Revival and emended description of 'Mycobacterium paraffinicum' Davis, Chase and Raymond 1956 as *Mycobacterium paraffinicum* sp. nov., nom. rev.

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The omission of the name '*Mycobacterium paraffinicum*' from the Approved Lists of Bacterial Names was due to phenotypic confusion surrounding a close relationship with *Mycobacterium scrofulaceum*. Correspondingly, '*M. paraffinicum*' strains grew slowly in >7 days, stained acid-alcohol-fast and produced yellow-pigmented, smooth, waxy colonies in the dark at an optimal temperature of 35 °C. However, '*M. paraffinicum*' strains demonstrated no activity for urease, nicotinamidase or pyrazinamidase and lacked growth at 42 °C, unlike *M. scrofulaceum*. The mycolic acid pattern, as determined by HPLC, clustered '*M. paraffinicum*' with *M. scrofulaceum*, *Mycobacterium avium* and *Mycobacterium parascrofulaceum*. Strains were fully susceptible to linezolid, rifabutin, clarithromycin and amikacin. Examination of the historical reference strain of '*M. paraffinicum*', ATCC 12670, and five additional isolates using comparative studies with 16S rRNA, *hsp65* and *rpoB* gene and concatenated sequences showed that they formed a tight taxonomic group that was distinct from similar non-tuberculous mycobacteria. Multilocus enzyme electrophoresis (MEE) analysis confirmed a close association of the five additional isolates with the reference strain of '*M. paraffinicum*' with a genetic distance of 0.12 and showed that all six strains were distinct from other closely related species. These genetic results provided unambiguous evidence of the uniqueness of this slowly growing, scotochromogenic species and supported the revival of the name as *Mycobacterium paraffinicum* (ex Davis, Chase and Raymond 1956) sp. nov., nom. rev. We propose the previously deposited reference strain ATCC 12670^T = DSM 44181^T = NCIMB 10420^T, located in collections worldwide, as the type strain.

In 1956, multiple strains of a soil bacterium were isolated below the level of grass roots in oily pools. They were characterized as Gram-positive, acid-alcohol-fast, non-motile, rod-shaped bacilli, with white colonies that became yellow with age and were waxy and wrinkled on selective isolation medium of mineral salts agar in a gaseous atmosphere of 40% ethane in air. Subsequently, the

bacteria were grown on ethanol- and acetate-containing medium and the name '*Mycobacterium paraffinicum*' was suggested to represent a paraffinic, gaseous hydrocarbon-utilizing, non-tuberculous mycobacterium (NTM). The proposed name was 'meant to connote the general utilization of gaseous and liquid paraffinic hydrocarbons' (Davis *et al.*, 1956). Furthermore, these mycobacteria were abundant in soil samples of petroliferous emanation, principally oilfields, and demonstrated specificity for ethane and higher paraffinic hydrocarbons (Davis *et al.*, 1959).

Abbreviations: IWGMT, International Working Group on Mycobacterial Taxonomy; MEE, multilocus enzyme electrophoresis; NTM, non-tuberculous mycobacterium.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA, *hsp65* and *rpoB* gene sequences of isolates 90683, 90750, 30760, 70769 and 80776 and '*M. paraffinicum*' ATCC 12670 are respectively GQ153265–GQ153270 (16S rRNA gene), GQ153283–GQ153288 (*hsp65*) and GQ153299–GQ153304 (*rpoB*).

A comparison of mycolic acid patterns, a dendrogram based on ETs and neighbour-joining trees based on *hsp65*, *rpoB* and concatenated sequences are available as supplementary material with the online version of this paper.

Taxonomy specialists affiliated with the International Working Group on Mycobacterial Taxonomy (IWGMT) conducted co-operative, numerical taxonomic open-ended studies of NTM that included '*M. paraffinicum*' strain ATCC 12670 = IWGMT OES 90139. An early study of slowly growing scotochromogenic mycobacteria of Runyon's group II used 140 characters to develop an $n \times n$ matrix table to demonstrate clustering behaviour of

