Mycobacterium eburneum sp. nov., a non-chromogenic, fast-growing strain isolated from sputum

Imen Nouioui,1,* Lorena Carro,1 Kanae Teramoto,2 José M. Igual,3 Marlen Jando,4
Maria del Carmen Montero-Calasanz,1 Iain Sutcliffe,5 Vartul Sangal,5 Michael Goodfellow1 and Hans-Peter Klenk1

Abstract

A polyphasic study was undertaken to establish the taxonomic position of a non-chromogenic, rapidly growing Mycobacterium strain that had been isolated from sputum. The strain, CECT 8775T, has chemotaxonomic and cultural properties consistent with its classification in the genus Mycobacterium and was distinguished from the type strains of closely related mycobacterial species, notably from Mycobacterium paraense DSM 46749T, its nearest phylogenetic neighbour, based on 16S rRNA, hsp65 and rpoB gene sequence data. These organisms were also distinguished by a broad range of chemotaxonomic and phenotypic features and by a digital DNA–DNA relatedness value of 22.8%. Consequently, the strain is considered to represent a novel species of Mycobacterium for which the name Mycobacterium eburneum sp. nov is proposed; the type strain is X82T (CECT 8775T=DSM 44358).

Mycobacterium [1] is the type genus of the family Mycobacteriaceae [2] in the order Corynebacteriales and can be distinguished from other genera in this taxon using a combination of chemotaxonomic and morphological properties [3]. At the time of writing, the genus contains more than 160 species with validly published names (http://www.bacterio.net); these taxa can be divided into two groups based on growth rate. Slowly growing mycobacteria require 7 or more days of incubation at optimal temperatures to form visible colonies from highly diluted inocula, whereas colonies of rapidly growing strains are seen in fewer than 7 days under comparable conditions [4]. Species assigned to the two groups can be distinguished using a range of genotypic and phenotypic criteria [5], including the characterisation of mycolic acids [6]. The genus encompasses obligate pathogens, saprophytes and non-tuberculous mycobacteria, which are common in the environment and can cause opportunistic infections in humans [7].

The aim of this study was to establish the taxonomic position of a rapidly growing mycobacterial strain that was isolated from sputum collected in Switzerland in 1998 and deposited in the Spanish Type Culture Collection as CECT 8775T, albeit without thorough taxonomic characterisation. During systematic studies on mycobacterial cultures, this organism was considered to be a presumptive new species of Mycobacterium. The results of the present polyphasic taxonomic study showed that strain CECT 8775T represents a novel species for which we propose the name Mycobacterium eburneum sp. nov.

Strains CECT 8775T and Mycobacterium paraense DSM 46749T [8] were obtained from the Spanish Type Culture Collection (CECT) and German Collection of Microorganisms and Cell Cultures (DSMZ), respectively. Both strains were maintained in 50 %, v/v glycerol at −80 °C. Strain CECT 8775T was examined for its ability to grow on glucose-yeast-malt extract (GYM; DSMZ Medium 65),

Keywords: polyphasic taxonomy; phylogeny; phenotyping; mycobacteria.

Author affiliations: 1School of Biology, Ridley Building, Newcastle University, Newcastle upon Tyne, NE1 7RU, UK; 2Advanced and Fundamental Technology Center, JECOL Ltd., 3–1–2 Musashino, Akishima, Tokyo 196–8558, Japan; 3Instituto de Recursos Naturales y Agrobiología de Salamanca, Consejo Superior de Investigaciones Científicas (IRNASA-CSIC), c/Cordel de Merinas 40–52, 37008 Salamanca, Spain; 4Leibniz Institute DSMZ – German Collection of Microorganisms and Cell Cultures, Inhoffenstraße 7B, 38124 Braunschweig, Germany; 5Faculty of Health and Life Sciences, Northumbria University, Newcastle upon Tyne NE1 8ST, UK.

