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Geodermatophilus chilensis sp. nov., from soil of the Yungay core-region of the Atacama Desert, Chile

Running title: *Geodermatophilus chilensis* sp. nov.

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The GenBank accession numbers for the 16S rRNA gene sequences of strains B12^T, B20 and B25 are KX943328, KX943329 and KX943330, respectively. The GenBank accession number for the draft genome sequence of strain B12^T is NVPT00000000.

Abstract

A polyphasic study was undertaken to establish the taxonomic status of three representative *Geodermatophilus* strains isolated from an extreme hyper-arid Atacama Desert soil. The strains, isolates B12^T, B20 and B25, were found to have chemotaxonomic and morphological

properties characteristic of the genus *Geodermatophilus*. The isolates shared a broad range of chemotaxonomic, cultural and physiological features, formed a well-supported branch in the *Geodermatophilus* 16S rRNA gene tree in which they were most closely associated with the type strain of *Geodermatophilus obscurus.* They were distinguished from the latter by BOX-PCR fingerprint patterns and by chemotaxonomic and other phenotypic properties. Average nucleotide identity, average amino acid identity and digital DNA-DNA hybridization values between the whole genome sequences of isolate B12^T and *G. obscurus* DSM 43160^T were 89.28%, 87.27% and 37.4%, respectively, metrics consistent with its classification as a separate species. On the basis of these data, it is proposed that the isolates be assigned to the genus *Geodermatophilus* as *Geodermatophilus chilensis* sp. nov. with isolate B12^T (CECT 9483^T= NCIMB 15089^T) as the type strain. Analysis of the whole genome sequence of *G. chilensis* B12^T with 5,341 open reading frames and a genome size of 5.5 Mb highlighted genes and gene clusters that encode for properties relevant to its adaptation to extreme environmental conditions prevalent in extreme hyper-arid Atacama Desert soils.

Keywords: *Geodermatophilus chilensis,* polyphasic taxonomy, Atacama Desert, whole-genome sequences.

Introduction

The genus *Geodermatophilus* [1] together with the genera *Blastococcus* [2], *Cumulibacter* [3], *Klenkia* [4] and *Modestobacter* [5] form the family *Geodermatophilaceae* [6, 7] of the order *Geodermatophilales* [8] which belongs to the class *Actinobacteria* [9]. Geodermatophili are recognised by using a combination of chemotaxonomic, morphological and physiological properties [10]. They are aerobic, Gram-stain positive actinobacteria which produce rudimentary substrate hyphae that develop into multilocular vesicles which release motile or non-motile propagules; have *meso*-diaminopimelic acid (*meso*-A_2pm) as the diamino acid of the peptidoglycan, fatty acids rich in *iso*-branched components, tetrahydrogenated menaquinones with nine isoprene units (MK-9[H_4]) as the major isoprenologue, glucose, galactose, mannose and ribose as diagnostic sugars; complex polar lipid patterns that generally contain diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, phosphatidylinositol and usually phosphatidylglycerol and a genomic DNA G+C content within the range 74.0–76.0 mol% [4, 11, 12].

At the time of writing, the genus *Geodermatophilus* encompasses 18 validly published species that form a well-supported clade in the *Geodermatophilaceae* 16S rRNA gene tree [11-13] and which can be distinguished using a broad range of phenotypic properties [11, 12, 14]. Nearly all of these taxa have been recognised within the last five years, as exemplified by *Geodermatophilus amargosae* [13], *Geodermatophilus aquaeductus* [15], *Geodermatophilus bullaregiensis* [11], *Geodermatophilus nigrescens* [16], *Geodermatophilus sabuli* [17] and *Geodermatophilus pulveris* [12]. Despite the recent surge in the number of *Geodermatophilus* species the genus remains underspeciated [18-20]. *Geodermatophilus* strains tend to be associated with soils, notably desert soils [1, 16, 21-30] though there is evidence that they are more widely distributed in the environment [15, 30, 31]. Geodermatophili tend to be resistant to adverse environmental conditions such as desiccation, ionizing radiation and UV light [11, 12, 15, 30, 32]. Analyses of the genome [33] and the proteome [34] of the type strain of *Geodermatophilus obscurus*, the type species of the genus, provide an insight into how geodermatophili adapt to extreme environmental conditions, relevant factors are considered to include pigmentation, catalase production and DNA repair mechanisms.

The present study was undertaken to establish the taxonomic provenance of three representative *Geodermatophilus* strains isolated from an extreme hyper-arid Atacama Desert soil. The isolates, strains B12^T, B20 and B25, were compared with the type strains of *Geodermatophilus* species using genotypic and phenotypic properties known to be of value in the delineation of species of *Geodermatophilus* [11, 12, 25] and found to form a novel species. The name proposed for this species is *Geodermatophilus chilensis* with isolate B12^T as the type strain. Analysis of the whole genome sequence generated for this strain gave an insight into how such strains may have adapted to the harsh environmental conditions prevalent in extreme hyper-arid Atacama Desert soils.

Materials and methods

Isolation of strains

Geodermatophilus strains were isolated from an extreme hyper-arid soil sample collected by one of us (ATB) in November 2010 from the Yungay core-region of the Atacama Desert (24° 06’18.6’’S/ 70° 01° 55.6W) at 1002 m above sea level. One gram of the soil sample was suspended in 4.5 ml of ¼ strength Ringer’s solution (Oxoid), shaken on a tumble shaker,

heated at 55°C for 6 minutes prior to preparation of 10^{-2} and 10^{-3} dilutions in Ringer's solution. Aliquots (100µl) of each of the tenfold dilutions were spread, in triplicate, over *Geodermatophilus obscurus* agar [35] supplemented with nalidixic acid (10µg/ml) and cycloheximide and nystatin (each at 25µg/ml). The isolation plates had been dried for 15 minutes at room temperature before incubation, as recommended by Vickers and Williams [36]. After incubation at 28°C for 2 weeks presumptive *Geodermatophilus* isolates were counted and expressed as the number of colony forming units (cfu) per gram dry weight soil.

