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Towards a high-resolution genotyping of the Latin American Mediterranean genotype of *Mycobacterium tuberculosis*

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INTRODUCTION & AIM

The Latin American Mediterranean (LAM) genotype of an important part of the Euro-American lineage of *Mycobacterium tuberculosis.* LAM strains are spread in most regions of the world although its different lineages show certain geographic specificity, e.g. LAM RD-Rio branch is more prevalent in South America and parts of Africa. In its turn, RD115/LAM-RUS branch is endemic to Russia and Northern Eurasia and is frequently multidrug-resistant.

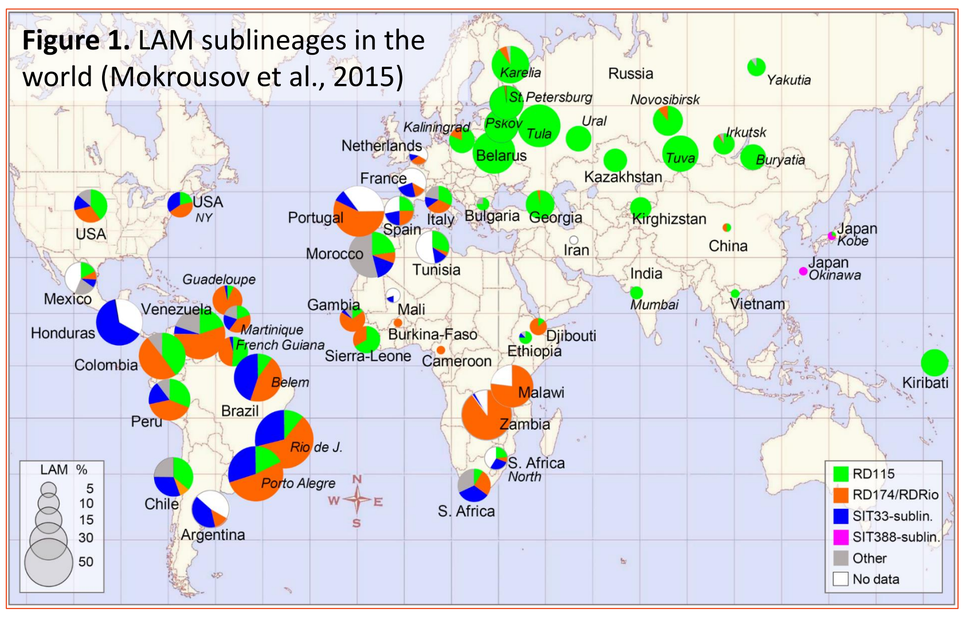


Figure 1. LAM sublineages in the world (Mokrousov et al., 2015)

