1 **Geographically widespread and novel hemotropic mycoplasmas and bartonellae in**
2 **Mexican free-tailed bats and sympatric North American bat species**
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24 **Keywords:** hemoplasmas, *Tadarida brasiliensis*, migration, One Health
25 **Running head:** Bacterial pathogens in Mexican free-tailed bats
26

27 **Abstract**
28 Bacterial pathogens remain poorly characterized in bats, especially in North America. We
29 describe novel (and in some cases panmictic) hemoplasmas (10.5% positivity) and bartonellae
30 (25.5% positivity) across three colonies of Mexican free-tailed bats (*Tadarida brasiliensis*), a
31 partially migratory species that can seasonally travel hundreds of kilometers. Molecular analyses
32 identified three novel *Candidatus* hemoplasma species most similar to another novel *Candidatus*
33 species in Neotropical molossid bats. We also detected novel hemoplasmas in sympatric cave
34 myotis (*Myotis velifer*) and pallid bats (*Antrozous pallidus*), with sequences in the latter 96.5%
35 related to *C.* Mycoplasma haemohominis. We identified nine *Bartonella* genogroups, including
36 those in cave myotis with 96.7% similarity to *C.* Bartonella mayotimonensis. We also detected
37 *Bartonella rochalimae* in migratory Mexican free-tailed bats, representing the first report of this
38 human pathogen in the Chiroptera. The seasonality and diversity of these bacteria observed here
39 suggest that additional longitudinal, genomic, and immunological studies in bats are warranted.
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41
42

Introduction

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 haemohominis and *C.* Bartonella mayotimonensis (9, 10). Greater characterization of these bacteria across global bat diversity (over 1,470 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 hundreds to over 1,000 kilometers between wintering grounds in Mexico to northern maternity colonies 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.

Material 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, Texas, 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*=20) 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, Oklahoma, 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

88   site, we also sampled four cave myotis (*Myotis velifer*), one hoary bat (*Lasiurus cinereus*), one
89   Townsend's big-eared bat (*Corynorhinus townsendii*), and two pallid bats (*Antrozous pallidus*).
90   Bats were captured with hand nets and mist nets while emerging from or returning to
91   roosts and placed in individual cloth bags. Bats were identified to species by morphology and
92   identified by sex, reproductive status, and age (23). Blood (<1% body mass) was sampled by
93   lancing the propatagial vein using 27G and 30G needles and collected with heparinized
94   capillaries. Blood was preserved on Whatman FTA cards and held at room temperature until
95   −20°C storage at the University of Oklahoma (OU). Sampling was approved by the Institutional
96   Animal Care and Use Committees of OU (2022-0198) and Southeastern Louisiana University
97   (0064), with permits from the Texas Parks and Wildlife Department (SPR-0521-063), Louisiana
98   Department of Wildlife and Fisheries (WDP-21-101), and Oklahoma Department of Wildlife
99   Conservation (ODWC, 10567389). All bats were released after sampling at the capture site.
100
101   *Molecular diagnostics*
102   We extracted genomic DNA from blood using QIAamp DNA Investigator Kits (Qiagen). To
103   determine hemoplasma presence, we used PCR targeting the partial 16S and 23S rRNA genes
104   (Table S1; (6, 7, 24, 25), with amplicons purified and sequenced at Psomagen. For DNA samples
105   positive for the 16S or 23S rRNA genes, we also attempted to amplify the partial *rpoB* gene,
106   using primers newly designed for this study (Table S1). To determine the presence of
107   bartonellae, we used nested PCR targeting the partial *gltA* gene (Table S1; (26)), with amplicons
108   purified with Zymo kits (DNA Clean & Concentrator-5, Zymoclean Gel DNA Recovery) and
109   sequenced at the North Carolina State University Genomic Sciences Laboratory. We included
110   blank FTA card punches and ultrapure water as extraction and negative controls, respectively, in
111   all PCRs. Hemoplasma PCRs used *Candidatus* Mycoplasma haemozalophi as a positive control,
112   but we did not include positive controls for *Bartonella* spp. to reduce cross-contamination risks
113   from nested PCR; instead, amplicons of expected size (~300 bp) were identified during gel
114   electrophoresis. Sequences are available on GenBank through accessions OQ407831–50,
115   OR783320–23, and PQ465198 (*Mycoplasma* spp. 16S rRNA); OQ359160–75 (*Mycoplasma* spp.
116   23S rRNA); OQ554332–38 (*Mycoplasma* spp. *rpoB*); and PP317862–72 (*Bartonella* spp. *gltA*).
117
118   *Statistical analysis*
119   We analyzed infection states using generalized linear models (GLMs) or generalized additive
120   models (GAMs) with binary response in R. All GLMs were fit using mean bias reduction
121   methods with the *brglm2* package (27), whereas GAMs were fit using restricted maximum
122   likelihood and the *mgcv* package (28). For each of our two pathogens, we fit the following four
123   GLMs. The first model was fit to all data and compared the odds of infection among Louisiana,
124   Texas, and Oklahoma bats. The second model was limited to the early-to-mid non-breeding
125   season and compared odds of infection between Texas and Louisiana bats. The third model was
126   limited to the late overwintering period and spring arrival to compare odds of infection between
127   Texas and that subset of Oklahoma bats. The fourth model was limited to Texas and included
128   reproductive status (only females were reproductive) and month to test demographic effects and
129   seasonality (i.e., maternity season through late overwintering). Lastly, for our GAMs, we fit a
130   similar model for each pathogen to the Oklahoma data, with a cyclic cubic smooth of month to
131   assess a different aspect of infection seasonality (i.e., spring arrival until onset of fall migration).
132
133   *Phylogenetic analysis*

