Mycobacterium malmesburyense sp. nov., a non-tuberculous species of the genus *Mycobacterium* revealed by multiple gene sequence characterization

Nomakorinte Gcebe,¹ ² ³ Victor Rutten,² ³ Nicolaas Gey van Pittius,⁴ Brendon Naicker⁵ and Anita Michel²

**Abstract**

Non-tuberculous mycobacteria (NTM) are ubiquitous in the environment, and an increasing number of NTM species have been isolated and characterized from both humans and animals, highlighting the zoonotic potential of these bacteria. Host exposure to NTM may impact on cross-reactive immune responsiveness, which may affect diagnosis of bovine tuberculosis and may also play a role in the variability of the efficacy of *Mycobacterium bovis* BCG vaccination against tuberculosis. In this study we characterized 10 NTM isolates originating from water, soil, nasal swabs of cattle and African buffalo as well as bovine tissue samples. These isolates were previously identified during an NTM survey and were all found, using 16S rRNA gene sequence analysis to be closely related to *Mycobacterium moriokaense*. A polyphasic approach that included phenotypic characterization, antibiotic susceptibility profiling, mycolic acid profiling and phylogenetic analysis of four gene loci, 16S rRNA, *hsp65*, * sodA* and *rpoB*, was employed to characterize these isolates. Sequence data analysis of the four gene loci revealed that these isolates belong to a unique species of the genus *Mycobacterium*. This evidence was further supported by several differences in phenotypic characteristics between the isolates and the closely related species. We propose the name *Mycobacterium malmesburyense* sp. nov. for this novel species. The type strain is WCM 7299T (=ATCC BAA-2759T=CIP 110822T).

Currently, more than 100 non-tuberculous mycobacterial species are listed in public databases (www.bacterio.net/). An increasing number of non-tuberculous mycobacteria (NTM) species have recently been isolated and characterized from both humans and animals, highlighting the zoonotic potential of these species (for review see [1–3]). An increased interest in these organisms has developed in view of the potential impact of host exposure to NTM on cross-reactive immune responsiveness, which may affect diagnosis of bovine tuberculosis [4–6] and may also play a role in the variability of the efficacy of *Mycobacterium bovis* BCG vaccination against tuberculosis [7–9]. NTM are ubiquitous in the environment, and there is no evidence of animal-to-animal or human-to-human transmission [10, 11]. Characterization of NTM isolates from environmental as well as clinical samples is important as these may also lead to identification of emerging pathogens. For instance, *Mycobacterium avium* was essentially a rare human pathogen before the acquired immune deficiency syndrome (AIDS) pandemic, and since then this NTM species has become a very important pathogen of AIDS patients [12]. The reference molecular method for the identification of mycobacteria is the sequencing of the 16S rRNA gene, which has contributed to the discovery of novel NTM isolates beyond possibilities offered by conventional methods such as phenotypic characterization [13]. However, this approach has its limitations as there are instances where the sequence of this gene has been found to be similar, if not identical, among species of the genus. It has been shown that multiple locus analysis provides a more detailed and accurate identification of species of the genus *Mycobacterium* than the use of a single locus [14]. We previously described the prevalence of NTM isolates in cattle, African buffaloes and their environments in South Africa and found a group of isolates (most closely related by analysis of the 16S rRNA gene to *Mycobacterium moriokaense*) that were not well described in the literature.

**Author affiliations:** ¹Tuberculosis, Laboratory, Agricultural Research Council – Onderstepoort Veterinary Institute, Onderstepoort, Gauteng 0110, South Africa; ²Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, Gauteng 0110, South Africa; ³Department of Infectious Diseases and Immunology, Division of Immunology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands; ⁴Centre of Excellence for Biomedical Tuberculosis Research, Division of Molecular Biology and Human Genetics, Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Stellenbosch University, Tygerberg, South Africa; ⁵Polymers and Composites, Materials Science and Manufacturing, Council for Scientific and Industrial Research, Brummeria, South Africa.

**Correspondence:** Nomakorinte Gcebe, gceben@arc.agric.za

**Keywords:** non-tuberculous mycobacteria; *Mycobacterium malmesburyense* sp. nov.; multiple gene sequence characterization.

**Abbreviation:** NTM, non-tuberculous mycobacteria.

One supplementary table is available with the online Supplementary Material.
at the species level [15]. The aim of the present study was to conduct an in-depth characterization of this group of isolates closely related to *M. moriokaense*.

