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TAXONOMIC DESCRIPTION
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Mangrovihabitans endophyticus gen. nov., sp. nov., a new member of the family *Micromonosporaceae* isolated from *Bruguiera sexangula*

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Abstract

A novel endophytic actinobacterium, designated strain S3Cf-2^T, was isolated from a surface-sterilized bark of *Bruguiera sexangula* collected from Dongzhaigang National Nature Reserve in Hainan Province, China. Phylogenetic analysis based on 16S rRNA gene sequences suggested that strain S3Cf-2^T fell within the family *Micromonosporaceae* and formed a distinct clade in the *Micromonosporaceae* phylogenetic tree. The 16S rRNA gene sequence similarity values between strain S3Cf-2^T and the type species of 30 genera in the family *Micromonosporaceae* were 91.55–97.45 %. Strain S3Cf-2^T formed extensively branched substrate mycelia without fragmentation. An oval or rod-like spore with a smooth surface was borne singly at the end of substrate mycelium. The novel isolate possessed *meso*-diaminopimelic acid as the diamino acid of the peptidoglycan, and glucose, galactose, mannose, arabinose, xylose and ribose as whole-cell sugars. The acyl type of the cell-wall peptidoglycan was glycolyl and mycolic acids were absent. The major polar lipids included diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol and an unknown aminolipid, corresponding to phospholipid type PII. The major menaquinones were MK-9(H_6) and MK-9(H_8). The major cellular fatty acids were iso-$C_{16:0}$, anteiso-$C_{15:0}$, anteiso-$C_{17:0}$ and iso-$C_{15:0}$. The G+C content of the genomic DNA was 71.4 mol%. On the basis of phylogenetic, phenotypic and chemotaxonomic analyses, strain S3Cf-2^T represents a novel species of a new genus within the family *Micromonosporaceae*, for which the name *Mangrovihabitans endophyticus* gen. nov., sp. nov. is proposed. The type strain of the type species is S3Cf-2^T (=DSM 100693^T=CGMCC 4.7299^T).

The family *Micromonosporaceae* with *Micromonospora* as the type genus was first proposed by Krasil'nikov [1], and the description was subsequently emended by Goodfellow *et al.* [2], Koch *et al.* [3], Stackebrandt *et al.* [4] and Zhi *et al.* [5] on the basis of 16S rRNA gene sequence analysis and chemotaxonomic data. The family encompasses a chemotaxonomically and morphologically diverse group of filamentous bacteria, and at the time of writing, 30 genera with validly published names have been reported within the family *Micromonosporaceae* [6]. Members of the family *Micromonosporaceae* are widely distributed in the environment and have been isolated from sediments, soils, rhizospheres, plant tissues, freshwater and marine habitats [6].

During a study on diversity of cultivable endophytic actinomycetes from mangrove plants, strain S3Cf-2^T was isolated from a surface-sterilized bark of *Bruguiera sexangula* collected from Dongzhaigang National Nature Reserve (19°56′59″N 110°34′32″E) in Hainan Province, China. Based on phylogenetic analysis, strain S3Cf-2^T should be assigned to the family *Micromonosporaceae*, and this taxonomic study using a polyphasic approach showed strain S3Cf-2^T was distinguishable from all existing genera within the family *Micromonosporaceae*. In this paper, we propose that strain S3Cf-2^{T}represents a novel species of a new genus within the family *Micromonosporaceae*.

The plant samples of *Bruguiera sexangula* were washed in running tap water to remove adhered epiphytes and soil debris, and then surface-sterilized according to the five-step sterilization procedure [7]. After drying under sterile conditions, the surface-sterilized bark was ground into

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Abbreviations: DAP, diaminopimelic acid; ISP, International *Streptomyces* Project.
The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain S3Cf-2^T is KT996126.
Three supplementary figures are available with the online Supplementary Material.

