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Mycobacterium arupense, Mycobacterium heraklionense, and a newly proposed species, "Mycobacterium virginiense" sp. nov., but not Mycobacterium nonchromogenicum, as species of the Mycobacterium terrae complex causing tenosynovitis and osteomyelitis

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Mycobacterium arupense, *Mycobacterium heraklionense*, and a Newly Proposed Species, "*Mycobacterium virginiense*" sp. nov., but Not *Mycobacterium nonchromogenicum*, as Species of the *Mycobacterium terrae* Complex Causing Tenosynovitis and Osteomyelitis

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***Mycobacterium terrae* complex has been recognized as a cause of tenosynovitis, with *M. terrae* and *Mycobacterium nonchromogenicum* reported as the primary etiologic pathogens. The molecular taxonomy of the *M. terrae* complex causing tenosynovitis has not been established despite approximately 50 previously reported cases. We evaluated 26 isolates of the *M. terrae* complex associated with tenosynovitis or osteomyelitis recovered between 1984 and 2014 from 13 states, including 5 isolates reported in 1991 as *M. nonchromogenicum* by nonmolecular methods. The isolates belonged to three validated species, one new proposed species, and two novel related strains. The majority of isolates (20/26, or 77%) belonged to two recently described species: *Mycobacterium arupense* (10 isolates, or 38%) and *Mycobacterium heraklionense* (10 isolates, or 38%). Three isolates (12%) had 100% sequence identity to each other by 16S rRNA and 99.3 to 100% identity by *rpoB* gene region V sequencing and represent a previously undescribed species within the *M. terrae* complex. There were no isolates of *M. terrae* or *M. nonchromogenicum*, including among the five isolates reported in 1991. The 26 isolates were susceptible to clarithromycin (100%), rifabutin (100%), ethambutol (92%), and sulfamethoxazole or trimethoprim-sulfamethoxazole (70%). The current study suggests that *M. arupense*, *M. heraklionense*, and a newly proposed species ("*M. virginiense*" sp. nov.; proposed type strain MO-233 [DSM 100883, CIP 110918]) within the *M. terrae* complex are the major causes of tenosynovitis and osteomyelitis in the United States, with little change over 20 years. Species identification within this complex requires sequencing methods.**

Mycobacterium terrae complex (MTC) was first characterized in 1981 by the International Working Group in Mycobacterial Taxonomy (IWGMT). The initial MTC consisted of two nonchromogenic slowly growing species: *M. terrae* and *Mycobacterium nonchromogenicum* (1, 2). Phenotypic separation within the group was often difficult, and molecular methods were not available, making establishment of species pathogenicity uncertain (3).

The complex is recognized as an environmental contaminant of sputum and a cause of tenosynovitis and osteomyelitis primarily of the fingers and wrist (3–35). Whether one or more members of the complex are true respiratory pathogens has not been established (1, 22).

The first published case report of tenosynovitis caused by the MTC was by Hirata and Tomiyama in 1976 (4). There have been approximately 34 additional case reports published since then, identified using nonmolecular methods (3–25), with 14 cases identified using molecular methods (26–28, 30–36) (Tables 1 and 2). With the exception of four isolates of *M. arupense*, including the original description of *M. arupense* (28), details of the methods and/or explicitly stating a 100% 16S rRNA gene sequence identity to recognized species for the remaining cases with molecular identifications have been absent (Table 2).

An excellent history and species update of the *M. terrae* complex based on multigene sequencing targets was published by Tortoli et al. (1). He noted that the presence of a two nucleotide insertion in helix 18 of the 16S rRNA gene (bp ~430 to 500; hypervariable region B or region V3) provided a consistent signature sequence for members of the MTC compared to other slowly growing mycobacteria (37, 38). He also characterized several new species in the complex, including *Mycobacterium heraklionense* and *Mycobacterium engbaekii* (1).

The greater availability of DNA sequencing (59) has resulted in a "boom" of new species of MTC, beginning with *Mycobacterium hiberniae* (39). An additional eight new species have been validly published since 2006: *Mycobacterium arupense* (28), *Mycobacterium kumamotonense* (36), *Mycobacterium heraklionense* (1), *Mycobacterium senuense* (40), *Mycobacterium minnesotense* (41), *My-*

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TABLE 1 Characteristics and species designations of 35 previously reported cases of tenosynovitis due to the *M. terrae* complex without molecular species identification[a]

No.	Age/sex	Site of tenosynovitis	Diagnosis or risk factor(s)s	Local steroid	Surgery	Granulomatous inflammation	AFB smear	Species	Reference	Location/yr of publication
1	65/M	Right foot (ankle)	Farmer; NA	+	NA	NA	NA	*M. nonchromogenicum*	4	Japan/1976
2	20/M	Left forefinger, wrist	NA	+	+	+	+	*M. terrae*	5	Texas/1978
3	23/M	Left index finger	Puncture from fish fin	+	+	+	−	*M. terrae*	6	NA/1979
4	57/M	Knee	RA	+	NA	NA	?	*M. terrae*	7	NA/1981
5	66/F	Right index finger	Puncture from blackberry thorn	−	+	+	+	*M. terrae*	8	NA/1981
6	60/M	Left hand, forearm	Puncture from wooden splinter	−	+	+	−	*M. terrae*	9	NA/1983
7	47/F	Right 4th and 5th fingers, palm, thumb	Puncture from straight pin	+	+	+	−	*M. terrae*	10	NA/1983
8	72/M	left forearm, right 5th finger, right thumb	Puncture wound	−	+	+	−	*M. terrae*	11	France/1984
9	75/M	Left index finger	Arteritis	+	+	+	−	*M. terrae*	12	NA/1985
10	NA	Knee	NA	+	NA	NA	NA	*M. terrae*	13	France/1987
11	54/M	Right 3rd finger, PIP joint	Puncture from fish fin	+	+	+	+	*M. terrae*	14	NA/1988
12	55/M	Right 3rd finger, MCP joint	Puncture from fish fin	−	−	NA	−	*M. terrae*	14	NA/1988
13	41/F	Hand, wrist	Renal transplant	+	NA	NA	NA	*M. terrae*	15	NA/1988
14	58/F	Right, 3rd finger	Nurse	+	+	+	−	*M. terrae*	16	Sweden/1989
15	28/F	Right, 2nd finger	Lab technician	−	+	−	−	*M. terrae*	16	Sweden/1989
16	71/F	Right, 2nd finger	Retired	+	+	−	−	*M. terrae*	16	Sweden/1989
17	47/F	Right 3rd finger	Cook	+	+	+	−	*M. terrae*	16	Sweden/1989
18	31/F	Right, 2nd finger	Office worker	+	+	+	−	*M. terrae*	16	Sweden/1989
19	38/F	Right, 3rd finger	Lab technician	+	+	+	−	*M. terrae*	16	Sweden/1989
20	48/M	3rd finger	NA	?	+	?	?	*M. terrae*	17	NA/1989
21	63/F	Wrist	Gardener	NA	NA	NA	NA	*M. terrae*	18	NA/1990
22	58/F	Right index finger	Gardening, cook (case 1)	−	+	+	−	*M. nonchromogenicum*	3	Maine/1991
23	60/M	Left wrist	Meat cutter (case 2)	−	+	+	−	*M. nonchromogenicum*	3	Illinois/1991
24	59/M	Left wrist	Maintenance work (case 3)	−	+	+	−	*M. nonchromogenicum*	3	Florida/1991
25	62/F	Right middle finger	NA (case 4)	−	+	+	−	*M. nonchromogenicum*	3	Texas/1991
26	40/M	Left hand, thumb	Dermatomyositis on steroids (case 5)	+	+	+	−	*M. nonchromogenicum*	3	Florida/1991
27	40/F	Right 5th finger	Fishing, gardening, prior surgery for carpal tunnel syndrome (case 6)	−	+	+	NA	*M. nonchromogenicum*	3	Virginia/1991
28	NA	NA	NA	?	?	?	?	*M. terrae*	19	France/1993
29	31/M	Knee	RA	?	?	?	?		20	NA/1994
30	36/M	Left 3rd finger, palm	Puncture glass sliver (mechanic)	+	+	+	−	*M. terrae*	21	Michigan/1999
31	37/F	Right 2nd finger	Maintained aquarium, some planting, no trauma	+	+	NA	NA	*M. terrae*	22	U.S./2000
32	42/F	Right index finger	Strain while repairing garden fence	+	+	+	NA	*M. terrae*	22	U.S./2000
33	31/M	Right knee	RA on steroids, methotrexate	−	−	NA	−	*M. terrae*	23	Taiwan/2009
34	76/M	Left hand	Retired fisherman, prior debridement for *M. marinum*	−	+	+	−	*M. terrae*	24	Hong Kong/2012
35	61/M	Right knee	Osteoarthritis	−	+	NA	NA	*M. terrae*	25	California/2012

[a] Abbreviations: M, male; F, female; AFB, acid-fast bacilli; PIP, proximal interphalangeal; NA, not available; RA, rheumatoid arthritis. Symbols: +, factor present; −, factor not present.

cobacterium longobardum (1), *Mycobacterium algericum* (42), and *Mycobacterium engbaekii* (1). Two other MTC species have been described that currently have no standing in nomenclature: "*Mycobacterium paraterrae*" (43) and "*Mycobacterium sinense*" (44).

