Received: 28 April 2020 | Revised: 3 August 2020 | Accepted: 4 August 2020

DOI: 10.1111/tbed.13780

ORIGINAL ARTICLE

Transboundary and Emerging Diseases WILEY

Genetic diversity of *Borrelia burgdorferi* sensu stricto: Novel strains from Mexican wild rodents

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Abstract

Borrelia burgdorferi s.s. is a Gram-negative spirochaete, the aetiological agent of Lyme disease, the most common vector-borne disease in the Northern hemisphere. Reports on the presence of *B. burgdorferi* in central Mexico have been strongly criticized, since these were based only on unspecific serological methods. Furthermore, the worldwide genetic diversity of *B. burgdorferi* s.s. has not been evaluated. For this reason, the aim of the present study was to confirm the presence of *B. burgdorferi* in the central area of Mexico and to evaluate its relationship with regard to the global genetic diversity of *B. burgdorferi* s.s. To achieve this, fragments of the *flagellin* and the *outer surface protein* A genes were amplified from ear biopsies of the arboreal wild endemic mice *Habromys schmidlyi*. With these sequences, a concatenated Bayesian analysis was performed to confirm the identity of *B. burgdorferi* s.s. Afterwards, the global genetic diversity of this bacterial species was evaluated using our sequences and those available in GenBank. A prevalence of 10.4% (5/48) of *H. schmidlyi* infected with *Borrelia* sp. was detected, and the phylogenetic analyses confirmed the identity of *B. burgdorferi* s.s. Using both genes, the genetic diversity was low. However, genetic structuring analyses revealed that populations of western United States and those from Mexico formed slightly different genetic groups, separated from the populations of the rest of the world. Our study not only confirms the presence of this bacterium in central Mexico, but also shows the most southern record of this bacterium so far. It also highlights the importance of *H. schmidlyi* as a new potential host of this bacterial species. Our study also provides first genetic data on an incipient process of divergence in *B. burgdorferi* s.s. populations of eastern United States and central Mexico.

KEYWORDS

Borreliella, flagellin, *Habromys schmidlyi*, Lyme disease, ospA

1 | INTRODUCTION

Borrelia burgdorferi s.l. is a species complex group of spirochaetes which encompass the aetiological agents of Lyme borreliosis, the most common vector-borne disease in the Northern hemisphere (Clark, Leydet, & Hartman, 2013; Kurtenbach et al., 2006; Saito et al., 2007). This species complex includes 22 validated genospecies, from which *Borrelia burgdorferi* sensu stricto (from now only *B. burgdorferi*) is the main causative agent of the Lyme Disease in North America, Europe and Asia (Margos et al., 2017, 2019; Steere et al., 2016).

The enzootic life cycle of this bacterial species is a complex network that encompass several tick species of the genus *Ixodes*, specifically, from the *Ixodes ricinus* complex (Güner et al., 2004; Kurtenbach et al., 2006). Ixodid ticks from this species complex feed on a broad spectrum of wild vertebrate hosts. Wild rodents of the genus *Peromyscus* have been reported as the main reservoirs of *B. burgdorferi* in North America (Kurtenbach et al., 2006; Schotthoefer & Frost, 2015; Steere et al., 2016).

The distribution and prevalence of *B. burgdorferi* have been widely studied in the Nearctic biogeographical region, especially in wild mammals (Coipan, Van Duijvendijk, Hofmeester, Takumi, & Sprong, 2018; Hovius, van Dam, & Fikrig, 2007; Huegli, Hu, Humair, Wilske, & Gern, 2002; Roome et al., 2017; Schotthoefer & Frost, 2015), including arboreal rodents such as the western gray squirrel *Sciurus griseus* (Lane, Mun, Eisen, & Eisen, 2005) and the eastern chipmunk *Tamias striatus* (Slajchert, Kitron, Jones, & Mannelli, 1997). In the tropical region, there are few records of *Borrelia*-like spirochaetes from arboreal marsupials (*Didelphis albiventris*, *Didelphis marsupialis* and *Phylander opossum*) and arboreal rodents (*Akodon montensis*) (Abel, Marzagão, Yoshinari, & Schumaker, 2000; da Costa, Bonoldi, & Yoshinari, 2002). This opens the possibility of considering arboreal mammals as potential reservoirs of other members of the *B. burgdorferi* s.l. complex in the Nearctic.

In Mexico, the *Borrelia burgdorferi* s.l. complex has been widely studied throughout the country. Yet, most of the studies have been widely criticized since they were carried out using only serological methods with unconfirmed results (Faccini-Martínez, 2019; Feria-Arroyo et al., 2014; Gordillo-Pérez et al., 2018; Norris, Barbour, Fish, & Diuk-Wasser, 2015; Norris, Barbour, Fish, & M Diuk-Wasser, 2014). Based on these serological studies, *Borrelia burgdorferi* has been suspected only in the northern part of the country (Gordillo-Pérez et al., 2009; Vargas et al., 2007). There is only one study made by molecular methods and with available sequences that has demonstrated this complex in *Ixodes kingi* recovered from *Vulpes macrotis* near the Mexican-US border (López-Pérez et al., 2019), but this has not been confirmed in wild mammals.

The central region of Mexico is characterized by a large and intricated mountainous system, the Transmexican Volcanic Belt, whose mountains have an average elevation of 2000 m, where *Quercus* oak forests and patches of primary cloud forest occur alternately in the higher parts throughout the entire region (Marines-Macías, Colunga-Salas, Verde-Arregoitia, Naranjo, & León-Paniagua, 2018). In this mountainous system, various species of ticks of the genus *Ixodes* are distributed (Guzmán-Cornejo, Robbins, & Pérez, 2007). Moreover, in one of the highest areas of this mountainous system, *Habromys schmidlyi*, an arboreal rodent, endemic and phylogenetically closely related to the genus *Peromyscus*, is the most abundant rodent species (León-Paniagua, Navarro-Sigüenza, Hernández-Baños, & Morales, 2007; Marines-Macías et al., 2018). Since the presence of *B. burgdorferi* in this mountainous area of Mexico was doubtful, one of the aims of this study was to analyse by molecular techniques whether this bacterium was present in *Habromys schmidlyi*.

Additionally, since previous attempts to evaluate the genetic diversity of *B. burgdorferi* have focused on several genes including *outer surface protein* and *flagellin* genes, but only at regional levels (Baranton, Seinost, Theodore, Postic, & Dykhuizen, 2001; Brisson, Vandermause, Meece, Reed, & Dykhuizen, 2010; Foretz, Postic, & Baranton, 1997; Mechai, Margos, Feil, Lindsay, & Ogden, 2015; Travinsky, Bunikis, & Barbour, 2010; Wallich et al., 1992), we now calculated the genetic diversity of *B. burgdorferi* at global scale to understand how the genetic diversity is distributed within this species.

2 | MATERIAL AND METHODS

2.1 | Animal sampling

During 2012–2013, an extensive fieldwork was done in the only two known regions harbouring populations of *H. schmidlyi*, in the State Park 'Cerro del Huixteco', within the Sierra de Taxco, Guerrero, Mexico, (18°36′N, 99°36′W), and the State Park 'Picacho de Oro y Plata', Zacualpan, Estado de México, Mexico, and surrounding areas (18°43′N, 99°46′W) (Figure 1). A total of 120 Sherman live-traps were used for rodent trapping in each locality: 60 traps were placed at the ground level, and the remaining 60 traps were placed on the tree trunks and branches, following the transect set on the ground. The arboreal trapping was done using climbing equipment to reach the highest possible point (20 m) [for further sampling information, see Marines-Macías et al., (2018)]. All captured individuals were identified in situ by their external morphology following Romo-Vázquez, León-Paniagua, and Sánchez (2005), and then tagged with an ear notch using a medical punch. Tissues were fixed individually in 96% ethanol. In order to avoid cross-contamination between tissues, the punch was disinfected with 10% chlorine and 96% ethanol between each use. All individuals were screened for the presence of ticks, yet none of the rodents had ticks. After these, all individuals were released in the same place where they had been collected (special permit SGPA/DGVS/12142/12 from the Secretaría del Medio Ambiente y Recursos Naturales).

2.2 | Borrelia molecular detection

For DNA extractions, the entire tissue (~2 cm of diameter) was manually cut in small pieces and placed in 1.5-µl tubes; in this step, also to avoid cross-contamination, the surgical equipment was disinfected (see above). We then used the QIAamp® DNA Mini Kit (QIAGEN, Hilden, Germany) following manufacturer's instructions with a single modification: we added 30 µl of proteinase K in each tube and incubated them at 56°C until the tissues were completely lysed.