powder by using micromill and then spread on the surface of chitin agar plates (2.0 g chitin, 0.7 g K_2HPO_4, 0.3 g KH_2PO_4, 0.5 g $MgSO_4.7H_2O$, 0.01 g $FeSO_4.7H_2O$, 0.001 g ZnSO4, 0.001 g $MnCl_2$, 15.0 g agar, 1000 ml distilled water, pH 7.0–7.5) supplemented with 1 % (v/v) plant tissue extract. The plant tissue extract was prepared as follows: approximately 5.0 g bark tissues of *Bruguiera sexangula*, washed with distilled water to remove the surface soils, were cut into pieces and ground in a mortar. The crumbled bark tissues were then poured into an erlenmeyer flask and immersed with 50 ml distilled water. After incubation on a rotary shaker (180 r.p.m.) at 28 °C for 12 h, the lixivium was filtered with gauzes and then added into the medium (1 %, v/v). Colonies of strain S3Cf-2^{T}appeared on the chitin agar plate after 4 weeks of incubation at 28 °C. The colonies were selected carefully and then transferred onto International *Streptomyces* Project (ISP) 2 agar [8] using the serial streaking technique. With observation of morphological characteristics of colonies on agar plates as well as the hyphal structure under the light microscope, a pure isolate was isolated and obtained. The purified isolate was maintained at 4 °C on ISP 2 agar slants and preserved in aqueous glycerol suspensions (20 %, v/v) at –80 °C.

Cultural characteristics were determined by observing growth of the strain at 28 °C for 2–6 weeks on ISP 2, ISP 3, ISP 4, ISP 5 and ISP 7 agars [8], nutrient agar [9], R2A agar (BD), tryptic soy agar (TSA; BD), Yeast-Starch agar [10] and Bennett's agar [11]. ISCC-NBS colour charts were used to assess colony colour and diffusible pigment [12]. Morphological characteristics of mycelia and spores were observed by light microscopy (BH2; Olympus) and scanning electron microscopy (Quanta 200; FEI) using gold-coated dehydrated specimens of cultures grown on ISP 2 agar at 28 °C for 21 days. For the spore motility test, cells were suspended in 0.1 ml sterile distilled water, and after incubation at 28 °C for 1 h, spore motility was observed under the light microscope. The Gram-staining test was performed as described by Magee *et al.* [13]. The temperature range for growth was determined by incubation of the strain on ISP 2 agar at 4, 15, 20, 25, 28, 30, 37, 42 and 45 °C for 2–4 weeks. The pH range for growth was measured in ISP 2 broth with various pH (pH 4.0–12.0, at intervals of 1.0 pH unit) for 2–4 weeks. For the pH experiments, the different buffers were used as described by Xu *et al.* [14]. Salt tolerance was tested in ISP 2 agar supplemented with 0, 1, 2, 3, 5, 7 and 9 % (w/v) NaCl for 2–4 weeks. Catalase activity was determined by bubble production in 3 % (v/v) H_2O_2. Oxidase activity was assessed by using 1 % (w/v) tetramethyl-*p*-phenylenediamine [15]. Hydrolysis of starch, gelatin, Tweens 20, 40 and 80, production of H_2S, milk coagulation and peptonization were examined as described by Gonzalez *et al.* [16]. Carbon-source utilization was tested using ISP 9 medium [8] as a basal medium, supplemented with a final concentration of 1 % (w/v) of the carbon sources. Other biochemical characteristics and enzyme activities were tested by using the API 20NE, API 50 CH and API ZYM kits (bioMérieux) according to the manufacturer's instructions.

Cells of strain S3Cf-2^T were aerobic and Gram-staining-positive. Strain S3Cf-2^T displayed good growth on ISP2, ISP 3, ISP 4, ISP 5 and ISP 7 agars, R2A agar, TSA, Yeast-Starch agar and Bennett's agar, but poor growth on nutrient agar. The substrate mycelium branched extensively, and the colour of colonies on different media was pale orange-yellow (ISP 2 agar), light yellow (ISP 3 agar/ISP 4 agar/Yeast-Starch agar), greenish-yellow (TSA/R2A agar) and yellowish-white (ISP 5 agar/ISP 7 agar/Bennett's agar). Aerial mycelia were absent on the media tested. Soluble pigment was not produced in any of the media tested. Scanning electron micrographs of a 21-day-old culture of strain S3Cf-2^T grown on ISP 2 agar are shown in Fig. 1. The substrate mycelia (approximately 0.4 µm in diameter) were extensively branched without fragmentation. Aerial hyphae or sporangia were not present. An oval or rod-like (approximately 0.5–0.9×0.8–1.4 µm in size) spore was borne singly at the end of substrate mycelium. The spores had a smooth surface and were non-motile. The strain was capable of growth on ISP 2 agar containing 0–2 % (w/v) NaCl. The temperature and pH range for growth were 20–37 °C and pH 6.0–9.0. Optimum growth occurred at 28–30 °C, pH 7.0 and without

