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Geographically widespread and novel hemotropic mycoplasmas and bartonellae in Mexican free-tailed bats and sympatric North American bat species

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ABSTRACT Bacterial pathogens remain poorly characterized in bats, especially in North America. We describe novel (and in some cases panmictic) hemoplasmas (10.1% positivity) and bartonellae (25.6% positivity) across three colonies of Mexican free-tailed bats (*Tadarida brasiliensis*), a partially migratory species that can seasonally travel hundreds of kilometers. Molecular analyses identified three novel *Candidatus* hemoplasma species most similar to another novel *Candidatus* species in Neotropical molossid bats. We also detected novel hemoplasmas in sympatric cave myotis (*Myotis velifer*) and pallid bats (*Antrozous pallidus*), with sequences in the latter 96.5% related to *Candidatus* Mycoplasma haematohominis. We identified nine *Bartonella* genogroups, including those in cave myotis with 96.1% similarity to *Candidatus* Bartonella mayotimonensis. We also detected *Bartonella rochalimae* in migratory Mexican free-tailed bats, representing the first report of this human pathogen in the Chiroptera. Monthly sampling of migratory Mexican free-tailed bats during their North American occupancy period also revealed significant seasonality in infection for both bacterial pathogens, with prevalence increasing following spring migration, peaking in the maternity season, and declining into fall migration. The substantial diversity and seasonality of hemoplasmas and bartonellae observed here suggest that additional longitudinal, genomic, and immunological studies in bats are warranted to inform One Health approaches.

IMPORTANCE Bats have been intensively sampled for viruses but remain mostly understudied for bacterial pathogens. However, bacterial pathogens can have significant impacts on both human health and bat morbidity and even mortality. Hemoplasmas and bartonellae are facultative intracellular bacteria of special interest in bats, given their high prevalence and substantial genetic diversity. Surveys have also supported plausible zoonotic transmission of these bacteria from bats to humans, including *Candidatus* Mycoplasma haematohominis and *Candidatus* Bartonella mayotimonensis. Greater characterization of these bacteria across global bat diversity (over 1,480 species) is therefore warranted to inform infection risks for both bats and humans, although little surveillance has thus far been conducted in North American bats. We here describe novel (and in some cases panmictic) hemoplasmas and bartonellae across three colonies of Mexican free-tailed bats and sympatric bat species. We find high genetic diversity and seasonality of these pathogens, including lineages closely related to human pathogens, such as *Bartonella rochalimae*.

KEYWORDS hemoplasmas, *Tadarida brasiliensis*, migration, One Health, *Bartonella*

Bats have been intensively sampled for viral pathogens, with species in this mammalian order hosting multiple viruses with high virulence in humans (1). However, bats remain understudied for bacterial pathogens, which can be significant for their impacts on both human health and bat morbidity and even mortality (2, 3). Hemotropic mycoplasmas (hemoplasmas) and bartonellae are facultative intracellular bacteria of special interest in bats, given their high prevalence and substantial genetic diversity (4, 5). For example, sampling of Neotropical bat communities has identified many common and co-circulating genotypes of these bacteria (6–8). Surveys in Oceania and Europe have also supported plausible zoonotic transmission of these bacteria from bats to humans, including *Candidatus* Mycoplasma haematohominis and *Candidatus* Bartonella mayotimonensis (9, 10). Greater characterization of these bacteria across global bat diversity (over 1,480 species) is therefore warranted to inform infection risks for both bats and humans, although little surveillance has thus far been conducted in North American bats (11, 12).

Although flight enables high mobility of bats, relatively few bat species undertake long-distance migrations (e.g., between maternity and wintering grounds) (13). In North America, Mexican free-tailed bats (*Tadarida brasiliensis*) display highly variable migratory strategies (14). The southwestern United States contains both non-migratory and migratory populations, with some individuals traveling 100–1,000 km between wintering grounds in Mexico to northern summer roosts in Oklahoma, Kansas, and Colorado (14–16). Other colonies across the species range include year-round residents (17, 18). This variation in migratory behavior could shape patterns of infection, including the seasonal dispersal of bacterial pathogens across landscapes to naïve hosts. Hemoplasmas have not yet been detected in this bat species (4, 19), and bartonellae have only been minimally described in the southernmost part of the bat species' geographic range (i.e., Chile and Argentina) (20).

Here, we conducted an initial characterization of hemoplasmas and bartonellae in Mexican free-tailed bats across multiple populations and seasons. Our goals were to identify novel pathogens in this bat species and to test for differences in prevalence among colonies that differ in migratory strategy and across the bat annual cycle. We also tested whether pathogen lineages were unique to each geography or if migration may facilitate panmixia. Lastly, we used this opportunity to perform a pilot characterization of these pathogens in sympatric bat species.

