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Research Article

Phylogenomics analysis of Mycobacterium bovis strains from Mexico: Insight report on its Phylogeography

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Abstract

Bovine Tuberculosis caused by *M. bovis* represents an economic and animal health problem worldwide. In Mexico, the biogeography of *M. bovis* stemmed from studies using genotyping through several genetic markers obtained from SNPs, RFLPs, VNTRs, and Spoligotyping. More recent studies worldwide have used a combination of genotying and whole genome sequencing, in order to cover evolutive history, behavior, phylogeography of the strains. Not whole genome studies in *M. bovis* from Mexico have been made in detail since in SRA Genbank are deposited only the "reads" of the sequences of the *M. bovis* isolates. Therefore, in the present study, we focused to carried out the genome assembly of the reads from the SRA database. Phylogenomic analysis show that different isolates of *M. bovis* strains cluster in the same clade independent of the region, suggesting a close relationship between them. Collectively the data provide an update in the *M. bovis* phylogeography in Mexico.

Keywords: *Bovine tuberculosis*; *Mycobacterium bovis*; Phylogenomics; Comparative Genomics; Mycobacterium Tuberculosis Complex

Introduction

Bovine Tuberculosis is a zoonotic disease caused by *Mycobacterium bovis* (bTB) that affects cattle, along with wild animal species that contribute to the dissemination and transmission of this disease, representing thus, a threat for ecology, agriculture, and human health [1-6]. In Mexico, there are states of high prevalence or endemics of bTB [3,7]. The transmission a spread of *M. bovis* strains in Mexico might be due to different factors, such as, interactions of cattle with the wildlife, direct contact with small droplets expelled from the infected animal, including coughing, or other contaminated material such as infected meat and milk with low or zero quality in the pasteurization process, transport of contaminated material, facilitates the spread and transmission of the *M. bovis* strains from one region to another. Current National Program for control and eradication of Bovine Tuberculosis involves slaughtering herds and movement restrictions on infected cattle for some stages [3,7], and the high cost in molecular tools in reference laboratories, the field test on livestock is still based on tuberculin test (PPD).

Intense efforts to determine the spread of bTB and the molecular epidemiology of *M. bovis* transmission status are worldwide [8-13]. Most of the reports on bovine tuberculosis (bTB) have focused on genotyping *M. bovis* strains using Restriction Fragment Length Polymorphism (RFLP'S), single nucleotide polymorphism (SNP'S) or Variable nucleotide tandem repeat (VNTR's), and/or Spoligotyping (forty-three sequences highly polymorphic repeats). These typing methodologies have allowed the construction of phylogeny in terms of the repeated sequences present through the genome [14-22]. Spoligotyping implementation and development

scale can support the National Program for control, surveillance, and eradication of bTB, avoiding the slaughtering of herds [3,23].

Milián., *et al*. 2012 [23,24] achieved a study of molecular patterns of *M. bovis* isolates between 2009 and 2010 to characterize outbreaks in infectious disease but is limited in endemic regions with no financial limitations. Two Spoligotyping studies have provided data of the most frequent spoligotypes patterns at regional, national and international levels. The transmission and the genomic dynamics [6,11,14,23-26]. Moreover, Nava., *et al*. 2015 [27] used VNTR's and spoligotyping to analyze and study *M. bovis* isolates of 16 states of the Mexican Republican [28-31]. Their study aimed to compare the genetic profiles using these two techniques. Moreover, Peréa-Razo., *et al*. 2018 [33] achieved Spoligotyping and SNP's through genome-wide association in a subpopulation of isolates of *M. bovis* from different regions of Mexico. Verdugo-Escárcega., *et al*. 2020 [28] showed the frequency of bTB using Spoligotyping and SNPs of the whole genome from the Jalisco State [28], Other reports referring to Baja California state [34,35], and Acapulco Gro [36]. In this context, genotyping of Mexican *M. bovis* isolates has revealed several groups of molecular markers.

A more recent approach has been used whole-genome sequencing (WGSs) [32,38-40] that can provide information of the localization of the polymorphism in only one nucleotide (SNP's) and higher resolution to survey infection sources. In fact, it is necessary for a higher power of resolution that allows an identification finer of the strains that contribute to the understanding of the disease transmission, the dynamics of the infectious disease, especially considering that bTB is a zoonotic disease [14,37-39]. Moreover, the combination of Genotyping and Phylogenomics can provide deep insight and unveil aspects of the origin, diversity, and the behavior of mycobacteria [4,6,11,14,37-39]. Furthermore, genome comparison can aid in the study of the relationships more precisely among taxons, especially within the members of the MTBC, which is known are almost identical, with a low amount of variation [25,27,37-39]. At this point, Kraemer., *et al*. 2020 [14] examined 1,960 genomes of *M. bovis* of 23 countries and at least 24 hosts, including humans. They performed a phylogenomic analysis combined with SNP's. From this analysis, they proposed four global lineages different Lb1, Lb2, Lb3, and Lb4). Mexican *M. bovis* isolates of cattle and humans is suggested that belong to the lineage I.b.3 (strains of the clonal complex Europe 2)(Eu2) and I.b.4 (strains of the clonal complex Europe 1)(Eu1) [6-8,10,11,14,37]. In addition, it is proposed that *M. africanum*, *M. microtti*, and *M. bovis*- are a separated lineage from *M. tuberculosis*, despite the 99.8% of homology [37,40-43]. They resemble an ancient progenitor that suffers a successive loss of DNA regions (RD), which gave rise to the lineages and sublineages of *M. bovis* strains [11,14,42-46]. It is also possible that in terms of the evolutive origin of these different lineages are favored in ancient times because the trade, colonization, and discovery of new continents (America) or new countries (New Zealand) [14].

