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TAXONOMIC DESCRIPTION
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Phylogenomics reveal that *Mycobacterium kansasii* subtypes are species-level lineages. Description of *Mycobacterium pseudokansasii* sp. nov., *Mycobacterium innocens* sp. nov. and *Mycobacterium attenuatum* sp. nov.

Florian Tagini,[1] Sébastien Aeby,[1] Claire Bertelli,[1] Sara Droz,[2] Carlo Casanova,[2] Guy Prod'hom,[1] Katia Jaton[1] and Gilbert Greub[1,3,*]

Abstract

Among the species *Mycobacterium kansasii*, seven subtypes have been previously reported based on the PCR and the restriction fragment length polymorphism of the gene *hsp65*. Here, we used whole-genome sequencing to refine *M. kansasii* taxonomy and correct multiple inconsistencies. Average nucleotide identity (ANI) values between *M. kansasii* subtypes ranged from 88.4 to 94.2 %, lower than the accepted 95–96 % cut-off for species delineation. In addition, *Mycobacterium gastri* was closer to the *M. kansasii* subtypes 1, 2, 3, 4 and 5 than *M. kansasii* subtype 6. The recently described species *Mycobacterium persicum* shared 99.77 % ANI with *M. kansasii* subtype 2. Consistent with the ANI results, the digital DNA–DNA hybridization value was below the 70 % threshold for species delineation between subtypes and above it within subtypes as well as between subtype 2 and *M. persicum*. Furthermore, core-genome phylogeny confirmed the current *M. kansasii* species to be polyphyletic. Hence, we propose (i) *Mycobacterium pseudokansasii* sp. nov., replacing subtype 3, with the type strain MK142^T (=CCUG 72128^T=DSM 107152^T), (ii) *Mycobacterium innocens* sp. nov., replacing subtype 5, with the type strain MK13^T (=CCUG 72126^T=DSM 107161^T), and (iii) *Mycobacterium attenuatum* sp. nov., replacing subtype 6, with the type strain MK41^T(=CCUG 72127^T=DSM 107153^T). Subtype 4 represents a new species-level lineage based on the genomic data but no strain was available. No genome sequence or strain was available for subtype 7. The proposed nomenclature will facilitate the identification of the most pathogenic subtype 1 as *M. kansasii* by clinicians while the new species names suggest the attenuated pathogenicity of the other subtypes.

The species *Mycobacterium kansasii*, a member of slow-growing non-tuberculous mycobacteria, is an environmental mycobacterium causing opportunistic infections in humans. *Mycobacterium kansasii* was first described in 1953 [1] and is one of the most frequent non-tuberculous mycobacteria isolated from patients [2–4]. Seven subtypes have been previously described based on the restriction fragment length polymorphism (RFLP) of the *hsp65* gene [5–8]. Furthermore, the *rpoB* and the *tuf* genes were also shown to successfully discriminate between subtypes [9, 10]. Subtype 1 is the most frequently isolated and most pathogenic subtype [6, 11, 12]. Subtype 2 is the second most common subtype recovered from patients, most of them with immunosuppression [62.5 % had a co-infection with human immunodeficiency virus (HIV) and 21 % were treated with corticosteroids], whereas subtype 3 is most often associated with colonization [6]. Subtypes 4–6 are very rarely isolated from patients and generally non-pathogenic [6]. Subtype 7 was – to our knowledge – only described by Taillard *et al.* and its pathogenicity remains unclear [6]. *Mycobacterium gastri*, a non-pathogenic and closely related species to *M. kansasii,* described in 1966

Author affiliations: [1]Institute of Microbiology, Department of Laboratory Medicine, Lausanne University Hospital and University of Lausanne, Lausanne, Switzerland; [2]Institute for Infectious Diseases, University of Bern, Bern, Switzerland; [3]Division of Infectious Diseases, Department of Medicine, Lausanne University Hospital, Lausanne, Switzerland.
***Correspondence:** Gilbert Greub, gilbert.greub@chuv.ch
Keywords: taxogenomics; non-tuberculous mycobacteria; atypical mycobacterium; virulence; comparative genomics; whole-genome sequencing.
Abbreviations: ANI, average nucleotide identity; DDH, digital DNA–DNA hybridations; HMMIS, high molecular mass internal standard; kb, kilobase; LMMIS, low molecular mass internal standard.
16S rRNA accession numbers: *Mycobacterium pseudokansasii* MK142, LS999932; *Mycobacterium innocens* MK13, LS999933; *Mycobacterium attenuatum* MK41, LS999934. Genome accession numbers: GCA_900565985.1, GCA_900566155.1, GCA_900565995.1, GCA_900566005.1, GCA_900566015.1, GCA_900566035.1, GCA_900566045.1, GCA_900566025.1, GCA_900566055.1, GCA_900566065.1, GCA_900566105.1, GCA_900566085.1, GCA_900566075.1.
One supplementary table and one supplementary figure are available with the online version of this article.