MATERIALS AND METHODS

Wild bat sampling

We sampled three North American colonies of Mexican free-tailed bats in 2021 and 2022 to compare infections among migratory strategies and provide an initial assessment of pathogen seasonality. We sampled non-migratory individuals in southeastern Louisiana (17), focusing on a colony in Pine Grove of approximately 1,000 bats, in the non-breeding season (October 2021, $n = 5$) and maternity season (July 2022, $n = 10$). We also sampled the partially migratory population of Bracken Cave near San Antonio, TX, which hosts tens of millions of this bat species in the maternity season and declines to approximately 10,000 bats in winter (16, 21). We sampled the maternity season (August 2021, $n = 19$) and mid-to-late winter (December 2021 and March 2022; $n = 9$ and $n = 19$). We also sampled a fully migratory colony at the Selman Bat Cave near Freedom, OK, where this maternity roost holds up to 100,000 bats during summer and is empty in winter (16, 22). We sampled bats monthly, from April to September 2022, spanning spring arrival, the maternity season, and fall migration ($n = 146$). In the same Oklahoma site, we also sampled four cave myotis (*Myotis velifer*), one hoary bat (*Lasiurus cinereus*), one Townsend's big-eared bat (*Corynorhinus townsendii*), and two pallid bats (*Antrozous pallidus*).

Bats were captured with hand nets and mist nets while emerging from or returning to roosts and placed in individual cloth bags. Bats were identified as species by morphology

and identified by sex, reproductive status, and age (23). Blood (<1% body mass) was sampled by lancing the propatagial vein using 27G and 30G needles and collected with heparinized capillaries. Blood was preserved on Whatman FTA cards and held at room temperature until −20°C storage at the University of Oklahoma. All bats were released after sampling at the capture site.

Molecular diagnostics

We extracted genomic DNA from blood using QIAamp DNA Investigator Kits (Qiagen). To determine hemoplasma presence, we used PCR targeting the partial 16S and 23S rRNA genes (Table S1) (6, 7, 24, 25), with amplicons purified and sequenced at Psomagen. For DNA samples positive for the 16S or 23S rRNA genes, we also attempted to amplify the partial *rpoB* gene, using primers newly designed for this study (Table S1). To determine the presence of bartonellae, we used nested PCR targeting the partial *gltA* gene (Table S1) (26), with amplicons purified with Zymo kits (DNA Clean and Concentrator-5, Zymoclean Gel DNA Recovery) and sequenced at the North Carolina State University Genomic Sciences Laboratory. We included blank FTA card punches and ultrapure water as extraction and negative controls, respectively, in all PCRs. Hemoplasma PCRs used *Candidatus* Mycoplasma haematozalophi as a positive control, but we did not include positive controls for *Bartonella* spp. to reduce cross-contamination risks from nested PCR; instead, amplicons of the expected size (~300 bp) were identified during gel electrophoresis.

Statistical analysis

We analyzed infection states using generalized linear models (GLMs) or generalized additive models (GAMs) with binary response in R. All GLMs were fit using mean bias reduction methods with the brglm2 package (27), whereas GAMs were fit using restricted maximum likelihood and the mgcv package (28). For each of our two pathogens, we fit the following four GLMs. The first model was fit to all data and compared the odds of infection among Louisiana, Texas, and Oklahoma bats. The second model was limited to the early-to-mid non-breeding season and compared odds of infection between Texas and Louisiana bats. The third model was limited to the late overwintering period and spring arrival to compare odds of infection between Texas and that subset of Oklahoma bats. The fourth model was limited to Texas and included reproductive status (only females were reproductive) and month-to-test demographic effects and seasonality (i.e., maternity season through late overwintering). Lastly, for our GAMs, we fit a similar model for each pathogen to the Oklahoma data, with a cyclic cubic smooth of month to assess a different aspect of infection seasonality (i.e., spring arrival until onset of fall migration).

Phylogenetic analysis

We used NCBI BLASTn to identify related *Mycoplasma* spp. (16S rRNA, 23S rRNA, and *rpoB*) and *Bartonella* spp. sequences (*gltA*), which we aligned with our sequences and reference sequences using MUSCLE. We used MrBayes for phylogenetic analysis, with each gene tree run for 20,000,000 generations using a GTR + I + G model. BLASTn was implemented in Geneious, whereas MUSCLE and MrBayes were implemented using NGPhylogeny.fr (29). We delineated genotypes of hemoplasmas and genogroups of bartonellae based on pairwise similarity among sequences and clustering on their phylogenies, using established criteria for defining novel bacterial lineages (6, 30). For hemoplasmas, we also used multi-loci data to propose novel *Candidatus* species when the same genotype was identified in at least two samples using 16S rRNA and one other marker (i.e., 16S rRNA and 23S rRNA, 16S rRNA, and *rpoB*) (7, 31).

We conducted two tests to assess if pathogen lineages were unique to each Mexican free-tailed bat colony, which would suggest geographically constrained transmission dynamics. First, we used χ^2 tests with *P* values generated by a Monte Carlo procedure

to quantify associations between geography and pathogen lineage assignments. Next, for any lineages identified in multiple colonies with sufficient sample size, we derived matrices of spatial and phylogenetic distance among sequenced PCR-positive samples and used Mantel tests with the *vegan* R package to assess isolation by distance (32). These tests used 1,000 randomizations.