Correspondence: Imen Nouioui, imen.nouioui@ncl.ac.uk or nouiouiimen@yahoo.fr

Acknowledgements: We would like to thank Dr. Peter Goodfellow, Dr. Michael Goodfellow, Dr. Rosario Pérez-Argüelles, Dr. Patricia Pérez-Argüelles and Dr. Paul Goodfellow for their helpful comments and criticism on the manuscript. This work was supported by the Spanish Ministry of Science and Innovation, Grant SAF2016-76284-P.
peptone-meat extract-glycerol (PMG; DSMZ Medium 250), Lowenstein-Jensen medium (LJ; [9]), Middelbrook 7H10 broth supplemented with oleic acid, albumin, dextrose and catalase (MB7H10; [10]) and tryptic soy agar (TSA; [11]) after incubation for 14 days at 10, 25, 37 and 45 °C. Growth was not observed under anaerobic conditions on PMG agar plates at 37 °C after 7 days using anaerobic atmosphere generation bags (Sigma-Aldrich 68061). Rough, non-pigmented ivory-coloured colonies of the strain were detected on the GYM, MB7H10, LJ and PMG plates at 37 °C after 14 days irrespective of whether the plates were incubated in the light or dark. Optimal growth was observed on the MB7H10 and PMG agar plates after 7 days at 37 °C. Growth was not observed on any of the media incubated at 10, 25 or 45 °C.

DNA extraction and PCR amplification on strain CECT 8775T were achieved using the method of Amaro et al. [12]. A BLAST search of the 16S rRNA gene sequence of the strain in the EzTaxon database [13] was performed and pairwise sequence similarities calculated using the method of Meier-Kolthoff et al. [14]. Maximum-likelihood (ML) and maximum-likelihood parsimony (MP) trees were constructed based on a comparison of the sequences of the strain and its close relatives. The DSMZ phylogenomics pipeline [15] accessible through the GGDC web server ([16]; available at http://ggdc.dsmz.de/) was used with RAxML [17] and TNT [18] to generate the ML and MP trees, respectively. Multiple sequence alignment was performed with MUSCLE [19]. Rapid bootstrapping replicates were used in conjunction with the auto maximal-relative-error (MRE) bootstrapping criterion [20] and tree bisection and reconnection branch swapping for the ML and MP analyses, respectively. The X2 test, as implemented in PAUP* [21], was applied to check the sequences for compositional bias. A neighbour-joining (NJ) tree [22] was constructed using MEGA software version 7.0 [23] and the evaluation of its robustness performed in a bootstrap analysis based on 1000 replicates [24]. The alignment of the sequences was achieved using CLUSTAL W [25].

In the BLAST analyses, strain CECT 8775T was found to be closely related to the type strain of Mycobacterium conceptionense [26], as these strains showed a 97.9 % 16S rRNA gene sequence similarity, which corresponds to 28 nucleotide (nt) differences. However, in the pairwise gene sequence analyses, the CECT 8775T strain was found to be closely related to the type strains of M. paraense and Mycobacterium kumamotonense [27], showing a 16S rRNA gene sequence similarity with each of these organisms of 98.0 %, a value that corresponded to 39 nt and 31 nt differences, respectively. It can been seen from Fig. 1 that strain CECT 8775T forms a well-supported subclade together with M. paraense DSM 46749T and that this taxon is loosely associated with a subclade that encompasses the type strains of Mycobacterium fermentans [28], Mycobacterium cookii [29], M. kumamotonense, Mycobacterium longobardum [30] and Mycobacterium moriokaense [31]. In contrast, the M. conceptionense type strain belongs to a well-supported subclade that includes the type strains of Mycobacterium boenickii [32], Mycobacterium farrigenes [33, 34], Mycobacterium neworleansense [32], Mycobacterium porcinum [35] and Mycobacterium septicum [36] (Fig. 1), and the type strains of Mycobacterium houstonense [32] and Mycobacterium senegalense [33, 34] are close relatives of these taxa [5] (Fig. 1). The topology of the ML tree is consistent with the one inferred from NJ (Fig. S1, available in the online Supplementary Material). In addition, strain CECT 8775T presents, as its close neighbour M. paraense, the 16S rRNA signature of rapidly growing mycobacteria as its sequence has a 14 consecutive nucleotide deletion at position 471–490 (Escherichia coli rRNA position) in hypervariable region B [37].