Test strains: maintenance and cultural conditions

Three representative *Geodermatophilus* strains, isolates B12^T, B20 and B25, were taken from the isolation plates and together with the type strain of *G. obscurus* DSM 43160^T maintained on slopes of GYM *Streptomyces* agar (DSMZ medium N° 65) at room temperature and as suspensions of cells in 20% v/v glycerol at –20°C and –80°C. Biomass for most of the chemotaxonomic analyses was harvested from GYM *Streptomyces* broths which had been shaken at 180 rpm in 500 ml baffled flasks for 7 days at 28°C following inoculation with 10 ml of the respective strains prepared in 50 ml of the same medium; the biomass preparations were washed twice in distilled water and freeze dried. Biomass for the corresponding fatty acid analyses was prepared from slopes of GYM *Streptomyces* agar that had been incubated for 4 days at 28°C then washed twice in distilled water and freeze dried. In turn, biomass for the molecular systematic studies was prepared from 5 ml GYM *Streptomyces* broths which had been incubated at 28°C for 10 days prior to washing twice in distilled water and stored at room temperature.

Phylogenetic analyses

Genomic DNA was extracted from isolates B12^T, B20 and B25 following the procedure described by Kieser et al. [37]. PCR mediated amplification of 16S rRNA gene sequences was performed in a final volume of 25 µl using primers 27f (5'AGAGTTTGATCMTGGCTCAG-3') and 1525r (5'-AAGGAGGTGATCCAGCC-3') [38], 100 ng of gDNA and MyFiM mix (Bioline, UK) following the protocol of the manufacturer; the PCR conditions were 5 minutes at 95°C followed by 30 cycles of 30 seconds at both 95°C and 55°C, and 23 seconds at 72°C. The PCR products were cleaned with exonuclease I and shrimp alkaline phosphatase (NEB, UK; #E2622S), sequenced (Geneius Ltd; Cramlington, UK) using Big Dye® terminator v3.1 cycle sequencing

(ThermoFisher Scientific Inc.) on an ABI sequence model 3730.1 (Applied Biosystems) and assembled using Pregap 4 and Gap4 software from the Staden package version 20.069 [39]. The resultant 16S rRNA gene sequences of isolates B12^T (1448 bp), B20 (1457 bp) and B25 (1443 bp) were compared against the database of 16S rRNA sequences of *Archaea* and *Bacteria* using the Basic Local Alignment Tool (BLAST) [40] at the National Center for Biotechnology Information (NCBI) website. The sequences of the isolates were compared against corresponding 16S rRNA gene sequences of the type strains of *Geodermatophilus* species retrieved from the EzTaxon server [41]. Phylogenetic trees were inferred using MEGA software version 7.0 [42] and the neighbour-joining [43], maximum-likelihood [44], and maximum-parsimony algorithms [45] with 1000 bootstrap repetitions [46]. Alignment of the nucleotide sequences was achieved with MUSCLE software from MEGA [47] and evolutionary distances calculated with the Kimura-2-parameter model [48]. The resultant trees were rooted using the 16S rRNA gene sequence of *Modestobacter multiseptatus* DSM 44406^T using FigTree version 1.4.2 (http://tree.bio.ed.ac.uk/software/Figtree/). This software was used to visualize the phylogenetic trees. All of the figures were edited in Inkscape version 0.9 (http://linkscape.org.en/). A similarity table was generated from the sequence alignment file using PHYDIT version 1.0 software.

BOX typing

BOX-PCR fingerprints from genomic DNA extracted from the isolates were generated using the BOX A1R primer [49] and experimental conditions described previously [50].

Chemotaxonomy

The isolates were examined for chemotaxonomic markers found to be of value in the systematics of *Geodermatophilus* species [11, 12]. Standard chromatographic procedures were used to establish the isomers of A_2pm [51], isoprenoid quinones [52], whole-cell sugars [53] and polar lipids [54] with minor modifications from Kroppenstedt and Goodfellow [55] followed by two-dimensional thin-layer chromatography; isoprenoid quinones extracted from *Micromonospora luteifusca* GUI2^T [56] were used as standards. In addition, fatty acids extracted from the isolates were methylated, analysed by following the protocol of the Sherlock Microbial Identification (MIDI) system, version 5 [57] and the resultant peaks identified using the ACTIN 6 database; the notation was according to that described in Montero-Calasanz et al. [30].

Cultural and morphological properties

Cultural features of the isolates were recorded on modified Bennett's (DSMZ medium N° 894), glucose-peptone-yeast extract-meat extract (GPHF; DSM medium N° 553), Luedemann's (DSMZ medium N° 877), potato dextrose (PD; DSMZ medium N° 129), peptone-yeast extract-glucose-vitamin solution (PYGV; DSMZ medium Nº 621) and Reasoner's 2A (DSMZ medium N° 830) agar plates and from tryptone-yeast extract, yeast extract-malt extract, oatmeal, inorganic-salts-starch, glycerol-asparagine, peptone-yeast extract-iron and tyrosine agar (ISP media 1–7 [58]) plates following incubation at 28°C for 3 weeks. Colony colours and those of diffusible pigments were recorded by comparison against chips from the Inter-Society Colour Council Natural Bureau of Standard Colour Charts [59]. In addition, isolate B12^T was examined for motility and morphological features using methods described by Trujillo et al. [60].

Phenotypic properties

The isolates and *G. obscurus* DSM 43160^T were examined for a broad range of phenotypic properties known to be of value in the circumscription of species classified in the family *Geodermatophilaceae* [11, 61]. Enzyme profiles were determined, in duplicate, using API galleries, according to the instructions of the manufacturer (BioMérieux), following incubation at 28°C for 24 hours. GENIII microplates and an Omnilog device (Biolog Inc, Hayward, CA, USA) were used to establish the ability of the isolates to oxidise carbon, and nitrogen compounds and to show their resistance to inhibitory agents; the microplates were inoculated, in duplicate, with cells suspended in a viscous inoculating fluid (IF-C) provided by the manufacturer at a cell density of 83% transmittance and incubated at 28°C. Data from the replicated cultures recorded in phenotypic mode were analysed using the opm package 1.0.6 [62] for R [63] and RStudio [64]; reactions showing distinctly different results between the duplicated cultures were scored as variable.