RESULTS

Migratory and seasonal effects on bat bacterial infection

We detected hemoplasmas in 21 of 208 Mexican free-tailed bats when targeting the partial 16S or 23S rRNA genes (10.1%, 95% confidence interval [CI]: 6.7%–14.9%). Sequencing of the 23S rRNA gene showed that three other bats (two from Texas and one from Oklahoma) had non-hemotropic mycoplasmas (OQ359169–OQ359170 and OQ359174) most related to *Mycoplasma muris* (97.5% sequence identity). We detected bartonellae in 53 of 207 tested bats (25.6%, 95% CI: 20.1%–32%). Only six Mexican free-tailed bats were coinfected by bartonellae and any mycoplasmas (2.9%, 95% CI: 1.3%–6.1%). Hemoplasmas were detected in all three Mexican free-tailed bat colonies, while bartonellae were only detected in the Texas and Oklahoma colonies. PCR positivity data are fully available in the Pathogen Harmonized Observatory (PHAROS): https://pharos.viralemergence.org/ (33).

Across all Mexican free-tailed bats (model 1), the odds of infection differed by colony for bartonellae ($\chi^2 = 9.6$, $P < 0.01$) but not hemoplasmas ($\chi^2 = 0.11$, $P = 0.95$; Fig. 1). When comparing only the resident and partially migratory populations in the non-breeding season (model 2), Louisiana and Texas bats did not differ in the odds of either infection (hemoplasmas: $\chi^2 = 1.21$, $P = 0.27$; bartonellae: $\chi^2 = 0$, $P = 1$). When comparing only the partially and fully migratory populations in spring (model 3), we did not detect colony differences for hemoplasmas ($\chi^2 = 0$, $P = 1$) or bartonellae ($\chi^2 = 0.11$, $P = 0.74$). When assessing risk factors of infection in the partially migratory Texas colony (model 4), we found no evidence of seasonal or demographic effects for either pathogen (Table S2). However, when assessing these predictors for the fully migratory Oklahoma colony across the full 2022 occupancy period, we identified significant seasonality in infection (Table S3). Prevalence increased from spring arrival and peaked in the maternity season (i.e., June 2022) for both bacteria, declining into fall migration (Fig. 1).

Genetic diversity of Mexican free-tailed bat hemoplasmas

Sequencing of 16S rRNA amplicons revealed four hemoplasma genotypes specific to Mexican free-tailed bats (i.e., TB1–4; Fig. 2; Table 1). The TB1 genotype was 97% similar to the MR1 genotype that we earlier isolated from another molossid bat (*Molossus nigricans*) in Belize (e.g., MH245174) (6), and TB1 was found in all three sampled populations. In contrast, TB2 was only detected in the Texas colony and was 96%–97% similar to hemoplasmas from carnivores and rodents and to the Belize bat MR1 genotype (6, 34, 35). Both TB3 and TB4 were only detected in Oklahoma and were ~99% similar to MR1. We also detected the PPM1 genotype, originally found in *Pteronotus mesoamericanus* and *P. fulvus* in Belize (6, 7), in the Oklahoma colony. Amplification of the 23S rRNA gene from two bats (one each from Texas and Oklahoma) also found a genotype we initially detected in cave myotis (i.e., MV1; Fig. S1) and the above *Mycoplasma muris*-like (non-hemotropic) genotype. These seven mycoplasma genotypes were not associated with geography ($\chi^2 = 13.07$; $P = 0.38$; Fig. S2). When considering the one genotype observed in multiple colonies and in more than one bat per site (TB1), we also found little support for isolation by distance with the 16S rRNA phylogeny (Mantel $r = 0.35$, $P = 0.10$).