Ten isolates used in this study were recovered from soil, water, nasal swabs and lymph nodes of cattle and pharyngeal swabs of buffalo, during a survey conducted between April 2010 and December 2012 to determine the prevalence and distribution of NTM, and analysis of isolates from routine diagnostic samples collected in different geographic and climatic regions of South Africa (Table 1). These isolates were characterized using phenotypic assays, antimicrobial susceptibility profiling, mycolic acid profiling and sequence-based analysis of four mycobacterial housekeeping genes, 16S rRNA, *hsp65*, *rpoB* and *sodA*.

All the cultures were maintained on either Middlebrook 7H11 agar plates (Becton Dickinson) supplemented with 0.1 % OADC (Merck Chemicals) and glycerol or on Löwenstein Jensen (LJ) slopes supplemented with glycerol and an antibiotic cocktail of polymyxin B, amphotericin B, carbenicillin and trimethoprim (PACT) (Becton Dickinson) at 37 °C for subsequent biochemical testing and mycolic acid analysis. Phenotypic characteristics of the isolates were compared with those of their closest relatives, *M. moriokaense*, *M. elephantis*, *M. novocastrense*, *M. flavescent* and *M. arupense*. Slopes and plates were incubated at 37, 45 and 25 °C in order to evaluate the ability of each isolate to grow at different temperatures. We also observed colony morphology (rough or smooth), pigmentation and acid fastness and different growth rates of the isolates. Sodium chloride (NaCl) tolerance (5 %) of each isolate was evaluated for 2–10 days. The isolates were tested for the ability to reduce nitrate, for urease activity and for niacin accumulation, using commercial test strips (Becton Dickinson) according to the manufacturer’s instructions. They were also tested for 3-day and 14-day arylsulphatase activity [16]. We tested the isolates for pyrazinamidase activity as described by Singh *et al.* [17], except that Middlebrook 7H11 agar was used and the culture incubated for 4 days. In addition, the isolates were also tested for the ability to hydrolyse Tween 80 using the method described by Kilburn *et al.* [18]. Semi-quantitative catalase activity of the isolates was tested by using 3 % hydrogen peroxide and observing formation of gas bubbles [19]. Finally, the isolates were tested for their ability to hydrolyse aesculin and for citrate utilization (National Health Laboratory Services, South Africa) as well as for their ability to utilize the following sugars as sole carbon sources: D-mannitol, inositol, L-rhamnose and L-arabinose (Selecta media). The differences identified in phenotypic characteristics of the isolates from those of the closely related species, i.e. *M. moriokaense*, *M. novocastrense*, *M. flavescent*, *M. elephantis* and *M. arupense*, as illustrated in Table 2, included pigmentation and growth rate as well as other biochemical traits. The most apparent feature that distinguished the isolates from both *M. moriokaense* and *M. arupense* was pigmentation, as all the novel isolates appeared to be scotochromogens whereas both these NTM were previously reported to be non-chromogenic [20–22]. Other characteristics that further segregated the isolates from *M. arupense* were their ability to degrade urea as well as to reduce nitrate to nitrite (except for isolate 242). *M. arupense* was previously reported to lack these traits [20]. Contrary to what has been reported for *M. novocastrense* and *M. flavescent*, as well as *M. elephantis*, all the isolates were unable to grow in the presence of 5 % NaCl [21–25]. In addition, the ability of the isolates to grow at 45 °C further separated them from *M. flavescent* as well as *M. arupense*, which were previously reported not to grow at this temperature [20, 25]. The phenotypic characteristics of the individual isolates are presented in Table S1 (available in the online Supplementary Material).

Antibiotic susceptibility to amikacin (30 µg), cefotixin (10 µg), ciprofloxacin (10 µg), clarithromycin (15 µg), imipenem (10 µg), amoxylin (30 µg) and tobramycin (10 µg) (Oxoid) was determined for all the isolates, using a modified Kirby–Bauer disc-diffusion method on Middlebrook 7H11 agar plates supplemented with 0.1 % OADC and incubated for 2–5 days at 37 °C, after which the zones of inhibition were measured [26]. If a minimum of 3 mm zone of inhibition was obtained, that was interpreted as inhibition. The isolates presented quite a uniform pattern with all being susceptible to amikacin, ciprofloxacin and doxycycline. With the exception of isolates Uyenvlei, Vryburg and Middledrift, all were also susceptible to