The isolates and the type strains of *G. obscurus* were also tested for their capacity to grow over a range of temperatures (4, 10, 18, 28, 32, 37, 40, 45 and 50°C) and pH regimes (pH 5.0-12.0 at single unit intervals) and in the presence of various concentrations of sodium chloride (1.0, 1.5, 3.0, 5.0, 7.0, 10.0, 15.0 and 20.0%, w/v) using GYM *Streptomyces* agar as the basal medium. pH values were adjusted using either concentrated hydrochloric acid or sodium hydroxide as the isolates did not grow in the presence of pH buffers, as previously observed by Montero-Calansanz et al. [30]. The GYM *Streptomyces* medium was used to test

the capacity of the strains to degrade casein (1%, w/v), cellulose (1%), elastin (0.3%), gelatin (0.3%), guanine (0.5%), hypoxanthine (0.4%), starch (1%), L-tyrosine (0.4%), uric acid (0.5%) and xanthine (0.4%); their ability to degrade tributyrin was determined using tributyrin agar (Sigma-Aldrich). All of these tests were recorded after incubation at 28˚C for 14 days. Aesculin (0.1%) and arbutin (0.1%) hydrolysis were carried out using media and methods described by Williams et al. [65], the hydrolysis of urea (0.2%, w/v) after Christensen [66] and nitrate reduction following Schaal et al. [67]. Catalase production was detected by the formation of bubbles after mixing a drop of 3% hydrogen peroxide to fresh growth of the cultures on glass slides. Oxidase activity was determined in a 1% w/v solution of *N-N-N'-N'*-tetramethyl-1,4-phenlydiamine (Sigma-Aldrich) and the development of a blue purple colour recorded as a positive result [68]. The degradation and tolerance tests were carried out using a cell suspension equivalent to 5 on the McFarland scale [69] in Replidishes (Sterilin) that were inoculated using a multipoint inoculator (Denley Instruments Ltd; UK). The tolerance tests were carried out in triplicate and incubated for 3 weeks at 28°C, apart from the temperature tests.

Whole genome sequencing of isolate B12^T and genomic analyses

A single colony of strain B12 ^{T} was used to inoculate 5 ml BHI broth (DSMZ medium N° 215) and incubated at 28°C in a shaking incubator for 48 hours. Genomic DNA extracted from 1.5 ml of the culture was sequenced on an MiSeq instrument (Illumina). The reads were assembled into contigs using SPAdes 3.9.0 [70] and contigs <1000 bp in size discarded. The draft assembly has been submitted to GenBank (accession no.: NVPT00000000) and is publicly available. The genome sequence of *G. obscurus* DSM 43160^T was obtained from GenBank (accession no.: NC_013757). The genomes of strains B12^T and *G. obscurus* DSM 43160^T were annotated using the RAST pipeline and a sequence based comparison made using the SEED Viewer [71, 72]. A two-way BLAST based average nucleotide identity (ANI) between the genomes of the strains and a two-way average amino acid identity (AAI) between their protein sequences were calculated using the online resource from the K. Konstantinidis group (http://enve-omics.ce.gatech.edu/; [73]). The digital DNA-DNA hybridization (dDDH) values between of the genome of the strains were calculated using the genome-to-genome distance calculator, GGDC 2.0 [74, 75]. Gene clusters for natural products were predicted using antiSMASH [76].

Results

Small numbers of strains growing on the isolation plates were assigned to the genus *Geodermatophilus* as they formed irregular, black colonies; the overall colony count of these isolates corresponded to 16.4 cfu per gram dry weight soil; strains B12^T, B20 and B25, taken to represent these colonies, were found to be Gram-stain positive and non-motile. They grew well on ISP media 2, 3, 5 and 7 but either scantly or poorly on ISP media 4 and 6, generally producing bluish-black colonies that turned completely black after a few weeks (Fig S1); diffusible pigments were not produced, apart from those formed by isolate B12^T on tyrosine agar (Table 1). Isolate B12^T displayed abundant growth on modified Bennett's and Luedemann's agar; very good growth was observed on GYM *Streptomyces*, R2A, GPHF, PYGV and PD agar plates. Overall, isolates B20 and B25 showed faster growth on GYM *Streptomyces* and modified Bennett's agar than isolate B12^T. The latter formed multilocular vesicles (Fig 1) and short-rod and coccoid-shaped cells that had a tendency to remain aggregated.

The three isolates formed a well-supported clade in the *Geodermatophilus* 16S rRNA gene tree (Fig 2). Isolate B12^T shared 16S rRNA gene sequence similarities with isolates B20 and B25 of 99.8 and 99.9% respectively, values equivalent to 3 and 2 nucleotide (nt) differences at 1438 and 1439 locations. The isolates were most closely related to *G. obscurus* DSM 43160^T sharing 16S rRNA gene sequence similarities with the latter of 98.96% (B12^T), 98.87% (B20) and 98.96% (B25), values that correspond to 15 nt differences at 1448, 1457 and 1443, respectively. *G. siccatus* DSM 45419^T and *G. poikilotrophus* DSM 44209^T were also closely related to the isolates sharing 16S rRNA gene similarities values with them that were in the range 98.86 and 98.68, values well above the threshold of 98.65% for recognising members of a novel species [77]. The 16S rRNA gene sequence similarities with the remaining type strains of *Geodermatophilus* species fell within the range 98.61 to 96.46%, values equivalent to 20 and 45 nt differences, respectively.

The BOX-PCR profiles of the isolates show that they are genetically diverse and hence are not clones (Fig 3).

Chemotaxonomy

The isolates were found to contain *meso*-A_2pm as the diamino acid of the peptidoglycan (Fig S2), MK-9(H_4) and MK-9(H_2) as predominant isoprenologues in a ratio of 10:1 (Fig S3),

polar lipid patterns consisting of diphosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphoglycolipid with either one or two additional unidentified lipids (Fig S4) and whole-organism hydrolysates rich in glucose and galactose with traces of ribose and xylose; the other trace sugars, arabinose and mannose, were discontinuously distributed (Fig S5). The detection of complex mixtures of saturated branched chain fatty acids in the isolates is in good agreement with previous reports [11, 12, 15, 17, 25, 26, 28]. In general, it is apparent from such studies that geodermatophili contain major amounts of iso-$C_{15:0}$ and iso-$C_{16:0}$ fatty acids though other predominant fatty acids may vary between species. Isolates B12^T, B20 and B25 were found to have similar proportions of *iso*-$C_{15:0}$ (19.0; 11.0; 14.5%), *iso*-$C_{16:0}$ (14.4; 13.0; 14.9%), *iso*-$C_{17:1}\omega 8c$ (9.7; 21.5; 19.1%) and *iso*-$C_{18:1}\omega 9c$ (10.8; 12.4; 13.2%), as shown in Table 2.

