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Worldwide occurrence of hemoplasmas in wildlife: insights into the patterns of infection, transmission, pathology, and zoonotic potential

Running title: Hemoplasmas of wildlife

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Abstract

Hemotropic mycoplasmas (hemoplasmas) have increasingly attracted the attention of wildlife disease researchers due to a combination of wide host range, high prevalence, and genetic diversity. A systematic review identified 75 articles that investigated hemoplasma infection in wildlife by molecular methods (chiefly targeting partial 16S rRNA gene sequences), which included 131 host genera across six orders. Studies were less common in the Eastern Hemisphere (especially Africa and Asia) and more frequent in the Artiodactyla and Carnivora. Meta-analysis showed that infection prevalence did not vary by geographic region nor host order, but wild hosts showed significantly higher prevalence than captive hosts. Using a taxonomically flexible machine learning algorithm, we also found vampire bats and cervids to have greater prevalence, whereas mink, a subclade of vesper bats, and true foxes all had lower prevalence compared to the remaining sampled mammal phylogeny. Hemoplasma genotype and nucleotide diversity varied little among wild mammals but were marginally lower in the Primates and Chiroptera. Coinfection with more than one hemoplasma species or genotype was always confirmed when assessed. Risk factors of infection identified were sociality, age, males, and high trophic levels, and both prevalence and diversity were often higher in undisturbed environments. Hemoplasmas likely use different and concurrent transmission routes and typically display enzootic dynamics when wild populations are studied longitudinally. Hemoplasma pathology is poorly known in wildlife but appears subclinical. *Candidatus* Mycoplasma haematohominis, that causes pathology in humans, probably has it natural host in bats. Hemoplasmas can serve as a model system in ecological and evolutionary studies, and future research on these pathogens in wildlife must focus on increasing the geographical range and taxa of studies and elucidating pathology, transmission, and zoonotic potential. To facilitate such work, we recommend universal PCR primers or NGS protocols to detect novel hemoplasmas and other genetic markers to differentiate among species and infer cross-species transmission.

Keywords: 16S rRNA, infection, meta-analysis, phylogeny, Mollicutes, wildlife.

Introduction

Studying how pathogens spread in natural populations and how they impact their hosts is critical for understanding infectious disease risks in wildlife and the potential for cross-species transmission (Plowright et al., 2019). Hemotropic mycoplasmas, widely known as hemoplasmas, are facultative intracellular erythrocytic bacteria or present as free-floating bacteria in the bloodstream and infect mammals, including domestic species, wildlife, and humans. Taxonomically, hemoplasmas were previously classified into the *Haemobartonella* and *Eperythrozoon* genera, but sequence analysis of the 16S rRNA gene showed that they belong to the class of Mollicutes of the family *Mycoplasmataceae* (Messick, 2004; Willi et al., 2007a). Phylogenetically, the hemotropic mycoplasmas cluster can be divided into two well-supported groups (i.e., suis and haemofelis groups) by bootstrap analysis (Volokhov et al., 2012). Most hemoplasma species have the *Candidatus* taxonomic status (Volokhov et al., 2012; Oren et al., 2020) because they have not yet been cultivated *ex vivo* in axenic culture, their species definitions are not yet fully established, there are no type strains available, and there are yet no established DNA bank(s) or international repositories for hemoplasmas (Firrao and Brown, 2013). As hemotropic mycoplasmas lack many of the metabolic pathways associated with energy production and synthesis of cell components found in other bacteria, they are fully dependent on host red blood cells and host growth factors, most of which are unknown (Citti and Blanchard, 2013; Guimaraes et al., 2014). Attempts to propagate some hemoplasmas (e.g., *M. suis* and *Ca.* M. haemohominis) *in vitro* in axenic culture have been made; however, their growth was either very slow or displayed short-term *in vitro* maintenance in culture without productive growth (Schreiner, 2011; Descloux et al., 2020). To date, no *in vitro* in axenic cultures are available for successful and efficient cultivation of hemoplasmas, and thus almost all known hemoplasma species are considered as uncultivable *in vitro*. At least a dozen of species of hemoplasma have been described in domestic animals (Supplementary File 1). Mixed infections with different hemoplasma species are common (Sykes and Tasker, 2013), including by hemoplasmas not yet taxonomically classified (e.g., Dieckmann et al., 2010).

Most if not all our knowledge about the pathology of these pathogens derives from domestic animals. Hemoplasmas can cause acute and chronic anemia, especially for immunocompromised hosts; however, many animals develop inapparent infections and are asymptomatic (Messick, 2004) (Supplementary File 1). Accumulating evidence shows that symptoms are generally more severe in immunocompromised, splenectomized, or immature individuals and when coinfected with different hemoplasmas and/or other pathogens (Sykes and Tasker, 2013). Some hemoplasmas are also more pathogenic than others. For example,

M. haemofelis (*Mhf*) is more pathogenic than the other feline hemoplasmas (Sykes and Tasker, 2013). Similarly, *M. ovis* can cause severe pathology and consequent economic losses in sheep (Neimark et al., 2004). Humans can also be infected by hemoplasmas. *Candidatus* Mycoplasma haematohominis (*C*Mhh) was first detected from a British patient and has subsequently been diagnosed in other rare cases of hemolytic anemia in humans worldwide (Steer et al., 2011; Alcorn et al., 2020; Descloux et al., 2020; Hattori et al., 2020).

Traditionally, diagnosis of hemotropic mycoplasmas was performed by examining blood smears. However, hemoplasmas are frequently absent on erythrocytes of infected animals, depending on the hemoplasma species (Sykes and Tasker, 2013). In addition, hemoplasmas cannot be cultured, further complicating diagnosis (Willi et al., 2007c). Currently, PCR is the test of choice for diagnosis in domestic animals (Sykes and Tasker, 2013). Most assays reported to date are based on detection and sequencing of the 16S rRNA gene and to a lesser extent of the 23S rRNA gene (Volokhov et al., 2011a), and/or the *RNase P RNA* gene (*rnpB*), the latter of which is sometimes used to discern between *Mycoplasma haemocanis* (*Mhc*) and *Mhf* (Birkenheuer et al., 2002), which are undifferentiable based on analysis of the 16S rRNA gene alone (Peters et al., 2008; Volokhov et al., 2017b). Although serologic tests have been developed for the detection of antibodies against the three feline hemoplasmas (Barker et al., 2010), those tests are not commercially available.

Owing to some of the above complications in diagnosing hemoplasmas, the routes of transmission for hemotropic mycoplasmas are far from being elucidated (Supplementary File 1). Fleas, ticks, and other arthropods have been implicated in the spread of some hemoplasmas (e.g., in felines; Willi et al., 2007a); however, in many cases, evidence for vector-borne transmission is circumstantial and lacks experimental confirmation. Other routes of transmission (direct, vertical, blood transfusion) have been demonstrated for some species (e.g., Museux et al., 2009; Cohen et al., 2018).

Although much research on hemoplasmas has traditionally concerned domestic animals, hemoplasmas have attracted the attention of wildlife disease specialists, especially during the last decade. Hemoplasma DNA has been detected in many wild species, showing in some cases remarkably high infection prevalence and 16S rRNA gene sequence diversity (e.g., Di Cataldo et al., 2020a). Closely related hemoplasmas are often detected in multiple host species, suggesting hemoplasmas may not strictly be host specialists. The combination of high prevalence, genetic diversity, and wide host range make hemotropic mycoplasmas attractive for the broader study of intra- and interspecific pathogen transmission (e.g., Becker

et al., 2020; Di Cataldo et al., 2020a). Here, we quantitatively synthesize the literature to date to determine geographic and taxonomic patterns in hemoplasma infection and genetic diversity in wild hosts. Next, we provide a critical review to identify knowledge gaps and prioritize key areas for future research.

Materials and Methods

Methodological considerations for hemoplasma species identification

Most of the studies included in the present review investigated hemoplasmas in wildlife on the basis of either full or partial 16S rRNA gene sequences, the latter of which comprise the majority of hemoplasma research. Therefore, the percentage of identity mentioned refers to that gene, unless otherwise indicated. Notwithstanding, the current genome-based taxonomy of Mollicutes, and especially uncultivated hemotropic mycoplasmas, is still under comprehensive investigation and is not yet well standardized (Gupta et al., 2018). The complete or draft (contigs) genomes of only nine hemoplasmas, *Mycoplasma parvum, M. suis, M. ovis, M. haemocanis, M. haemofelis, Ca.* M. haematolamae, *Ca.* M. haematominutum, *Ca.* M. haematohominis, and *Ca.* M. haematobovis, are currently available in GenBank, and current information on intraspecies and interspecies genomic differences of different hemoplasmas is very limited in comparison to other bacterial taxa. We only consider as valid the *Candidatus* species included in the most recent taxonomic consensus (Oren et al., 2020).

Search strategy and study selection

We performed a systematic search following the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guideline for systematic reviews (Moher et al., 2009). Our search included the databases Google Scholar, PubMed, Web of Science Core Collection, and Scopus, using the following query: ((“wildlife” OR “wild” OR “synanthropic”) AND (hemoplasma OR haemoplasma OR hemotropic mycoplasma OR haemotropic mycoplasma)).

References identified by the search were screened for inclusion criteria and relevance to the review question by two of the co-authors (JM and DJB). Discrepancies were resolved by consensus. Studies were selected using the following inclusion criteria: studies published from inception to November 1st, 2020, studies in English, published in peer-reviewed journals, that investigated non-domestic hosts (either wild, synanthropic, or captive) for hemoplasmas, and that used PCR and sequencing. Non-English studies, as well as grey literature, books, reviews, conference papers, and poster abstracts were excluded.

Quantitative data analysis

To compare estimates of hemoplasma infection prevalence, we only considered studies based on molecular tools such as PCR analysis and amplicon sequencing, as conventional light microscopy can generate false-negatives for PCR-positive individuals (e.g., Volokhov et al., 2011a; Suksai et al., 2016; Di Cataldo et al., 2020b) and also false positive results (e.g., Howel-Jolly bodies, artefacts, staining precipitates, etc.). From all studies, we extracted the number of sampled and hemoplasma-positive animals per country, host species, and population (i.e., wild or captive).

To first assess variation in study effort, we fit a generalized linear model (GLM) with the number of studies per combination of mammal host order and country as a Poisson-distributed response, with host order, geographic region (Western and Eastern Hemisphere), and their interaction as predictors in R. To next assess if hemoplasma prevalence varied geographically, across host orders, and between captive and wild animals, we used a phylogenetic meta-analysis. Because approximately 21% of all sampled host genera were not resolved to species (e.g., for bat species of the *Eidolon* genus), and most genera included only one (54%) or two (29%) species, we aggregated data to host genus, producing 223 records of prevalence (Supplementary file 2). We matched our 131 host genera against a recent mammal phylogeny (Upham et al., 2019) and used the *ape* and *treespace* packages to simplify the tree (Paradis et al., 2004; Jombart et al., 2017). Using the *metafor* package, we calculated the Freeman-Tukey double arcsine transformed proportion of hemoplasma-positive hosts and their sampling variances (Viechtbauer, 2010). We then fit hierarchical meta-analysis models with observation-level random effects, alongside the genus-level phylogeny and country (Konstantopoulos, 2013).