134   We used NCBI BLASTn to identify related mycoplasma (16S rRNA, 23S rRNA, *rpoB*) and
135   bartonellae sequences (*gltA*), which we aligned with our sequences and reference sequences
136   using MUSCLE. We used MrBayes for phylogenetic analysis, with each gene tree run for
137   20,000,000 generations using a GTR+I+G model. BLASTn was implemented in Geneious,
138   whereas MUSCLE and MrBayes were implemented using NGPhylogeny.fr (29). We delineated
139   genotypes of hemoplasmas and genogroups of bartonellae based on pairwise similarity among
140   sequences and clustering on their phylogenies, using established criteria for defining novel
141   bacterial lineages (6, 30). For hemoplasmas, we also used multi-loci data to propose novel
142   *Candidatus* species when the same genotype was identified in at least two samples using 16S
143   rRNA and one other marker (i.e., 16S rRNA and 23S rRNA, 16S rRNA and *rpoB*) (7, 31).
144   We conducted two tests to assess if pathogen lineages were unique to each Mexican free-
145   tailed bat colony, which would suggest geographically constrained transmission dynamics. First,
146   we used chi-squared tests with *p* values generated by a Monte Carlo procedure to quantify
147   associations between geography and pathogen lineage assignments. Next, for any lineages
148   identified in multiple colonies with sufficient sample size, we derived matrices of spatial and
149   phylogenetic distance among sequenced PCR-positive samples and used Mantel tests with the
150   *vegan* R package to assess isolation by distance (32). These tests used 1,000 randomizations.
151
152   **Results**
153   *Migratory and seasonal effects on bat bacterial infection*
154   We detected hemoplasmas in 21 of 209 Mexican free-tailed bats when targeting the partial 16S
155   or 23S rRNA genes (10.5%, 95% CI: 7.1−15.4%). Sequencing of the 23S rRNA gene showed
156   three other bats (two from Texas and one from Oklahoma) had non-hemotropic mycoplasmas
157   (OQ359169–70, OQ359174) most related to *Mycoplasma muris* (97.5% sequence identity). We
158   detected bartonellae in 53 of 208 tested bats (25.5%, 95% CI: 20–31.8%). Only six Mexican
159   free-tailed bats were coinfected by bartonellae and any mycoplasmas (2.9%, 95% CI: 1.3–6.1%).
160   Hemoplasmas were detected in all three Mexican free-tailed bat colonies, while bartonellae were
161   only detected in the Texas and Oklahoma colonies. PCR positivity data are fully available in the
162   Pathogen Harmonized Observatory (PHAROS): https://pharos.viralemergence.org/ (33).
163   Across all Mexican free-tailed bats (model 1), the odds of infection differed by colony for
164   bartonellae ($\chi^2 = 9.72$, $p < 0.01$) but not hemoplasmas ($\chi^2 = 0.02$, $p = 0.99$; Figure 1). When
165   comparing only the resident and partially migratory populations in the non-breeding season
166   (model 2), Louisiana and Texas bats did not differ in the odds of either infection (hemoplasmas:
167    $\chi^2 = 1.21$, $p = 0.27$; bartonellae: $\chi^2 = 0$, $p = 1$). When comparing only the partially and fully
168   migratory populations in spring (model 3), we did not detect colony differences for hemoplasmas
169   ($\chi^2 = 0$, $p = 1$) or bartonellae ($\chi^2 = 0.11$, $p = 0.74$). When assessing risk factors of infection in the
170   partially migratory Texas colony (model 4), we found no evidence of seasonal or demographic
171   effects for either pathogen (Table S2). However, when assessing these predictors for the fully
172   migratory Oklahoma colony across the full 2022 occupancy period, we identified significant
173   seasonality in infection (Table S3). Prevalence increased from spring arrival and peaked in the
174   maternity season (i.e., June 2022) for both bacteria, declining into fall migration (Figure 1).
175
176   *Genetic diversity of Mexican free-tailed bat hemoplasmas*
177   Sequencing of 16S rRNA amplicons revealed four hemoplasma genotypes specific to Mexican
178   free-tailed bats (i.e., TB1–4; Figure 2, Table 1). The TB1 genotype was 97% similar to the MR1
179   genotype that we earlier isolated from another molossid bat (*Molossus nigricans*) in Belize (e.g.,

