

Newly Identified *Mycobacterium africanum* Lineage 10, Central Africa

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Analysis of genome sequencing data from >100,000 genomes of *Mycobacterium tuberculosis* complex using TB-Annotator software revealed a previously unknown lineage, proposed name L10, in central Africa. Phylogenetic reconstruction suggests L10 could represent a missing link in the evolutionary and geographic migration histories of *M. africanum*.

The traditional view of restricted diversity among bacterial agents causing human and animal tuberculosis is being revised thanks to wide use of whole-genome sequencing (WGS). Besides *Mycobacterium canettii*, representative of exceptional, nonclonal, early-evolution branching lineages of tubercle bacilli in eastern Africa, several previously unknown lineages of *M. tuberculosis* complex have been identified in Africa during the past decade. *M. tuberculosis* complex lineage 7 (L7) was discovered in the Horn of Africa and L8 in the African Great Lakes region (1,2). *M. africanum* L9 was found only in Djibouti and Somalia. In contrast, 2 other major *M. africanum*-affiliated lineages contributing substantially to the tuberculosis burden, L5 and L6, are found mostly in western Africa (3). The pathway between eastern and western Africa in the evolutionary history of the bacillus remains unclear. We describe a newly identified sister lineage of L6 and L9 associated with central Africa and discuss implications for determining the evolutionary history of related *M. africanum* lineages L5, L6, and L9. We based research on publicly available data and thus required no ethics approval.

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The Study

We used the TB-Annotator platform (G. Senelle, unpub. data, <https://www.biorxiv.org/content/10.1101/2023.06.12.526393v1>) to integrate WGS data from 102,001 *M. tuberculosis* complex isolates in the National Center for Biotechnology Information (NCBI) public domain. This platform identifies genetic variations, including single-nucleotide polymorphisms (SNPs), regions of difference (RDs), and IS6110 insertions, differentiating selected genomes from *M. tuberculosis* H37Rv. The TB-Annotator database also contains information on genotypic drug resistance and geographic location of variant isolation.

SNPs from an exploratory set comprising 15,699 isolates largely of Africa origin were used to build a phylogenetic tree. Our analysis identified a lineage sister to *M. africanum* L6 and L9, branching between these lineages and the animal lineage A1 (La_A1) (3). The newly identified lineage is represented by only 2 genomes: ERR2707158, obtained from a strain isolated in 2008 from a patient residing in Kinshasa, Democratic Republic of the Congo (DRC), now incorporated under reference ITM-501386 (CT2008-03226) in the coordinated collections of microorganisms of the Institute of Tropical Medicine (Antwerp, Belgium); and ERR2516384, obtained from a strain isolated in Belgium in 2013 (V. Mathys, pers. comm., email, 2023 Jul 5). The genomes of the new lineage carried none of the SNP markers described in the latest *M. tuberculosis* complex lineage classification scheme (4) and no SNPs that confer drug resistance.

To confirm the phylogenetic position of those 2 genomes, we identified SNPs from 132 isolates covering the genetic and geographic diversity of L5 and L6 and including representatives of all other lineages using the Genotube pipeline (A. Le Meur, pers. comm., email, 2023 Sep 15) and TB-Profiler (5). Resulting phylogenetic reconstruction confirmed the clustering of ERR2707158 and ERR2516384 in a branch between L6 and L9 and animal lineage

La_A1 (Figure). The newly designated L10 samples shared 375 specific SNPs with isolates from our selected set of 132 samples; 243/375 specific SNPs were not detected in any of the 102,001 genomes included in TB-Annotator. Among those specific SNPs, 91 were synonymous (Appendix 1, <https://wwwnc.cdc.gov/EID/article/30/3/23-1466-App1.xlsx>). The pairwise distance between the 2 samples of interest was 382 SNPs (SNPs outside of repetitive regions, manually checked when discordant between 2 pipelines), much shorter than the dis-

tance to the other samples of our selection (minimum 1,137 SNPs; average 1,591 ±222 SNPs) (Appendix 2, Figure 1, <https://wwwnc.cdc.gov/EID/article/30/3/23-1466-App2.pdf>).

