Z96093)
- Pectobacterium carotovorum NCPPB 312T (JQHJ01000001)
- Rohrkolberia cinguli 2T (FR729479)
- Sanssonia erythinae CFBP 5236T (AF273037)
- Lonsdalea quercina ATCC 29281T (JIBO010000012)
- Brenneria salicis DSM 30166T (MIMA01000033)

- Bruguierivorax albus BGMRC 2031T (MN059649)

- Biostraticolata ofi DSM 19580T (AM74412)
- Candidatus Sodalis baculum HBA7(LT897836)
- Sodalis glossinidius DSM 16929T (M99060)
- Candidatus Sodalis melophagi CZT1(JN872637)

- Sodalis praecaptivus HS1T(CP006569)

- Adiaceo aphidicola 13A2T (AY692362)
- Hamiltonella defense 5A7 (CP001277)

- Thorsellia anophelis CCUG 49520T (AY837748)
- Coetzee brasiliensis Braz8T (KU748636)

Family Enterobacteriaceae and Erwiniaceae

- Arsenophonus nasoniae ATCC 49151T (AY264674)
- Phlomobacter fragariae FranceT(U91515)

- Geobacter metallireducens GS-15T (CP000148)
the strain BGMRC 2031T shared the presence of a riboflavin synthesis gene cluster with the strain S. preeapctius HS7, S. glossinidius, and Candidatus S. baculum. Furthermore, the new type strains shared the lack of genes encoding soluble cytochrome b562 with those strains. Genes putatively encoded for the aminopeptidases and anaerobic respiratory reductases were only found in the genomes of the new taxon.

**Effects on Lifespan of Caenorhabditis elegans**

The mean survival times (% vs DMSO) of the worms pretreated with strain BGMRC 2031T and blank control are shown in Fig S5. The lifespan of worms treated with BGMRC 2031T extract did not differ significantly from that of the worms treated with the blank control (0.1% DMSO), which extended the mean lifespan and maximum lifespan by 4.5% and 12.5%, respectively.

In summary, the unique phenotypic characteristics, principal fatty acid composition (C16:0 and iso-C16:1 and/or C14:0 4.5% and 12.5%, respectively. which extended the mean lifespan and maximum lifespan by 4.5% and 12.5%, respectively.

In summary, the unique phenotypic characteristics, principal fatty acid composition (C16:0 and iso-C16:1 and/or C14:0 4.5% and 12.5%, respectively.

**Description of Bruguierivorax gen. nov.**

*Bruguierivorax* (Bru.gui.e.ri.vo’rax. N.L. n. Bruguiera a mangrove plant genus; L. masc. adj. vorax devouring, ravenous, voracious; N.L. masc. n. Bruguiervorax, Brugiéra devouring).

Cells are Gram-negative, aerobic, motile, rod-shaped, catalase positive, and oxidase negative. Acid is produced from 2-ketogluconate, dulcitol, δ-adonitol and δ-mannose. The major respiratory quinones are MK-8 and Q-8. The major polar lipids are phosphatidyl methyl ethanolamine, phosphatidyl glycerol, diphasphatidyl glycerol, phosphatidyl inositol, one unidentified phospholipid and one unknown polar lipid. The type species is *Bruguierivorax albus*.

**Description of Bruguierivorax albus sp. nov.**

*Bruguierivorax* albus (al’bus. L. masc. adj. albus white, referring to the color of the colonies).