180   MH245174) (6), and TB1 was found in all three sampled populations. In contrast, TB2 was only
181   detected in the Texas colony and was ~96–97% similar to hemoplasmas from carnivores and
182   rodents and to the Belize bat MR1 genotype (6, 34, 35). Both TB3 and TB4 were only detected
183   in Oklahoma and were ~99% similar to MR1. We also detected the PPM1 genotype, originally
184   found in *Pteronotus mesoamericanus* and *P. fulvus* in Belize (6, 7), in the Oklahoma colony.
185   Amplification of the 23S rRNA gene from two bats (one each from Texas and Oklahoma) also
186   found a genotype we initially detected in cave myotis (i.e., MV1; Figure S1) and the above
187   *Mycoplasma muris*–like (non-hemotropic) genotype. These seven mycoplasma genotypes were
188   not associated with geography ($\chi^2 = 13.07$ $p = 0.38$; Figure S2). When considering the one
189   genotype observed in multiple colonies and in more than one bat per site (TB1), we also found
190   little support for isolation by distance with the 16S rRNA phylogeny (Mantel $r = 0.35$, $p = 0.10$).
191   Amplification of paired partial 23S rRNA (Figure S1) and/or *rpoB* (Figure S3) genes for
192   samples belonging to these 16S rRNA genotypes suggested at least three novel *Candidatus*
193   hemoplasma species circulate in Mexican free-tailed bats. Based on 100% identity of two *rpoB*
194   sequences (OQ554335–36) and 100% identity of paired 16S rRNA sequences included in the
195   TB1 genotype, first detected in Bracken Cave (OQ407846, OQ407848), we propose the name *C.*
196   Mycoplasma haematobrackenitadaridae sp. nov. Similarly, given 99.98% identity among seven
197   23S rRNA sequences (OQ359161–65, OQ359168, OQ359172) and 100% identity in paired 16S
198   rRNA sequences included in the TB3 genotype from the Selman Bat Cave (OQ407832,
199   OQ407836–39, OQ407843–44), we propose the name *C.* M. haematoselmanitadaridae sp. nov.
200   Lastly, given 100% identity of two 23S rRNA sequences (OQ359160, OQ359166) and 99.8%
201   identity in paired 16S rRNA sequences from the TB4 genotype (also identified from the Selman
202   Bat Cave; OQ407831, OQ407840), we propose the name *C.* M. haematotraderitadaridae sp. nov.
203   (Figures 2 and S1), based on the stream running adjacent to the bat cave (Traders Creek) (36).
204   Given the similarity of Mexican free-tailed bat hemoplasma 16S rRNA sequences to
205   those from molossid bats sampled in Belize (6), we also attempted to amplify the 23S rRNA and
206   *rpoB* genes from *Molossus nigricans* sampled in 2017 and 2018 in Belize that previously tested
207   positive for the MR1 and MR2 genotypes (6). We re-extracted DNA from four FTA cards and
208   applied the same additional PCR protocols described earlier. We obtained partial 23S rRNA and
209   *rpoB* sequences for two (OQ518943–44) and three (OQ554329–31) *M. nigricans*, respectively.
210   Based on 100% inter-sequence similarity of the *rpoB* sequences and high ($\bar{x} = 99.98\%$) identity
211   of paired 16S rRNA sequences (MH245122, MH245172, MH245174), we propose the name *C.*
212   Mycoplasma haematomolossi sp. nov. to designate this novel hemoplasma (Figures 2 and S3).
213
214   *Genetic diversity of Mexican free-tailed bat bartonellae*
215   Sequencing of *gltA* amplicons next revealed at least six *Bartonella* genogroups circulating in
216   Mexican free-tailed bats (Figure 3, Table 1). The first genogroup was detected in both Texas and
217   Oklahoma, with sequences from all three sampled months across 2021 and 2022 from Bracken
218   Cave but only from May and June in the Selman Bat Cave. The *gltA* sequences were ≥99.6%
219   similar to those recently detected in Mexican free-tailed bats in Argentina (KX986617) (20),
220   such that we consider these sequences to all form the TB1 genogroup. The TB2–4 genogroups
221   were only found in the Oklahoma colony. TB2 was identical to sequences from streblid bat flies
222   in Mexico (e.g., ≥99.7% identity to MF988072) (37), while TB3 represents a novel genogroup
223   with ~92% similarity to *Bartonella vinsonii* (e.g., MK984790) (38). Likewise, TB4 was novel
224   and distantly related (~91%) to *Bartonella quintana* (i.e., Z70014) (39). Oklahoma bats also
225   harbored g*ltA* sequences with 97–100% identity to *Bartonella rochalimae* in fleas from foxes

