INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY

TAXONOMIC DESCRIPTION
Kim et al., Int J Syst Evol Microbiol 2017;67:3882–3887
DOI 10.1099/ijsem.0.002217

MICROBIOLOGY SOCIETY

Description of *Mycobacterium chelonae* subsp. *bovis* subsp. nov., isolated from cattle (*Bos taurus coreanae*), emended description of *Mycobacterium chelonae* and creation of *Mycobacterium chelonae* subsp. *chelonae* subsp. nov.

Byoung-Jun Kim,[1] Ga-Na Kim,[1] Bo-Ram Kim,[1] Che Ok Jeon,[2] Joseph Jeong,[3] Seon Ho Lee,[3] Ji-Hun Lim,[3] Seung-Heon Lee,[4] Chang Ki Kim,[4] Yoon-Hoh Kook[1] and Bum-Joon Kim[1,*]

Abstract

Three rapidly growing mycobacterial strains, QIA-37^T, QIA-40 and QIA-41, were isolated from the lymph nodes of three separate Korean native cattle, Hanwoo (*Bos taurus coreanae*). These strains were previously shown to be phylogenetically distinct but closely related to *Mycobacterium chelonae* ATCC 35752^T by taxonomic approaches targeting three genes (16S rRNA, *hsp6* and *rpoB*) and were further characterized using a polyphasic approach in this study. The 16S rRNA gene sequences of all three strains showed 99.7 % sequence similarity with that of the *M. chelonae* type strain. A multilocus sequence typing analysis targeting 10 housekeeping genes, including *hsp65* and *rpoB*, revealed a phylogenetic cluster of these strains with *M. chelonae*. DNA–DNA hybridization values of 78.2 % between QIA-37^T and *M. chelonae* indicated that it belongs to *M. chelonae* but is a novel subspecies distinct from *M. chelonae*. Phylogenetic analysis based on whole-genome sequences revealed a 95.44±0.06 % average nucleotide identity (ANI) value with *M. chelonae*, slightly higher than the 95.0 % ANI criterion for determining a novel species. In addition, distinct phenotypic characteristics such as positive growth at 37 °C, at which temperature *M. chelonae* does not grow, further support the taxonomic status of these strains as representatives of a novel subspecies of *M. chelonae*. Therefore, we propose an emended description of *Mycobacterium chelonae*, and descriptions of *M. chelonae* subsp. *chelonae* subsp. nov. and *M. chelonae* subsp. *bovis* subsp. nov. are presented; strains ATCC 35752^T(=CCUG 47445^T=CIP 104535^T=DSM 43804^T=JCM 6388^T=NCTC 946^T) and QIA-37^T (=KCTC 39630^T=JCM 30986^T) are the type strains of the two novel subspecies.

Non-tuberculous mycobacteria (NTM), which are ubiquitous in the environment and reside in soil and water, can be opportunistic pathogens in humans and animals [1, 2]. In particular, rapidly growing mycobacteria (RGM) strains capable of growth within 7 days on solid agar, including *Mycobacterium abscessus*, *Mycobacterium chelonae* and *Mycobacterium fortuitum*, have increased in clinical importance, due to their increased isolation frequency from clinical specimens [3, 4]. *M. chelonae* is one of the most frequently encountered species of RGM strains. These ubiquitous organisms are present in many different environments, including water, soil and dust [5]. They can cause a variety of diseases including respiratory tract infections, skin and soft tissue infections, and biomaterial-related infections [6]. From a taxonomic view, *M. chelonae* consists of only one subspecies represented by the strain ATCC 35752^T [7], which is phylogenetically closest to the *M. abscessus* complex, in which three subspecies have recently been