A more recent study by Loisseau., *et al*. [41] infered the evolutive origin of *M. bovis* from East and West Africa. From these regions is hypothesized that other *M. bovis* groups are distributed and dispersed driven the cattle movements worldwide. Interestingly, Loisseau., *et al*. 2020 [41] in addition to use spoligotyping, they used other genomic markers such as deletions and single-nucleotide polymorphisms (SNPs). While most of the large globally representative collections of whole- genome sequences (WGS) have been toward the biogeography, evolutive origin of *M. tuberculosis*; studies on other ecotypes animal adapted are lacking, such as *M. bovis*. The genetic markers have allowed to define four major groups of genotypes within *M. bovis* populations, known as clonal complexes European 1 and 2 (Eu1 and Eu2) and African 1 and 2 (Af1 and Af2). In particular, TB in Europe and in the Americas is caused by clonal complex Eu1 including the British islands and former trading countries of the IK, North America, Central America and Mexico), while Eu2 is prevalent mostly in the Iberian Peninsula and Brazil [41].

Since in Mexico, studies on *M. bovis* isolates from different states of the Mexican Republic have doing since long time ago toward the use of different genetic tools such as Spoligotpyping, RFLPs, VNTRs, SNPs and WGS (REFS) which have provided knowledge about that strains vary by geography, and have defined strain families based on the presence or absence of spacers in the direct repeat region of the MTBC genome. But spoligotyping diversity is determined at a single locus and not evolutive origin and phylogenetic distances can be reliably infered or calculated [19,21-23,31,32,36]. A deeper insight into the analysis of whole-genome sequences (WGS), may allow to enhance understanding of the global population structure, phylogeography and evolutionary history [13,14,28,39-41].

In the present study, a Phylogenomic analysis of the *M. bovis* genomes of isolates of important economical regions in livestock. Previous studies reported by other authors have demonstrated the molecular and geographical distribution of the genetic markers, based mostly in genotyping. However, more recent studies have used combined genetic and genomic tools that allow to approach and inferred evolutive origin, diversity and global distribution, behavior, Phylogeography, Phylogenetic and Phylogenomic

relationships. Herein, we are reporting data regarding the phylogeography of the genomes of *M. bovis* strains. Collectively, the in silico phylogenomic analysis suggest that the movements of herds and of dairy products have a role in the phylogeography of *M. bovis* of Mexico.

Methods

Data collection

The sequence of *M bovis* is available in the SRA database from NCBI, which was downloaded from: https://trace.ncbi.nlm.nih.gov/Traces/sra/sra.cgi?view=software. Software that allows conversion of SRA files to FASTA files was installed: cd bin_sra ls./ fastaq-dump –x 5 –z SRR7236189 (where –x indicates the first five files that contain the sequence y-z, which is the site shown. The metadata table built with the files of the sequences retrieved from the NCBI database includes some relevant features about the strains as code, biosample code, geographical localization, genotype, host, description, spots, size (Mb), and G-C content. In addition, into account for the table, the percentage of quality of the sequence per base. Thus, Four hundred and seventy-one DNA sequences were selected based on two main criteria parameters: host (human or bovine), and geographical location (state), for the *in silico* analysis.

The assembly of the *Mycobacterium bovis* genomes, using the program HybridSapdes(Supp Mat). The grade of the assembled genomes obtained is composed of *contigs*, located in an interval of 140-200. Few *contigs* are nine units under 140, and in most of the cases are higher than 200 (with numbers of 300-450). Except for the states of Aguascalientes, Hidalgo, Queretaro, and the State of Mexico. The *contigs* in the strain of the town of Ensenada have values higher to the thousands with 1430, 3686, 2972, 1387, and 1222, respectively. These values don't represent a high percentage since only 14 strains of the total (n = 471). The program provides global statistics. Among the most representative statistics, more representative is the number of scaffolds or *contigs,* the length of this, and the value of N50. Together they represent the quality of the assembly in terms of the contiguity, where N50 represents the assembly more complete if the value is higher (Suppl Mat).

For the phylogenomics analysis all the concentrated files (*.fasta) originated from the file of assembled genomes were used. Since the sample size was too big, the study was divided in five economic zones of the country, which coincide with the zones of high bTB prevalence in Mexico: North Zone, North-Center Zone, Center Zone, and Center-South zone. The North Zone includes Ensenada, Mexicali, Tijuana, Tecate, Coahuila (Figure 1A). It includes the samples registered only as Baja California, with a total of 189 sequences. The Center-North Zone includes Aguascalientes, Guanajuato, and Jalisco and contained 69 samples. For the Center Zone, there were only 29 samples, corresponding to the State of Mexico. The Center-South Zone was composed of 184 sequences, representing the Hidalgo, Queretaro, and Veracruz states. The phylogenomics trees built using REALPHY. The most representative genomes (n = 169 genomes) were incorporated in one single file, and analyzed. To compare the genomes of Mexican *M. bovis* with the genomes from other countries, genomes available in the NCBI Database from Canada (1), Corea (2), United Kingdom (2), Brasil (2), Tokyo (1), Thailand (1), Bulgary (1), Mexico (1), Russia (2), Denmark (2), China (4) and France (1). A Phylogenomic tree built with 169 genomes representative of different states of Mexico and the 20 genomes of foreign countries along with the reference genome of *M. bovis.* A tree with a higher quality and structured view using iTOL tool (Suppl Mat).