226 (OQ436435) (40); these *B. rochalimae* sequences were only detected in summer months. Lastly,
227 Oklahoma bats had *gltA* sequences in the same clade as bartonellae originally found in vampire
228 bats (*Desmodus rotundus*; DR8 genogroup) (26). These six *Bartonella* genogroups were not
229 associated with geography ($\chi^2 = 8.20$, $p = 0.16$; Figure S4), and we also found no support for
230 isolation by distance for the TB1 genotype with our *gltA* phylogeny (Mantel $r = -0.04$, $p = 0.59$).
231

Bacterial infections of sympatric bat species
233 Opportunistic sampling of other bats in Oklahoma revealed further bacterial diversity (Table 1).
234 Three of the four cave myotis and both pallid bats tested positive for hemoplasmas, whereas
235 three of three cave myotis, both pallid bats, and the single Townsend's big-eared bat tested
236 positive for bartonellae; the single hoary bat tested negative for both bacterial pathogens.
237 For hemoplasmas (Figure 2), we identified a single novel hemoplasma genotype in each
238 PCR-positive species (i.e., MV1 and AP1), with 16S rRNA sequences most closely related (i.e.,
239 ≥98% similarity) to previously detected genotypes in vesper bats from Chile and Belize (e.g.,
240 EF1 and MYE) (6, 19). Notably, 16S rRNA sequences of the AP1 genotype were 96.5% similar
241 to those of *Candidatus* Mycoplasma haemohominis (i.e., GU562823), whereas those of the MV1
242 genotype were only ~94% similar. 23S rRNA and *rpoB* sequences from cave myotis
243 (OQ359173, OQ554337) were entirely novel (<85% similarity to GenBank sequences), with the
244 former 100% similar to select 23S rRNA sequences identified from our Mexican free-tailed bats.
245 For bartonellae (Figure 3), all three positive cave myotis had their own genogroup. One
246 *gltA* sequence was ~98% similar to that first identified in little brown bats (*Myotis lucifugus*)
247 elsewhere in North America (i.e., KX807172), here denoted the ML1 genogroup. Another cave
248 myotis had the TB4 genotype. The final cave myotis *gltA* sequence clustered within a clade of
249 *Candidatus* Bartonella mayotimonensis sequences (~96%), isolated from a human endocarditis
250 patient in Iowa, USA (FJ376732) and other little brown bats (KX807177–9) (11, 41). For pallid
251 bats, one sequence also belonged to the TB4 genogroup, whereas the other formed the novel AP1
252 genogroup most related (~95%) to bartonellae from Natal long-fingered bats (*Miniopterus*
253 *natalensis*) and their bat flies in South Africa (e.g., MW007711 and MW007707) (42). Lastly,
254 the single positive Townsend's big-eared bat hosted a unique genogroup (CT1) only ~94%
255 similar to bartonellae found from *Myotis nigricans* in Guatemala (e.g., MN529509) (5).
256

