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TAXONOMIC DESCRIPTION

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Chitinophaga extrema sp. nov., isolated from subsurface soil and leaf litter in a tropical peat swamp forest

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Abstract

A Gram-negative, filamentous aerobic bacterium designated as strain Mgbs1^T was isolated on 12 April 2017 from the subsurface soil and leaf litter substrate at the base of a *Koompassia malaccensis* tree in a tropical peat swamp forest in the northern regions of the state of Selangor, Malaysia (3° 39′ 04.7′ N 101° 17′ 43.7″ E). Phylogenetic analyses based on the full 16S rRNA sequence revealed that strain Mgbs1^T belongs to the genus *Chitinophaga* with the greatest sequence similarity to *Chitinophaga terrae* KP01^T (97.65%), *Chitinophaga jiangningensis* DSM27406^T (97.58%), and *Chitinophaga dinghuensis* DHOC24^T (97.17%). The major fatty acids of strain Mgbs1^T (>10%) are iso-$C_{15:0}$, $C_{16:1}$ $\omega5c$ and iso-$C_{17:0}$ 3-OH while the predominant respiratory quinone is menaquinone-7. Strain Mgbs1^T has a complete genome size of 8.03 Mb, with a G+C content of 48.5 mol%. The DNA–DNA hybridization (DDH) score between strain Mgbs1^T and *C. jiangningensis* DSM27406^T was 15.9%, while *in silico* DDH values of strain Mgbs1^T against *C. dinghuensis* DHOC24^T and *C. terrae* KP01^T were 20.0 and 19.10% respectively. Concurrently, Average Nucleotide Identity (ANI) scores between strain Mgbs1^T against all three reference strains are 73.2%. Based on the phenotypic, chemotaxonomic, and phylogenetic consensus, strain Mgbs1^T represents a novel species of the genus *Chitinophaga*, for which the name *Chitinophaga extrema* sp. nov. is proposed (=DSM 108835^T=JCM 33276^T).

BRIEF INTRODUCTION

The genus *Chitinophaga* was originally coined by Sangkhobol and Skerman in 1981 [1] to describe chitinolytic, elongated, filamentous bacteria often isolated from diverse environmental palates. To date, there has been on average, one newly discovered *Chitinophaga* species each year since the first discovery of the type strain *Chitinophaga pinensis* in 1981 [2]. Currently, there have been a total of 31 officially recognized *Chitinophaga* species [3] by 2018, with another six new species alone discovered in 2019. Known strains of *Chitinophaga* were each found from diverse environments including river sediments, rhizospheres, rhizoplanes, vermicomposts, tree bark, plant roots, weathered rocks, sludges, and soils of various conditions [3]. Members of the *Chitinophaga* genus usually possess $C_{16:1}$ $\omega5c$ and $C_{15:0}$ iso as their major fatty acids, menaquinone-7 (MK-7) as their major respiratory quinone, as well as homospermidine as their major polyamine [4].

ISOLATION AND ECOLOGY

Strain Mgbs1^T was isolated from a tropical peat swamp forest (3° 39′ 04.7′ N 101° 17′ 43.7″ E), which is part of the Sungai Karang Forest Reserve located in the northern regions of the state of Selangor, Malaysia. This key reserve in Peninsular Malaysia protects pristine peatlands while acting as a hotspot for Hornbill conservation efforts. The region has a tropical climate with an average annual precipitation and temperature

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Keywords: *Chitinophaga extrema*; Malaysia; Phylogeny; Taxonomy; Tropical Peat Swamp; *Koompassia malaccensis*.
Abbreviations: DDH, DNA–DNA hybridization; EUCAST, European Committee on Antimicrobial Susceptibility Testing; GGDC, Genome-to-Genome Distance Calculator; ME, minimum-evolution; ML, maximum-likelihood; MP, maximum-parsimony; NJ, neighbour-joining; R2A, Reasoner's 2A; UPGMA, unweighted pair group method with arithmetic mean.
The GenBank/EMBL/DDBJ accession numbers for the complete 16S rRNA gene sequence and genome sequence of strain Mgbs1^T are MT363191 and RIAR00000000 respectively.
One supplementary table and eight supplementary figures are available with the online version of the article.

of 2061 mm and 27.2 °C respectively [5]. Strain Mgbs1^T was isolated from a patch of subsurface soil at the base of a *Koompassia malaccensis* tree that was partially submerged in peat water. The site was chosen primarily as *K. malaccensis* is a common emergent species in peat forests, and its presence as a native species indicates a pristine environment [6]. The sampled soil area is littered with newly fallen and partially degraded leaves from the surrounding flora.