Results

Phylogenomic analysis of the assembled genomes of *M. bovis* from Mexico

The phylogenomic analysis of the assembled genomes (this work) (Suppl mat) of all the "reads" of *M. bovis* retrieved from the SRA database confimed and strengthen that the genomes of the different *M. bovis* isolates from the four important economical regions (Figure 1A) in livestock are closely related since clustering in the same clade, independent of the region (Figure 2A) Yes, it is true, previous studies reported by several authors have shown that geographical distribution of the spoligotypes in the Mexican Republic. However, this distribution is refered to one single locus or single pattern. Our results are not opposed to this, on the contrary, support and strengthened the biogeographical distribution of the whole genomes of *M. bovis* isolates in Mexico. For all the *M: bovis* "reads" retrieved from the SRA database, from the different regions of Mexico (Figure 1A), briefly, the assembly of the *Mycobacterium bovis* genomes was carried out as follow: Using the HybridSapdes program (Genome Asembly Suppl mat).. The grade of the assembled genomes obtained is composed of *contigs*, located in an interval of 140-200 bases. Only a few *contigs* are nine units under 140, and in most of the cases, they are higher than 200 (with numbers of 300-450), except for in the states of Aguascalientes, Hidalgo, Queretaro, and the State of Mexico (Figure 2A-B). In total, 429 genomes were assembled from states of Baja California (Ensenada, Mexicali, Tijuana, Tecate and El Rosarito), Coahuila, Hidalgo, Aguascalientes, Jalisco, Guanajuato, Estado de

México, Querétaro and Veracruz (Figure ¡). Other metadata table were also built which included several characteristics of each isolate (Table 1) (Suppl Mat). In addition, a filtering of the sequences based on different parameters, such as host, and sequence length was also carried out (Table 2) (Suppl Mat). The Phylogenomic tree for each zone shows a close relationship among the strains (only shown two representative regions, Center-North (Figure 3A) and Center South (Figure 3A). This relationship also is observed for the *M. bovis* isolates from the different regions, and a tendency towards a total grouping by tree zone (North and Center Region, Suppl Mat, Figure 2C-D). One explanation might be that groups are formed with strains from two or more states, or different towns, except for group 1 and group 3 that concentrate isolates of *M. bovis* of the same state/town: Jalisco and Ensenada, respectively. Groups 2 and 4 comprised strains of two different states: Coahuila and Queretaro. Coahuila, group 2 [spadesq_145, spadesq_112 y spadesc_378_], and Queretaro, group 4 [spadesc_193 y spadesq_131]. Both isolates come from distantly states. Furthermore, we observed the presence of *M. bovis* isolates from the states of Hidalgo (spadesh_269, spadesh_231 y spadesh_232), Querétaro (spadesq_404 y spadesq464) and Jalisco (spadesj_380 y spadesj_381) with higher differences in relation to the rest of the isolates separated of the formed groups (Figure 2A-B).

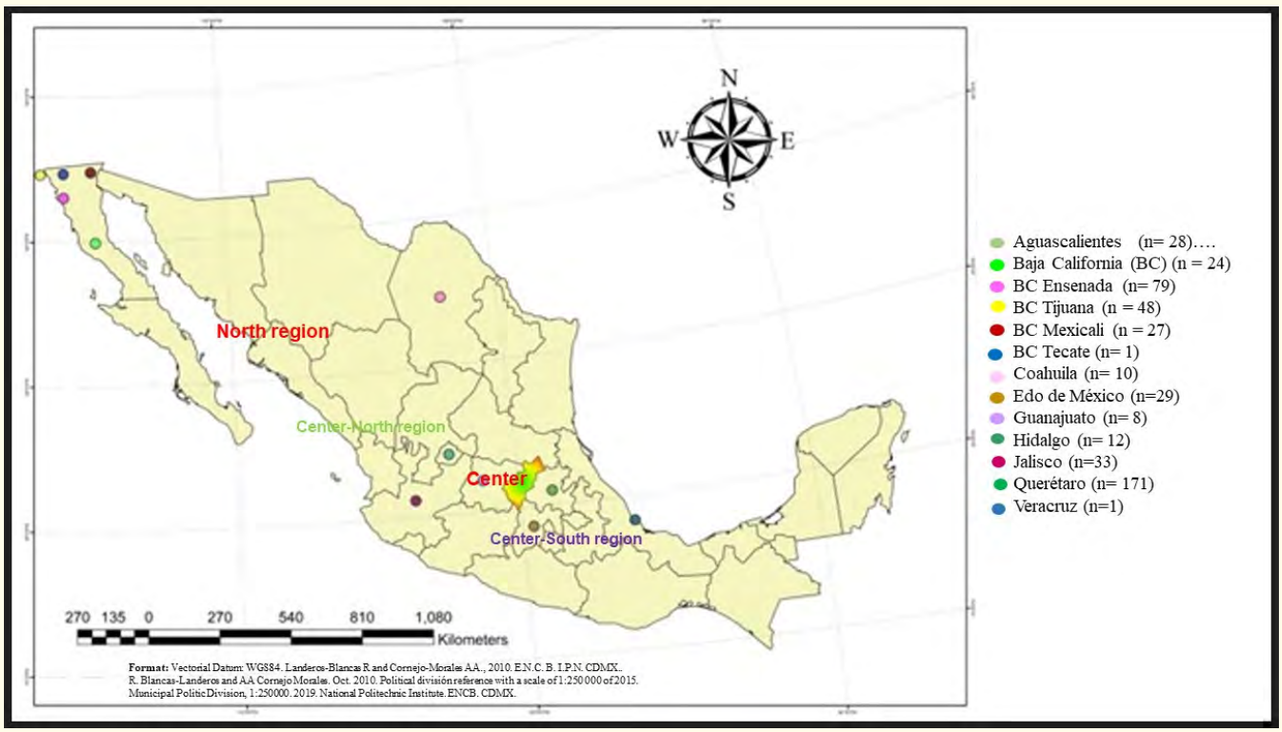


Figure 1: Geographical localization in Mexico of the *M. bovis* sampling. A Political division reference with a scale of 1:250 000 of 2015. Municipal Politic Division, 1:250000. The states shown in the map comprise the four regions, North, Center-North, Center and Center-South used for the study. The different states (colored), and the number of *M. bovis* isolates from each state (right panel).