Discussion
258 Hemoplasmas and bartonellae are emerging as model systems for studying bacterial infections in
259 bats (5, 7), but their infection dynamics and diversity remain poorly characterized, notably in
260 North American systems (11, 12). We here demonstrate novel diversity of hemoplasmas and
261 bartonellae in bats in the south-central United States, including the circulation of lineages of both
262 pathogens with clear infection seasonality in a migratory colony of Mexican free-tailed bats.
263 Such work provides the foundation for further empirical studies to elucidate the transmission
264 dynamics of these bacteria, their pathogenicity in bats, and their possible zoonotic risk.
265 Our findings suggest relatively common infection with site-specific and panmictic
266 bacterial lineages. Within both bacterial genera, most lineages found in Mexican free-tailed bats
267 were restricted to a single site, indicating spatially constrained transmission. However, we also
268 detected lineages in multiple sites, such as the TB1 hemoplasma genotype in Louisiana, Texas,
269 and Oklahoma; TB1 and MV1 hemoplasma genotypes as well as the *Mycoplasma muris*–like
270 genotype in Texas and Oklahoma; and TB1 *Bartonella* genogroup in Texas and Oklahoma (for
271 which sequences were nearly identical to those from *Tadarida brasiliensis* in Argentina) (20).

272 Such results may be explained by migratory connectivity in Mexican free-tailed bats, for which
273 regional migrations spanning hundreds to over 1,000 kilometers have been well-characterized in
274 North America, including between the Selman Bat Cave and Bracken Cave (14, 15, 43); this
275 suggests the migratory behavior of this species can enhance bacterial dispersal. However, given
276 the presence of these bacterial lineages in migratory and non-migratory populations (i.e.,
277 Louisiana) and at the extremes of the bat range (e.g., over 5,000 km between the Selman Bat
278 Cave and the Argentina site for the TB1 *Bartonella* genogroup), these results also suggest the
279 ancestral spread of these bacteria and limited selection pressure on lineages across the bat range.
280 Future studies are needed to identify the migratory routes of Mexican free-tailed bats,
281 especially for understanding the origins of possibly zoonotic bacterial lineages and the potential
282 for these bats to disperse infection during spring and fall migrations. Researchers could capitalize
283 on advances in tracking small vertebrates for long periods, such as use of absorbent sutures, to
284 ensure lightweight radiotags stay attached to bats for the duration of migration and winter (44).
285 Such work is also needed to assess if these infections negatively impact bat migration trajectory
286 and success, as observed for blood pathogens in migratory songbirds (45). Longitudinal studies
287 would also inform such analyses, as our data from one full occupancy season in the Oklahoma
288 colony suggest bacterial prevalence peaks in the maternity season. Additional seasonal sampling
289 is needed to assess how infection risk varies across the full migratory cycle, if prevalence tracks
290 bat population size and/or ectoparasite intensity, and whether infections are sufficiently common
291 in autumn to facilitate their dispersal with migration. Further genetic analyses could also inform
292 patterns of bat connectivity and pathogen spread. For example, the TB2 *Bartonella* genogroup
293 from Oklahoma Mexican free-tailed bats showed 100% identity to bartonellae from bat flies
294 from Morelos, Hidalgo, and Jalisco in central Mexico (37), spanning the likely wintering sites of
295 this bat species (14, 15). Similarly, previous analyses of *Trypanasoma cruzi* from this same
296 Oklahoma bat population detected lineages similar to those along the Texas–Mexico border,
297 further showing possible southern origins of infection and high pathogen dispersal capacity (46).
298 Additional molecular and immunological studies are also needed to better characterize
299 these novel bat bacterial pathogens and their health impacts. We identified 16S rRNA and *gltA*
300 sequences with moderate-to-high similarity to zoonotic pathogens such as *C.* Mycoplasma
301 haemohominis, *C.* Bartonella mayotimonensis, and *Bartonella rochalimae* (9, 41, 47). For the
302 former two pathogens, our bat sequences were ~96% similar to zoonotic lineages, likely
303 indicating divergence from a common ancestor at least tens of million of years ago (7, 48). *B.*
304 *rochalimae* has been found in cats and dogs (49), and our detections in Mexican free-tailed bats
305 indicate a broadening of host range into bats. Our sequences showed ~97–100% similarity to
306 those from fox fleas (40), relevant given likely flea transmission (49) and detection of fleas in the
307 Oklahoma bat population where these sequences were found (Dyer, personal communication).
308 Generation of whole genomes for our novel bat pathogens could inform their zoonotic risk, both
309 by better linking them to cryptic human infections (9) and by facilitating machine learning
310 models that predict zoonotic potential from genomic composition, as applied for viruses (50).
311 Other -omics analyses could also elucidate whether these bacterial infections are pathogenic in
312 bats themselves. In addition to assessing impacts of infection on migration outcomes as noted
313 above, approaches such as transcriptomics and proteomics could test if bats have a pronounced
314 immune response to these bacterial infections or appear largely tolerant (51). Such studies could
315 be especially informative when comparing immunity between migratory and non-migratory
316 periods, which could test whether long-distance migration may disrupt immune tolerance in bats.