Table 1. Details of the strains used for the genomic analysis

Old species name	Strain	Assembly/analysis accession	Genome size (Kb)	Number of contigs	N50 (bp)	Sequencing depth	Genome sequence Source	*M. kansasii* subtype	New species proposition
Mycobacterium kansasii	ATCC 12478^T	GCF_000157895.3	6577	2	Complete	80x	NCBI	1	*Mycobacterium kansasii*
Mycobacterium kansasii	1010001495	GCF_001632965.1	6358	140	91 256	28.2x	NCBI	1	*Mycobacterium kansasii*
Mycobacterium kansasii	MK22	GCA_900565985.1	6406	187	71 547	219x	This study	1	*Mycobacterium kansasii*
Mycobacterium kansasii	MK40	GCA_900566155.1	6607	199	68 849	235x	This study	1	*Mycobacterium kansasii*
Mycobacterium kansasii	MK7	GCA_900565995.1	6414	165	87 134	331x	This study	1	*Mycobacterium kansasii*
Mycobacterium kansasii	1010001469	GCF_001632975.1	6266	165	85 083	33.8x	NCBI	2	*Mycobacterium persicum*
Mycobacterium persicum	AFPC-000227^T	GCF_002086675.1	6172	387	44 952	11x	NCBI	2	–
Mycobacterium kansasii	MK15	GCA_900566005.1	6241	190	72 560	253x	This study	2	*Mycobacterium persicum*
Mycobacterium kansasii	MK42	GCA_900566015.1	6119	188	67 218	188x	This study	2	*Mycobacterium persicum*
Mycobacterium kansasii	MK4	GCA_900566035.1	6424	195	72 167	251x	This study	2	*Mycobacterium persicum*
Mycobacterium kansasii	1010001468	GCF_001632915.1	6142	164	65 195	24.1x	NCBI	3	*Mycobacterium pseudokansasii*
Mycobacterium kansasii	MK142^T	GCA_900566075.1	6426	2	Complete	130x	This study	3	*Mycobacterium pseudokansasii*
Mycobacterium kansasii	MK21	GCA_900566045.1	6288	220	65 394	209x	This study	3	*Mycobacterium pseudokansasii*
Mycobacterium kansasii	MK35	GCA_900566025.1	6295	218	65 269	204x	This study	3	*Mycobacterium pseudokansasii*
Mycobacterium kansasii	1010001458	GCF_001632895.1	6027	188	69 082	37x	NCBI	4	Undefined at present
Mycobacterium kansasii	1010001493	GCF_001632885.1	5627	173	54 964	29.9x	NCBI	5	*Mycobacterium innocens*
Mycobacterium kansasii	1010001454	GCF_001632905.1	6172	346	41 206	25x	NCBI	5	*Mycobacterium innocens*
Mycobacterium kansasii	MK13^T	GCA_900566055.1	6187	361	35 686	189x	This study	5	*Mycobacterium innocens*
Mycobacterium kansasii	MK136	GCA_900566065.1	6345	196	72 849	232x	This study	6	*Mycobacterium attenuatum*
Mycobacterium kansasii	MK191	GCA_900566105.1	6357	221	57 930	118x	This study	6	*Mycobacterium attenuatum*
Mycobacterium kansasii	MK41^T	GCA_900566085.1	6528	230	54 408	231x	This study	6	*Mycobacterium attenuatum*
Mycobacterium gastri	DSM 43505^T	GCF_002102175.1	5817	154	85 262	105x	NCBI	–	–
Mycobacterium colombiense	CECT 3035^T	GCF_002105755.1	5582	1	Complete	340x	NCBI	–	–
Mycobacterium szulgai	DSM 44166^T	GCF_002116635.1	6673	178	95 710	122x	NCBI	–	–
Mycobacterium parascrofulaceum	ATCC BAA-614^T	GCF_000164135.1	6564	405	36 222	53x	NCBI	–	–
Mycobacterium tuberculosis	H37Rv^T	GCF_000195955.2	4412	1	Complete	–	NCBI	–	–
Mycobacterium intracellulare	ATCC 13950^T	GCF_000277125.1	5402	1	Complete	–	NCBI	–	–
Mycobacterium marinum	E11	GCF_000723425.2	6451	2	Complete	400x	NCBI	–	–
Mycobacterium avium subsp. *avium*	DJO-44271	GCF_000770235.1	5011	1	Complete	107.5x	NCBI	–	–
Mycobacterium gordonae	DSM 44160^T	GCF_002101675.1	7602	260	62 676	120x	NCBI	–	–
Mycobacterium	DSM	GCF_002102015.1	4933	146	71 256	104x	NCBI	–	–

Table 1. cont.

Old species name	Strain	Assembly/analysis accession	Genome size (Kb)	Number of contigs	N50 (bp)	Sequencing depth	Genome sequence Source	*M. kansasii* subtype	New species proposition
xenopi	43995^T								
Mycobacteroides abscessus	ATCC 19977^T	GCF_000069185.1	5090	2	Complete	–	NCBI	–	–

[13], is phenotypically distinguishable from *M. kansasii* because it is not photochromogenic. Despite *M. gastri* sharing the same 16S rRNA gene sequence as *M. kansasii* ATCC 12498^T (subtype 1), it was shown to differ in a phylogeny based on average nucleotide identity (ANI)-divergent values [14]. In 2017, *Mycobacterium persicum* was described as a new closely related species of *M. kansasii* and *M. gastri*, altogether forming the *M. kansasii* complex [15].