For the molecular identification of *Borrelia*, a fragment (~300 bp) of the 16S rDNA region was amplified using the primers and conditions previously reported by Skotarczak et al., (2005). From all positive samples, two additional fragments of ~280 bp of the *flagellin* gene and ~794 bp of the *osp*A gene were amplified, using the primers

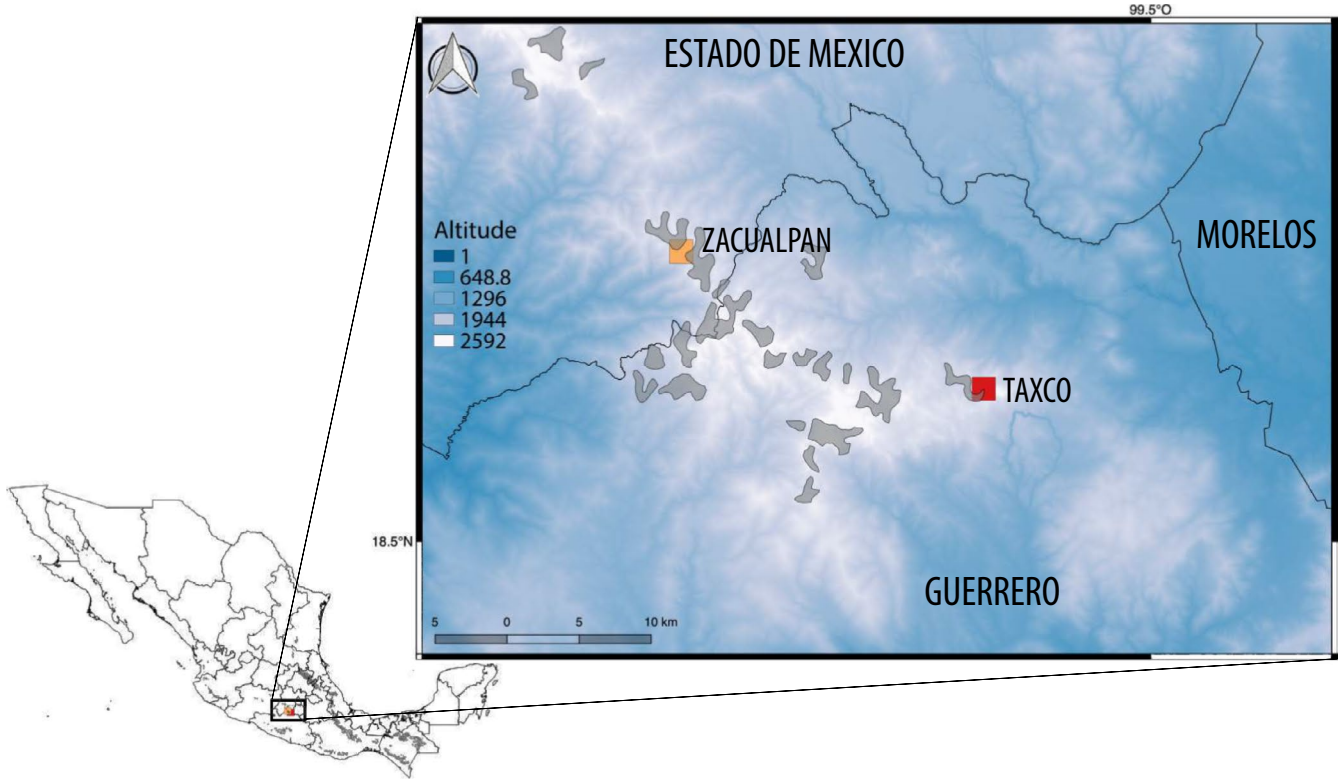

FIGURE 1 Sampling sites for *Habromys schmidlyi*. The gradient of altitude in Mexican mountains is shown in blue scales and distribution of the cloud forest in Mexico according to CONABIO (http://www.conabio.gob.mx/informacion/gis/) in grey shading

and conditions of Zore et al., (1999), and Bunikis et al., (2004), respectively. The reaction mixtures consisted of 12.5 μl of GoTaq® Green Master Mix, 2 × of Promega Corporation (Madison, WI, USA), the pair of primers (100 ng each), 6.5 μl nuclease-free water and 150–250 ng DNA (~2 μl from the total elution of 200 μl) in a final volume of 25 μl. We followed the PCR conditions specified previously for the *flagellin* (Zore et al., 1999) and *osp*A genes (Bunikis et al., 2004). In all reactions, we included a negative (the same reaction mixture with water) and a positive control (DNA from a *Borrelia* sp. previously isolated from *Amblyomma dissimile* ticks attached to *Rhinella horribilis* in Veracruz, Mexico, accession number: KY389373 (as previously reported by Morales-Diaz et al. (2020)).

PCR products were visualized in 2% agarose gels on the ODYSSEY CLx Imaging System (LI-COR Biosciences). All positive PCR products were prepared and sequenced at Laboratorio de Secuenciación Genómica de la Biodiversidad y de la Salud, Instituto de Biología, Universidad Nacional Autónoma de México (UNAM). The DNA sequences were edited and aligned using 4 Peaks V1.8 (Nucleobytes B.V.) and MEGA X (Kumar, Stecher, Li, Knyaz, & Tamura, 2018), conducting visual inspections of all sequences.

2.3 | Molecular characterization of Borrelia

To identify the species of *Borrelia* recovered from *H. schmidlyi*, sequences of the 16S rDNA region, the *flagellin* and *osp*A genes from all species of the *B. burgdorferi* s.l. complex available in GenBank were included into a concatenated alignment in MEGA X (Kumar et al., 2018) and aligned with the Muscle algorithm (Edgar, 2004). The best scheme of partition and substitution model of the concatenated data was calculated in Partition Finder 2 (Guindon et al., 2010; Lanfear, Calcott, Ho, & Guindon, 2012; Lanfear, Frandsen, Wright, Senfeld, & Calcott, 2017).

A phylogenetic analysis was performed by the Bayesian inference approach in MrBayes 3.2.7 (Ronquist et al., 2012), using the MCMC algorithm and the substitution model calculated previously. Three hot and one cold chains in two independent runs of 10 million generations, sampling every 1,000 generations, were used. The final topology was obtained using a majority consensus, considering a burn-in of 20%. The convergence of results and good sampling (ESS> 200) was checked in Tracer 1.7.1 (Rambaut, Drummond, Xie, Baele, & Suchard, 2018).

Additionally, all available sequences of *Borrelia burgdorferi* were obtained from GenBank, by manual searching and by a BLAST tool searching only for *flagellin* and *osp*A genes. In order to corroborate the correct identification, a global alignment and a phylogenetic analysis were performed, as previous described, for each gene. Only those sequences clustered in a monophyletic clade were included for further genetic analyses (Figures S1, S2). The 16S rDNA region was not included in the genetic analyses, since this region did not vary within the *B. burgdorferi* s.s. phylogenetic analysis (data not shown).