The isolate was obtained by means of a modified isolation chip (iChip) based on a design by Nichols *et al.* 2010 [7]. Briefly, approximately 1 g of oxic peat matter was weighed and reconstituted homogeneously in warm M9 minimal agar (0.8% w/v agar; Lab M, UK) to a dilution of approximately 10^3 cells ml^{-1}. Thereafter, the central plate of the iChip was dipped into the molten agar so that each through-hole of the plate receives approximately one bacterium in an agar plug. The iChip assembly was then placed back in the soil of origin, at approximately 20 cm deep within the oxic peat layer for an *in situ* incubation for 2 weeks. Microcolonies within each agar plug were microscopically screened and streaked onto tryptone soy agar (10% w/v) (Oxoid, UK) supplemented with 50 µg ml^{-1} of cycloheximide to prevent fungal contamination. All isolates were then tested for their antimicrobial activity against *Candida albicans* (ATCC 10231), *Staphylococcus aureus* (ATCC 700699), *Enterococcus faecalis* (ATCC 700802), *Acinetobacter baumannii* (ATCC BAA1605), *Escherichia coli* (ATCC 2523), and *Pseudomonas aeruginosa* (ATCC 10145); isolates producing activity against at least five of the six test pathogens have been selected for 16S rRNA sequencing for identification. Isolate Mgbs1^T was selected based on its ability to secrete broad-spectrum antimicrobial compounds while having the lowest 16S rRNA sequence similarity to its most closely related species among all isolates tested. Strain Mgbs1^T was then preserved in 25% (v/v) glycerol solution at −80 °C for long-term storage and use.

16S rRNA PHYLOGENY

The 16S rRNA gene of strain DSM 108835^T was amplified using the universal primers 27 f (5′- AGAGTTTGATCMTGGCTCAG −3′) and 1492 r (5′- TACGGYTACCTTGTTACGACTT −3′) [8]. Briefly, the PCR reaction mixture contains 1X MyTaq Red Buffer, 1.25 U MyTaq DNA polymerase (Bioline, UK), 0.5 µM of 27 f primer, 0.5 µM of 1492 r primer, 5.0 µl of genomic DNA, with sterile H_2O to make up the remaining volume to 50.0 µl. The PCR condition was set to an initial denaturation step at 95 °C for 5 min, followed by 30 cycles of denaturation at 95 °C for 45 s, annealing at 60 °C for 45 s, and elongation at 72 °C for 2 min, followed by a final elongation step at 72 °C for 10 min. The amplicons were then subjected to Sanger sequencing and the resulting reads trimmed and combined to obtain a 1431 nt long sequence.

A complete 16S rRNA gene sequence (1532 nt) was also obtained from the hybrid genome assembly and was found to have 1% nucleotide difference when compared to the 16S rRNA PCR amplicon. The two sequences were then combined to create a full length consensus which was then used to search against all available relevant 16S rRNA sequences in the EzBioCloud (http://eztaxon-e.ezbiocloud.net/) [9] and the NCBI (www.ncbi.nlm.nih.gov) databases. The 16S rRNA sequence has been uploaded to GenBank under the accession MT363191. Thereafter, the 16S rRNA sequence was used to construct the maximum-likelihood (ML) [10], neighbour-joining (NJ) [11], maximum-parsimony (MP) [12], minimum-evolutionary (ME) [13], and UPGMA [14] phylogenetic trees via the MEGA X software [15]. The Kimura's two-parameter model was used to determine evolutionary distances with bootstrap analysis (1000 resamples) to evaluate tree topology.

The 16S rRNA gene sequence analysis indicated that strain Mgbs1^T was most closely related to *Chitinophaga terrae* KP01^T (97.58%), *Chitinophaga jiangningensis* DSM 27406^T (97.58%), *Chitinophaga vietnamensis* VP7442^T (97.31%), and *Chitinophaga dinghuensis* DHOC24^T (97.17%) as well as a sequence similarity of 92.81% to the genus type strain *Chitinophaga pinensis* DSM2588^T. Furthermore, the ML phylogenetic construct confirms that strain Mgbs1^T resides in the genus *Chitinophaga* and forms a cluster with *C. terrae* KP01^T, *C. jiangningensis* DSM27406^T, and *Chitinophaga dinghuensis* DHOC24^T (Fig. 1). Applying the remaining four evolutionary distance models NJ (Fig. S1, available in the online version of this article), MP (Fig. S2), UPGMA (Fig. S3) and ME (Fig. S4) topology construct methods yielded similar relatedness. Collectively, *C. terrae* KP01^T, *C. jiangningensis* DSM27406^T and *C. dinghuensis* DHOC24^T were acquired from the DSMZ collection of microorganisms and were used as reference strains for further phenotypic and biochemical assessments. All reference strains were grown in the same conditions and media as that of strain Mgbs1^T.

GENOME FEATURES

The genomic DNA of strain Mgbs1^T was extracted using the Wizard Genomic DNA Purification (Promega, USA) following the manufacturer's protocol. Thereafter, the obtained DNA was sequenced separately on the Illumina MiSeq (50× coverage) and MinION by Oxford Nanopore Technologies platforms. The raw reads of both sequencing platforms were subjected to hybrid *de novo* genome assembly using Unicycler [16] which yielded seven contigs with a total size of 8.03 Mb, with the largest contig being 7989002 bp long. (Table 1). Notably, contig two was found to be a complete circular plasmid with a size of 49483 bp. The full genomic sequence has been submitted to the NCBI database (www.ncbi.nlm.nih.gov/genome) under the accession RIAR00000000. The genomic DNA has a G+C content of 48.5 mol%.

