# Expanded diversity of hemoplasmas in rare and undersampled Neotropical bats

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**Abstract**

Hemotropic mycoplasmas are emerging as a model system for studying bacterial pathogens in bats, but taxonomic coverage of sampled host species remains poor. We leveraged a long-term field program in Belize to uncover novel hemoplasma diversity in bats by analyzing samples from species infrequently encountered in typical surveys. PCR targeting the partial 16S rRNA and 23S rRNA genes and subsequent phylogenetic analyses identified two putative novel host shifts of hemoplasmas, four entirely novel hemoplasma genotypes, and the first detections of hemoplasmas in four bat species. Our analyses demonstrate that additional genetic diversity of hemoplasmas in bats can be uncovered by targeting rare or otherwise undersampled host species.

**Main text**

Several decades of research have characterized the diversity of viruses in bats, for which some species host viruses of notable human health concern [1]. Although bats remain relatively understudied for other pathogens, the diversity of their bacterial pathogens has been of increasing interest [2]. Hemotropic mycoplasmas (hemoplasmas) are facultative intracellular bacteria that are emerging as a model group of bat pathogens [3]. Sampling globally has uncovered substantial genetic diversity of bat hemoplasmas [4–6], including high similarity to human hemoplasmas that may indicate zoonotic transmission [7,8]. However, most sampling has focused on relatively common bat species, leaving taxonomic gaps in understanding how hemoplasma diversity is distributed globally and which bats may harbor potentially zoonotic hemoplasmas [2,3]. Here, we leveraged an ongoing field program from which we previously identified 24 new hemoplasma genotypes in 23 bat species to test additional under- and unsampled species for these bacteria [4,9].

41 During April–May 2019, November 2021, and April–May 2022, we sampled a diverse
42 bat community in Orange Walk District, Belize [10]. Bats were captured using mist nets and harp
43 traps along flight paths and at roosts, held in individual bags until processing, and identified to
44 species based on morphology [11]. Blood volumes under 1% body mass were sampled by lancing
45 the propatagial vein using sterile needles and collected with heparinized capillary tubes. Blood
46 was preserved on Whatman FTA cards and held at room temperature until storage at -20C at the
47 University of Oklahoma. Field methods were approved by the Institutional Animal Care and Use
48 Committees of the American Museum of Natural History (AMNHIACUC-20190129 and -
49 20210614) and University of Oklahoma (2022-0197). Bat sampling was authorized by the Belize
50 Forest Department by permits FD/WL/1/19(06), FD/WL/1/19(09), and FD/WL/1/21(12).

51 We used Qiagen QIAamp DNA Investigator Kits to extract genomic DNA from 61 blood
52 samples from 17 bat species. Our prior studies and those of other groups had mostly minimally
53 surveyed 14 of these species [4,6,12], whereas *Lophostoma nicaraguae*, *Micronycteris microtis*, and
54 *Molossus alvarezi* remained wholly unsampled. To determine the presence of hemoplasmas, we
55 used PCR targeting the partial 16S rRNA gene with primers and procedures described previously
56 [4,9]. We also attempted to amplify the partial 23S rRNA gene for 16S rRNA–positive samples to
57 establish a broader library of this gene for hemoplasmas [13]. Amplicons were purified and directly
58 sequenced at Psomagen. We used NCBI BLASTn to identify related sequences, followed by
59 MUSCLE for sequence alignment and MrBayes for phylogenetic analysis (10,000,000
60 generations with a GTR+G+I model) using the NGPhylogeny.fr platform [14]. We delineated
61 hemoplasma genotypes based on analysis of the partial 16S rRNA gene (850–860 bp) and
62 clustering on the hemoplasma phylogeny, using previously established criteria for novelty [4,9].

We detected hemoplasmas via amplification of the 16S rRNA gene in 41% of samples (95% CI = 30–54%; Table S1 and Figure 1), with additional amplification of the 23S rRNA gene for six bats (GenBank: OQ456384–86, OQ456392, OR055987–88). Of the 11 bat species that tested positive, four had identical genotypes to those we earlier described in Belize [4]: *Myotis elegans* and the MYE genotype, *Carollia sowelli* and genotypes CS1 and CS2, *Glossophaga mutica* (formerly *G. soricina*) and the GLS genotype, and *Pteronotus fulvus* and the PPM1 genotype. All three *Phyllostomus discolor* had sequences 100% identical to partial sequences from *P. discolor* in Brazil [6], which we classify as the PD1 genotype. Finding the same genotype at the extremes of the *P. discolor* range (sites 5,000 km apart) and in two subspecies (*P. discolor discolor* and *verrucosus*) suggests the ancestral spread of this pathogen across the Americas.