Almost all published cases of these 11 new species of the MTC have been respiratory except for the two previously mentioned isolates of *M. arupense* (including the type strain, ATCC BAA-1242), which were associated with tenosynovitis or hand infections (28). The distribution of these 11 newer species of the complex as well as the two established members (*M. terrae* and *M. nonchromogenicum*) among clinical isolates from synovial fluid, tissue, or bone based on DNA sequencing is not known. Thus, we collected tendon, synovial, and bone isolates of the *M. terrae* complex recovered over 30 years, including isolates previously identified as *M. nonchromogenicum* using nonmolecular methods, and subjected them to molecular identification (3). We also present a

TABLE 2 Characteristics of 14 previously reported cases of tenosynovitis due to the *M. terrae* complex based on DNA sequencing[a]

Case	Age/sex	Site of tenosynovitis	Underlying disease or source of trauma	Treatment with steroids	Surgery	Granulomatous inflammation	Species	DNA Sequence	% identity with type strain	Location/yr	Reference
1	67/M	Right index finger	None	NA	+	NA	*M. nonchromogenicum*	16S	NA	Norway/2006	26
2	19/F	Right 2nd finger	NA	NA	NA	NA	*M. nonchromogenicum*	NA	NA	Texas/2006	27
3	60/F	Left 3rd finger	None	–	+	+	*M. nonchromogenicum*	NA	NA	Texas/2006	27
4	NA	Tendon	NA	NA	NA	NA	*M. arupense*	com 16S	100	NA/2006	28
5	NA	Finger wound[b]	NA	NA	NA	NA	*M. arupense*	com 16S	100	NA/2006	28
6	54/F	Left 3rd finger, palm	Diabetes, motorcycle accident	–	+	+	*M. arupense*	850 bp	99.2	Taiwan/2008	29
7	68/M	Right index finger	None	+	+	+	*M. arupense*	DNA-DNA hybridization	NA	NA/2011	30
8	58/M	Total right hand	None	+	+	+	*M. arupense*	16S, 478 bp	100	Minnesota/2011	31
9	35	Wrist, osteo	Cut hand with glass	–	–	+	*M. arupense*	"16S," "*hsp65*"	NA, NA	France/2012	32
10	71/M	Left elbow	Diabetes	–	+	+	*M. longobardum*	com16S	99.20	NA//2013	54
11	76/M	Right forearm	NA	NA	+	?	*M. arupense*	NA	NA	Japan/2014	33
12	37/M	Left middle finger	Tree thorn	–	+	+	*M. heraklionense*	"16S"	NA	NA/2014	34
13	56/F	Right 2nd finger	Puncture wound from crab	+	+	+	*M. arupense*	16S, 500 bp, *hsp65*, 415 bp	100, 100	Korea/2014	35
14	68/M	NA	NA	NA	+	NA	*M. arupense*	16S, 1,432 bp, *hsp65*	99.8, 100	Japan/2006	36

[a] M, male; F, female; com 16S, complete 16S sequence; "16S," base pair not specified; "*hsp65*," base pair not specified.
[b] Data not provided as to whether tenosynovitis was present. However, cellulitis without tenosynovitis has not been described for this group of isolates.

case report of osteomyelitis due to a previously unrecognized taxa within the MTC.

CASE REPORT

The patient is a 75-year-old previously healthy male who sustained a chainsaw injury to his leg. He developed a secondary wound infection and suspected osteomyelitis of the underlying tibia. He underwent an incision and debridement of the leg. Gram stain and routine cultures were negative. Special stains, including acid-fast bacillus (AFB) stains, were negative, but AFB cultures were positive in broth for a nonpigmented slowly growing organism initially identified by high-performance liquid chromatography (HPLC) patterns and phenotypic characteristics as being in the *M. terrae* complex. Histopathology on the tissue was not performed. The patient was treated initially with clarithromycin and doxycycline because of a history of a rash with sulfonamides. Subsequent susceptibility tests showed the isolate to be susceptible to clarithromycin, ethambutol, rifabutin, linezolid, and trimethoprim-sulfamethoxazole (TMP-SMX) but resistant to doxycycline. The patient was treated with clarithromycin and ethambutol, and his wound healed without incident. The isolate (MO-4693) was subsequently shown by complete 16S rRNA gene and partial *rpoB* gene sequencing to be a previously unrecognized member of the *M. terrae* complex.

MATERIALS AND METHODS

Previous cases. All previously published cases of the association of *M. terrae* complex with tenosynovitis or osteomyelitis were sought in the medical literature. Identification obtained by nonsequence-based versus sequence-based methods was highlighted.

Current isolates. All synovial tissue, joint fluid, or bone biopsy isolates of the *M. terrae* group submitted to the Mycobacteria/Nocardia Research Laboratory at The University of Texas Health Science Center at Tyler (UTHSCT) between 1984 and 2014 for identification and/or susceptibility testing were sought. This number included five isolates identified as *M. nonchromogenicum* based on phenotypic features and HPLC patterns from a 1991 publication (3), including strain MO-233. Clinical information was reviewed at the time of presentation. Isolates had been stored at −70°C in tryptic soy broth with 15% glycerol and were subcultured to Middlebrook 7H10 agar for molecular testing.

Reference strains included in the study were *M. terrae* ATCC 15755^T, *M. nonchromogenicum* ATCC 19530^T, *M. kumamotonense* DSM 45093^T, *M. arupense* ATCC BAA-1242^T, and "*M. paraterrae*" DSM 45127^T.

This study was approved by the Institutional Review Board of UTHSCT.

DNA extraction. A small loopful of bacteria from isolated colonies was suspended in 100 μl of preparation reagent (PrepMan Ultra, Life Technologies, Carlsbad, CA). Samples were held for 30 s and then heat killed for 10 min at 100°C, and then the samples were cooled down at room temperature for 2 min and centrifuged at maximum speed in a microcentrifuge for 2 min. The DNA was extracted by transferring 50 μl of the supernatant.

16S rRNA complete gene PCR and sequencing. 16S rRNA complete gene sequencing was performed as previously described by Edwards et al. (45).

PCR was performed in a 20-μl reaction mixture using a 10 μM concentration of each of the primers (pA and pH) (45), 1× FailSafe Premix I, and 1.25 U of FailSafe enzyme mix (Epicentre, Madison, WI). The PCR product (5 μl) was run on a 2% agarose gel (Promega, Madison, WI) with EZ safe stain (1 μl) (eEnzyme, Gaithersburg, MD) and visualized under UV light using a SYBR gold emission filter.

After purifying the amplicon using USB ExoSap-IT reagent (Affymetrix, Santa Clara, CA), sequencing was performed using primers pC,

TABLE 3 Twenty-six clinical cases of tenosynovitis or osteomyelitis due to *M. terrae* complex and their causative species based on complete 16S rRNA gene sequence

Organism and isolate no.	Strain name	Source	Complete 16S rRNA gene sequence base pair match	% identity[a]	Location/yr
M. arupense ($n = 10$)					
1	MO-3556	Right elbow synovial fluid	1,475/1,475	100	Missouri/2010
2	MO-2220	Fourth finger fluid	1,475/1,475	100	Iowa/2006
3	MO-1791	Right wrist	1,475/1,475	100	Texas/2004
4	MO-4448	Finger	1,482/1,482	100	Arkansas/1999
5	MO-1082, pigmented	Right elbow synovial fluid	1,475/1,475	100	Florida/1999
6	MO-1089	Synovial fluid	1,482/1,482	100	Kansas/1998
7	MO-49	Hand	1,482/1,482	100	Florida/1985
8	MO-86, pigmented	Left wrist, synovium	1,474/1,474	100	Maine/1986
9	MO-3744	Left index finger	1,474/1,474	100	Missouri/2011
10	MO-4781	Hand	1,465/1,465	100	Texas/2013
M. heraklionense ($n = 10$)					
1	MO-3474	Right index finger	1,427/1,427	100	Texas/2010
2	MO-4449	Tissue, left hand	1,427/1,427	100	Massachusetts/1996
3	MO-786	Finger, tissue	1,427/1,427	100	North Carolina/1996
4	MO-778	Finger	1,427/1,427	100	California/1996
5	MO-7	Right hand, synovium	1,427/1,427	100	Texas/1984
6	MO-51	Hand	1,427/1,427	100	California/1985
7	MO-4967	Right index finger	1,422/1,422	100	Washington/2014
8	MO-5013	Right index finger	1,422/1,422	100	Texas/2013
9	MO-5024	Right index finger	1,422/1,422	100	Illinois/2014
10	MO-5209	Index finger	1,422/1,422	100	Washington/2015
M. kumamotonense ($n = 1$)					
1	MO 2762	Right hand tendon	1,423/1,424 (1 gap)	99.93	Massachusetts/2008
Proposed new species ($n = 5$)					
1	MO 1300	Knee	1,474/1,474	100	Florida/2001
2	MO-233[b]	Flexor tendon	1,474/1,474	100	Virginia/1991
3	MO-5116	Elbow	1,474/1,474	100	North Carolina/2014
4	MO-3559	Flexor tendon	1,359/1,359	100	Unknown
5	MO-4693	Tibia	1,359/1,359	100	Missouri/2013

[a] % identity is the identity of organism against the corresponding type strain. In the case of the final two values, the strains both have same single base pair mismatch.
[b] Proposed type strain.

pD, and pE (45) and a BigDye Terminator v3.1 cycle sequencing kit on an ABI 3500 genetic analyzer according to the manufacturer's instructions (Life Technologies, Carlsbad, CA).