Author affiliations: [1]Department of Biomedical Sciences, Microbiology and Immunology, Cancer Research Institute, Institute of Endemic Diseases, and Liver Research Institute, Seoul National University Medical Research Center (SNUMRC), Seoul National University College of Medicine, Seoul, Republic of Korea; [2]School of Biological Sciences, Chung-Ang University, Seoul, Republic of Korea; [3]Department of Laboratory Medicine, Ulsan University Hospital, University of Ulsan College of Medicine, Ulsan, Republic of Korea; [4]Korean Institute of Tubercuosis, Chungbuk, Republic of Korea.
***Correspondence:** Bum-Joon Kim, kbumjoon@snu.ac.kr
Keywords: rapid growing mycobacteria; multilocus sequence typing; *Mycobacterium chelonae*; *Mycobacterium chelonae* subsp. *chelonae*; *Mycobacterium chelonae* subsp. *bovis*.
Abbreviations: ADC, albumin-dextrose-catalase; ANI, average nucleotide identity; MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight; MLST, multi-locus sequence typing; NTM, non-tuberculous mycobacteria; OADC, oliec-albumin-dextrose-catalase; RGM, rapidly growing mycobacteria.
The Genbank/EMBL/DDBJ accession numbers for the partial 16S rRNA, *argH*, *cya*, *glpK*, *gnd*, *murC*, *pgm*, *pta* and *purH* genes from strains QIA-37^T, QIA-40 and QIA-41 are KY593892 ~ KY593894 (16S rRNA), KY593895 ~ KY593897 (*argH*), KY593898 ~ KY593900 (*cya*), KY593901 ~ KY593903 (*glpK*), KY593904 ~ KY593906 (*gnd*), KY593907 ~ KY593909 (*murC*), KY593910 ~ KY593912 (*pgm*), KY593913 ~ KY593915 (*pta*) and KY593916 ~ KY593918 (*purH*), respectively. The accession numbers are listed in Table S3.
Sixteen supplementary tables and seven supplementary figures are available with the online Supplementary Material.

proposed as *M. abscessus* subsp. *abscessus*, *M. abscessus* subsp. *bolletii* and *M. abscessus* subsp. *massiliense* [8].

Recently, the application of molecular biology techniques to the identification of isolates from environmental sources and clinical specimens has fueled an increased awareness of the diversity within NTM [9]. To date, a variety of novel NTM species have been successfully introduced, mainly from Korean pulmonary patients [10–13]. Furthermore, we have recently reported on the existence of some phylogenetically distinct mycobacterial strains isolated from Korean native cattle, Hanwoo (*Bos taurus coreanae*), via combinatorial molecular taxonomic approaches targeting three genes, the 16S rRNA (1.2–1.3 kb), *hsp65* (603 bp) and *rpoB* (711 bp) genes [14].

In this study, three strains, designated QIA-37^T, QIA-40, and QIA-41, were isolated from the lymph nodes of three independently slaughtered Hanwoo cattle (*Bos taurus coreanae*) in Korea (The Animal and Plant Quarantine Agency) as described previously [14]. Isolated strains were subcultured in both Middlebrook 7H9 broth (Becton Dickinson) supplemented with albumin-dextrose-catalase (ADC) and Middlebrook 7H10 agar (Becton Dickinson) plate supplemented with oleic-albumin-dextrose-catalase (OADC) for 3 to 5 days.

The phenotypic characteristics of strains QIA-37^T, QIA-40 and QIA-41 were analysed by determination of colony morphologies and growth patterns at various temperatures (25, 30, 37 and 45 °C) on Middlebrook 7H10 agar plates (supplemented with OADC) or in Middlebrook 7H9 broth (supplemented with ADC) for 3 to 5 days. In addition, the enzyme activities of alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase were analysed using an API ZYM kit (bioMérieux) following the manufacturer's instructions and comparing the results with those of the related RGM reference strains, *M. abscessus* subsp. *abscessus* ATCC 19977^T, *M. abscessus* subsp. *massiliense* CIP 108297^T and *M. chelonae* ATCC 35752^T. Antimicrobial susceptibilities were also determined by the agar proportion method on Muller–Hinton agar medium (supplemented with OADC) [15]. Antimicrobial reagents tested included amikacin, cefoxitin, ciprofloxacin, clarithromycin, doxycycline, imipenem, moxifloxacin, rifampicin, trimethoprim/sulfamethoxazole, tobramycin, ethambutol and linezolid.

All three strains (QIA-37^T, QIA-40 and QIA-41) showed rod-shaped morphologies and were acid-fast bacilli with no spores or filaments. Typically, rough and white mature colonies of these strains developed in about 3 to 5 days on the Middlebrook 7H10 agar plates. Additionally, all the strains showed similar growth patterns and grew well at both 30 °C and 37 °C, unlike *M. chelonae* ATCC 35752^T, which could not grow at 37 °C (Table 1). None of the strains could be grown at 45 °C.