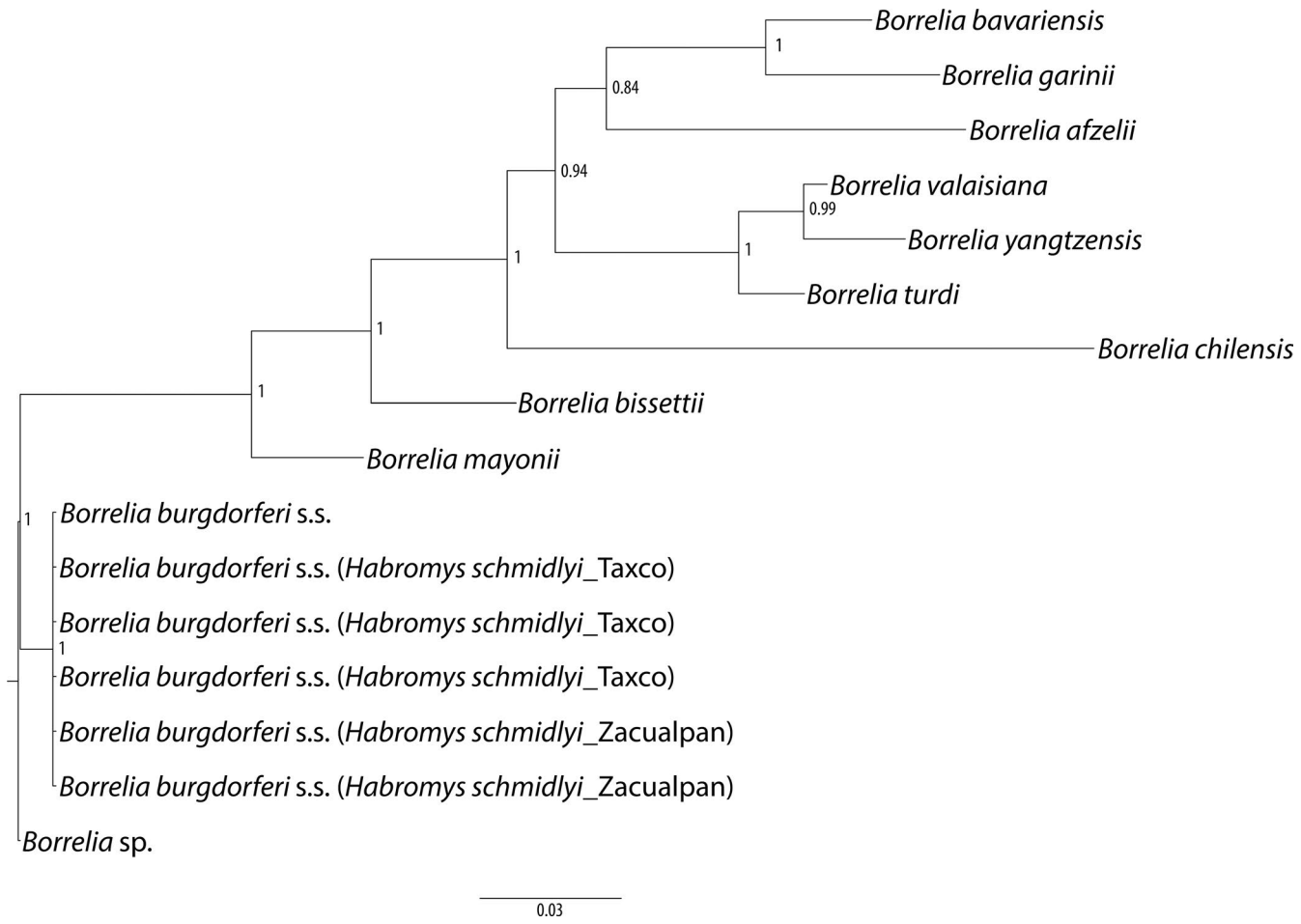

FIGURE 2 Phylogenetic tree inferred from the concatenated alignment (16S rDNA + *flagellin* + *osp*A genes) for the genus *Borrelia*. The phylogenetic relationships were inferred based on the HKY + I substitution model (Hasegawa, Kishino, & Yano, 1985) for a total of 1,309 bp. Posterior probabilities are indicated at each node. Scale bar indicates number of nucleotide substitutions. Sequences generated in this study were submitted to GenBank under accession numbers: MT378393-MT378397 for 16S rDNA region, MG934557-MG934561 for *flagellin* and MN461274-MN461278 for *osp*A

2.4 | Genetic analyses

In order to provide a more detailed account of the genetic diversity and to be able to detect possible populations and subpopulations, a principal coordinates analysis (PCoA) in PAST3 (Hammer, David, & Paul, 2001) was performed for each gene, based on Euclidean distances.

Hierarchical genetic differentiation analysis was tested for both genes by the molecular variance (AMOVA) method in Arlequin 3.5.2.2 (Excoffier & Lischer, 2010). The hierarchical analyses were conducted at three levels: (a) among isolates, equivalent to each sequence; (b) among populations, which were defined by their geographic distribution; and (c) among groups, which were defined by previous analyses. For this, a matrix of pairwise differences, using 10,000 permutations to test the significance of our results, was performed in Arlequin.

Haplotype networks with mutational step estimations were constructed for each gene using the median-joining network in POPART 1.7 (Leigh & Bryant, 2015).

Finally, to detect the geographic and genetic structures for each gene, Geneland analyses were done (Guillot, Renaud, Ledevin, Michaux, & Claude, 2012). From one to six populations were tested, following the *prior* distribution of samples, according to the sampling site referred by each author. In order to do this, one million generations with a thinning of 100, with no coordinate uncertainty, were used.

3 | RESULTS

A total of 48 *H. schmidlyi* individuals were sampled, 29 from Taxco and 19 from Zacualpan. The presence of *Borrelia* DNA was found in biopsies of five individuals (5/48 = 10.4%), three individuals from Taxco and two from Zacualpan. From the five positive individuals, a total of 292 bp were obtained for the 16S rDNA region, 225 bp for the *flagellin* gene, and 792 bp for the *osp*A. According to the phylogenetic analyses, sequences obtained from *H. schmidlyi* grouped together in a monophyletic clade with other sequences of *B. burgdorferi* s.s. from North America and Europe (Figure 2, Figures S1, S2).

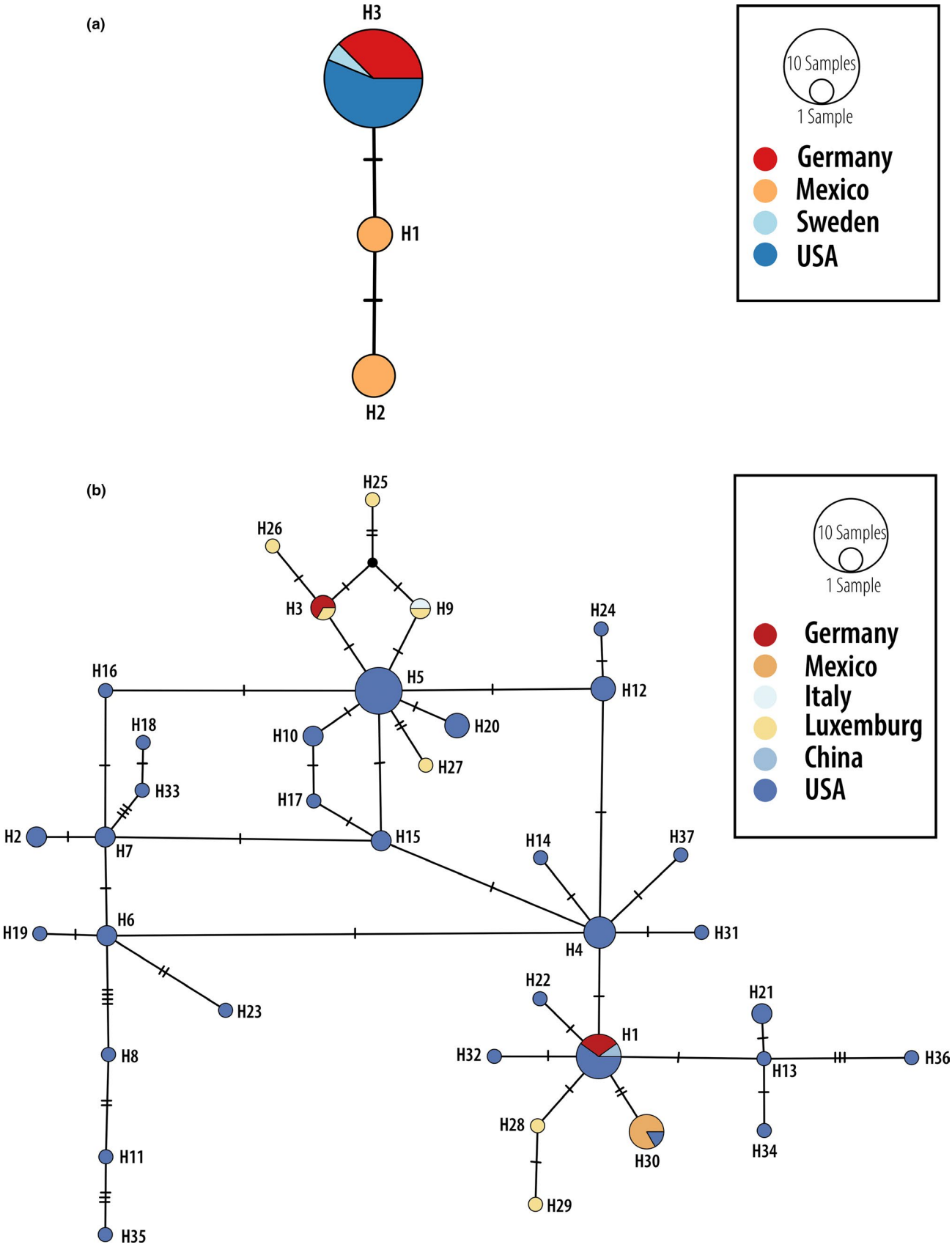

FIGURE 3 Haplotype network analyses for *Borrelia burgdorferi* s.s. (a) Haplotype network inferred with the *flagellin* gene including all available and validated sequences (see Figure S1). (b) Haplotype network inferred with the *ospA* gene including all available and validated sequences (see Figure S2). The colours correspond to the country of origin of each haplotype. Black lines represent the mutational steps between each haplotype