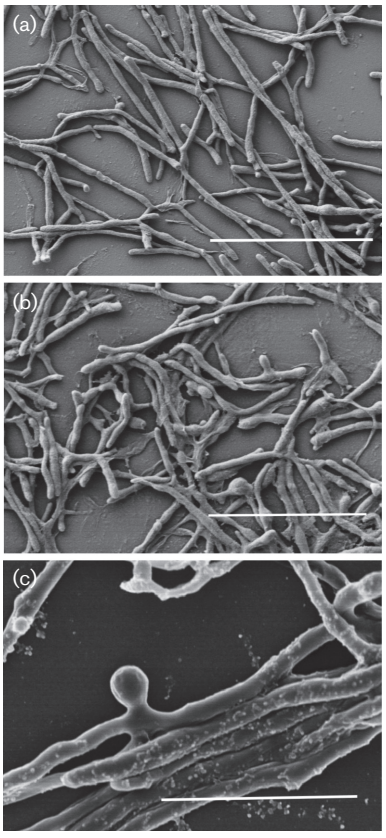

Fig. 1. Scanning electron micrographs of strain S3Cf-2^T grown on ISP 2 agar for 21 days at 28 °C. (a) Non-fragmented substrate mycelium; (b, c) oval or rod-like spore developed on the substrate mycelium. Bars, (a, b) 10 µm; (c) 3 µm.

NaCl. No growth occurred at 15 °C, 42 °C, pH 5.0, pH 10.0 or in the presence of 3 % (w/v) NaCl. Other physiological and biochemical characteristics of strain S3Cf-2^T are given in the species description.

Biomass for the chemotaxonomic studies except fatty acid analysis was obtained from cultures grown in yeast extract-glucose broth [1.0 % (w/v) yeast extract, 1.0 % (w/v) glucose, pH 7.0] on a rotary shaker at 28 °C for 7 days. The polar lipids were extracted and analysed by two-dimensional TLC on silica gel 60 F_{254} plates (Merck) as described by Minnikin *et al.* [17]. The solvent systems of the first and the second dimension were chloroform/methanol/water (64 : 27 : 5, by vol.) and chloroform/methanol/acetic acid/water (80 : 18 : 12 : 5, by vol.), respectively. For analyses of the diagnostic isomers of diaminopimelic acid (DAP) in the whole-cell hydrolysates, cells were hydrolysed with 6M HCl at 120 °C for 12 h, dried in vacuo, and then the hydrolysates were analysed on a cellulose TLC plate (Merck) as described by Hasegawa *et al.* [18]. The solvent system for the ascending chromatography was methanol/pyridine/acetic acid/water (10 : 1 : 0.25 : 5, by vol.). Whole-cell sugars of strain S3Cf-2^T were analysed as described previously [19]. The *N*-acyl type of muramic acid were determined by the method of Uchida *et al.* [20]. The presence of mycolic acids was examined by TLC following Tomiyasu [21]. Menaquinones of strain S3Cf-2^T were extracted according to the method of Collins *et al.* [22], then analysed and confirmed by HPLC coupled with a single quadrupole mass spectrometer LCMS-2020 (Shimadzu) as described by Guo *et al.* [23]. For analysis of whole-cell fatty acids, cell mass of strain S3Cf-2^T was harvested from yeast extract-glucose agar cultivated at 28 °C for 9–10 days, when the bacterial communities reached the late-exponential stage of growth. The whole-cell fatty acids were saponified, methylated and extracted according to the standard protocols described by Sasser [24], and analysed by using an Agilent 7890A gas chromatograph coupled with an Agilent 5975C single quadrupole mass spectrometer equipped with the Nist08 Library software database [25]. For DNA G+C content, genomic DNA of strain S3Cf-2^T was prepared according to the method described by Marmur [26] and the G+C content was determined by reversed-phase HPLC as described by Mesbah *et al.* [27].