Biochemical phenotypic traits were almost similar between the three strains (QIA-37^T, QIA-40 and QIA-41) and the three reference strains (*M. abscessus* subsp. *abscessus* ATCC 19977^T, *M. abscessus* subsp. *massiliense* CIP 108297^T and *M. chelonae* ATCC 35752^T). They proved to be positive for the production of 10 enzymes, including esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, α-glucosidase, β-glucosidase and α-mannosidase. However, they tested negative for the production of alkaline phosphatase, trypsin, α-chymotrypsin, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase and α-fucosidase. In the case of β-galactosidase, except for *M. abscessus* subsp. *massiliense* CIP 108297^T, all the strains showed weakly positive activities (Table 1).

Although variations in antibiotics resistance patterns were found between the three novel strains (QIA-37^T, QIA-40 and QIA-41), they showed a higher level of resistance to most antibiotics than *M. chelonae* ATCC 35752^T. It is noteworthy that these three strains showed a similar level of inducible resistance to clarithromycin as observed in *M. chelonae* (≤0.5–1 μg ml^{-1}), which is lower than that observed in *M. abscessus* subsp. *abscessus* ATCC 19977^T, which shows inducible resistance to clarithromycin at more than 64 μg ml^{-1} (Table S1, available in the online Supplementary Material).

Table 1. Comparison of the culture and enzyme activity characteristics among the *M. abscessus – M. chelonae* group strains

Strains: 1, QIA-37^T; 2, QIA-40; 3, QIA-41; 4, *M. abscessus* subsp. *abscessus* ATCC 19977^T; 5, *M. abscessus* subsp. *massiliense* CIP 108297^T; 6, *M. chelonae* ATCC 35752^T. +, Growth/positive; −, no growth/negative; ±, weakly positive. All strains were positive for growth at 25 and 30 °C, detectable growth in <7 days and after >7 days, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, α-glucosidase, β-glucosidase and α-mannosidase. All strains were negative for growth at 45 °C, alkaline phosphatase, trypsin, α-chymotrypsin, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase and α-fucosidase. R, rough; S, smooth; W, white.

Characteristic	1	2	3	4	5	6
Growth at 37 °C	+	+	+	+	+	−
Morphology	RW	RW	RW	RW	SW	RW
β-Galactosidase (API ZYM)	±	±	±	±	−	±

To analyse the total lipids from strains QIA-37^T, QIA-40 and QIA-41, lipids were extracted with $CHCl_3/CH_3OH$ (1 : 1, v/v, adding 0.5 µl 2,5-dihydroxybenzoic acid) from 30 ml of 7H9 broth cultures. Total lipids from other RGM strains (*M. abscessus* subsp. *abscessus* ATCC 19977^T, *M. abscessus* subsp. *massiliense* CIP 108297^T and *M. chelonae* ATCC 35752^T) were also extracted. Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry analysis was carried out on the samples extracted by using a Voyager DE-STR MALDI-TOF instrument (Perseptive Biosystems) equipped with a pulse nitrogen laser emitting at 337 nm as reported by Pérez *et al.* [16].

The mass spectra of the three strains (QIA-37^T, QIA-40 and QIA-41) and the other reference strains (*M. abscessus* subsp. *abscessus* ATCC 19977^T, *M. abscessus* subsp. *massiliense* CIP 108297^T and *M. chelonae* ATCC 35752^T) exhibited two distinct clusters of peaks ranging from *m/z* 1205.29±8.11 to *m/z* 1300.44±12.52 and from *m/z* 1354.10±7.91 to *m/z* 1461.46±8.58. The first and second clusters represent diglycosylated glycopeptidolipid (DG-GPL) and triglycosylated GPL (TG-GPL), respectively [17, 18]. Although these strains showed similar MALDI-TOF profiles, the three novel strains showed a higher intensity of the second cluster (corresponding to TG-GPL) than of the first cluster, which was distinct from *M. chelonae* ATCC 35752^T (Fig. S1).