Phylogenies based on partial sequences of the 65 kilodalton heat shock protein (hsp65) and rpoB (rRNA polymerase β subunit) genes are proving to be useful in mycobacterial systematics [38–41]. In the present study, hsp65 (392 nt) and rpoB (407 nt) sequences of strain CECT 8775T, amplified following the protocols of McNabb et al. [38] and Adékambi et al. [39], respectively, were compared with the corresponding sequences of their close phylogenetic neighbours and a concatenated multilocus sequence tree generated based on 16S rRNA, hsp65 and rpoB genes sequences, using the maximum-likelihood algorithm and the Kimura two-parameter model [42] from MEGA version 7 software; the accession numbers of all the sequences are displayed in Table S1. It can be seen from Fig. 2 that strain CECT 8775T belongs to a well-supported subclade in the multilocus sequence typing (MLST) tree together with the type strain of M. paraense; this taxon lies next to a subclade that encompasses M. algericum, M. cookii, M. kumamotonense and M. longobardum. In turn, M. conceptionense belongs to a distantly related subclade that contains M. boenickii, M. farrigenes, M. neworleansense, M. porcinum and M. septicum.

The digital DNA–DNA hybridization (dDDH) value between the draft genome of strain CECT 8775T (unpublished data) and that of M. paraense DSM 4674T, its closest phylogenetic neighbour, was calculated using formula 2 of the GGDC web server available at http://ggdc.dsmz.de/phylo_form.php. The two strains were shown to have a dDDH similarity of 23.8 %, a value well below the 70 % cut-off point recommended for the delineation of closely related prokaryotic species [43].

Strains CECT 8775T and M. paraense DSM 46747T were examined for chemotaxonomic properties known to be of value in mycobacterial systematics [5]. Biomass harvested from Pombe minimal glutamate broth after 7 days at 37 °C was washed three times in 0.9 % (w/v) sodium chloride solution and freeze-dried. Cellular fatty acids were extracted from the biomass of these strains and fatty acid methyl esters (FAMES) prepared following saponification and methylation using the protocol introduced by Miller [44], as modified by Kuykendall et al. [45]. The FAMES were analysed by gas chromatography (Agilent 6890 N instrument) and the resultant peaks automatically integrated; fatty acid
names and properties were determined using the standard Microbial Identification (MIDI) system, version 4.5, and the Myco 6 database [46]. Mycolic acid methyl esters prepared according to Minnikin and Goodfellow [47] were analysed by matrix-assisted laser desorption/ionization (MALDI) time-of-flight mass spectrometry (TOF-MS) using spiral ion trajectory (MALDI spiral-TOFMS), according to Teramoto et al. [6]. Standard chromatographic procedures were used to determine the isomers of diaminopimelic acid [48], while whole cell sugar extracts [49] were examined according to Staneck and Roberts [48]. Isoprenoid quinones were extracted after Collins [50] and identified by high pressure liquid chromatography following Kroppenstedt [51] and polar lipids extracted and identified using the protocol established by Minnikin et al. [52], as modified by Kroppenstedt and Goodfellow [53]. Functional groups were revealed

**Fig. 1.** Maximum-likelihood (ML) phylogenetic tree based on almost complete 16S rRNA gene sequences showing relationships between strain CECT 8775T and the type strains of closely related mycobacteria. The branches are scaled in terms of the expected number of substitutions per site. The numbers above the branches are bootstrap support values greater than 60%.
using spray reagents, namely α-naphthol-sulphuric acid [40.5 ml ethanol, 4 ml distilled water, 6.5 ml concentrated sulfuric acid and 10.5 ml α-naphthol (15 g l⁻¹ ethanol)] to detect sugar containing lipids, Dragendorff’s solution (Merck) to identify choline-containing lipids [54], ninhydrin reagent (0.2 % ninhydrin in acetone) to reveal amino groups [55], molybdenum blue (Sigma 119KG123) to detect lipids with phospho-groups and molybdatophosphoric acid (Sigma P1518) to determine total lipid content.