Amplification of paired partial 23S rRNA (Fig. S1) and/or *rpoB* (Fig. S3) genes for samples belonging to these 16S rRNA genotypes suggested that at least three novel *Candidatus* hemoplasma species circulate in Mexican free-tailed bats. Based on 100% identity of two *rpoB* sequences (OQ554335 and OQ554336) and 100% identity of paired
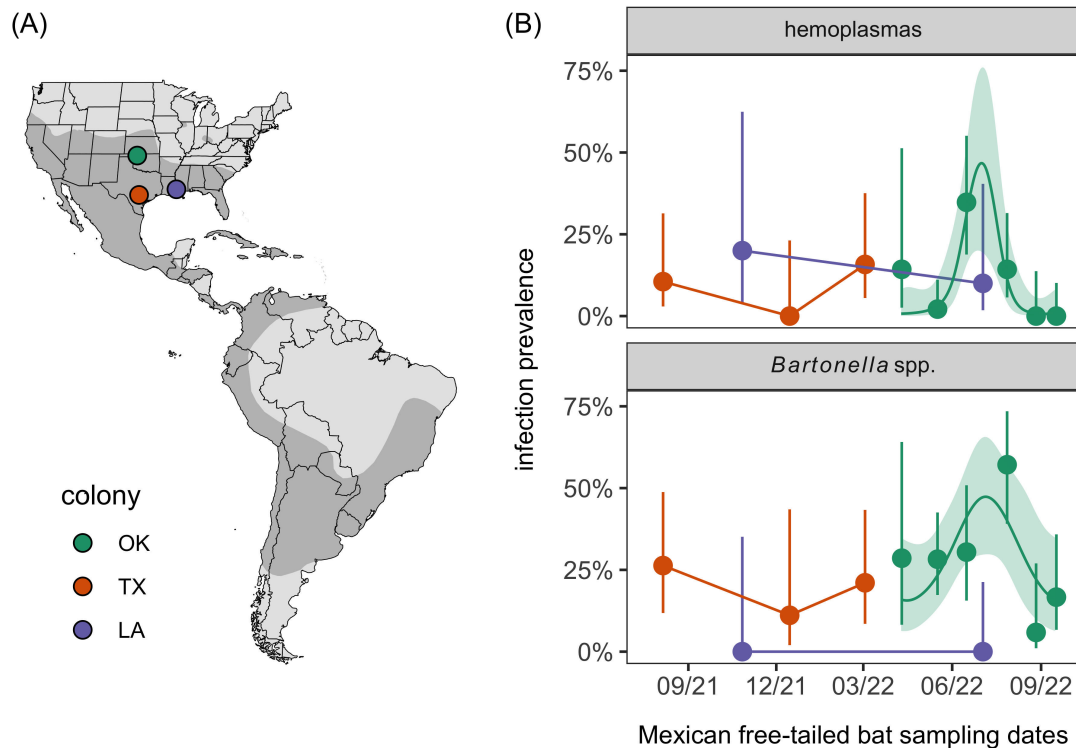
FIG 1 (A) Sampled Mexican free-tailed bat (*Tadarida brasiliensis*) colonies relative to the host distribution in the Americas. (B) Hemoplasma and *Bartonella* spp. infection prevalence across months and colonies; segments denote 95% CIs using Wilson's method. For Oklahoma bats, curves and bands display the GAM trends and 95% confidence intervals.

16S rRNA sequences included in the TB1 genotype, first detected in Bracken Cave (OQ407846 and OQ407848), we propose the name *Candidatus* Mycoplasma haemato-brackenitadaridae sp. nov. Similarly, given 99.98% identity among seven 23S rRNA sequences (OQ359161–OQ359165, OQ359168, and OQ359172) and 100% identity in paired 16S rRNA sequences included in the TB3 genotype from the Selman Bat Cave (OQ407832, OQ407836–OQ407839, OQ407843, and OQ407844), we propose the name *Candidatus* M. haematoselmanitadaridae sp. nov. Lastly, given 100% identity of two 23S rRNA sequences (OQ359160 and OQ359166) and 99.8% identity in paired 16S rRNA sequences from the TB4 genotype (also identified from the Selman Bat Cave; OQ407831 and OQ407840), we propose the name *Candidatus* M. haematotraderitadaridae sp. nov. (Fig. 2; Fig. S1), based on the stream running adjacent to the bat cave (Traders Creek) (36).

Given the similarity of Mexican free-tailed bat hemoplasma 16S rRNA sequences to those from molossid bats sampled in Belize (6), we also attempted to amplify the 23S rRNA and *rpoB* genes from *Molossus nigricans* sampled in 2017 and 2018 in Belize that previously tested positive for the MR1 and MR2 genotypes (6). We re-extracted DNA from four FTA cards and applied the same additional PCR protocols described earlier. We obtained partial 23S rRNA and *rpoB* sequences for two (OQ518943 and OQ518944) and three (OQ554329–OQ55432931) *M. nigricans*, respectively. Based on 100% inter-sequence similarity of the *rpoB* sequences and high (x̄ = 99.98%) identity of paired 16S rRNA sequences (MH245122, MH245172, and MH245174), we propose the name *Candidatus* Mycoplasma haematomolossi sp. nov. to designate this novel hemoplasma (Fig. 2; Fig. S3).

Genetic diversity of Mexican free-tailed bat bartonellae

Sequencing of *gltA* amplicons next revealed at least six *Bartonella* genogroups circulating in Mexican free-tailed bats (Fig. 3; Table 1). The first genogroup was detected in both