317   Lastly, Mexican free-tailed bats and their sympatric bat species provide several important
318 ecosystem services, including but not limited to predating on crop pests and contributing to the
319 tourism economy from bat flight watching (52, 53). Understanding the prevalence, genetic
320 diversity, and pathogenicity of bacterial pathogens in bats can inform One Health approaches
321 that emphasize conservation measures to promote bat, domestic animal, and human health (54).
322

Tables

Table 1. *Mycoplasma* spp. (A) and *Bartonella* spp. (B) lineages identified from Louisiana, Texas, and Oklahoma bats during this study (2021–2022). 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.

	Lineage	States	Bat species	Mean intra-lineage similarity (%)
(A)	TB1[*]	LA, TX, OK	*Tadarida brasiliensis*	99.6[i], 100[iii]
	TB2[*]	TX, OK	*Tadarida brasiliensis*	100[i]
	TB3[*]	OK	*Tadarida brasiliensis*	100[i], 100[ii]
	TB4[*]	OK	*Tadarida brasiliensis*	100[i], 100[ii]
	PPM1	OK	*Tadarida brasiliensis*	NA[¶]
	MV1[*]	TX, OK	*Myotis velifer*, *Tadarida brasiliensis*	100[i], 100[ii]
	AP1[*]	OK	*Antrozous pallidus*	100[i]
	M. muris–like[†]	TX, OK	*Tadarida brasiliensis*	99.3[ii]
(B)	TB1	TX, OK	*Tadarida brasiliensis*	96.8[iv]
	TB2[*]	OK	*Tadarida brasiliensis*	NA[¶]
	TB3[*]	OK	*Tadarida brasiliensis*	97.2[iv]
	TB4[*]	OK, TX	*Tadarida brasiliensis*, *Myotis velifer*, *Antrozous pallidus*	97.4[iv]
	Bartonella rochalimae	OK	*Tadarida brasiliensis*	97.5[iv]
	DR8	OK	*Tadarida brasiliensis*	98.2[iv]
	ML1	OK	*Myotis velifer*	NA[¶]

C. Bartonella mayotimonensis–like	OK	*Myotis velifer*	NA[¶]
AP1[*]	OK	*Antrozous pallidus*	NA[¶]
CT1[*]	OK	*Corynorhinus townsendii*	NA[¶]