To assess the genomic differences between *M. kansasii* subtypes and its closely related species, *M. persicum* and *M. gastri*, we performed whole-genome sequencing of 13 strains belonging to five different *M. kansasii* subtypes (1, 2, 3, 5 and 6). We compared these genomes with publicly available whole-genome sequences of the *M. kansasii* complex (*n*=9), of widespread slow-growing mycobacterial species (*n*=9) and of the rapid-growing *Mycobacteroides abscessus* (*n*=1) (Table 1). The subsequent phylogenomic analyses indicated that each *M. kansasii* subtype corresponds to a new species-level lineage.

M. kansasii strains isolated from patients at the Lausanne University Hospital (Table 1) were grown in Mycobacterial Growth Indicator Tubes BD BACTEC MGIT, supplemented with MGIT OADC (oleic acid, albumin, dextrose and catalase) enrichment and MGIT PANTA antibiotic mixture (polymyxin B, amphotericin B, nalidixic acid, trimethoprim and azlocillin) (Becton Dickinson). DNA extraction is described in the supplementary materials. Whole genome sequencing was performed with a MiSeq (Illumina) sequencer using either 150 or 250 bp paired-end protocols. In addition, sequencing was done with a Pacific Biosciences RS II sequencer for the strain MK142^T using one SMRT cell version P6-C4 (Pacific Biosciences).

Read quality was assessed with FastQC version 0.11.4 before and after trimming (available online at: www.bioinformatics.babraham.ac.uk/projects/fastqc). Reads were trimmed with Trimmomatic version 0.35 using parameters: 'MINLEN:60, LEADING:9, TRAILING:9, SLIDING-WINDOW:3 : 15'. Assemblies were performed using SPAdes assembler version 3.9.0 [16]. For PacBio, *de novo* assembly of the subread sequences of strain MK142^T was done using the Hierarchical Genome Assembly Process (HGAP) workflow (PacBio DevNet; Pacific Biosciences), as available in SMRT Analysis version 2.3.0. Annotation was done using Prokka version 1.11 for all strains sequenced using the MiSeq sequencer and version 1.12 for strain MK142^T. For strain MK142^T, one complete chromosomal sequence as well as one plasmid sequence were obtained.

Pairwise average nucleotide identity (ANI) values were calculated using JSpecies version 1.2.1 [17]. Pairwise digital DNA–DNA hybridization (DDH) values were calculated using the Genome-to-Genome Distance Calculator 2.1 [18]. Interestingly, based on both the ANI and the DDH values, *M. gastri* is genetically less distant to subtypes 1–5 than *M. kansasii* subtype 6, suggesting that the species *M. kansasii* may be polyphyletic. Regardless of the source of the strain (NCBI or this study), both the ANI and the DDH values between strains of the same subtype were above the species cutoff of 95–96 and 70 %, respectively (Fig. 1). Conversely, between *M. kansasii* strains of different subtypes, both the ANI and the DDH values were below that cutoff, thus defining new species-level lineages. Surprisingly, *M. persicum* AFPC-000227^T shared high ANI and DDH values with *M. kansasii* subtype 2, as seen between strains of the same subtype. This finding supports the definition of *M. persicum* as a new mycobacterial species and we suggest that it should replace *M. kansasii* subtype 2 denomination.

The 16S rRNA gene alone and a concatenated nucleotide sequence of the 16S rRNA, *rpoB* and *hsp65* genes were aligned using MAFFT version 7.310 [19] and used for phylogenetic reconstruction with FastTree version 2.1.8 with double precision and parameters '–nt –gamma –spr 4 –mlacc 2 –slownni' [20]. Phylogenetic trees were rooted on *M. abscessus* ATCC 19977^T, a rapid-growing mycobacterium, using Archaeopteryx 0.9921 [21] and visualized using Figtree version 1.4.2 [22]. As expected, *M. kansasii* subtypes 1 and 4 presented the same 16S rRNA sequence as *M. gastri*. However, subtypes 2, 3, 5 and 6 presented distinct unique 16S rRNA gene sequences (Fig. 2), sharing 99.61, 99.61, 99.87 and 99.54 % nucleotide identity with the 16S rRNA gene sequence of subtype 1, respectively (BLAST analysis). The phylogeny based on the concatenated 16S rRNA–*rpoB*–*hsp65* genes could distinctly separate each subtype as well as *M. gastri* (Fig. S1, available in the online version of this article). Both the 16S rRNA and the concatenated 16S rRNA–*rpoB*–*hsp65* genes also clustered *M. persicum* very tightly with *M. kansasii* subtype 2.

