Mycobacterium arosiense sp. nov., a slowly growing, scotochromogenic species causing osteomyelitis in an immunocompromised child

Didi Bang,[1] Troels Herlin,[2] Marc Stegger,[1] Aase Bengaard Andersen,[3] Pirjo Torkko,[4,5] Enrico Tortoli[6] and Vibeke Ostergaard Thomsen[1]

Correspondence
Didi Bang
DVB@ssi.dk

[1]International Reference Laboratory of Mycobacteriology and National Center for Antimicrobials and Infection Control, Statens Serum Institut, Artillerivej 5, DK-2300 Copenhagen, Denmark

[2]Department of Pediatrics, Aarhus University, Brendstrupgaardsvej, Skejby Hospital, DK-8200 Aarhus N, Denmark

[3]Department of Infectious Diseases, Rigshospitalet, University of Copenhagen, Blegdamsvej 9, DK-2100 Copenhagen, Denmark

[4]Laboratory of Environmental Microbiology, Department of Environmental Health, National Public Health Institute, PO Box 95, FI-70701 Kuopio, Finland

[5]Department of Clinical Microbiology, Kuopio University Hospital, PO Box 1777, FI-70211 Kuopio, Finland

[6]Mycobacteriology Reference Center, Microbiology Laboratory, Careggi Hospital, viale Morgagni 85, I-50134 Florence, Italy

A yellow-pigmented, scotochromogenic, slowly growing mycobacterial strain, designated T1921[T], was isolated from the disseminated osteomyelitic lesions of a 7-year-old child with an underlying partial gamma interferon receptor alpha-1 deficiency. Hybridization by the line probe assay indicated the presence of a *Mycobacterium* species. Sequencing of the 16S rRNA gene, the internally transcribed spacer (ITS) region and the *hsp65* and *rpoB* genes revealed that strain T1921[T] could be differentiated from all recognized species of the genus *Mycobacterium*. Phylogenetic analysis based on the 16S rRNA gene indicated that strain T1921[T] was related most closely to *Mycobacterium intracellulare*, whereas analysis based on the ITS and *hsp65* and *rpoB* genes indicated that it was most closely related to *Mycobacterium avium*. Phenotypic tests were not able to differentiate strain T1921[T] from similar slowly growing mycobacteria. Strain T1921[T] is considered to represent a novel species of the genus *Mycobacterium*, for which the name *Mycobacterium arosiense* sp. nov. is proposed. The type strain is T1921[T] (=DSM 45069[T] =ATCC BAA-1401[T]).

Current techniques for genetic sequencing and mycolic-acid analyses have greatly enhanced the ability to differentiate among organisms within mycobacterial taxonomy (Tortoli *et al.*, 2001; Janda & Abbott, 2002). Pathogenic, slowly growing, non-tuberculous mycobacteria (NTM) can cause a wide range of diseases, including osteoarticular disease (Heifets, 2004). *Mycobacterium avium* and *Mycobacterium intracellulare* have been described in case reports to be the causative agents of osteomyelitis in both immunocompetent and immuno-compromised children (Raszka *et al.*, 1994; Villella *et al.*, 2001; Arend *et al.*, 2001; Collert *et al.*, 1983). In most cases, they cause disease that is not severe, but, in immunocompromised individuals, disseminated disease may be life threatening. Here we characterize and describe a slowly growing *Mycobacterium*-like strain, isolated from different sites of osteomyelitic lesions from a young girl, and show that this represents a novel species of the genus.

A 7-year-old girl with a hereditary partial gamma interferon (IFN-γ) receptor alpha-1 deficiency presented with fatigue, low-grade fever, weight loss (2.5 kg) and pain in the left clavicle. The patient was found to have an