A Genome-to-Genome Distance Calculator (GGDC, v.2.1) approach [17] was used to assess the intergenomic distances of the closely related species of strain Mgbs1^T via genomic sequences as a step up from 16S rRNA sequence similarities. Briefly, the whole genome assembly of strain Mgbs1^T was compared to the genomic sequences of the closely related strains obtained from the GenBank database. In that regard, strain Mgbs1^T had *in silico* DNA–DNA

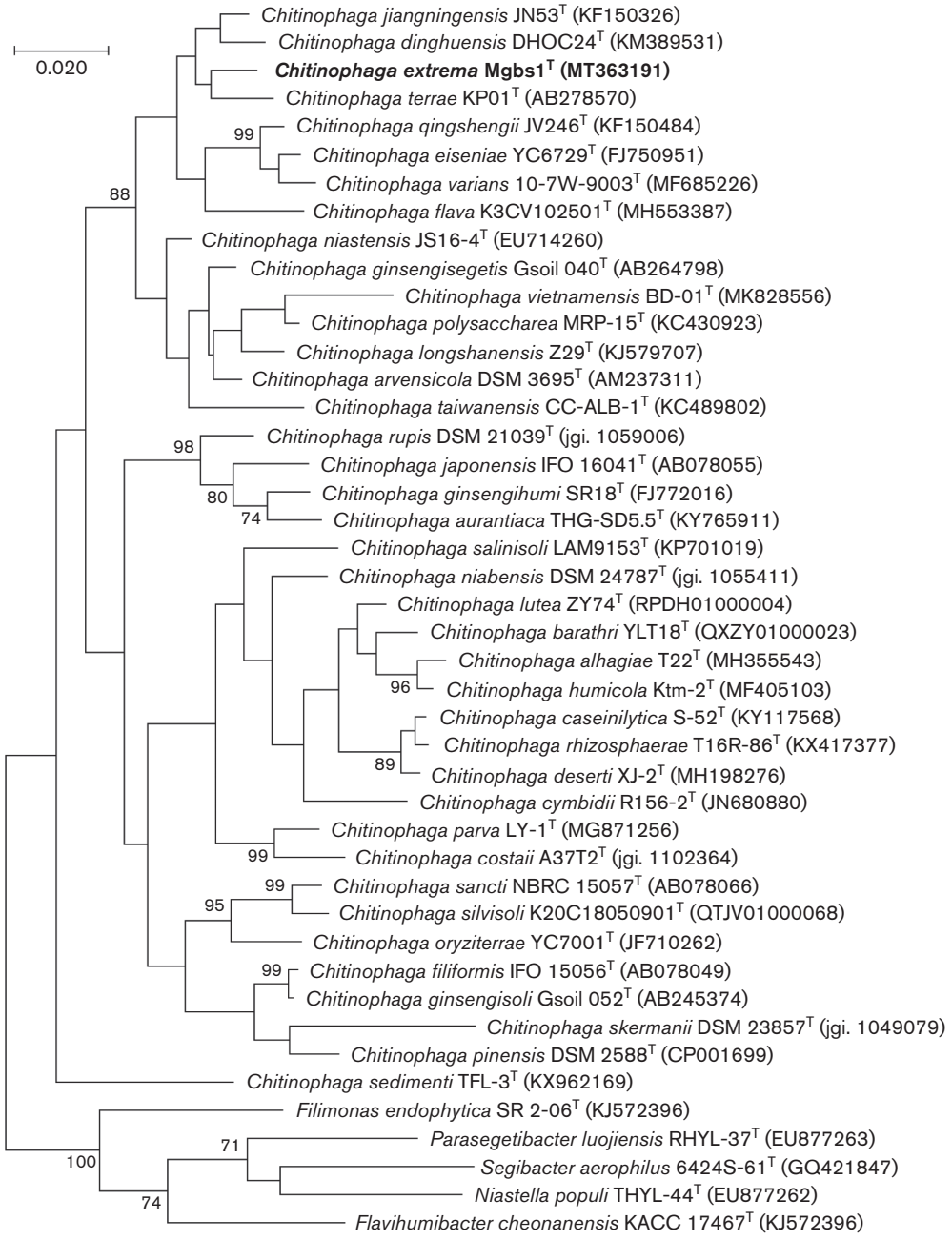

Fig. 1. Maximum-likelihood phylogenetic tree based on 16S rRNA gene sequences of strain Mgbs1^T and its relation to other species of the genus *Chitinophaga* and representatives of other taxa. GenBank accession numbers are shown in parenthesis. All bootstrap values are shown. Bar, 2 nt substitutions per 100 nt.

hybridization (DDH) values of 20.20, 20.10, 20.00, 19.50, 19.10% with *Chitinophaga pinensis* DSM2588^T, *Chitinophaga vietnamensis* VP7442^T, *Chitinophaga dinghuensis* DHOC24^T, *Chitinophaga jiangningensis* DSM 27406^T and *Chitinophaga terrae* KP01^T, respectively. In addition, a traditional DDH assay was also conducted on strain Mgbs1^T against the closely related species *C. jiangningensis* DSM27406^T in consideration of both GGDC-DDH and 16S rRNA similarities. DDH revealed a similarity of 15.90% between the two species, thus indicating that strain Mgbs1^T is separate from *C. jiangningensis* DSM27406^T. Furthermore, the Average Nucleotide Identity (ANI) of strain Mgbs1^T assessed against *Chitinophaga dinghuensis* DHOC24^T, *Chitinophaga jiangningensis* DSM 27406^T and *Chitinophaga terrae* KP01^T are 73.23, 73.17 and 73.23% respectively [18]. DDH values of less than 70% and ANI values of less than 95% are indicative of a strain belonging to a new species [17, 19].