Gene sequence analysis was performed using RipSeq software (Isentio AS, Bergen, Norway). Primer regions were excluded, yielding a final sequence of 1,489 bp for all strains (as is the case for members of the *M. terrae* complex). Sequences were compared to those for validated type strains and all available sequences using RipSeq and NCBI BLAST version 2.3.0+. Three separate NCBI databases were used for BLAST analyses: (i) 16S rRNA sequences (*Bacteria* and *Archaea*), (ii) the nucleotide collection (nr/nt), and (iii) whole-genome shotgun contigs (wgs). A separate BLAST analysis using only the first 500 bp from the 5 new species was performed to better assess the presence of similar sequences in the public domain. Interpretation was in accordance with the Clinical and Laboratory Standards Institute (CLSI) interpretive criteria for DNA target sequencing (46) (Table 3).

***rpoB* partial gene sequencing.** Sequencing of region V and region III of the *rpoB* gene (720 bp and 315 bp, respectively, excluding the primer regions) was performed on selected isolates, including isolates whose complete 16S rRNA gene sequence was not a 100% match to a validated species type strain as described previously (47, 48). The nucleotide collection (nr/nt) NCBI database was used for BLAST analyses.

***hsp65* partial gene sequencing.** A 441-bp region of the *hsp65* gene was amplified (49) and used for molecular analysis of a 401-bp sequence (excluding the primer regions). Sequencing was done on some of the isolates that are not 100% to a validated type strain by 16S rRNA gene sequencing using the BigDye Terminator v3.1 cycle sequencing kit on an ABI 3500 genetic analyzer. Both the nucleotide collection (nr/nt) and whole-genome shotgun contig (wgs) NCBI databases were used for BLAST analyses.

Phylogenetic analyses. For all gene targets, sequencing alignments of strains from this study and chosen sequences from GenBank were created and phylogenetic analyses were conducted using MEGA version 6 (50). A web-accessible database of *hsp65* sequences from *Mycobacterium* reference strains was also used to populate the *hsp65* alignment (51).

Members of the *M. terrae* complex are known to harbor two copies of the ribosomal operon that may contain differences in the 16S rRNA gene (52, 53), seen as ambiguous bases in sequence electropherograms. Variations in positions of ambiguity were not considered in 16S phylogenetic analyses in this study.

Susceptibility testing. Susceptibility testing of the *M. terrae* complex isolates was performed using broth microdilution according to CLSI guidelines (54).

Nucleotide sequence accession numbers. The complete 16S rRNA sequences of MO-233, MO-1300, MO-5116, MO-2762, MO-3559, MO-4693 and *Mycobacterium* sp. strain DSM 45127 ("*M. paraterrae*") were deposited in GenBank (accession numbers KR025879, KR025880, KR025881, KR025882, KR025883, KR025884, and KT861785). The accession numbers for *rpoB* region V sequences deposited in GenBank for strains MO-233, MO-1300, MO-5116, MO-2762, MO-3559, MO-4693, *M. arupense* ATCC BAA-1242^T (AR 30097) are KR025885, KR025886, KR025887, KR025888, KR025889, KR025890, and KT861786, respectively. The accession numbers for *rpoB* region III sequences deposited in GenBank for strains MO-233, MO-1300, MO-5116, MO-2762, MO-3559, and MO-4693 are KR025891 through KR025896.

RESULTS

Previous reported cases. A total of 35 previously published cases of tenosynovitis and osteomyelitis due to members of the *M. terrae* complex and identified using nonmolecular methods since 1976 were reviewed. Biochemical testing and HPLC were primarily used in the species identification. All cases were previously reported to be due to *M. terrae* or *M. nonchromogenicum*. Most (80%) cases involved the hand, fingers, or wrists, with histopathologic findings of granuloma and growth of the organism from operative materials (Table 1).

A total of 14 cases of tenosynovitis and osteomyelitis due to the *M. terrae* complex identified using molecular methods were investigated (Table 2). In three cases (cases 1, 9, and 12) 16S rRNA gene sequencing was used but did not provide a percent match with a type strain, in one case (case 7) DNA-DNA hybridization was used, and in three cases (cases 2, 3, and 11) no molecular details were given. The first three cases (cases 1 to 3) (Table 2) were all reported as *M. nonchromogenicum*. Two isolates (cases 4 and 5, Table 2) underwent complete 16S rRNA gene sequencing as well as secondary gene target sequencing as part of the original study describing *M. arupense* by Cloud et al. in 2006 (28). One of these (AR 30097^T) is the recognized type strain of *M. arupense*, and both isolates were a 100% match of the complete 16S rRNA gene to each other and other identified sputum isolates (28). In two other cases partial 16S rRNA gene sequencing was used, with 100% match to the type strain of *M. arupense* (cases 8 and 13) (31, 35). Two additional case isolates underwent 16S rRNA partial gene sequencing (cases 6 and 10); one reported as *M. arupense* and the other as *M. longobardum*, although both were only a 99.2% match to the type strain. The isolate in the last case (no. 14) underwent complete 16S sequencing and was a 99.8% (1,429/1,432 bp) match to *M. arupense* (36). By current CLSI standards these last three isolates are grouped as "most closely related") (46, 55). Overall, based on the provided information, only four isolates met current CLSI sequencing criteria for a specific species identification (cases 4, 5, 8, and 13), and all were identified as *M. arupense* (46).

Current isolates. A total of 26 patients with available isolates for study were identified (Table 3). Five of these isolates were identified in the presequencing era (all as *M. nonchromogenicum*) (3), and 21 were new isolates. The 25 patients from known locations were from 13 states: Texas (5), Missouri (3), Florida (3), Massachusetts (2), California (2), Washington (2), Iowa (1), Arkansas (1), Kansas (1), Maine (1), North Carolina (2), Illinois (1), and Virginia (1). Isolates were from the finger, hand, or wrist (18) (69%), elbow (3) (11.5%), knee (1) (4%), flexor tendon (2) (8%), tibia (1) (4%), and synovial fluid (1) (4%).

16S rRNA complete gene sequencing. By complete 16S rRNA gene sequencing, a 100% identity to a validated type strain sequence (Table 3) was obtained for three species: *M. arupense* (10 isolates or 38%), *M. heraklionense* (10 isolates or 38%), and *M. kumamotonense* (one isolate or 4%). There were no matches to *M. terrae* or *M. nonchromogenicum*. Of note, strain MO-2762, identified as *M. kumamotonense*, presented with a gap in the 3′ end compared to the type strain sequence. For a clinical respiratory isolate of *M. kumamotonense* from Canada (unpublished data; strain B0621B018392 [Fig. 1]), a sequence electropherogram of this region revealed the presence of two 16S copies where one sequence contained the gap and the other did not. This resulted in a single base pair shift at that position, making all subsequent sequence data uninterpretable. We considered, then, that this gap could be a feature of some strains of *M. kumamotonense* and therefore was not considered a true base pair difference.

There were three isolates (MO-233, MO-1300, and MO-5116) with 100% identity to each other but no match to any validated species or other GenBank sequence ("new species") using the top 250 matches from nucleotide collection database. The closest established species were *M. arupense*, with a mismatch of 5 bp (99.7%), followed by *M. nonchromogenicum* and *M. heraklionense*, with mismatches of 9 bp (99.4%) and 10 bp (99.3%), respectively. These isolates and their relationship to other isolates of the *M. terrae* complex using the complete 16S rRNA gene are shown in Fig. 1.

The two other isolates, MO-3559 and MO-4693, did not match any known species or the proposed new species and (after excluding positions of ambiguity) have 100% identity with each other. The closest validated species for MO-3559 is *M. arupense* (7-bp mismatch [99.5%]) followed by *M. nonchromogenicum* and *M. heraklionense*, with mismatches of 9 bp (99.4%) and 10 bp (99.3%), respectively. For MO-4693, the closest validated species is *M. arupense* (5-bp mismatch [99.7%]), followed by *M. nonchromogenicum* and *M. heraklionense*, with mismatches of 6 bp (99.6%) and 8 bp (99.4%), respectively. MO-3559 has two base pair differences compared to the proposed new species MO-233, and MO-4693 presents with only one base pair change from MO-233 after excluding ambiguous bases (the second base pair change in 16S rRNA gene between MO-3559 from MO-233 is an ambiguous base in the 16S rRNA gene sequence of MO-4693). This indicates that some of the 5 ambiguous bases in the 16S rRNA gene of MO-4693 might be true base pair mismatches with MO-3559, which is also supported by significant sequence variations observed between the two isolates by other genes (*rpoB* and *hsp65*).