elevated erythrocyte sedimentation rate (102 mm h^{-1}), C-reactive protein (384 nmol l^{-1}) and leukocytosis (11.3 × 10^9 l^{-1}) with neutrophilia (58 %). A bone (technetium-99m, ^{99m}Tc) scan, magnetic resonance imaging and radiography confirmed changes consistent with disseminated osteomyelitis of the left clavicle, chest and extremities. Three isolations, obtained from different sites of the osteomyelitic bone lesions, revealed the presence of acid-fast bacilli based on auramine-rhodamine-stained fluorescence microscopy, with smear grades of 2+, 1+ and 3+, respectively, as described by Smithwick (1979). All the samples fulfilled the American Thoracic Society criteria for clinical significance (American Thoracic Society, 1997). Therapy was initiated with rifabutin, ethambutol, isoniazid and clarithromycin for a period of 1 year and there were no signs of relapse 18 months later. Drug susceptibility testing was performed by using the BACTEC 460 radiometric system (Siddiqi, 1996) and minimal inhibitory concentrations were determined in Middlebrook 7H9 broth. The organism was found to be susceptible to clarithromycin, rifamycins, amikacin, moxifloxacin, linezolid and clofazimine by using the two methods above (Table 1). Resistance was observed to isoniazid, ciprofloxacin, ofloxacin and streptomycin. Ethambutol gave variable results with the two methods, probably due to the lack of a standardized procedure.

Single bacterial strains were isolated from different locations: one from clavicle biopsy material (T1921^T) and two from bone marrow samples (T1919 and T1920). The biopsy specimen was subjected to standard decontamination with N-acetyl-L-cysteine/NaOH and cultured on Löwenstein–Jensen and BACTEC MGIT 960 liquid media (Becton Dickinson). The bone marrow specimens were seeded directly in a BACTEC MGIT 9050 (Becton Dickinson) system and on Löwenstein–Jensen slants without N-acetyl-L-cysteine/NaOH pretreatment. All three isolations were subjected to repeated genotypic and phenotypic analyses and revealed similar results, indicating that they represented a single strain. Testing by the commercial line probe system (INNO LiPA MYCOBACTERIA v 2; Innogenetics) used for mycobacterial species identification was performed according to the manufacturer's instructions. Hybridization with the genus-specific probe and failure to hybridize with species-specific probes in the 16–23S rRNA gene internally transcribed spacer region (ITS) indicated the presence a *Mycobacterium* strain that did not belong to any species identifiable with the system.

Genetic sequencing of the almost-complete 16S rRNA gene and partial *rpoB* and 65-kDa heat-shock protein (*hsp65*) genes and the ITS between the 16S and 23S rRNA genes was carried out by using standard procedures (Kirschner *et al.*, 1993; Roth *et al.*, 1998; McNabb *et al.*, 2004; Adekambi *et al.*, 2003). Phylogenetic analysis was carried out via alignment by using BLAST searches (GenBank). The gene sequences of strain T1921^T were compared with the most closely related mycobacterial species present in major international nucleotide sequence databases (GenBank, EMBL, DDBJ).

The almost-complete 16S rRNA gene sequence of strain T1921^T did not match that of any known mycobacterial species (GenBank database; http://www.ncbi.nlm.nih.gov/BLAST/). When the sequence of the 16S rRNA gene of strain T1921^T was compared with those of the most closely related species, there were six (0.42 %) mismatches with the type strain of *M. intracellulare* in a 1441 bp stretch and 11 mismatches (0.74 %) with that of *M. avium* in a 1495 bp stretch. In the hypervariable region A of the 16S rRNA gene, two mismatches occurred, with a substitution of A for T at position 203 and a T for A at position 205 (*Escherichia coli* 16S rRNA gene positions). In the hypervariable region (420 bp) of the *hsp65* gene, the closest match was to the type strain of *M. avium*, which

Table 1. Susceptibility testing of strain T1921^T by the microdilution and BACTEC 460 methods

Drug susceptibility test results are denoted as: S, susceptible; R, resistant; ND, not done. MIC, Minimal inhibitory concentration.

