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, Sébastien Aeby, Claire Bertelli, Sara Droz, Carlo Casanova, Guy Prod’hom, Katia Jaton and Gilbert Greub

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 95–98% 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<sup>T</sup> (=CCUG 72128<sup>T</sup> = DSM 107152<sup>T</sup>), (ii) *Mycobacterium innocens* sp. nov., replacing subtype 5, with the type strain MK13<sup>T</sup> (=CCUG 72126<sup>T</sup> = DSM 107161<sup>T</sup>), and (iii) *Mycobacterium attenuatum* sp. nov., replacing subtype 6, with the type strain MK41<sup>T</sup> (=CCUG 72127<sup>T</sup> = DSM 107153<sup>T</sup>). 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: 1Institute of Microbiology, Department of Laboratory Medicine, Lausanne University Hospital and University of Lausanne, Lausanne, Switzerland; 2Institute for Infectious Diseases, University of Bern, Bern, Switzerland; 3Division 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 hybridizations; HMMIS, high molecular mass internal standard; kb, kilobase; LMMIS, low molecular mass internal standard.

The 16S rRNA accession numbers: *Mycobacterium pseudokansasii* MK142, LS999932; *Mycobacterium innocens* MK13, LS999933; *Mycobacterium attenuatum* MK41, LS999934. Genome accession numbers: GCA_900565985.1, GCA_900565995.1, GCA_900566005.1, GCA_900565805.1, GCA_900565605.1, GCA_900566035.1, GCA_900566045.1, GCA_900566055.1, GCA_900566065.1, GCA_900566075.1, GCA_900566085.1, GCA_900566095.1.

One supplementary table and one supplementary figure are available with the online version of this article.

This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
| 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<sup>T</sup> | GCF_000157895.3 | 6577 | 2 | Complete | 80x | NCBI | subtype | Mycobacterium kansasii |
| Mycobacterium kansasii | 1010001495 | GCF_001632965.1 | 6358 | 140 | 91 256 | 28.2x | NCBI | | Mycobacterium kansasii |
| Mycobacterium kansasii | MK22 | GCA_900565985.1 | 6406 | 187 | 71 547 | 219x | This study | | Mycobacterium kansasii |
| Mycobacterium kansasii | MK40 | GCA_900566155.1 | 6607 | 199 | 68 849 | 235x | This study | | Mycobacterium kansasii |
| Mycobacterium kansasii | MK7 | GCA_900565995.1 | 6414 | 165 | 87 134 | 331x | This study | | Mycobacterium kansasii |
| Mycobacterium kansasii | 1010001469 | GCF_001632975.1 | 6266 | 165 | 85 083 | 33.8x | NCBI | | Mycobacterium kansasii |
| Mycobacterium persicum | AFPC-000227<sup>T</sup> | GCF_002086675.1 | 6172 | 387 | 44 952 | 11x | NCBI | | Mycobacterium persicum |
| Mycobacterium kansasii | MK15 | GCA_900566005.1 | 6241 | 190 | 72 560 | 253x | This study | | Mycobacterium persicum |
| Mycobacterium kansasii | MK42 | GCA_900566015.1 | 6119 | 188 | 67 218 | 188x | This study | | Mycobacterium persicum |
| Mycobacterium kansasii | MK4 | GCA_900566035.1 | 6424 | 195 | 72 167 | 251x | This study | | Mycobacterium persicum |
| Mycobacterium kansasii | 1010001468 | GCF_001632915.1 | 6142 | 164 | 65 195 | 24.1x | NCBI | | Mycobacterium pseudokansasii |
| Mycobacterium kansasii | MK142<sup>T</sup> | GCA_900566075.1 | 6426 | 2 | Complete | 130x | This study | | Mycobacterium pseudokansasii |
| Mycobacterium kansasii | MK21 | GCA_900566045.1 | 6288 | 220 | 65 394 | 209x | This study | | Mycobacterium pseudokansasii |
| Mycobacterium kansasii | MK35 | GCA_900566025.1 | 6295 | 218 | 65 269 | 204x | This study | | Mycobacterium pseudokansasii |
| Mycobacterium kansasii | 1010001458 | GCF_001632895.1 | 6027 | 173 | 54 964 | 29.9x | NCBI | | Undefined at present |
| Mycobacterium kansasii | 1010001493 | GCF_001632885.1 | 5627 | 346 | 41 206 | 25x | NCBI | | Mycobacterium innocens |
| Mycobacterium kansasii | MK13<sup>T</sup> | GCA_900566055.1 | 6187 | 361 | 35 686 | 189x | This study | | Mycobacterium innocens |
| Mycobacterium kansasii | MK136 | GCA_900566065.1 | 6345 | 196 | 72 849 | 232x | This study | | Mycobacterium attenuatum |
| Mycobacterium kansasii | MK191 | GCA_900566105.1 | 6357 | 221 | 57 930 | 118x | This study | | Mycobacterium attenuatum |
| Mycobacterium kansasii | MK41<sup>T</sup> | GCA_900566085.1 | 6528 | 230 | 54 408 | 231x | This study | | Mycobacterium attenuatum |
| Mycobacterium gastri | DSM 43505<sup>T</sup> | GCF_000164135.1 | 6564 | 405 | 36 222 | 53x | NCBI | | |
| Mycobacterium colombiense | CECT 3035<sup>T</sup> | GCF_002105755.1 | 5582 | 1 | Complete | 340x | NCBI | | |
| Mycobacterium szulgai | DSM 44166<sup>T</sup> | GCF_000164135.1 | 6564 | 405 | 36 222 | 53x | NCBI | | |
| Mycobacterium paratuberculosis | ATCC BAA-614<sup>T</sup> | GCF_000195955.2 | 6412 | 1 | Complete | – | NCBI | | |
| Mycobacterium tuberculosis | ATCC 13950<sup>T</sup> | GCF_000277125.1 | 5402 | 1 | Complete | – | NCBI | | |
| Mycobacterium marinum | E11 | GCF_000723425.1 | 4412 | 1 | Complete | 400x | NCBI | | |
| Mycobacterium avium subsp. avium | DJO-44271 | GCF_000770235.1 | 5011 | 1 | Complete | 107.5x | NCBI | | |
| Mycobacterium intracellulare | DSM 44160<sup>T</sup> | GCF_002101675.1 | 5817 | 154 | 85 262 | 105x | NCBI | | |
| Mycobacterium marinum | DSM 44160<sup>T</sup> | GCF_002101675.1 | 5817 | 154 | 85 262 | 105x | NCBI | | |
| Mycobacterium szulgai | DSM 44160<sup>T</sup> | GCF_002101675.1 | 5817 | 154 | 85 262 | 105x | 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           | ATCC 43995³ | GCF_000069185.1-1                     | 5090          | 2                        | Complete | –               | NCBI                   | –                  | –                     |
| abscessus        | ATCC 19977³ |                                |                 |                             |         |                 |                       |                    |                       |