To first assess heterogeneity in prevalence, we fit a random-effects model (REM; intercept only). We used restricted maximum likelihood to obtain unbiased estimates of the variance components, from which we derived I^2 to quantify the contribution of true heterogeneity to total variance in prevalence (Senior et al., 2016). We used these estimates to partition the variance attributed to each random effect; for host genus, we derived phylogenetic heritability (*H2*) as a measure of phylogenetic signal (Nakagawa and Santos, 2012). We used Cochran's Q to test if such heterogeneity was greater than expected by sampling error alone. We then fit a mixed-effects model (MEM) with the same random effects and fixed effects of geographic region, host order, and if sampled animals were wild or captive.

Because taxonomic patterns in hemoplasma infection can arise at other resolutions besides order (Becker et al., 2020), we ran several phylogenetic analyses. We used the *caper* package to assess phylogenetic signal in binary infection status (*D*; Orme et al., 2013) and prevalence (Pagel's λ; Pagel, 1999). As the odds of pathogen detection often increase with sampling effort (Shaw et al., 2001), we used a phylogenetic GLM and phylogenetic generalized least squares (PGLS) model to test whether sample size per genus predicted infection status (i.e., apparent resistance or susceptibility) and prevalence, respectively. Lastly, we applied a novel graph partitioning algorithm, phylogenetic factorization, to flexibly identify host clades with significantly infection outcomes. With a standardized taxonomy from our mammal supertree (Upham et al., 2019), we used the *phylofactor* package to partition infection status and prevalence as binomial responses in a series of GLMs (Washburne et al., 2019). We included the total number of hosts sampled in the former as weights to account for uneven sampling effort (Crowley et al., 2020), and we determined the number of significant mammal clades in both models using Holm's sequentially rejective test with a 5% family-wise error rate.

To assess hemoplasma diversity, we analyzed nucleotide polymorphisms of 16SrRNA sequences using DnaSP.5 (Librado and Rozas, 2009) to obtain genotype diversity (Gd) and nucleotide diversity (π) (only from studies that used universal primers for hemoplasmas). Hemoplasma sequences were obtained from GenBank and grouped by host genus. We included only host genera with a sample size of at least three sequences. Before conducting analyses in DnaSP, we aligned sequences using ClustalW executed in Geneious Prime 2019.2.1 (https://www.geneious.com/prime; Biomatters Ltd.). As in our phylogenetic analyses of infection prevalence, we assessed phylogenetic signal in both diversity metrics per host genus. We then fit two, independent PGLS models to test whether genotype diversity and nucleotide diversity varied with sample size (the number of sequences per host genus) and between host orders.

Results and Discussion

Quantitative analyses of hemoplasma prevalence and diversity in wildlife

Our literature search identified 75 studies fulfilling our selection criteria of wildlife hemoplasmas, spanning the Western and Eastern Hemisphere and 131 host genera across six orders: Artiodactyla, Carnivora, Primates, Rodentia, Marsupialia, and Chiroptera. The number of hemoplasma studies varied strongly by geography (χ^2=16.46, p<0.001) and marginally by host order (χ^2=9.29, p=0.10) but not their interaction (χ^2=1.85, p=0.76). Wildlife hemoplasma studies were less common in the Eastern Hemisphere (RR=0.39), and

both bats (RR=0.40, p=0.01) and primates (RR=0.48, p=0.09) had generally fewer studies compared to more well-studied orders such as the Artiodactyla and Carnivora (Figure 1).

When considering hemoplasma surveillance across host genera, countries, and both wild and captive animals, we detected significant heterogeneity in infection prevalence (I^2=0.96, Q_{222}=3229, p<0.001). Host phylogeny accounted for most of this variation ($H2$=60%), and the REM estimated an adjusted mean prevalence of 25.6% (95% Confidence Intervals: 3.03%-59.9%). Our MEM found that hemoplasma prevalence did not vary by geographic region (Q_1=0.36, p=0.55) nor by host order (Q_5=1.68, p=0.89), but wild hosts, in general, did have significantly higher hemoplasma prevalence than captive hosts (β=0.14, z=2.37, p=0.02; Figure 2).

We next identified moderate phylogenetic signal in both the host genus-level infection status (D=0.81) and infection prevalence (λ=0.53). The odds of a genus being found to be infected by hemoplasmas increased with sample size (OR=1.02, p<0.01), but prevalence did not vary with sample size (β<-0.001, t=0.21, p=0.83). Phylogenetic factorization identified no taxonomic patterns in infection status (Figure 3A) but strong taxonomic patterns in prevalence beyond the resolution of mammal order (Figure 3B). For prevalence, we identified five clades with significantly different hemoplasma prevalence. The sampled Desmodontinae (i.e., *Desmodus* and *Diphylla*) and Cervidae (i.e., *Rangifer, Odocoileus, Mazama, Blastocerus, Ozotoceros*) had greater mean prevalence than the remaining mammal phylogeny (clade 1 and 2, respectively; x̄=0.66 and 0.62; Figure 3B). In contrast, the genus *Neovison*, a subclade of the Vespertilioninae (*Pipistrellus, Nyctalus, Vespertilio, Hypsugo*), and the genus *Vulpes* all had lower prevalence compared to the remaining sampled mammals (clade 3, 4, and 5, respectively; x̄=0.02, 0.02, and 0.04; Figure 3B).

Lastly, genotype diversity varied little among wild mammal genera ($\bar{x}$=0.75 ± 0.05 SE), and showed little phylogenetic signal (λ=0.17, Figure 4A). After excluding marsupials (n=1; *Didelphis*, which also had very low genotype diversity), we found a weak effect for diversity to decline with sample size (β=-0.01, t=1.77, p=0.09) and that primates had marginally lower diversity than other orders (β=-0.34, t=1.74, p=0.09); however, lower diversity in primates was driven by the genus *Sapajus*. Similarly, we observed that nucleotide diversity also varied little among mammal genera ($\bar{x}$=0.07 ± 0.02 SE) and showed no phylogenetic signal (λ=0); however, nucleotide diversity was not related to sample size (β<-0.001, t=0.15, p=0.88) but was marginally lower in primates (β=-0.16, t=1.80, p=0.08) and the Chiroptera (β=-0.13, t=1.71, p=0.10; Figure 4B)

Insights into hemoplasmas from terrestrial and aquatic mammals

Our literature search illustrated that hemoplasmas have been reported in non-chiropteran mammals in 21 countries (Figure 1), chiefly in the Americas (n=7; 5 in South America and 2 in North America) and Europe (n=7), with few reports from Asia (n=4) and Africa (n=3), and no information available from Australia. Hemoplasma infection has only been investigated in five out of the 18 orders (excluding the Chiroptera) that compose the class Mammalia (Supplementary File 2). Although we found weak variation in the number of studies per host order, the intensity of sampling has been disproportionate. In wildlife, hemoplasmas have been studied in 35 species of Carnivora, 13 species of Artiodactyla, eight species of Primates, and four species of Marsupialia. Although around 50 species of Rodentia have been investigated, almost all individuals were sampled in Brazil, primarily in a single study. Some other host species have been investigated in captivity, but carnivores and ungulates have again been the most studied taxa in such contexts. This bias is probably related to the genetic proximity of carnivores and artiodactyls to domestic species and thus the likelihood that these wild hosts could pose risks of pathogen spillover.

Infection with hemoplasmas has been detected in most wild animals when over 20 individuals were sampled. There are some notable exceptions, such as the 186 specimens of European water vole (*Arvicola terrestris*) analyzed by Willi et al. (2007), all of which were negative. However, it is important to note that these rodents were studied with the aim of detecting feline hemoplasmas, and thus only primers specific for known feline hemoplasmas were used. This likely prevented authors from detecting other hemotropic *Mycoplasma* species in rodents (see "Methodological considerations"). We here review key findings across studies from each sampled terrestrial host order.

Artiodactyls

Eighteen species of artiodactyls have been investigated to date for hemoplasmas, primarily in the Western Hemisphere, and mostly in the Cervidae, although some bovids and suids have also been studied. In particular, our analysis identified cervids as having significantly greater mean hemoplasma prevalence than the remaining mammal phylogeny (Figure 3B). In some studies with larger sample sizes, as many as 89% of white-tailed deer (*Odocoileus virginianus*) (Maggi et al., 2013a) and 72% of marsh deer (*Blastocerus dichotomus*) (A. Grazziotin et al., 2011) were infected. Three studies failed to detect hemoplasmas in ungulates, all of which sampled captive populations: one study included a very small sample of Barbary sheep (*Ammotragus lervia*) (Santos et al., 2017) whereas the other two sampled peccaries (*Tayassu* spp.) (da Costa

Vieira et al., 2011; Dias et al., 2019). However, these studies used *M. suis*-specific primers, which prevented detecting other hemotropic *Mycoplasma* species.

Hemoplasmas that have been detected in cervids are most closely related to those of sheep and cattle, such as *M. ovis*, *Ca.* M. haematovis, and *M. wenyonii*. The first description in a non-domestic ungulate species was in reindeers (*Rangifer tarandus*) kept in captivity in Michigan and Tennessee, USA (Stoffregen et al., 2006). Reindeers were infected by three genotypes, most closely related, respectively, with *M. ovis*, *M. wenyonii*, and *Mhc*/*Mhf*. The latter was designated as hemotropic *Mycoplasma* sp. clone 107LSIA based on 16S rRNA gene divergence (Stoffregen et al., 2006). Farmed white-tailed deer (*Odocoileus virginianus*) were found infected by a hemoplasma with 98.5% sequence identity with *M. ovis* (Boes et al., 2012). The first study dealing with free-ranging wild cervids described two *Candidatus* species in sika deer (*Cervus nippon*): *Ca.* M. erythrocervae, which was most closely related with hemotropic *Mycoplasma* sp. clone 107LSIA when described, and *Ca.* M. haematocervi. Although this species showed closed relatedness to *M. ovis* and *M. wenyonii* based on the 16S rRNA gene sequence, it was provisionally designated as a new species based on the differences of the *rnpB* gene (Watanabe et al., 2010). It was later shown that sika deer can be co-infected by both *Candidatus* species (Tagawa et al., 2013). Posterior studies have found different deer species in central Europe (Hornok et al., 2018), Brazil (Grazziotin et al., 2011; André et al., 2020) and the USA (Maggi et al., 2013a) infected with hemoplasmas that phylogenetically clustered with *M. ovis*, *M. wenyonii,* and/or *Ca.* M. haematobovis. Similarly, the only studies including an ovine (Hornok et al., 2012) and a caprine (Ootake et al., 2010) species identified, respectively, *M. wenyonii* and *Ca.* M. haematobovis, and *M. ovis*. The only study in a bovine included 97 wild buffaloes (*Syncerus caffer*) from Mozambique using species-specific primers, which detected both *M. wenyonii* and *Ca.* M. haematobovis, including four cases of coinfection (Gonçalves et al., 2018).