Table 1. Differential phenotypic characteristic comparisons of strain Mgbs1^T against its most phylogenetically related species of *Chitinophaga*

Strains: 1, Mgbs1^T; 2, *C. dinghuensis* DSM 29821^T; 3, *C. jiangningensis* DSM 27406^T; 4, *C. terrae* DSM 23920^T. All listed data were obtained in the present study. Data indicated within parentheses were taken from the appropriate literature [28–30]. +, Positive; w, weakly positive; –, negative; ND, data not available.

Characteristics	1	2	3	4
Cell Length (μm)	8.5–10.7	(0.5–30.0)	(2.3–2.6)	(0.6–0.8)
Catalase	+	(+)	(+)	(+)
Oxidase	+	(–)	(+)	(+)
Urease	+	+	+	–
Temperature range for growth (°C)	15–40	(10–37)	(15–42)	(15–42)
Growth at 37 °C	+	(+)	(+)	(+)
Optimal temperature for growth (°C)	28–32	(28–33)	(28.0)	NA
pH range for growth	3.0–8.5	(5.5–8.5)	(5.0–10.0)	(6.0–9.0)
Optimal pH for growth	3.5–7.0	(6.5–7.5)	(7.0)	NA
Max. NaCl for growth (% w/v)	2.5	2.5	6.0	2.5
Optimal NaCl for growth (% w/v)	1.0–1.5	(0.0–0.5)	NA	NA
Growth on Reasoner's 2A agar	+	(+)	(+)	(+)
Assimilation of (API 20 NE and BIOLOG III):				
Carbon Sources:				
D-Cellubiose	+	w	w	+
Dextrin	–	–	w	w
D-Fructose	w	–	–	+
D-Galactose	+	+	w	+
D-Melibioise	w	–	–	+
Raffinose	–	–	–	w
D-Salicin	+	–	w	+
Trehalose	+	–	–	+
Turanose	w	–	–	+
Gentiobiose	+	w	+	+
Glycerol	+	–	–	–
L-Arabinose	–	–	+	+
L-Fucose	–	–	w	+
L-Rhamnose	+	–	–	+
N-Acetyl-D-Galactosamine	+	w	–	w
Pectin	–	–	–	+
Stachyose	–	–	–	w
Sucrose	w	–	–	+
α-Lactose	w	–	–	+
β-Methyl-D-Glucoside	w	–	–	+
Amino Acids:				

Continued

Table 1. Continued

Characteristics	1	2	3	4
D-Serine	–	–	w	–
Gelatin	+	+	w	w
Glycyl-L-Proline	+	w	w	–
L-Aspartic Acid	+	–	w	–
L-Glutamic Acid	+	–	w	–
L-Histidine	w	w	–	–
L-Serine	w	–	–	–
Acids:				
D-Galacturonic Acid	w	–	w	–
L-Galactonic Acid Lactone	w	–	–	–
Acetoacetic Acid	–	–	w	–
Propionic Acid	+	–	–	–
Acetic Acid	+	–	–	–
Sodium butyrate (butyric acid)	–	–	w	–
Growth in the presence of (BIOLOG III):				
1% Sodium Lactate	+	+	w	w
Guanidine HCl	–	w	+	–
Tetrazolium Violet	+	w	w	w
Nalidixic Acid	+	–	–	+
Enzymatic activity (API 20 NE and API ZYM):				
Cystine-Arylamidase	w	w	w	+
Trypsin	+	–	–	+
Chymotrypsin	–	–	–	w
α-Galactosidase	–	w	–	+
β-Galactosidase	+	+	w	+
α-Mannosidase	–	+	w	+
α-Fucosidase	–	+	w	+
Arginin dihydrolase	–	–	+	–
Urease	w	+	+	–
Protease	+	+	+	–
Genomic Overview:				
Genome size (Mb)	8.03	7.12*	7.18*	6.63*
Number of contigs	7	26*	25	70
Contig N_{50} (bp)	7989002	747558*	635715	183407
DNA G+C content (mol%)	48.5	44.7*	47.4*	45.8*

*Data retrieved from the NCBI database (www.ncbi.nlm.nih.gov/genome) under the following genomic ID: 70222, *C. dinghuensis* DHOC24^T; 50668, *C. jiangningensis* DSM 27406^T; 49654, *C. terrae* KP01^T.

