

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

Table S1. PCR primers and amplification parameters used in this study

Table S2. GLM results for Mexican free-tailed bats from Bracken Cave, Texas

Table S3. GLM results for Mexican free-tailed bats from Selman Bat Cave, Oklahoma

Figure S1. Consensus Bayesian phylogeny of mycoplasma 23S rRNA sequences

Figure S2. Consensus Bayesian phylogeny of mycoplasma *rpoB* sequences

Table S1. PCR primers and amplification parameters used in this study.

Primers	Sequence	Organism and gene	Expected product (bp)	Temperature (°C) / time (seconds)				Cycles	Ref
				Initial denaturation	Denaturation	Annealing	Extension		
CS 443f	GCTATGTCTGCATTCTATCA	Bartonellae <i>gltA</i>	~700	95/120	95/30	48/30	72/120	40	1
CS 1210r	GATCYTCAATCATTCTTTCCA								
Bhcs 781p	GGGACCAGCTCATGGTGG		~300	95/180	95/30	55/30	72/120	40	2
Bhcs 1137n	AATGCAAAAAGAACAGTAAACA								
UNI_16S_hemoFnew	TGAATAAGTGACAGCWAACATATGTGCC	Hemoplasma 16S rRNA	~850–900	95/300	95/50	60/60	72/60	55	3* This study
UNI_16S_hemoR	GACGGGCGGTGTGTACAAGACCTG								
UNI_rpoB_hemoF1	CCTAAYTTRARYATWMGKGACGTTCACTATT C	Hemoplasma <i>rpoB</i> (primer set 1)**	~785	95/300	95/50	55/60	72/60	55	This study
UNI_rpoB_hemoR1_1	GAAGAMARRATAATDGCATCYTCATAGTTGT A								
UNI_rpoB_hemoF1	CCTAAYTTRARYATWMGKGACGTTCACTATT C	Hemoplasma <i>rpoB</i> (primer set 2)**	~1280	95/300	95/50	55/60	72/90	55	This study
UNI_rpoB_hemoR1_2	ACAGGAGTWCCATCYTCYARRTAWGGCAT								
UNI_23S_Myc_Ur_cladeF	CCCAGACCATKGGGYAAGCCTA	Hemoplasma 23S rRNA	~1500–80	95/300	95/50	58/60	72/90	55	3
UNI_23S_Myc_Ur_cladeR	GAGACAGTCAAGAGATGGTTACAC								

*Primers were slightly modified based on available hemoplasma 16S rRNA gene data

**Both sets of primers were used to amplify the hemoplasma *rpoB* gene. The *rpoB* primers were designed based on the conserved sequences found within the *rpoB* gene sequences of known hemotropic mycoplasmas and closely related *Mycoplasma* species.

1. Birtles RJ, Raoult D. Comparison of partial citrate synthase gene (*gltA*) sequences for phylogenetic analysis of *Bartonella* species. *International Journal of Systematic and Evolutionary Microbiology*. 1996;46(4):891-7.
2. Norman AF, Regnery R, Jameson P, Greene C, Krause D. Differentiation of *Bartonella*-like isolates at the species level by PCR-restriction fragment length polymorphism in the citrate synthase gene. *Journal of Clinical Microbiology*. 1995;33(7):1797-803.
3. Volokhov DV, Norris T, Rios C, Davidson MK, Messick JB, Gulland FM, Chizhikov VE. Novel hemotropic mycoplasma identified in naturally infected California sea lions (*Zalophus californianus*). *Veterinary Microbiology*. 2011;149(1-2):262-8.

Table S2. Results of GLMs with mean bias reduction for hemoplasma and *Bartonella* spp. positivity in Mexican free-tailed bat samples from Bracken Cave in Texas ($n = 48$ and $n = 45$, respectively; model 4). Reference levels include bats sampled in August 2021, males, non-reproductive bats, and pre-adult bats (juveniles and subadults).

Parameter	hemoplasmas			<i>Bartonella</i> spp.		
	OR	z	<i>p</i>	OR	z	<i>p</i>
Intercept		1.79	0.07		1.29	0.20
December 2021	0.05	1.42	0.16	0.77	0.12	0.91
March 2022	0.26	0.83	0.41	3.49	0.58	0.56
Female	0.64	0.36	0.72	0.23	1.32	0.19
Reproductive	0.25	0.76	0.44	5.93	0.77	0.44
Adult	18.35	1.45	0.15	1.16	0.07	0.95

Table S3. Results of GLMs with mean bias reduction for hemoplasma and *Bartonella* spp. positivity in Mexican free-tailed bat samples from Selman Bat Cave in Oklahoma ($n = 77$ and $n = 73$, respectively; model 5). Reference levels include bats sampled in June 2022, males, non-reproductive bats, and pre-adult bats (juveniles and subadults).

Parameter	hemoplasmas			<i>Bartonella</i> spp.		
	OR	z	<i>p</i>	OR	z	<i>p</i>
Intercept		0.65	0.51		1.01	0.31
May 2022	0.08	2.76	< 0.01	0.08	3.15	< 0.01
April 2022	0.16	1.38	0.17	0.07	1.51	0.13
Female	4.86	0.81	0.42	0.77	0.19	0.85
Reproductive	0.29	1.25	0.21	0.74	0.33	0.74
Adult	1.76	0.35	0.73	0.34	1.05	0.29

Figure S1. Consensus Bayesian phylogeny of partial 23S rRNA mycoplasma 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 16S rRNA sequences in Figure 1.



Figure S2. Consensus Bayesian phylogeny of partial *rpoB* mycoplasma 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 16S rRNA sequences in Figure 1.