---

**Table 1.** Origin of NTM isolates used in the study

<table>
<thead>
<tr>
<th>NTM isolate ID</th>
<th>Sample type</th>
<th>Climatic region in South Africa</th>
<th>Location and GPS co-ordinates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Balasi</td>
<td>Water</td>
<td>Escarpment</td>
<td>Bischo: 32° 50′ 58″ S 27° 26′ 17″ E</td>
</tr>
<tr>
<td>242</td>
<td>Nasal swab</td>
<td>Moderate Eastern Plateau</td>
<td>Frankfort: 27° 30′ 14″ S 27° 35′ 58″ E</td>
</tr>
<tr>
<td>Middledrift</td>
<td>Nasal swab</td>
<td>Escarpment</td>
<td>Middledrift: 32° 49′ 7″ S 26° 59′ 15″ E</td>
</tr>
<tr>
<td>WCM 7299&lt;sup&gt;7&lt;/sup&gt;</td>
<td>Bovine asal swab</td>
<td>Mediterranean climate</td>
<td>Malmesbury: 33° 27′ 4.7″ S 18° 43′ 19.06″ E</td>
</tr>
<tr>
<td>Vryburg</td>
<td>Bovine nasal swab</td>
<td>Semi-arid</td>
<td>Vryburg: 26° 58′ S 24° 54″ E</td>
</tr>
<tr>
<td>Uyenvlei</td>
<td>Bovine nasal swab</td>
<td>Moderate eastern plateau</td>
<td>Lephalale: 23° 40′ S 2° 45′ E</td>
</tr>
<tr>
<td>C28</td>
<td>Buffalo pharyngeal swab</td>
<td>Subtropical coast</td>
<td>Hluhluwe Imfolozi park: 28° 13′ 11″ S 31° 57′ 07″ E</td>
</tr>
<tr>
<td>C4</td>
<td>Buffalo pharyngeal swab</td>
<td>Subtropical coast</td>
<td>Hluhluwe Imfolozi park: 28° 13′ 11″ S 31° 57′ 07″ E</td>
</tr>
<tr>
<td>TB 5612</td>
<td>Bovine lymph node</td>
<td>Subtropical coast</td>
<td>Bergville: 28° 43′ 48″ S 29° 21′ 0.2″ E</td>
</tr>
<tr>
<td>TB 5960A</td>
<td>Bovine lymph node</td>
<td>Escarpment</td>
<td>East London: 32° 59′ S 27° 52″ E</td>
</tr>
</tbody>
</table>
Table 2. Comparison of phenotypic characteristics of the NTM isolates with those of the closely related species

<table>
<thead>
<tr>
<th>NTM ID/species</th>
<th>Source</th>
<th>Growth rate (days)</th>
<th>Pigment</th>
<th>Growth at:</th>
<th>5% NaCl tolerance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>25°C</td>
<td>37°C</td>
</tr>
<tr>
<td>M. moriokaense ATCC 43059&lt;sup&gt;†&lt;/sup&gt; (tested in the lab)</td>
<td>Ref</td>
<td>&lt;7</td>
<td>–</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>M. moriokaense (e, f)&lt;sup&gt;*&lt;/sup&gt;</td>
<td>Ref</td>
<td>&lt;7</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>M. novocastrense (a, c)</td>
<td>Ref</td>
<td>&lt;7</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>M. flavescens (b, g, h)</td>
<td>Ref</td>
<td>&lt;7</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>M. arupense (d)</td>
<td>Ref</td>
<td>&lt;7</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>M. elephantis (a, b)</td>
<td>Ref</td>
<td>&lt;7</td>
<td>+</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>NTM isolates (n=10)</td>
<td>D</td>
<td>&lt;7</td>
<td>+</td>
<td>+</td>
<td>+++</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>NTM ID/species</th>
<th>Semi-quantitative catalase</th>
<th>Tween 80 hydrolysis (14 days)</th>
<th>Aryl sulphatase (3 days)</th>
<th>Urease</th>
<th>Niacin</th>
<th>Nitrate reduction</th>
<th>Pyrazinamidase activity</th>
<th>Citrate</th>
<th>Aesculin</th>
<th>D-Mannitol</th>
<th>Inositol</th>
<th>L-Rhamnose</th>
<th>L-Arabinose</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. moriokaense ATCC 43059&lt;sup&gt;†&lt;/sup&gt; (tested in the lab)</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. moriokaense (e, f)</td>
<td>–, +</td>
<td>ND</td>
<td>ND</td>
<td>–, +</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+, –</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>M. novocastrense (a, c)</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>V</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>M. flavescens (b, g, h)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>M. arupense (d)</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>+, V</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>M. elephantis (a, b)</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>–</td>
<td>–</td>
<td>ND</td>
</tr>
<tr>
<td>NTM isolates (n=10)</td>
<td>D (n=8/10)&lt;sup&gt;†&lt;/sup&gt;</td>
<td>D (n=9/10)&lt;sup&gt;†&lt;/sup&gt;</td>
<td>D (n=1/10)&lt;sup&gt;†&lt;/sup&gt;</td>
<td>D (n=2/10)&lt;sup&gt;†&lt;/sup&gt;</td>
<td>D (n=9/10)&lt;sup&gt;†&lt;/sup&gt;</td>
<td>+</td>
<td>–</td>
<td>D (n=1/10)&lt;sup&gt;†&lt;/sup&gt;</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