Among suids, only wild boars (*Sus scrofa*) have been investigated. In all cases, *M. suis* was the causative pathogen (Hoelzle et al., 2010; Dias et al., 2019). However, all studies used *M. suis*- and/or *M. parvum*-specific primers, which limits knowing whether wild suids can be infected with other hemoplasmas.

Unfortunately, no studies have yet compared hemoplasmas sequences from sympatric domestic and wild ungulates to determine the potential for cross-species transmission.

Carnivores

Carnivores have received much attention in proportion to their diversity, probably due to their close phylogenetic relationship with the two most common pet species (i.e., dogs and cats), and to the endangered status of many of them. The first study of wildlife hemoplasmas included a variety of wild cats "to investigate wild felid species as possible reservoirs of feline hemoplasmas" (Willi et al., 2007b). In contrast, other researchers perceived this relationship as a facilitator of cross-species transmission from domestic cats and dogs to endangered carnivores (e.g. Sacristán et al., 2019; Di Cataldo et al., 2020a).

From our literature search, carnivores were clearly the most intensively sampled order in terms of the number of studies (Figure 1) and have been sampled in both wild and captive contexts (Figure 2). Up to 47 species belonging to the families (in order of the number of studies) *Felidae, Canidae, Mustelidae, Procyonidae, Ursidae*, and *Viverridae* have been investigated. As in ungulates, studies including a sample size of at least five animals generally have found positive individuals, confirming that hemoplasma infection is also frequent in carnivores. However, observed prevalences are seemingly more variable. For example, a total of 353 red foxes (*Vulpes vulpes*) were investigated in three different studies in Japan, Slovakia, and Spain, with only 15 positives in total (Sasaki et al., 2008; Koneval et al., 2017; Millán et al., 2018); further, only three of 78 crab-eating foxes (*Cerdocyon thous*) were found hemoplasma-positive in Brazil (de Sousa et al. 2017). Indeed, our phylogenetic analyses found that animals in the genus *Vulpes* tended to have lower prevalence than other mammals (Figure 3B). In contrast, prevalences around 50-60% have been reported among Darwin's foxes (*Lycalopex fulvipes*) in Chile (Cabello et al., 2013; Di Cataldo et al., 2020a); raccoons (*Procyon lotor*), bobcats (*Lynx rufus*), and pumas (*Puma concolor*) (Kellner et al., 2018), and black bears (*Ursus americanus*) (Westmoreland et al., 2017) in USA; and Eurasian badgers (*Meles meles*) in Spain (Millán et al., 2018). Up to 78% of coatis (*Nasua nasua*) were positive in Brazil (de Sousa et al. 2017), but the most striking prevalence was found among Serengeti lions (*Panthera leo*), where 44 out of 45 individuals were positive (Willi et al., 2007b). Whether these differences correspond with true variations in susceptibility and not to methodological issues cannot be inferred without experimental studies; however, it is compelling that, in the same region and period, using the same tissues and protocols, prevalence was remarkably higher in badgers (58%) than in red foxes (2.5%) (Millán et al., 2018). Coinfection was a common finding in carnivores whenever it was investigated (see below).

Hemoplasmas sequences obtained from felids corresponded almost exclusively with known feline hemoplasmas, namely *Mhf*, *Candidatus* Mycoplasma haematominutum (*CMhm* and/or *Ca.* M. turicense (*C*Mt). However, species-specific primers were used in most studies, which prevented detecting other

hemoplasma species or genotypes. Indeed, sequences not related to the typical feline hemoplasmas have been described in two host species when universal primers were used. A sequence closely related to a rodent hemoplasma was detected in a guigna (*Leopardus guigna*) and a domestic cat in Chile (Sacristán et al., 2019). This sequence, hemotropic *Mycoplasma* sp. clone ZD019, was first found in a Darwin's fox also in Chile, and had 99-100% identity with a sequence detected in a South American grey fox (*L. griseus*) in Argentina (Millán et al., 2019b), raising the question of whether this is a new carnivore hemoplasma or the result of a spillover from prey to predators (or vice versa). Similarly, a sequence closely related to another rodent hemoplasma (*M. haemomuris*) was detected in two leopard cats (*Prionailurus bengalensis*) in Korea (Hwang et al., 2015). Indeed, it has been proposed that some feline hemoplasmas resulted from interspecies transmission between rodents and cats (Willi et al., 2005). However, none of the three typical feline hemoplasmas have been confirmed to infect rodents.

Similarly, wild canids appear to be infected by any of the two known dog hemoplasmas (more frequently *Mhc*), even when universal primers were used (e.g. Millán et al., 2019b). The only exceptions include the above-mentioned hemotropic *Mycoplasma* sp. clone ZD019 in two *Lycalopex* spp., the finding of *Mhf* in a Darwin's fox according to analysis of the *rnpB* gene (Cabello et al., 2013), and some intriguing findings in red foxes. Here, sequences showing highest identity to *C*Mt were reported in the only positive fox in Spain (Millán et al., 2018) and in six out of 13 positives in Slovakia (Koneval et al., 2017); additionally, sequences related to raccoon hemoplasmas were reported in two other red foxes in that country (Koneval et al., 2017).

Although our analyses did not find variation in hemoplasma genotype diversity among host orders, members of the families *Mustelidae, Procyonidae*, and *Viverridae* have shown especially high sequence variability. In the USA, six different genotypes were detected in a sample of 95 raccoons (Volokhov et al., 2017a). Two of these genotypes were closely related with *Mhc*/*Mhf* based on analysis of the 16S rRNA gene, but analysis of other genes indicated that these genotypes were unlikely to belong to the known species *Mhc* or *Mhf*. The other four genotypes were only 86-96% similar to previously described hemoplasmas, suggesting the presence of new species or genotypes (Volokhov et al., 2017a). In Brazil, several hemoplasma genotypes were found among coatis: ten sequences were closely related to *Mhc*/*Mhf* based on phylogenetic analyses of the 16S rRNA and RnaseP genes. Additionally, a new *Candidatus* Mycoplasma species was proposed for the hemoplasma detected in 14 coatis and one crab-eating fox, which grouped in a separate branch with high values of clade support and was closely related to a new hemoplasma genotype detected in a capybara (de

Sousa et al. 2017). In a study in Spain including several species of these Carnivora families, up to eleven genotypes were identified according to partial 16S rRNA sequences (Millán et al., 2018). Some genotypes appeared to be host specific, but many were shared between host species. Two genotypes, found in three different mustelid species, were closely (99-100%) related to different *Candidatus* Mycoplasma haematoparvum (*C*Mhp) sequences. One of these was prevalent in the pine marten (*Martes martes*; five out of nine readable sequences), raising the question of whether *C*Mhp may have originated in a wild carnivore (suborder Caniformia). Another genotype was frequently detected in common genets (*Genetta genetta*; four out of five readable sequences) but was also found in one Eurasian badger (*Meles meles*); this showed 99.3% identity with published *C*Mhm sequences, which again suggests that the genet (suborder Feliformia) might be a natural host for this hemoplasma. Four genotypes, found predominantly in badgers, showed between 98-99% identity with hemoplasmas from raccoons. One of these genotypes were markedly frequent in badgers, corresponding with 29 out of 37 readable sequences. A sequence similar to *Ca.* M. haemomeles was found in two badgers, and a further sequence showing only 88% identity with a raccoon hemoplasma was found in a badger. The authors proposed that this might correspond with a new species. Interestingly, six individuals from three species were infected with three sequences most closely related with two different hemoplasmas from Spanish bats, suggesting a possibly predatory route of infection. Unfortunately, the work by Millán et al. (2018) only reported sequences of approximately 380 bp, letting all these hypothesis unsolved. Two recent studies evaluated the presence of hemoplasmas in invasive Amercan minks (*Neovison vison*) in southern Chile. Whereas one study found no hemoplasma DNA in a sample of 50 individuals (Sepúlveda-García et al., In press), Zapararte et al. (2020) detected five infected individuals out of 246. This low prevalence might be related with the fact that American mink populations of Chile originated from individuals that escaped from fur farms, and we have shown that captive animals have lower prevalence that wild animals. Sequencing of the almost entire 16S rRNA gene yielded two different sequences, one most closely related with hemoplasmas from Brazilian rodents and the other with *C*Mhp and *C*Mhm. Analysis of the RNaseP gene in the latter case revealed a sequence with 95% identity with *Mhf* str. Ohio2.

There are only two studies of hemoplasmas in ursids. One study in Japan detected hemoplasma sequences in *Ursus thibetanus japonicus* most closely related to but distinct from *Mhc*/*Mhf* via analysis of the 16S rRNA gene and the ITS region (Iso et al., 2013). Another study found higher diversity among a sample of 68 *Ursus americanus* in North America (Westmoreland et al., 2017). Four genotypes were detected among 29 sequenced amplicons, 17 of which were most closely related to *C*Mhp. Eleven belonged to a genotype most

closely related to the sequence from the Japanese bear and, in consequence, with *Mhc*/*Mhf*. One sample was most closely related to a *Mycoplasma* sp. detected in an Australian dog (Westmoreland et al., 2017).

Hemoplasmas have also been studied in a limited sample of pinnipeds. *Ca.* M. haematozalophi was described in wild California sea lions (*Zalophus californianus*) admitted for rehabilitation in California, USA, or live captured in Oregon, USA (Volokhov et al., 2011b). Phylogenetic analysis of the resulting 16S rRNA sequences revealed that hemoplasma was only 92.1% similar to a previously described hemoplasma of alpacas, *Ca.* M. haematolamae. Hemoplasmas were detected in 12% of California sea lions and appeared to be asymptomatic. In contrast, hemoplasmas were not detected in northern elephant seals (*Mirounga angustirostris*; *n*=20) in the same study. Hemoplasma infections in other pinnipeds or other marine mammals (e.g., cetaceans) remain unstudied.