Strain CECT 8775ᵀ and the type strain of M. paraense were found to have chemotaxonomic properties in accordance with their classification in the genus Mycobacterium [5]. They produced whole organism hydrolysates rich in meso-2,6-diaminopimelic acid, arabinose, galactose, glucose and ribose (wall chemotype IV; [49]); straight chain saturated, unsaturated and 10 methyl-octadecanoic (tuberculostearic) fatty acids; mycolic acids; dihydrogenated menaquinones with nine isoprene units as the sole menaquinone; and polar lipid patterns that included diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, aminolipids, glycolipids (GL₁-5) and unidentified lipids (L₁-₃) (Fig. S2; phospholipid pattern 2 according to Lechevalier et al. [56]). However, the two strains were distinguished by several chemotaxonomic features. The fatty acid profile of strain CECT 8775ᵀ showed major amounts of C₁₆₀ (38.2 %), C₁₀Me-C₁₈₀ (25.5 %) and summed feature 3 (16.8 %), which contains 2₀-AUC 18.838 ECL/2₀ alcohol/19:0 Cycloprop ω1₀c/19:0 Cycloprop ω8c, while M. paraense DSM 46747ᵀ exhibited C₁₈₀, higher amounts of C₁₈₀, ω9c (39.4 %) and C₁₆₀ (32.1 %), a lower amount of C₁₀Meₐ C₁₈₀ (8.1 %) and lacked summed feature 3. Similarly, only the polar lipid profile of strain CECT 8775ᵀ included phosphatidylglycerol (PG) and an unidentified phospholipid whereas in contrast the M. paraense type strain contained two aminolipids (AL₁,₂) and lacked PG. Finally, strain CECT 8775ᵀ was found to have dicarboxy-mycolic acids with 64 carbons and the M. paraense type strain α-keto-mycolic acids with 87 carbons (Fig. 3).

---

Fig. 2. MLST phylogenetic tree inferred from 2396 nt concatenated sequences of 16S rRNA, hsp65, rpoB genes using the maximum likelihood method showing relationships between strain CECT 8775ᵀ and the type strains of closely related mycobacteria. The numbers above the branches are bootstrap support values when larger than 50 %.

---

Mycobacterium porcinum
Mycobacterium boenickei
Mycobacterium septicum
Mycobacterium neworleansense
Mycobacterium conceptionense
Mycobacterium farcinogenes
Mycobacterium houstonense
Mycobacterium insubricum
Mycobacterium pallens
Mycobacterium senegalense
Mycobacterium brisbanense
Mycobacterium fluoranthenivorans
Mycobacterium mucogenicum
Mycobacterium rufum
Mycobacterium chubuense
Mycobacterium psychrotolerans
Mycobacterium poriferae
Mycobacterium paraense
Mycobacterium moriokaense
Mycobacterium eburneum
Mycobacterium cookii
Mycobacterium kumamotonense
Mycobacterium algericum
Mycobacterium longobardum
Mycobacterium poriferae
Mycobacterium moriokaense
Mycobacterium paraense
Mycobacterium eburneum
Mycobacterium cookii
Mycobacterium longobardum
Mycobacterium poriferae
Mycobacterium kumamotonense
Mycobacterium algericum
Mycobacterium longobardum
Mycobacterium poriferae
Mycobacterium kumamotonense
Mycobacterium algericum
Mycobacterium longobardum
Mycobacterium poriferae
Mycobacterium kumamotonense
Mycobacterium algericum
Mycobacterium longobardum
Mycobacterium poriferae
Mycobacterium kumamotonense
Mycobacterium algericum
Mycobacterium longobardum
Mycobacterium poriferae
Mycobacterium kumamotonense
Mycobacterium algericum
Mycobacterium longobardum
Mycobacterium poriferae
Mycobacterium kumamotonense
Mycobacterium algericum
Mycobacterium longobardum
Mycobacterium poriferae
Mycobacterium kumamotonense
Mycobacterium algericum
Mycobacterium longobardum
Mycobacterium poriferae
Mycobacterium kumamotonense
Mycobacterium algericum
Mycobacterium longobardum
Mycobacterium poriferae
Mycobacterium kumamotonense
Mycobacterium algericum
Mycobacterium longobardum
Mycobacterium poriferae
Mycobacterium kumamotonense
Mycobacterium algericum
Mycobacterium longobardum
Mycobacterium poriferae
Mycobacterium kumamotonense
Mycobacterium algericum
Mycobacterium longobardum
Mycobacterium poriferae
Mycobacterium kumamotonense
Mycobacterium algericum
Mycobacterium longobardum
Mycobacterium poriferae
Mycobacterium kumamotonese
Mycobacterium algericum
Mycobacterium longobardum
Mycobacterium poriferae
Mycobacterium kumamotonense
Mycobacterium algericum
Mycobacterium longobardum
Mycobacterium poriferae
Mycobacterium kumamotonense
Mycobacterium algericum
Mycobacterium longobardum
Mycobacterium poriferae
Mycobacterium kumamotonense
Mycobacterium algericum
Mycobacterium longobardum
Mycobacterium poriferae
Mycobacterium kumamotonense
Mycobacterium algericum
Mycobacterium longobardum
Mycobacterium poriferae
Mycobacterium kumamotonense
Mycobacterium algericum
Mycobacterium longobardum
Mycobacterium poriferae
Mycobacterium kumamotonense
Mycobacterium algericum
Mycobacterium longobardum
Mycobacterium poriferae
Mycobacterium kumamot
The two strains were examined for a broad range of phenotypic properties. To this end, they were tested for their ability to use a range of carbon compounds, to metabolise at several pH values and in the presence of sodium chloride and antibiotics using GENIII, PM 11 and PM 12 microplates and an Omnilog device (BIOLOG, Hayward, CA). All of the microplates were inoculated with mycobacterial suspensions in IPC solution (inoculating fluid provided by the manufacturer) for the GENIII microplates and in MB 7H10 broth supplemented with Dye mix G for the PM11 and PM 12 microplates following the protocol of Khatri et al. [57]; the inoculation solutions showed 71% transmittance. All of these tests were carried out in triplicate using freshly prepared inocula harvested at mid-logarithmic phase (OD$_{600}$ nm 0.30–0.60) [57]. The plates were incubated at 37°C for 7 days and the resultant data were exported and analysed using the opm package version 1.3.36 [58, 59]. The strains were also tested for their ability to produce catalase [60], heat stable catalase [61], arylsulfatase after 3 and 20 days [62], niacin accumulation [63], nitrate reductase [64], growth in presence of potassium tellurite [63, 65], degradation of Tween 80 [66], and urea hydrolysis [60]. Additional enzymatic features were determined using API ZYM kits, following the manufacturer’s instructions (BioMérieux, France).