the study species (Wayne *et al.*, 1971). The IWGMT noted that cluster 4 included *Mycobacterium scrofulaceum* ATCC 19981^T and '*M. paraffinicum*' ATCC 12670 (Davis *et al.*, 1956). They found that the fit of '*M. paraffinicum*' was poor in the cluster and concluded that its status as a separate species was uncertain. In 1976, the Judicial Commission of the International Committee on Systematic Bacteriology suggested a comprehensive review of names of bacteria to eliminate confusing, illegitimate or invalid names and recommended the retention of names for taxa with complete descriptions (Skerman *et al.*, 1980). The effective date of publication of these Approved Lists of Bacterial Names was 1 January 1980 in the *International Journal of Systematic Bacteriology*. In preparation for this change, taxonomy experts representing the genus *Mycobacterium* published a preliminary list of species for comment in 1976 (Kubica, 1978). The name '*M. paraffinicum*' appeared on this list under the heading 'commonly encountered mycobacterial species names without legitimate standing' and noted 'synonymous with *Mycobacterium scrofulaceum*; status as a separate species still to be determined'. When the Approved Lists were published in 1980, '*M. paraffinicum*' was excluded because it was not clearly distinguishable from other species with the standard methods of analysis available at that time. Shortly thereafter, the IWGMT published on slowly growing mycobacteria with numerical taxonomic methods using 178 study characters (Wayne *et al.*, 1981). They detected 10 clusters, and '*M. paraffinicum*' ATCC 12670 was positioned in cluster 7 and was distinguished from *M. scrofulaceum* by differences in pyruvate utilization, urease, nicotinamidase, pyrazinamidase and tellurite reduction. Also, the intracluster matching score suggested the species to be a unique taxon. The IWGMT agreed that proposing the revival of the name was outside the purpose of their group and recommended that the individuals who had introduced the strain should do the work. However, the IWGMT continued to include '*M. paraffinicum*' in subsequent numerical taxonomic studies, but noted that it was premature to revive the name as the status of the species was unclear because it could not be distinguished with confidence from *M. scrofulaceum* (Wayne *et al.*, 1983, 1989). The IWGMT recommended semantide-based comparative studies to determine whether the name '*M. paraffinicum*' should be revived or placed as an outlier to known species. Another IWGMT study concluded that the characteristics of '*M. paraffinicum*' conformed moderately to strains of the third intermediate category of scotochromogenic slow growers associated with '*M. avium*–*Mycobacterium intracellulare*–*M. scrofulaceum* (MAIS) intermediate terminology' (Wayne *et al.*, 1991). However, the species was phenotypically dissimilar to *Mycobacterium avium*–*Mycobacterium intracellulare* (MAC) species, and it was recommended that the strains be referred to by an informal vernacular term, 'the *paraffinicum* group' (Wayne *et al.*, 1991). IWGMT studies consistently acknowledged that the study strain OES 90139 represented a distinct taxon but, despite the evidence for retaining the name, it was judged

insufficient due to the study of a single reference isolate. In 1992, a review of infrequently encountered mycobacterial diseases acknowledged that semantide studies had not been applied to the species '*M. paraffinicum*' but noted that the isolate had obviously different biochemical responses from *M. scrofulaceum* and possibly belonged to a discrete environmental species (Wayne & Sramek, 1992).

The 16S rRNA gene sequence for '*M. paraffinicum*' ATCC 12670 was deposited in the NCBI nucleotide database as GenBank accession number X88925, albeit with ambiguities (Springer *et al.*, 1995). This study showed that '*M. paraffinicum*' was closely related to *M. scrofulaceum*, with a Hamming distance of 3 for the 16S rRNA gene sequence. However, the IWGMT concluded that the single reference strain did not exhibit unequivocal taxonomic relatedness and that the name should not be revived until polyphasic studies were completed (Wayne *et al.*, 1996). Subsequent comparative sequencing studies showed that the '*M. paraffinicum*' ATCC 12670 16S rRNA gene was unique but genetically close to that of *M. scrofulaceum* ATCC 19981^T (Turenne *et al.*, 2001). Hybridization of distinct signature regions within the first 500 bp of the 5' end of the 16S rRNA gene with commercial test kits for Line probe technology positioned '*M. paraffinicum*'–*M. scrofulaceum* in a complex (Russo *et al.*, 2006). However, matrix-assisted laser desorption ionization-time of flight mass spectrometry analysis of base-specific cleavage products of the PCR-amplified 16S rRNA gene unambiguously distinguished '*M. paraffinicum*' from *M. scrofulaceum* (Lefmann *et al.*, 2004). Species ambiguity remained, with other methods such as *hsp65* restriction fragment length polymorphisms being problematic for '*M. paraffinicum*', as this technique identified '*M. paraffinicum*' as *M. avium*, despite a suggestion that '*M. paraffinicum*' was 'perhaps synonymous to *M. scrofulaceum*' (Hafner *et al.*, 2004). Conversely, sequence analysis of this same *hsp65* gene region produced a separate branch distribution for '*M. paraffinicum*' compared with *M. scrofulaceum* (McNabb *et al.*, 2004), but doubt persisted regarding the validity of the name.