A close relationship of *M. bovis* strains with Canadian *M. bovis* strain

Comparative genomics analysis was carried out using 145 representative genomes of each of the four regions analyzed (Figure 1), and 19 genomes from different countries (rNCBI database). We found that the strains from France, GCF_902459825.2, and Brazil GCF_000934325.9 were related more to the Mexican strains that showed the higher differences among the Mexican strains analyzed: spadesh_269, spadesh_231, spadesh_232, spadesq_404, spadesq464, spadesj_380 and spadesj_381) (Figure 3A). A large group comprises 86.89% group 5, which is divided into two large groups, named group 5.1 and group 5.2. In group 5.2. we can find the reference strain AF122/97 isolated in the United Kingdom, which is highly related to the genomes of Querétaro (spadesq_293, spadesq_144 y spadesq_324), and to the genomes corresponding to the state of Mexico (spadesed_190). Despite the formation of the subgroups in groups 5.1 and 5.2, there are differences between them. In group 5.2, the subgroups are formed by strains from different economic zones and different states of the Mexican Republic, among them: Aguascalientes and Guanajuato, or Jalisco and Aguascalientes together with Tijuana Town. Group 5.1,

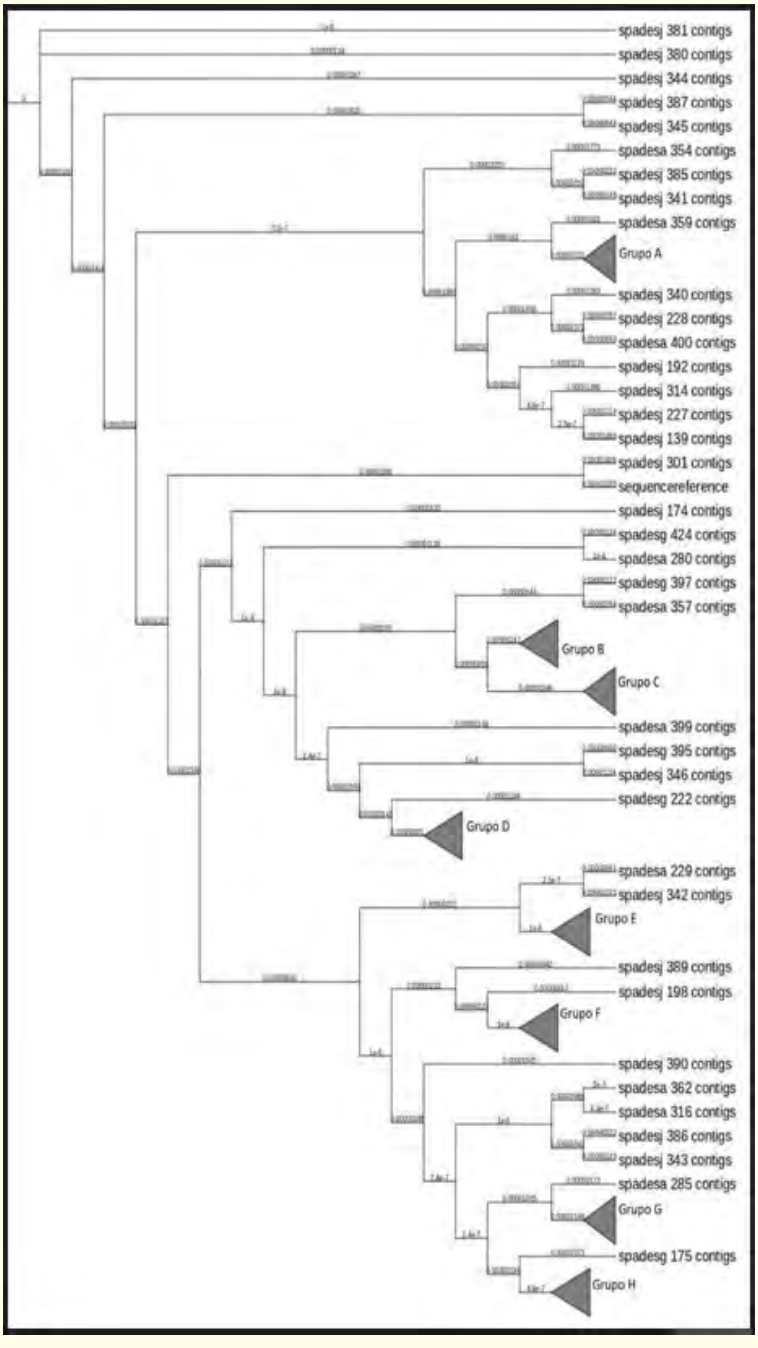


Figure 2A: Representative Phylogenomic tree of the Mexican strains of Mycobacterium bovis of the Center and North region. The tree was built using the REALPHY web server 1.1.3 of the Swiss Institute of Bioinformatics. Sequence Alignment was performed with Bowtie2, dividing the genomes in subsequences of 50 pd and a value of *k*mer of 22; while the reconstruction of the phylogeny was generated through PhML inside the server with the reversible general model in the time (GTR) of evolution of nucleotides and the variation of the velocity of gamma distribution. Eight groups were obtained, generated based in the nodes collapse, denoted from A-H. The group A correspond to 2 genomes (j339, g348), group B represent 4 genomes (a279, a331, a403, a402), group C 2 genomes (g394, a271), group D 11 genomes (j384, j382, j396, j127, a284, a352, a355, a283, a353, a356, a358), group E 3 (a414, j126, j235), group F 5 (j225, j197, j238, j226 y a398), group G 2 (a286, a272). Group H, 3 (j230, j383, a401). The letter following to the Word "spades" represent the initial of the name of the state to which the simple belong with a scale of 0.00001 for the tree.