Compound	Microdilution		BACTEC 460	
	MIC (µg ml^{-1})	Interpretation	Critical concentration (µg ml^{-1})	Interpretation
Clarithromycin	2	S	2.0	S
Rifampicin	0.25	S	2.0	S
Rifabutin	⩽0.06	S	1.0	S
Amikacin	2	S	4.0	S
Moxifloxacin	0.5	S	ND	—
Ofloxacin	ND	—	2.0	R
Ciprofloxacin	>16	R	ND	—
Ethambutol	2	S	2.5	R
Linezolid	4	S	ND	—
Streptomycin	>32	R	ND	—
Clofazimine	ND	—	1.0	S
Isoniazid	ND	—	1.0	R

differed by 10 bp (95 % similarity). In the region (730 bp) of the *rpoB* gene recently proposed (Adekambi *et al.*, 2003) as a gold standard for the genetic differentiation of rapidly growing mycobacteria, the closest match was again with the type strain of *M. avium*, but which was characterized by 40 mismatches (94 % similarity). Finally, in the ITS, on which the differentiation of *M. avium* complex (MAC) sequevars is based (Mijs *et al.*, 2002), strain T1921^T differed, in a fragment of 309 bp, by 11 bp (96 % similarity) from the type strain of *M. avium*.

Phylogenetic trees were constructed for all the above genetic regions, with the exception of the ITS region, based on the neighbour-joining method (Saitou & Nei, 1987) and with the type strain of *Mycobacterium tuberculosis* as an outgroup. In the tree based on the 16S rRNA gene, strain T1921^T fell within the slowly growing *Mycobacterium* species (Fig. 1). Phylogenetic trees based on the *hsp65* and *rpoB* genes are available as Supplementary Fig. S1 in IJSEM Online. The tree based on *rpoB* gene sequences turned out to be poorly significant as almost all database sequences of this gene are for rapid growers, with the only entries related to strain T1921^T being a number of *M. avium* strains.

GLC analysis of fatty acids, fatty alcohols and mycolic acid cleavage products was performed as described by Torkko *et al.* (2003). All strains were grown on Middlebrook 7H11 agar. Fatty acid analysis of strain T1921^T by GLC (Fig. 2) showed a profile with close similarity to that of *M. intracellulare* (not shown). Fatty acids typical of mycobacteria were present. The major fatty acids of strain T1921^T were 16 : 0 and 10-methyl 18 : 0 (tuberculostearic acid). The main mycolic acid cleavage product was 24 : 0. In addition, alcohols 2-OH 20 : 0 and 2-OH 18 : 0 were present, but the latter was only a minor component. HPLC analysis was performed by using a System Gold instrument (Beckman) with Ultrasphere-XL column according to standard procedures (Butler *et al.*, 1996). Mycolic acids were prepared by saponification and extraction of whole cells and then derivatized to bromophenacyl esters. Low- and high-molecular-mass standards were included for peak identification. Analysis of mycolic acids by HPLC showed the typical three-clustered pattern shared by MAC species and by *Mycobacterium scrofulaceum*. Minor differences in the second and third cluster of peaks (Fig. 3) were within the variability typically reported when using this method.

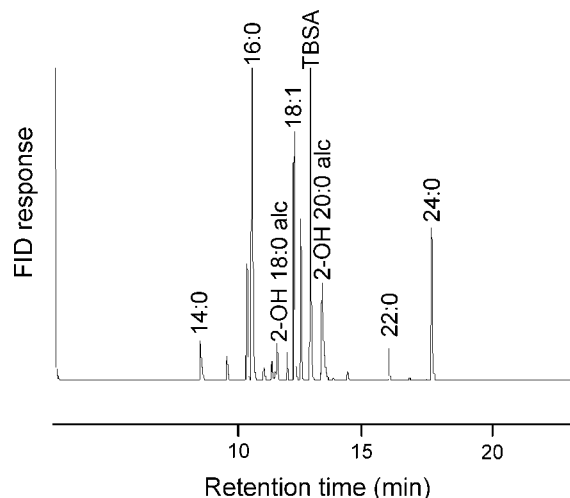Acid–alcohol-fastness was verified by Ziehl–Neelsen-stained smears from colonies grown on Löwenstein–

Fig. 2. GLC fatty acid profile of strain T1921^T. FID, Flame ionization detector; TBSA, tuberculostearic acid.