Comparative polyphasic studies were impeded because of a lack of strains necessary to clarify the taxonomic position of '*M. paraffinicum*'. Researchers would not name the species based upon a single reference isolate, even though the reference strain was shown to occupy a unique taxonomic position in the genus *Mycobacterium*. We used phylogenetic and comparative genetic analysis of six strains to demonstrate conclusively that '*M. paraffinicum*' is a distinct species. In this report, we propose the revival of '*Mycobacterium paraffinicum*' in accordance with the rules of the International Code of Nomenclature of Prokaryotes (formerly the International Code of Nomenclature of Bacteria; the Bacteriological Code) (Lapage *et al.*, 1992).

Type strains were recovered from our collection at –70 °C storage. Some strains originated from the former Trudeau Mycobacteria Culture Collection (TMC) and, for clarification, current ATCC designations are included. Control

strains included the reference strain of '*M. paraffinicum*', ATCC 12670, *M. avium* subsp. *avium* TMC 724^T = ATCC 25291^T, *M. scrofulaceum* TMC 1323^T = ATCC 19981^T, *Mycobacterium parascrofulaceum* CIP 108112^T and *Mycobacterium chimaera* CIP 107892^T. An additional five isolates (strains 90683, 70769, 80776, 30760 and 90750) sent from various US public health departments were identified in our laboratory by matching 16S rRNA gene sequences (Rogall *et al.*, 1990; Turenne *et al.*, 2001; Charles *et al.*, 2005; Wang *et al.*, 2008) with the '*M. paraffinicum*' reference strain and grouping by mycolic acid analysis with HPLC (Butler & Guthertz, 2001). Strain 90750 was from a bronchial lavage and the remaining isolates were isolated from sputum. For subsequent testing of slowly growing mycobacteria, cells were incubated at 35 °C in Middlebrook 7H9 broth or on 7H11 plates or Löwenstein–Jensen (LJ) medium (Lévy-Frébault & Portals, 1998).

Investigation of antimicrobial susceptibility was done using broth microdilution with Sensititre SLOWMYCO plates (Trek Diagnostic Systems) according to the manufacturer's instructions. Individual plates included both positive and negative growth controls. *M. avium* ATCC 25291^T was included as a control for validation of results (Woods *et al.*, 2003; Wallace *et al.*, 1986; Heifets, 1988). Susceptibility testing was done with '*M. paraffinicum*' ATCC 12670 and the five additional isolates. Susceptibilities were determined by microdilution broth MICs (CLSI, 2009; NCCLS, 2003). For comparative analysis, similar mycobacteria controls included *M. avium* ATCC 25291^T, *M. scrofulaceum* ATCC 19981^T and *M. parascrofulaceum* CIP 108112^T. Antimicrobials tested were amikacin, rifabutin, ciprofloxacin, clarithromycin, ethambutol, gatifloxacin, linezolid, minocycline, moxifloxacin, rifampicin, streptomycin and trimethoprim/sulfamethoxazole.

Mycolic acids were examined by HPLC according to previously published procedures (Butler & Guthertz, 2001).

Electrophoretic mobilities of six cellular enzymes were determined using multilocus enzyme electrophoresis (MEE) by previously published methods for typing of NTM (Yakrus *et al.*, 1992). The enzymes studied were adenylate kinase, glutamate oxaloacetate transaminase, phosphoglucosomerase, benzyl alcohol dehydrogenase, aconitase and phosphoglucomutase.