The remaining six bat species were infected with novel genotypes in that host (i.e., host shifts or a new host-specific genotype) or represented the first hemoplasma detections for that species. For the former, we detected two genotypes in *Dermanura phaeotis* that were previously identified as specific to *Carollia sowelli* (CS1) and *Sturnira parvidens* (SP1, group B); the later genotype was also previously detected once in *Artibeus lituratus* [4], and we recently identified the group A variant in *Pteronotus mesoamericanus* [15]. Whereas the former four bat species belong to the family Phyllostomidae, *Dermanura* and *Carollia* belong to different subfamilies in that clade, suggesting putative inter-generic host shifts likely restricted by broader evolutionary history [4].

Novel hemoplasmas were detected in *Eptesicus furinalis*, *Molossus alvarezi*, *Lophostoma nicaraguae*, *Gardnerycteris keenani*, and *Noctilio leporinus*, with the latter four representing the first hemoplasma detections in these species and the latter three representing entirely novel hemoplasma genotypes. Although we previously characterized two genotypes in *Eptesicus furinalis* (EF1 and EF2, with the former also identified here) [4], we identified a third, novel

genotype (EF3) in a single *E. furinalis*, with 99.4% identity to EF1. A single *Molossus alvarezi* tested positive for the MR1 genotype (99.8% identity), previously identified only in the closely related *M. nigricans* in Belize [4]. We identified the novel NL1 genotype in *Noctilio leporinus*, one of two members of the family Noctilionidae; this genotype shared only 96.1% identity to the MYE genotype found in *Myotis elegans*, a distantly related vespertilionid [4]. We identified the novel LN1 genotype in a single *Lophostoma nicaraguae*, which shared 99.2% identity to the LE genotype previously identified in the closely related *L. evotis*[4] and 99.1% identity to the PD1 genotype in *P. discolor*[6]; all three bats are members of the Phyllostominae subfamily. Lastly, we detected a novel genotype (GK1) in a single additional phyllostomine (*Gardnerycteris keenani*), but GK1 was most related to the GLS genotype (97.6%) earlier found in *Glossophaga mutica* [4].

Our analyses demonstrate additional genetic diversity of hemoplasmas in bats uncovered by targeting less-sampled species. We encourage additional analyses of hemoplasmas in other unsampled bat taxa and geographies [2,3]. Further, longitudinal studies are needed to understand how these hemoplasmas circulate in bats, transmission risk to other species, and impacts on bats.

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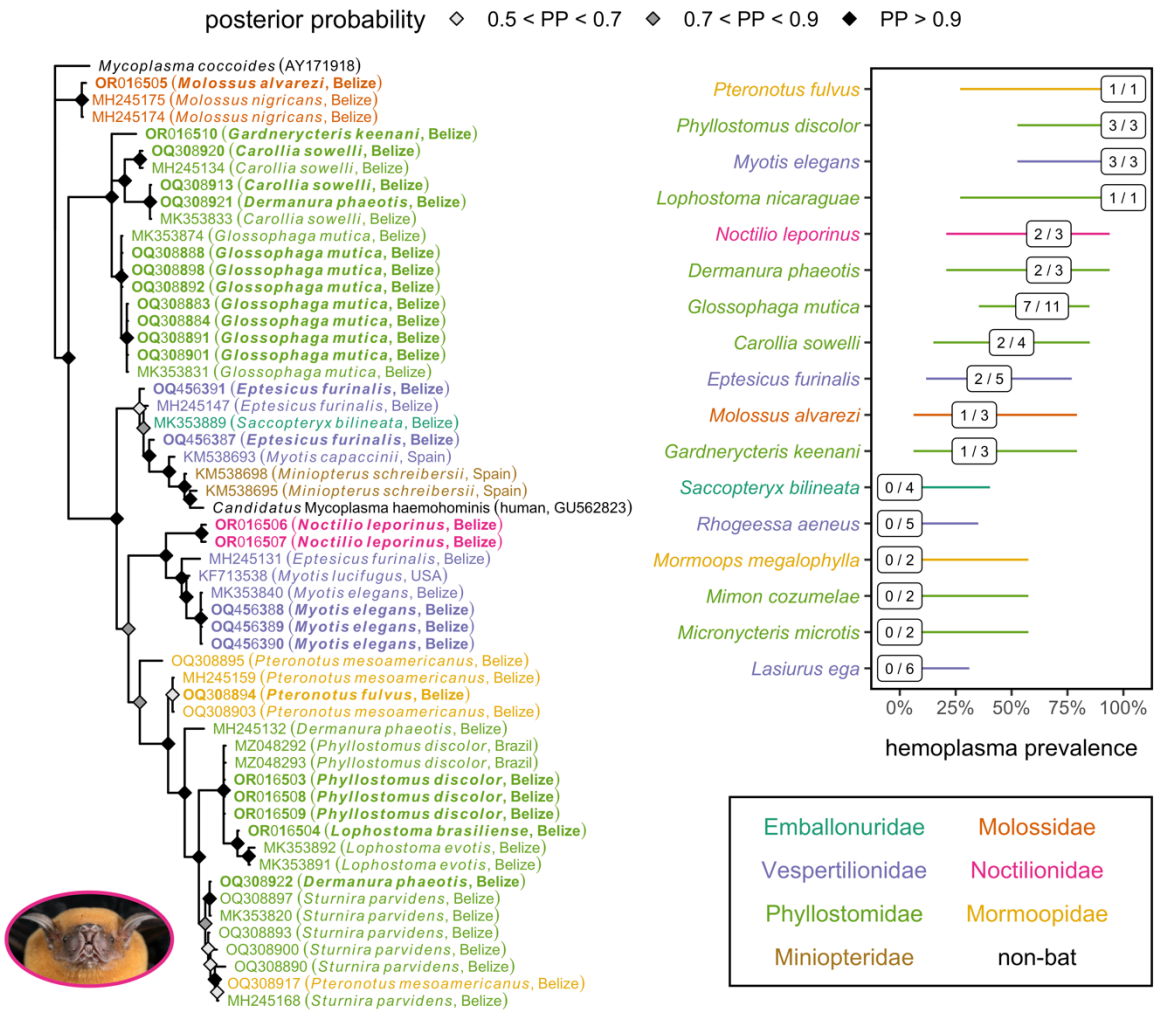
## Conflicts of interest