Jensen slants. Tests for growth and biochemical characteristics were carried out by using conventional techniques (Lévy-Frébault & Portaels, 1992; Torkko *et al.*, 1998). *β*-Galactosidase activity was determined by using commercial discs according to the manufacturer's instructions (Rosco). After growth for 14 days on Middlebrook 7H11 agar medium, strain T1921^T formed small, smooth, yellow scotochromogenic colonies with heavy inocula; the strain did not grow well on egg glycerol medium at temperatures of 36–42 °C. Growth was observed at temperatures of 30–42 °C, with optimum growth at 42 °C. No growth was observed after 8 weeks at 22 or 45 °C. Strain T1921^T was positive for thermotolerant catalase, pyrazinamidase, acid phosphatase and alkaline phosphatase, but negative for Tween 80 hydrolysis, catalase (semi-quantitative analysis) and urease, arylsulfatase (after 14 days), α-galactosidase and *β*-galactosidase. The nitrate reductase test was positive, in contrast to results for *M. avium*, *M. intracellulare* and *M. scrofulaceum* (Table 2) (Murray *et al.*, 2007). However, these phenotypic features were not sufficient to differentiate strain T1921^T from other closely related slowly growing mycobacteria.

The MAC traditionally consists of 28 serotypes (serovars) belonging to the species *M. avium* and *M. intracellulare*. *M. scrofulaceum* is no longer recognized as part of this
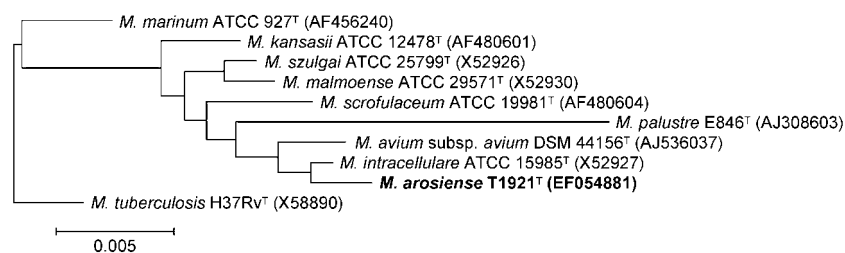

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences of strain T1921^T and the type strains of selected mycobacterial species. Bar, 0.005 substitutions per nucleotide position.
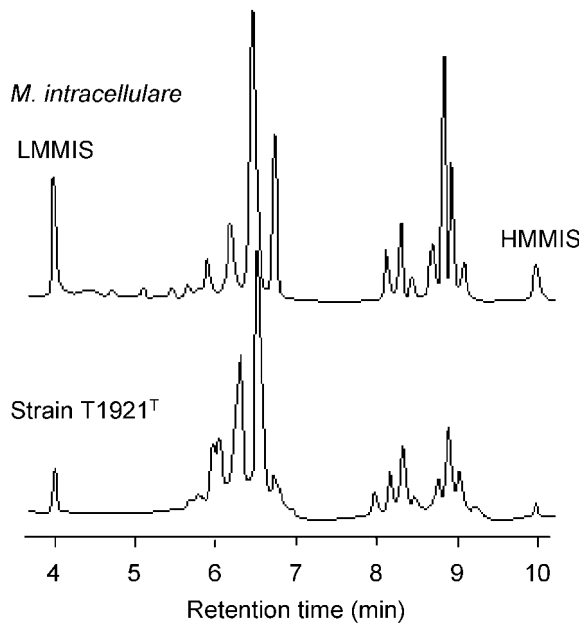
Fig. 3. HPLC fatty acid profile of strain T1921[T] compared with that of *M. intracellulare*. LMMIS, Low-molecular-mass internal standard; HMMIS, high-molecular-mass internal standard.

complex (Murray *et al.*, 2007). The MAC has recently been extended with the description of *Mycobacterium chimaera* (Tortoli *et al.*, 2004), and evidence for a fourth species within the MAC has recently been presented with the description of *Mycobacterium colombiense* (Murcia *et al.*, 2006). Strain T1921[T] possesses unique 16S rRNA, *hsp65*, ITS and *rpoB* gene sequences, and can readily be distinguished from all other known mycobacteria on this basis.