DNA was extracted from 200 µl of a 7H9 bacterial suspension by bead beating in a 2 ml tube containing 250 µl 0.1 mm zirconium beads suspended in 300 µl TE buffer (10 mM Tris/HCl, 1 mM EDTA, pH 8.0). Cells were lysed with a FastPrep FP120 instrument (Qbiogene) set at 6.0 ms⁻¹ for 45 s and a biphasic separation was achieved by adding 300 µl chloroform. The resulting solution was centrifuged in a microcentrifuge at 14 000 r.p.m. for 5 min to separate the layers. The DNA-containing aqueous TE layer was transferred to a sterile tube, heated to 90 ± 5 °C for 30 min and stored at 4 °C until used in subsequent PCR amplification. The 16S rRNA gene was amplified and sequenced with the same primer pair, fd1/rp2 (Weisburg

et al., 1991). Two additional housekeeping genes, *hsp65* and *rpoB*, were amplified and sequenced using, respectively, primer pairs TB11/TB12 (Telenti *et al.*, 1993) and MycoF/MycoR (Adékambi *et al.*, 2003). Products of sequencing reactions were recorded with an ABI Prism 3130 xl DNA sequencer following the standard protocol of the supplier (Perkin Elmer Applied Biosystems). The percentage of similarity between the sequences was determined using the CLUSTAL W program supported by the PBIL website (<http://pbil.univ-lyon1.fr>). For phylogenetic analysis, sequences were trimmed in order to start and finish at the same nucleotide position for all the strains under study. Multisequence alignment was performed by using the CLUSTAL_X program, version 1.81, with the PHYLIP software package (Thompson *et al.*, 1997). Phylogenetic trees were compared using the maximum-parsimony, maximum-likelihood and neighbour-joining methods. One neighbour-joining tree was based upon concatenated 16S rRNA, *hsp65* and *rpoB* gene sequences. The maximum-likelihood trees were reconstructed using PhyML software with GTR, gamma distribution and invariable sites as the substitution model and 100 bootstrap replications (Guindon & Gascuel, 2003). Phylogenetic trees were obtained from DNA sequences by using the maximum-parsimony and neighbour-joining methods with 1000 bootstrap replications in MEGA version 3.1 (Kumar *et al.*, 2004).

Strains were fully grown on mycobacteria media in 14 days at an optimum temperature of 35 °C and stained acid–alcohol-fast, characteristic of the genus *Mycobacterium*. Extensive IWGMT polyphasic studies had suggested an affiliation of the so-called '*M. paraffinicum*' reference strain ATCC 12670 within the genus *Mycobacterium* and emphasized the uniqueness of this species. IWGMT results of biochemical testing clustered this reference strain close to the scotochromogenic species *M. scrofulaceum*, having negative test results for Tween 80 hydrolysis, niacin production, nitrate reduction, tolerance of 5% NaCl and acid phosphatase. Conversely, the taxonomic position of '*M. paraffinicum*' was distinct, with positive tests for Tween opacity and negative results for nicotinamidase, pyrazinamidase and urease compared with *M. scrofulaceum* (Table 1).

The six '*M. paraffinicum*' strains were susceptible to rifabutin, linezolid, clarithromycin and amikacin. Amikacin susceptibility was different compared with *M. scrofulaceum*, but the drug susceptibility patterns were generally not distinctive (Table 2).

'*M. paraffinicum*' cells assimilated triglycerides, glycolipids and mycolic acids in the outer layers of the cell wall, similar to species of the genus *Mycobacterium* (Gusev *et al.*, 1978). Differentiation of the cellular lipid components by TLC analysis showed a similar but slightly different lipid pattern compared with *M. scrofulaceum* (Sehrt *et al.*, 1976). Mycolic acid analysis with HPLC was not conclusive but demonstrated late, double-cluster HPLC patterns representative of mycobacteria. Study isolates shared characteristics of alpha,

Table 1. Phenotypic characteristics of '*M. paraffinicum*' ATCC 12670 and type strains of closely related species

Strains: 1, '*M. paraffinicum*' ATCC 12670; 2, *M. scrofulaceum* ATCC 19981^T; 3, *M. parascrofulaceum* CIP 108112^T; 4, *M. avium* ATCC 25291^T; 5, *M. nebraskense* ATCC BAA-837^T. Data were compiled for type strains from Davis *et al.* (1956), Kent & Kubica (1985), Wayne *et al.* (1991, 1996), Turenne *et al.* (2004), Tsukamura (1984) and Mohamed *et al.* (2004). +, Positive; –, negative; v, variable; ND, no data available.