posterior probability   ◇   0.5 < PP < 0.7   ◆   0.7 < PP < 0.9   ◆   PP > 0.9
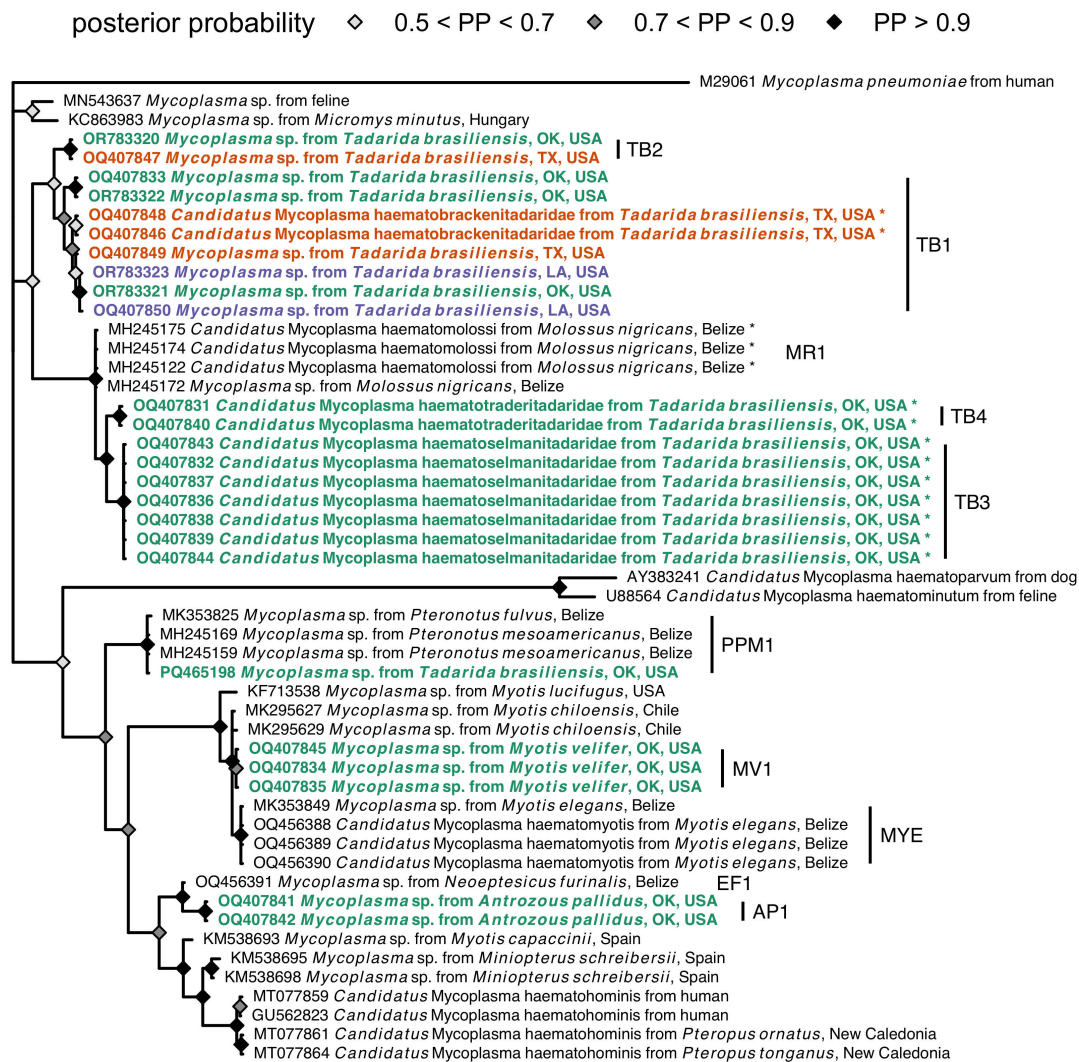

FIG 2 Consensus Bayesian phylogeny of the partial 16S rRNA hemoplasma sequences from this study (highlighted in bold and colored by geography; see Table 1 for genotype assignments) and reference sequences from bats and other mammals. Nodes are colored by posterior probability (nodes with less than 50% support are not shown). Hemoplasmas with *Candidatus* species names proposed here are indicated by asterisks and have paired 23S rRNA or *rpoB* sequences (see Fig. S1 and S3).

Texas and Oklahoma, with sequences from all three sampled months across 2021 and 2022 from Bracken Cave but only from May and June in the Selman Bat Cave. These *gltA* sequences were ≥99.6% similar to those recently detected in Mexican free-tailed bats in Argentina (KX986617) (20), such that we consider them to all form the TB1 genogroup. The TB2 and TB3 genogroups were only found in the Oklahoma colony and both represented novel bartonellae. TB2 showed ≤93% similarity to *Bartonella vinsonii* (e.g., MK984790) (37), while TB3 was distantly related (~90%) to *Bartonella quintana* (i.e., CP091505). Oklahoma bats also harbored *gltA* sequences with 100% identity to *Bartonella rochalimae* in fleas from foxes (e.g., OQ436435) (38); these *B. rochalimae* sequences were only detected in summer months. Oklahoma bats also had a *gltA* sequence in the same clade as sequences from streblid bat flies in Mexico (e.g., MF988072), vampire bats (*Desmodus rotundus*) in Mexico and Peru (e.g., MG799396), and Parnell's mustached bats (*Pteronotus parnellii*; KY629828) in Mexico (DR1 genogroup) (26, 39). Oklahoma bats also had *gltA* sequences in the same clade as other bartonellae first found in vampire bats (DR8) (26). These six *Bartonella* genogroups were not associated

TABLE 1 *Mycoplasma* spp. and *Bartonella* spp. lineages identified from Louisiana, Texas, and Oklahoma bats during this study (2021–2022)[h]