The case history confirms that the novel mycobacterium was the cause of a serious disseminated osteomyelitis in a

child suffering from a partial IFN-γ receptor alpha-1 deficiency. The father of the child was likewise diagnosed with the previously undescribed defect at position 794delT in the IFN-γ gene, and he had previously been susceptible to severe mycobacterial infections (Herlin *et al.*, 1981; Storgaard *et al.*, 2006). Rare hereditary syndromes of both partial and complete IFN-γ deficiencies that render patients susceptible to infections with NTM have previously been described (Villella *et al.*, 2001; Arend *et al.*, 2001). These patients often remain at life-long risk of the recurrence of infections with NTM, stressing the importance of reporting unusual strains in order to increase awareness of and improve therapeutic strategies for these rare infections. The pathogenicity of the proposed slowly growing mycobacterial species was supported by the presence of clinical signs of osteomyelitis, laboratory findings and response to treatment. It is proposed that strain T1921[T] represents a novel species of the genus *Mycobacterium*, for which the name *Mycobacterium arosiense* sp. nov. is proposed.

Description of *Mycobacterium arosiense* sp. nov.

Mycobacterium arosiense (a.ro.si.en′se. N.L. neut. adj. *arosiense* pertaining to Arosia, the Latin name of Aarhus, the city in Denmark where the type strain was isolated).

Cells are rod-shaped and acid-fast. Produces small, smooth, yellow scotochromogenic colonies after 14 days at 36–42 °C. Optimum temperature for growth is 42 °C. Positive for thermotolerant catalase activity, nitrate reductase, pyrazinamidase, acid phosphatase and alkaline phosphatase; negative for catalase in the semi-quantitative test (<45 mm foam). Cannot be differentiated from other closely related slowly growing mycobacteria based on phenotypic test results. Susceptible *in vitro* to clarithromycin, rifamycins, amikacin, moxifloxacin, linezolid and clofazimine, but resistant to isoniazid, fluoroquinolones and streptomycin. The fatty acid profile is similar to that of *M. intracellulare* via GLC and is indistinguishable from that of *M. intracellulare* via HPLC. The sequences of the 16S

Table 2. Growth and biochemical characteristics of strain T1921[T] in comparison with those of closely related slowly growing mycobacterial species

+, Present; −, absent; ±, variable result. For *M. scrofulaceum*, the percentage of strains positive in each test is given in parentheses, and the test result is based on these percentages.

Characteristic	Strain T1921[T]	*M. avium*	*M. intracellulare*	*M. scrofulaceum*
Optimal temperature (°C)	42	35–37	35–37	37
Pigmentation*	S	S/N	S/N	S (97)
Tween 80 hydrolysis	−	−	−	− (2)
Semi-quantitative catalase (45 mm foam)	−	−	−	+ (84)
Catalase (68 °C)	+	±	±	+ (94)
Urease	−	−	−	+
Nitrate reductase	+	−	−	− (5)
Pyrazinamidase	+	+	+	±

*S, Scotochromogenic; N, non-chromogenic.