Physiological tests

The isolates and *G. obscurus* DSM 43160^T, their closest phylogenetic neighbour, gave identical results for most of the replicated phenotypic tests, the exceptions were some of the GENIII microplates data (Table 2). In turn, the isolates have almost identical phenotypic profiles though only strains B12^T, B20 and B25 gave positive results for uric acid degradation, allantoin hydrolysis and gelatin degradation, respectively; three out of the four strains were positive for α-chymotrypsin, the exception was isolate B12^T; the *G. obscurus* strain was positive for α-chymotrypsin and gelatin and negative for all of the other aforementioned tests. Isolates B20 and B25, but not B12^T, metabolised *N*-acetyl-D-glucosamine while isolates B12^T and B20, but not B25, used stachyose as a sole carbon source. The isolates and *G. obscurus* DSM 43160^T were oxidase and nitrate reductase negative, degraded starch and tributyrin but not casein, elastin, guanine, L-tyrosine, but grew from pH 5-12, from 18 to 37°C, optimally at 28°C, only the isolates grew up to 40°C; all four strains tolerated the presence of 1.5%, w/v sodium chloride, but only the isolates grew in the presence of 3.0% w/v sodium chloride. In contrast, the isolates, unlike the *G. obscurus* strain, produced β–galactosidase; hydrolysed aesculin, degraded arbutin, utilised D-glucose-6-phosphate, 3-*O*-methyl-D-glucose and β-methyl-D-glucoside as sole carbon sources and contained MK-9(H_2) as opposed to MK-8(H_4) as the predominant isoprenologue. In contrast, *G. obscurus* DSM 43160^T, unlike the isolates, metabolised D-mannitol and contained MK9-(H_0) and MK8-(H_4). The isolates can be distinguished from the type strains of other close phylogenetic neighbours using a combination of chemotaxonomic and other phenotypic data (Table 2), the data on

these strains were acquired using the same BIOLOG microplate procedure employed in the present experiments.

Genomic analysis: resolving the taxonomic status of isolate B12^T

The draft assembly of the genome (GenBank accession no.: NVPT00000000) of strain B12^T, which has a digital guanine and cytosine content of 74 mol %, was compared with the genome sequences of *G. obscurus* DSM 43190^T, *G. siccatus* DSM 45419^T and *G. poikilotrophus* DSM 44209^T. The dDDH value between strain B12^T and these type-strains were 37.40, 44.50 and 37.80%, respectively, values well below the recommended cut-off value of 70% for assigning strains to the same species [78]. Similarly, low ANI and AAI values of 89.28% and 87.27% were observed between the genomes of isolate B12^T and its nearest phylogenetic neighbour, *G. obscurus*, indicating that it belongs to a different species [79, 80].

Detection of genes associated with stress responses

The SEED analyses of the B12^T genome identified 163 genes that are associated with stress responses, including two genes involved in carbon starvation, a gene encoding carbon starvation protein A and another carbon storage regulator protein (Table S1). Carbon starvation protein A activates peptide uptake and helps bacteria to survive in low carbon environments [81-83]. The majority of the other stress response genes (67/163 genes) are associated with oxidative stress involved in different biosynthetic/metabolic activities (Table S1). Twenty-six genes belonging to "Choline and Betaine Uptake and Betaine Biosynthesis" pathways, including a *sox* gene cluster, while additional copies of genes encoding different subunits of sarcosine oxidase are present in the B12^T genome (Table S1) and are associated with responses to osmotic stress [84-88]. Five genes of the *cspA* family and 15 genes assigned to the *dnaK* gene cluster were identified and are involved in responses to cold [22] and heat shock [89], respectively (Table S1). Specific desiccation stress genes were not detected in the B12^T genome even though actinobacteria from hyper-arid soil need to cope with a dearth of available water [90]. However, as for *Modestobacter caceresii* KNN45.2b^T [61], several genes associated with the biosynthesis and uptake of trehalose were detected in the genome of isolate B12^T; this sugar has been linked with tolerance to heat and desiccation in bacteria [91].

A copy of the *uvrA* gene along with a paralogue, two *uvrB* genes and a copy of the *uvrC* gene were detected in the B12^T genome; these genes belong to the *uvrABC* DNA repair

system and encode subunits A–C of the exonuclease ABC enzyme. Two copies of the *uvrD* gene present in the genome of strain B12^T encode ATP dependent DNA helicase; these genes are involved in UV resistance in *Modestobacter marinus* strain BC501 [92]. In addition, eight genes, including *recQ*, belonging to the RecFOR DNA repair pathway were identified, these genes are involved in stabilising the genome [93]. Two *coxGSML* gene clusters along with a *coxSML* cluster, a *coxD* protein, two genes encoding xanthine and CO dehydrogenase maturation factors (XdhC/CoxF family) and a gene encoding molybdopterin cytidylyltransferase were detected in the B12^T genome; carbon-monoxide dehydrogenases are associated with chemolithoautotrophic lifestyles in bacteria though the use of CO as a carbon and energy source [94].