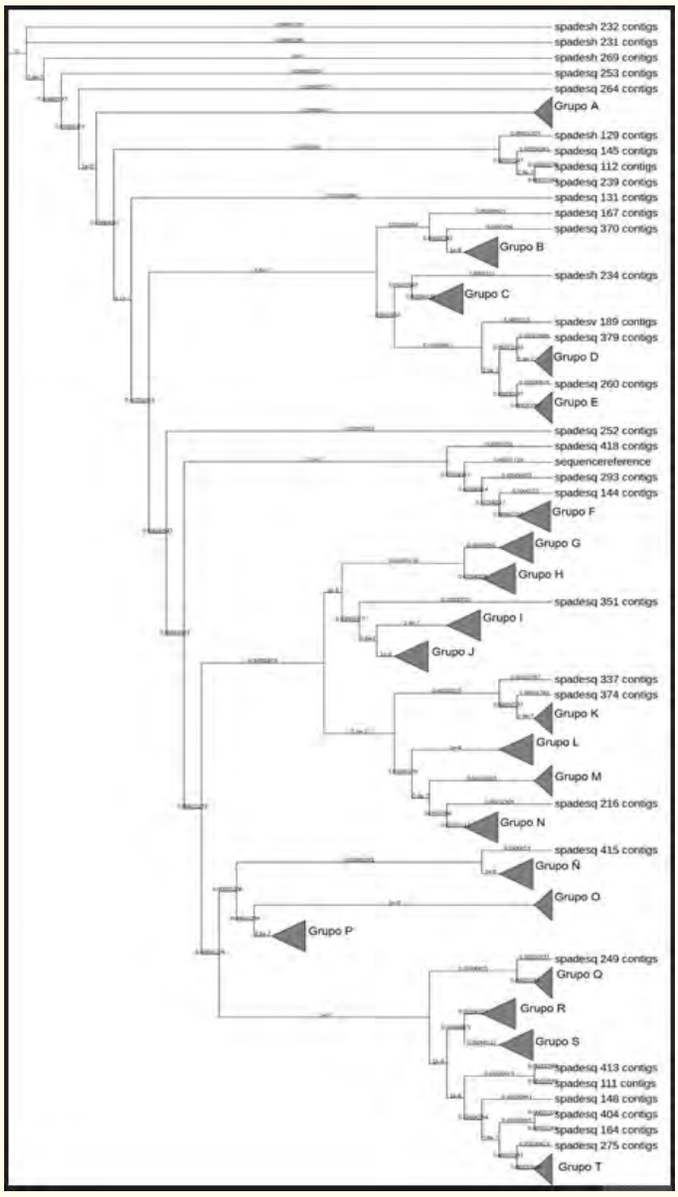
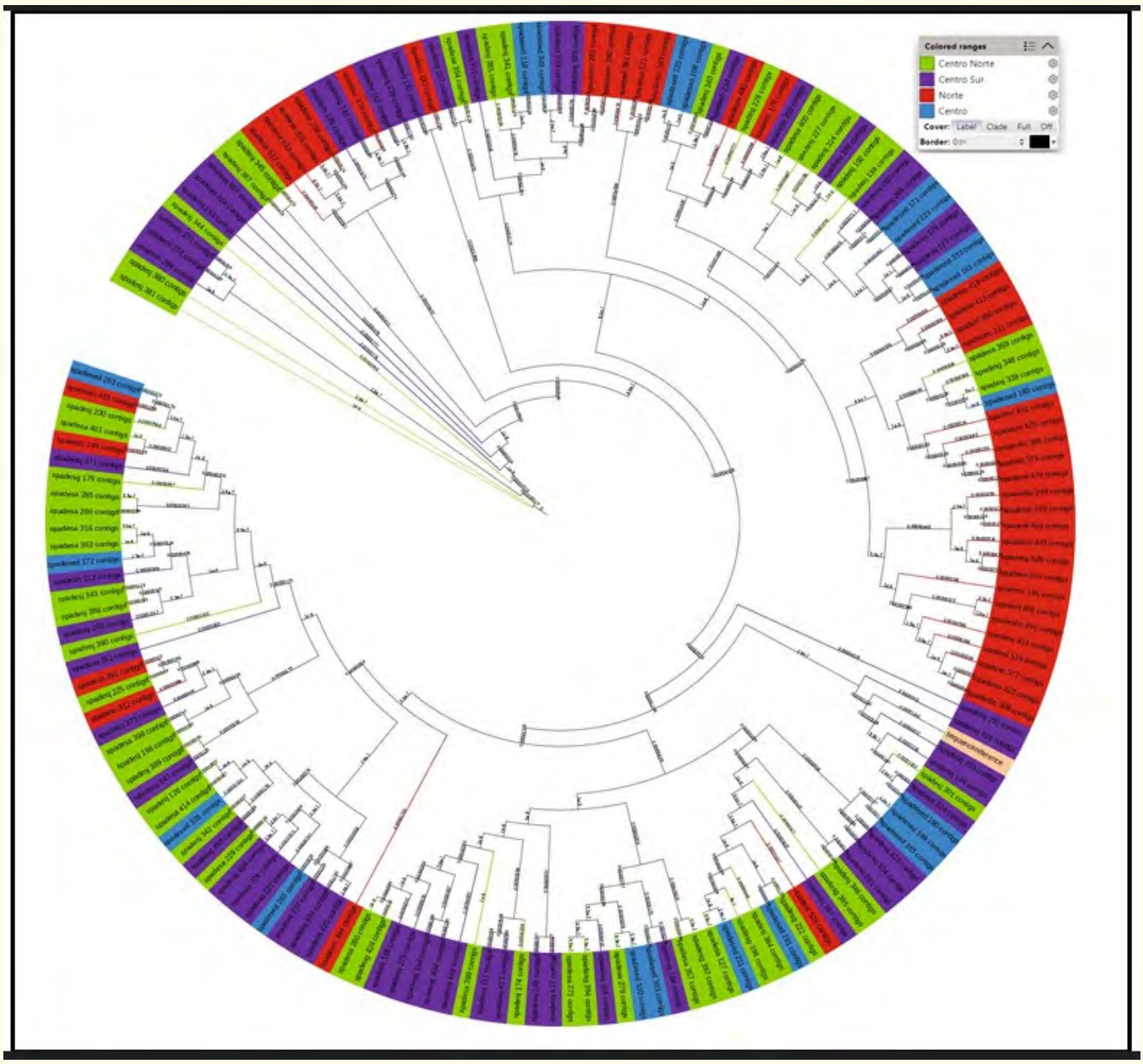