Cells are usually 0.4–0.6 μm wide and 1.0–1.6 μm long. After 2 days of incubation on ISP2 agar at 28 °C, colonies are circular, smooth, white and round and 0.5–1.5 mm in diameter. Noval strain grew well on ISP2 agar, LB agar and R2A agar, but no growth occurred on ISP3, ISP4, ISP5, ISP7, nutrient agar or trypticase soy agar plates. Optimum growth occurred at 28 °C, at pH 7.0–8.0 and in the presence of 0–1% (w/v) NaCl. The strain was negative for gelatin hydrolysis, nitrate reduction, hydrolysis of cellulose, starch, and Tween 20, 40, and 80 tests, while it was positive for milk coagulation and peptoni- sation tests. The strain was positive for O-nitrophenyl-β-D-galactopyranoside, VP, glucose fermentation, mannitol fermentation, sorbitol fermentation, amygdalin, rhamnose, and NO₃. The alkaline phosphatase, leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase and β-galactosidase activities were positive of new strain. New strain produced acid from δ-arabinitol, δ-arabinitol, δ-adonitol, 5-ketogluconate, 2-ketogluconate, δ-glucose, δ-arabinose, δ-arabinose, δ-mannitol, dulcitol, δ-mannose, δ-ribose, δ-trehalose, δ-fucose, δ-fructose, δ-sorbitol, δ-galactose, δ-rhamnose, δ-mannose, gluconate, δ-xylose and N-acetyl-D-glucosamine. The major fatty acids of new strain were C₁₆:₀, summed feature 2 (iso-C₁₆:₁ and/or C₄₀:₃-ΟΗ), summed feature 3 (C₁₆:₁ω7c and/or C₁₆:₁ω6c), C₁₂:₀, C₁₇:₀ cyclo, and C₁₄:₀. rod-shaped, catalase positive, and oxidase negative, and do not produce hydrogen disulfide. Members of this family produce acid from N-acetylglucosamine and are negative for ornithine decarboxylase lysine decarboxylase. The family *Bruguierivoracaceae* belongs to the order *Enterobacteriales* of the class *Gamma proteobacteria*.  

**Description of Bruguierivoracaceae fam. nov.**

*Bruguierivoracaceae* (Bru. gui.e.ri.vo’ra.ce’ae. N.L. fem. pl. n. Bruguierivoracaceae, Brugierivoracaceae, Brugierivorax, Brugierivorax devouring).

The major fatty acids of family *Bruguierivoracaceae* are C₁₆:₀ and feature 3 (C₁₆:₁ω7c and/or C₁₆:₁ω6c). Major respiratory quinones are MK-8 and Q-8. The 16S rRNA gene-based and phylogenomic analysis showed that the genus *Bruguierivorax*, *Biostraticola* and *Sodalis* forms a separate phylogenetic clade. The family *Bruguierivoracaceae* contains the type genus *Sodalis* and the genera *Biostraticola* and *Bruguierivorax*. These bacteria are motile
Fig. 2 Whole-genome-based phylogenetic tree constructed using UBCGs (concatenated alignment of 92 core genes) showing the phylogenetic relationship of BGMRC 2031T with reference species in the order Enterobacteriales of Enterobacteriales. Gene support indices (GSIs) are given at branching points. Bar, 0.05 substitution per position.
This type strain BGMRC 2031\textsuperscript{T} was isolated from the sediment of *B. gymnorrhiza* root collected from Guangxi Province (= NBRC 111907\textsuperscript{T} = KCTC 52119\textsuperscript{T}). The GenBank accession number assigned for the 16S rRNA gene sequence of strain BGMRC 2031\textsuperscript{T} was MN059649. The Whole-Genome Shotgun project of strain BGMRC 2031\textsuperscript{T} has been deposited in DDBJ/ENA/GenBank under accession number SZPQ00000000.

Table 3  Genome characteristics of related strains and BGMRC 2031\textsuperscript{T}

| Characteristic          | 1     | 2     | 3     | 4     | 5     | 6     |
|-------------------------|-------|-------|-------|-------|-------|-------|
| 16S similarity (%)      | 100.0 | 95.49 | 95.56 | 95.42 | 91.28 | nd    |
| Contigs                 | 188   | 32    | 2     | 4     | 1     | 1     |
| Total length (bp)       | 566,116 | 429,087 | 515,942 | 430,208 | 16,224 | 307,533 |
| ANI (%)                 | 100   | 73.16 | 74.71 | 74.18 | 71.24 | 77.69 |
| DDH (%)                 | 100   | 29.9  | 26.5  | 26.2  | 26.4  | 26.8  |
| NS0 value (bp)          | 147,949 | 391,830 | 4,709 | 4,171,874 | 1,622,395 | 11,593 |
| L50 values              | 12    | 4     | 1     | 1     | 1     | 80    |
| Genome size (Mb)        | 5.66  | 4.29  | 5.16  | 4.31  | 1.62  | 3.08  |
| G+C content (mol%)      | 55.4  | 53.9  | 57.1  | 54.4  | 36.8  | 56.4  |