Biosynthetic gene clusters for specialized metabolites

The draft genome of isolate B12^T was the subject of genome mining using the antiSMASH server [76]. Several putative biosynthesis gene clusters were revealed including two containing protein homologues to type III polyketide synthase (T3PKS) while others were associated with the synthesis of terpenes and siderophores. The two T3PKSs were highly similar (~96% sequence identity) to Gobs_4821 of *G. obscurus* DSM 43160^T, a protein containing a chalcone synthase domain (Pfam accessions [95]: PF02797 and PF00195). Sequence alignment (not shown) between the two putative T3PKSs of isolate B12^T, Gobs_4821 and RppA of *Streptomyces griseus* (a known T3PKS involved in the biosynthesis of the pentaketide 1,3,6,8-tetrahydroxynaphthalene [96]) showed that those of B12^T correspond to fragments of this enzyme while the three residues of the catalytic triad were scattered across these fragments. Interestingly, one of the T3PKS proteins was encoded at the end of a contig (Node_324) of the B12^T genome and the other at the beginning of another contig (Node_55) suggesting that these two fragments might form a single T3PKS unit and, hence, a predicted biosynthesis gene cluster. An improved genome alignment is essential for a more comprehensive interpretation of predicted biosynthesis gene clusters in the genome of strain B12^T.

Discussion

Strains B12^T, B20 and B25 contained *meso*-A_2pm as the wall diamino acid, MK-9(H_4) as the predominant isoprenologue, galactose as the diagnostic sugar, fatty acids rich in branched-chain components and complex polar lipid patterns, properties consistent with their classification in the genus *Geodermatophilus* [7, 11, 12, 25, 26, 28]. This assignment is

underpinned by the observation that isolate B12^T produces multilocular vesicles (thalli) and has a DNA base composition rich in guanine and cytosine. The isolates formed a clade in the *Geodermatophilus* 16S rRNA gene tree that was supported by all of the tree-making algorithms and by a 98% bootstrap value. In turn, the three strains were most closely, albeit loosely, associated with the type strain of *G. obscurus.*

A range of genomic metrics are now available to distinguish between orthologous genes of closely related prokaryotic species [97], including the calculation of ANI and AAI values [98-100]. In addition, genome-to-genome sequence comparisons have been widely used to delineate prokaryotic species while digital DDH values have been found to be highly correlated with genetic distances based on variations in 16S rRNA genes [74, 101]. In light of these developments it is encouraging that ANI, AAI and of DNA values between isolate B12^T and *G. obscurus* DSM 43160^T showed that these strains belong to a distinct genome species within the genus *Geodermatophilus*.

It is evident from the wealth of data considered above that isolates B12^T, B20 and B25 have many chemotaxonomic, cultural and physiological features in common, some of which can be weighted to distinguish them from their nearest phylogenetic neighbours, notably from *G. obscurus* DSM 43160^T, as exemplified by their fatty acid, polar lipid, and phenotypic profiles. It is, therefore, clear that the three representative isolates form a new centre of taxonomic variation within the genus *Geodermatophilus* that merits recognition as a new species. It is proposed that the isolates be recognised as *Geodermatophilus chilensis* sp. nov.

Description of *Geodermatophilus chilensis* sp. nov.

Geodermatophilus chilensis (chi.len'sis. N.L. adj. *chilensis* referring to Chile, country from which the strains were isolated).

Aerobic, Gram-stain positive, catalase positive, oxidase negative actinobacteria that form multilocular vesicles that release non-motile, short-rod, coccoid shaped cells. Growth occurs between 18°C and 40°C, optimally ~28°C, from pH 5 to pH 12, optimally pH ~7.5 and in presence of 3.0 %, w/v sodium chloride. Grows well on glycerol-asparagine, oatmeal and yeast extract-malt extract agar producing circular, black-blue colonies with entire margins. Oxidises D-cellobiose, D-glucose-6-phosphate and D-maltose. Does not degrade hypoxanthine. Additional phenotypic features are cited in the text and in Tables 1 and 2. The predominant fatty acids are *iso*-$C_{15:0}$, *iso*-$C_{16:0}$, *iso*-$C_{17:1}\omega 8c$ and *iso*-$C_{18:1}\omega 9c$, and the major

polar lipids diphosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol with a phosphatidylgycolipid. Additional chemotaxonomic properties are typical of the genus. The *in silico* G+C content of the draft genome type strain is 74 mol %.

The type strain, B12^T (=CECT 9483 ^{T}= NCIMB 15089^T), and isolates B20 and B25 were isolated from an extreme hyper-arid soil from the Yungay core-region of the Atacama Desert, Chile.

This first report of *Geodermatophilus* strains from Atacama Desert soil provides further evidence that members of this poorly studied genus are present in habitats characterised by low water and nutrient availability, high solar radiation and marked variations in temperature [18, 21, 32, 102, 103]. It seems likely that additional geodermatophili isolated from Atacama Desert soils will be found to be new species [104]; culture-independent studies show that these organisms are an integral component of the actinobacterial core microbiome of such soils [105]. It is also very interesting that genes and gene clusters identified in the genome of isolate B12^T encode for properties relevant to its ability cope with harsh environmental conditions found in extreme hyper-arid Atacama Desert soils, as exemplified by genes encoding for carbon starvation, osmotic stress, response to cold and heat shocks and to UV light. Digital Protologue Taxonumber: TA00343.