Figure 2B: Representative Phylogenomic tree of the Mexican strains of *Mycobacterium bovis* of the Center and South region. The tree was built using the REALPHY web server 1.1.3 of the Swiss Institute of Bioinformatics. Sequence Alignment was performed with Bowtie2, dividing the genomes in subsequences of 50pd and a value of *k*mer of 22; while the reconstruction of the phylogeny was generated through PhML inside the server with the reversible general model in the time (GTR) of evolution of nucleotides and the variation of the velocity of gamma distribution. Eight groups were obtained, generated based in the nodes collapse, denoted from A-T. The group A correspond to a 2 genomes (q407 y q149), group B represent 7 genomes (q328, q205, q133, q160, qh233, h168, h128), group C 12 genomes (q300, q156, q273, q137, q277, q406, q405, q256, q372, q297, q147, q116), group D 2 genomes (q117, q115), group E 2 (q289, q287), group F 3 (q324, q421, q106), group G 6 (q347, q248, q217, q290, q266, q162), group H 7 (q373, q369, q296, q130, h199, q338, q146), group I 16 (q371, q274, q117, q360, q412, q292, q291, q201, q420, q200, q423, q422, q270, q204, q202), group J 18 (q258, q361, q368, q366, q419, q329, q259, q219, q173, h122, q409, q163, q123, q185, q179, q187, q186, group K 2 (q425, q154), group L 6 (q365, q294, q288, q158, q250, q109), group M 2 (q327, q141), group N 8 (q408, q325, q278, q159, q120, q121, q157, q113), group Ñ 4 (q334, q364, q251, q107), group O 32 (q367, q110, q152, q426, q166, q132, q410, q157, q326, q118, q276, q114, q151, q254, q255, q411, q257, q323, q319, q210, q245, q125, q247, q207, q318, q243, q246, q134, q241, q237, q135, q244, q242, q208, q236, q214, q212, q213 y q321), group Q 2 (q417, q376), group R 5 (q155, q262, q140, q143, q150), group S 5 (q184, q183, q188, q182, q181), Group T 2 (h196 and h142). The letter following to the Word "spades" represent the initial of the name of the state to which the simple belong with a scale of 0.00001 for the tree.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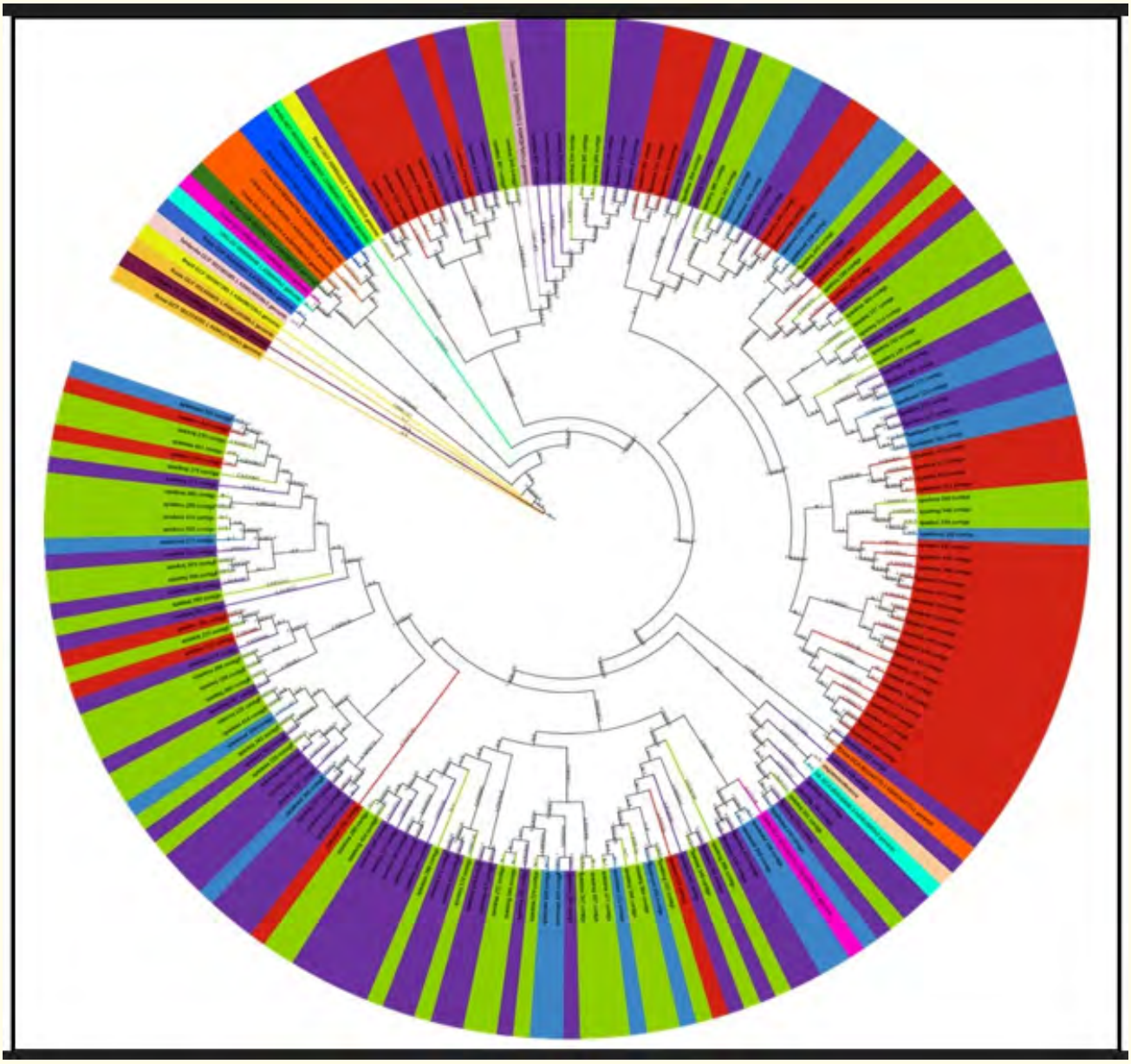