The isolate MO-4693 presented with 5 ambiguous bases that could not be resolved upon repeat sequencing from a single colony and are presumed to be due to 2 differing copies of the 16S rRNA gene.

Comparing against nonvalidated species, the closest match for the 5 new species strains was "*M. paraterrae*" (GenBank accession number EU919229.1), with four base pair mismatches over the full gene. For this reason, the proposed type strain for the species was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) (DSM 45127) for confirmation and comparison. However, it was determined that the isolate submitted to DSMZ as the type strain of "*M. paraterrae*" has a strikingly different 16S rRNA gene sequence than that deposited in GenBank, differing from it by 47 bp (96.8%). This was also confirmed in the DSMZ (with a 100% sequence identity to that determined in our facility) and stated on their website along with strain

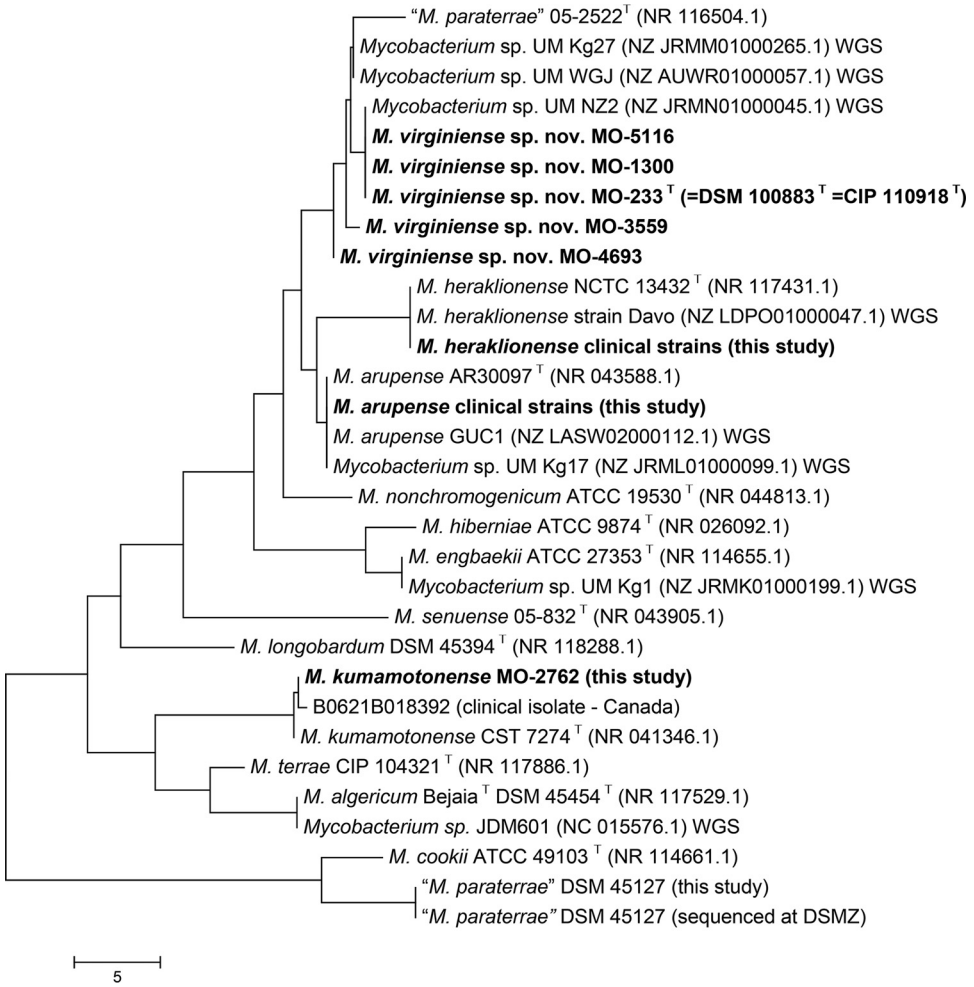

FIG 1 Near-complete 16S rRNA gene sequence dendrogram of both established and nonvalid species of the *Mycobacterium terrae* complex, representative sequences for clinical strains of *M. arupense*, *M. heraklionense*, and *M. kumamotonense* described in this study, and the proposed new species based on neighbor-joining and partial deletion analysis using a site coverage cutoff of 95%. Sequences derived from available whole-genome sequences of members of the *M. terrae* complex (indicated by "WGS") were also included. *Mycobacterium cookii* and "*Mycobacterium paraterrae*" are included as outliers. The bar represents the number of nucleotide differences.

info for DSM 45127 (https://www.dsmz.de/catalogues/details/culture/DSM-45127.html?tx_dsmzresources_pi5%5BreturnPid%5D=304). The complete 16S rRNA gene sequence determined for the strain of "*M. paraterrae*" presently in the DSMZ collection (DSM 45127) appears to represent a novel species closest to *M. cookii* ATCC 49103^T (GenBank accession number AF480598) (9-bp difference [99.4% identity]). Interestingly, a BLAST analysis of the corresponding *rpoB* sequence of "*M. paraterrae*" previously deposited in GenBank (accession number EU919230) reveals a closest match (283/298 bp [95.0%]) with that of *M. cookii* CIP105396^T (accession number AY544904). Similarly, BLAST analysis of the corresponding *hsp*65 sequence of "*M. paraterrae*" deposited in GenBank (accession number EU919228) reveals a close match only to a single entry (579/583 bp) described as *M. cookii*-like (accession number JX566891).

Of the five isolates from the 1991 publication by Ridderhof et al. (3) identified by nonmolecular methods as *M. nonchromogenicum*, two were identified by complete 16S rRNA gene sequencing as *M. arupense*, two were *M. heraklionense*, and one belonged to the proposed new species, "*M. virginiense*" (MO-233).

***rpoB* partial gene sequencing region V.** Two of the five members of the proposed new species (MO-233 and MO-1300) had 100% sequence identity to each other by *rpoB* region V partial gene sequencing but differed by 21 bp (97.1%) from its closest species, *M. nonchromogenicum*. The sequence of the third member (MO-5116) differed by 5 bp (99.3%) from the first two isolates. The last two isolates (MO-3559 and MO-4693) differed from each other by 29 bp (96.0%) of the *rpoB* region V sequence and from the proposed type strain MO-233 by 28 bp (96.1%) and 18 bp (97.5%), respectively. Their relationship to other available members of the *M. terrae* complex is shown in Fig. 2.

Clinical isolate MO-2762, with a single deletion in the 3′ end of the 16S rRNA gene sequence in comparison with the type strain sequence of *M. kumamotonense*, corresponded also to *M. kumamotonense* NCTC 1342^T (accession no. JN571251) by *rpoB* region V (3-bp difference [99.6%]) and by *rpoB* region III (see below). This high degree of similarity further confirms that MO-2762 is a strain of *M. kumamotonense*. To our knowledge, this is the first reported case of tenosynovitis due to this new species, first described in 2006 (36).

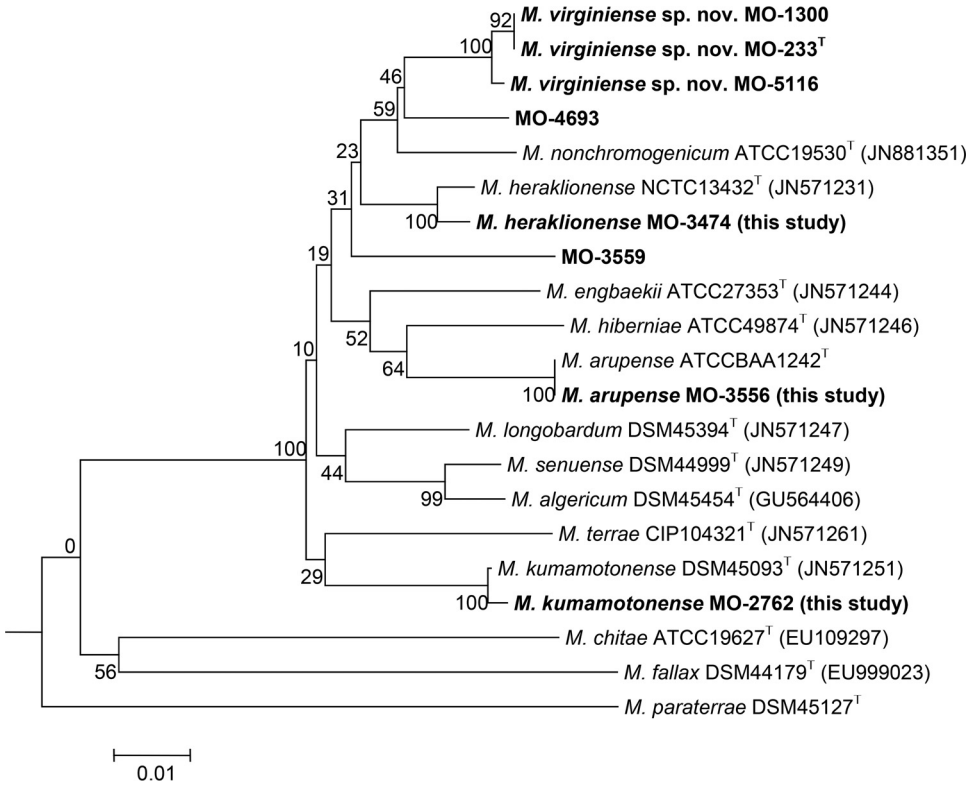

FIG 2 *rpoB* partial gene sequence dendrogram of region V (47) for the three members of the proposed new species, "*M. virginiense*" (MO-233, MO-1300, and MO-5116), the unidentified isolates MO-3559 and MO-4693, and the isolate that by 16S rRNA complete gene sequencing differed by a single deletion from *M. kumamotonense* (MO-2762). There is no region V sequence for *M. arupense* ATCC BAA1242^T in GenBank, so the *rpoB* V region was sequenced and the sequence was used; the sequence was submitted to GenBank. The strain relationships are based on neighbor-joining and complete deletion analysis.