PHYSIOLOGY AND CHEMOTAXONOMY

Phenotypic assessments of strain Mgbs1^T were performed under the same conditions in which all cells were incubated on either nutrient agar (NA) (Oxoid, UK) or Reasoner's 2A (R2A) (DSMZ Media 830; 0.50 g yeast extract, 0.50 g protease peptone (Difco No. 3), 0.50 g casamino acids, 0.50 g glucose, 0.50 g soluble starch, 0.30 g sodium pyruvate, 0.30 g K_2HPO_4, 0.05 g $MgSO_4 \bullet 7H_2O$, 15 g bacteriological agar, 1.0 l distilled water, pH 7.2). Cellular morphology was observed using cells incubated at 30 °C on R2A agar after 3 days. Growth was also assessed on additional media including horse blood agar (HBA) (Oxoid, UK), tryptic soy agar (TSA) (Oxoid, UK), bromocresol lactose agar (BLA) (HiMedia, IN), xylan agar (XA) [20], pectin agar (PA) (HiMedia, IN), MacConkey agar (Oxoid, UK) and colloidal chitin agar (CCA; DSMZ Media 1681).

For light microscopy observations (Motic BA200 Binocular Microscope, HK), Gram-staining was first performed using a Gram-stain kit (bioMérieux) according to the manufacturer's suggested protocol. For cells observed using field emission scanning electron microscopy (FESEM; Hitachi SU8010, JP), several colonies were first obtained and washed in phosphate-buffered saline (Oxoid, UK). Thereafter, a bacterial smear was prepared on a coverslip which was fixated in filtered (0.22 um) 2.5% gluteraldehyde (Merck, US) for 18 h followed by dehydration with a gradual increasing gradient of ethanol (Fisher Scientific, US) over 1 h before drying under a laminar flow. The specimen was then sputter coated (Quorum Q150R S, UK) with platinum before viewing.

The growth characteristics of strain Mgbs1^T were tested on nutrient agar or broth (Oxoid, UK). Growth temperature was tested on nutrient agar at varying temperatures (10, 15, 20, 25, 30, 35, 37, 40, 45 °C) over 5 days. A pH range of growth was tested in nutrient broth from pH 2.0 to pH 11.0 at 0.5 intervals at the optimal growth temperature of 30 °C over 5 days. Salt tolerance was assessed in nutrient broth within a range of 0.0–3.0% (w/v) NaCl with 0.5% intervals, at 30 °C over 5 days. Oxidase activity was tested using BactiDrop oxidase reagent (Thermo Scientific, UK) while catalase activity was tested using 3% (v/v) hydrogen peroxide (Merck, US). Briefly, these reagents were dispensed separately onto two sets of three separate colonies. Motility was tested using SIM media (Oxoid, UK) and via the hanging drop method using nutrient broth. Flexirubin-type pigments were tested by a rapid colour change of the colony from yellow to red in aqueous 20% KOH solution [21]. Other biochemical and enzymatic traits were determined using the API 20NE and API ZYM systems (bioMérieux) according to the manufacturers' instructions. Utilization of various carbon sources were assessed using Biolog Gen III MicroPlates system. All test systems were carried out in accordance with the manufacturer's protocol and incubated at 30 °C.

The sensitivity of strain Mgbs1^T towards antibiotics was evaluated using antibiotic impregnated discs (Oxoid, UK) containing ciprofloxacin (5 µg), tetracycline (30 µg), gentamicin (120 µg), streptomycin (300 µg), amikacin (30 µg), linezolid (30 µg), cefepime (30 µg), cefotaxime (30 µg), cefazolin (30 µg), tazobactam/piperacillin (11 µg), ampicillin-sulbactam (10–10 µg), imipenem (10 µg), vancomycin (30 µg), teicoplanin (30 µg), erythromycin (15 µg), sulfamethoxazole-trimethoprim (23.75–1.25 µg), chloramphenicol (30 µg), colistin (10 µg), polymyxin B (300 µg), and nitrofurantoin (300 µg). The antibiotic sensitivity test was performed in accordance to EUCAST standards [22]. Strain Mgbs1^T exhibited varying degrees of susceptibility only to streptomycin, linezolid, chloramphenicol, and sulfamethoxazole-trimethoprim (Table S1). However, all other tested antibiotics had no effect on strain Mgbs1^T. There are currently no established antibiotic sensitivity breakpoints available for *Chitinophaga* spp. in any widely accepted standards of antibiotic testing. Therefore, the antibiotic susceptibility breakpoints of *Enterobacterales* by EUCAST recommendations was used as an approximate comparator for strain Mgbs1^T.

Cellular fatty acid fingerprinting was carried out on the cell mass of strain Mgbs1^T and its closely related species *C. terrae* KP01^T, *C. jiangningensis* DSM27406^T and *C. dinghuensis* DHOC24^T that were grown in nutrient broth at 30 °C, over 3 days and shaken at 150 r.p.m. The grown cultures were then harvested, washed, and freeze dried prior to analyses. The dry cell mass was then saponified, methylated, and the fatty acids extracted in accordance to the methods of Miller *et al.* [23] and Kuykendall *et al.* [24]. The fatty acid methyl esters (FAMES) were then separated by gas chromatography (Agilent, 6890 N, US), detected by a flame ionisation detector and subsequently identified via using the Sherlock Microbial Identification System (version 6.1; MIDI, US). The complete fatty acid composition of strain Mgbs1^T in comparison to its closely related reference strains are provided in Table 2. The major cellular fatty acids of strain Mgbs1^T were iso-$C_{15:0}$ (38.31%), $C_{16:1}5c$ (28.82%), and iso-$C_{17:0}$ 3-OH (11.16%), which was similar to the reference strains tested.