We next explored other features of the genomes to corroborate SNP-based phylogenetic inferences. In addition to the deletion of RD9 shared with the L5/L6 branch and animal-associated lineages, the 2 L10 genomes lacked RD7, RD8, and RD10 (3). However, they did not show the RD702 (L6/L9) or RD713 (L5) deletions. In contrast, the 2 unclassified

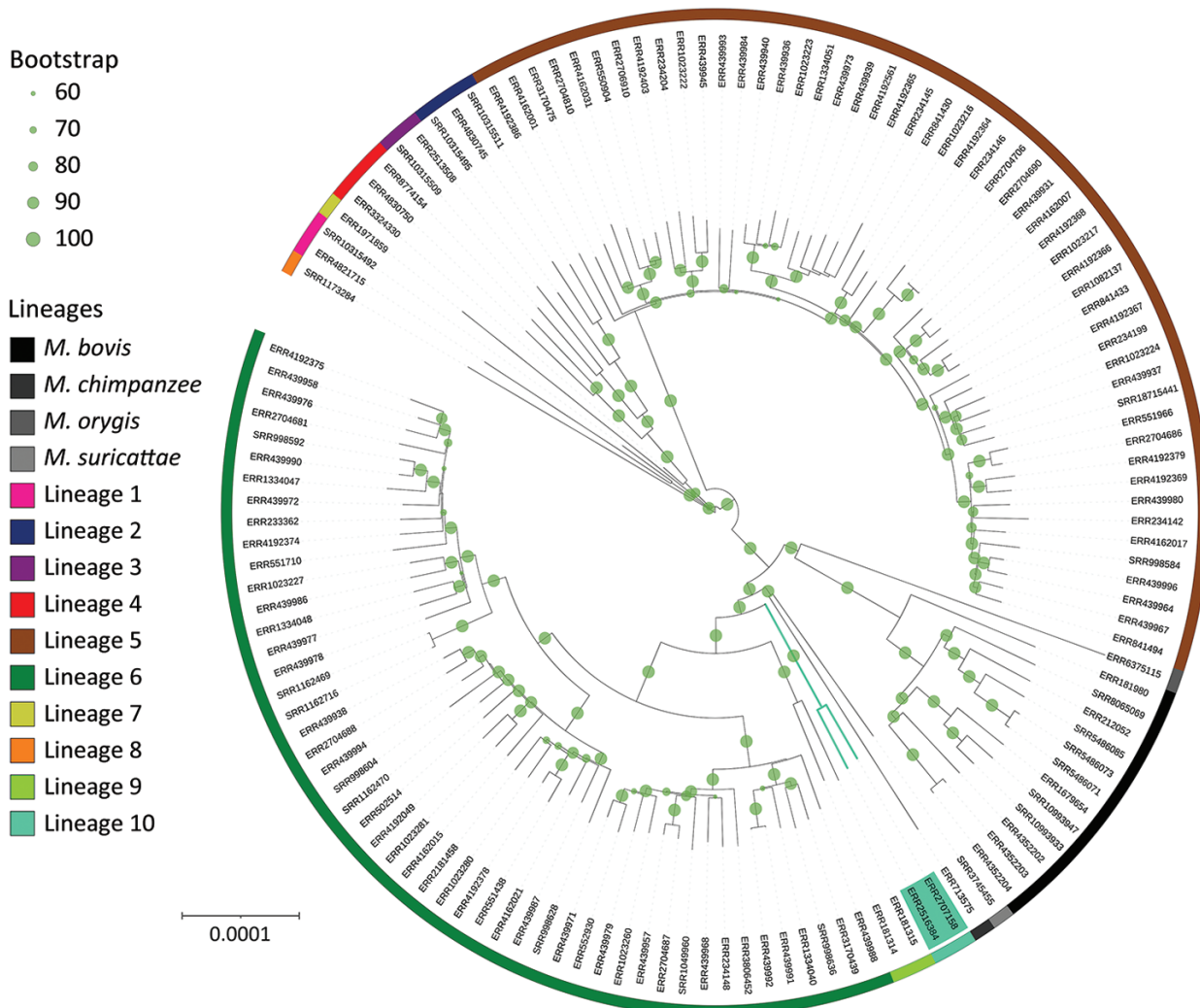


Figure. Global *Mycobacterium* phylogeny including newly identified *M. africanum* L10 (proposed) strains (green shading). We selected *M. africanum* samples for harboring RD9 deletion, having documented country of origin (for the purpose of additional analyses; Appendix 2, Figure 2, <https://wwwnc.cdc.gov/EID/article/30/3/23-1466-App2.pdf>), and refined our selection to retain a sole representative of each sublineage for each country. This sample represents the genetic and geographic diversity of *M. africanum* in Africa. Specifically for this phylogenetic reconstruction, single-nucleotide polymorphisms were identified in comparison with an *M. tuberculosis* ancestor (11) and reincorporated into the whole genome to avoid biases in the molecular model or need for Lewis correction. Phylogeny was rooted with *M. canettii*, subsequently removed for better visualization. Bootstrap