***rpoB* partial gene sequencing region III.** A 315-bp fragment of *rpoB* region III was analyzed (analysis with a shorter fragment depending on available matches is otherwise indicated). Two of the five members of the new species (MO-233 and MO-1300) had 100% sequence identity to each other by *rpoB* region III sequencing but differed by 15/305 bp (95.1%) from the type strain of its closest established species, *M. arupense*. The sequence of the third member (MO-5116) differed from the other two by 15 bp (95.2%) and only by 7/312 bp (97.8%) from its closest species, *M. heraklionense*. This does not represent the type strain (no examples are available for this region); however, this GenBank entry is from the same reference (and authors) that described the species (1). The sequences of the last two (MO-3559 and MO-4693) differed from the "new species" proposed type strain MO-233 by 17 bp (94.6%) and 16 bp (95.0%), respectively. Their relationship to other members of the *M. terrae* complex is shown in Fig. 3.

The isolate with a 1-bp difference (a deletion) in complete 16S rRNA gene sequence from *M. kumamotonense* (MO-2762) had an *rpoB* gene region III sequence that differed by only 1 bp (99.7% identity) from *M. kumamotonense* NCTC 12342^T.

***hsp65* partial gene sequencing.** *hsp65* gene sequencing was done on isolates MO-233, MO-1300, MO-5116, MO-3559, and MO-4693 using the primers used by Telenti et al. (49). A 401-bp *hsp65* sequence (within-primer region) was analyzed for all 5 strains. MO-1300 and MO-5116 differed from the proposed type strain, MO-233, by 1 bp and 3 bp, respectively, representing 99.8% and 99.3% similarities. The closest match for MO-233 to the type strain of an established species was with *M. engbaekii* (8 bp [98.0%]), followed by *M. arupense* (13 bp [96.8%]). Strain MO-4693 diverged from MO-233 by 10 bp (97.5%), and its closest established species was *M. engbaekii* (12 bp [97.0%]). Strain MO-3559 diverged by 20 bp (95.0%) from MO-233, and its closest established species was *M. heraklionense* (7 bp [98.3%]). Their relationship to other members of the *M. terrae* complex is shown in Fig. 4.

Sequence comparisons with non-type strains by BLAST analysis. To assess the presence of the novel species elsewhere, a BLAST analysis was also performed using only the first 500 bp of the 16S rRNA gene, allowing for comparison with GenBank sequences closer to 500 bp in length, as is performed in many clinical laboratories. Sequences of clinical isolates presenting with a 100% match were strains FI-10193 (accession number JN571170.1) (1) and N177 (accession number AY215361.1), both indicated as members of the *M. terrae* complex. Upon BLAST analysis of the 16S rRNA gene (full) against the whole-genome shotgun contigs (wgs) database, a 99.9 to 100% match (0 to 1 bp) was achieved with 3 of 4 strains obtained from the trunk washes of captive elephants. These strains were described as new genomospecies within the *M. terrae* complex (56).

Further investigation against non-type strain sequences was also done using *hsp65*. With the large number of *hsp65* sequences related to the MTC deposited in public sequence databases, many of which are either not identified or misidentified, comparison was restricted to only those with a 100% match. A BLAST analysis

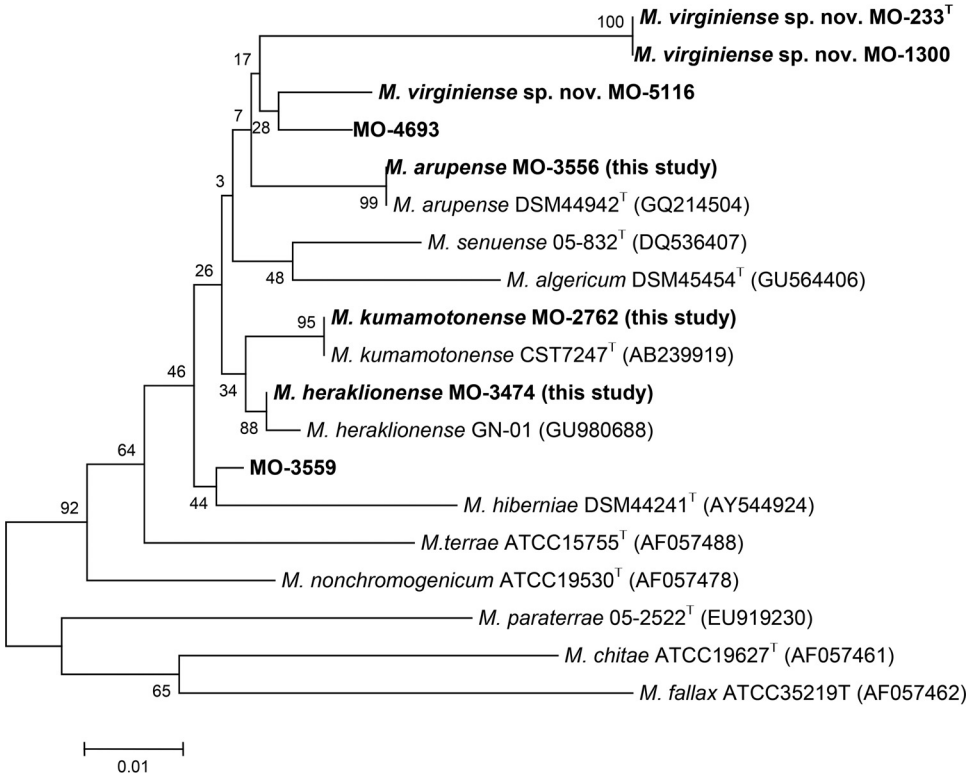

FIG 3 *rpoB* partial gene sequence dendrogram of region III (48), including the three members of the proposed new species, "*M. virginiense*" (MO-233, MO-1300, and MO-5116), the unidentified isolates MO-3559 and MO-4693, and the isolate that by 16S rRNA complete gene sequencing was closest to *M. kumamotonense* (MO-2762). The strain relationships are based on neighbor-joining and complete deletion analysis.

of 401 bp of the *hsp65* of strain MO-233 against the nr (nucleotide collection) revealed a 100% match with 4 sequences in GenBank: (i) strain InDRE Chiapas1942, a clinical isolate from Mexico (accession number JX154109.1); (ii) strain IEC35, a pulmonary specimen from Brazil (accession number HM056146.1); (iii) strain P51, a clinical isolate from Brazil (accession number GQ478699.1); and (iv) strain FI-10193, a clinical specimen from Italy and described as an unassigned strain of the *M. terrae* complex (accession number JN571212.1) (1). Strain MO-4693 revealed a 100% match with "*M. terrae*" variant MS699 (accession

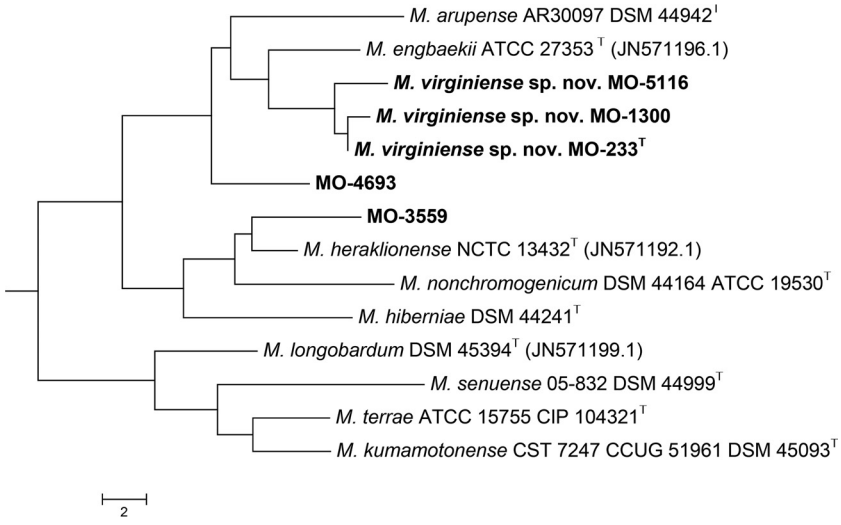

FIG 4 *hsp65* gene sequence (fragment highlighted by Telenti et al.) (49) phylogeny, including the three members of the proposed new species, "*M. virginiense*" (MO-233, MO-1300, and MO-5116) and the unidentified isolates MO-3559 and MO-4693. Representative type strain sequences were obtained from the curated *hsp65* database created by Dai et al., a curated database (51). The tree is rooted using *M. crocinum* and *M. rhodesiae*, the two closest species to MO-233 outside the *M. terrae* complex. The strain relationships are based on neighbor-joining and pairwise deletion analysis. The bar represents the number of base pair differences.