<sup>v</sup>, Variable results based on data from Tortoli [22] and Shojaei [23]; +, positive; –, negative; +++ optimum growth; D, strain dependant.

<sup>*</sup>Data retrieved from: a, Tortoli [22]; b, Turenne et al. [21]; c, Shojaei et al. [23]; d, Cloud et al. [20]; e, Tsukamura et al. [36]; f, Adékambi et al. [37]; g, Tortoli et al. [25]; h, Bojalil et al. [24].

<sup>†</sup>Ratio of positive results.
clarithromycin. With the exception of WCM 7299, which was susceptible to tobramycin, as well as TB 5960, TB 5612 and C4, which were susceptible to amoxylin, all the other isolates were resistant to imipenem, cefoxinin, tobramycin and amoxylin.

Genetic characterization included PCR and sequence analysis of a 1400 bp fragment of the 16S rRNA gene [13], a 439 bp region of the gene encoding the 65 kDA heat shock protein (hsp65) [27], part (764 bp) of the RNA polymerase beta subunit (rpoB) gene [28, 29] and part (464 bp) of the superoxide dismutase (sodA) gene [30]. Boiled culture suspensions from individual isolates were used as DNA template in the various PCR protocols. The amplicons were sequenced at the Central Analytical Facility of Stellenbosch University and Inqaba biotechnologies (South Africa) using the same primer sequences that were used for amplification of the respective gene fragments. Sequences from both strands were edited manually, and pairwise alignments were undertaken using the BioEdit Sequence alignment editor (version 7.1.9) and Molecular Evolutionary Genetics Analysis (MEGA) platform (www.megasoftware.net) (version 6) [31]. The resulting consensus sequences were analysed on the NCBI BLAST platform for species identification.

Fig. 1. Phylogenetic tree reconstructed using the neighbour-joining method, illustrating the genetic position of the isolates. Genbank accession numbers for the sequences are shown in parentheses. The tree is based on the partial 16S rRNA gene sequences. The percentages of replicate trees (more than 50 %) in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches [38]. Mycobacterium moriokaense was used as an outgroup sequence. Bar, 0.01 substitutions per nucleotide position.
(www.blast.ncbi.nlm.nih.gov/Blast.cgi) by megablast. For phylogenetic analysis, all the sequences were first trimmed at both the 5' and the 3' ends to encompass the most highly corresponding gene fragment sequences of mycobacteria deposited in the Genbank database. The resulting fragment used for the concatenated nucleotide sequences was in the following order (16S rRNA gene, hsp65, rpoB and sodA).

Multiple sequence alignments of the individual genes as well as the concatenated sequences were performed using Clustalw [32] from MEGA (version 6), to determine the percentage similarity between each of the gene sequences. Evolutionary divergences between the sequences were estimated using the maximum composite likelihood method. Phylogenetic trees resulting from individual gene fragment sequences as well as concatenated sequences of the isolates and those of other mycobacteria (both slow and rapidly growing) retrieved from Genbank (https://www.ncbi.nlm.nih.gov/nucleotide/) were reconstructed using the neighbour-joining method [33] and validated using the maximum composite likelihood method. One thousand bootstrap replicates were run, and Nocardia farcinica and Nocardia brasiliensis were used as outgroups.

Sequence analysis of the isolates’ hsp65 gene fragments showed 95 % sequence similarity to M. novocastrense. Partial sequence analysis of the rpoB gene identified these isolates to share 95 % sequence similarity with M. novocastrense, M. flavesces or M. arupense. Characterization of the isolates by sequencing of the partial fragment of the sodA gene showed 8/10 isolates to share between 92 and 93 % sequence similarity with M. flavescescens and M. novocastrense. Two isolates, Balasi and TB 5612, could not be amplified with the sodA primers used. Sequence analysis of the partial 16S rRNA gene showed the isolates to share between 97 and 98 % similarity with M. moriokaense.