342 [*] Novel lineages
343 [†] Non-hemotropic mycoplasma
344 [i] 16S rRNA sequence
345 [ii] 23S rRNA sequence
346 [iii] *rpoB* sequence
347 [iv] *gltA* sequence
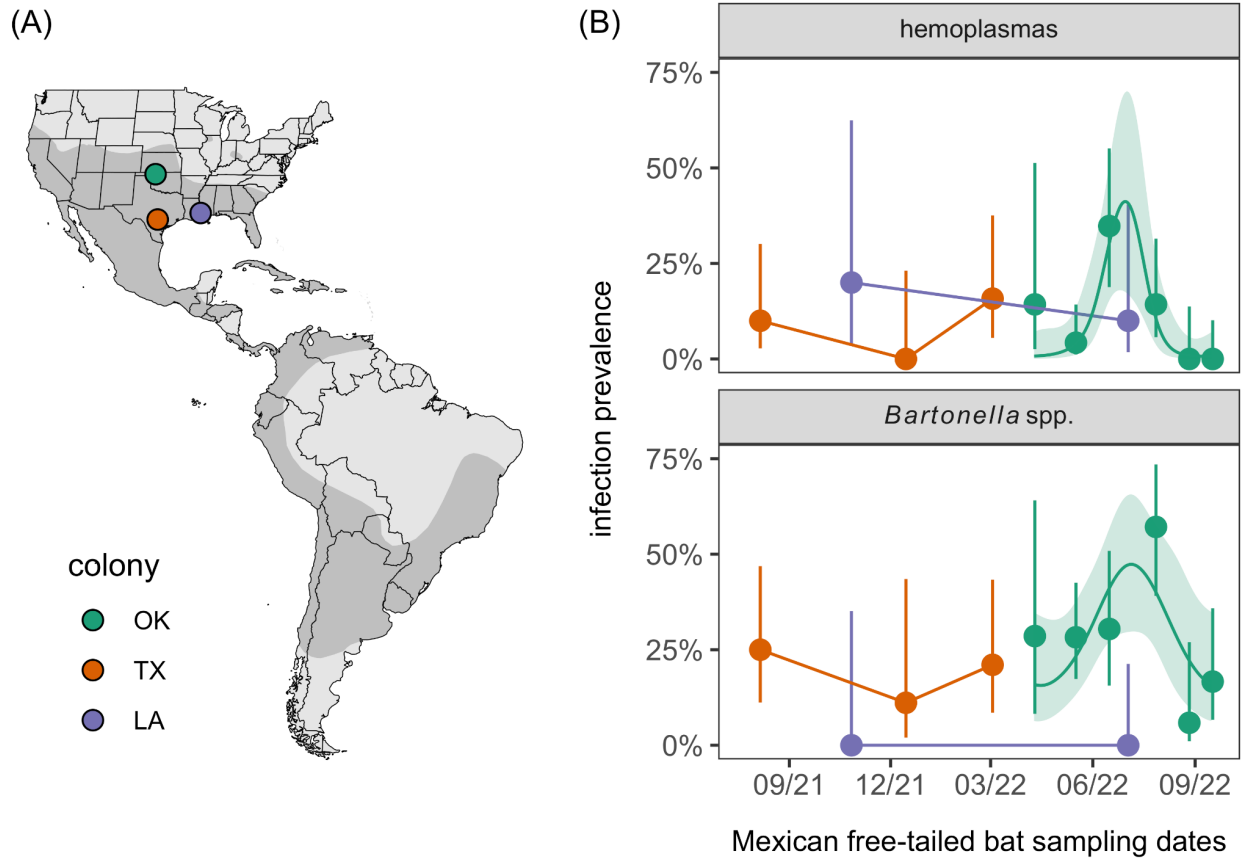348 [¶] Single sequence
349

350 **Figures**

351

352 Figure 1. (A) Sampled Mexican free-tailed bat (*Tadarida brasiliensis*) colonies relative to the

353 host distribution in the Americas. (B) Hemoplasma and *Bartonella* spp. infection prevalence

354 across months and colonies; segments denote 95% confidence intervals using Wilson's method.