TABLE 4 Antimicrobial susceptibilities of isolates of species within the *M. terrae* complex producing tenosynovitis or osteomyelis

Organism and isolate no.	MO strain designation	MIC (μg/ml)[a]											
		AMK	RMP	RBT	EMB	CIP	MOX	CLA	DOX	LZD	MIN	SMX	TMP-SMX
M. arupense													
1	49	16	8		1	>8		2				32	
2	1082	>64	16	≤0.25	0.5	>16	>8	2	>16	16	>32		2/38
3	1791		4	≤0.25	1	>16	>32	2		16	16	64	
4	3556	>64	2	≤0.25	≤0.5	>16	>8	0.25	16	16			2/38
5	3744	>64	8	≤0.25	≤0.5	>16	>8	1	>16	8			8/152
6	4448	>64	2	≤0.25	≤0.5	>16	>8	0.25	>16	8			0.25/4.25
7	2220	32	4	0.12	≤0.5	16	>8	2			8		0.5/9.5
8	86	64	4	≤0.25	≤0.5	>16	>8	0.5	16	8			4/76
9	1089	>64	4	≤0.25	≤0.5	>16	>8	0.12	16	16			1/19
10	4781	2	1	≤0.25	≤0.5	8	>8	1	8	8			8/152
M. heraklionense													
1	7	8	2		4	8		2			>32	8	
2	51	8	8		16	>8		1			>32	16	
3	778	64	8	≤0.25	2	>16	>8	1, 0.25	>16	8			1/19
4	786	64	>8	1, 1	4	>16	>8	1	>16	32			2/38
5	3474	16	4	0.5	2	>16	>8	2	>16	64			1/19
6	4449	16	1	≤0.25	2	>16	>8	0.5	>16	32			2/38
7	4967	64	0.5	≤0.25	8	16	>8	0.5	>16	>64			≤0.12/2.38
8	5013	>64	8	0.5	2	>16	>8	4	>16	64			
9	5024	16	>8	≤0.25	2	>16	>8	0.5	8	16			1/19
10	5210	32	>8	0.5	2	>16	>8	0.5	>16	32			1/19
M. kumamotonense													
1	2762	64	>8	1	≤0.5	16	4	1		4	16		0.25/9.75
Newly proposed species													
1	233[b]	>8	1	1	4	>16	>8	1	>16	16			1/19
2	1300	>64	64	≤0.25	2	>16	>8	1	16	64	>16		2/38
3	5116	>64	>8	1	2	>16	>8	1	>16	32	>8		2/38
Unique strains													
1	3559	>64	>8	≤0.25	2	>16	>8	0.5	>16	32			2/38
2	4693	>64	4	≤0.25	1	8	8	0.25	16	2			1/19

[a] Abbreviations: AMK, amikacin; RMP, rifampin; RBT, rifabutin; EMB, ethambutol; CIP, ciprofloxacin; MOX, moxifloxacin; CLA, clarithromycin; DOX, doxycycline; LZD, linezolid; MIN, minocycline; SMX, sulfamethoxazole; TMP-SMX, trimethoprim-sulfamethoxazole.
[b] Original MICs determined in 1988 on MO-233.

number AY550212.1) (57) and FI-11039 (accession number JN571213.1) (1). No identical matches were found for the remaining 3 strains, though close matches (1 to 3 bp) were found.

Susceptibility testing. Antimicrobial agents active against the six species of *M. terrae* complex associated with tenosynovitis or osteomyelitis included clarithromycin (26/26, or 100%), ethambutol (24/26, or 92%), rifabutin (26/26, or 100%), and sulfamethoxazole (3/4) or trimethoprim-sulfamethoxazole (19/22, or 86%). The isolates were almost all resistant to rifampin (23/26) and the quinolones ciprofloxacin (26/26) and moxifloxacin (23/23) (Table 4).

DISCUSSION

This study clearly demonstrates the inability of phenotypic tests and mycolic acid analysis (HPLC) to recognize newer mycobacterial species defined by DNA sequencing, including members of the *M. terrae* complex. Tenosynovitis or osteomyelitis caused by members of the *M. terrae* complex was believed on the basis of phenotypic testing by mycolic acid analysis (HPLC) to be due to *M. nonchromogenicum* or *M. terrae* for more than 30 years. The recognition of *M. arupense* as a cause of tenosynovitis in 2006 (28) was the first indication that other species might be responsible. The current study suggests that neither *M. terrae* nor *M. nonchromogenicum* is a cause of tenosynovitis and that earlier isolates identified as these species by nonsequencing methods were misidentified (3).

There is no treatment of choice for *M. terrae* complex tenosynovitis. Previous reports have noted the benefits of a macrolide combined with one or more additional agents that included ethambutol, rifabutin, and/or a sulfonamide, including trimethoprim-sulfamethoxazole (TMP-SMX) (3, 31). The major pathogens defined in the current study are *M. arupense* and *M. heraklionense*. These species are generally susceptible to clarithromycin, ethambutol, rifabutin, and TMP-SMX. A recent report of susceptibilities of 40 isolates of *M. arupense* by Beam et al. gave similar results to the current study (31) with 100% of tested isolates susceptible to clarithromycin, ethambutol, and rifabutin, and approximately 50% susceptible to TMP-SMX (31).

Five of these 26 isolates (19%) belonged to a previously unrecognized species. Three of the five isolates had 100% sequence identity for their complete 16S rRNA gene and differed by 5 bp from its closest validated species. Three isolates, including the proposed type strain (MO-233, MO-1300, and MO-5116), exhibited >99% sequence identity for region V of the *rpoB* gene, the sequence of the *hsp65* gene highlighted by Telenti et al., and two-thirds of region III of the *rpoB* gene. Strain MO-3559, however, exhibited <96.2% identity for these sequences, and MO-4693 exhibited from 97.9% to 95.3% identity for the same sequences. The high degree of variance of the strains MO-3559 and MO-4693 from each other and from the other three strains of the proposed new species possibly indicates emergence of new species that is beyond the scope of this study. The proposed name for the three isolates, "*M. virginiense*," refers to the geographic location of the first recognized case.

Like the other previous 11 members of the *M. terrae* group, this new species has a two-nucleotide insertion in helix 18 of the 16S rRNA gene (37, 58) characteristic of members of the *M. terrae* complex. It also shares other culture features of this group, including lack of pigmentation and growth rate of more than 7 days.

In the current study, one isolate of *M. kumamotonense*, whose complete 16S rRNA gene sequence differed by one deletion from the type strain, was identified. Given the likelihood of the presence of two ribosomal operons and the high degree of similarity of region V of the *rpoB* between the current strain and the type strain (99.6%), it is highly likely that the current isolate is *M. kumamotonense*. The extra base pair occurs within 20 bp of the 3′ end of the sequence in GenBank, while the current sequence with the gap is approximately 80 bp longer (1). Recent studies have shown this species to have two copies of the 16S rRNA gene, and this is the most likely explanation of this single base pair difference (53).

Description of new species. Isolates of the newly proposed species "*M. virginiense*" were acid fast, slowly growing, and nonpigmented on Middlebrook 7H10 agar. The isolates were buff colored and grew in >7 days. They did not grow at 42°C, and their optimal growth temperature was 35°C.

By CLSI guidelines, the isolates were susceptible to clarithromycin, ethambutol, rifabutin, and TMP-SMX and resistant to rifampin, the quinolones, including moxifloxacin, amikacin, and the tetracycline analogues doxycycline and minocycline (54). Their complete 16S rRNA gene, the *hsp65* fragment highlighted by Telenti et al., and regions III and V of the *rpoB* gene are different from those of other members of the *M. terrae* complex.

The proposed type strain is MO-233, which is an acid fast slowly growing, nonchromogenic isolate on Middlebrook agar that produced tenosynovitis in a 58-year-old woman from Virginia (case 1 in the paper by Ridderhof et al. [3]). The isolate was niacin negative, had a strongly positive (5+) nitrate score, had a semiquantitative catalase score of >45 mm, had a negative arylsulfatase at 3 and 14 days, was urease negative, was positive for Tween hydrolysis, and did not reduce tellurite (3). The isolate has been submitted to the Collection de l'Institut Pasteur (CIP) (CIP110918) and the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) (DSM 100883).