rRNA gene, ITS, and *hsp65* and *rpoB* genes differ from all recognized mycobacteria. Related most closely to *M. intracellulare* based on phylogenetic analysis of the 16S rRNA gene.
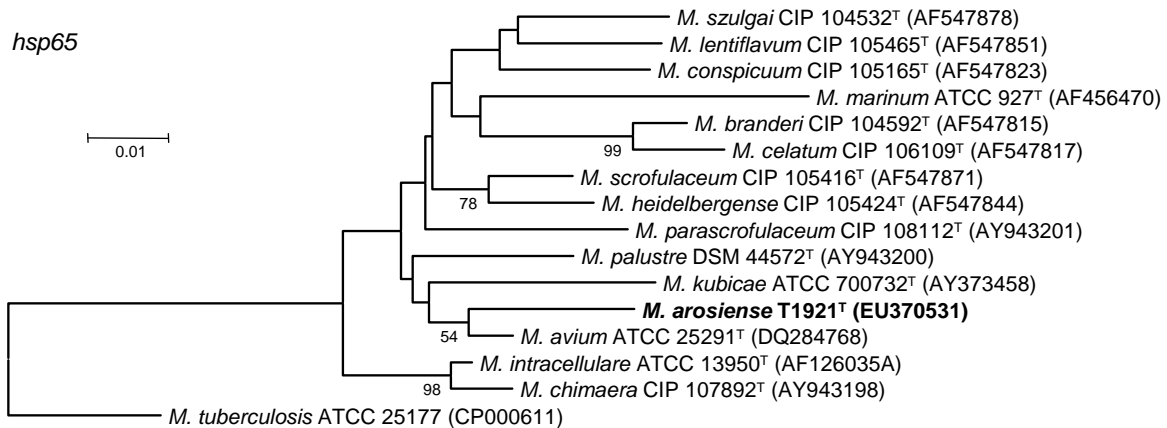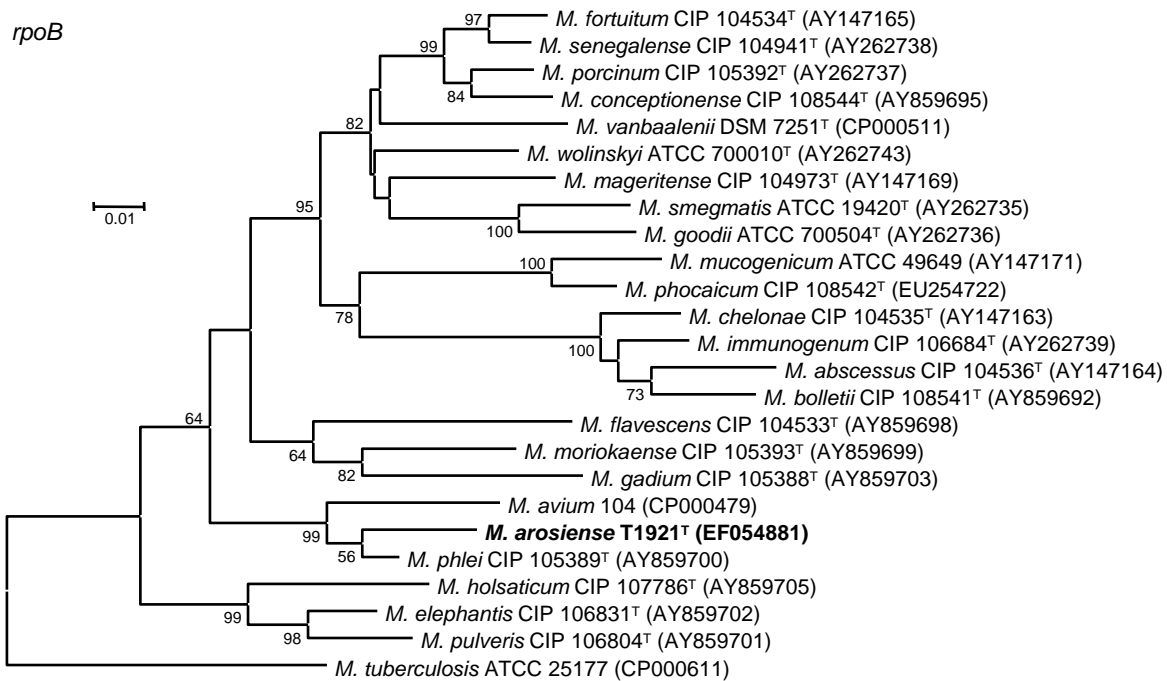
The type strain, T1921^T (=DSM 45069^T =ATCC BAA-1401^T), was isolated from a bone biopsy from the clavicle of a young girl with osteomyelitis.

Acknowledgements

References

Adekambi, T., Colson, P. & Drancourt, M. (2003). *rpoB*-based identification of nonpigmented and late-pigmenting rapidly growing mycobacteria. *J Clin Microbiol* **41**, 5699–5708.

American Thoracic Society (1997). Diagnosis and treatment of disease caused by nontuberculous mycobacteria. *Am J Respir Crit Care Med* **156**, S1–S25.

Arend, S. M., Janssen, R., Gosen, J. J., Waanders, H., de Boer, T., Ottenhoff, T. H. & van Dissel, J. T. (2001). Multifocal osteomyelitis caused by nontuberculous mycobacteria in patients with a genetic defect of the interferon-gamma receptor. *Neth J Med* **59**, 140–151.

Butler, W. R., Floyd, M. M., Silcox, V., Cage, G., Desmond, E., Duffey, P. S., Guthertz, L. S., Gross, W. M., Jost, K. C. & other authors (1996). *Standardized Method for HPLC Identification of Mycobacteria*. Atlanta, GA: US Department of Public Health Service Centers for Disease Control and Prevention.