Conflict of interest

The authors declare no conflicts of interest

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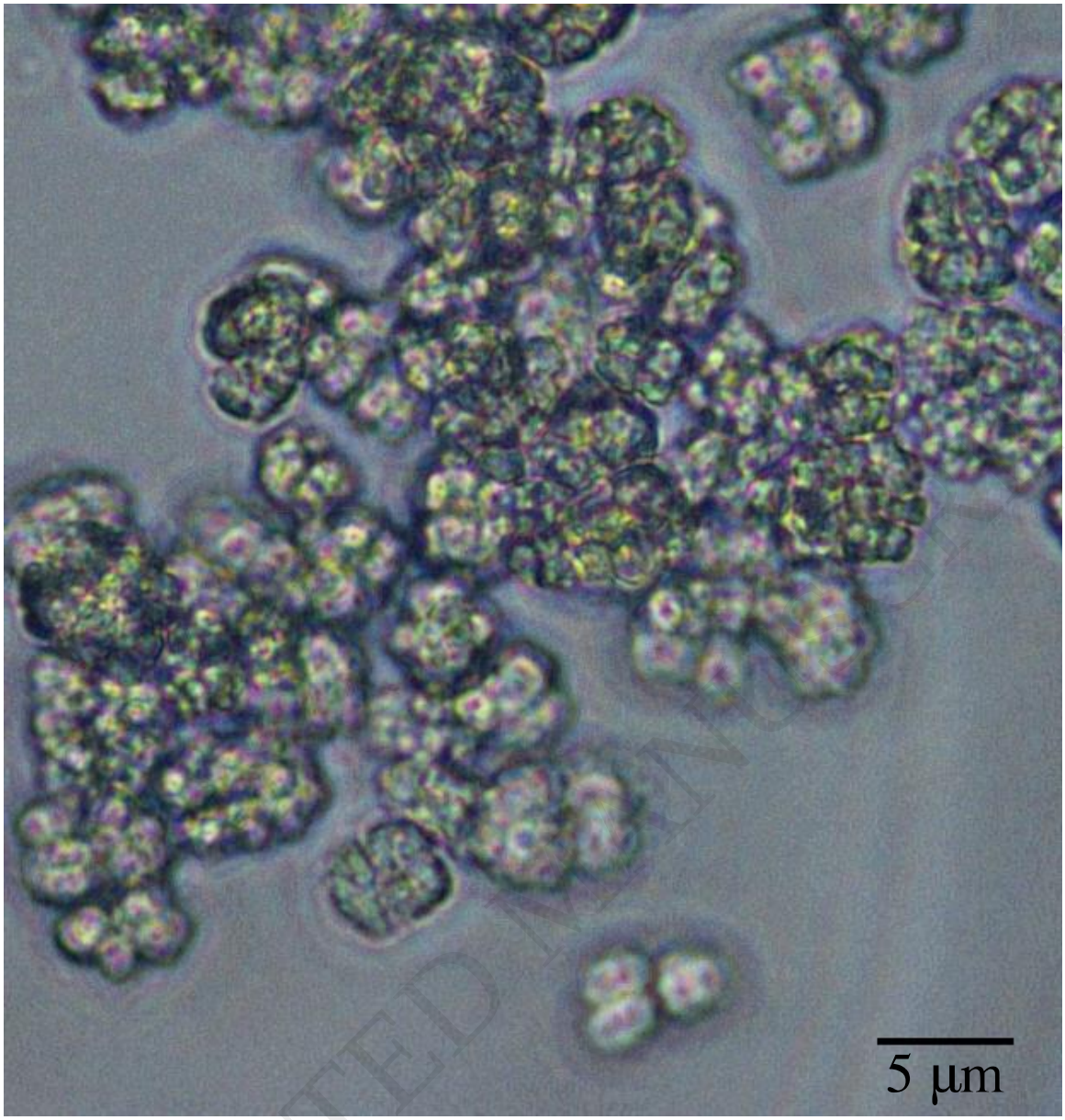
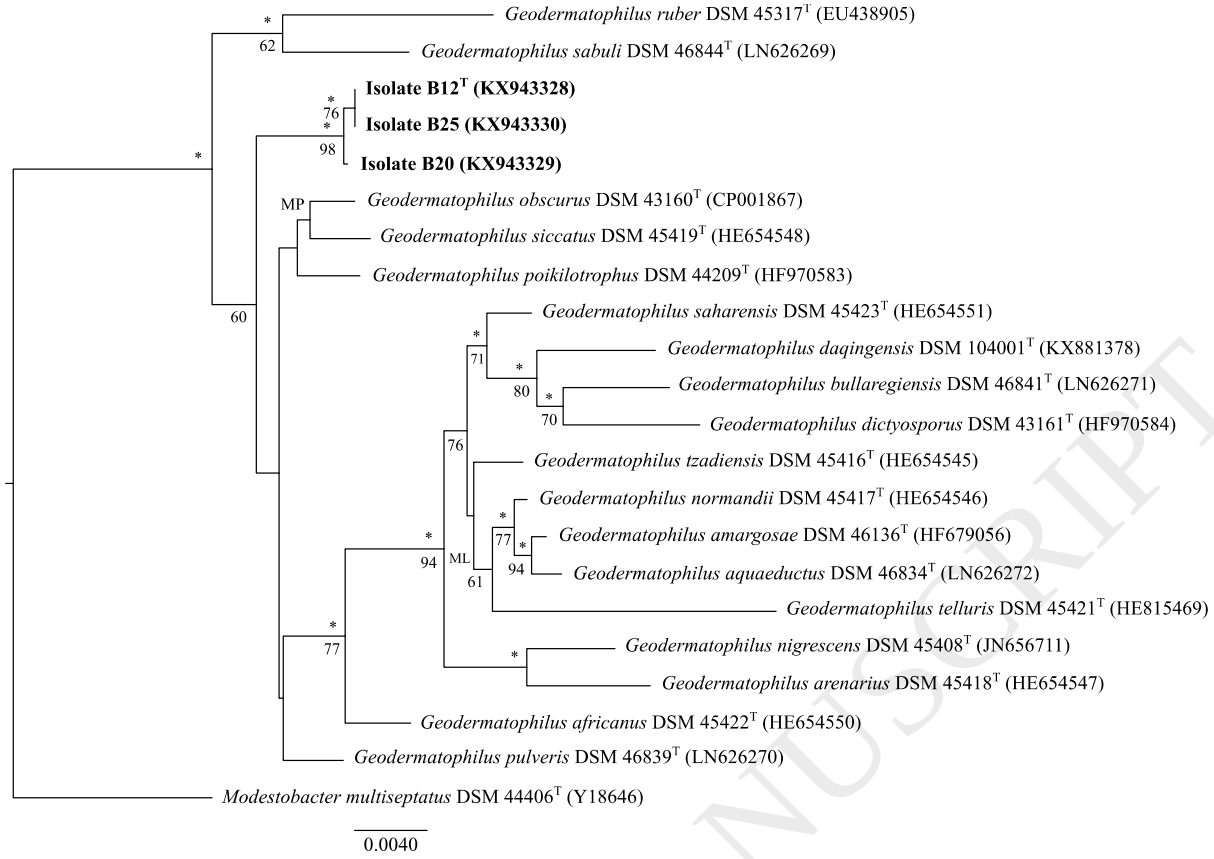
Legends for Figures

Fig. 1. Phase contrast image of isolate B12^T following growth on GYM *Streptomyces* agar at 28°C for 7 days showing multilocular vesicles.