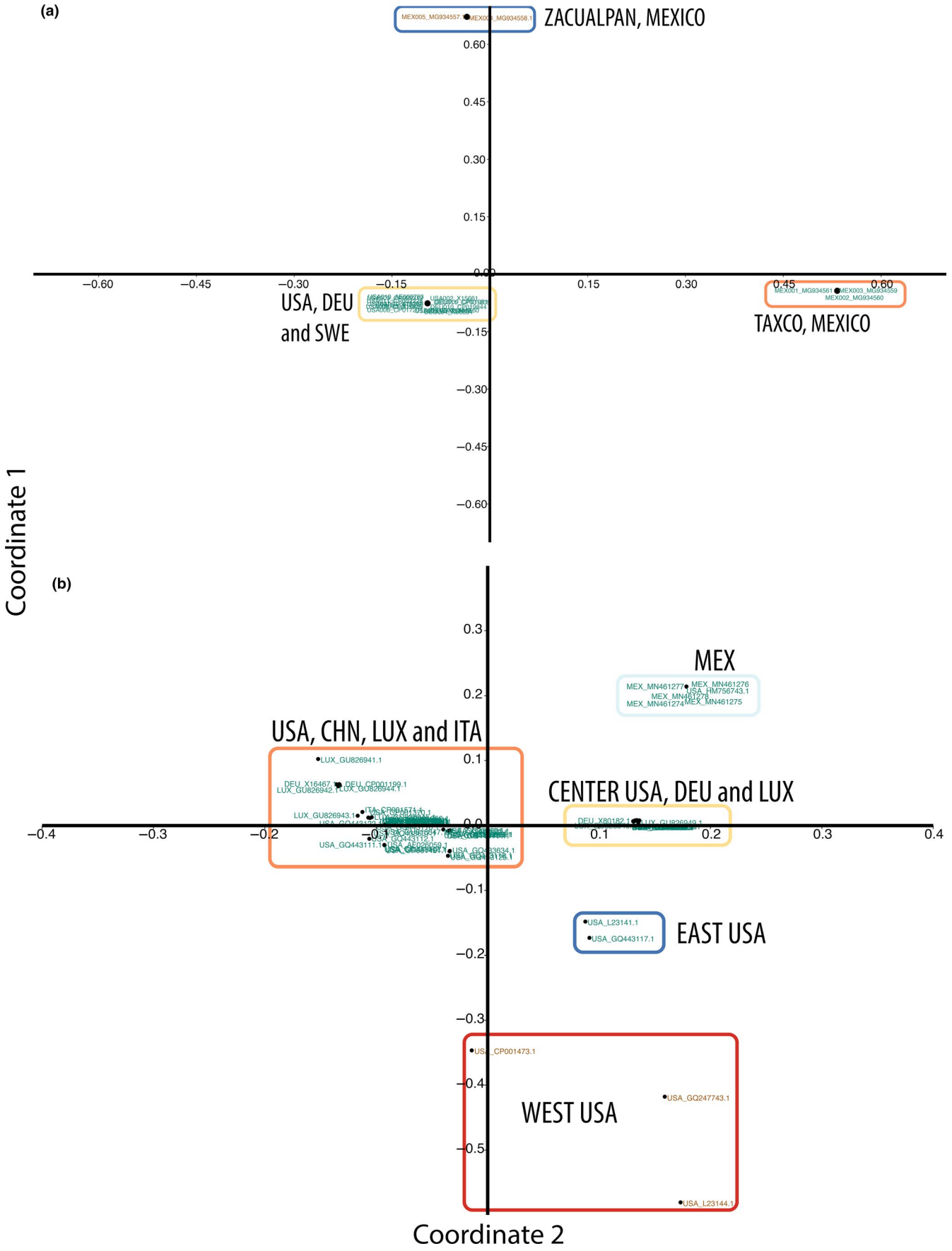
(a)
ZACUALPAN, MEXICO
USA, DEU and SWE
TAXCO, MEXICO
Coordinate 1
(b)
MEX
USA, CHN, LUX and ITA
CENTER USA, DEU and LUX
EAST USA
WEST USA
Coordinate 2

FIGURE 4 PCoA plot of the first and second axes based on Euclidean distances for all available sequences of *B. burgdorferi* s.s. (a) PCoA plot for the partial nucleotide sequence of the *flagellin* gene. The proportion of total variance along the two axes was 93.97% and 6.02%, respectively. (b) PCoA plot for the complete *osp*A gen. The proportion of total variance along the two axes was 37.96 and 10.48%, respectively. CHN, China; DEU, Germany; ITA, Italy; LUX, Luxembourg; Mex, Mexico. Colour squares show the genetic groups obtained from each analysis

TABLE 1 Analysis of molecular variance results for the *flagellin* partial gene

Source of variation	Percentage of variation	Fixation index	*p*-value
Among groups (Mexican populations *vs* the rest of the world)	92.64	$\phi_{CT} = 0.92$	>.001
Among populations within groups	−2.22	$\phi_{SC} = -0.3$	>.001
Within populations	9.58	$\phi_{ST} = 0.9$	**<.001**

Values in bold were statistically significant.

3.1 | Genetic analyses

From the *flagellin* gene, three haplotypes were obtained, with a haplotype diversity of 0.4095 and nucleotide diversity of 0.00224. The haplotype network shows the 'H3' haplotype, both as the most frequent haplotype and the differentiation centre, which includes all samples with the exception of isolates from Mexico, H1 from Taxco and H2 from Zacualpan, both populations were the sites where *H. schmidlyi* was sampled (Figure 3a).

On the other hand, from the haplotype network recovered from the *osp*A gene, 37 haplotypes were recovered, with a haplotype diversity of 0.9530 and nucleotide diversity of 0.00522. This network