The analysis of strain Mgbs1^T respiratory quinones were carried out by the Identification Service and Dr. Brian Tindall, DSMZ, Germany. Briefly, respiratory quinones from the dry cell mass was extracted using hexane, followed by silica-based solid phase extraction [25, 26]. The purified quinones were separated by high performance liquid chromatography (HPLC) using a reverse phase column followed by analysis by methods of Tindall [23]. The only respiratory quinone identified in strain Mgbs1^T was menaquinone-7, which is in line to that of other *Chitinophaga* species.

The polar lipids of strain Mgbs1^T were analysed by the methods of Bligh & Dyer [27] with minor modifications. Briefly, the polar lipids were extracted from the dry cell mass using a chloroform: methanol: 0.3% aqueous NaCl mixture. The polar lipids were then obtained from the chloroform phase and purified by the methods of Tindall [23] on 2D silica gel thin layer chromatography. Total lipid content and specific functional groups were detected with varying reagent sprays. Briefly, the total lipid content was detected using molybdatophosphoric acid followed by heating at 150 °C for 10 min. Aminolipids were detected using a 0.2% (w/v) solution of ninhydrin in

Table 2. Cellular fatty acid profiles of strain Mgbs1^T and closely related species of the genus *Chitinophaga*.

Strains: 1, Mgbs1^T; 2, *C. dinghuensis* DSM 29821^T; 3, *C. jiangningensis* DSM 27406^T; 4, *C. terrae* DSM 23920^T. All listed data were obtained in the present study. Data indicated within parentheses were taken from the appropriate literature [28–30]. Fatty acid analyses are presented in % and were conducted in duplicates. N.A., data not available; –, not detected or trace detection of <1.0%.

Fatty acid	1	2	3	4
Unbranched chains				
$C_{13:1}$	N.A.	N.A.	(0.6)	N.A.
$C_{14:0}$	1.3	–	–	–
$C_{14:0}$ 2-OH	–	N.A.	(0.2)	N.A.
$C_{15:0}$	–	N.A.	–	–
$C_{15:0}$ 2-OH	–	N.A.	(0.2)	N.A.
$C_{16:0}$	2.3	3.2	3.8	2.3
$C_{16:0}$ 2-OH	–	N.A.	–	N.A.
$C_{16:0}$ 3-OH	2.8	2.2	1.8	1.5
$C_{17:0}$	–	N.A.	(0.1)	N.A.
$C_{17:0}$ 2-OH	–	–	(0.1)	N.A.
$C_{18:0}$	–	N.A.	N.A.	N.A.
$C_{18:0}$ 3-OH	–	N.A.	–	N.A.
$C_{18:1}$ 2-OH	–	N.A.	(0.6)	N.A.
Branched chains				
Iso-$C_{15:0}$	38.3	42.2	35.4	42.9
Anteiso-$C_{15:0}$	–	–	(0.1)	N.A.
Iso-$C_{15:0}$ 3-OH	4.4	3.1	3.0	3.0
Iso-$C_{16:0}$	–	–	–	–
Iso-$C_{16:0}$ 3-OH	–	(0.7)	–	–
$C_{16:1}5c$	28.8	30.9	34.9	27.7
$C_{16:1}11c$	–	1.5	–	1.6
Iso-$C_{17:0}$	–	–	–	1.2
Anteiso-$C_{17:0}$	–	N.A.	(0.1)	N.A.
Iso-$C_{17:0}$ 3-OH	11.2	10.6	11.2	14.0
$C_{17:1}c$	–	N.A.	(0.2)	N.A.
$C_{17:1}c$	–	N.A.	(0.1)	N.A.
$C_{18:1}9c$	–	N.A.	N.A.	N.A.
Summed features*				
2	–	–	–	–
3	7.2	2.6	4.8	2.1
4	–	–	–	1.4
8	–	N.A.	(0.6)	N.A.
Unidentified fatty acids†				
ECL 13.565	–	–	–	(3.2)
ECL 14.959	–	–	N.A.	N.A.
ECL 16.582	–	1.0	–	(1.1)

*Summed features are groups of two or three fatty acids that are treated together for the purpose of evaluation in the MIDI system and include both peaks with discrete ECLs as well as those where the ECLs are not reported separately. Summed feature 2 includes $C_{12:0}$ aldehyde; Summed feature 3 includes $C_{16:1}7c$ and/or iso-C15:0 2-OH; Summed feature 4 includes iso-$C_{17:1}$ iso I and/or Anteiso B; Summed feature 8 includes $C_{18:1}$ $\omega 6c$ and or $C_{16:1}$ $\omega 7c$.
†ECL, equivalent chain length.

butanol saturated with water followed by heating at 105 °C for 10 min. The Zinzadze reagent of Dittmer and Lester was used for the detection of phospholipids while glycolipids were detected with 1-naphthol reagent followed by heating at 100 °C for 3 min. The polar lipid profile of strain Mgbs1^T contains phosphatidylethanolamine as the major component followed by several other unidentified lipids, glycolipids, and aminolipids (Fig. S5). Strain Mgbs1^T shares the same major polar lipid with most members of the genus *Chitinophaga*.