support was computed using 100 replicates and shown when ≥0.6. Circles confirm the large support of almost all branches, especially of L10 and its sister branches. L10 branching point lies between L9 and the La_A1 lineage grouping chimpanzee and Dassie bacillus. Scale bar indicates nucleotide substitutions per site.

Conclusions

Through the extensive mining of WGS and genotyping databases, we newly identified a thus far rare *M. tuberculosis* complex lineage, L10 (proposed), present in central Africa. The lineage is characterized by a new region of deletion, IS6110 insertions, and 243 SNPs, including *gyrA* G7901T, *recN* C1920096T, and *dnaG* C2621730T. L10 represents a sister clade to L6, found mainly in western Africa, and L9, specifically in eastern Africa, and reveals a putative previously missing piece in the evolutionary history and migrations of *M. africanum*. Our findings extend the known diversity of *M. africanum* in Africa.

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About the Author

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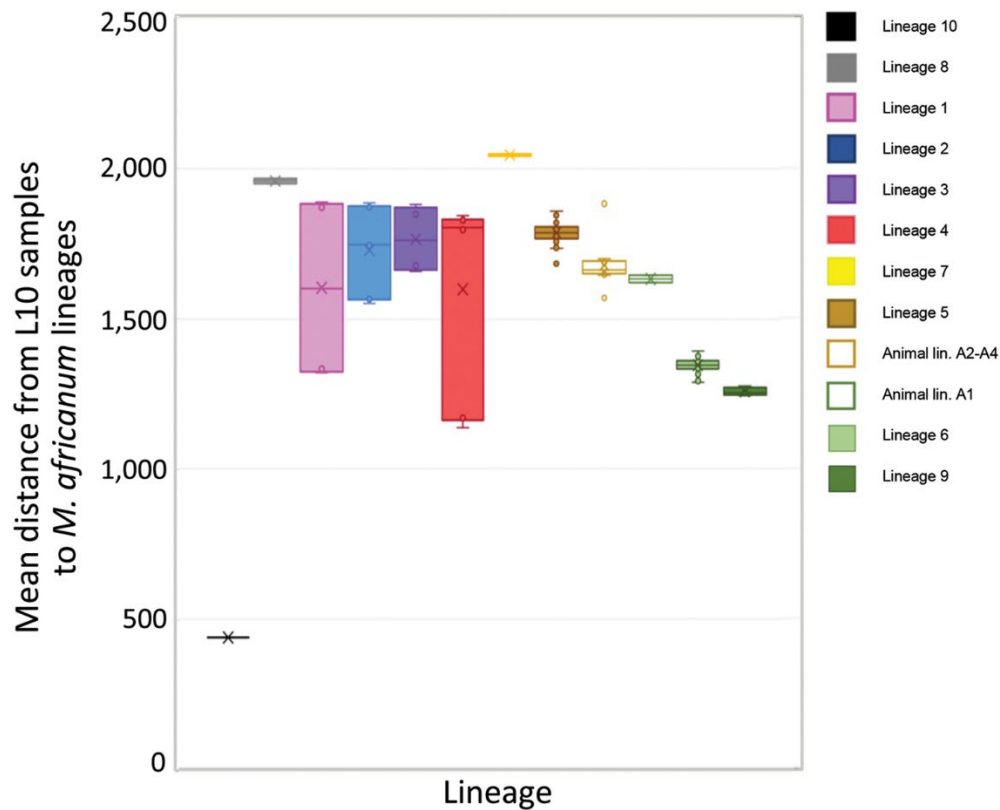
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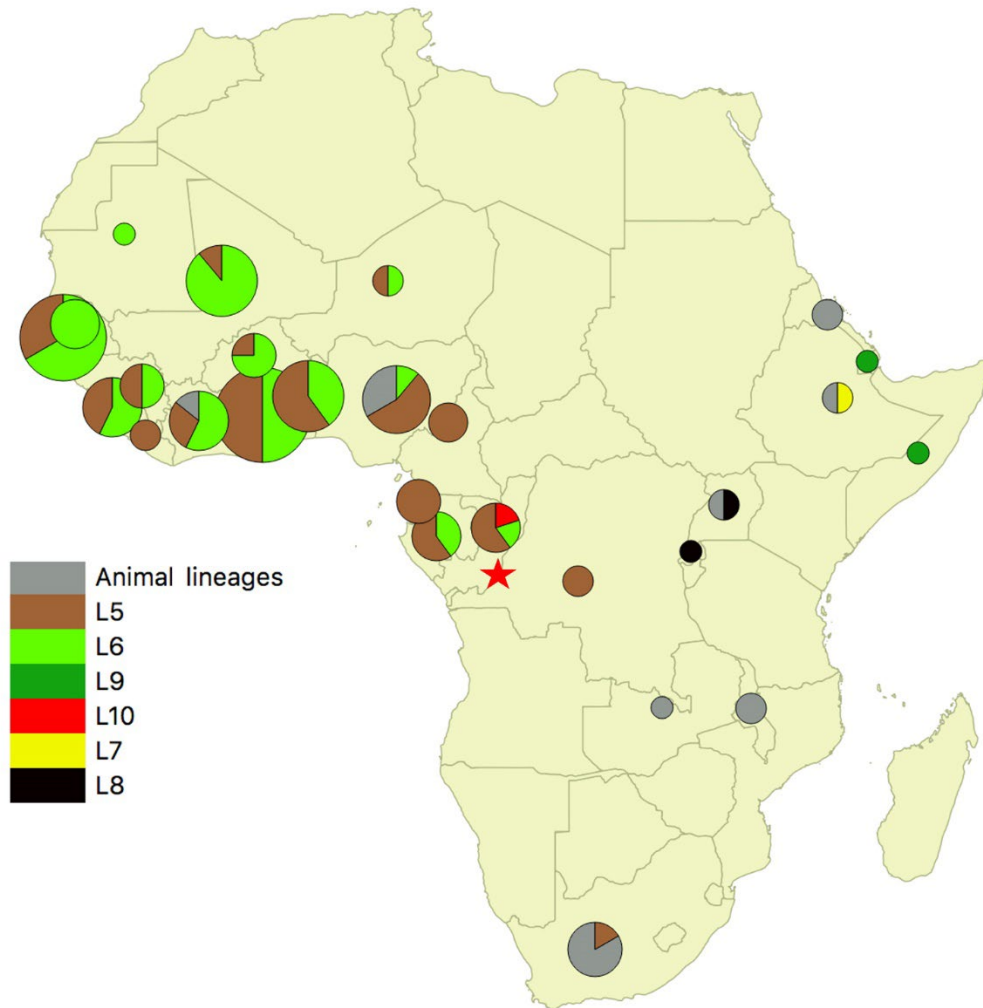
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Appendix 2