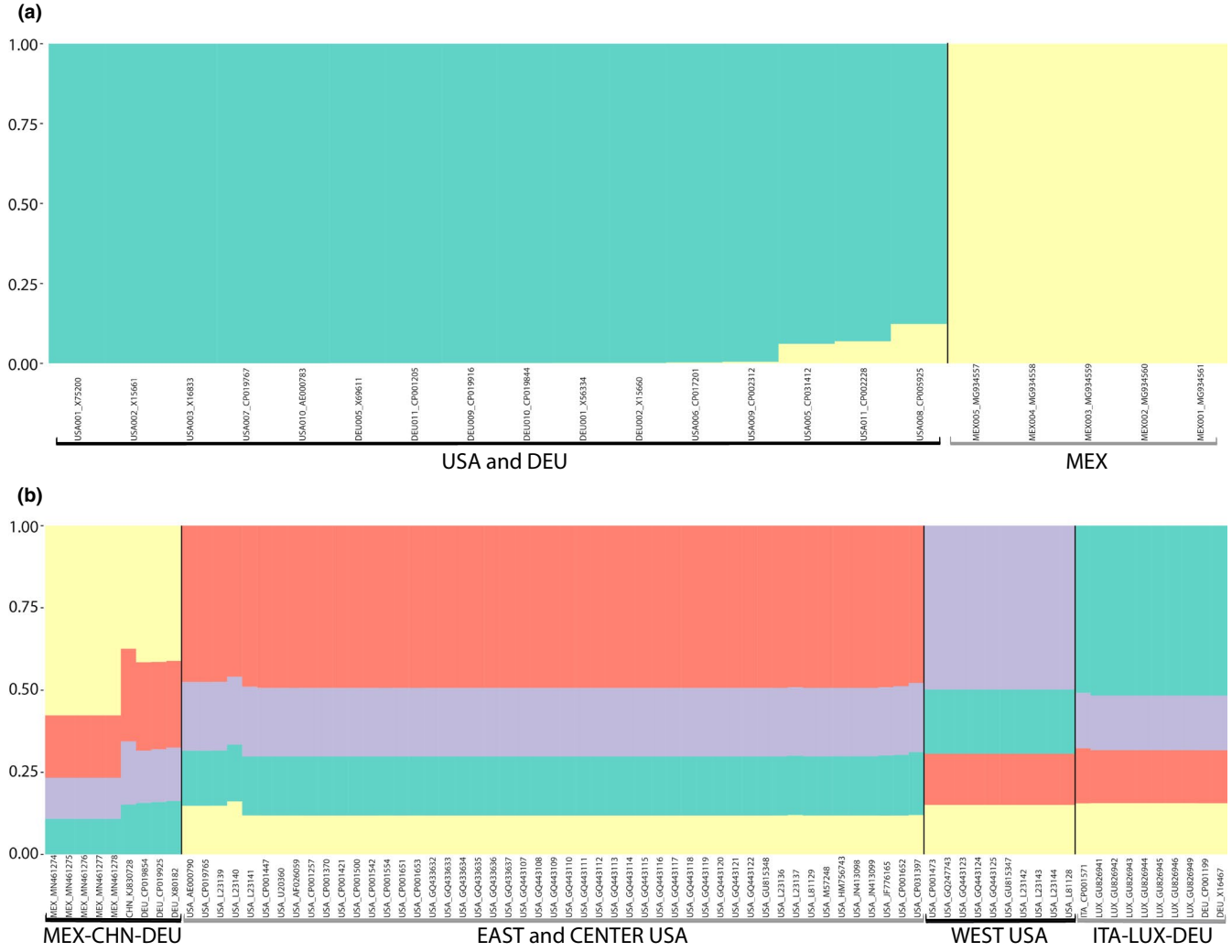

FIGURE 5 Genetic structure of *Borrelia burgdorferi* s.s. samples representing the maximum number of clusters (K). The y-axis represents the identity percentage of each sequence, and black lines separate each population. (a) Geneland analysis results for the partial *flagellin* gen with two populations (K = 2). (b) Geneland analyses for the complete *osp*A gen showing four genetic populations (K = 4). CHN, China; DEU, Germany; ITA, Italy; LUX, Luxemburg; Mex, Mexico

shows intricate relationships among all isolates and two main centres of diversification, H1 and H5, which include isolates from America, Europe and Asia. Meanwhile, haplotypes from North American and Luxemburg isolates are widely distributed along the network and most of them as tip haplotypes (Figure 3b).

The ordination analysis showed three genetic groups for the *flagellin* gene, two of them represent each Mexican population and the last one conformed by the North American-European samples (Figure 4a). The *osp*A sequences formed at least five main genetic groups, where it seems that western United States is the most different group (Figure 4a).

The analysis of molecular variance with the partial *flagellin* gene revealed that 92.64% of variation was among groups (*i.e.* among the Mexican populations and North American-European population), 9.58% was within populations, and − 2.22% was among populations but within groups (Table 1). However, ϕ-statistics obtained with AMOVA, revealed significant variation only within populations [$\phi_{ST} = 0.9$; $p < .0001$], but variation among groups was not statistically significant ($\phi_{CT} = 0.92$; $p > .001$; Table 1). Additionally, the Geneland analysis confirms the presence of two genetic populations with high probability values: Mexican populations in one group, and the rest of the sequences were included in the second group (Figure 5a).

On the other hand, the AMOVA analysis, using the complete *osp*A gene, revealed that 54.31% of variation among groups (*i.e.* western United States and the rest of the world [samples from eastern United States, as well as Mexican, European and Asian samples]), 34.19% was within populations, and 11.44% was among populations but within groups (Table 2). ϕ-statistics revealed a subtle genetic structure among groups, which was not significant ($\phi_{CT} = 0.54$; $p > .0001$; Table 2). With Geneland analysis, four genetic populations were obtained: (a) Mexican-Chinese-eastern German populations, (b) eastern and central USA, (c) western USA, and (d) Italian-Luxemburg-western German populations; however, the individual probability of identity to each group was low (Figure 5b).

4 | DISCUSSION

This is the first confirmatory record of *B. burgdorferi* s.s. in the central region of Mexico and, also, the first record of this bacterium in wild Mexican mammals. Furthermore, this is the most southern record of *B. burgdorferi* s.s. so far, in the transition zone of the Nearctic and Neotropical biogeographical regions. Our report now represents the first association of *B. burgdorferi* with a species of arboreal mouse in Mexico and highlights the importance of arboreal mammals as potential hosts of this bacterium in the wild.

TABLE 2 Analysis of molecular variance results for the *osp*A gene

Source of variation	Percentage of variation	Fixation index	*p*-value
Among groups (Western United States *vs* the rest of the world)	54.37	$\phi_{CT} = 0.54$	>.001
Among populations within groups	11.44	$\phi_{SC} = 0.25$	**<.001**
Within populations	34.19	$\phi_{ST} = 0.66$	**<.001**

Values in bold were statistically significant.

Some studies have tried to relate several species of mammals and various genera of hard ticks from Mexico (*e.g. Amblyomma*, *Dermacentor* and *Ixodes*) as hosts and potential reservoirs of this bacterium (Gordillo-Pérez et al., 2009; López-Pérez et al., 2019; Vargas et al., 2007). However, studies done by Gordillo-Pérez et al. (2009) and Vargas et al. (2007) were based on serological methods to test the infection and, although these were subsequently confirmed by identification of the bacterium by PCR, doubts have arisen. The published sequences have been questioned and presumed to be a probable contamination since they are identical to the sequence of isolate B31, which was used as a positive control (Norris et al., 2015).

Is important to note that transmission of *B. burgdorferi* has been associated with hard ticks of the genus *Ixodes*, specifically of the species from the *I. ricinus* complex (Güner et al., 2004; Kurtenbach et al., 2006). In North America, the main vectors for *B. burgdorferi* are *Ixodes scapularis*, *Ixodes pacificus* and *Ixodes affinis* (Kurtenbach et al., 2006) are generalist ectoparasites feeding from many different vertebrates species (Kurtenbach et al., 2006). Although those hard ticks have not been previously associated with *H. schmidlyi*, it is possible to think that there might be a member of the genus *Ixodes*, not yet recovered, that parasitized this rodent species, thus maintaining the enzootic transmission in the wild, since this tick genus is found mainly inside burrows and nests (Durden, 2006).

The study of the Mexican *Ixodes* is still in its initial period (Guzmán-Cornejo et al., 2007), and many taxonomic and ecological studies are still needed to allow us to understand, which species are involved in the maintenance of enzootic cycles of pathogens, as is the case of the cycle involving *B. burgdorferi* and *H. schmidlyi*. This rodent species has been described as arboreal, living mainly between 1 and 10 m above ground level, and probably makes nests in the understory stratum (Marines-Macías et al., 2018). Despite its arboreal habits, it was shown that this rodent uses holes inside tree trunks to reach the floor and feed; thus, tick search should be focused on these areas.

In the last stage of the life cycle of species from the *Ixodes ricinus* complex, the adults feed mainly on large mammals, which serve as maintenance hosts (Hayes & Piesman, 2003; Kurtenbach et al., 2006). The most important maintenance host in North America for *Borrelia* vectors is *O. virginianus*, since one individual can support large numbers of adult *I. scapularis* (Piesman & Gern, 2004; Spielman, Wilson, Levine, & Piesman, 1985). However, other records show that *I. pacificus* adults also feed on medium-sized mammals, such as foxes of genus *Urocyon*, which can serve as maintenance hosts (Castro & Wright, 2007; Crooks et al., 2001). These two mammals (*O. virginianus* and the grey fox, *Urocyon cinereoargenteus*) are commonly found in cloud

forest of central Mexico and in the Sierra de Taxco and Zacualpan (Ceballos, 2013; León-Paniagua & Romo-Vázquez, 1993), which could be infested by some species of the *I. ricinus* complex, that in turn, keep the populations of *B. burgdorferi* s.s. in circulation.

4.1 | Genetic diversity

To our knowledge, this is the first study using all the available online sequences of the genes *osp*A and *flagellin* to evaluate the worldwide genetic diversity of *B. burgdorferi*. When comparing both genes, the *flagellin* gene seems to be less diverse, with only three haplotypes, compared with the 37 haplotypes of the *osp*A gene. This finding is supported by previous studies where several authors have reported that the outer surface proteins, such as *osp*A, *osp*B and *osp*C, exhibit a higher degree of heterogeneity compared to other genes such as the *flagellin* gene (Baranton et al., 2001; Brisson et al., 2010; Foretz et al., 1997; Travinsky et al., 2010; Wallich et al., 1992).