Fig. 2. Neighbour joining tree based on almost complete 16S rRNA gene sequences showing relationships between isolates B12^T, B20, and B25 and between them and closely related *Geodermatophilus* type strains. Asterisks indicate branches of the tree that were also found using the maximum-likelihood (ML) and maximum-parsimony (MP) tree-making algorithms. Only bootstrap values above 50% are shown. Scale bar, indicates the number of substitutions per nucleotide position.

Fig. 3. BOX-PCR fingerprints of isolates B12^T, B20 and B25. Lane 1: GeneRuler 100bp Plus DNA Ladder (Thermo Scientific); 2, isolate B12^T; 3, isolate B20; 4, isolate B25; 5, GeneRuler 1kbp Plus DNA Ladder (Thermo Scientific). The size of bands for the DNA ladders are shown in kbp.





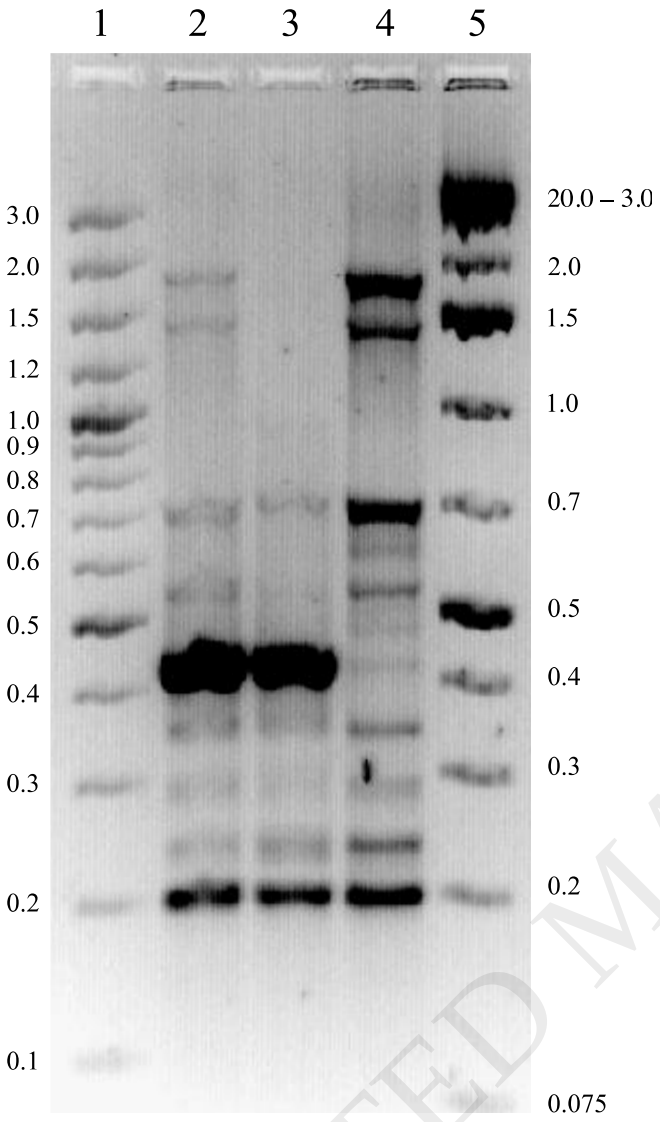


Table 1. Growth and cultural characteristics of isolates B12^T, B20 and B25 on ISP media after incubation for 14 days at 28°C.

	Growth			**Colony colour**			**Diffusible pigment**		
Media	B12^T	B20	B25	B12^T	B20	B25	B12^T	B20	B25
Tryptone-yeast extract (ISP 1)	+	+	+	Medium grey	Dark grey	Dark grey	None	None	None
Yeast extract-malt extract agar (ISP 2)	++	+++	+++	Black-blue	Black-blue	Black-blue	None	None	None
Oatmeal agar (ISP 3)	++	++	++	Black-blue	Dark grey	Black-blue	None	None	None
Inorganic salts-starch agar (ISP 4)	+	+	±	Black-blue	Dark grey	N.R.	None	None	None
Glycerol-asparagine agar (ISP 5)	+++	++	+++	Black-blue	Black-blue	Black-blue	None	None	None
Peptone-yeast extract-iron agar (ISP 6)	+	±	+	Light yellow-pink	N.R.	Light yellow-pink	None	None	None
Tyrosine agar (ISP 7)	++	±	++	Black-blue	N.R.	Black-blue	Brown-orange	None	Brown-orange

+++ = abundant growth; ++ = very good growth; + = poor growth; ± = scant growth; N.R. = not recorded due to scant growth.

Table 2. Phenotypic properties that differentiate *Geodermatophilus chilensis* isolates. B12^T, B20 and B25 from closely related type-strains of the genus *Geodermatophilus*.

Strains: 1, B12^T, B20 and B25 (data from this study); 2, *G. africanus* DSM 45422^T [58]; 3, *G. obscurus* DSM 43160^T (data from this study, unless indicated); 4, *G. poikilotrophus* DSM 44209^T [64, 73]; 5, *G. pulveris* DSM 46829^T [79]; 6, *G. ruber* DSM 45317^T [55]; 7, *G. sabuli* DSM 46844^T [31]; 8, *G. siccatus* DSM 45419^T [29].