GenBank accession number: SZPQ00000000 SMCR00000000 CP006569.1 GCA_000010085.1 LT897836 SJOI00000000

Strains: 1, BGMRC 2031\textsuperscript{T}; 2, *Biostraticola tofi* DSM 19580\textsuperscript{T}; 3, *Sodalis praecaptivus* HST\textsuperscript{T}; 4, *Sodalis glossinidius* DSM 16929\textsuperscript{T}; 5, Candidatus *Sodalis baculum* HBA; 6, *Sodalis lignotolerans* 159R, nd, not determined

Table 4  Comparison of the presence and absence of selected genes in related strains and BGMRC 2031\textsuperscript{T}

| Genes putatively encoding                                      | 1     | 2     | 3     | 4     | 5     | 6     |
|---------------------------------------------------------------|-------|-------|-------|-------|-------|-------|
| **Oxidative phosphorylation/energy metabolism**               |       |       |       |       |       |       |
| Phosphate metabolism                                          | +     | −     | +     | +     | −     | +     |
| Anaerobic respiratory reductases                              | +     | −     | −     | −     | −     | −     |
| Aminopeptidases                                               | +     | −     | −     | −     | −     | +     |
| **Motility**                                                  |       |       |       |       |       |       |
| Flagellar motility                                            | +     | −     | −     | +     | −     | +     |
| **Electron transport chain**                                  |       |       |       |       |       |       |
| Terminal cytochrome d ubiquinol oxidases                       | +     | +     | −     | +     | −     | −     |
| Terminal cytochrome oxidases                                  | +     | +     | −     | +     | +     | −     |
| Biogenesis of c-type cytochromes                              | +     | +     | −     | +     | −     | +     |
| **Other**                                                     |       |       |       |       |       |       |
| Trehalose biosynthesis                                        | +     | +     | +     | +     | −     | +     |
| Denitrifying reductase gene clusters                          | +     | −     | −     | +     | −     | −     |
| Non-mevalonate branch of isoprenoid biosynthesis              | +     | −     | −     | −     | −     | +     |
| Ammonia assimilation                                          | +     | −     | −     | −     | −     | −     |
| Common pathway for synthesis of aromatic Compounds (DAHP synthase to chorismate) | +     | −     | +     | +     | +     | −     |
| Lysine biosynthesis DAP pathway                                | +     | −     | −     | +     | +     | +     |
| Riboflavin synthesis cluster                                  | +     | −     | +     | +     | +     | +     |
| Pyridoxin (Vitamin B6) biosynthesis                           | +     | +     | +     | +     | +     | +     |
| Flavodoxin                                                    | +     | −     | +     | +     | +     | +     |
| Nitrogen fixation                                             | +     | −     | −     | −     | −     | −     |
| Biotin biosynthesis                                           | +     | −     | +     | −     | −     | −     |
| Soluble cytochrome b562                                       | −     | −     | −     | −     | −     | −     |

Strains: 1, BGMRC 2031\textsuperscript{T}; 2, *Biostraticola tofi* DSM 19580\textsuperscript{T}; 3, *Sodalis praecaptivus* HST\textsuperscript{T}; 4, *Sodalis glossinidius* DSM 16929\textsuperscript{T}; 5, Candidatus *Sodalis baculum* HBA; 6, *Sodalis lignotolerans* 159R
Brugiervorax albus gen. nov. sp. nov. Isolated from Mangrove Sediment and Proposal of...

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