Lineage	States	Bat species	Mean intra-lineage similarity (%)
Mycoplasma spp.			
TB1[a]	LA, TX, OK	*Tadarida brasiliensis*	99.6,[c] 100[e]
TB2[a]	TX, OK	*Tadarida brasiliensis*	100[c]
TB3[a]	OK	*Tadarida brasiliensis*	100,[c] 100[d]
TB4[a]	OK	*Tadarida brasiliensis*	100,[c] 100[d]
PPM1	OK	*Tadarida brasiliensis*	NA[g]
MV1[a]	TX, OK	*Myotis velifer* and *Tadarida brasiliensis*	100,[c] 100[d]
AP1[a]	OK	*Antrozous pallidus*	100[c]
Mycoplasma muris-like[b]	TX, OK	*Tadarida brasiliensis*	99.3[d]
Bartonella spp.			
TB1	TX, OK	*Tadarida brasiliensis*	99.6[f]
TB2[a]	OK	*Tadarida brasiliensis*	97.4[f]
TB3[a]	OK, TX	*Tadarida brasiliensis*, *Myotis velifer*, and *Antrozous pallidus*	98.7[f]
Bartonella rochalimae	OK	*Tadarida brasiliensis*	100[f]
DR1	OK	*Tadarida brasiliensis*	NA[g]
DR8	OK	*Tadarida brasiliensis*	NA[g]
ML1	OK	*Myotis velifer*	NA[g]
Ca. Bartonella mayotimonensis-like	OK	*Myotis velifer*	NA[g]
AP1[a]	OK	*Antrozous pallidus*	NA[g]
CT1[a]	OK	*Corynorhinus townsendii*	NA[g]

[a]Novel lineages.
[b]Non-hemotropic mycoplasma.
[c]16S rRNA sequence.
[d]23S rRNA sequence.
[e]*rpoB* sequence.
[f]*gltA* sequence.
[g]Single sequence.
[h]Lineages are given with their host species, locations, and mean intra-genotype (mycoplasmas) or intra-genogroup (bartonellae) sequence similarity from the partial 16S rRNA, 23S rRNA, *rpoB*, or *gltA* gene sequences identified here.

with geography ($\chi^2 = 8.20$, $P = 0.16$; Fig. S4), and we also found no support for isolation by distance for the TB1 genotype with our *gltA* phylogeny (Mantel $r = -0.02$, $P = 0.53$).

Bacterial infections of sympatric bat species

Opportunistic sampling of other bats in Oklahoma revealed further bacterial diversity (Table 1). Three of the four cave myotis and both pallid bats tested positive for hemoplasmas, whereas three of three cave myotis, both pallid bats, and the single Townsend's big-eared bat tested positive for bartonellae; the single hoary bat tested negative for both bacterial pathogens.

For hemoplasmas (Fig. 2), we identified a single novel hemoplasma genotype in each PCR-positive species (i.e., MV1 and AP1), with 16S rRNA sequences most closely related (i.e., ≥98% similarity) to previously detected genotypes in vesper bats from Chile and Belize (e.g., EF1 and MYE) (6, 19). Notably, 16S rRNA sequences of the AP1 genotype were 96.5% similar to those of *Candidatus* Mycoplasma haematohominis (i.e., GU562823), whereas those of the MV1 genotype were only ~94% similar. 23S rRNA and *rpoB* sequences from cave myotis (OQ359173 and OQ554337) were entirely novel (<85% similarity to GenBank sequences), with the former 100% similar to select 23S rRNA sequences identified from our Mexican free-tailed bats.