The major polar lipids detected in strain S3Cf-2^T were diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol and an unidentified aminolipid (AL). Phosphatidylglycerol, four unidentified phospholipids (PL1–PL4), an unidentified glycolipid (GL) and two unidentified lipids (L1, L2) were also present as minor components (Fig. S1, available in the Supplementary Material). This polar lipid profile corresponded to phospholipid type PII of Lechevalier *et al.* [28], a common pattern among members of the family *Micromonosporaceae*. The whole-cell hydrolysates of strain S3Cf-2^T contained *meso*-DAP as diagnostic diamino acids and glucose, galactose, mannose, arabinose, xylose and ribose as whole-cell sugars, indicating a whole-cell sugar pattern D according to Lechevalier and Lechevalier [29]. The *N*-acyl type of the muramic acid was glycolyl. Mycolic acids were not detected. The major menaquinones were MK-9(H_6) (83.3 %) and MK-9(H_8) (12.2 %), with MK-10(H_6) (2.1 %) and MK-9(H_4) (1.8 %) as minor components. The predominant cellular fatty acids (>10 % of the total) of strain S3Cf-2^T were iso-$C_{16:0}$ (30.8 %), anteiso-$C_{15:0}$ (23.2 %), anteiso-$C_{17:0}$ (15.6 %) and iso-$C_{15:0}$ (12.0 %), with small amounts (>1 %) of $C_{17:1}\omega 9c$ (4.6 %), iso-$C_{14:0}$ (3.0 %), iso-$C_{17:0}$ (3.0 %), $C_{16:0}$ (1.9 %), $C_{17:0}$ (1.6 %), $C_{16:1}\omega 9c$ (1.2 %) and $C_{15:0}$ (1.1 %), indicating fatty acid type 2d as described by Kroppenstedt [30]. The DNA G+C content of strain S3Cf-2^T was 71.4 mol%.

The extraction of genomic DNA from strain S3Cf-2^T and PCR amplification of the 16S rRNA gene were performed as described by Li *et al.* [31]. The purified PCR products were cloned using the pEASY-T1 Cloning kit (TransGen Biotech) according to the manufacturer's instructions, and sequenced by an ABI PRISM 3730XL DNA Analyzer. The 16S rRNA gene sequence similarity values between strain S3Cf-2^T and related species were obtained from the EzTaxon e-server (www.ezbiocloud.net; [32]). Multiple alignments were made using CLUSTAL X [33]. Evolutionary distances were calculated using Kimura's two-parameter model [34]. Phylogenetic trees were reconstructed using neighbour-joining [35], maximum-parsimony [36] and maximum-likelihood [37] methods with MEGA software version 6.0 [38]. The topologies of the phylogenetic trees were evaluated by using the bootstrap method of Felsenstein [39] with 1000 repeats.

The nearly full-length 16S rRNA gene sequence (1484 bp) of strain S3Cf-2^T showed a close relationship with members of family *Micromonosporaceae*. 16S rRNA gene sequence similarity values between strain S3Cf-2^T and the type species of all genera with validly published names belonging to the family *Micromonosporaceae* were 91.55–97.45 %. The recognized species or subspecies showing the highest 16S rRNA gene sequence similarity values to strain S3Cf-2^T were *Couchioplanes caeruleus* subsp. *azureus* DSM 44103^T (97.45 %) and *Krasilnikovia cinnamomea* 3-54(41)^T (97.36 %). Lower sequence similarities (<97.0 %) were found with all other recognized species of the family *Micromonosporaceae*. A neighbour-joining phylogenetic tree (Fig. 2) indicated that the novel isolate fell within the lineage of the family *Micromonosporaceae* and formed a distinct phyletic line, clustered with the some members of the genera *Actinoplanes* (96.46–96.88 % 16S rRNA gene sequence similarity), *Krasilnikovia* (97.36 %), *Couchioplanes* (96.56–97.45 %) and *Pseudosporangium* (96.88 %). This relationship was supported by a bootstrap value of 81 % and was also found in trees obtained by using the maximum-likelihood and maximum-parsimony algorithms (Figs S2 and S3).