Identical results were recorded for all of the phenotypic tests that were carried out in triplicate. It can be seen from Table 1 that the two type strains can be distinguished by a broad range of phenotypic features, although they also have many such properties in common. In general, strain CECT 8775$^T$ showed more activity against sugars than $M$. paraense DSM 46749$^T$ and, unlike the latter, was positive for arylsulfatase after 3 and 20 days, degraded Tween 80, produced esterase (C4), utilised L-alanine, $\alpha$-keto-butyric acid, L-glutamic acid, and D-saccharic acid and was resistant to several antibiotics, including amikacin, carbenicillin, cephalothin, chlorotetracycline, spiramycin, tetracycline and tobramycin. In contrast, only the $M$. paraense strain produced $\alpha$-chymotrypsin, reduced nitrate, accumulated niacin, utilised acetocacetic acid, citric acid, dextrin, D- and L-malic acid, and was resistant to amoxicillin, cefazolin, ceftriaxone, demeclocycline, enoxacin, gentamicin, lomefloxacin, minocycline, troleandomycin and vancomycin.

In conclusion, strain CECT 8775$^T$ can be distinguished from the type strains of closely related mycobacteria based on 16S rRNA, hsp65 and rpoB gene sequence data. In particular, this strain can be distinguished from $M$. paraense DSM 46749$^T$ using a broad range of chemotaxonomic and phenotypic properties and by a digital DNA–DNA relatedness value of only 23.8%. It is evident, therefore, from this comprehensive polyphasic study that the strain forms a new centre of taxonomic variation in the genus Mycobacterium and thereby merits recognition as a new species. It is proposed that strain CECT 8775$^T$ be recognised as the type strain of a new taxon, namely $Mycobacterium$ eburneum sp. nov.