Primates

Compared to ungulates and carnivores, non-human primates have been less frequently sampled for hemoplasmas (Figure 1). Eleven species of non-human primates have been investigated for these pathogens. However, only eight were free-living and, of those, seven were studied in Brazil. Although it has been known that primates are susceptible to hemoplasmas (see Contamin and Michel, 1999 and references therein), initial molecular detections of hemoplasmas in a primate occurred in French Guyana with captive squirrel monkeys (*Saimiri sciureus*) (Neimark et al., 2002). Nevertheless, analysis of archived monkey samples allowed Barker et al. (2011) to identify hemoplasmas in the blood of an owl monkey (*Aotus trivirgatus*) imported into the United Kingdom from Colombia in the late 1960s. Provisional hemoplasma species in primates include *Ca.* M. kahanei in squirrel monkeys (Neimark et al., 2002), *Ca.* M. aoti in owl monkeys (Barker et al., 2011), and *Ca.* M. haematomacacae in cynomolgus monkeys (Maggi et al., 2013c), all of which were detected in captive animals.

Only five studies have examined hemoplasmas in wild primates, one in Japan and four in Brazil. A sequence showing 94% identity with *Ca.* M. kahanei was found in a howler monkey (*Alouatta caraya*) in Brazil (Santos et al., 2013). Sashida et al. (2014) found a single sequence in all nine Japanese monkeys (*Macaca fuscata*) sampled, which showed 98% identity with *Ca.* M. haematomacacae. Sequences retrieved from *Sapajus apella* (both wild and captive) from Brazil also showed high identity with *Ca.* M. haematomacacae and with sequence of the Japanese monkeys (Bonato et al., 2015). In that same study, a different sequence, 99% similar to *Ca.* M. kahanei, was found in two squirrel monkeys (Bonato et al., 2015). Cubilla et al. (2017b) reported the detection of two sequences from black howler monkeys showing ~94% identity with *Ca.* M.

kahanei sequences, which coincides with the finding of Santos et al. (2013). Finally, de Melo et al. (2019) studied 68 captive howler monkeys (chiefly *A. caraya*) from different institutions in Sao Paulo, finding hemoplasma DNA in 18 individuals. The sequenced 16S rRNA gene amplicons were closely related with previously reported sequences from South American primates. According to the authors, sequences from South American primates described so far belong to five genotypes, two belonging to the *Mhf* group, and three to the *M. suis* group (de Melo et al., 2019).

Rodents

Many species of wild rodents have been investigated for hemoplasmas, chiefly in Brazil (Figure 1). However, most wild rodent data belong to a single study, with generally small sample sizes per species (Gonçalves et al., 2015). Wild rodents were also investigated in Switzerland with the goal of determining their roles as natural hosts for feline hemoplasmas (Willi et al., 2007a). Prevalence in wild species with representative (>30) sample sizes range from 0% in *Arvicola terrestris* (0/186) and *Thricomys fosteri* (0/77) to 35% in *Necromys lasiurus* (18/51) and 47% in *Apodemus* sp. (24/51). Synanthropic rodents (especially rats *Rattus* spp. and house mice *Mus musculus*) have been also investigated in Brazil, Japan, Switzerland, Hungary, and Chile. In these species, hemoplasma prevalence was generally around or above 50% (Willi et al., 2007a; Sashida et al., 2013; de Oliveira Conrado et al., 2015; Gonçalves et al., 2015; Hornok et al., 2015), with the exception of a recent study in Chile (8%; Alabí et al., 2020).

Two species have been described in rodents, namely *Mycoplasma coccoides* and *Ca.* M. haemomuris. Both are largely known to infect laboratory and wild mice (Thurston, 1954; Kreier and Hall, 1968) and belong to the haemofelis group. Although it is almost impossible to determine which was the natural hosts of these species originally, it is worth mentioning that one of the first molecular studies of *Ca.* M. haemomuris was based in the small Japanese field mouse (*Apodemus argenteus*; Rikihisa et al., 1997). This suggests that some wild rodents are natural hosts for these species. In fact, most of the sequences retrieved from rodents from Brazil, Japan, and Switzerland are phylogenetically clustered with any of these two species. However, there are some exceptions. For example, capybaras (*Hydrochoerus hydrochaeris*) appear to have their own hemoplasma (uncultured *Mycoplasma* sp. clone 1), whose 16S rRNA sequences showed only 92% identity with the closest related sequence (*M. coccoides*) (Vieira et al., 2009). This genotype was later found in capybaras in another study (Gonçalves et al., 2020). Recently, a novel hemoplasma was described in hairy dwarf porcupines (*Sphiggurus villosus*) from Southern Brazil (Valente et al., 2020). According to phylogenetic

analysis of the 16S and 23S rRNA gene amplicons, this hemoplasma belongs to the *Mhf* group and clusters together with hemoplasmas from capybaras and carnivores (Valente et al. 2020). In Japan, a sequence showing 87% identity with *Ca.* M. hemomuris was detected in *Rattus norvegicus* (*Mycoplasma* sp. Japan N008; Sashida et al., 2013). In Hungary, a potentially novel species was detected in a sample of wild and synanthropic rodents. In that study, two 16S rRNA sequences showing 98% and 92-93% homology to *C*Mt were found in a harvest mouse (*Micromys minutus*) and a brown rat (*Rattus norvegicus*; Hornok et al., 2015). In that same study, another brown rat was infected with a hemoplasma closely related (99%) to the abovementioned *Mycoplasma* sp. Japan N008 (Hornok et al., 2015). In a larger study in Brazil, 10 genotypes were identified among several species of wild rodents and showed between 86-99% identity with known murine hemoplasmas (Gonçalves et al., 2015). In Chile, two variants were described in rodents with high identity to variants detected in wild rodents from Brazil (Alabí et al. 2020).

Marsupials

In contrast to the other terrestrial mammals described above, marsupials have received limited attention for hemoplasmas (Figure 1). The first report of a hemoplasma in a marsupial is of *Ca.* M. haematodidelphidis from the North American opossum (*Didelphis virginianus*) in the USA (Messick et al., 2002). Since hat study, representatives of four species of marsupials have been tested for the presence of hemoplasmas, all in Brazil. First, seven out of eight white-eared opossums (*Didelphis albiventris*) captured in a city park in Maringá (Paraná State) were infected with a hemoplasma showing 99% identity with *Ca.* M. haematodidelphidis. These sequences were included in the same well-supported branch with one of the sequences detected in raccoons in the USA (Massini et al., 2019). Identical sequences have since been confirmed in 14 out of 43 white-eared opossums from urban forests in Mato Grosso do Sul (Gonçalves et al., 2020). This hemoplasma has been recently proposed to be a novel species, named *Ca.* M. haemoalbiventris, according to sequence analysis of 16S and 23S rRNA gene amplicons (Pontarolo et al., 2020). In another study, none out of 30 specimens of four species, including some white-eared opossums, were infected by hemoplasmas (de Sousa et al., 2017).

Emerging hemoplasma patterns in bats

Although bats have been heavily studied for zoonotic viruses, increasing efforts have also focused on studying bacterial pathogens in the Chiroptera (Mühldorfer, 2013; Brook and Dobson, 2015). This is especially true for

hemoplasmas, for which bats have been increasingly sampled over the past decade, with research conducted in 12 countries (Figure 1) spanning the Americas (Costa Rica, Chile, Belize, Peru, Brazil, the United States), parts of Europe (Germany, Switzerland, Spain), and Oceania (Australia and New Caledonia); only one study has examined bat hemoplasmas in Africa (Nigeria). Initial work demonstrated that hemoplasmas were relatively common in little brown bats (*Myotis lucifugus*) sampled across the northeastern USA (Mascarelli et al., 2014), and subsequent studies have confirmed frequent hemoplasma detections in a wide variety of bat species, including 97% of Schreibers' bats (*Miniopterus schreibersii*) in Spain (Millán et al., 2015), 67% of vampire bats (*Desmodus rotundus*) in Peru and Belize (Volokhov et al., 2017b), ~90% of molossids (*Molossus molossus* and *M. nigricans*) in Belize and Brazil (Ikeda et al., 2017; Becker et al., 2020), and 60% of *Eidolon* fruit bats in Nigeria (Di Cataldo et al., 2020b). Indeed, our phylogenetic analyses found that the Desmodontinae (i.e., *Desmodus* and *Diphylla*) had significantly greater hemoplasma prevalence compared with other mammals (Figure 3B). However, approximately half of all sampled bat species have instead displayed no evidence of hemoplasma infection. Some apparently negative species have clearly been undersampled (i.e., *n*=1-2), such as *Eumops nanus* and *Noctilio leporinus* in Belize (Becker et al., 2020), *Hypsugo savii* in Switzerland (Fritschi et al., 2020), *Lasiurus cinereus* in Chile (Millán et al., 2019a), captive *Megaderma lyra* and *Rousettus aegyptiacus* in Germany (Fritschi et al., 2020), *Phyllostomus discolor* and *Natalus espiritosantensis* in Brazil (Ikeda et al., 2017), *Diphylla ecaudata* in Peru (Volokhov et al., 2017b), and *Epomophorus* species in Nigeria (Di Cataldo et al., 2020b). However, some more robustly sampled bats, such as *Pipistrellus pipistrellus* (*n*=123) and *P. kuhlii* (*n*=28), have also consistently tested negative, which suggests some species may be resistant to infection or have ecological characteristics that reduce their exposure (Ikeda et al., 2017; Millán et al., 2019a; Becker et al., 2020; Fritschi et al., 2020). Our phylogenetic analysis also found that a subclade of the Vespertilioninae (including the genera *Pipistrellus, Nyctalus*, *Vespertilio*, and *Hypsugo*) had significantly lower prevalence than other mammals. However, no experimental tests of hemoplasma susceptibility have yet been performed in bats.

Several sequences phylogenetically related to known hemoplasma species have been identified in bats. Sequences with 93-96% similarity to *M. coccoides* (known to infect rodents and felids; Thurston (1954), Kreier and Hall (1968)) were detected in *Molossus molossus* from Brazil (Ikeda et al., 2017). Sequences from bats have also shown strong phylogenetic similarity to hemoplasmas detected in primates. In other cases, bat hemoplasmas have been closely related to human hemoplasmas, including 97% similarity of sequences from *Miniopterus schreibersii* in Spain to *C*Mhh (Millán et al., 2015). Similar sequences have also been detected in

Eidolon fruit bats from Nigeria (Di Cataldo et al., 2020b) and *Myotis chiloensis* from Chile (Millán et al., 2019a). Recently, sequences with up to 100% identity to *C*Mhh were identified in three *Pteropus* species in New Caledonia (Descloux et al. 2020). However, many other sequences represent novel genotypes with variable similarity to previously identified hemotropic *Mycoplasma* species in other bats, ticks, rodents, primates, and carnivores (Becker et al., 2020).

Coinfection with hemoplasmas

Few studies have assessed coinfection of wildlife with different hemoplasma genotypes, precluding a more systematic quantitative analysis. However, all studies using diagnostic protocols capable of detecting coinfection have confirmed this whenever a sufficient sample size was tested (Table 1). In the seminal study by Willi et al. (2007) with wild felids, the authors showed frequent coinfection by up to the three feline hemoplasmas. For example, as many as 87% of the lions were coinfected, with 56% concurrently infected by sequences closely related to *Mhc*, *C*Mhm, and *C*Mt (Willi et al., 2007b). Studies in other animals have shown diverse frequency of coinfections, with these being particularly uncommon in bobcats and pumas studied in California, USA (Kellner et al., 2018).