Strain S3Cf-2^T should be classified as a member of the family *Micromonosporaceae* on the basis of both the phylogenetic and the chemotaxonomic characteristics, in particular the muramic acid acyl type (*N*-glycolyl), the absence of mycolic acids, the phospholipid type (type II) and the

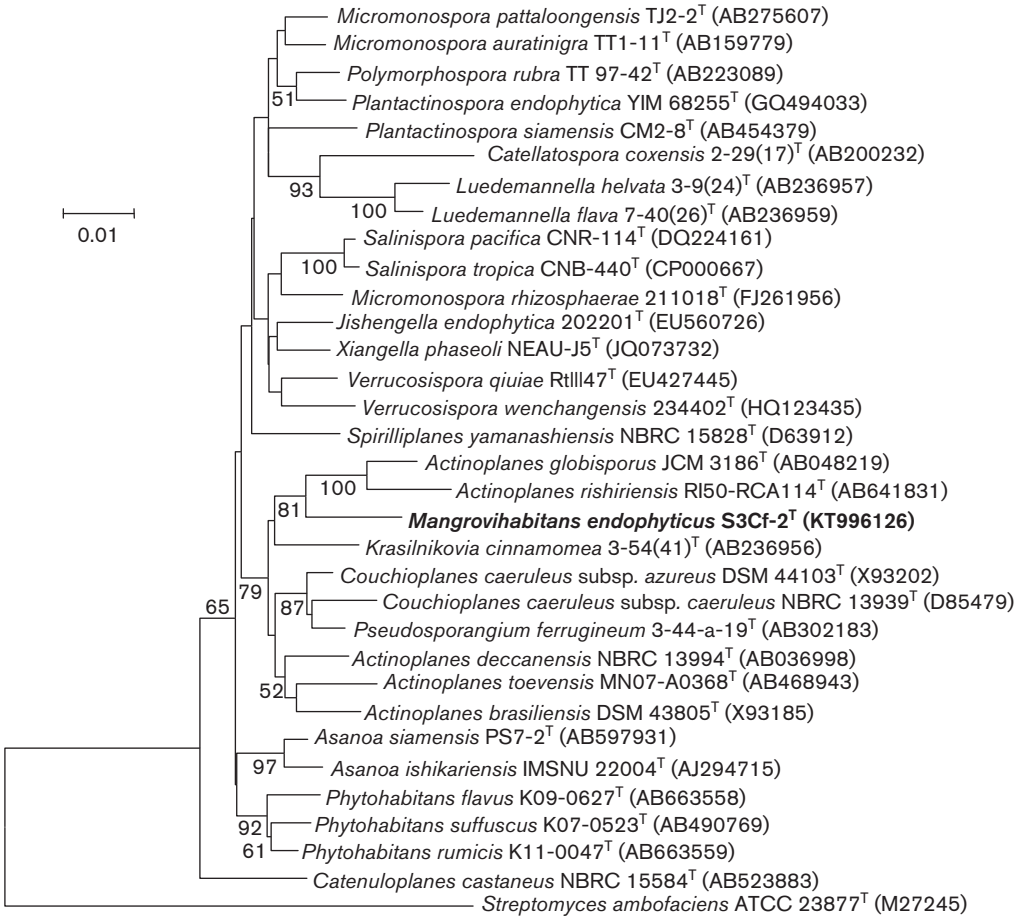

Fig. 2. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationships between strain S3Cf-2^T and the type strains of species of related genera in the family *Micromonosporaceae*. *Streptomyces ambofaciens* ATCC 23877^T was used as an outgroup. Numbers at nodes refer to bootstrap values (based on 1000 replicates); only values >50 % are shown. Bar, 0.01 substitutions per nucleotide position.