To compare the mycolic acid profiles of strains QIA-37^T, QIA-40, QIA-41 and additional RGM strains (*M. abscessus* subsp. *abscessus* ATCC 19977^T, *M. abscessus* subsp. *massiliense* CIP 108297^T and *M. chelonae* ATCC 35752^T), extracted mycolic acids were analysed by HPLC as described by Butler *et al.* [19]. Additionally, to identify and compare the mycolic acid profiles, an online HPLC mycobacterium library (available at http://www.MycobacToscana.it) was used.

Generally, HPLC profiles of mycolic acids from all the strains assayed exhibited similar patterns, showing two late-emerging clusters of peaks, a trait suggesting that these strains are related to each other as a *M. abscessus – M. chelonae* group. The profiles were characterized by continuous peaks between 3.7 and 6.4 min, with the two most prominent clusters occurring at approximately 4.9 and 6.0 min (Fig. S2).

DNA–DNA hybridization was carried out to verify the DNA relatedness between strains *M. chelonae* ATCC 35752^T and QIA-37^T as previously described [11, 13, 20, 21]. The hybridization signals were detected using a scanner (HP Scanjet 3770) and analysed using the Adobe Photoshop program (version 7.0). The signal produced by the hybridization of the probe to the homologous target DNA was taken to be 100 %, and signal intensities by the self-hybridization of the series of dilutions were used for calculating the levels of DNA relatedness between strains *M. chelonae* ATCC 35752^T and QIA-37^T.

The DNA–DNA hybridization was confirmed by cross-hybridization. The DNA–DNA relatedness between *M. chelonae* ATCC 35752^T and QIA-37^T was 78.2±0.90 %, above the 70 % DNA–DNA relatedness that is generally used as a standard for species delineation [22], suggesting that QIA-37^T belongs to the same species as *M. chelonae* ATCC 35752^T.

For the multi-locus sequence typing (MLST) based phylogenetic analysis, genomic DNA was extracted from cultured colonies of three strains, QIA-37^T, QIA-40 and QIA-41, using a previously reported bead-beater phenol extraction method [23]. The sequence data of three target genes, 16S rRNA (1431 bp), heat shock protein 65 (*hsp65*) (603 bp) and RNA polymerase β-subunit (*rpoB*) (711 bp), which had already been analysed in our previous study [14], were used after extraction from the GenBank database. The sequences of eight MLST genes, including argininosuccinate lyase (*argH*) (453 bp), adenylate cyclase (*cya*) (452 bp), glycerol kinase (*glpK*) (554 bp), 6-phosphogluconate dehydrogenase (*gnd*) (513 bp), UDP *N*-acetylmuramate-L-Ala ligase (*murC*) (405 bp), phosphoglucomutase (*pgm*) (495 bp), phosphate acetyltransferase (*pta*) (486 bp) and phosphoribosylaminoimidazole carboxylase ATPase subunit (*purH*) (502 bp) [24], were further analysed in this study. All the primer sequences are listed in Table S2. For the taxonomical analysis, the sequences obtained were aligned using the multiple alignment algorithm in the MegAlign software package as described previously [23]. Evolutionary distance matrices were generated according to the Jukes and Cantor model [25]. Phylogenetic trees were inferred from the 11 target gene sequences using the neighbour-joining [26] and maximum-parsimony [27] methods in the MEGA software (version 4.0) [28]. All the sequences obtained were deposited in the GenBank database, and the details are listed in Table S3.

In all cases, the three strains (QIA-37^T, QIA-40 and QIA-41) had sequences that were identical to each other. Levels of sequence similarity in the 16S rRNA gene among the three strains (QIA-37^T, QIA-40 and QIA-41) and additional RGM strains, including *M. abscessus* subsp. *abscessus* ATCC 19977^T, *M. abscessus* subsp. *bolletii* CIP 108541^T, *M. abscessus* subsp. *massiliense* CCUG 48898^T and *M. chelonae* ATCC 35752^T, ranged from 99.8 % (compared with *M. chelonae*) to 99.9 % (compared with *M. abscessus* subsp. *abscessus*, *M. abscessus* subsp. *massiliense* and *M. abscessus* subsp. *bolletii* type strains) (Table S4). The three novel strains (QIA-37^T, QIA-40 and QIA-41) showed no differences in hypervariable regions of the 16S rRNA gene sequence compared with other RGM strains (*M. abscessus* subsp. *abscessus* ATCC 19977^T, *M. abscessus* subsp. *bolletii* CIP 108541^T, *M. abscessus* subsp. *massiliense* CCUG 48898^T and *M. chelonae* ATCC 35752^T) (Fig. S3). However, three different nucleotides (those corresponding to the 967th, 977th and 1006th nucleotides of the 16S rRNA gene sequence of *M. abscessus* ATCC 19977^T) were found in the other regions of the 16S rRNA gene sequences compared between the three strains and *M. chelonae* ATCC 35752^T. The neighbour-joining tree generated from the 16S rRNA gene sequences indicated that the three strains were clustered