355 For Oklahoma bats,

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

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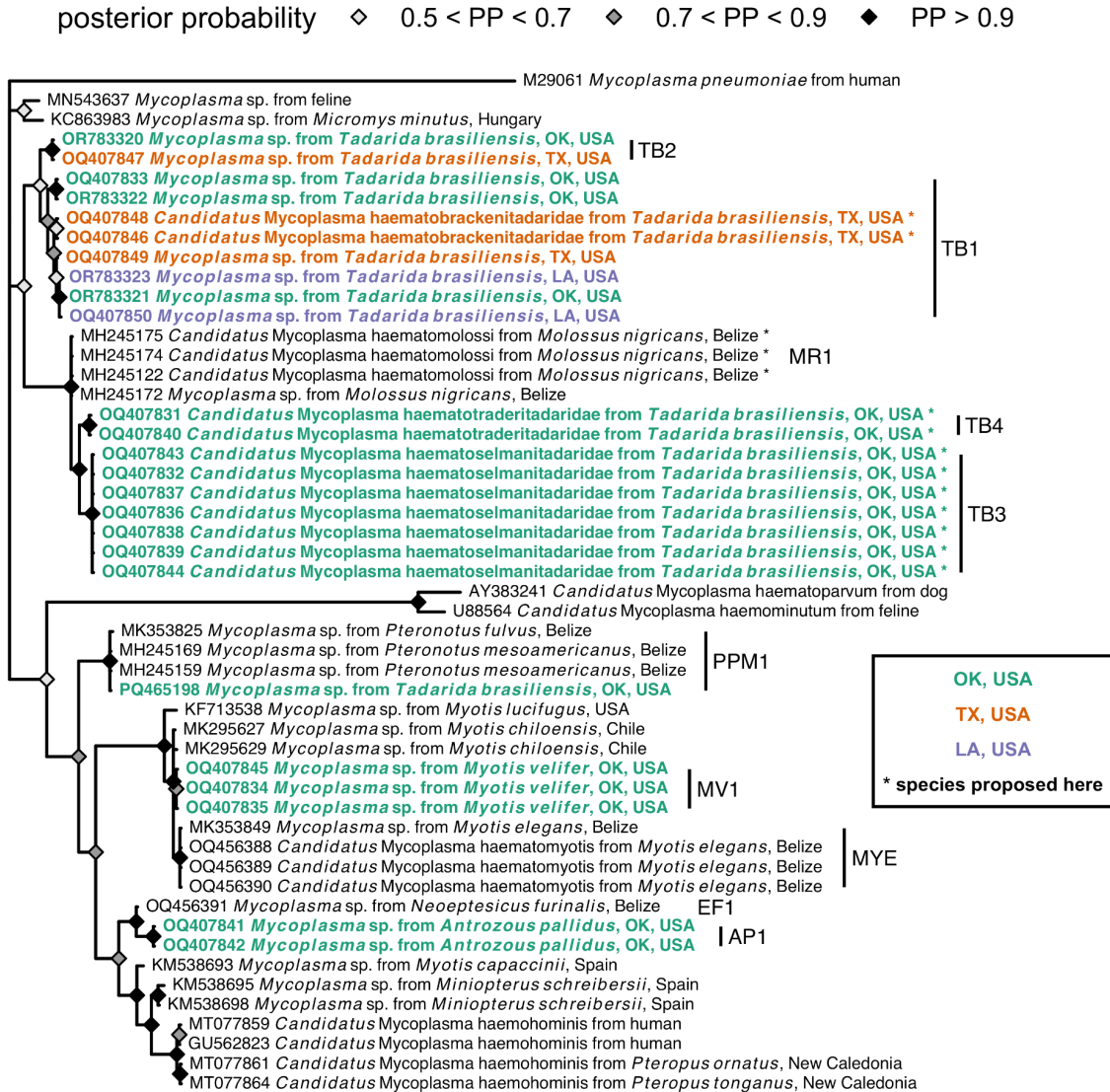
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369 Figure 3. Consensus Bayesian phylogeny of the partial *gltA Bartonella* spp. sequences from this
370 study (highlighted in bold and colored by geography; see Table 1 for genogroup assignments)
371 and reference sequences from bats, other mammals,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