	1	2	3	4	5	6	7	8
GYM agar								
Colony colour	Light orange, black-blue	Black	Black	Light red, greenish black	Black	Light red, red	Pale pink	Light red, black
Colony texture	Dry	Dry	Dry	Moist	Dry	Moist	Dry	Moist
GENIII BIOLOG microplate tests								
(a) Oxidation of amino acids								
L-Alanine	–	–	–	–	–	+	v	–
L-Histidine	–	–	–	–	–	v	v	v
D-Serine	–	–	–	–	–	+	–	–
L-Serine	–	–	–	–	v	+	v	–
(b) Oxidation of sugars								
D-Cellobiose	+	v	+	+	–	+	+	–
D-Fructose	+	–	+	+	+	+	+	+
β-Gentiobiose	+	–	v	v	v	+	v	–
D-Glucose-6-phosphate	+	–	–	–	–	–	v	–
Glycerol	+	–	+	+	+	–	–	+
3-*O*-Methyl-D-Glucose	+	–	–	–	–	–	v	–
myo-Inositol	+	–	+	+	+	–	v	+
D-Maltose	+	–	+	+	v	+	v	+
D-Mannitol	–	–	+	+	+	–	+	+
D-Salicin	+	–	v	–	v	–	v	–
D-Sorbitol	+	–	+	+	v	–	+	+
Stachyose	+	–	v	+	–	–	v	–
Sucrose	+	–	+	+	–	+	v	+
N-acetyl-D-glucosamine	+	–	–	–	–	v	v	–
β-methyl-D-glucoside	+	–	–	–	–	+	v	–
(c) Oxidation of organic acids								
β-Hydroxy-Butyric acid	+	+	+	+	+	–	+	+
p-Hydroxy-phenyl acetic acid	–	v	–	–	v	–	–	+
Mucic acid	–	–	–	–	–	+	v	–
(d) Utilisation of:								
Inosine	–	–	–	–	v	+	v	–

Tween 40	+	v	+	v	v	v	–	+
Major fatty acids[a]	iso-$C_{15:0}$, iso-$C_{16:0}$, iso-$C_{17:1}\omega 8c$ iso-$C_{18:1}\omega 9c$	iso-$C_{16:0}$	iso-$C_{15:0,}$ iso-$C_{16:0}$, iso-$C_{17:1}\omega 8c$[d]	iso-$C_{15:0,}$ iso-$C_{16:0}$, iso-$C_{17:1}\omega 8c$	iso-$C_{16:0}$, iso-$C_{16:1}\omega 7c$	iso-$C_{15:0}$, anteiso-$C_{15:0}$, iso-$C_{16:0}$, anteiso-$C_{17:0}$, $C_{17:1}\omega 8c$	iso-$C_{15:0}$, iso-$C_{16:0}$, anteiso-$C_{15:0}$, anteiso-$C_{17:0}$	iso-$C_{15:0}$, iso-$C_{16:0}$, iso-$C_{17:1}\omega 8c$
Predominant menaquinones MK[b]	$9(H_4)$, $9(H_2)$	$9(H_4)$	$9(H_4)$, $9(H_2)$, $9(H_0)$, $8(H_4)$[e]	$9(H_4)$	$9(H_4)$, $9(H_2)$	$9(H_4)$	$9(H_4)$, $9(H_6)$	$9(H_4)$, $8(H_4)$, $9(H_0)$
Phospholipids[c]	PGL, L (2 L, $B12^T$ and B25)	PG	PG, G[e]		PE-OH	PG, 2 PL, G[e]		PG

; +, positive; -, negative; v, variable; [a], only fatty acids making up ≥ 10% of each area are shown; [b], only menaquinones making up ≥ 5% of each area are shown; [c], all of the strains contain: DPG, diphosphatidylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; remaining abbreviations: PE-OH, hydroxy-phosphatidylethanolamine; PG, phosphatidylglycerol; PGL, phosphoglycolipid; PL, unidentified phospholipid; G, unidentified glycolipid; L, unidentified lipid; [d], data from Montero-Calasanz et al. [23]; [e], data from Montero-Calasanz et al. [4].

Isolates $B12^T$, B20, B25 and *G. obscurus* DSM 43160^T were positive for: API ZYM tests: acid phosphatase, alkaline phosphatase, cystine arylamidase esterase (C 4), esterase lipase (C 8), *N*-acetyl-β-glucosaminidase, α-glucosidase, β-glucosidase, leucine arylamidase, naphthol-AS-BI-phosphohydrolase, trypsin and valine arylamidase. GEN III BIOLOG microplate tests: D-cellobiose, D-fructose, *myo*-inositol, D-maltose, D-sorbitol, sucrose and D-trehalose (sugars), glycerol (polyol), acetoacetic acid, β-hydroxy-butyric acid and L-malic acid, (organic acids) and Tween 40 (polymer). Other phenotypic tests: starch and tributyrin. All organisms grew from pH 5-12, from 18°C-37°C, in the presence of 1.5% NaCl and were catalse positive.

Isolates $B12^T$, B20, B25 and *G. obscurus* DSM 43160^T were negative for: API ZYM tests: α-fucosidase, α-galactosidase, β-glucuronidase, lipase (C 14) and α-mannosidase. Gen III BIOLOG microplate tests: L-alanine, L-histidine, inosine and L-serine, (amino acids), mucic acid, *p*-hydroxy-phenylacetic acid and D-saccharic acid, (organic acids), resistance to fusidic acid, guanidine hydrochloride, lincomycin, niaproof, D-serine #1, sodium bromate, troleandomycin and vancomycin. Other phenotypic tests: casein, cellulose, elastin, guanine, L-tyrosine and xanthine; nitrate reductase and oxidase.

Variable results recorded for isolates $B12^T$, B20, B25 and *G. obscurus* DSM 43160^T: GEN III BIOLOG microplates tests: D-arabitol, dextrin, D-fructose-6-phosphate, L-fucose, *N*-acetyl-D-galactosamine, galactose, β-gentiobiose, D-glucose, α-D-lactose, D-mannitol, *N*-acetyl-β-D-mannosamine, D-mannose, D-melibiose, D-raffinose, L-rhamnose, D-salicin, turanose, stachyose (sugars); acetic acid, γ-amino-*n*-butyric acid, α-hydroxy-butyric acid, α-*keto*-butyric acid, citric acid, α-*keto*-glutaric acid, L-lactic, D-lactic acid methyl ester, D-malic acid, methyl pyruvate, *N*-acetyl neuraminic acid, propionic acid, quinic acid, bromo succinic acid (organic acids); pectin (polymers); glycyl-proline (dipeptide); L-arginine, L-aspartic acid, L-glutamic acid, L-pyroglutamic acid, D-aspartic acid (amino acids); D-

galacturonic acid, D-gluconic acid, L-galactonic acid-γ-lactone (sugars acids); glucuronamide (amine hexose) and resistance to aztreoman, nalidixic acid, potassium tellurite and tetrazolium blue.