For bartonellae (Fig. 3), all three positive cave myotis had their own genogroup. One *gltA* sequence was 98.4% similar to that first identified in little brown bats (*Myotis*
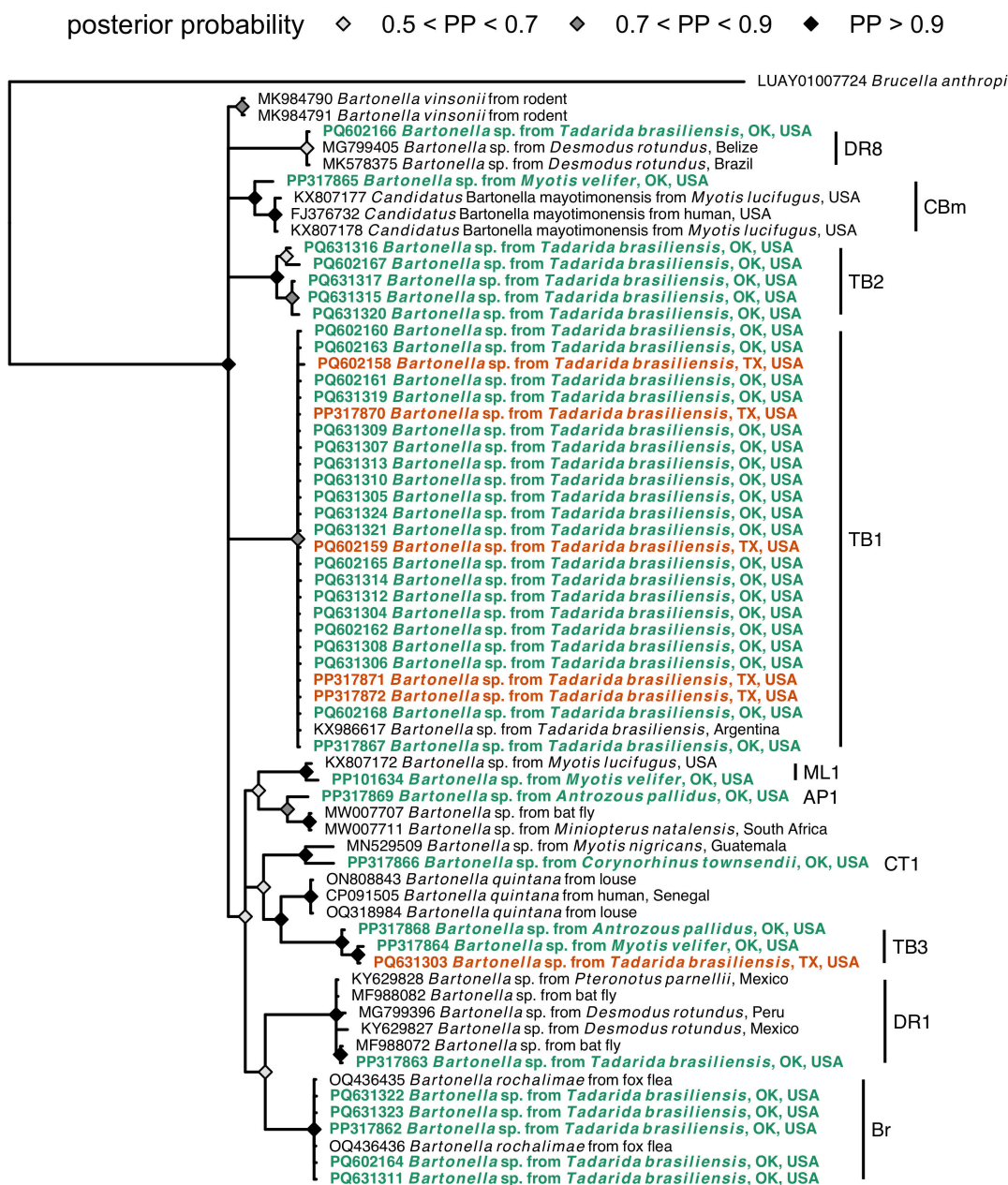
posterior probability ◇ 0.5 < PP < 0.7 ◆ 0.7 < PP < 0.9 ◆ PP > 0.9

FIG 3 Consensus Bayesian phylogeny of the partial *gltA Bartonella* spp. sequences from this study (highlighted in bold and colored by geography; see Table 1 for genogroup assignments) and reference sequences from bats, other mammals, and ectoparasites. Nodes are colored by posterior probability (nodes with less than 50% support are not shown).

lucifugus) elsewhere in North America (i.e., KX807172), here denoted the ML1 genogroup. Another cave myotis had the TB3 genotype. The final cave myotis *gltA* sequence was 96.1% similar to *Candidatus* Bartonella mayotimonensis isolated from a human endocarditis patient in Iowa, USA (FJ376732) and showed analagous similarity to related sequences from other little brown bats (KX807177–KX8071779) (11, 40). For pallid bats, one sequence also belonged to the TB3 genogroup, whereas the other formed the novel AP1 genogroup most related (95.6%) to bartonellae from Natal long-fingered bats (*Miniopterus natalensis*) and their bat flies in South Africa (e.g., MW007711 and MW007707) (41). Lastly, the single positive Townsend's big-eared bat hosted a unique genogroup (CT1) only 94.3% similar to bartonellae found from *Myotis nigricans* in Guatemala (e.g., MN529509) (5).

DISCUSSION

Hemoplasmas and bartonellae are emerging as model systems for studying bacterial infections in bats (5, 7), but their infection dynamics and diversity remain poorly characterized, notably in North American systems (11, 12). We demonstrate novel diversity of hemoplasmas and bartonellae in bats from the south-central United States, including the circulation of lineages of both pathogens with clear infection seasonality in a migratory colony of Mexican free-tailed bats. Such work provides the foundation for further empirical studies to elucidate the transmission dynamics of these bacteria, their pathogenicity in bats, and their possible zoonotic risk.