together and with the *M. abscessus* – *M. chelonae* group (Fig. S4). Unlike the 16S rRNA-based tree showing 99.9 % sequence similarity with the three subspecies of *M. abscessus*, the three strains were closely clustered with *M. chelonae* ATCC 35752^T (=CCUG 47445^T) in the *hsp65* and *rpoB* gene based phylogenetic analyses (Fig. S5). In the partial *hsp65* gene (603 bp) sequence, the three strains showed 5 bp differences with *M. chelonae* ATCC 35752^T, resulting in a 99.2 % sequence similarity (Table S5). In the partial *rpoB* gene (711 bp) sequence, the three strains showed 4 bp differences with *M. chelonae* CCUG 47445^T (99.4 %) (Table S6).

Using the eight MLST gene sequences, including *argH*, *cya*, *glpK*, *gnd*, *murC*, *pgm*, *pta* and *purH* genes, single-gene-based phylogenetic trees were also reconstructed. All the trees showed that the three strains (QIA-37^T, QIA-40 and QIA-41) were clustered together with *M. chelonae*. These results were also supported by high bootstrap values (98~100 %) and maximum-parsimony trees (Fig. S6). The sequence similarity between the three strains and *M. chelonae* was highest with *gnd* and lowest with *argH*. In the case of the partial *gnd* gene sequence, similarity was 97.0 % between the three strains and *M. chelonae*, but in the partial *argH* sequence, sequence similarity was 94.3 % (Tables S7 and S8). With the other partial *cya*, *glpK*, *murC*, *pgm*, *pta* and *purH* sequences, sequence similarities were 94.7, 96.0, 96.5, 94.5, 97.1 and 94.6 % between the three strains and *M. chelonae*, respectively (Tables S9–S14).

A phylogenetic tree based on the concatenated sequences (5173 bp) of a total of 10 genes, including *hsp65*, *rpoB* and the eight MLST gene fragments, also showed the phylogenetic location of the three strains (QIA-37^T, QIA-40 and QIA-41) clustered with *M. chelonae*, having a high bootstrap value of 100 %, and this node was also supported with maximum-parsimony-based topology (Fig. 1). Sequence similarity based on the concatenated sequence was 96.5 % between the three strains and *M. chelonae*, but it was 87.9 ±0.4 % between the three strains and other RGM strains (Table S15). Concatenated sequence similarity between the cluster of *M. chelonae* (96.5 % sequence similarity between the three strains and *M. chelonae*) was lower than those between the three subspecies of *M. abscessus* (*M. abscessus* subsp. *abscessus*, *M. abscessus* subsp. *massiliense* and *M. abscessus* subsp. *bolletii*; 97.8±0.4 %) (Table S15). This result suggested that the three strains, QIA-37^T, QIA-40 and QIA-41, were close phylogenetically to *M. chelonae* but should be differentiated from the pre-described *M. chelonae* ATCC 35752^T (=CCUG 47445^T) as representatives of a novel subspecies.