Figure 3: A. Comparative genomic tree of the *Mycobacterium bovis* grouping four genomes of the economical regions in livestock in Mexico. North region (red), Center-Norht región (green), Center región (blue) and Center-South (violet) (A). The label genome in beige represent the genome of reference of *Mycobacterium bovis* AF122/97. *B. M. bovis* genomes of representative regions of Mexico are also related to *M. bovis* genomes of other countries. Twenty genomes de *M. bovis* were integrated, taken from the NCBI base date from the countries Russia (2), Bulgary (1), Brasil (2), Thailandy (1), Tokyo (1), UK (2), Corea (2), Denmark (2), México (1), Canadá (1), France (1), and China (4)(B). The tree was built using the REALPHY web server 1.1.3 of the Swiss Institute of Bioinformatics. Sequence Alignment was performed with Bowtie2, dividing the genomes in subsequences of 50pd and a value of kmer of 22; while the reconstruction of the phylogeny was generated through PhML inside the server with the reversible general model in the time (GTR) of evolution of nucleotides and the variation of the velocity of gamma distribution. he letter following to the Word "spades" represent the initial of the name of the state to which the simple belong with a scale of 0.00001 for the tree.

however, forms subgroups with a combination of states. However, the formation of two groups is notorious when members are composed of the strains in the state towns of Baja California and Coahuila. Moreover, the genome of Korea GCF_001078615 was found to be related to the State of Mexico (spadesed_165 and spadesed_169) and Querétaro (spadesq_334 y spadesq_415). Furthermore, the genomes of *M. bovis* of Canada GCF, 002975475.1, were related to the Mexican strains of *M. bovis* of groups 2, 3, and 4, forming one same clade (Figure 3B).

Discussion

Herein, we are reporting the Phylogenomic analysis of the *M. bovis* strains isolated from cattle in Mexico. The data obtained provide an update of the *M. bovis* phylogeography in Mexico.

Although molecular markers (SNPs, VNTRs, RFLPS, Spoligoytping) [19,21,22,27] provide useful information related to the biogeography of these markers determined by each other of these genetic tools. These are based on the location of specific DNA target molecules that are not representative of the entire genome, so a higher resolving power is needed to allow finer identification. of the strains and contribute to the understanding of the propagation of the disease [28,29,33]. However, to give insight the evolutive history, global population structure, phylogeography, and the phylogenetic relationships among different *M. bovis.* It is the whole genome sequencing analysis combined with genotyping tools, that are being carried out [13,14,27,39-41]. It is true, several studies have shown about the molecular epidemiology but also of the geographical distribution of the ecotypes of the MTBC, mostly using the spoligotypes patterns, or a combination of whole sequencing (WGS) and spoligotypes [8,23,24,29,33,34]. Both conclude that there is for one side herds and dairy products, or even migration of individuals that disperse *M. bovis* and/or *M. tuberculosis* animal-adapted ecotypes [26]. By another hand, the combination of analysis between Genotyping and Phylogenomics [28,33], can provide deep insight and unveil aspects of the origin, diversity, behavior of mycobacteria [13,14,27,39-41].

The phylogenomic analysis of *M.* bovis strains from Mexico (Figure 1) was carried out using several bioinformatic tools. (Suppl mat). The phylogeographical distribution of the *M. bovis* strains were not at the level of single locus patterns such as spoligoytpes, VNTR'S, SNP's but throughout the whole genome sequences analysis. To perform this, genome assembly of *M. bovis* were carried out (Supl Mat). Thereafter, phylogenomic tree for the different region in livestock was built. Interestingly, we found that *M. bovis* genomes cluster in same clade independent of the region, and implying the phylogenetic relatedness among the (Figure 2A-B, 3A). Of relevance is that the analysis showed close relationships between strains from the same area, as well as between strains from different areas (Figure 2A-B; 3A).