fatty acid type (2d). However, the morphological and chemotaxonomic characteristics of strain S3Cf-2^T can be readily distinguished from the established genera of this family (Table 1). Diagnostic morphological features of strain S3Cf-2^T are the production of non-motile spores singly borne at the end of substrate mycelium, and it does not produce aerial hyphae or sporangia. This morphological characteristic is clearly different from members of the genera *Actinoplanes*, *Krasilnikovia*, *Couchioplanes* and *Pseudosporangium*, which are close phylogenetic neighbours clustered with strain S3Cf-2^T on the phylogenetic trees. Species of the genus *Actinoplanes* form characteristic reproductive structures, sporangia, on substrate mycelia, and the spores produced within the sporangia are actively motile by flagella. Species of the genera *Couchioplanes*, *Krasilnikovia* and *Pseudosporangium* form pseudosporangial structures by aggregated spore chains, and spores are motile in the genus *Couchioplanes*. In addition, members of the genus *Couchioplanes*, sharing the highest 16S rRNA gene sequence similarity with strain S3Cf-2^T, are clearly distinguished from the novel isolate by having L-lysine instead of diaminopimelic acid in the cell wall, MK-9(H_4) as the major menaquinone component, 2c fatty acid type, and by forming a deep blue vegetative mycelial pigment [40]. Members of the closest genus on phylogenetic trees, *Actinoplanes*, clearly differ from strain S3Cf-2^T by the major menaquinones [MK-9(H_4) and MK-10(H_4)]. The genus *Pseudosporangium* can also be differentiated from strain S3Cf-2^T by containing 3-OH-DAP in the cell wall. Other genera in the family *Micromonosporaceae* are clearly distinguishable from strain S3Cf-2^T in morphological and chemotaxonomic characteristics (Table 1).

On the basis of the phylogenetic, morphological and chemotaxonomic data presented here, strain S3Cf-2^T is considered to represent a novel species of a new genus within the family *Micromonosporaceae*, for which the name *Mangrovihabitans endophyticus* gen. nov., sp. nov. is proposed.

Table 1. Morphological features and chemotaxonomic characteristics of strain S3Cf-2^T (*Mangrovihabitans* gen. nov.) and recognized genera of the family *Micromonosporaceae*

Data for reference genera were taken from Ørskov [41], Couch [42], Kane [43], Thiemann *et al.* [44], Asano and Kawamoto [45], Yokota *et al.* [46], Rheims *et al.* [47], Kudo *et al.* [48], Tamura *et al.* [40, 49–51], Lee and Hah [52], Matsumoto *et al.* [53, 54], Maldonado *et al.* [55], Thawai *et al.* [56, 57], Ara and Kudo [10, 58], Ara *et al.* [59, 60], Wiese *et al.* [61], Monciardini *et al.* [62], Qin *et al.* [63], Inahashi *et al.* [64], Lee and Lee [65], Xie *et al.* [66], Li *et al.* [67], Wang *et al.* [68] and Mingma *et al.* [69]. +, Present; –, absent; *m*-DAP, *meso*-diaminopimelic acid; Ara, arabinose; Gal, galactose; Glc, glucose; Man, mannose; Rha, rhamnose; Rib, ribose; Xyl, xylose. Fatty acid types are classified according to Kroppenstedt [30] and phospholipid types according to Lechevalier *et al.* [28].