Coinfections may be common owing to hemoplasmas sharing transmission routes, such as the same vector (see below). This hypothesis has been supported by coinfections being far more common in free-living than in captive animals, the latter of which are often less exposed to arthropods and even treated against them. In canids, the combination of hemoplasmas reported included sequences closely related to *Mhc* and *C*Mhp (Di Cataldo et al., 2020a). In felids, the most common combinations are composed of sequences closely related to *Mhf* and *C*Mhm, *C*Mhm and *C*Mt, or *Mhf* and *C*Mhm and *C*Mt, but strikingly never by *Mhf* and *C*Mt (Willi et al., 2007a; Kellner et al., 2018; Sacristán et al., 2019). *C*Mhm and *C*Mt have been proposed to share the same arthropod vector (Willi et al., 2007a), but such frequency of triple infections, for example in lions, may be more likely related to exposure through multiple and different transmission routes during the host's lifespan or to other life traits such as social behavior (see below).

Risk factors

Relatively few studies have gathered sufficient information to allow inferring potential risk factors for hemoplasma infection in wildlife. Nevertheless, some ecological and evolutionary factors appear to increase hemoplasma risk. Some species may have higher risk of infection than others, facilitated by species traits. In

the abovementioned study in Spain, infection prevalence was significantly higher in badgers than foxes (Millán et al., 2018). A higher rate of intraspecific contacts due to the social lifestyle of badgers may explain these differences. Social behavior may also explain the high prevalence found in raccoons (Volokhov et al., 2017a) and coatis (de Sousa et al. 2017), and the high genotype richness and/or coinfection rate detected in both badges and racoons (Volokhov et al., 2017a; Millán et al., 2018). Heavier bat species and those with larger colonies also had higher prevalence, which could further support density-dependent transmission (Becker et al., 2020). However, high prevalence has also been reported in solitary bat species (e.g., Di Cataldo et al., 2020b).

Feeding habits of mammal species, in particular predatory behavior, may also increase hemoplasma infection risk, especially in carnivores. For example, ancestral state analyses showed that wild felids in California may become infected after predating upon domestic cats (Kellner et al., 2018). Similarly, a sequence from a potentially new hemoplasma species, closely related to rodent hemoplasmas, has been found in different species of South American carnivores (Cabello et al., 2013; Millán et al., 2019b; Sacristán et al., 2019) and probably originated from a predator-prey transmission route. Indeed, exposure of a predator to prey hemoplasmas was suggested for the domestic cat, although this hypothesis has not yet been confirmed (Willi et al., 2007a).

At the individual level, age also appears to be a risk factor of infection. For example, prevalence was higher in adult than in juvenile jaguars (Furtado et al., 2018) and Darwin's foxes (Di Cataldo et al., 2020a). In the latter system, authors considered that this may reflect the fact that hemoplasma infection is enzootic in the fox population. However, within wild vampire bat populations, subadults had marginally higher odds of hemoplasma infection, and non-reproductive individuals also had greater infection prevalence (Volokhov et al., 2017b). Sex biases in hemoplasma infection have also been observed in various mammal taxa. Male racoons had higher prevalence than females in the USA (Volokhov et al., 2017a). Across a diverse Neotropical bat community in Belize, males also had consistently higher odds of an infection (Becker et al., 2020). However, other authors (e.g. Hoelzle et al., 2010; Sacristán et al., 2019; Di Cataldo et al., 2020a) have not found differences between males and females, suggesting that sex biases in infection may vary across mammalian taxa.

Some environmental factors may also affect wildlife exposure or susceptibility to hemoplasmas. Volokhov et al. (2017) found habitat-related differences in hemoplasma prevalence and genotype richness in raccoons: heavier raccoons had greater odds of infection in an undisturbed island but had lower odds of

infection on an urban island, and raccoons from undisturbed habitat had more individuals coinfected with multiple hemoplasma genotype than those from the urban habitat. These results may be attributable to different environments more readily supporting vectors that may transmit hemoplasmas and/or reduced aggressive behavior in urban areas due to the presence of supplemental feeding (e.g., garbage). Sacristán et al. (2019) also observed higher prevalence in guignas captured in continuous forests than in fragmented landscapes. Authors suggested that higher guigna densities in forests (the preferred habitat), and therefore higher rates of intraspecific contact, explained these differences. On the other hand, vampire bats had lower hemoplasma prevalence in habitats with more livestock prey, suggesting increased food availability may improve immune defense against these pathogens (Becker et al., 2018a). Similarly, high food availability in urban areas was suggested to explain lower hemoplasma prevalence in rodents compared to those in forest fragments owing to more robust immune defense or to less frequent aggressive interactions from reduced food competition (Gonçalves et al., 2020). Lastly, Neotropical bats with higher dietary exposure to mercury, a heavy metal, generally had lower odds of hemoplasma infection, suggesting that contaminants might eliminate bacterial habitat through anemia or cause mortality of possible arthropod vectors (Becker et al., 2020b).

The living condition of animals (i.e. sampled in the wild or in captivity) also affect the odds of hemoplasma infection. Few studies have explicitly compared wild and captive counterparts. Significantly higher prevalence was found in free-ranging capybaras (80%) compared to captive animals (30%) (Vieira et al., 2009). In broader support of this pattern, our meta-analysis identified significantly lower prevalence in captive animals compared to those sampled in the wild (Figure 2). This most likely reflects differences in exposure more than in susceptibility. Owing to support for direct and vector-borne transmission (see below), differences in host density and/or exposure to potential vectors between captive and wild populations might explain these results.

Transmission routes

Although transmission routes have been best investigated in domestic animals, some insights can be made from wildlife studies. For example, some studies have analyzed ectoparasites retrieved from wild hosts for hemoplasma DNA. Gonçalves et al. (2020) detected hemoplasma DNA in ticks (*Amblyomma* sp.) and lice (*Polyplax spinulosa*) retrieved from rodents, Hornok et al., (2019) in ticks (*Ixodes simplex*) retrieved from bats, and Descloux et al. (2020) in bat flies (*Cyclopodia horsfieldi*). However, neither study could determine

whether the detected DNA originated from the tick's blood meal and thus belonged to the host. Millán et al. (2019) also detected hemoplasma DNA in *Pulex irritans* fleas from *Lycalopex* foxes, but not in *Amblyomma tigrinum* ticks from the same hosts, which would be expected if the DNA originated from the host blood. However, this study found no strong association between hemoplasma presence in grey foxes and their fleas, with fleas being positive in one third of non-infected foxes. Moreover, the amplicons from hosts and fleas were identical in only one of the three foxes positive for hemoplasmas with ectoparasites (Millán et al., 2019b). Hemoplasma coinfection with *Bartonella henselae* and/or *B. vinsonii berkhoffi* is relatively frequent (dos Santos et al., 2008; Yuan et al., 2009; Sykes, 2010; Maggi et al., 2013b; Millán et al., 2019b), which could support a role of fleas in hemoplasma transmission given that these vectors play key roles in the spread of *Bartonella*.

Nevertheless, other studies have failed to detect hemoplasma DNA in the arthropods retrieved from infected hosts (de Sousa et al., 2017) or have not found differences in prevalence between flea-positive and -negative animals (Sacristán et al., 2019). Interestingly, when comparing prevalence in guignas captured in different bioclimatic regions in Chile, Sacristán et al. (2019) found that prevalence was higher in the rainy temperate area. Ticks are absent in such cold regions, ruling out their possible role as hemoplasma vectors. Similarly, Darwin's foxes investigated by Di Cataldo et al. (2020a), which presented a prevalence of almost 60%, were captured in a tick-free region of Chile. Lastly, *Mhc*-positive wolves were sampled in wet regions from northern Spain (Millán et al., 2018), where these animals do not host *Rhipicephalus sanguineus* (the putative vector for this hemoplasma species), but do host other tick species (Sobrino et al., 2012).

Using epidemiological models, Kellner et al. (2018) suggested that vectors (among other pathways) may play a role in hemoplasma transmission among wild felids. Therefore, the role of fleas as vectors (including mechanical) remains an open question. Similarly, although some hemoplasma sequences detected in bats display relatively high similarity to those from bat ticks (e.g., Volokhov et al., 2017b; Millán et al., 2019a; Becker et al., 2020), ectoparasite presence did not strongly predict hemoplasma infection status across Neotropical bats (Becker et al., 2020). Future work comparing hemoplasma sequences between hosts and their fed ectoparasites (i.e., ticks, bat flies, mites, fleas) can help elucidate the transmission roles of arthropod vectors, but experimental transmission trials are the ultimate method of confirming vector-borne transmission.

Taking into account the high prevalence of hemoplasma infection in many of the wildlife species investigated (e.g., 97% *of Miniopterus schreibersii*; Millán et al., 2015), hemoplasmas are most likely using

different and probably concurrent transmission routes that can include ticks, fleas, or other vectors (not excluding a mechanical participation); direct transmission during social contact or aggressive interactions; and perhaps vertical transmission. Metagenomic studies of vampire bats identified *Mycoplasma* species in saliva, which could facilitate transmission both within and between species owing to feeding and grooming behavior (Volokhov et al., 2017b). Experimental studies of rodent hemoplasmas also support direct transmission (Cohen et al., 2018).

Studies of bat hemoplasmas also suggest generally little variation in infection prevalence over time (e.g., Volokhov et al., 2017b; Becker et al., 2020), which could support enzootic dynamics over more sporadic epidemic dynamics. However, seasonal sampling is necessary to elucidate more fine-scale temporal hemoplasma dynamics (Plowright et al., 2019), but such studies remain uncommon for hemoplasmas. In one rare example, Di Cataldo et al., (2020a) studied Darwin's foxes in Chiloé Island (southern Chile) for seven years and in all four seasons. This study found no interannual or inter-seasonal variation in *Mhc* prevalence, suggesting that the infection is probably enzootic in the species. Likewise, no differences in prevalence were found in guignas captured in different seasons in Chile (Sacristán et al., 2019).

Pathology

Unfortunately, few studies have investigated the potential pathological effect of hemoplasma infection in wild hosts. Obvious pathology has been noted in a few cases, always in captive animals. Even in such cases, these studies acknowledge the impossibility of establishing a cause-effect relationship between the symptoms and hemoplasma infection (Dillberger et al., 1994).