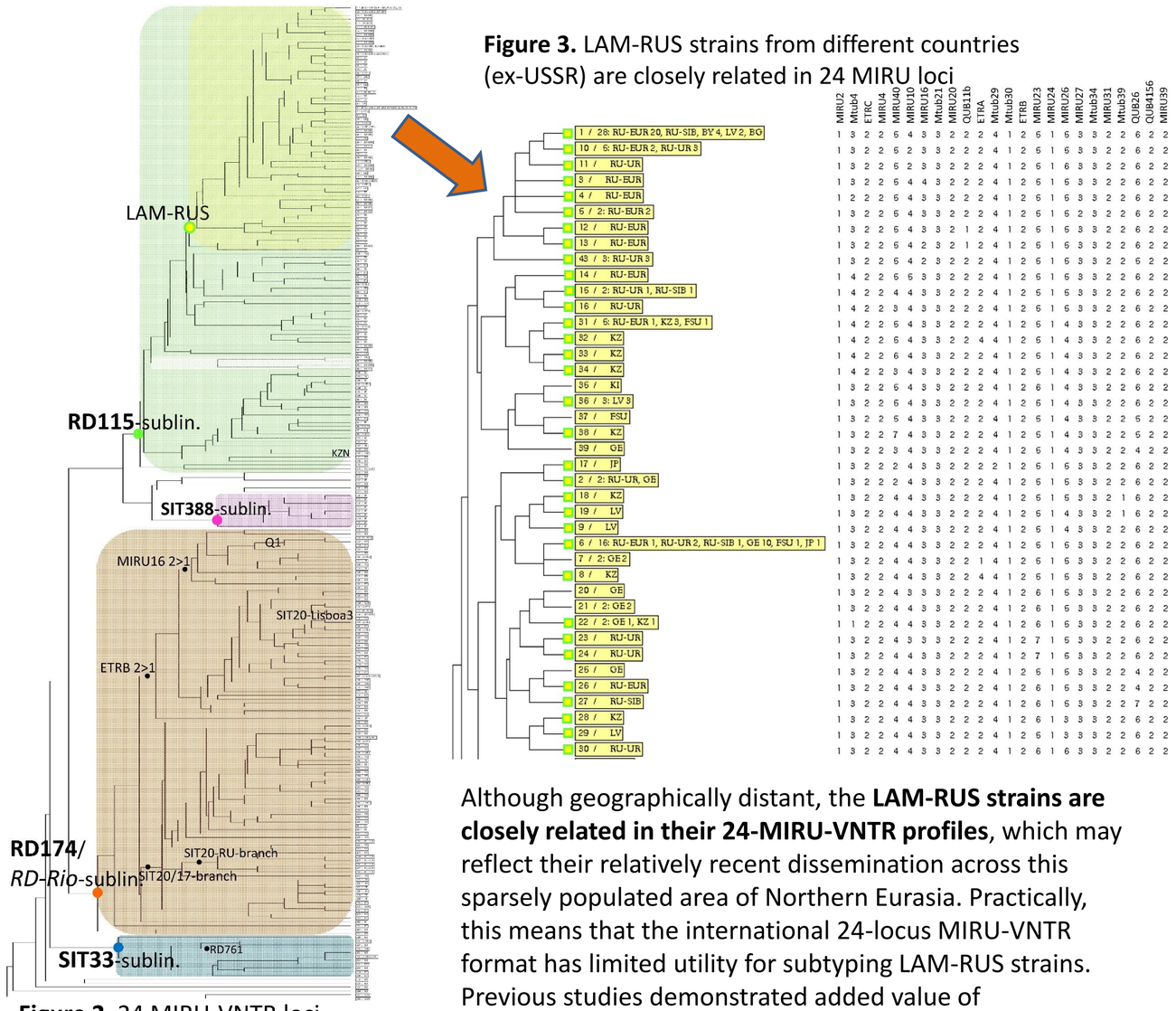


Figure 3. LAM-RUS strains from different countries (ex-USSR) are closely related in 24 MIRU loci

Figure 2. 24 MIRU-VNTR loci phylogeny of global collection of LAM strains (Mokrousov et al., 2015)

Although geographically distant, the **LAM-RUS strains are closely related in their 24-MIRU-VNTR profiles**, which may reflect their relatively recent dissemination across this sparsely populated area of Northern Eurasia. Practically, this means that the international 24-locus MIRU-VNTR format has limited utility for subtyping LAM-RUS strains. Previous studies demonstrated added value of hypervariable (HV) VNTR loci to discriminate within closely related Beijing genotype strains (Allix-Beguec et al., 2014).

AIM: to evaluate seven hypervariable VNTR loci for their discriminatory power among LAM-RUS strains.

METHODS

A sample of 133 *M. tuberculosis* LAM strains from different locations was subjected to spoligotyping (followed by comparison with SITVIT2) and 24-loci MIRU-VNTR typing.

Strains were assigned to LAM based on spoligotyping, detection of LAM specific SNP in Rv0129c or by clustering with LAM reference profiles of MIRU-VNTRplus.org tool.

Seven hypervariable VNTR loci (3820, 4120, 3232, 1982, 2136a, 3155, 3336) were additionally genotyped. MIRU-VNTRplus.org and PAUP software were used for phylogenetic analysis of the VNTR data, treated as discrete variables. The Hunter-Gaston Index (HGI) was used to assess the discriminatory power of individual loci and their combinations.

RESULTS & DISCUSSION

Based on spoligotyping, 22 different spoligotypes were identified, of which SIT42 was the most prevalent (n=37), followed by SIT254 (n=26) and SIT252 (n=22).

Thirty-locus typing revealed 14 clusters (2–15 isolates) and 58 unique profiles. The Hunter-Gaston Index (HGI) for the 30-locus set was 0.979. HGI values for individual loci ranged from 0 (MIRU4, MIRU24, MIRU39, Mtub29) to 0.5595 (VNTR3820) and 0.6165 (MIRU40).

The combined discriminatory power of the 11 most polymorphic loci was 0.968 (13 clusters of 2–17 isolates; 67 unique profiles), which is nearly equivalent to that of the full 30-locus VNTR set.