For the whole-genome sequence based phylogenetic comparison between QIA-37^T and other RGM strains, the whole-genome sequence of QIA-37^T was determined in this study as described previously [29, 30]. Briefly, the genomic DNA of strain QIA-37^T was sequenced on a Pacific Biosciences RS sequencer (450 876 and 290 607 reads) and an Illumina Hi-Seq sequencer (77 158 074 reads). PacBio raw data were assembled *de novo* with the hierarchical genome assembly process (HGAP) of the single-molecule real-time (SMRT) analysis software (Pacific Biosciences). After the *de novo* assembly, a total of 82 contigs were obtained, which were corrected by mapping using the CLC reference assembler compared with Illumina Hi-Seq raw data. Total mapped reads were 57 314 928, representing ~1145.1× coverage for the estimated 4.8 Mb genome size. All the remaining gaps between contigs were completely filled by gap-filling PCR amplification. After obtaining the genome sequence, gene prediction was performed using the RAST server (Rapid Annotation using Subsystems Technology, http://rast.nmpdr.org/rast.cgi). Additionally, tRNA and rRNA predictions were performed using the tRNAscan-SE (tRNAscan-SE 1.21, http://lowelab.ucsc.edu/tRNAscan-SE/) and RNAmmer (RNAmmer 1.2 server, http://www.cbs.dtu.dk/services/RNAmmer/) programs, respectively. Sequence analysis was performed in the National Instrumentation Centre for Environmental Management (NICEM) (Genome analysis unit) at Seoul National University.

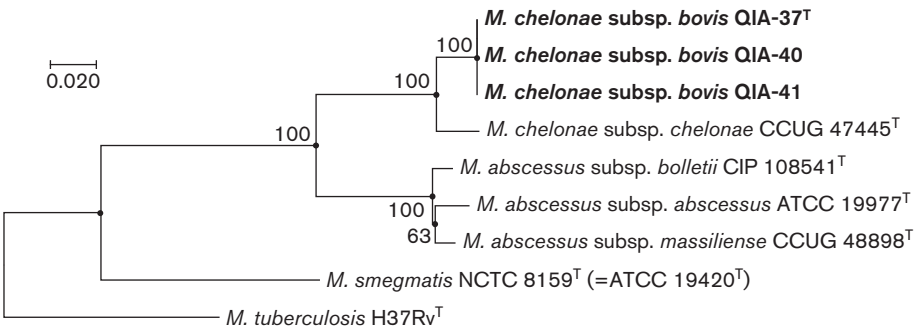

Fig. 1. Phylogenetic relationship of the three strains (QIA-37^T, QIA-40 and QIA-41) among other species of the genus *Mycobacterium* based on concatenated sequences (*hsp65*, *rpoB* and eight MLST genes; 5173 bp). These trees were reconstructed using the neighbour-joining method. Bootstrap values were calculated from 1000 replications. Values <50 % are not shown. Filled circles indicate that the corresponding groups were supported in the maximum-parsimony trees. Sequences of *M. tuberculosis* were used as an outgroup. Bar, substitutions per nucleotide position.

Using the whole-genome sequences of strains *M. chelonae* CCUG 47445^T (NZ_CP007220) and QIA-37^T (NZ_CP010071), genome-based multiple alignments were performed by the Mauve alignment algorithm (http://asap.genetics.wisc.edu/software/mauve/) and visualized by the TreeViewX program (http://darwin.zoology.gla.ac.uk/~rpage/treeviewx/). In addition, the average nucleotide identity (ANI), which may be used to replace DNA–DNA hybridization [31], was measured using the CLgenomics program (http://chunlab.com/software_clgenomics).

The reconstructed tree showed that strain QIA-37^T was closely clustered with *M. chelonae* CCUG 47445^T (Fig. S7), as shown in our MLST-based phylogenetic analysis. The ANI value between strain QIA-37^T and *M. chelonae* CCUG 47445^T (95.44 %±0.06) was slightly higher than the recommended cut-off point of 95 % ANI for species delineation [31], but was always lower than those comparing the type strains of the three subspecies of *M. abscessus*, *M. abscessus* subsp. *abscessus* ATCC 19977^T, *M. abscessus* subsp. *massiliense* CIP 108297^T and *M. abscessus* subsp. *bolletii* CIP 108541^T (*M. abscessus* subsp. *abscessus* vs *M. abscessus* subsp. *massiliense*=96.95±0.11; *M. abscessus* subsp. *abscessus* vs *M. abscessus* subsp. *bolletii*=97.15±0.02; *M. abscessus* subsp. *massiliense* vs *M. abscessus* subsp. *bolletii*=97.00±0.03) (Table S16), strongly supporting that QIA-37^T represents a novel subspecies of *M. chelonae*.