Groups of orthologous sequences were defined using OrthoFinder version 2.1.2 [23]. A total of 1351 single-copy orthologous groups were identified and aligned using MAFFT version 7.310. Then, each alignment was concatenated into a core-genome alignment of 489 835 amino acids. A maximum-likelihood core-genome phylogeny was reconstructed using FastTree (as mentioned above but without '–nt'

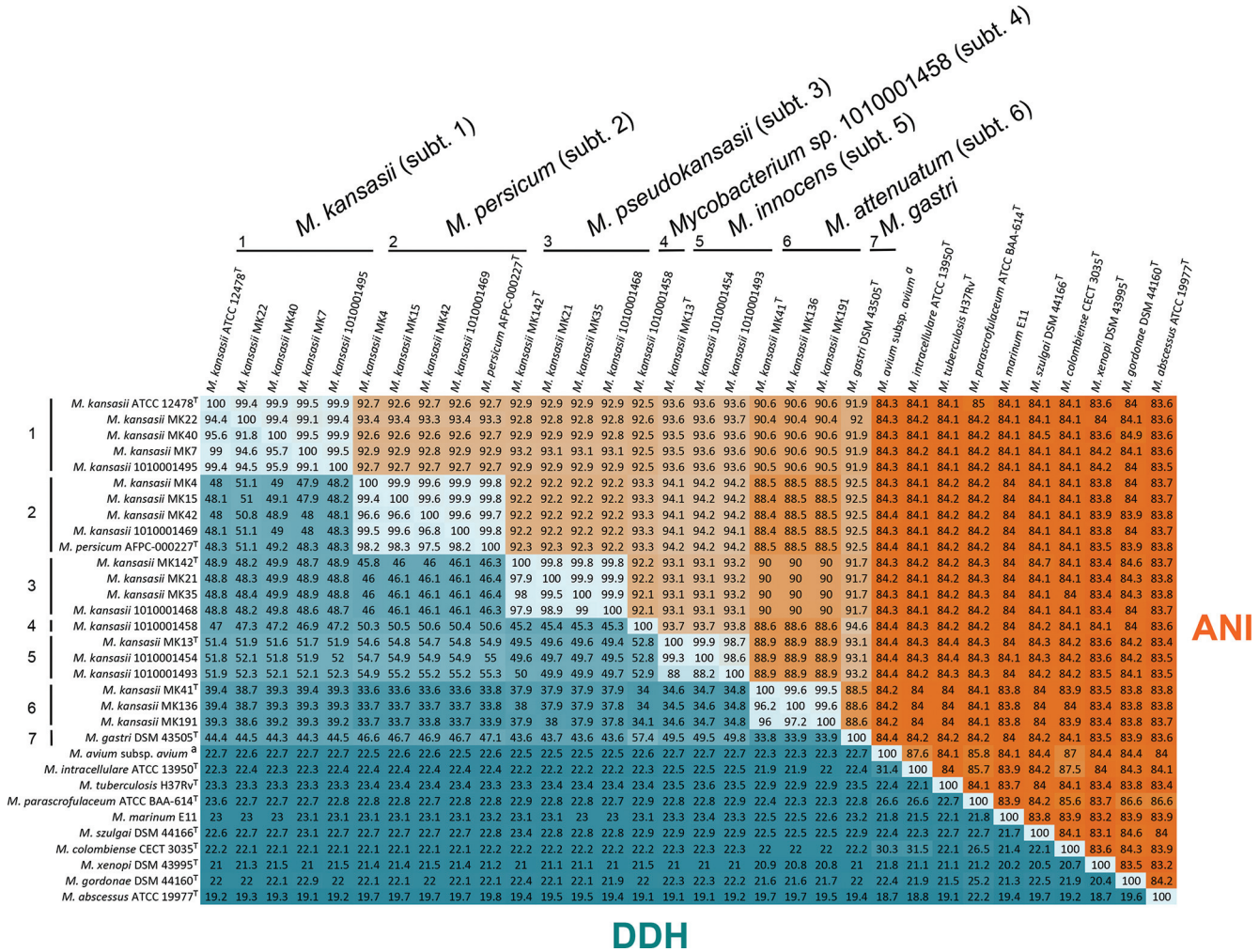

Fig. 1. Heat map of pairwise average nucleotide identity (ANI) and digital DNA–DNA hybridations (DDH) values. *M. kansasii* strains present a mean ANI value of 99.6 % (range 98.6–99.9%) and a mean DDH value of 96.8 % (range 88–99.6%) within each subtype, whereas an ANI value of 91.9 % (range 88.4–94.2%) and a DDH of 45.6 % (range 33.6–55.3%) was observed between subtypes. The proposed nomenclature for all the species of the *M. kansasii* complex is indicated. [a]Strain DJO-44271.

parameter). The phylogenetic tree root was determined as mentioned in the precedent paragraph. On the core-genome phylogeny, *M. gastri* and – as expected – *M. persicum* (*M. kansasii* subtype 2) branch among other *M. kansasii* subtypes, confirming that the species *M. kansasii* is not monophyletic and new species lineages need to be defined for each subtype, as proposed in Fig. 3.

RFLP analysis of the hypervariable fragment of the gene *hsp65* after *in silico* amplification (with Tb11, 5′-ACCAACGATGGTGTGTCCAT; Tb12, 5′-CTTGTCGAACCGCATACCCT) and digestion with *Bst*EII and *Hae*III [5] was performed using Geneious version 9.1.8 [24]. Results (Table S1) were compared with the findings of Richter *et al.* [7]. Subtypes 1, 3, 4 and 5 respectively displayed the expected same pattern for *Bst*EII and *Hae*III digestion. Contrarily to what was reported by Shahraki *et al.* [15], *M. persicum* had the same restriction fragment lengths as all *M. kansasii* subtype 2 strains when comparing to the results of Richter *et al.* [7]. Interestingly, strains MK41^T and MK136 had the same restriction profile as subtype 6, whereas MK191 had a slightly different pattern due to a mutation C307T located at a *Hae*III restriction site (the coordinates refer to the *hsp65* gene, locus_tag: LAUMK191_03491). Thus, differences in RFLP profiles do not always reflect genome differences because MK191, MK41^T and MK136 shared more than 99.5 % average nucleotide identity. Hence, RFLP cannot be considered as a reliable method to type mycobacteria of the *M. kansasii* complex and genomics should be preferred when possible.