However, despite the close degree of relationship between the analyzed strains, the National phylogenomic tree (Figures 2A-B; 3A) showed that there is no total grouping trend by zone, since groups are formed with two different states and/or municipalities or with more than two, with the exception of group 1 and 3 indicated in the tree that concentrate strains from the same state/ municipality: Jalisco and Ensenada respectively, while group 2 and 4 are made up of strains from two different states: Coahuila and Querétaro, spadesq_145, spadesq_112 and spadesc_378 for group 2; spadesc_193 and spadesq_131 for group 4, which come from relatively distant states (Figure 3A-B). The presence of strains from the states of Hidalgo (spadesh_269, spadesh_231 and spadesh_232), Querétaro (spadesq_404 and spadesq464) and Jalisco (spadesj_380 and spadesj_381) was also observed, with a greater difference in relation to the rest that are separated from the groups formed (Figure 2A-B), whose particular characteristics can be studied, as well as analyze what has led these strains to move away from the rest within the same geographical area (Figure 3A) [8,23,24,29,33].

Moreover, comparative genomics of *M. bovis* strains with isolates from different countries have provided insight the evolutive origin and phylogenetic relationships among the strains [14,39,40]. In the present study, we carried out this type of anaylisis between the representative *M. bovis* genomes of different regions of Mexico with those *M. bovis* strains from other parts of the world (Figure 3B). From the data, we found that France GCF_902459825.2, China_GCA_000194075.3 and Brazil GCF_000934325.9 *M. bovis* genomes were related to those genomes that in the national tree presented the largest group (group 5). In addition, the genomes from Brazil and France are even more related to each other, while the strain Canada GCF_002975475.1 was related with the strains that present a greater difference within the national tree, being closer to strains from the state of Querétaro. the Korea *M. bovis* genome

s GCF_001078615.1 was related to strains corresponding to the State of Mexico (spadesed_165 and spadesed_169), and Querétaro (spadesq_334; spadesq_415) (Figure 3B). Collectively these data strenghten the theory that after African Origin of *M. bovis*, the clonal complex of this pathogen disperse and distribuited worlwide due to the trade of cattle and therefore movement between countries. This economical activity dated since colonization times [14,41]. The question that arise from the phylogenomic analysis carried out in the present study, is about the implications in terms of the trade of dairy products or even of cattle in the phylogeography and the relationships between the *M. bovis* isolates?.

It is well known that livestock activity in Mexico has a great influence on the loss of biodiversity, both in plant areas and in fauna species, for the year 2014, the number of heads of cattle in 23 entities of the country exceeded the capacity of their livestock areas and their natural ecosystems to maintain them[3]. In particular, livestock overpopulation is greater in the center of the country (SENASICA 2021) [3] where we have the largest number of samples and given the proximity between ranches, dairy areas specialized in meat and slaughterhouses, there could be an exchange of strains. Indeed, cattle are sold between farms, transport of cattle from one farm to another without registering or without respective reports [24,28]. This is reflected in the Phylogenomic relationship between strains from different regions such as the State of Mexico, Jalisco, Querétaro, Guanajuato and Aguascalientes that are observed more related and whose distance between states is not short (Figure 3A). Another factor that influences the mobilization of cattle from small livestock which contributes as an infection factor. In addition the lack of availability of complete epidemiological information [23,36]. On the other hand, as mentioned before, there is a tendency for *M. bovis* strains from the towns of the northern state of Baja California to group together, the same data that is comparable with previous works based on typing, since in them there is also a single grouping trend of spoligotypes in that state which could indicate a low entry of livestock or external products to this place [23,33,36].

Furthermore, at the international level, it is known that the illegal transfer of cattle occurs. In November 2019, Mexico and Guatemala signed a memorandum of legal understanding to regulate the introduction into our country of cattle, for immediate slaughter and terminal fattening, originating and coming from Central America (SADER, 2019). Besides, there is legal importation through other countries such as Nicaragua, Belize, Canada, the United States, and a market was recently opened for the purchase of meat from Argentina (CEDRSSA, 2018; Morales, 2021, ergonomista; Opportimes, 2021, Consejo Mexicano de la Carne, 2021), it is also worth mentioning that Mexico purchases various products from the United States, mainly meat, and purchases cattle and meat from countries such as Nicaragua, Canada, Brazil, Mexico and New Zealand (CEDRSSA, 2018), so this exchange of herds and dairy products can also be a factor of spread despite the good surveillance practices that the different countries carry out.

Conclusion

Phylogeography is based on the whole genomes assembly of the *M. bovis* isolates. Indeed, a combination of these genomic tool and genotyping (SNPs, Spoligotyping) allowed to infer the African origin of *M. bovis* [41]. As Millan., *et al*. 2016 [26] reported on the molecular distribution of *M. bovis* in Mexico [23,33]. In this report, the Phylogenomic analysis of the *M. bovis* isolates from cattle of Mexico show a close relationships among the strains independent of the important economical region in livestock. It strengthen previous data on genotyping showing the molecular distribution and epidemiology of the isolates from cattle, and even from human [31]. Moreover, the Phylogenomic analysis of *M. bovis* strains from Mexico strengthen the fact that transportation and cattle movement play a role in the phylogeography of *M. bovis.*

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Conflict of Interest Statement

The authors declare “No conflict of interest”.

Author Contribution Statement

G.G.G.M. conceptualization, data analysis, writing of the manuscript. R.B.L. Bioinformatic work, Data analysis. C.J.S.V. and G.E.O.R. Bioinformatic work. All authors have read and approved the manuscript.

Data Availability Statement

Gabriela Edith Olguin-Ruiz and Carlos Javier Sanchez-Vallejo are employed by the E.N.C.B. IPN, CD MEX. Rebeca Blancas-Landeros is a bachelor degree in Biology by ENCB.IPN. She is now graduate student in Mexico. There are no patents, products in development, or marketed products to declare. This does not alter the author’s adherence to all the policies on sharing data (Metadata Tables 1and 2) and materials with whom correspond.

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