Appendix 2 Figure 1. Intra- versus inter-lineage genetic distance of L10 strains. The distances between L10 strains and all other samples shown in the tree of Figure 1 were computed using snp-dist tool excluding PE-PPE regions. The legend is provided in the same order as they appear on the X-axis. Note that L10 strains are much more similar to one another than to strains from other lineages.



Appendix 2 Figure 2. Map of Africa showing distribution of *Mycobacterium africanum* sublineages. This map locates the African samples from our 132-sample diversity set (Figure 1 in the main text), as annotated by Genotube. Animal lineages include La_A1 and other animal lineages. The sampling of these lineages should not be considered representative because of lack of sublineage definition among animal lineages in Genotube. In contrast, because 1 sample from each known *M. africanum* sublineage was retained for each country, sampling can be considered as representative of L5 and L6 diversity. The map was built with QGIS version 3.4.6 (Madeira on Mac-OS10.13.6, using an open source shapefile with African boundaries obtained from <https://hub.arcgis.com>). The single localized L10 strain (from Democratic Republic of Congo) reported in the NCBI SRA database is shown in red. The red asterisk shows the Republic of Congo where another L10 strain was isolated according to SITVITWEB database. Of note, Belgium, where the third L10 strain was collected, has a history of colonizing DRC; the strain was isolated from a patient with an African name which is not indicative but compatible with the actual origin of this strain in central Africa (DRC). This map also highlights the presence of L5 in central Africa.