The genetic diversity analyses of the *flagellin* gene revealed a subtle genetic differentiation between Mexican populations and the rest of the world. This finding is consistent with previous studies obtained from other conserved genes, such as that of the 16S rDNA fragment, from which a restricted gene flow and no evidence of genetic structure were observed among the USA populations of *B. burgdorferi* (Humphrey, Caporale, & Brisson, 2010), as well as with RFLPs analyses from worldwide isolates (Wallich et al., 1992). The Mexican sequences obtained in this study correspond to the first record of *B. burgdorferi* in a cloud forest environment worldwide. These sequences could be associated with unique haplotypes, and additionally, they could also reflect adaptations to the potential vectors and hosts present in the cloud forest ecosystem. The latter makes the search for ticks essential for linking this genetic structuration with ecological and evolutionary drivers.

In contrast, the genetic diversity analysis of the *osp*A gene shows subtle genetic structuration around the world, especially in western USA. An explanation for this subtle genetic structure could be that this protein is necessary for the primary establishment and persistence in the vector, avoiding the immunological system of the ticks (Hovius et al., 2007; Yang, Pal, Alani, Fikrig, & Norgard, 2004). Since *B. burgdorferi* is transmitted by several *Ixodes* species throughout its distribution range, it is likely that this promotes a high variability in the *osp*A gene throughout the different vector species. Additionally, local polymorphism could be maintained by ecological barriers limiting gene flow within unsuitable host species, as has been demonstrated after comparing the genotypes of *B. burgdorferi* in ticks and vertebrates from eastern versus western United States, which showed that these two populations appear to be in a genetic differentiation process (Humphrey et al., 2010).

So far, there are two hypotheses on the origin of *B. burgdorferi* s.s.: some authors have proposed that the centre of origin could be North America (Foretz et al., 1997; Ras, Postic, Foretz, & Baranton, 1997), although the most recent hypothesis proposes that its origin is European (Margos et al., 2008). Our study, using the *flagellin* gene, now supports that the origin of *B. burgdorferi* is possibly in the Holarctic, since Mexican populations represent the youngest populations in more recently colonized regions, based on Bayesian inference and haplotype network. However, with *osp*A gene, the haplotype and phylogenetic relationships among individuals are more complex, likely due to its high variability that could erase historical information. It is important to notice that considering the *osp*A data, the USA and Luxemburg concentrate the largest number of unique haplotypes, with 18 and 5 haplotypes, respectively. The differences in the number of unique haplotypes at each sampling region can be explained by a negative frequency-dependent mechanism, where no area has a single or few unique haplotypes, as has been shown when comparing genetic genotypes of the *osp*C, IGS and *dbp*A genes of *B. afzelii* with the initial infection in its hosts (Barthold, 1999; Coipan et al., 2018). Additional sampling mainly in non-studied areas, genomic data and ancestral reconstruction area analyses could address the interesting question related to the origin of these bacteria.

The *flagellin* gene has been demonstrated to be useful for species delimitation and molecular identification (López-Pérez et al., 2019; Potkonjak et al., 2016), whereas the *osp*A gene has proven to be useful for fine-scale genetic diversity studies, especially to test questions related to local adaptations in different environments.

To conclude, the findings of this study suggest the possibility of an enzootic focus of *B. burgdorferi* s.s. in two populations within the Transmexican Volcanic Belt. The infection of *H. schmidlyi* now reveals a new wild host of this spirochaete and represents the most southern population of *B. burgdorferi* in a region located in the transitional zone between the Nearctic and the Neotropical of high altitude and associated with a cloud forest. Additionally, an incipient genetic differentiation between western United States and Mexico was found, probably due to historical and ecological processes such as an unsuitable host, the adaptation to new vector species, as well as to environmental conditions.

ACKNOWLEDGEMENTS

Pablo Colunga-Salas is a doctoral student from the Programa de Doctorado en Ciencias Biomédicas, Universidad Nacional Autónoma de México (UNAM) and has received CONACyT fellowship 463798. Giovani Hernández-Canchola was supported by NSF DEB-1754393, DEB-1441634 and CONACyT. The authors thank Tania Marines-Macías, Miguel Ángel Salas-Téllez, Andrea Cerón-Acosta, Héctor Ortega-Salas, Héctor Olguín-Monrroy, David Ortíz, Martín Cabrera-Garrido, Mónica Sánchez and Alfredo A. Gutiérrez-González for their assistance during field work and Laura Márquez Valdelamar for processing samples for sequencing, and to two anonymous reviewers for their comments in the earlier manuscripts. The sponsorship for this work was supported by grants CONACyT 239482, PAPIIT IN216713, PAPIIT IN2175152 and PAPIIT IN211418.

CONFLICT OF INTEREST

The authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest, non-financial interest in the subject matter or materials discussed in this manuscript.

ETHICAL APPROVAL

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. Animals were handled according to National Legislation and with the Guidelines of the American Society of Mammalogists for the Use of Wild Mammals in Research.

DATA AVAILABILITY STATEMENT

The data obtained in this study were submitted in GenBank, under accession numbers: MT378393-MT378397 for 16S rDNA region, MG934557-MG934561 for *flagellin* and MN461274-MN461278 for *osp*A. Furthermore, sequences used in genetic diversity analyses were downloaded from GenBank and accession numbers are shown in all figures.

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REFERENCES

Abel, I. S., Marzagão, G., Yoshinari, N. H., & Schumaker, T. T. S. (2000). Borrelia-like spirochetes recovered from ticks and small mammals collected in the atlantic forest reserve, Cotia County, State of São Paulo, Brazil. *Memórias do Instituto Oswaldo Cruz*, *95*, 621–624. https://doi.org/10.1590/S0074-02762000000500006

Baranton, G., Seinost, G., Theodore, G., Postic, D., & Dykhuizen, D. (2001). Distinct levels of genetic diversity of Borrelia burgdorferi are associated with different aspects of pathogenicity. *Research in Microbiology*, *152*, 149–156. https://doi.org/10.1016/S0923-2508(01)01186-X

Barthold, S. W. (1999). Specificity of infection-induced immunity among Borrelia burgdorferi sensu lato species. *Infection and Immunity*, *67*, 36–42.

Brisson, D., Vandermause, M. F., Meece, J. K., Reed, K. D., & Dykhuizen, D. E. (2010). Evolution of northeastern and midwestern Borrelia burgdorferi, United States. *Emerging Infectious Diseases*, *16*, 911–917. https://doi.org/10.3201/eid1606.090329

Bunikis, J., Garpmo, U., Tsao, J., Berglund, J., Fish, D., & Barbour, A. G. (2004). Sequence typing reveals extensive strain diversity of the Lyme borreliosis agents Borrelia burgdorferi in North America and Borrelia afzelii in Europe. *Microbiology*, *150*, 1741–1755. https://doi.org/10.1099/mic.0.26944-0

Castro, M. B., & Wright, S. A. (2007). Vertebrate hosts of Ixodes pacificus (Acari: Ixodidae) in California. *Journal of Vector Ecology*, *32*, 140–149. https://doi.org/10.3376/1081-1710(2007)32[140:vhoipa]2.0.co;2

Ceballos, G. (2013). *Mammals of Mexico*. Baltimore, MD: Johns Hopkins University Press, pp. 957.

Clark, K. L., Leydet, B., & Hartman, S. (2013). Lyme borreliosis in human patients in Florida and Georgia, USA. *International Journal of Medical Sciences*, *10*, 915–931. https://doi.org/10.7150/ijms.6273

Coipan, C. E., Van Duijvendijk, G. L. A., Hofmeester, T. R., Takumi, K., & Sprong, H. (2018). The genetic diversity of Borrelia afzelii is not maintained by the diversity of the rodent hosts. *Parasites and Vectors*, *11*, 1–7. https://doi.org/10.1186/s13071-018-3006-2

Crooks, K. R., Scott, C. A., Angeloni, L., Bowen, L., Kimsey, R. B., & Van Vuren, D. H. (2001). Ectoparasites of the island fox on Santa Cruz island. *Journal of Wildlife Diseases*, *37*, 189–193. https://doi.org/10.7589/0090-3558-37.1.189

da Costa, I. P., Bonoldi, V. L. N., & Yoshinari, N. H. (2002). Search for Borrelia sp. in Ticks collected from potential reservoirs in a urban forest reserve in the State of 111 Mato Grosso do Sul, Brasil: a short report. *Memórias do Instituto De Butantan*, *97*, 631–635.