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REFERENCES

1. **Tortoli E, Gitti Z, Klenk H-P, Lauria S, Mannino R, Mantegani P, Mariottini A, Neonakis I.** 2013. Survey of 150 strains belonging to the *Mycobacterium terrae* complex and description of *Mycobacterium engbaekii* sp. nov., *Mycobacteirum heraklionense* sp. nov., and *Mycobacterium longobardum* sp. nov. Int J Syst Evol Microbiol **63:**401–411. http://dx.doi.org/10.1099/ijs.0.038737-0.
2. **Wayne LG, Good RC, Bottger EC, Butler R, Dorsch M, Ezaki T, Gross W, Jonas V, Kilburn J, Kirschner P, Krichevsky MI, Ridell M, Shinnick TM, Springer B, Stackebrandt E, Tarnok I, Tarnok Z, Tasaka H, Vincent V, Warren NG, Knott CA, Johnson R.** 1996. Semantide- and chemotaxonomy-based analyses of some problematic phenotypic clusters of slowly growing mycobacteria, a cooperative study of the International Working Group on Mycobacterial Taxonomy. Int J Syst Bacteriol **46:**280–297. http://dx.doi.org/10.1099/00207713-46-1-280.
3. **Ridderhof JC, Wallace RJ, Jr, Kilburn JO, Butler WR, Warren NG, Tsukamura M, Steele LC, Wong ES.** 1991. Chronic tenosynovitis of the hand due to *Mycobacterium nonchromogenicum*: use of high-performance liquid chromatography for identification of isolates. Rev Infect Dis **13:** 857–864. http://dx.doi.org/10.1093/clinids/13.5.857.
4. **Christensson B, Olsson B, Arner M, Miorner H.** 1993. Subcutaneous infection of the hand due to *Mycobacterium nonchromogenicum*. J Hand Surg (Am) **18:**130–132. http://dx.doi.org/10.1016/0363-5023(93)90257-4.
5. **Edwards MS, Huber TW, Baker CJ.** 1978. *Mycobacterium terrae* synovitis and osteomyelitis. Am Rev Respir Dis **117:**161–163.
6. **Halla JT, Gould JS, Hardin JG.** 1979. Chronic tenosynovial hand infection from *Mycobacterium terrae*. Arthritis Rheum **22:**1386–1390. http://dx.doi.org/10.1002/art.1780221211.
7. **Dijkmans BAC, Mouton RP, Macfarlane JD, Reynvaan-Groendijk A, Rozing P, van den Broek PJ, van der Meer JWM.** 1981. Bacterial arthritis caused by *Mycobacterium terrae*. Infection **9:**204–207. http://dx.doi.org/10.1007/BF01640981.
8. **Huskisson EC, Doyle DV, Fowler EF, Shaw EJ.** 1981. Sausage digit due to radish bacillus. Ann Rheum Dis **40:**90–91. http://dx.doi.org/10.1136/ard.40.1.90.
9. **Mehta JB, Hovis WM.** 1983. Tenosynovitis of the forearm due to *Mycobacterium terrae* (radish bacillus). South Med J **76:**1433–1435. http://dx.doi.org/10.1097/00007611-198311000-00028.
10. **May DC, Kutz JE, Howell RS, Raff MJ, Melo JC.** 1983. *Mycobacterium terrae* tenosynovitis: chronic infection in a previously healthy individual. South Med J **76:**1445–1446. http://dx.doi.org/10.1097/00007611-198311000-00033.
11. **Janier M, Dupont B, Achach PC, David H, Lapresle C.** 1984. Tenosynovities digitales a mycobacteries non tubercueuses: a propos due 4 cas à *Mycobacterium terrae*. Presse Med **13:**269–271.
12. **Love G, Melchior E.** 1985. *Mycobacterium terrae* tenosynovitis. J Hand Surg (Am) **10:**730–732. http://dx.doi.org/10.1016/S0363-5023(85)80221-5.
13. **Fournié B, Dabernat H, Vaudet B, Jalby H, Fournie A.** 1987. Arthrite due genou à *Mycobacterium terrae*. Rev Rhum Mal Osteoartic **54:**49–51.
14. **Kremer LB, Rhame FS, House JH.** 1988. *Mycobacterium terrae* tenosynovitis. Arthritis Rheum **31:**932–934. http://dx.doi.org/10.1002/art.1780310721.
15. **Deenstra W.** 1988. Synovial hand infection from *Mycobacterium terrae*. J Hand Surg (Br) **13:**335–336. http://dx.doi.org/10.1016/0266-7681(88)90105-2.
16. **Petrini B, Svartengren G, Hoffner SE, Unge G, Widstrom O.** 1989. Tenosynovitis of the hand caused by *Mycobacterium terrae*. Eur J Clin Microbiol Infect Dis **8:**722–724. http://dx.doi.org/10.1007/BF01963759.
17. **Rougraff BT, Reeck CC, Slama TG.** 1989. *Mycobacterium terrae* osteomyelitis and septic arthritis in a normal host. Clin Orthop **238:**308–310.
18. **Karthigasu KT, Spagnolo DV, Gow BL.** 1990. *Mycobacterium terrae* tenosynovitis. Pathology **22:**106–107. http://dx.doi.org/10.3109/00313029009063789.
19. **Katz D.** 1993. *Mycobacterium terrae* tenosynovitis of the hand. Ann Chir