Property	1	2	3	4	5
Temperature range for growth (°C)	22–37	22–37	25–37	22–45	25–35
Pigment production*	s	s	s	N	s
Catalase tests:					
Semi-quantitative (>45 mm)	+	+	v	–	–
Heat-stable (68 °C)	+	+	+	+	+
Tween opacity (5 weeks)	+	–	ND	+	ND
Tween 80 hydrolysis	–	–	–	–	v
Iron uptake	ND	–	–	–	ND
Growth on MacConkey agar	ND	–	–	–	–
Acid phosphatase	–	–	–	–	ND
Nicotinamidase	–	+	ND	+	ND
Pyrazinamidase	–	+	+	+	–
Urease	–	+	+	–	–
Arylsulfatase (3/14 days)	–/v (10 days)	–/–	–/v	–/v	–/ND

*N, Non-chromogen; s, scotochromogen.

keto and epoxy wax ester mycolic acids, closely akin to species related to *M. avium* (Supplementary Fig. S1, available in IJSEM Online). Therefore, unambiguous species identification required molecular sequence determinations.

The MEE strain typing method was used to generate a dendrogram of genetic relationships among the isolates (Supplementary Fig. S2). Genetic distance was measured with enzyme electrophoretic types (ET) and demonstrated

a highly related group with a distance of 0.124 and isolates clustered closely with the reference strain of '*M. paraffinicum*', ATCC 12670. An exact correlation of ET was found for two of the study isolates, 90750 and 80776. One of the study isolates, 90683, matched the reference strain of '*M. paraffinicum*'. Three of the study isolates and the reference strain of '*M. paraffinicum*' united at a shallow genetic distance of 0.07. In contrast, the tightly clustered isolates of '*M. paraffinicum*' were separated from *M. scrofulaceum*

Table 2. Antimicrobial susceptibility patterns of study strains

Strains: 1, *M. avium* ATCC 25291^T; 2, *M. scrofulaceum* ATCC 19981^T; 3, *M. parascrofulaceum* CIP 108112^T; 4, '*M. paraffinicum*' ATCC 12670; 5, *M. chimaera* CIP 107892^T; 6, isolate 90750; 7, isolate 30760; 8, isolate 70769; 9, isolate 80776; 10, isolate 90683. CLSI or NCCLS breakpoint guidelines (CLSI, 2009; NCCLS, 2003) were used to determine susceptibilities by microdilution broth MICs (µg ml^{–1}). Modified MIC drug susceptibility breakpoints were rifampicin (≥2 µg ml^{–1}), ethambutol (≥4 µg ml^{–1}), trimethoprim/sulfamethoxazole (4/76 µg ml^{–1}) and minocycline (≥8 µg ml^{–1}). Intermediate breakpoints were interpreted as drug resistant. Underlined MICs demonstrate drug susceptibility.

Antimicrobial	1	2	3	4	5	6	7	8	9	10
Ciprofloxacin	2	8	>16	8	16	<u>1</u>	8	<u>1</u>	<u>1</u>	4
Gatifloxacin	<u>1</u>	<u>2</u>	4	8	>8	<u>0.25</u>	8	<u>0.5</u>	<u>0.5</u>	<u>2</u>
Moxifloxacin	<u>1</u>	<u>0.5</u>	<u>2</u>	<u>2</u>	8	<u>0.5</u>	4	<u>0.5</u>	<u>0.5</u>	<u>1</u>
Linezolid	16	<u>8</u>	<u>8</u>	<u>8</u>	52	<u>4</u>	<u>8</u>	<u>2</u>	<u>4</u>	<u>4</u>
Rifampicin	<u>1</u>	<u>1</u>	<u>0.25</u>	4	>8	<0.06	<u>1</u>	<u>0.25</u>	<u>1</u>	<u>0.5</u>
Rifabutin	<0.06	<0.06	<u>0.12</u>	<u>0.5</u>	<u>1</u>	<0.06	<u>0.12</u>	<u>0.12</u>	<0.06	<u>0.12</u>
Trimethoprim/sulfamethoxazole	<u>2/38</u>	>8/152	<u>4/76</u>	>8/152	8/152	<u>0.5/9.5</u>	<u>2/38</u>	<u>1/19</u>	<u>1/19</u>	<u>4/76</u>
Minocycline	16	>32	<u>8</u>	32	32	<u>4</u>	16	<u>4</u>	32	<u>8</u>
Ethambutol	>32	>32	32	16	>32	<u>8</u>	8	8	32	<u>16</u>
Clarithromycin	<0.5	<u>8</u>	<0.5	<0.5	<u>8</u>	<0.5	<0.5	<0.5	<0.5	<0.5
Amikacin	<u>8</u>	32	<u>4</u>	<u>8</u>	32	<u>1</u>	<u>2</u>	<u>8</u>	<u>16</u>	<u>2</u>
Streptomycin	<u>8</u>	32	<u>8</u>	<u>8</u>	16	<u>4</u>	<u>1</u>	<u>8</u>	<u>16</u>	<u>2</u>