Phylogenetic relatedness of the isolates and other species of the genus Mycobacterium, based on the 16S rRNA gene as well as the concatenated sequences of the four gene fragments (16S rRNA, hsp65, rpoB and sodA) is illustrated by the phylogenetic trees in Figs 1 and 2 respectively. Phylogenetic analysis of the isolates based on the 16S rRNA gene sequences as well as the concatenated sequences revealed the isolates to form a single cluster. The highest level of bootstrap support value (up to 100 %) supporting the clustering identified these isolates as belonging to the same species. The genus Mycobacterium. Despite that these isolates showed 97–98 % 16S rRNA gene sequence similarity to M. moriokaense, they did not cluster phylogenetically with this species and neither did they cluster with the other closest relatives. These isolates clearly represented a novel species not previously described in the literature.

Mycolic acid profile determination of isolate WCM 7299T was done and the results compared to those of Mycobacterium fortuitum ATCC 6841T and that of M. moriokaense ATCC 43059T. Triplicate culture plates of each of the three mycobacterial strains were analysed separately by LC-MS. The comparative mass spectrometric analysis between M. fortuitum, M. moriokaense and isolate WCM 7299T is presented in the 1000–1300 m/z range as this is the typical mass range in which mycolic acids are found when analysed using LC-MS [34]. The number of molecular ions of significantly high intensity was ten for M. fortuitum, seven for M. moriokaense and eight for WCM 7299T (Fig. 3). Although the ion clusters between all three isolates showed a different m/z pattern from their respective Flow Injection Analysis (FIAs), further studies using liquid or gas chromatography may be required to determine their mycolic acid class ratio (Fig. 3). Gcebe et al. [35] determined the DNA G+C content of the type strain WCM 7299T in a separate study as 67.4 mol% [35].

The data presented here revealed that the 10 isolates belong to a novel NTM species, and we propose Mycobacterium malmesburyense sp. nov. as the name for the novel species.
DESCRIPTION OF MYCOBACTERIUM MALMESBURYENSE SP. NOV.

*Mycobacterium malmesburyense* [mal.mes.bu.ry.en’se. N.L. neut. adj. *malmesburyense* pertaining to Malmesbury, after a town (Malmesbury) in South Africa, where one of the isolates (the type strain) of this species originated from].

Acid-fast bacillus. Colonies form a yellow–orange pigment in the dark, and grow on L) slants supplemented with polymyxin B, amphotericin B, carbenicillin and trimethoprim (PACT), as well as on Middlebrook 7H11 at 25, 37 and 45 °C, within 2–5 days but optimally at 37 °C. Positive for the semi-quantitative catalase test (>45 mm foam), Tween 80 hydrolysis, and nitrate reductase, urease and pyrazinamidase activity tests. Different isolates show variable activity for the 3-day and 14-day arylsulphatase tests, as well as niacin production, but most are negative for the 3-day test, positive for the 14-day test and negative for niacin accumulation. Does not grow in the presence of 5 % NaCl and does not utilize D-mannitol, inositol, L-rhamnose or L-arabinose as sole carbon sources. Negative for the aesculin hydrolysis and citrate activity tests. Positive or negative reactions are regarded as those exhibited by 80 % or more strains. Growth of most strains is not inhibited by imipenem, tobramycin, cefoxitin or amoxylin, but is inhibited by ciprofloxacin, clarithromycin and doxycycline. Has unique 16S rRNA, *hsp65*, *sodA* and *rpoB* gene sequences that are clearly different from any other mycobacterial species with *M. moriokaense*, *M. flavescens*, *M. novocastrense* and *M. arupense* being the most closely related species. Phenotypic differences that separate this species from its closest relatives include pigmentation, biochemical characteristics and growth characteristics as well as the mycolic acid profile. Mainly found in the environments at the escarpment, semiarid plateau, moderate eastern plateau and subtropical coastal regions, as well as areas with a mediterranean climate, in South Africa. Isolates from bovine tissue samples have been encountered in multiple parts of the country. No clinical relevance has been defined for this species.

The type strain is WCM 7299T (=ATCC BAA-2759T=CIP 110822T), which was isolated from a bovine nasal swab. Isolates Balasi, Vryburg, Uyenvlei, TB 5612, TB 5960A, 242, Middledrift, C4 and C8 are additional strains of this species.

Funding information
We are grateful to WOTRO Science for global development, grant number W01.65.321.00, for funding.

Acknowledgements
The authors would like to thank Ms Levina Ramkumar from Zoonotic Diseases Laboratories of ARC-Onderstepoort Veterinary Institute for her technical support.

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

References