Our findings suggest relatively common infection with site-specific and panmictic bacterial lineages. Within both bacterial genera, most lineages found in Mexican free-tailed bats were restricted to a single site, indicating spatially constrained transmission. However, we also detected lineages in multiple sites, such as the TB1 hemoplasma genotype in Louisiana, Texas, and Oklahoma; the MV1 hemoplasma genotype and *Mycoplasma muris*-like genotype in Texas and Oklahoma; and the TB1 *Bartonella* genogroup in Texas and Oklahoma (for which sequences were nearly identical to those from Mexican free-tailed bats in Argentina) (20). Such results may be explained by migratory connectivity in Mexican free-tailed bats, for which regional migrations spanning 100–1,000 km have been well-characterized in North America, including between the Selman Bat Cave and Bracken Cave (14, 15, 42); this suggests that the migratory behavior of this species can enhance bacterial dispersal. However, given the presence of these bacterial lineages in migratory and non-migratory populations (i.e., Louisiana) and at the extremes of the bat range (e.g., over 5,000 km between the Selman Bat Cave and the Argentina site for the TB1 *Bartonella* genogroup), these results also suggest the ancestral spread of these bacteria and limited selection pressure on lineages across the bat range.

Future studies are needed to identify the migratory routes of Mexican free-tailed bats, especially for understanding the origins of possibly zoonotic bacterial lineages and the potential for these bats to disperse infection during spring and fall migrations. Researchers could capitalize on advances in tracking small vertebrates for long periods, such as the use of absorbent sutures, to ensure lightweight radiotags stay attached to bats for the duration of migration and winter (43). Such work is also needed to assess if these infections negatively impact bat migration trajectory and success, as observed for blood pathogens in migratory songbirds (44). Longitudinal studies would also inform such analyses, as our data from one full occupancy season in the Oklahoma colony suggest bacterial prevalence peaks in the maternity season. Additional seasonal sampling is needed to assess how infection risk varies across the full migratory cycle, if prevalence tracks bat population size and/or ectoparasite intensity, and whether infections are sufficiently common in autumn to facilitate their dispersal with migration. Further genetic analyses could also inform patterns of bat connectivity and pathogen spread. For example, the DR1 *Bartonella* genogroup identified in Oklahoma Mexican free-tailed bats showed over 99.7% identity to bartonellae from bat flies from Morelos, Hidalgo, and Jalisco in central Mexico (39), spanning the likely wintering sites of this bat species (14, 15). Similarly, previous analyses of *Trypanasoma cruzi* from this same Oklahoma bat population detected lineages similar to those along the Texas–Mexico border, further showing possible southern origins of infection and high pathogen dispersal capacity (45).

Additional molecular and immunological studies are also needed to better characterize these novel bat bacterial pathogens and their health impacts. We identified 16S rRNA and *gltA* sequences with moderate-to-high similarity to zoonotic pathogens such as *Candidatus* Mycoplasma haematohominis, *Candidatus* Bartonella mayotimonensis, and *Bartonella rochalimae* (9, 40, 46). For the former two pathogens, our bat sequences were ~96% similar to zoonotic lineages, likely indicating divergence from a common ancestor at least tens of millions of years ago (7, 47). *Bartonella rochalimae* has been found in cats and dogs (48), and our detections in Mexican free-tailed bats indicate a

broadening of host range into bats. Our sequences showed 100% similarity to those from fox fleas (38), which is relevant given the likely flea transmission (48) and the detection of fleas in the Oklahoma bat population where these sequences were found (K. E. Dyer, unpublished data). Generation of whole genomes for our novel bat pathogens could inform their zoonotic risk by better linking them to cryptic human infections (9) and by facilitating machine learning models that predict zoonotic potential from genomic composition, as applied for viruses (49). Other -omics analyses could also elucidate whether these bacterial infections are pathogenic in bats themselves. In addition to assessing the impacts of infection on migration outcomes as noted above, approaches such as transcriptomics and proteomics could test if bats have a pronounced immune response to these bacterial infections or appear largely tolerant (50). Such studies could be especially informative when comparing immunity between migratory and non-migratory periods, which could test whether long-distance migration may disrupt immune tolerance in bats.

Lastly, Mexican free-tailed bats and their sympatric bat species provide several important ecosystem services, including but not limited to predating on crop pests and contributing to the tourism economy from bat flight watching (51, 52). Understanding the prevalence, genetic diversity, and pathogenicity of bacterial pathogens in bats can inform One Health approaches that emphasize conservation measures to promote bat, domestic animal, and human health (53).

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DATA AVAILABILITY

Sequences are available on GenBank through accessions OQ407831–OQ407850, OR783320–OR783323, and PQ465198 (*Mycoplasma* spp. 16S rRNA); OQ359160–OQ359175 (*Mycoplasma* spp. 23S rRNA); OQ554332–OQ554338 (*Mycoplasma* spp. *rpoB*); and PP101634, PP317862 PP317862–PP317872, PQ602158–PQ602168, and PQ631303–PQ631324 (*Bartonella* spp. *gltA*).

ETHICS APPROVAL

Bat sampling was approved by the Institutional Animal Care and Use Committees of the University of Oklahoma (2022-0198) and Southeastern Louisiana University (0064), with permits from the Texas Parks and Wildlife Department (SPR-0521-063), Louisiana Department of Wildlife and Fisheries (WDP-21-101), and Oklahoma Department of Wildlife Conservation (10567389).

ADDITIONAL FILES

The following material is available online.

Supplemental Material

Supplemental Material (mSphere00116-24-s0001.pdf). Supplemental tables and figures.

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