The authors declare no conflicts of interest.

**Figure 1.** Consensus Bayesian phylogeny of 16S rRNA hemoplasma sequences from this study (highlighted in bold; see Table S1 for genotype assignments) and reference sequences from bats and other host species. Nodes are colored by posterior probability (nodes with less than 50% support are not shown), and sequences from bats are colored by bat family. The inset shows hemoplasma prevalence per bat species sampled here ($n$ = 17) and associated 95% confidence intervals (Wilson score interval). Photograph of *Noctilio leporinus* by Sherri and Brock Fenton.



## References


1 Letko *et al*. Bat-borne virus diversity, spillover and emergence. *Nat Rev Microbiol* 2020; **18**: 461–471.

2 Szentivanyi *et al*. Trends in bacterial pathogens of bats: global distribution and knowledge gaps. *Transbound Emerg Dis* 2023; **2023**.

3 Millán *et al*. Worldwide occurrence of haemoplasmas in wildlife: insights into the patterns of infection, transmission, pathology and zoonotic potential. *Transbound Emerg Dis* 2020.

4 Becker *et al.* Ecological and evolutionary drivers of haemoplasma infection and bacterial genotype sharing in a Neotropical bat community. *Mol Ecol* 2020; **29**: 1534–1549.

5 Wang *et al.* High prevalence and genetic diversity of hemoplasmas in bats and bat ectoparasites from China. *One Health* 2023; **16**: 100498.

6 Ikeda *et al.* Molecular detection and genotype diversity of hemoplasmas in non-hematophagous bats and associated ectoparasites sampled in peri-urban areas from Brazil. *Acta Trop* 2022; **225**: 106203.

7 Millán *et al*. Widespread infection with hemotropic mycoplasmas in bats in Spain, including a hemoplasma closely related to '*Candidatus* Mycoplasma hemohominis'. *Comp Immunol Microbiol Infect Dis* 2015; **39**: 9–12.

8 Descloux *et al.* Flying fox haemolytic fever, description of a new zoonosis caused by '*Candidatus* Mycoplasma haemohominis'. *Clin Infect Dis* 2020.

9 Volokhov *et al*. Novel hemotropic mycoplasmas are widespread and genetically diverse in vampire bats. *Epidemiology & Infection* 2017.

10 Herrera *et al*. Disassembly of fragmented bat communities in Orange Walk District, Belize. *Acta Chiropterologica*. 2018; **20**: 147.

11 Reid. A field guide to the mammals of Central America and Southeast México: Oxford University Press. *New York* 1997; **334**.

12 Ikeda *et al.* Evidence and molecular characterization of *Bartonella* spp. and hemoplasmas in neotropical bats in Brazil. *Epidemiol Infect* 2017; **145**: 2038–2052.

13 Volokhov *et al.* Novel hemotrophic mycoplasma identified in naturally infected California sea lions (*Zalophus californianus*). *Vet Microbiol* 2011; **149**: 262–268.

14 Lemoine *et al.* NGPhylogeny.fr: new generation phylogenetic services for non-specialists. *Nucleic Acids Res* 2019; **47**: W260–W265.

15 DeAnglis *et al.* Bat cellular immunity varies by year and dietary habit in an increasingly fragmented landscape. *bioRxiv*. 2023.