Genus	Single spore	Sporangia	Spore motility	Diamino acid (s)	Whole-cell sugars	Fatty-acid type	Major menaquinone(s)	Phospholipid type
Mangrovihabitans	+	–	–	*m*-DAP	Gal, Glc, Man, Ara, Xyl, Rib	2d	9($H_{6,8}$)	II
Actinoplanes	–	+	+	*m*-DAP	Ara, Xyl	2d	9(H_4), 10(H_4)	II
Krasilnikovia	–	Pseudosporangia	–	*m*-DAP	Gal, Glc, Man, Ara, Xyl, Rib	2d	9($H_{6,4,8}$)	II
Couchioplanes	–	Pseudosporangia	+	L-Lys	Ara, Gal, Xyl	2c	9(H_4)	II
Pseudosporangium	–	Pseudosporangia	–	*m*- and 3-OH-DAP	Ara, Gal, Glc, Man, Xyl, Rib	2d	9(H_6)	II
Actinocatenispora	–	–	–	*m*-DAP	Gal, Glc, Man, Ara, Xyl, Rib	3b	9($H_{4,6}$)	II
Actinorhabdospora	–	–	–	*m*-DAP	Gal, Glu, Man, Rib	3b	10($H_{4,6}$)	II
Allocatelliglobosispora	–	–	–	3-OH-DAP	Glc, Rha, Rib, Xyl, Ara, Gal, Man	3b	10($H_{4,6}$), 9(H_4)	II
Asanoa	–	–	–	*m*-DAP	Ara, Rha, Rib, Xyl, Gal, Man, Glc	2d	10($H_{6,8}$)	II
Catellatospora	–	–	–	*m*- and 3-OH-DAP	Ara, Xyl, Gal	3b	9($H_{4,6}$), 10(H_4)	II
Catelliglobosispora	–	–	–	*m*-DAP	Rha, Rib, Gal, Xyl, Man, Glc	3b	10(H_4)	II
Catenuloplanes	–	–	+	L-Lys	Xyl	2c	9(H_8), 10(H_8)	III
Dactylosporangium	–	+	+	*m*-DAP	Ara, Xyl	3b	9($H_{4,6,8}$)	II
Hamadaea	–	–	–	*m*- and 3-OH-DAP	Xyl, Gal, Man, Rib, Ara, Rha	3b	9(H_6)	II
Jishengella	+	–	–	*m*-DAP	Xyl, Man, Ara, Rib, Glc	3a	9($H_{4,6,8}$)	II
Longispora	–	–	–	*m*-DAP	Ara, Gal, Xyl	2d	10($H_{4,6}$)	II
Luedemannella	–	+	–	*m*-DAP	Gal, Glc, Man, Rha, Rib, Xyl, Ara	2d	9($H_{6,4}$)	II
Micromonospora	+	–	–	*m*-DAP	Ara, Xyl	3b	10($H_{4,6}$), 9($H_{4,6}$)	II
Phytohabitans	–	–	–	*m*-DAP, L-Lys	Gal, Glc, Man, Rib, Xyl	2d	9(H_6), 10($H_{4,6}$)	II
Phytomonospora	+	–	–	*m*-DAP	Gal, Glc, Rib, Man	2d	8(H_2), 9(H_2), 10($H_{2,4,6}$)	III
Pilimelia	–	+	+	*m*-DAP	Ara, Xyl	2d	9($H_{2,4}$)	II
Planosporangium	–	+	+	*m*-DAP	Ara, Xyl	3b	9(H_4), 10(H_4)	II
Plantactinospora	+	–	–	*m*-DAP	Ara, Xyl, Gal, Glc	2d	10($H_{6,8,4}$)	II
Polymorphospora	–	–	–	*m*-DAP	Xyl	2a	9($H_{4,6}$), 10($H_{4,6}$)	II
Rhizocola	–	–	–	3,4-OH-DAP	Gal, Xyl, Man, Rib	2d	9($H_{4,6}$)	II
Rugosimonospora	+	–	–	3-OH-DAP	Ara, Gal, Xyl	2c	9($H_{8,6}$)	II
Salinispora	+	–	–	*m*-DAP	Ara, Gal, Xyl	3a	9(H_4)	II
Spirilliplanes	–	–	+	*m*-DAP	Man, Glc, Xyl, Gal	2d	10(H_4)	II
Verrucosispora	+	–	–	*m*-DAP	Man, Xyl, Rib	2d	9(H_4)	II
Virgisporangium	–	+	+	3-OH-DAP	Gal, Glc, Man, Rha, Xyl	2d	10($H_{4,6,8}$)	II
Xiangella	–	–	–	*m*-DAP	Man, Glc, Gal	3a	9($H_{4,6}$)	III

DESCRIPTION OF *MANGROVIHABITANS* GEN. NOV.

Mangrovihabitans (Man.gro.vi.ha′bi.tans. N.L. n. *mangrovum* mangrove; L. pres. part. *habitans* inhabitant; N.L. masc. n. *Mangrovihabitans* an inhabitant of mangrove).