Collert, S., Petrini, B. & Wickman, K. (1983). Osteomyelitis caused by *Mycobacterium avium*. *Acta Orthop Scand* **54**, 449–451.

Heifets, L. (2004). Mycobacterial infections caused by nontuberculous mycobacteria. *Semin Respir Crit Care Med* **25**, 283–295.

Herlin, T., Thelle, T., Kragballe, K., Borregaard, N. & Thestrup-Pedersen, K. (1981). Sustained depression of monocyte cytotoxicity in a boy with disseminated nontuberculous mycobacteriosis. *J Pediatr* **99**, 264–267.

Janda, J. M. & Abbott, S. L. (2002). Bacterial identification for publication: when is enough enough? *J Clin Microbiol* **40**, 1887–1891.

Kirschner, P., Springer, B., Vogel, U., Meier, A., Wrede, A., Kiekenbeck, M., Bange, F. C. & Böttger, E. C. (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.

Lévy-Frébault, V. V. & Portaels, F. (1992). Proposed minimal standards for the genus *Mycobacterium* and for description of new slowly growing *Mycobacterium* species. *Int J Syst Bacteriol* **42**, 315–323.

McNabb, A., Eisler, D., Adie, K., Amos, M., Rodrigues, M., Stephens, G., Black, W. A. & 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.

Mijs, W., de Haas, P., Rossau, R., Van der Laan, T., Rigouts, L., Portaels, F. & van Soolingen, D. (2002). Molecular evidence to support a proposal to reserve the designation *Mycobacterium avium* subsp. *avium* for bird-type isolates and '*M. avium* subsp. *hominissuis*' for the human/porcine type of *M. avium*. *Int J Syst Evol Microbiol* **52**, 1505–1518.

Murcia, M. I., Tortoli, E., Menendez, M. C., Palenque, E. & Garcia, M. J. (2006). *Mycobacterium colombiense* sp. nov., a novel member of the *Mycobacterium avium* complex and description of MAC-X as a new ITS genetic variant. *Int J Syst Evol Microbiol* **56**, 2049–2054.

Murray, P. R., Baron, E. J., Jorgensen, J. H., Landry, M. L. & Pfaller, M. A. (2007). *Manual of Clinical Microbiology*, 9th edn. Washington, DC: American Society for Microbiology.

Raszka, W. V., Jr, Trinh, T. T. & Zawadsky, P. M. (1994). Multifocal *M. intracellulare* osteomyelitis in an immunocompetent child. *Clin Pediatr (Phila)* **33**, 611–616.

Roth, A., Fischer, M., Hamid, M. E., Michalke, S., Ludwig, W. & Mauch, H. (1998). Differentiation of phylogenetically related slowly growing mycobacteria based on 16S–23S rRNA gene internal transcribed spacer sequences. *J Clin Microbiol* **36**, 139–147.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Siddiqi, S. H. (1996). *BACTEC 460 TB SYSTEM. Product and Procedure Manual*. Sparks, MD: Becton Dickinson Microbiology Systems.

Smithwick, R. W. (1979). *Laboratory Manual for Acid-Fast Microscopy*, 2nd edn. Atlanta, GA: US Public Health Service.

Storgaard, M., Varming, K., Herlin, T. & Obel, N. (2006). Novel mutation in the interferon-gamma-receptor gene and susceptibility to mycobacterial infections. *Scand J Immunol* **64**, 137–139.

Torkko, P., Suutari, M., Suomalainen, S., Paulin, L., Larsson, L. & Katila, M. L. (1998). Separation among species of *Mycobacterium terrae* complex by lipid analyses: comparison with biochemical tests and 16S rRNA sequencing. *J Clin Microbiol* **36**, 499–505.

Torkko, P., Katila, M. L. & Kontro, M. (2003). Gas-chromatographic lipid profiles in identification of currently known slowly growing environmental mycobacteria. *J Med Microbiol* **52**, 315–323.

Tortoli, E., Bartoloni, A., Böttger, E. C., Emler, S., Garzelli, C., Magliano, E., Mantella, A., Rastogi, N., Rindi, L. & other authors (2001). Burden of unidentifiable mycobacteria in a reference laboratory. *J Clin Microbiol* **39**, 4058–4065.