In conclusion, our DNA–DNA relatedness analysis, whole-genome sequence and 10 MLST gene sequence based phylogenetic analyses indicated that the three *M. chelonae*-related strains (QIA-37^T, QIA-40 and QIA-41) isolated from Korean native cattle, Hanwoo (*Bos taurus coreanae*), represent a novel subspecies of *M. chelonae* distinct from *M. chelonae* ATCC 35752^T (=CCUG 47445^T) rather than a mere variant of *M. chelonae*. In addition, their distinct phenotypic traits such as positive growth at 37 °C, which is not possible for *M. chelonae* ATCC 35752^T, and their distinct MALDI-TOF MS profile further support the above assertion. Therefore, we propose that *M. chelonae* should be divided into two subspecies. An emended description of *Mycobacterium chelonae* and descriptions of *M. chelonae* subsp. *chelonae* subsp. nov., and *Mycobacterium chelonae* subsp. *bovis* subsp. nov. are presented; strains ATCC 35752^T and QIA-37^T are the type strains of the two new subspecies.

EMENDED DESCRIPTION OF *MYCOBACTERIUM CHELONAE*

M. chelonae (che.lo′na.e. N.L. gen. n. *chelonae* of a tortoise).

The description is as those given for *Mycobacterium chelonei* subsp. *chelonei* in 1972 [32] and *M. chelonae* in 1992 [7]. It grows well at lower temperatures (~30 °C) than other mycobacterial species. It shows greater susceptibility to several antibiotics than *M. abscessus* and *M. massiliense*.

The type strain is ATCC 35752^T(=CCUG 47445^T=CIP 104535^T=DSM 43804^T=JCM 6388^T=NCTC 946^T).

DESCRIPTION OF *MYCOBACTERIUM CHELONAE* SUBSP. *CHELONAE* SUBSP. NOV.

M. chelonae subsp. *chelonae* (che.lo′na.e. N.L. gen. n. *chelonae* of a tortoise).

The description is as that given for *Mycobacterium chelonei* with the following addition. It grows poorly at 37 °C.

The type strain is ATCC 35752^T(=CCUG 47445^T=CIP 104535^T=DSM 43804^T=JCM 6388^T=NCTC 946^T).

DESCRIPTION OF *MYCOBACTERIUM CHELONAE* SUBSP. *BOVIS* SUBSP. NOV.

M. chelonae subsp. *bovis* (bo′vis. L. gen. n. *bovis* of the ox, pertaining to hanwoo, a Korean native cattle from which the type strain was isolated).

Cells are acid-alcohol-fast, aerobic, non-motile, and non-spore forming rods with frequently curved, Gram-stain-positive bacilli. It grows well at both 30 °C and 37 °C. On Middlebrook 7H10 agar medium, white and rough mature colonies develop within 5 days. Cells show positive or weakly positive responses for esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, β-galactosidase, α-glucosidase, β-glucosidase and α-mannosidase. Cells show negative responses for alkaline phosphatase, trypsin, α-chymotrypsin, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase and α-fucosidase activities. The MALDI-TOF MS profiles of total lipids exhibit two clusters of peaks, the first cluster ranging from *m/z* 1205.29±8.11 to *m/z* 1300.44±12.52 and the second cluster ranging from *m/z* 1354.10±7.91 to *m/z* 1461.46 ±8.58. HPLC profiles of mycolic acids represent the two late-emerging clusters of peaks. Phylogenetic analysis based on *hsp65*, *rpoB*, eight MLST genes and whole-genome sequence shows that it is closely related to *M. chelonae* within the rapidly growing mycobacteria group. Genetically, all the gene sequences determined (16S rRNA, *hsp65*, *rpoB*, *argH*, *cya*, *glpK, gnd*, *murC*, *pgm*, *pta* and *purH*) are unique. Phylogenetic analyses based on whole-genome sequences and concatenated gene sequences show that it belongs to the rapidly growing mycobacteria and is closely related to *Mycobacterium chelonae* subsp. *chelonae* ATCC 35752^T.

The type strain is QIA-37^T (=KCTC 39630^T=JCM 30986^T), isolated from Korean native cattle, Hanwoo (*Bos taurus coreanae*).

Funding information
This research was supported by the National Research Foundation of Korea (NRF) funded by the Ministry of Education (grant no. NRF-2016R1A2B4011847).

Acknowledgements
Bo-Ram Kim received a scholarship from the BK21-plus education program provided by the National Research Foundation of Korea.

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

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