Based on the consensus of the genotypic, phylogenic, phenotypic and biochemical analyses, strain Mgbs1^T represents a novel species of the genus *Chitinophaga*, for which the name *Chitinophaga extrema* is proposed.

PROTOLOGUE

Chitinophaga extrema [ex.tre'ma., L. fem. adj. *extrema* extreme, pertaining to the ability of the type strain to persist within a harsh ecological niche of a tropical peat swamp forest where *Chitinophaga* is not normally found].

Bacterial cells of strain Mgbs1^T stain Gram-negative, are rod-shaped (3.5–3.6×8.5–10.7 µm), strictly aerobic, non-motile and non-sporulating (Table 1, Fig. S6). Colonies grown on TSA, NA and R2A agars appear similar and are small (1.0–4.0 mm) sticky, irregularly shaped, have raised elevation, slightly wavy margins and with a characteristic bright yellow hue (Fig. S7); furthermore, strain Mgbs1^T colonies are also beta haemolytic and are larger in diameter (4.0 mm to 7.0 mm) on HBA. However, colonies grown in BLA do not cause a significant media colour change, indicating weak lactose utilization. Colonies did not produce clearing zones in both XA and PA agars as well, indicating an unexpected negative utilization of xylan and pectin which are commonly found in soils saturated with vegetation (Fig. S7). Notably, MacConkey and CCA media do not support the growth of strain Mgbs1^T.

Cells of strain Mgbs1^T are oxidase- and catalase-positive, and positive for the production of flexirubin-type pigments. Cell

growth can be observed at temperatures 15–40 °C (optimum, 30 °C), pH 3.0 to 8.5, (optimum, pH 3.5 to 7.0), and NaCl concentrations of 0.0–2.5% (w/v; Table 1).

The major fatty acids (>10%) of strain Mgbs1^T are iso-$C_{15:0}$, $C_{16:1}$ ω5c, Iso-$C_{17:0}$ 3-OH. The major respiratory quinone is menaquinone-7 while the major polar lipid of strain Mgbs1^T is phosphatidylethanolamine. In terms of carbon sources, strain Mgbs1^T is positive for the assimilation of D-cellulobiose, D-galactose, D-glucose, maltose, D-mannose, D-salicin, trehalose, gentiobiose, glycerol, L-rhamnose, N-acetylgalactosamine, N-acetyleglucosamine, and α-lactose (Table 1). Strain Mgbs1^T also assimilates gelatin, proline, aspartic acid, glutamic acid, propionic acid and acetic acid. In terms of enzymatic activity, strain Mgbs1^T possesses acid and alkaline phosphatase, trypsin, phosphohydrolase, β-galactosidase, α-glucosidase, β-glucosidase, β-glucosaminidase, protease, as well as leucine, valine and cystine arylamidases. Strain Mgbs1^T is resistant to ciprofloxacin, tetracycline, gentamicin, amikacin, imipenem, vancomycin, teicoplanin, erythromycin, colistin, polymyxin B, nitrofurantoin, as well as to all tested penicillins and cephalosporins; however, strain Mgbs1^T exhibits some degree of susceptibility against streptomycin, linezolid, sulfamethoxazole-trimethoprim, chloramphenicol (Table S1) and nalidixic acid (Table 1, Biolog Gen III).

The type strain, Mgbs1^T (DSM 108835^T=JCM 33276^T), was isolated from a tropical peat swamp in the Northern regions of the state of Selangor, Malaysia (3° 39′ 04.7′ N 101° 17′ 43.7″ E). Strain Mgbs1^T has a genomic size of 8.03 Mb with a G+C content of 48.5 mol%. The GenBank accession numbers of the full 16S rRNA gene sequence and complete genome assembly of strain Mgbs1^T are MT363191 and RIAR00000000, respectively.

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Author contributions
J. B. L. T., S. M. L. and P. P., were part of the supervisory team for the present study, while JBLT and SML managed the execution and coordination of the study. J. B. L. T., S. M. L., P. P. and C. B. S. G., conceptualized and formulated the goals of the study. C. B. S. G., carried out the investigatory work, with C. M. Y., contributing in the sampling aspect of the investigation. Formal analysis and validation on the genomic data of the strain was carried out by S. P., H. R., S. L., L. C. and C. B. S. G. The writing of the original draft was done by C. B. S. G., while S. P., L. W. W., P. P. and J. B. L. T. were involved in the review and editing of the manuscript. L. W. W., S. M. L. and J. B. L. T., validated the overall findings of the present study. The acquisition of study funds was carried out by C. B. S. G., S. M. L. and J. B. L. T.

