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

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

Figure S2. Consensus Bayesian phylogeny of mycoplasma rpoB sequences

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

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*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 hemotrophic 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).

	hemoplasmas			Bartonella spp.			
Parameter	OR	z	р	OR	z	р	
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	

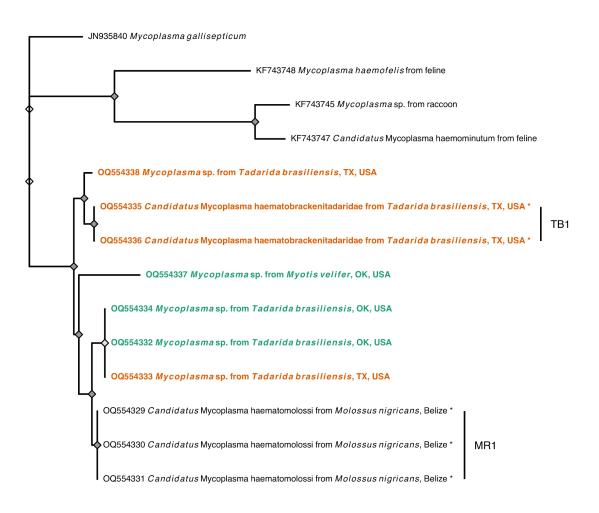
Table S3. Results of GLMs with mean bias reduction for hemoplasma and *Bartonella* spp. positivity in Mexican free-tailed bat samples from Selman Bat Cave in Oklahoma (n = 77 and n = 73, respectively; model 5). Reference levels include bats sampled in June 2022, males, non-reproductive bats, and pre-adult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

	he	moplasm	nas	Bartonella spp.			
Parameter	OR	z	р	OR	<i>z</i>	р	
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.

posterior probability \diamond 0.7 < PP < 0.9 \diamond PP > 0.9 NR_077056 Mycoplasma pneumoniae LR214962 Mycoplasma fermentans , LR215023 *Mycoplasma iowae* HM135466 Mycoplasma microti HM135461 Mycoplasma muris OQ359174 Mycoplasma sp. from Tadarida brasiliensis, OK, USA OQ359170 Mycoplasma sp. from Tadarida brasiliensis, TX, USA M. muris-like YOQ359169 Mycoplasmasp. from Tadarida brasiliensis, TX, USA NR_076944 Mycoplasma haemocanis from dog NR_103993 Mycoplasma haemofelis from feline OR055988 Candidatus Mycoplasma haematophyllostomi from Phyllostomus discolor, Belize OQ456392 Mycoplasma sp. from Neoeptesicus furinalis, Belize CQ359167 Mycoplasma sp. from Antrozous pallidus, OK, USA OQ518943 Mycoplasmasp. from Molossus nigricans, Belize OQ456384 Candidatus Mycoplasma haematomyotis from Myotis elegans, Belize OQ359173 Mycoplasma sp. from Myotis velifer, OK, USA • OQ359175 *Mycoplasma* sp. from *Tadarida brasiliensis*. OK. USA MV1 OQ359171 Mycoplasmasp. from Tadarida brasiliensis, TX, USA OQ518944 Mycoplasma sp. from Molossus nigricans, Belize OQ359160 Candidatus Mycoplasma haematotraderitadaridae from Tadarida brasiliensis, OK, USA * TB4 . OQ359166 *Candidatus* Mycoplasma haematotraderitadaridae from *Tadarida brasiliensis*, OK, USA * OQ359172 Candidatus Mycoplasma haematoselmanitadaridae from Tadarida brasiliensis, OK, USA * OQ359168 Candidatus Mycoplasma haematoselmanitadaridae from Tadarida brasiliensis, OK, USA * OQ359161 Candidatus Mycoplasma haematoselmanitadaridae from Tadarida brasiliensis, OK, USA * OQ359162 Candidatus Mycoplasma haematoselmanitadaridae from Tadarida brasiliensis, OK, USA * TB3 OQ359163 Candidatus Mycoplasma haematoselmanitadaridae from Tadarida brasiliensis, OK, USA * OQ359164 Candidatus Mycoplasma haematoselmanitadaridae from Tadarida brasiliensis, OK, USA * OQ359165 Candidatus Mycoplasma haematoselmanitadaridae from Tadarida brasiliensis, OK, USA *

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.



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