Durden, L. A. (2006).Taxonomy, host associations, life cycles and vectorial importance of ticks parasitizing small mammals. In S. Morand, B. R. Krasnov, & R. Poulin (Eds.), *Micromammals and Macroparasites*. (pp. 91–102). Tokio: Springer.

Edgar, R. C. (2004). MUSCLE: A multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics*, *5*, 1–19. https://doi.org/10.1186/1471-2105-5-113

Excoffier, L., & Lischer, H. E. L. (2010). Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, *10*, 564–567. https://doi.org/10.1111/j.1755-0998.2010.02847.x

Faccini-Martínez, Á. A. (2019). Call for Caution to Consider Lyme Neuroborreliosis as A Frequent Neurological Disease in Mexico. *Archives of Medical Research*, *50*, 18. https://doi.org/10.1016/j.arcmed.2019.03.005

Feria-Arroyo, T. P., Castro-Arellano, I., Gordillo-Perez, G., Cavazos, A. L., Vargas-Sandoval, M., Grover, A., … Esteve-Gassent, M. D. (2014). Implications of climate change on the distribution of the tick vector Ixodes scapularis and risk for Lyme disease in the Texas-Mexico transboundary region. *Parasites and Vectors*, *7*, 199. https://doi.org/10.1186/1756-3305-7-199

Foretz, M., Postic, D., & Baranton, G. (1997). Phylogenetic analysis of Borrelia burgdorferi sensu stricto by arbitrarily primed PCR and pulsed-field gel electrophoresis. *International Journal of Systematic Bacteriology*, *47*, 11–18. https://doi.org/10.1099/00207713-47-1-11

Gordillo-Pérez, G., Solorzano, F., Cervantes-Castillo, A., Sanchez-Vaca, G., García-Ramirez, R., Díaz, A. M., … Torres, J. (2018). Lyme neuroborreliosis is a severe and frequent neurological disease in Mexico. *Archives of Medical Research*, *49*, 399–404. https://doi.org/10.1016/j.arcmed.2018.11.007

Gordillo-Pérez, G., Vargas, M., Solórzano-Santos, F., Rivera, A., Polaco, O. J., Alvarado, L., … Torres, J. (2009). Demonstration of Borrelia burgdorferi sensu stricto infection in ticks from the northeast of Mexico. *Clinical Microbiology and Infection*, *15*, 496–498. https://doi.org/10.1111/j.1469-0691.2009.02776.x

Guillot, G., Renaud, S., Ledevin, R., Michaux, J., & Claude, J. (2012). A unifying model for the analysis of phenotypic, genetic, and geographic data. *Systematic Biology*, *61*, 897–911. https://doi.org/10.1093/sysbio/sys038

Guindon, S., Dufayard, J. F., Lefort, V., Anisimova, M., Hordijk, W., & Gascuel, O. (2010). New algorithms and methods to estimate maximum-likelihood phylogenies: Assessing the performance of PhyML 3.0. *Systematic Biology*, *59*, 307–321. https://doi.org/10.1093/sysbio/syq010

Güner, E. S., Watanabe, M., Hashimoto, N., Kadosaka, T., Kawamura, Y., Ezaki, T., … Masuzawa, T. (2004). *Borrelia turcica* sp. nov., isolated from the hard tick Hyalomma aegyptium in Turkey. *International Journal of Systematic and Evolutionary Microbiology*, *54*, 1649–1652. https://doi.org/10.1099/ijs.0.03050-0

Guzmán-Cornejo, C., Robbins, R. G., & Pérez, T. M. (2007). The Ixodes (Acari: Ixodidae) of Mexico: parasite-host and host-parasite checklists. *Zootaxa*, *1553*, 47–58.

Hammer, Ø., David, A. T. H., & Paul, D. R. (2001). Past: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica*, *4*, 1–9.

Hasegawa, M., Kishino, H., & Yano, T.-A. (1985). Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution*, *22*, 160–174. https://doi.org/10.1007/BF02101694

Hayes, E. B., & Piesman, J. (2003). How can we prevent Lyme disease? *New England Journal of Medicine*, *348*, 2424–2430. https://doi.org/10.1056/NEJMra021397

Hovius, J. W. R., van Dam, A. P., & Fikrig, E. (2007). Tick-host-pathogen interactions in Lyme borreliosis. *Trends in Parasitology*, *23*, 434–438. https://doi.org/10.1016/j.pt.2007.07.001

Huegli, D., Hu, C. M., Humair, P. F., Wilske, B., & Gern, L. (2002). Apodemus species mice are reservoir hosts of Borrelia garinii OspA serotype 4 in Switzerland. *Journal of Clinical Microbiology*, *40*, 4735–4737. https://doi.org/10.1128/JCM.40.12.4735-4737.2002

Humphrey, P. T., Caporale, D. A., & Brisson, D. (2010). Uncoordinated phylogeography of borrelia burgdorferi and its tick vector, Ixodes scapularis. *Evolution* (N., Y). *64*, 2653–2663. https://doi.org/10.1111/j.1558-5646.2010.01001.x

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, *35*, 1547–1549. https://doi.org/10.1093/molbev/msy096

Kurtenbach, K., Hanincová, K., Tsao, J. I., Margos, G., Fish, D., & Ogden, N. H. (2006). Fundamental processes in the evolutionary ecology of Lyme borreliosis. *Nature Reviews Microbiology*, *4*, 660–669. https://doi.org/10.1038/nrmicro1475

Lane, R. S., Mun, J., Eisen, R. J., & Eisen, L. (2005). Western gray squirrel (Rodentia: Sciuridae): a primary reservoir host of Borrelia burgdorferi in Californian oak woodlands? *Journal of Medical Entomology*, *42*, 388–396. https://doi.org/10.1603/0022-2585(2005)042[0388:WGSRSA]2.0.CO;2

Lanfear, R., Calcott, B., Ho, S. Y. W., & Guindon, S. (2012). PartitionFinder: Combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution*, *29*, 1695–1701. https://doi.org/10.1093/molbev/mss020

Lanfear, R., Frandsen, P. B., Wright, A. M., Senfeld, T., & Calcott, B. (2017). Partitionfinder 2: New methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution*, *34*, 772–773. https://doi.org/10.1093/molbev/msw260

Leigh, J. W., & Bryant, D. (2015). POPART: Full-feature software for haplotype network construction. *Methods in Ecology and Evolution*, *6*, 1110–1116. https://doi.org/10.1111/2041-210X.12410

León-Paniagua, L., Navarro-Sigüenza, A. G., Hernández-Baños, B. E., & Morales, J. C. (2007). Diversification of the arboreal mice of the genus Habromys (Rodentia: Cricetidae: Neotominae) in the Mesoamerican highlands. *Molecular Phylogenetics and Evolution*,, *42*, 653–664. https://doi.org/10.1016/j.ympev.2006.08.019

León-Paniagua, L., & Romo-Vázquez, E. (1993). Mastofauna de la Sierra de Taxco, Guerrero. In R. A. Medellín, & G. Ceballos (eds), *Avances en el Estudio de los Mamíferos de México* (pp. 45–64). Mexico City, Mexico: Asociación Mexicana de Mastozoología, AC.

López-Pérez, A. M., Sánchez-Montes, S., Foley, J., Guzmán-Cornejo, C., Colunga-Salas, P., Pascoe, E., … Suzan, G. (2019). Molecular evidence of Borrelia burgdorferi sensu stricto and Rickettsia massiliae in ticks collected from a domestic-wild carnivore interface in Chihuahua, Mexico. *Ticks and Tick-Borne Diseases*, *10*, 1118–1123. https://doi.org/10.1016/j.ttbdis.2019.05.018

Margos, G., Fedorova, N., Becker, N. S., Kleinjan, J. E., Marosevic, D., Krebs, S., … Lane, R. S. (2019). Borrelia maritima sp. nov., a novel species of the Borrelia burgdorferi sensu lato complex, occupying a basal position to North American species. *International Journal of Systematic and Evolutionary Microbiology*, online ver, *70*(2), 849–856. https://doi.org/10.1099/ijsem.0.003833

Margos, G., Fedorova, N., Kleinjan, J. E., Hartberger, C., Schwan, T. G., Sing, A., & Fingerle, V. (2017). Borrelia lanei sp. nov. extends the diversity of Borrelia species in California. *International Journal of Systematic and Evolutionary Microbiology*, *67*, 3872–3876. https://doi.org/10.1099/ijsem.0.002214