The high-performance liquid chromatography (HPLC) profiles of the cell-wall mycolic acids [25] of strains MK22 (subtype 1), MK15 (subtype 2), MK142^T (subtype 3),

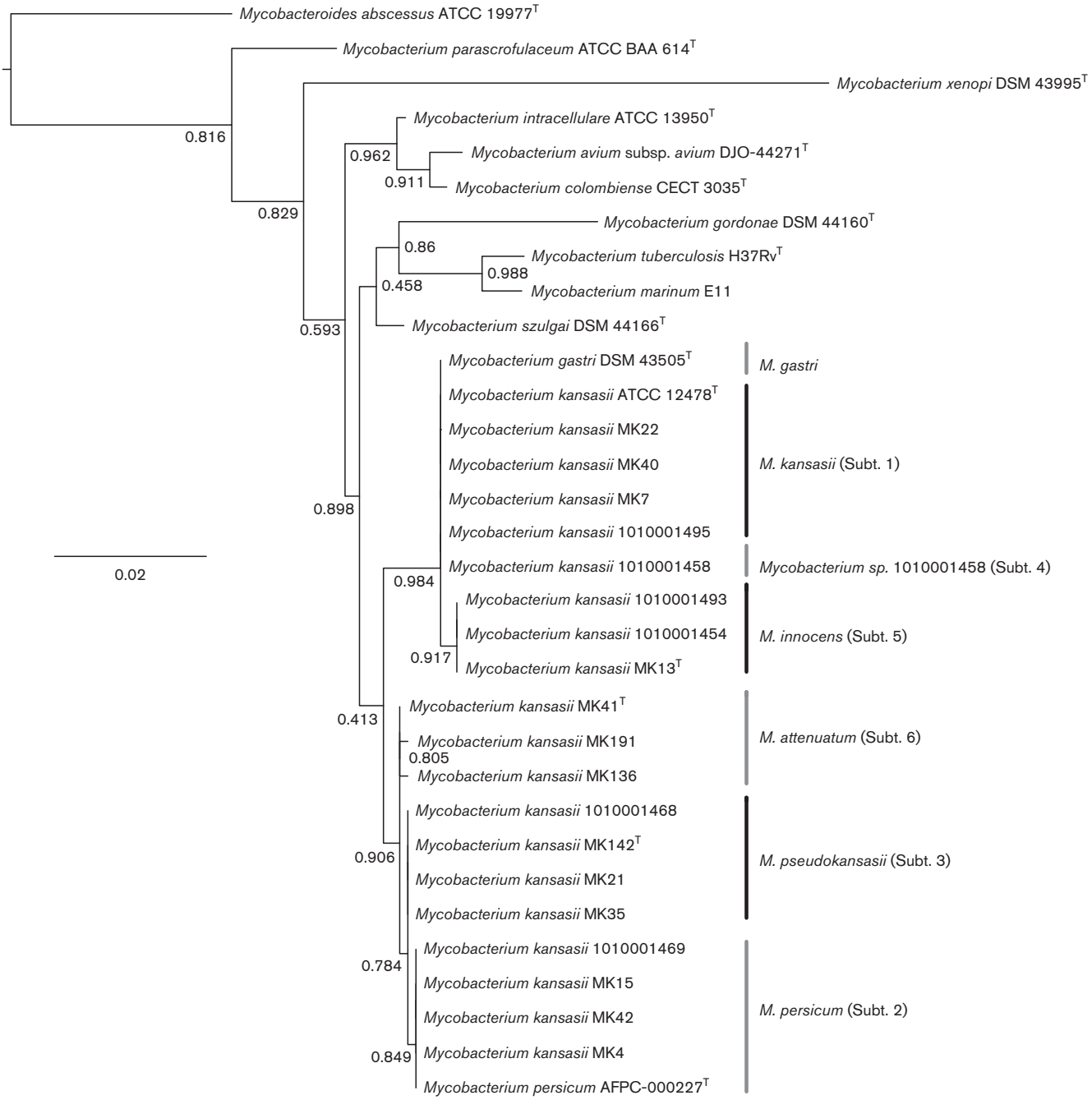

Fig. 2. Maximum-likelihood phylogenetic tree based on the nucleotide alignment of full-length 16S rRNA genes. Proposed new species names as well as former *M. kansasii* subtypes are indicated on the right. *M. kansasii*, *M. gastri* and *Mycobacterium* sp. 1010001458 (subtype 4) share the same 16S rRNA gene sequences, whereas *M. innocens*, *M. attenuatum*, *M. pseudokansasii* and *M. persicum* all had specific 16S rRNA gene sequences. Interestingly, *M. attenuatum* showed some diversity in its 16S rRNA gene. Bar, number of nucleotide substitutions per site alongside the branches. Node supports are based on the Shimodaira–Hasegawa test.