Table. Spoligotypes of the studied LAM strains

SIT	spoligoprofile	number of isolates
20	■■□■■■■■■■■■■■■■■■■□□□□■■■■■■■■□□□□■■■■■■■	3
42	■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■■□□□□■■■■■■■	37
60	■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■■□□□□■■■□■■■	1
150	■■■■■■■■■■■■■□■■■■■□□□□■■■■■■■■□□□□■■■■■■■	2
252	■■■■■■■■■■□□■■■■■■■■□□□□■■■■■■■■□□□□■■■■■■■	22
254	■■■■■■■■■■■■■□□□□□□□□□■■■■■■■■□□□□■■■■■■■	26
264	■■■■■■■■■■■■□□□□□□□□□□■■■■■■■■□□□□■■■■■■■	7
266	■■■■■■■□■■■■■□□□□□□□□□□■■■■■■■■□□□□■■■■■■■	1
402	■■■□□	1
444	■■■■■■■■■■■■■□□□□□□□□□□■■■■■■■■□□□□■■■■■■■	3
492	■■■■■■■□■■■■■■■■■■■■□□□□■■■■■■■■□□□□■■■■■■■	2
496	■■■■■■■■■■□■□□□□□□□□□□■■■■■■■■□□□□■■■■■■■	5
500	■■■■■■■■■■□□□□□□□□□□□□□■■■■■■■□□□□■■■■■■■	3
560	■■■■■■■■■□□□□□□□□□□□□□□□□□□□□□□□□□□■■■■■■■	1
803	■■■■■■■■■■■■□□□□□□□□□□■■■■■■■■□□□□■■■■■■■	4
1176	■■■■■■■■■■■■□■■■■■□□□□■■■■■■■■□□□□■■■■■■■	4
1337	■■■■■■■■■■■■□□□□□□□■□□□□■■■■■■■■□□□□■■■■■■■	2
1451	■■■■■■■■■■■■■□□□□□□□□□□□□□□□□□□□□□□□□□□□□□	7

Figure 4. Dendrogram of the studied LAM strains based on the complete panel of 30 VNTR loci.

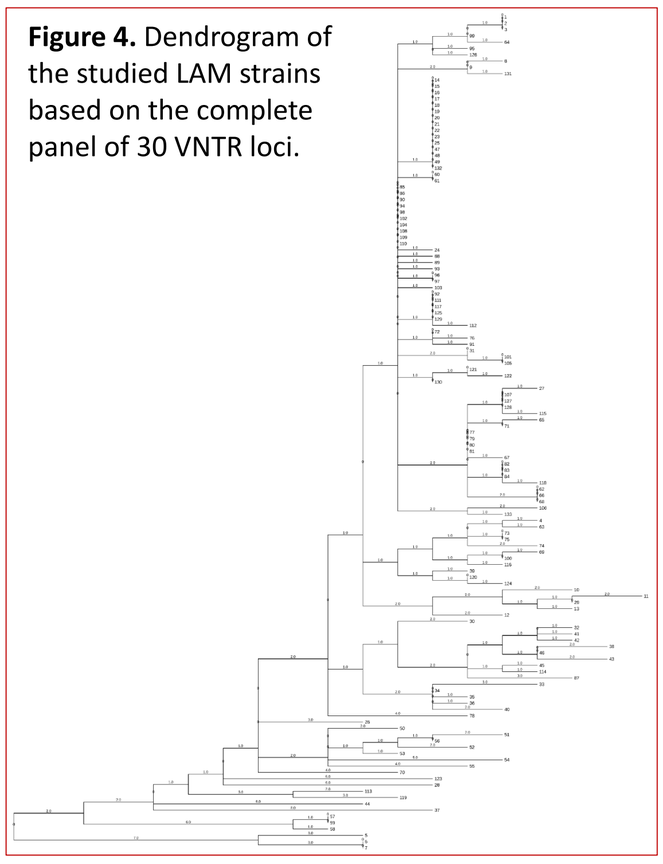
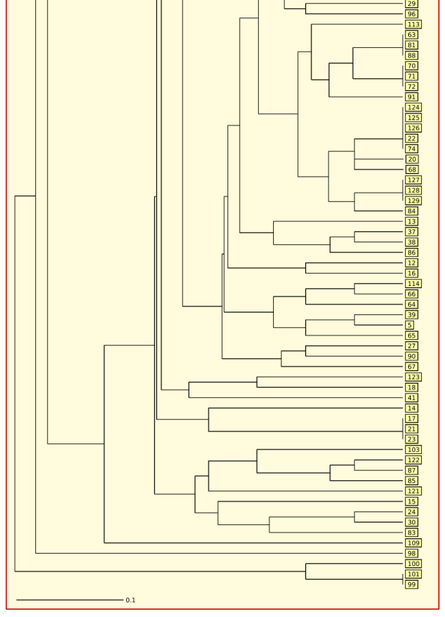


Figure 5. Dendrogram of the studied LAM strains based on 11 most polymorphic VNTR loci.



CONCLUSION

The optimized 11-locus panel (7 classical Mtub04 MIRU40 MIRU10 MIRU16 MIRU23 MIRU26 QUB26 and 4 hypervariable 3820, 4120, 3232, 3336) provides sufficient discriminatory power for LAM genotype strains and could serve as a cost-effective tool for primary epidemiological surveillance of this important *M. tuberculosis* lineage.

Future research will evaluate this method with more strains from more world regions.

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