In free-living hemoplasma-infected animals, some studies have simply reported that captured individuals were in good condition and/or did not show any clinical signs of anemia (e.g. Krengel et al., 2013; Volokhov et al., 2017a; Westmoreland et al., 2017), whereas other studies using hematological and/or serum chemistry parameters have shown mostly inconclusive results. For example, 11 out of 12 hemoplasma-infected captive African lions showed normal hematocrit and hemoglobin values, but one animal with hypochromic normocytic anemia was PCR negative for hemoplasmas (Ghazisaeedi et al., 2017). Based on packed cell volume (PCV), Ramalho et al. (2017) reported that two out of 12 captive capuchin monkeys were anemic, of which one was infected by *Mycoplasma*. Similarly, Silva et al. (2007) and Grazziotin et al. (2011) did not find differences in PCV among PCR-positive and -negative individuals. Vieira et al. (2009) investigated PCV and total plasma protein levels in capybaras. Although some differences were found between PCR-positive

and -negative animals, these values also differed significantly between captive and free-ranging individuals, making the comparison spurious. The PCV from two captive hemoplasma-infected coatis were within the reference values for the species (Cubilla et al., 2017a). de Melo et al. (2019) reported higher monocyte and lymphocyte counts and lower platelet counts in captive *Alouatta* sp. monkeys PCR-positive for hemoplasmas than in PCR-negative individuals, whereas red blood cell counts and serum chemistry did not differ between groups. Other studies have failed to detect differences in several hematological and/or serum chemistry parameters among PCR-positive and -negative individuals (e.g. Suksai et al., 2016; Cubilla et al., 2017b; Sacristán et al., 2019; Di Cataldo et al., 2020a) or in comparison with the published reference values (e.g. Volokhov et al., 2011b; Maggi et al., 2013b), which suggests that most hemoplasma infections are asymptomatic. Additionally, vampire bats with more robust innate immune function (i.e., bacterial killing ability) displayed lower odds of hemoplasma infection (Becker et al., 2018a), suggesting that wild mammals likely do mount innate immune responses against hemoplasmas. On the other hand, little brown bats (*Myotis lucifugus*) with and without clinical signs of white-nose syndrome did not vary in hemoplasma prevalence (Mascarelli et al., 2014).

Studies with jaguars (Furtado et al., 2018) and Darwin's foxes (Di Cataldo et al., 2020a) observed cases of sustained infection, with some individuals infected by the same genotype in capture events separated for months or even years. This suggests these species may have high tolerance to chronic infection or be easily re-infected due to a potentially imperfect immune response to these pathogens. Moreover, the high genetic variability of 16S rRNA hemoplasmal genotypes in the Darwin's fox population, with up to six different genotypes detected in 25 individuals, may suggest this species has some degree of tolerance to hemoplasmas (Di Cataldo et al., 2020a). Limited recaptures among vampire bats in Peru and Belize also suggested individuals remained infected between sampling years, although authors were unable to distinguish whether these represent persistent infections or new transmission events (Volokhov et al., 2017b). Future work is needed to assess whether such patterns hold in other wild species. Given these findings, and with the exception of some hemoplasmas that can be more pathogenic (e.g., *M. ovis*), in captivity situations (e.g. Dillberger et al., 1994; Messick et al., 2000; Haefner et al., 2003; Stoffregen et al., 2006; Boes et al., 2012) or during coinfections (e.g. Iberian lynx coinfected with *Cytauxzoon felis*; Willi et al. (2007)), hemoplasmas most likely primarily have a subclinical course in wildlife hosts.

Zoonotic potential of hemoplasmas

Lastly, there are purported cases of human infection with possible animal hemoplasmas. In one study, people with animal and/or arthropod contact was shown to be at significantly greater risk of infection with hemoplasmas (Maggi et al., 2013b). In a small cohort of individuals positive for hemoplasmas, primarily veterinarians and a wildlife manager, the partial 16S rRNA gene sequences obtained were closely related to *M. ovis* (Maggi et al., 2013d). A high prevalence of infection with a hemoplasma closely related to *M. suis* was also confirmed among asymptomatic swine farmers and veterinarians (Yuan et al., 2009). Other PCR-based reports of animal hemoplasmas in humans include *Mhf*-like infection in a HIV-positive patient (dos Santos et al., 2008) and a veterinarian infected with a *CMhp*-like hemoplasma (Maggi et al., 2013d). Even fewer examples exist of zoonotic transmission from wildlife. *C*Mhh-like hemoplasmas have been implicated in very rare cases of severe anemia in humans (Steer et al., 2011; Alcorn et al., 2020; Hattori et al., 2020; Descloux et al. 2020). In a recent study, Descloux et al. (2020) reported *C*Mhh in 15 human patients in New Caledonia who suffered from fever, weight loss, fatigue, splenomegaly, and accidental spleen rupture, with a lethality rate of 27%. Fourteen of the 15 positive cases exhibited febrile autoimmune hemolytic anemia and hemophagocytosis. Comparative genome sequencing revealed that the average nucleotide identity (ANI) value between genomes of *C*Mhh strain SWG34-3 reported by Hattori et al. (2020) from a Japanese patient and *C*Mhh strain Neocaledonica described by Descloux et al. (2020) is ~98%, which is above the threshold for bacterial species delineation of ≥95% (Goris et al., 2007; Richter and Rosselló-Móra, 2009; Yoon et al., 2017), and therefore, demonstrates these two strains likely belong to the same species. Interestingly, most of the positive patients reported hunting and eating flying foxes, and closely related (up to 100%) 16s rRNA and *dnaK* genes sequences were detected in the flying foxes analyzed. On this basis, Descloux et al. 2020 named the disease as “flying fox haemolytic fever”. In addition, hemoplasma sequences similar to *C*Mhh have also being found in Microchiroptera bat species (Millán et al., 2015; Di Cataldo et al., 2020b). However, such patterns could also be the result from host shifts over evolutionary time rather than contemporary cross-species transmission. Because most studies, except for two recent publications (Hattori et al., 2020 and Descloux et al., 2020), have not used other genetic markers (e.g., housekeeping genes, complete or partial genome sequencing) in addition to the 16S rRNA gene to identify hemoplasmas to the species level, possible zoonotic transmission of hemoplasmas remains largely unresolved. Assessing zoonotic potential of hemoplasmas warrants more comprehensive genomic identification criteria alongside systematic screening of persons in close contact with wildlife.

Future directions

Diagnostic considerations

The use of a solitary universal genetic marker such as the 16S rRNA gene cannot provide an accurate genetic identification of phylogenetically closely related mycoplasma isolates to the species level. The application of other genetic markers (e.g., housekeeping genes, complete or partial genome sequencing) in addition to the 16S rRNA gene provides reliable identification of the Mollicutes isolates and significantly reduce the risk of misidentification (Volokhov et al., 2012, 2017). Therefore, hemoplasmas detected in animal samples and in wild hosts in particular on the basis of only a single, evolutionary conservative genetic marker, such as full or partial 16S rRNA gene sequences found to be very similar (≥97%) or identical to a known hemoplasma species, cannot be automatically assigned to the known species and should be referred to as "species-like". Because most studies of wildlife hemoplasmas only sequence a portion of the 16S rRNA gene, such hosts cannot be definitively linked with the hemoplasma species to which these sequences were most closely related. Here, we frequently referred to novel hemoplasmas sequences as genotypes, and criteria for hemoplasma genotype assignment have been published elsewhere (Becker et al., 2020). However, we caution that genotype is not synonymous with species in the context of identifying *Mycoplasma* spp. (Volokhov et al., 2012).

To advance studies of wildlife hemoplasmas, other methodological aspects should be considered. Many diagnostic protocols and PCR primers exist in the literature, some of which are specific for a given hemoplasma species. However, as our review demonstrates, wild mammals are mostly infected by hemoplasmas that have not yet been described. We thus strongly recommend that researchers investigating hemoplasmas in wildlife first run a protocol that is universal for most hemoplasmas and which is based on *in silico* bioinformatic analysis to detect potentially new hemoplasma species. All PCR-positive results based on the 16S rRNA gene (using a universal PCR protocol) should always be confirmed by direct sequencing of amplicons, because almost all published primers for detection of hemoplasmas may cross-amplify the 16S rRNA genes of other closely related bacterial species that could be present in a sample. Based on subsequent findings, these diagnostics can then be followed by species-specific protocols to confirm early findings and/or coinfections. The development of primers specific for new genotypes detected in a given species, especially if such genotypes are unique and prevalent, as performed by Volokhov et al. (2017), is encouraged. Researchers must also consider that some hemoplasmas, such as *Mhc* and *Mhf*, are indistinguishable based on 16S rRNA sequences alone (Sykes and Tasker, 2013). As emphasized throughout this review, other genetic markers (eg.,

rpoB, rpoC, *gyrB, dnaK,* 23S rRNA) are necessary to differentiate some closely related hemoplasma species (Kämpfer and Glaeser, 2011; Volokhov et al., 2012, 2017; Descloux et al. 2020, Valente et al. 2020).

Another possibility to detect known and novel hemoplasmas is next-generation sequencing (NGS or metagenomic [untargeted] deep sequencing) techniques (Horiba et al., 2018; Gu et al., 2019). NGS can randomly amplify and comprehensively detect potentially all microorganisms present in a given sample, enabling universal unbiased pathogen detection. However, in some cases, NGS detection sensitivity can be lower than species-specific PCR assays (Anis et al., 2018); therefore, NGS may not work well for detection of pathogens that present with a very low titer (e.g., bacteria in blood). The untargeted nature of NGS also amplifies all nucleic acids in a sample, including those of the host, which are more abundant than those of the pathogens. Therefore, millions of NGS reads need to be analyzed using appropriate bioinformatic tools to identify the pathogen(s) of interest (Hattori et al., 2020). Currently, only two studies have used metagenomic deep sequencing to detect hemoplasmas in human clinical specimens (Hattori et al., 2020 and Descloux et al. 2020). In the former, a high copy number of the *C*Mhh genome was detected in human serum and bone marrow samples using NGS. Additionally, metagenomic deep sequencing of DNA of saliva samples from *Desmodus rotundus* bats in Peru identified non-hemotropic *Mycoplasma* species alongside hemoplasma genotypes phylogenetically similar to those identified in blood of these bats, which provided indirect evidence for potential direct transmission of hemoplasmas through biting or social contacts (Volokhov et al., 2017b).

An alternative to metagenomic untargeted sequencing is targeted NGS (Gu et al., 2019). Targeted NGS refers to the selective amplification of specific genomic regions of interest prior to massive parallel sequencing, e.g., conserved 16S rRNA, 23S rRNA, and/or the 16S-23S internal transcribed spacer sequences. Compared to metagenomic sequencing, targeted sequencing has more limited potential to discover novel pathogens. However, selectively sequencing pathogens of interest provides better sensitivity, better specificity, ease of downstream analysis, and lower cost by allowing more samples to be tested in one run (Anis et al., 2018). As an example of such approach, NGS-based targeted sequencing of 16S rRNA amplicon metabarcodes has been used to detect hemoplasmas and confections with other blood pathogens in canines from Thailand (Huggins et al., 2019). Thus, different NGS approaches can be applied to identify either known or novel hemoplasmas in domestic and wild animals and to obtain genomic sequences for these pathogens. However, the selection and use of each NGS approach for hemoplasma detection in wildlife should be decided on case-by-case basis, because universal NGS protocols for routine diagnostics are not yet established.