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

Ethical statement
No ethical approval is required for the present study publication.

References

1. Sangkhobol V, Skerman VBD. Chitinophaga, a new genus of chitinolytic myxobacteria. *Int J Syst Bacteriol* 1981;31:285–293.
2. Glavina Del Rio T, Abt B, Spring S, Lapidus A, Nolan M *et al*. Complete genome sequence of Chitinophaga pinensis type strain (UQM 2034). *Stand Genomic Sci* 2010;2:87–95.
3. Parte AC. LPSN--list of prokaryotic names with standing in nomenclature. *Nucleic Acids Res* 2014;42:D613–D616.
4. Whitman WB, Bergey's Manual Trust. *Bergey's Manual of Systematics of Archaea and Bacteria*, 2015. Wiley; 2015. In press.
5. Sungai Besar climate. Sungai Besar climate: Average Temperature, weather by month, Sungai Besar weather averages - Climate-Data.org. https://en.climate-data.org/asia/malaysia/selangor/sungai-besar-765916/ [accessed accessed 20 October 2019].
6. Page SE, Rieley JO, Shotyk ØW, Weiss D. Interdependence of peat and vegetation in a tropical peat swamp forest. *Philos Trans R Soc Lond B Biol Sci* 1999;354:1885–1897.
7. Nichols D, Cahoon N, Trakhtenberg EM, Pham L, Mehta A *et al*. Use of ichip for high-throughput in situ cultivation of "uncultivable" microbial species. *Appl Environ Microbiol* 2010;76:2445–2450.
8. Monciardini P, Sosio M, Cavaletti L, Chiocchini C, Donadio S. New PCR primers for the selective amplification of 16S rDNA from different groups of actinomycetes. *FEMS Microbiol Ecol* 2002;42:419–429.
9. Yoon SH, Ha S-M, 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.
10. Huelsenbeck JP, Crandall KA. Phylogeny estimation and hypothesis testing using maximum likelihood. *Annu Rev Ecol Syst* 1997;28:437–466.
11. Bruno WJ, Socci ND, Halpern AL. Weighted neighbor joining: a likelihood-based approach to distance-based phylogeny reconstruction. *Mol Biol Evol* 2000;17:189–197.
12. Sourdis J, Nei M. Relative efficiencies of the maximum parsimony and distance-matrix methods in obtaining the correct phylogenetic tree. *Mol Biol Evol* 1988;5:298–311.
13. Rzhetsky A, Nei M. Theoretical foundation of the minimum-evolution method of phylogenetic inference. *Mol Biol Evol* 1993;10:1073–1095.
14. Gronau I, Moran S. Optimal implementations of UPGMA and other common clustering algorithms. *Inf Process Lett* 2007;104:205–210.
15. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol Biol Evol* 2018;35:1547–1549.
16. Wick RR, Judd LM, Gorrie CL, Holt KE. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. *PLoS Comput Biol* 2017;13:e1005595.
17. Meier-Kolthoff JP, Auch AF, Klenk HP, Göker M. Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics* 2013;14:60–14.
18. Yoon SH, Ha S-M, Lim J, Kwon S, Chun J. A large-scale evaluation of algorithms to calculate average nucleotide identity. *Antonie van Leeuwenhoek* 2017;110:1281–1286.
19. Kim M, H-S O, Park S-C, Chun J. Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes.
20. Kalim B, Ali NM. Optimization of fermentation media and growth conditions for microbial xylanase production. *3 Biotech* 2016;6:122.
21. Güde H. Occurrence of cytophagas in sewage plants. *Appl Environ Microbiol* 1980;39:756–763.
22. Matuschek E, Brown DFJ, Kahlmeter G. Development of the EUCAST disk diffusion antimicrobial susceptibility testing method

and its implementation in routine microbiology laboratories. *Clin Microbiol Infect* 2014;20:O255–O266.

23. Miller LT. Single derivatization method for routine analysis of bacterial whole-cell fatty acid methyl esters, including hydroxy acids. *J Clin Microbiol* 1982;16:584–586.
24. Kuykendall LD, Roy MA, O'neill JJ, Devine TE. Fatty acids, antibiotic resistance, and deoxyribonucleic acid homology groups of *Bradyrhizobiurn japonicum* 1988.
25. Tindall BJ. A comparative study of the lipid composition of Halobacterium saccharovorum from various sources. *Syst Appl Microbiol* 1990;13:128–130.
26. Tindall BJ. Lipid composition of *Halobacterium lacusprofundi* 1990.
27. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* 1959;37:911–917.
28. Kim MK, Jung HY. *Chitinophaga terrae* sp. nov., isolated from soil. *Int J Syst Evol Microbiol* 2007;57:1721–1724.
29. Lv YY, Wang J, You J, Qiu LH. *Chitinophaga dinghuensis* sp. nov, isolated from soil. *Int J Syst Evol Microbiol* 2015;65:4816–4822.
30. Wang Q, Cheng C, He LY, Huang Z, Sheng XF. *Chitinophaga jiangningensis* sp. nov., a mineral-weathering bacterium. *Int J Syst Evol Microbiol* 2014;64:260–265.