[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 12498² (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 MK142² 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 MK142² 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 MK142². For strain MK142², 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-000227⁷ 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 19977⁷, 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’
The phylogenetic tree root was determined as mentioned in the precedent paragraph. On the core-genome phylogeny, *M. gastri* and *M. persicum* (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′-ACCAAC-GATGGTGTGTCCAT; Tb12, 5′-CTTGTCGAACCGCTTACCCT) and digestion with *BstE* II 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 *BstE* II 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 MK41T 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, MK41T 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), MK142T (subtype 3),
MK13<sup>T</sup> (subtype 5) and MK41<sup>T</sup> (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 Mycobacterium persicum AFPC-000227<sup>T</sup>.
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. szulgai 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-Pajaes 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 Löwenstein–Jensen medium with 10µg ml$^{-1}$ thiosemicarbazone or 5% NaCl or on MacConkey agar without violet crystal, whereas they all grew on Löwenstein–Jensen medium supplemented with 5µg ml$^{-1}$ thio-phen-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. 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.
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. pseudokanssii* 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. pseudokanssii* 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. pseudokanssii* 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. pseudokanssii* to be closely related to the other members of the *M. kansasii* complex.

The type strain is MK142T (=CCUG 72128T=DSM 107152T) 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 LS999934 and GCA_900566085.1, 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 reductase 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 MK41T (=CCUG 72127T=DSM 107153T) 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.

**Funding information**

The authors received no specific grant from any funding agency.

**Acknowledgements**

We would like to thank René Brouillet, Gregory Gonzalez, Virginie Martin and Carmen Perroulaz for their technical help. We thank Trestan Pillonel for providing the scripts used to build the core-genome alignment as well as Onya Opota for the helpful discussions about the project.

**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.

**References**

1. Buhrer VB, Pollak A. Human infection with atypical acid-fast organisms; report of two cases with pathologic findings. *Am J Clin Pathol* 1953;23:363–374.
2. Field SK, Cowie RL. Lung disease due to the more common non-tuberculous mycobacteria. *Chest* 2006;129:1653–1672.
3. Prevots DR, Marras TK. Epidemiology of human pulmonary infection with non-tuberculous mycobacteria: a review. *Clin Chest Med* 2015;36:13–34.
4. Hoeftsoot W, van Ingen J, Andrejak C, Angeby K, Bauriaud R et al. The geographic diversity of nontuberculous mycobacteria isolated from pulmonary samples: an NTM-NET collaborative study. *Eur Respir J* 2013;42:1604–1613.

**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 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, MK13T (=CCUG 72126T=DSM 107161T), 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.
Five reasons to publish your next article with a Microbiology Society journal

1. The Microbiology Society is a not-for-profit organization.
2. We offer fast and rigorous peer review – average time to first decision is 4–6 weeks.
3. Our journals have a global readership with subscriptions held in research institutions around the world.
4. 80% of our authors rate our submission process as ‘excellent’ or ‘very good’.
5. Your article will be published on an interactive journal platform with advanced metrics.

Find out more and submit your article at microbiologyresearch.org.