The type of tissue analyzed by molecular methods should be also be taken into consideration, especially if comparisons are to be made between host species, habitats, and years. The efficiency of published PCR protocols is not equal for hemoplasma detection in all organs or tissues, and hemoplasma loads in tissues are inversely associated with the time since infection. For example, Koneval et al. (2017) detected DNA in four of nine fox blood samples but only in four of 49 liver and five of 242 spleen samples. In contrast, Hornok et al., (2018) found higher prevalence in red and fallow deer spleens compared to blood samples. According to the authors, such differences may be caused by the quick elimination of hemoplasma DNA in the spleen following phagocytosis. (Sepúlveda-García et al., In press) found divergent results depending in the tissue tested: while one American mink was positive in a blood sample and negative in spleen, another mink presented negative PCR results in blood but detectable hemoplasma DNA in spleen. Systematic sampling of hosts for different tissues, where possible, would facilitate improved diagnosis of hemoplasmas and allow for assessing systemic infections. However, relatively non-invasive sampling (i.e., blood) may often be the only tissue available for diagnosis when conducting longitudinal studies or in threatened wildlife species. To determine whether hemoplasmas are viable organisms in the bloodstream or saliva, which could facilitate transmission among susceptible hosts, analysis of RNA isolated from blood or saliva using RT-PCR or NGS for detection of 16S and 23S rRNA will be essential (Volokhov et al., 2011a; Hattori et al., 2020). For more detailed studies of hemoplasma pathology in wildlife, electron microscopy and *in situ* hybridization techniques could also be helpful for studying the distribution of these pathogens in different tissues and assessing systemic infections (Hattori et al., 2020). For pathological studies, the absence of reliable hematologic, immunological, and biochemical reference intervals for many wildlife (including for different ages, sexes, geographic regions, diet habits, and seasons) is another obstacle for rigorously assessing the possible pathogenic effects of hemoplasmas on the health of their wildlife hosts.

Another possibility to investigate hemoplasmas in wildlife, especially in felids and canids, is serological studies. This approach has not been thoroughly developed. However, the published data on serology is promising for future pathogenesis studies and may have application in diagnostics, potentially for wildlife such as Iberian lynxes or other wild felids infected with *Mhf* (Willi et al., 2007b; Wolf-Jäckel et al., 2010).

Knowledge gaps

As emphasized in Figures 1 and 2, there are clear geographical biases in wildlife hemoplasma research, including areas where sampling is scarce or inexistent, such as Africa, Asia (excluding Japan), and Australia

(excluding limited work on bats). Additionally, many taxa are underrepresented, especially considering their overall species richness (e.g., rodents), whereas other have not yet been investigated (e.g., cetaceans, insectivorous). The Eastern Hemisphere is clearly underrepresented across wildlife taxa, but this is especially so in marsupials and primates. More comprehensive sampling across taxa and geographies is needed for robust comparative analyses to gain insights into the environmental drivers of infection, frequency of host shifts versus codivergence over evolutionary timescales, and species differences in infection prevalence.

The pathology associated with hemoplasma infection in wildlife should also be addressed, given that most insights are from domestic animals. Such work will require larger sample sizes and different approaches to quantifying pathology. From the evidence to date, the study of blood parameters is likely insufficient to determine the pathological potential of hemoplasmas. However, applying immune assays to wildlife or comparing genome-wide patterns between uninfected and infected hosts (e.g., using RNA-Seq of blood or spleen) could better address consequences of hemoplasma infection for wildlife health (Becker et al., 2018b; Zylberberg, 2019).

Similarly, our understanding of hemoplasma transmission is poor, both in domestic and wild animals. Identifying likely transmission pathways is necessary to establish preventive measures when needed, either for threatened wildlife or to limit zoonotic transmission. In particular, experimental studies remain the best option for differentiating exposure routes (e.g., Willi et al., 2007a; Museux et al., 2009; Cohen et al., 2018). However, given logistical constraints, application of epidemiological models (e.g., susceptible-infected-recovered frameworks) could be applied to even sparsely collected longitudinal infection prevalence data to elucidate the relative contribution of different hypothesized transmission routes (e.g., Becker et al., 2018c).

Likewise, our knowledge about the zoonotic potential of hemoplasmas is scarce. Wildlife professionals frequently handle wild animals, but limited information is available about the exposure to hemoplasmas in this group. Future surveys of wildlife veterinarians, biologists, and professionals at rescue centers and zoos could determine if individuals are at risk of infection with hemoplasmas of wildlife, as has been proposed for domestic animal practitioners (Maggi et al., 2013d). In other cases where humans may have accidental close contact with wildlife (e.g., predation by vampire bats), comprehensive genetic or genomic studies of similarity between human and wildlife hemoplasmas will be essential for inferring the likelihood of cross-species transmission events. Currently, the evaluation of ANI and/or average amino acid identity (AAI) is well-suited for delineation of multiple bacterial species, including some known *Mycoplasma* species. However, universal cut-off value(s) to delineate bacterial species based on the ANI and AAI do not

exist for most Mollicutes species, including for hemoplasmas, which are still insufficiently studied for genomic sequences. Future assessment of intra- and interspecies variation in ANI and AAI values will require the availability of high-quality whole genome sequences of multiple (diverse) field isolates for each hemoplasma species or genotype.

Because almost all known hemoplasma species are still considered as uncultivable *in vitro*, complete or partial genome sequencing is usually performed on DNA extracted from blood of either naturally or experimentally infected animals at the peak of bacteremia (eg. Nascimento et al., 2012). This genome sequencing approach can be challenging for most wildlife because (i) hemoplasma loads in blood of persistently infected animals are usually low, (ii) experimental infections to produce high-titer bacteremia are infrequent, and (iii) possible co-infection with other blood-borne pathogens can restrict "clear" sequencing of the target hemoplasma DNA from field-based blood samples.

Beyond the potential for hemoplasmas to have pathological effects on wildlife and to be zoonotic, these pathogens can also serve as a model system in ecological and evolutionary studies (e.g., Becker et al., 2020; Di Cataldo et al., 2020b). The often-high prevalence, genetic diversity, and occasionally wide host range of hemotropic mycoplasmas makes them well-suited to studying infection risks both within and between host species over ecological and evolutionary timescales. Further study of wildlife hemoplasmas could thus facilitate basic insights into the factors that structure pathogen prevalence, diversity, and cross-species transmission more generally.

Data Availability Statement

The data supporting the findings of this study are available in the Supplementary File 2.

Conflict of interests

The authors declare no conflict of interest.

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Figures legends

Figure 1. Maps of hemoplasma study effort (number of unique studies identified from our literature search) across mammals, stratified by host order. All maps use the Gilbert projection.

Figure 2. Boxplots show the median and interquartile range of hemoplasma prevalence per host genus, stratified by geography (Western or Eastern Hemisphere) and population (captive or wild). Points are colored by infection prevalence, with cases of no detection shown in white.

Figure 3. Phylogenetic distribution of binary infection status (A) and infection prevalence (B), with host clades detected by phylogenetic factorization identified by number and shown through shading. Red clades are those with higher odds of infection or prevalence compared to the rest of the phylogeny (i.e., Desmodontinae [1] and Cervidae [2]), whereas blue displays clades with lower odds of infection or prevalence (i.e., *Neovision* [3], subclade of the Vespertilioninae [4], and *Vulpes* [5]).

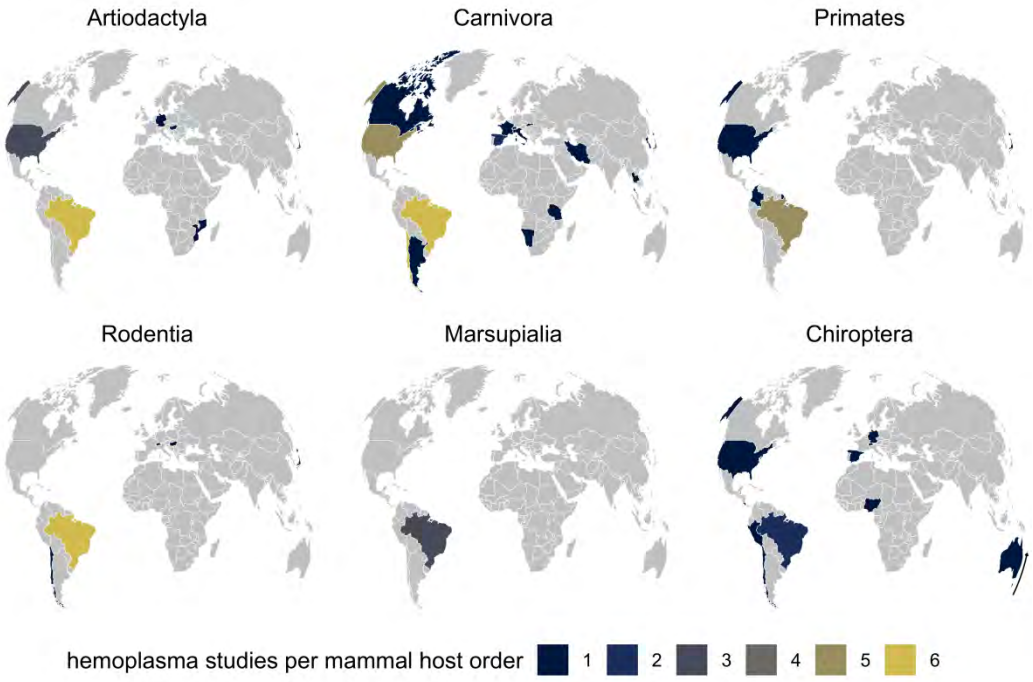
Figure 4. Phylogenetic patterns in hemoplasma genotype diversity (A) and nucleotide diversity (B) among mammal genera. Points are colored by order and scaled by genotype diversity. Our analyses identified little phylogenetic signal in either diversity metric among hosts (Pagel's λ=0.17 and 0, respectively). However, primates had marginally lower genotype diversity whereas both primates and bats had marginally lower nucleotide diversity.

Table 1. Coinfection by more than one hemoplasma species detected in free-living wildlife, based on the study of partial 16S rRNA gene sequences.