ATCC 19981^T and *M. parascrofulaceum* CIP 108112^T by a genetic distance of 0.29. Since reference isolates *M. scrofulaceum* ATCC 19981^T and *M. parascrofulaceum* CIP 108112^T were separated by a relative genetic distance of 0.216, the MEE results provided evidence that the cluster of '*M. paraffinicum*' isolates represented a separate species.

The IWGMT semantide-based study emphasized a variety of characteristics that were definitive for '*M. paraffinicum*', including 16S rRNA gene sequencing, alpha-antigen analysis, T-catalase analysis and results from different DNA–DNA hybridization tests (Wayne *et al.*, 1996). The fluorimetric DNA–DNA hybridization results were correlated with the recommended 70 % DNA cut-off value for species and defined the reference strain of '*M. paraffinicum*' as unique. They demonstrated that the reference strain was 'an isolated strain that exhibits no unequivocal taxonomic relatedness to any other known culture' (Wayne *et al.*, 1996). Although a whole-genome reference DNA probe hybridization method showed a positive reaction with *M. scrofulaceum*, it was acknowledged that the control probe set did not contain a DNA probe for *M. paraffinicum* and only included an *M. scrofulaceum* probe. We confirmed the genetic affiliation with phylogenetic analysis of the 16S rRNA gene for the reference strain and for the five additional strains and demonstrated the distinct monophyletic tree branching of '*M. paraffinicum*' within the pigmented, slowly growing mycobacteria. Using almost-complete 16S rRNA gene sequences (1483 bp), '*M. paraffinicum*' isolates exhibited a unique sequence on a separate phylogenetic tree branch with 99.8, 98.7 and 98.4 % similarity to the type strains of *M. scrofulaceum*, *Mycobacterium nebraskense* and *Mycobacterium malmoense*, respectively (Fig. 1). No intraspecies variation was found. Further comparative study using *hsp65* sequencing (401 bp) showed that the '*M. paraffinicum*' isolates exhibited 96.0, 96.0, 95.0 and 96.5 % similarity, respectively, to the type strains of *M. scrofulaceum*, *M. parascrofulaceum*, *M. nebraskense* and *M. malmoense* and that these species were on a distinct branch (Supplementary Fig. S3). An analogous result was revealed by comparing 745 bp of the *rpoB* gene; the '*M. paraffinicum*' isolates exhibited

94.7, 96.2 and 90.7 % similarity, respectively, to the type strains of *M. scrofulaceum*, *M. nebraskense* and *M. malmoense*, and these species were on a separate branch (Supplementary Fig. S4). No intraspecies variation was found. The *rpoB* nucleotide sequence similarity was below the 97.1 % cut-off value recommended for new species recognition (Adékambi & Drancourt, 2004). A phylogenetic tree consisting of concatenated 16S rRNA, *hsp65* and *rpoB* housekeeping gene sequences (2629 bp) demonstrated genetic robustness and clearly discriminated the '*M. paraffinicum*' isolates on a separate branch (Supplementary Fig. S5).

The recent description of *Mycobacterium seoulense* indicated a close 16S rRNA gene relationship (>99 % sequence similarity) with *M. scrofulaceum* and *M. nebraskense* and implied a possible association with '*M. paraffinicum*'. However, GenBank comparative BLAST alignments of the 16S rRNA gene of *M. seoulense* 03-19^T (accession no. DQ536403; Mun *et al.*, 2007) with '*M. paraffinicum*' ATCC 12670 (GQ153270) demonstrated differences in 12 positions, and the branching of the 16S rRNA gene tree showed a divergent position for *M. seoulense* (Fig. 1).