An aerobic and Gram-staining-positive actinomycete that forms extensively branched substrate mycelia without fragmentation. An oval or rod-like spore is borne singly at the end of substrate mycelium. Spores have a smooth surface and are non-motile. Aerial hyphae or sporangia are not produced. The cell wall contains *meso*-DAP as the diamino acid. Glucose, galactose, mannose, arabinose, xylose and ribose are detected as whole-cell sugars. The acyl type of the cell-wall peptidoglycan is glycolyl. Mycolic acids are absent. The major polar lipids include diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol and an unknown aminolipid, corresponding to phospholipid type PII. Some additional polar lipids may be present. The major menaquinones are MK-9(H_6) and MK-9(H_8). Predominant

cellular fatty acids are iso-$C_{16:0}$, anteiso-$C_{15:0}$, anteiso-$C_{17:0}$ and iso-$C_{15:0}$. The DNA G+C content of the type species is 71.4 mol%.

The type species is *Mangrovihabitans endophyticus*.

DESCRIPTION OF *MANGROVIHABITANS ENDOPHYTICUS* SP. NOV.

Mangrovihabitans endophyticus (en.do.phy′ti.cus. Gr. pref. *endo* within; Gr. n. *phyton* plant; L. masc. suff. *-icus* adjectival suffix used with the sense of belonging to; N.L. masc. adj. *endophyticus* within plant, endophytic, pertaining to the isolation from plant tissues).

General morphological and chemotaxonomic characteristics are as given in the genus description. Displays good growth on ISP 2, ISP 3, ISP 4, ISP 5 and ISP 7 agars, R2A agar, TSA, Yeast-Starch agar and Bennett's agar, but poor growth on nutrient agar. The colour of colonies on different media is light yellow to greenish-yellow. Soluble pigment is not produced on any of the tested media. Growth occurs at 20–37 °C (optimum 28–30 °C), pH 6.0–9.0 (optimum pH 7.0) and in the presence of 0–2 % (w/v) NaCl (optimum 0 %). Positive for catalase but negative for oxidase reaction. Tweens 20, 40, 80, casein and gelatin are hydrolysed, but not starch. Coagulation and peptonization of milk, nitrate reduction and H_2S production are negative. Aesculin degradation is observed. Urease and arginine dihydrolase activities are absent. D-Arabinose, D-galactose, D-glucose, cellobiose, maltose, melibiose, lactose, D-mannose, D-fructose, D-mannitol, raffinose, D-sorbitol, *myo*-inositol, glycerol and sucrose are utilized as sole carbon sources, but dextrin, L-fucose, L-rhamnose, ribose and xylose are not. Acid is produced from methyl α-D-glucoside, *N*-acetyl glucosamine, amygdalin, arbutin, aesculin, salicin, cellobiose, maltose, lactose, starch and D-gentiobiose (API 50CH). Positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase, leucine arylamidase, valine arylamidase, cysteine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, α-glucosidase, β-glucosidase and *N*-acetyl-β-glucosaminidase, but negative for α-chymotrypsin, α-galactosidase, β-glucuronidase, α-mannosidase and α-fucosidase (API ZYM). The major menaquinones are MK-9(H_6) and MK-9(H_8), and small amounts of MK-10 (H_6) and MK-9(H_4) are also present. The main polar lipids include diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol and an unidentified aminolipid. Phosphatidylglycerol, four unidentified phospholipids, an unidentified glycolipid and two unidentified lipids are also present as minor components. The major cellular fatty acids are iso-$C_{16:0}$, anteiso-$C_{15:0}$, anteiso-$C_{17:0}$ and iso-$C_{15:0}$. Other cellular fatty acids detected as minor components are $C_{17:1}\omega9c$, iso-$C_{14:0}$, iso-$C_{17:0}$, $C_{16:0}$, $C_{17:0}$, $C_{16:1}\omega9c$ and $C_{15:0}$.

The type strain, S3Cf-2^T (=DSM 100693^T=CGMCC 4.7299^T), was isolated from a surface-sterilized bark of *Bruguiera sexangula* collected from Dongzhaigang National Nature Reserve in Hainan Province, China. The G+C content of the genomic DNA of the type strain is 71.4 mol%.

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

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