Host species	**Coinfected/ total infected**	**Hemoplasma spp. (*n*)***	**Reference**
Felidae			
Lion (*Panthera leo*)	39/44	*Mhf* + *C*Mhm *(5)*	Willi et al., 2005
		*C*Mhm + *C*Mt *(9)*	
		Mhf + *C*Mhm + *C*Mt *(25)*	
Jaguar (*Panthera onca*)	7/22	*Mhf/Mhc* + *C*Mhm *(5)*	Furtado et al., 2018
		*C*Mhm + *C*Mt *(1)*	
		Mhf/Mhc + *C*Mhm + *C*Mt *(1)*	
Iberian lynx (*Lynx pardinus*)	5/13	*Mhf* + *C*Mhm *(3)*	Willi et al., 2005
		*C*Mhm + *C*Mt *(1)*	
		Mhf + *C*Mhm + *C*Mt *(1)*	
Bobcat (*Lynx lynx*)	7/113	*Not reported*	Kellner et al., 2018
European wildcat (*Felis silvestris silvestris*)	5/12	*C*Mhm + *C*Mt *(4)*	Willi et al., 2005
		Mhf + *C*Mhm + *C*Mt *(1)*	
Fishing cat (*Prionailurus viverrinus*)	3/5	*Mhf* + *C*Mhm	Suksai et al., 2016
Guigna (*Leopardus guigna*)	4/24	*Mhf* + *C*Mhm *(3)*	Sacristán et al., 2019
		Mhf + *Mycoplasma sp. (1)*	
Puma (*Puma concolor*)	2/84	Not reported	Kellner et al., 2018
Procyonidae			

Raccoon (*Procyon lotor*)	47/57	Combinations of 6 genotypes.	Volokhov et al., 2017a
South American coati (*Nasua nasua*)	2/2	*Mhf* + *C*Mt *(1)*	Cubilla et al., 2017a
		M sp. + *C*Mt *(1)*	
Canidae			
Darwin's fox (*Lycalopex fulvipes*)	9/30	*Mhc* + *C*Mhp *(9)*	Di Cataldo et al., 2020a
Andean fox (*Lycalopex culpaeus*)	2/23	*Mhc* + *C*Mhp *(2)*	Cevidanes et al. (Unpublished)
Cervidae			
Sika deer (*Cervus nippon yesoensis*)	6/23	*Ca.* M. haematocervi + *Ca.* M. erythrocervae (*6*)	Tagawa et al., 2013
Red deer (*Cervus elaphus*)	Not reported	*M. wenyonii* + *Ca.* M. haematobovis	Hornok et al., 2018
Bovidae			
African buffalo (*Syncerus caffer*)	4/97	*M. wenyonii* + *Ca.* M. haematobovis	Gonçalves et al., 2018

**Mhf*: *Mycoplasma haemofelis*; *Mhc*: *Mycoplasma haemocanis*; C*Mhm*: *Candidatus* Mycoplasma haematominutum; C*Mhp*: *Candiatus* Mycoplasma haematoparvum; *C*Mt: *Candidatus* Mycoplama turicense.



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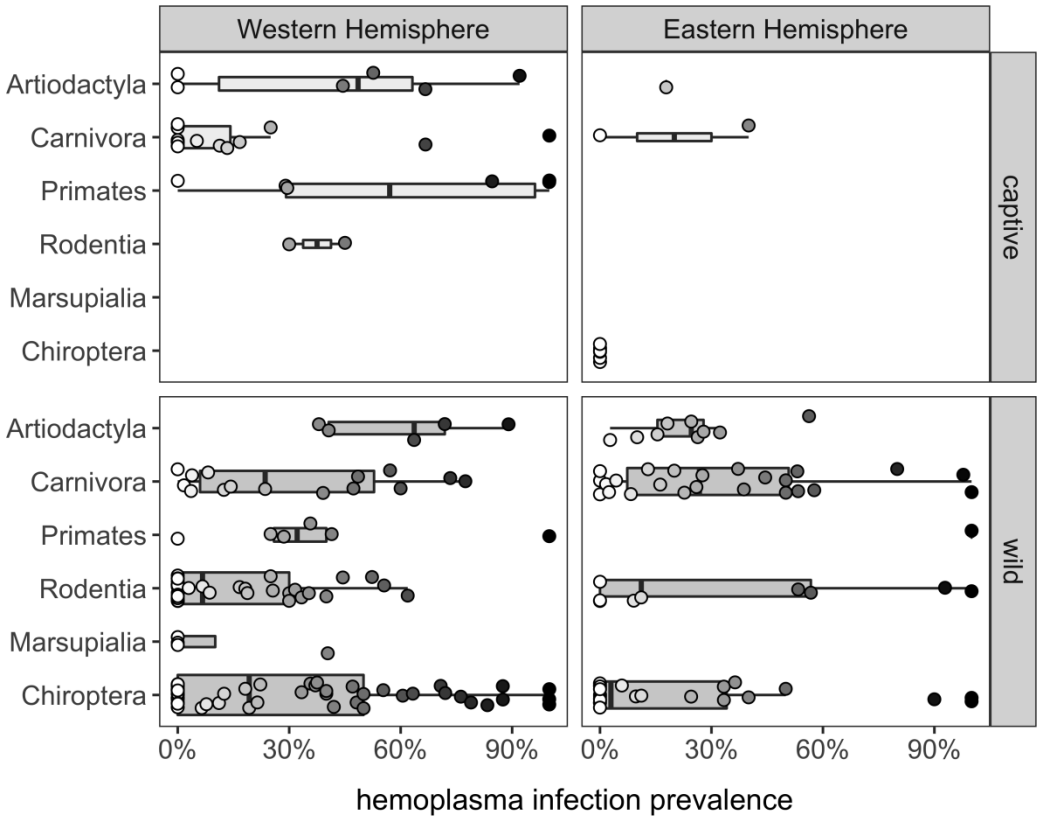

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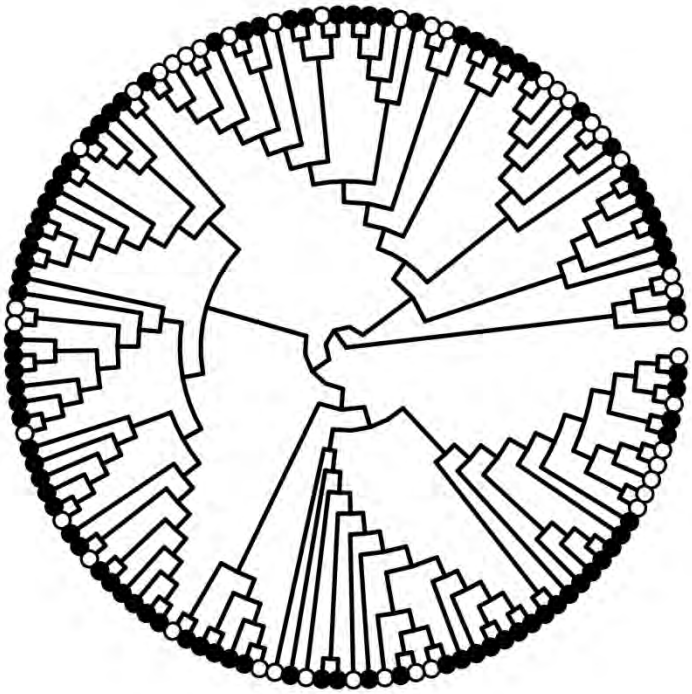
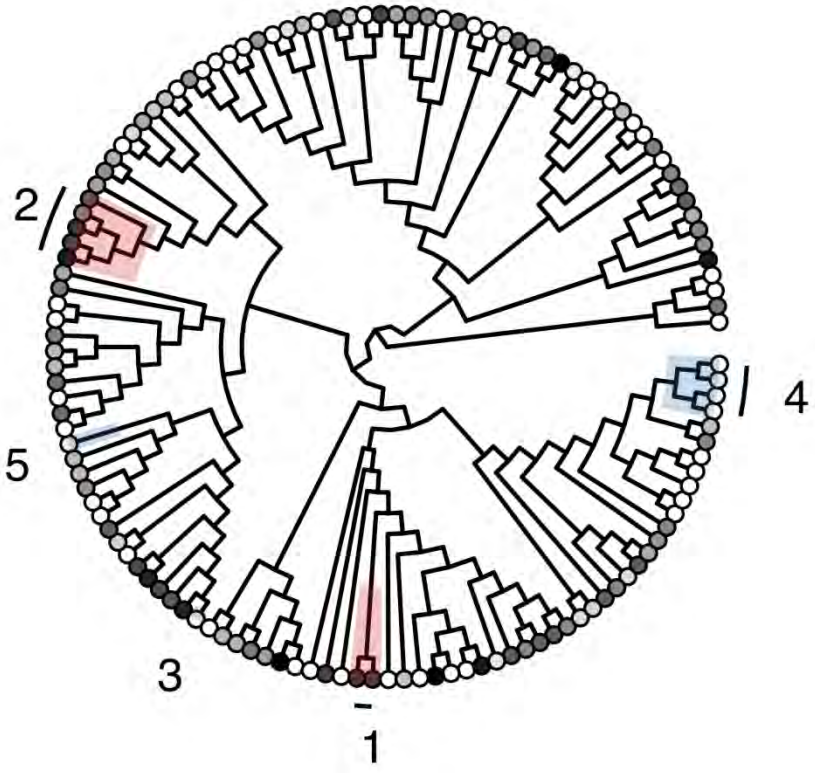
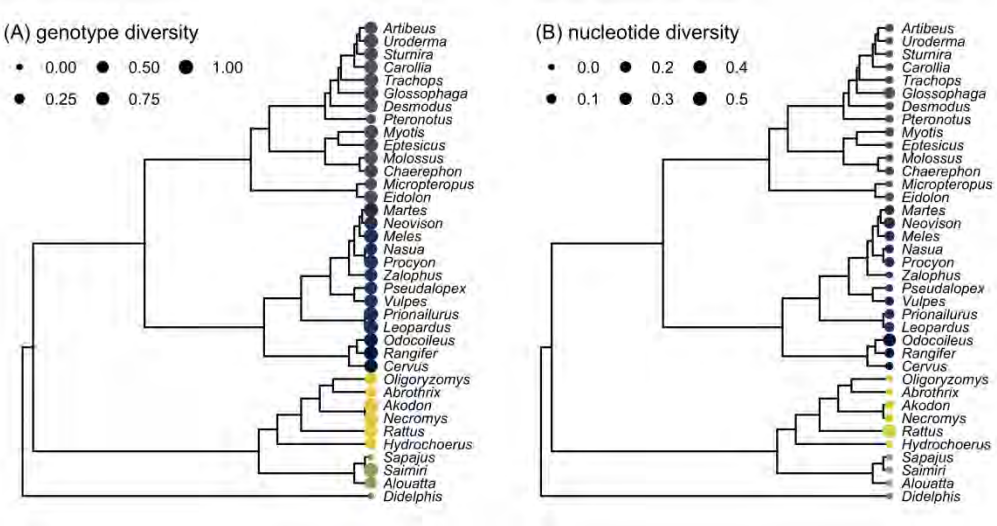
(A) infection status



(B) infection prevalence





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