MK13^T (subtype 5) and MK41^T (subtype 6) were generated after growth on Middlebrook 7H10 agar. Cells were saponified, extracted and derivatised following the recommendations of the Sherlock Mycobacteria Identification System (SMIS, MIDI). Mycolic acids were separated with a gradient of methanol and 2-propanol on an Agilent ChemStation 1100/1200 HPLC system and analysed with the MIDI Sherlock Software version 4.0. All strains produced similar profiles with a single late-eluting peak cluster (Fig. 4). The MIDI software successfully identified strain MK22 (subtype 1) as

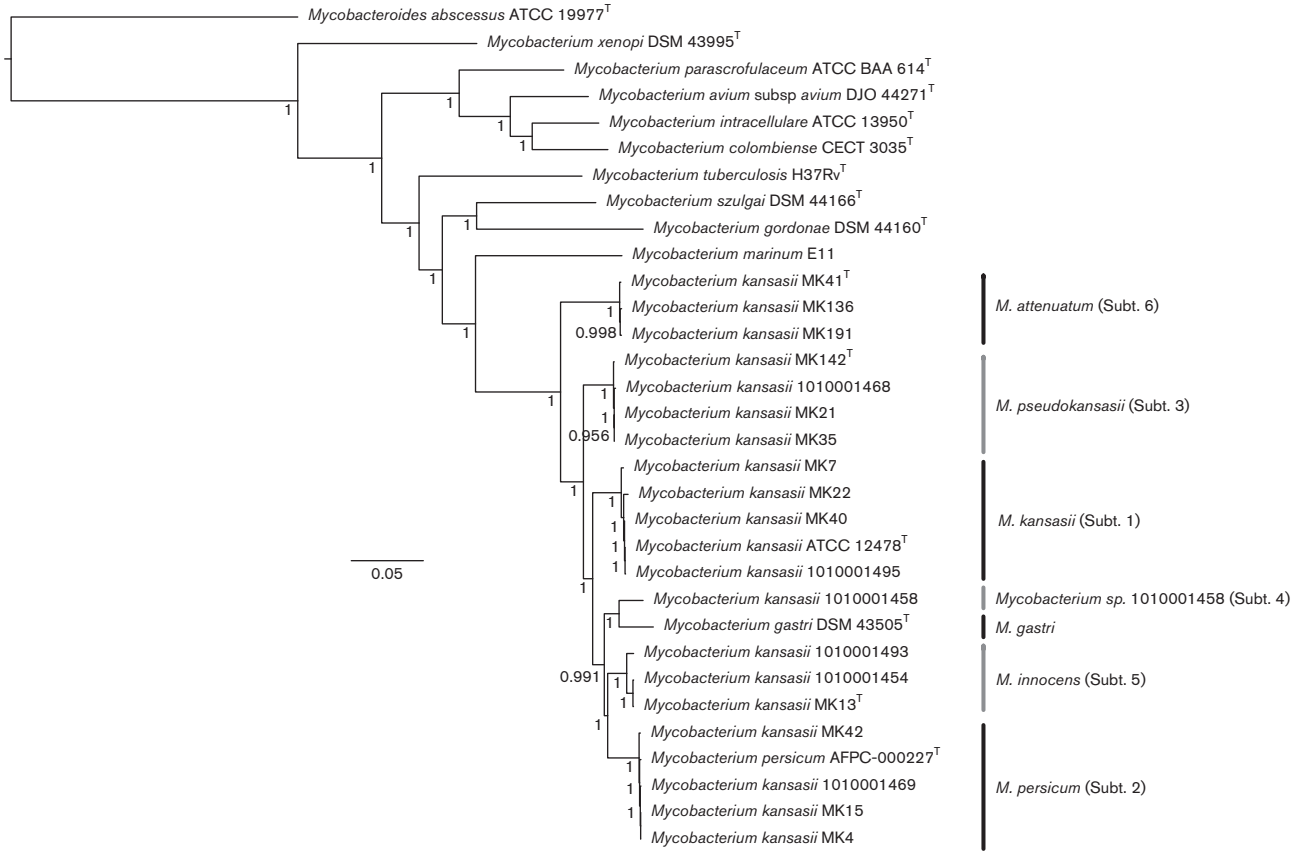

Fig. 3. Maximum-likelihood phylogenetic tree based on the amino acid alignment of concatenated single-copy orthologous genes. Proposed new species names as well as *M. kansasii* subtypes are indicated on the right. *M. gastri* is closely related to *Mycobacterium* sp. 1010001458 (subtype 4) and *M. persicum* clusters among – former – subtype 2 strains. Bar, number of amino acid substitutions per site alongside the branches. Node supports are based on the Shimodaira–Hasegawa test.

M. kansasii. Despite their genetic distance, strain MK15 (subtype 2) and strain MK41^T (subtype 6) were also identified as *M. kansasii* with high similarity indexes (>8.0). However, the identification of strains MK142^T (subtype 3) and MK13^T (subtype 5) was unsuccessful using the Sherlock criteria; the profiles showed similarities not only to *M. kansasii*, but also to *M. szuglai* and *M. asiaticum* for strains MK142^T (subtype 3), and *M. bovis* (BCG) for MK13^T (subtype 5; data not shown).