'*M. paraffinicum*' was described as an environmental NTM specifically associated with hydrocarbon-laden soil beneath sludge ponds in oil and gas production sites, but a lack of study cultures prevented semantide studies as recommended by IWGMT (Wayne & Sramek, 1992). The strain that provided the sequence deposited in GenBank was designated the reference strain of '*M. paraffinicum*' but the name has not been validly published. However, the name is not on the list of rejected names (*nomina rejicienda*; Rule 56a of the Bacteriological Code; Lapage *et al.*, 1992) and is therefore available for valid publication. Molecular systematics of multiple strains clearly supported the revival of the name. Therefore, after more than five decades of taxonomic confusion, we propose the revival of the name '*Mycobacterium paraffinicum*' as a *nomen revictum* (Rules 28a and 33c of the Bacteriological Code) for the same organism to which the name was originally applied. It is our intent that the revival of the name be perpetuated in

Fig. 1. Neighbour-joining phylogenetic tree based on 1483 bp of the 16S rRNA gene sequence from multiple strains of '*M. paraffinicum*'. Asterisks at nodes indicate common groupings also demonstrated in the maximum-parsimony and maximum-likelihood trees (not shown). The tree is unrooted and shows closely related species. *Mycobacterium* species with known nomenclature are noted with strain designations and GenBank accession numbers. Bootstrap percentages are provided at branch points. Bar, 0.2 % difference in nucleotide sequences.

subsequent revisions to include the original authors (revised Rule 33c; Young & Euzéby, 2008). The original 1956 isolate that was deposited as ATCC 12670 is designated the type strain of *Mycobacterium paraffinicum*. Strains in other international collections originated from this original ATCC deposit and are listed in this publication. This type strain has been deposited in multiple collections as noted below and satisfies the requirements of the Bacteriological Code for deposit of type cultures in two or more collections in different countries.

Extensive physiological characteristics are given in earlier studies (Davis *et al.*, 1956; Wayne *et al.*, 1989, 1991, 1996).

Description of *Mycobacterium paraffinicum* (ex Davis *et al.* 1956) sp. nov., nom. rev.

Mycobacterium paraffinicum (par.af.fi'ni.cum. N.L. n. *paraffinicum* paraffin; L. neut. suff. -icum suffix used with the sense of pertaining to; N.L. neut. adj. *paraffinicum* pertaining to paraffin).

The original description of '*Mycobacterium paraffinicum*' (Davis *et al.*, 1956) is emended to reflect genetic properties, drug MICs and the mycolic acid profile, as presented here. The description is as given by Davis *et al.* (1956) for a slowly growing, soil-dwelling, scotochromogenic bacterium that uses paraffinic hydrocarbons other than methane and also incorporates additional characteristics described by IWGMT studies (Wayne *et al.*, 1989, 1991, 1996; Wayne & Sramek, 1992). Bacilli are long, slender rods, 0.5–0.7 µm wide and 3.0–7.0 µm long. Cells are non-motile, Gram-positive and acid–alcohol-fast, with terminal stained granules. Colonies are yellow, waxy and smooth or wrinkled on LJ medium. Grows on Middlebrook 7H10 and 7H11 agar and in 7H9 broth. Growth occurs slowly, in >7 days. Growth at 22 °C is stunted; optimum growth at 35 °C and no growth at 45 °C. Typical biochemical characteristics of the species are negative tests for urease, nicotinamidase and pyrazinamidase and positive results for Tween opacity (5 weeks). Strains are susceptible to amikacin, rifabutin, clarithromycin and linezolid and resistant to ethambutol. Cell walls contain alpha, keto and wax ester mycolic acids characteristic of species associated with the *Mycobacterium avium* complex. Conclusive identification is by molecular sequence analysis of the 16S rRNA, *hsp65* or *rpoB* gene.

The type strain is ATCC 12670^T (=DSM 44181^T =NCIMB 10420^T), deposited by J. B. Davis, Magnolia Petroleum Co., Easton, LA, USA, in the ATCC in 1956.

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