**Fig. 3.** Mycolic acid profiles of $M$. eburneum strain CECT 8775$^T$ (a) and $M$. paraense DSM 46749$^T$ (b) using high throughput matrix-assisted laser desorption/ionization time of flight (MALDI TOF).
Table 1. Phenotypic features that distinguish strain CECT 8775$^T$ from the type strain of \textit{M. paraense}

<table>
<thead>
<tr>
<th>Biochemical tests</th>
<th>Strain CECT 8775$^T$</th>
<th>\textit{M. paraense} DSM 46749$^T$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arylsulfatase 3 and 20 days, esterase (C4),</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>α-Chymotrypsin, nitrate reduction, niacin accumulation</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Degradation test</td>
<td>Tween 80</td>
<td>+</td>
</tr>
<tr>
<td>Biolog GENIII microplates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Utilisation of sugars*</td>
<td>Cellobiose, N-fructose, D- and L-fucose, D-galactose, D-glucose-6-phosphate, myo-inositol, N-acetyl-D-glucosamine, maltose, methyl β-D-glucoside, starchyose, sucrose, turanose</td>
<td>+</td>
</tr>
<tr>
<td>Dextrin, glucuronamidase, raffinose, D-sorbitol</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Utilisation of amino acids*</td>
<td>L-Alanine, L-glutamic acid, D-Serine</td>
<td>+</td>
</tr>
<tr>
<td>Resistance to*</td>
<td>Sodium bromate, sodium chloride (1 %, w/v)</td>
<td>–</td>
</tr>
<tr>
<td>Sodium formate</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Growth in presence of*</td>
<td>Fusicid acid, guanidine hydrochloride, lincomycin, lithium chloride, nalidixic acid, niaproof, tetrazolium blue, troleandomycin, vancomycin</td>
<td></td>
</tr>
<tr>
<td>Biolog PM 11 microplates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resistance to*</td>
<td>Amikacin, cephalothin, chlorotetracycline</td>
<td>+</td>
</tr>
<tr>
<td>Aminocillin, cefazolin, ceftriaxone, demeclocycline, enoxacin, gentamicin, lomefloxacin, minocycline, neomycin</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Biolog PM 12 microplates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Growth in presence of*</td>
<td>Carbenicillin, spiramycin, tetracycline, tobramycin, L-aspartic-β-hydroxamate, 2,4-diamino-6,7-diisopropylpterdine, 5-fluoroorotic acid, polymyxin B, D,D-serine dihydroxamate, spectinomycin, sulfamethazine, sulfamethoxazole, sulfathiazole</td>
<td></td>
</tr>
</tbody>
</table>

*Concentrations of antibiotics as specified for Biolog.

**DESCRIPTION OF MYCOBACTERIUM EBURNEUM SP. NOV.**

\textit{Mycobacterium eburneum} (\textit{eburneum} N. L. neut. adj. \textit{eburneus} referring to the ivory-coloured colonies produced by the organism).

Gram-positive, strictly aerobic, acid-alcohol fast, non-motile, non-spore-forming organism which produces ivory-coloured colonies on Middelbrook 7H10 and peptone meat extract glycerol agar after 7 days at 37 °C in both the dark and light. Grows from 28 to 37 °C, optimally at 37 °C, from pH 7 to 8, optimally at pH 7.0. Produces acid and alkaline phosphatase, β-glucosidase, catalase, catalase (68 °C), esterase lipase (C8), esterase (C4), lipase (C14), leucine arylamidase, naphthol-AS-BI-phosphohydrolase, urease and valine arylamidase, degrades Tween 80, is arylsulfatase positive after 3 and 20 days and is not inhibited by potassium tellurite. Major cellular fatty acids (>20 %) are C₁₆:₀ and C₁₀MeC₁₈:₀. Contains dicarboxy mycolic acids with 64 carbons; the major menaquinone is MK-9 (H$_2$) and the
phospholipid pattern is composed of diphosphatidylglycerol, phosphatidyethanolamine, phosphatidylglycerol and phosphatidylinositol. The in silico DNA G+C content of the strain is 68.7 %.

The type strain X82T (\text{CETC} 8775\textsuperscript{T} = DSM 44358\textsuperscript{T}) was isolated from a sputum specimen. The GenBank accession number of the 16S rRNA, hsp65 and rpoB genes sequences are KX879093, KY63441 and KY630442, respectively.

Funding information
This project was supported by the School of Biology (Newcastle University). I. N. and L. C. thank Newcastle University for postdoctoral fellowships.

Acknowledgements
The authors are indebted to Enrico Tortoli (Emerging Bacterial Pathogens Unit, IRCCS San Raffaele Scientific Institute, Milan, Italy) for providing the draft genomic data on the type strain of \textit{M. paraenae}.

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

References