Margos, G., Gatewood, A. G., Aanensen, D. M., Hanincová, K., Terekhova, D., Vollmer, S. A., … Kurtenbach, K. (2008). MLST of housekeeping genes captures geographic population structure and suggests a European origin of Borrelia burgdorferi. *Proceedings of the National Academy of Sciences of the United States of America*, *105*, 8730–8735. https://doi.org/10.1073/pnas.0800323105

Marines-Macías, T., Colunga-Salas, P., Verde-Arregoitia, L. D., Naranjo, E. J., & León-Paniagua, L. (2018). Space use by two arboreal rodent species in a Neotropical cloud forest. *Journal of Natural History*, *52*, 1417–1431. https://doi.org/10.1080/00222933.2018.1459921

Mechai, S., Margos, G., Feil, E. J., Lindsay, L. R., & Ogden, N. H. (2015). Complex population structure of Borrelia burgdorferi in southeastern and south central Canada as revealed by phylogeographic analysis. *Applied and Environmental Microbiology*, *81*, 1309–1318. https://doi.org/10.1128/AEM.03730-14

Morales-Diaz, J., Colunga-Salas, P., Romero-Salas, D., Sánchez-Montes, S., Estrada-Souza, I. M., Ochoa-Ochoa, L. M., … Cruz-Romero, A. (2020). Molecular detection of reptile-associated Borrelia in Boa constrictor (Squamata: Boidae) from Veracruz, Mexico. *Acta Tropica*, *205*, 105422. https://doi.org/10.1016/j.actatropica.2020.105422

Norris, S. J., Barbour, A. G., Fish, D., & Diuk-Wasser, M. A. (2015). Response to Esteve-Gassent et al: FlaB sequences obtained from Texas PCR products are identical to the positive control strain Borrelia burgdorferi B31. *Parasites and Vectors*, *8*, 1–7. https://doi.org/10.1186/s13071-015-0899-x

Norris, S. J., Barbour, A. G., Fish, D., M Diuk-Wasser, M. A. Analysis of the intergenic sequences provided by Feria-Arroyo et al does not support the claim of high Borrelia burgdorferi tick infection rates in Texas and northeastern Mexico. *Parasites & Vectors*, *7*(1), 3–7. https://doi.org/10.1186/s13071-014-0467-9

Piesman, J., & Gern, L. (2004). Lyme borreliosis in Europe and North America. *Parasitology*, *129*, S191–S220. https://doi.org/10.1017/S0031182003004694

Potkonjak, A., Kleinerman, G., Gutiérrez, R., Savić, S., Vračar, V., Nachum-Biala, Y., … Baneth, G. (2016). Occurrence of Borrelia burgdorferi Sensu Lato in Ixodes ricinus Ticks with First Identification of Borrelia miyamotoi in Vojvodina, Serbia. *Vector-Borne and Zoonotic Diseases*, *16*, 631–635. https://doi.org/10.1089/vbz.2016.2008

Rambaut, A., Drummond, A. J., Xie, D., Baele, G., & Suchard, M. A. (2018). Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology*, *67*, 901–904. https://doi.org/10.1093/sysbio/syy032

Ras, N. M., Postic, D., Foretz, M., & Baranton, G. (1997). Borrelia burgdorferi sensu stricto, a bacterial species "made in the U.S.A".? *International Journal of Systematic Bacteriology*, *47*, 1112–1117. https://doi.org/10.1099/00207713-47-4-1112

Romo-Vázquez, E., León-Paniagua, L., & Sánchez, O. (2005). A new species of Habromys (Rodentia: Neotominae) from México. *Proceedings of the Biological Society of Washington*, *118*, 605–618. https://doi.org/10.2988/0006-324X(2005)118[605:ANSOHR]2.0.CO;2

Ronquist, F., Teslenko, M., Van Der Mark, P., Ayres, D. L., Darling, A., Höhna, S., … Huelsenbeck, J. P. (2012). Mrbayes 3.2: Efficient bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, *61*, 539–542. https://doi.org/10.1093/sysbio/sys029

Roome, A., Hill, L., Al-Feghali, V., Murnock, C. G., Goodsell, J. A., Spathis, R., & Garruto, R. M. (2017). Impact of white-tailed deer on the spread of Borrelia burgdorferi. *Medical and Veterinary Entomology*, *31*, 1–5. https://doi.org/10.1111/mve.12191

Saito, K., Ito, T., Asashima, N., Ohno, M., Nagai, R., Fujita, H., … Kawabata, H. (2007). Case report: Borrelia valaisiana infection in a Japanese man associated with traveling to foreign countries. *The American Journal of Tropical Medicine and Hygiene*, *77*, 1124–1127. https://doi.org/10.4269/ajtmh.2007.77.1124

Schotthoefer, A. M., & Frost, H. M. (2015). Ecology and epidemiology of lyme borreliosis. *Clinics in Laboratory Medicine*, *35*, 723–743. https://doi.org/10.1016/j.cll.2015.08.003

Skotarczak, B., Wodecka, B., Rymaszewska, A., Sawczuk, M., Maciejewska, A., Adamska, M., … Swierzbinska, R. (2005). Prevalence of DNA and antibodies to Borrelia burgdorferi sensu lato in dogs suspected of borreliosis. *Annals of Agricultural and Environmental Medicine*, *12*, 199–205.

Slajchert, T., Kitron, U. D., Jones, C. J., & Mannelli, A. (1997). Role of the Eastern Chipmunk (Tamias Striatus) in the Epizootiology of Lyme Borreliosis in Northwestern Illinois, USA. *Journal of Wildlife Diseases*, *33*, 40–46. https://doi.org/10.7589/0090-3558-33.1.40

Spielman, A., Wilson, M. L., Levine, J. F., & Piesman, J. (1985). Ecology of Ixodes dammini-borne human babesiosis and Lyme disease. *Annual Review of Entomology*, *30*, 439–460.

Steere, A. C., Strle, F., Wormser, G. P., Hu, L. T., Branda, J. A., Hovius, J. W. R., … Mead, P. S. (2016). Lyme Borreliosis. *Nature Reviews Disease Primers*, *2*, e16090. https://doi.org/10.1016/B978-0-444-63596-9.00022-0

Travinsky, B., Bunikis, J., & Barbour, A. G. (2010). Geographic differences in genetic locus linkages for Borrelia burgdorferi. *Emerging Infectious Diseases*, *16*, 1147–1150. https://doi.org/10.3201/eid1607.091452

Vargas, M., Gordillo-Pérez, G., Solórzano, F., Rivera, A., Polaco, O. J., Muñoz, O., & Torres, J. (2007). Evidences of Borrelia burgdorferiin ticks of the northeast of Mexico. In: E. G. Estrada-Venegas, A. Equihua-Martínez, C. Luna-León, & J. L. Rosas-Acevedo (eds), *Entomología Mexicana* (Vol. *6*, pp. 830–835). Mexico City, Mexico: Sociedad Mexicana de Entomología.

Waddell, P. J., & Steel, M. A. (1997). General time-reversible distances with unequal rates across sites: Mixing Γ and inverse gaussian distributions with invariant sites. *Molecular Phylogenetics and Evolution*, *8*, 398–414. https://doi.org/10.1006/mpev.1997.0452

Wallich, R., Helmes, C., Schaible, U. E., Lobet, Y., Moter, S. E., Kramer, M. D., & Simon, M. M. (1992). Evaluation of genetic divergence among Borrelia burgdorferi isolates by use of OspA, fla, HSP60, and HSP70 gene probes. *Infection and Immunity*, *60*, 4856–4866.

Yang, X. F., Pal, U., Alani, S. M., Fikrig, E., & Norgard, M. V. (2004). Essential role for OspA/B in the life cycle of the lyme disease spirochete. *Journal of Experimental Medicine*, *199*, 641–648. https://doi.org/10.1084/jem.20031960

Zore, A., Petrovec, M., Prosenc, K., Trila, T., Ruzic-Sabljic, E., & Avsic-Zupanc, T. (1999). Infection of small mammals with Borrelia burgdorferisensu lato in Slovenia as determined by polymerase chain reaction (PCR). *Wiener Klinische Wochenschrift*, *22–23*, 997–999.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Colunga-Salas P, Hernández-Canchola G, Sánchez-Montes S, Lozano-Sardaneta YN, Becker I. Genetic diversity of *Borrelia burgdorferi* sensu stricto: Novel strains from Mexican wild rodents. *Transbound Emerg Dis*. 2020;00:1–12. https://doi.org/10.1111/tbed.13780