Strains MK22 (subtype 1), MK15 (subtype 2), MK142^T (subtype 3), MK13^T (subtype 5) and MK41^T (subtype 6) presented mature colonies on 7H10 medium after 2 weeks of growth at 37 °C in aerobic conditions. Photochromogenicity on Löwenstein–Jensen culture medium (37 °C) was also confirmed. No strain of subtypes 4 and 7 was available in our laboratory. Regarding phenotypic properties, Jiménez-Pajares *et al,* characterized 298 *M. kansasii* strains (subtypes 1–6) [26]. In their study, all strains were reported to grow in more than 1 week at an optimal temperature of 37 °C on Löwenstein–Jensen medium. Growth was inhibited at 25 and 45 °C. Furthermore, no growth was detected on Löwenstrein–Jensen medium with 10 µg ml^{-1} thiosemicarbazone or 5 % NaCl or on MacConKey agar without violet crystal, whereas they all grew on Löwenstein–Jensen medium supplemented with 5 µg ml^{-1} thiophen-2-carboxylic acid hydrazide. All strains presented strong catalase activity at 68 °C but none was able to reduce potassium tellurite or exhibited an arylsulfatase activity after 3 days. However, a high degree of variability of several phenotypic tests – niacin production, nitrate reduction, Tween 80 hydrolysis and urease activity – was reported within and between each former subtype of *M. kansasii* (described in the species description) [26]. Therefore, phenotypic testing should not be recommended to achieve reliable identifications of the species of the *M. kansasii* complex.

Existing species-level lineages include *M. kansasii* (subtype 1), *M. persicum* (subtype 2) as well as *M. gastri*. In this study, we propose to define three new species-level lineages of the *M. kansasii* complex, corresponding to subtypes 3, 5 and 6: *Mycobacterium pseudokansasii* sp. nov., *Mycobacterium innocens* sp. nov. and *Mycobacterium attenuatum* sp. nov., respectively. This new taxonomical classification is

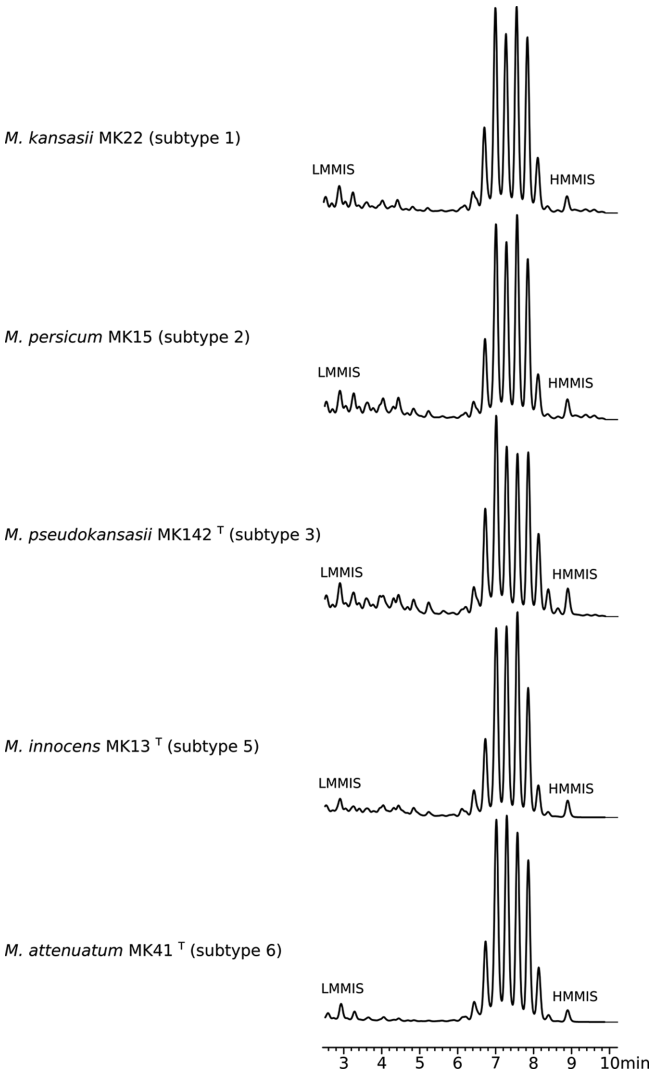

Fig. 4. High-performance liquid chromatography results of the mycolic acid patterns of each available former *M. kansasii* subtype. Despite the genetic distance reported, each species of the complex shared similar spectra. LMMIS, low molecular mass internal standard; HMMIS, high molecular mass internal standard.

necessary to conserve the monophyly of each species (Figs S1 and 3) and corroborates common cut-offs for species using genetic distances. Furthermore, our results are congruent with the Genome Taxonomy Database (GTDB), in which the *M. kansasii* species was split into six species-level lineages [27], as well as with three recently published WGS phylogenies by Tortoli *et al.* [14], Gupta *et al.* [28] and Nouioui *et al.* [29]. No type strain for the former subtype 4 was available but the clear-cut genomic findings of *Mycobacterium* sp. 1010001458 suggest that a new species name should be defined as soon as a type strain is available. No strain or genome of *M. kansasii* subtype 7 was available and this subtype was described only in one study [6]. The gel electrophoresis technique used at that time lacks precision and this subtype might have been misidentified with a subtype 3 that share a very similar restriction profile. Given the absence of available genomic sequence for this subtype, we cannot infer any recommendation on its taxonomic classification. Defining new species names may help clinicians discriminating between all members of the *M. kansasii* complex which present drastic differences in their pathogenicity.