Main Memb Super **12:**136–139. http://dx.doi.org/10.1016/S0753-9053(05)80089-4.
20. **Kozin SH, Bishop AT.** 1994. *Mycobacterium* infections of the upper extremity. J Hand Surg (Am) **19:**480–487. http://dx.doi.org/10.1016/0363-5023(94)90067-1.
21. **Fodero J, Chung KC, Ognenovski VM.** 1999. Flexor tenosynovitis in the hand caused by *Mycobacterium terrae*. Ann Plast Surg **42:**330–332. http://dx.doi.org/10.1097/00000637-199903000-00017.
22. **Smith DS, Lindholm-Levy P, Huitt GA, Heifets LB, Cook JL.** 2000. *Mycobacterium terrae*: case reports, literature review, and in vitro antibiotic susceptibility testing. Clin Infect Dis **30:**444–453. http://dx.doi.org/10.1086/313693.
23. **Chen HW, Lai CC, Tan CK.** 2009. Arthritis caused by *Mycobacterium terrae* in a patient with rheumatoid arthritis. Int J Infect Dis **13:**e145–e147. http://dx.doi.org/10.1016/j.ijid.2008.09.002.
24. **Omoruyi OJ, Ip WY, Fung BK.** 2012. Metachronous *Mycobacterium terrae* complex tenosynovitis of the hand. J Hand Surg Eur Vol **37:**573–574. http://dx.doi.org/10.1177/1753193412441123.
25. **Lembo G, Goldstein EJ, Troum O, Mandelbaum B.** 2012. Successful treatment of *Mycobacterium terrae* complex infection of the knee. J Clin Rheumatol **18:**359–362. http://dx.doi.org/10.1097/RHU.0b013e31826d1e11.
26. **Eskesen AN, Skråmm I, Steinbakk M.** 2007. Infectious tenosynovitis and osteomyelitis caused by *Mycobacterium nonchromogenicum*. Scand J Infect Dis **39:**179–180. http://dx.doi.org/10.1080/00365540600798817.
27. **Olsen RJ, Cernoch PL, Land GA.** 2006. Mycobacterial synovitis caused by slow-growing nonchromogenic species and a review of the literature. Arch Pathol Lab Med **130:**783–791.
28. **Cloud JL, Meyer JJ, Pounder JL, Jost KC, Jr, Sweeney A, Carrol KC, Woods GL.** 2006. *Mycobacterium arupense* sp. nov., a novel moderately growing non-chromogenic bacterium isolated from clinical specimens. Int J Syst Evol Microbiol **56:**1413–1418. http://dx.doi.org/10.1099/ijs.0.64194-0.
29. **Tsai T-F, Lai C-C, Tsai I-C, Chang C-H, Hsiao C-H, Hsueh P-R.** 2008. Tenosynovitis caused by *Mycobacterium arupense* in a patient with diabetes mellitus. Clin Infect Dis **47:**861–863. http://dx.doi.org/10.1086/591281.
30. **Senda H, Muro H, Terada S.** 2011. Flexor tenosynovitis caused by *Mycobacterium arupense*. J Hand Surg Eur **36:**72–73. http://dx.doi.org/10.1177/1753193410381825.
31. **Beam E, Vasoo S, Simner PJ, Rizzo M, Mason EL, Walker RC, Deml SM, Brown-Elliott BA, Wallace RJ, Jr, Wengenack NL, Sia IG.** 2014. *Mycobacterium arupense* flexor tenosynovitis: case report and review of antimicrobial susceptibility profiles for 40 clinical isolates. J Clin Microbiol **52:**2706–2708. http://dx.doi.org/10.1128/JCM.00277-14.
32. **Legout L, Ettahar N, Massongo M, Veziris N, Ajana F, Beltrand E, Senneville E.** 2012. Osteomyelitis of the wrist caused by *Mycobacterium arupense* in an immunocompetent patient: a unique case. Int J Infect Dis **16:**e761–e762. http://dx.doi.org/10.1016/j.ijid.2012.05.007.
33. **Ogawa K, Satou S, Hurukawa M, Minegishi M, Kikkawa Y, Tanaka T, Nakanaga K, Ishii N.** 2014. A case of flexor tenosynovitis caused by *Mycobacterium arupense.* J Jpn Soc Clin Microbiol **24:**17–22.
34. **Abedalthagafi M, Rosenberg O, Miller S.** 6 January 2014. First report of tenosynovitis in an immunocompetent person caused by *Mycobacterium heraklionense*. JMM Case Rep http://dx.doi.org/10.1099/jmmcr.0.002071.
35. **Lee SJ, Hong SK, Park SS, Kim E-C.** 2014. First Korean case of *Mycobacterium arupense* tenosynovitis. Ann Lab Med **34:**321–324. http://dx.doi.org/10.3343/alm.2014.34.4.321.
36. **Masaki T, Ohkusu K, Hata H, Fujiwara N, Iihara H, Yamada-Noda M, Nhung PH, Hayashi M, Asano Y, Kawamura Y, Ezaki T.** 2006. *Mycobacterium kumamotonense* sp. nov. recovered from clinical specimen and the first isolation report of *Mycobacterium arupense* in Japan: novel slowly growing, nonchromogenic clinical isolates related to *Mycobacterium terrae* complex. Microbiol Immunol **50:**889–897. http://dx.doi.org/10.1111/j.1348-0421.2006.tb03865.x.
37. **Kirschner P, Springer B, Vogel U, Meier A, Wrede A, Kiekenbeck M, Bange FC, Böttger EC.** 1993. Genotypic identification of mycobacteria by nucleic acid sequence determination: report of a 2-year experience in a clinical laboratory. J Clin Microbiol **31:**2882–2889.
38. **Springer B, Stockman L, Teschner K, Roberts GD, Böttger EC.** 1996. Two-laboratory collaborative study on identification of mycobacteria: molecular versus phenotypic methods. J Clin Microbiol **34:**296–303.
39. **Kazda J, Cooney R, Monaghan M, Quinn PJ, Stackebrandt E, Dorsch M, Daffe M, Müller K, Cook BR, Tarnok ZS.** 1993. *Mycobacterium hiberniae* sp. nov. Int J Syst Bacteriol **43:**352–357. http://dx.doi.org/10.1099/00207713-43-2-352.
40. **Mun H-S, Park J-H, Kim H, Yu H-K, Park Y-G, Cha C-Y, Kook Y-H, Kim B-J.** 2008. Mycobacterium senuense sp. nov., a slowly growing, non-chromogenic species closely related to the Mycobacterium terrae complex. Int J Syst Evol Microbiol **58:**641–646. http://dx.doi.org/10.1099/ijs.0.65374-0.
41. **Hannigan GD, Krivogorsky B, Fordice D, Welch JB, Dahl JL.** 2013. *Mycobacterium minnesotense* sp. nov., a photochromogenic bacterium isolated from sphagnum peat bogs. Int J Syst Evol Microbiol **63:**124–128. http://dx.doi.org/10.1099/ijs.0.037291-0.
42. **Sahraoui N, Ballif M, Zelleg S, Yousfi N, Ritter C, Friedel U, Amstutz B, Yala D, Boulahbal F, Guetarni D, Zinsstag J, Keller PM.** 2011. *Mycobacterium algericum* sp. nov., a novel rapidly growing species related to the *Mycobacterium terrae* complex and associated with goat lung lesions. Int J Syst Evol Microbiol **61:**1870–1874. http://dx.doi.org/10.1099/ijs.0.024851-0.
43. **Lee H, Lee SA, Lee IK, Yu HK, Park YG, Jeong J, Lee SH, Kim SR, Hyun JW, Kim K, Kook Y-H, Kim B-J.** 2010. *Mycobacterium paraterrae* sp. nov. recovered from a clinical specimen: novel chromogenic slow growing mycobacteria related to *Mycobacterium terrae* complex. Microbiol Immunol **54:**46–53. http://dx.doi.org/10.1111/j.1348-0421.2009.00184.x.
44. **Zhang ZY, Sun ZQ, Wang ZL, Hu HR, Wen ZL, Song YZ, Zhao JW, Wang HH, Guo XK, Zhang Zhang SL.** 2013. Identification and pathogenicity analysis of a novel non-tuberculous mycobacterium clinical isolate with nine-antibiotic resistance. Clin Microbiol Infect **19:**91–96.
45. **Edwards U, Rogall T, Blöcker H, Ende M, Böttger EC.** 1989. Isolation and direct complete nucleotide determination of entire genes. Characterization of a gene coding for 16S ribosomal RNA. Nucleic Acid Res **17:**7843–7853.
46. **Clinical and Laboratory Standards Institute.** 2008. Interpretive criteria for identification of bacteria and fungi by DNA target sequencing: approved guideline. CLSI document MM18-A. Clinical and Laboratory Standards Institute, Wayne, PA.
47. **Adékambi T, Colson P, Drancourt M.** 2003. *rpoB*-based identification of nonpigmented and late-pigmenting rapidly growing mycobacteria. J Clin Microbiol **41:**5699–5708. http://dx.doi.org/10.1128/JCM.41.12.5699-5708.2003.
48. **Kim B-J, Lee SH, Lyu MA, Kim SJ, Bai GH, Chae GT, Kim EC, Cha CY, Kook YH.** 1999. Identification of mycobacterial species by comparative sequence analysis of the RNA polymerase gene (*rpoB*). J Clin Microbiol **37:**1714–1720.
49. **Telenti A, Marchesi F, Balz M, Bally F, Bottger EC, Bodmer T.** 1993.Rapid identification of mycobacteria to the species level by polymerase chain reaction and restriction enzyme analysis. J Clin Microbiol **31:**175–178.
50. **Tamura K, Stecher G, Peterson D, Filipski A, Kumar S.** 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol Biol Evol **30:**2725–2729. http://dx.doi.org/10.1093/molbev/mst197.
51. **Dai J, Chen Y, Lauzardo M.** 2011. Web-accessible database of *hsp65* sequences from *Mycobacterium* reference strains. J Clin Microbiol **49:**2296–2303.
52. **Ninet B, Monod M, Emler S, Pawlowski J, Metral C, Rohner P, Auckenthaler R, Hirschel B.** 1996. Two different 16S rRNA genes in a mycobacterial strain. J Clin Microbiol **34:**2531–2536.
53. **Menéndez MC, Jiménez MS, Yubero J, García MJ.** 2014. Mycobacterium kumamotonense, another member of the Mycobacterium terrae complex unusually carrying two copies of the ribosomal RNA operon. Mycobact Dis **4:**6.
54. **Clinical and Laboratory Standards Institute.** 2011. Susceptibility testing of mycobacteria, nocardiae, and other aerobic actinomycetes: approved standard, 2nd ed. CLSI document M24-A2. Clinical and Laboratory Standards Institute, Wayne, PA.
55. **Hong SK, Sung JY, Lee HJ, Oh M-D, Park SS, Kim E-C.** 2013. First case of *Mycobacterium longobardum* infection. Ann Lab Med **33:**356–359. http://dx.doi.org/10.3343/alm.2013.33.5.356.
56. **Ngeow YF, Wong YL, Tan JL, Hong KW, Ng HF, Ong BL, Chan KG.** 2015. Identification of new genomospecies in the Mycobacterium terrae complex. PLoS One **10**(4):e0120789. http://dx.doi.org/10.1371/journal.pone.0120789.
57. **McNabb A, Eisler D, Adie K, Amos M, Rodrigues M, Stephens G, Black WA, Isaac-Renton J.** 2004. Assessment of partial sequencing of the 65-

kilodalton heat shock protein gene (*hsp65*) for routine identification of *Mycobacterium* species isolated from clinical sources. J Clin Microbiol **42:**3000–3011. http://dx.doi.org/10.1128/JCM.42.7.3000-3011.2004.

58. **Springer B, Wu WK, Bodmer T, Haase G, Pfyffer GE, Kroppenstedt RM, Schröder KH, Emler S, Kilburn JO, Kirschner P, Telenti A, Coyle MB, Böttger EC.** 1996. Isolation and characterization of a unique group of slowly growing mycobacteria: description of *Mycobacterium lentiflavum* sp. nov. J Clin Microbiol **34:**1100–1107.
59. **Rogall T, Wolters J, Flohr T, Bottger EC.** 1990. Towards a phylogeny and definition of species at the molecular level within the genus *Mycobacterium*. Int J Syst Bacteriol **40:**323–330. http://dx.doi.org/10.1099/00207713-40-4-323.