Tortoli, E., Rindi, L., Garcia, M. J., Chiaradonna, P., Dei, R., Garzelli, C., Kroppenstedt, R. M., Lari, N., Mattei, R. & other authors (2004). Proposal to elevate the genetic variant MAC-A, included in the *Mycobacterium avium* complex, to species rank as *Mycobacterium chimaera* sp. nov. *Int J Syst Evol Microbiol* **54**, 1277–1285.

Villella, A., Picard, C., Jouanguy, E., Dupuis, S., Popko, S., Abughali, N., Meyerson, H., Casanova, J. L. & Hostoffer, R. W. (2001). Recurrent *Mycobacterium avium* osteomyelitis associated with a novel dominant interferon gamma receptor mutation. *Pediatrics* **107**, E47.

rpoB

97 — *M. fortuitum* CIP 104534^T (AY147165)
99 — *M. senegalense* CIP 104941^T (AY262738)
— *M. porcinum* CIP 105392^T (AY262737)
84 — *M. conceptionense* CIP 108544^T (AY859695)
82 — *M. vanbaalenii* DSM 7251^T (CP000511)
— *M. wolinskyi* ATCC 700010^T (AY262743)
95 — *M. mageritense* CIP 104973^T (AY147169)
— *M. smegmatis* ATCC 19420^T (AY262735)
100 — *M. goodii* ATCC 700504^T (AY262736)
100 — *M. mucogenicum* ATCC 49649 (AY147171)
— *M. phocaicum* CIP 108542^T (EU254722)
78 — *M. chelonae* CIP 104535^T (AY147163)
100 — *M. immunogenum* CIP 106684^T (AY262739)
— *M. abscessus* CIP 104536^T (AY147164)
73 — *M. bolletii* CIP 108541^T (AY859692)
64 — *M. flavescens* CIP 104533^T (AY859698)
64 — *M. moriokaense* CIP 105393^T (AY859699)
82 — *M. gadium* CIP 105388^T (AY859703)
— *M. avium* 104 (CP000479)
99 — **M. arosiense T1921^T (EF054881)**
56 — *M. phlei* CIP 105389^T (AY859700)
— *M. holsaticum* CIP 107786^T (AY859705)
99 — *M. elephantis* CIP 106831^T (AY859702)
98 — *M. pulveris* CIP 106804^T (AY859701)
— *M. tuberculosis* ATCC 25177 (CP000611)

0.01

hsp65

— *M. szulgai* CIP 104532^T (AF547878)
— *M. lentiflavum* CIP 105465^T (AF547851)
— *M. conspicuum* CIP 105165^T (AF547823)
— *M. marinum* ATCC 927^T (AF456470)
— *M. branderi* CIP 104592^T (AF547815)
99 — *M. celatum* CIP 106109^T (AF547817)
— *M. scrofulaceum* CIP 105416^T (AF547871)
78 — *M. heidelbergense* CIP 105424^T (AF547844)
— *M. parascrofulaceum* CIP 108112^T (AY943201)
— *M. palustre* DSM 44572^T (AY943200)
— *M. kubicae* ATCC 700732^T (AY373458)
— **M. arosiense T1921^T (EU370531)**
54 — *M. avium* ATCC 25291^T (DQ284768)
— *M. intracellulare* ATCC 13950^T (AF126035A)
98 — *M. chimaera* CIP 107892^T (AY943198)
— *M. tuberculosis* ATCC 25177 (CP000611)

0.01

Supplementary Fig S1. Neighbour-joining phylogenetic trees based on the *hsp65* and *rpoB* gene sequences of strain T1921^T compared with selected mycobacterial species. Bars, 0.01 substitutions per nucleotide position.

Bang, D., Herlin, T., Stegger, M., Andersen, A. B., Torkko, P., Tortoli, E. & Thomsen, V. O. (2008). *Mycobacterium arosiense* sp. nov., a slowly growing, scotochromogenic species causing osteomyelitis in an immunocompromised child. *Int J Syst Evol Microbiol* **58**, 2398–2402.