DESCRIPTION OF *MYCOBACTERIUM PSEUDOKANSASII* SP. NOV.

Mycobacterium pseudokansasii (pseu.do.kan.sas'i.i. Gr. adj. *pseudes* false; N.L. gen. n. *kansasii* the specific epithet of *Mycobacterium kansasii*; N.L. gen. n. *pseudokansasii* the false (*Mycobacterium*) *kansasii*).

This species corresponds to the former *M. kansasii* subtype 3. The name was chosen because it is rarely pathogenic

despite being the third most common subtype recovered and could help suggesting the clinicians that it has higher chances of being only a colonizer. *M. pseudokansasii* grows in approximately 2 weeks into rough photochromogenic colonies on Löwenstein–Jensen media at 37 °C. Beige colonies can be obtained on 7H10 medium after 2 weeks' growth in the same culture conditions. *M. pseudokansasii* exhibits a nitrate reductase activity and is able to hydrolyse Tween 80 after 1–5 days. However, it does not produce niacin and has a variable urease activity. Other phenotypic features shared by all the former *M. kansasii* subtypes are reported in the main text. *M. pseudokansasii* shares a very similar HPLC profile with *M. kansasii* and the other members of the complex, characterized by six major peaks eluting between 6.5 and 8.5 min. Reliable molecular identification can be achieved using PCR-RFLP of the *hsp65* or the *tuf* gene, or using PCRs and sequencing of various genes including *hsp65*, 16S rRNA and *rpoB* genes. The maximum-likelihood core-genome phylogeny shows *M. pseudokansasii* to be closely related to the other members of the *M. kansasii* complex.

The type strain is MK142^T (=CCUG 72128^T=DSM 107152^T) and was isolated from a blood culture of a patient with a disseminated mycobacterial infection. 16S rRNA gene and whole-genome sequence data are available under the accession numbers LS999932 and GCA_900566075.1, respectively.

DESCRIPTION OF *MYCOBACTERIUM INNOCENS* SP. NOV.

Mycobacterium innocens (in′no.cens. L. neut. adj. *innocens* innocent or inoffensive).

This species, whose name highlights its rare pathogenicity, corresponds to the former *M. kansasii* subtype 5. It is a slow-grower and generally displays photochromogenicity on Löwenstein–Jensen media after growth at 37 °C. Beige colonies can be obtained on 7H10 medium after a 2 weeks' growth (37 °C). *M. innocens* does not produce niacin but has variable nitrate reductase, Tween 80 hydrolysis and urease activities. Other phenotypic features are described in the main text (shared by all former *M. kansasii* subtypes). *M. innocens* exhibits a similar HPLC profile with the other members of the *M. kansasii* complex. PCR-RFLP of the *hsp65* or the *tuf* gene, or PCRs and sequencing of various genes including *hsp65*, 16S rRNA and *rpoB* genes, allow reliable molecular identification. The maximum-likelihood core-genome phylogeny shows *M. innocens* to be closely related to *M. persicum* and to the other members of the *M. kansasii* complex.

The type strain, MK13^T (=CCUG 72126^T=DSM 107161^T), was isolated from an expectoration of a patient. 16S rRNA gene and whole-genome sequence data are available under the accession numbers LS999933 and GCA_900566055.1, respectively.

DESCRIPTION OF *MYCOBACTERIUM ATTENUATUM* SP. NOV.

Mycobacterium attenuatum (at.te.nu.a′tum. L. part. adj. *attenuatum,* attenuated).

Formerly *M. kansasii* subtype 6, this species is non-pathogenic, as suggested by its name, and very rarely isolated from patients. *M. attenuatum* is a slow-grower and displays photochromogenicity on Löwenstein–Jensen media (37 °C). Beige colonies can also be observed on 7H10 medium after 2 weeks' growth at 37 °C. *M. attenuatum* phenotypically exhibits variable niacin production, nitrate reducase and urease activities. However, Tween 80 hydrolysis is observed after 1–3 days. Other general phenotypic features are shared with the other former *M. kansasii* subtypes. *M. attenuatum* exhibits the same HPLC profile as the other members of the *M. kansasii* complex. Reliable molecular identification can be done using PCR-RFLP of the *hsp65* or the *tuf* gene, or using PCRs and sequencing of various genes including *hsp65*, 16S rRNA and *rpoB* genes. The maximum-likelihood core-genome phylogeny shows *M. attenuatum* to be the most distant deep-branching species of the *M. kansasii* complex.

The type strain is MK41^T (=CCUG 72127^T=DSM 107153^T) and was isolated from bronchial secretions (aspiration of bronchial secretions) of a patient known for Still's disease and reported as a non-pathogenic colonizer. 16S rRNA gene and whole-genome sequence data are available under the accession numbers LS999934 and GCA_900566085.1, respectively.

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

Ethical statement
All human clinical data were collected in accordance with the ethical standards of the regional and national research committee (part of protocol 2017–00194) and with the 1964 Helsinki declaration and its later amendments or similar ethical standards.

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