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MOLECULAR SURVEY OF HAEMOSPORIDIAN PARASITES IN HAWKS, FALCONS, AND OWLS (ACCIPITRIFORMES, FALCONIFORMES, STRIGIFORMES) FROM MINNESOTA AND NORTH DAKOTA, WITH REMARKS ON THE PHYLOGENETIC RELATIONSHIPS OF HAEMOSPORIDIANS IN NORTH AMERICAN RAPTORS

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ABSTRACT

Avian haemosporidians are a diverse group of apicomplexan parasites that are globally distributed and infect almost all avian orders. Haemosporidian surveys of raptors (birds of prey) are underrepresented compared to those of songbirds, perhaps because of the greater difficulty in capturing and handling raptors. In this study, we captured raptors over a 7-yr period from northeastern North Dakota and northwestern Minnesota. Using standard molecular methods, we successfully screened 595 individuals representing 5 species of hawks (Accipitriformes), 3 species of falcons (Falconiformes), and 7 species of owls (Strigiformes). The overall infection prevalence averaged 41.5%, ranging from 31.6% in falcons (n = 38) to 85.7% in owls (n = 14). Thirty-one (12.6%) of the 247 infected raptors were infected concurrently with 2 or more haemosporidian genera. *Leucocytozoon* was the most common parasite genus identified. A total of 27 haemosporidian lineages were identified composed of 8 *Leucocytozoon*, 6 *Parahaemoproteus*, and 13 *Plasmodium* lineages. Twelve lineages (44%) were novel lineages identified for the first time. Raptor host order showed a significant phylogenetic signal within the tree topology of haemosporidian lineages from North American raptors. A significant effect of host order was also identified in the phylogenetic reconstructions of *Haemoproteus*, *Leucocytozoon*, and *Parahaemoproteus* lineages, with large clades restricted to mostly Accipitriformes and Strigiformes. Similar host specificity was not evident within the *Plasmodium* phylogeny, with most lineages infecting multiple raptor host orders and some lineages not restricted to raptors. Our results demonstrate that raptors support a unique and diverse community of haemosporidian parasites, many of which are distinct to raptor species. Studying haemosporidians within raptors expands our knowledge of host–parasite evolutionary relationships, species diversity, and cryptic speciation within this ubiquitous group of parasites.

Avian haemosporidians (Apicomplexa, Haemosporida) are vector-borne parasites, comprised of 4 genera: *Leucocytozoon*, *Haemoproteus*, *Parahaemoproteus*, and *Plasmodium*. These parasites are globally distributed, highly diverse, and infect almost all avian orders (Valkiūnas, 2005; Clark et al., 2014; Galen et al., 2018; Fecchio et al., 2020b, 2021). Each parasite genus is transmitted by a different family of hematophagous fly (Order: Diptera). *Leucocytozoon* parasites (with 1 exception) are transmitted by black flies (Simuliidae), *Haemoproteus* parasites are transmitted by hippoboscid flies (Hippoboscidae), *Parahaemoproteus* parasites are transmitted by biting midges (Ceratopogonidae), and avian *Plasmodium* parasites are transmitted by culicine mosquitoes (Culicidae) (Valkiūnas, 2005; Santiago-Alarcon et al., 2012; Fecchio et al., 2020b). The development of a standard barcode utilizing the cytochrome b gene (Bensch et al., 2000; Fallon et al., 2003; Hellgren et al., 2004; Waldenström et al., 2004; Bell et al., 2015) has revealed an astounding diversity of avian haemosporidian parasites represented by over 270 named species (Fecchio et al., 2020b) and over 5,000 genetic lineages deposited within MalAvi, the largest database of avian haemosporidian parasites (Bensch et al., 2009). Historically, sampling has been biased towards passerines (Passeriformes), but broader sampling of nonpasserines is warranted if we are to appreciate the entire diversity of avian haemosporidians. This is especially true as recent work has shown nonpasserine hosts can harbor their own unique parasite

clades (Outlaw and Ricklefs, 2009; Greiner et al., 2011; Bertram et al., 2017; Yabsley et al., 2018; Harl et al., 2022, 2024; Vanstreels et al., 2022; Svobodová et al., 2023).

Raptors (Accipitriformes, Cathartiformes, Falconiformes, Strigiformes) are highly mobile apex predators that play a key role in ecological stability and serve as indicators of environmental stress (McClure et al., 2018; Badry et al., 2020, 2022; Gomez et al., 2022; Negro et al., 2022; Ozaki et al., 2023). However, raptors can be difficult to capture and handle, and some species are protected. Not surprisingly, raptors are underrepresented in avian haemosporidian surveys (see Harl et al., 2022, 2024; Svobodová et al., 2023). There are few haemosporidian species known to infect raptors, with only 22 species known to infect the largest order Accipitriformes (Valkiūnas, 2005; Harl et al., 2022, 2024). Of the thousands of avian haemosporidian genetic lineages within MalAvi, few are reported from raptors. For example, of the almost 18,000 host records within MalAvi, only 585 are from raptors—211 from Accipitriformes (hawks, eagles), 6 from Cathartiformes (New World vultures), 76 from Falconiformes (falcons), and 298 from Strigiformes (owls). Within North American raptors, sampling is even sparser, with only 134 records within MalAvi, the majority of which (i.e., 94) are from owls. The raptor host fauna in North America is diverse, comprised of 4 orders (Accipitriformes, Cathartiformes, Falconiformes, Strigiformes) and 124 species (Chesser et al., 2024). However, the known diversity of North American raptor haemosporidians is based on only 17 raptor species.

Increased haemosporidian surveys of North American raptors can provide insights at various levels. From the perspective of parasite biodiversity, increased sampling may reveal a hidden diversity of haemosporidians, uncovering unique clades and species (Sehgal et al., 2006; Krone et al., 2008; Ortego and Cordero, 2009; Outlaw and Ricklefs, 2009; Valkiūnas et al., 2010; Greiner et al., 2011; Hanel et al., 2016; Walther et al., 2016; Bertram et al., 2017; Barino et al., 2021; Harl et al., 2022, 2024; Svobodová et al., 2023). From the perspective of host–parasite evolution, increased sampling over a broad range of raptor species will allow a deeper understanding of whether haemosporidian phylogeny is linked more closely with host phylogeny or similarities in host ecology. This comparison is possible because raptors are not a monophyletic group; that is, falcons (Falconiformes) are more closely related to songbirds (Passeriformes) and parrots (Psittaciformes) than to hawks (Accipitriformes) (Hackett et al., 2008; McCormack et al., 2013; Jarvis et al., 2014). Lastly, from the perspective of conservation, some raptor species are endangered and haemosporidian infections may affect raptor survival (Raidal et al., 1999; Raidal and Jaensch, 2000; Ishak et al., 2008; Leppert et al., 2008; Niedringhaus et al., 2018; Giorgiadis et al., 2020; Yoshimoto et al., 2021; Briggs et al., 2022). Thus, it is important to understand the level of haemosporidian parasitism in various raptor groups.

To examine the prevalence, distribution, and diversity of haemosporidian parasites within North American raptors, we used molecular methods to screen 595 blood samples collected from 15 raptor species over a 7-yr period (2011–2017) from northeastern North Dakota (ND) and northwestern Minnesota (MN). We describe infection prevalence and genetic diversity of haemosporidians parasitizing raptors within this region. Using phylogenetic analyses, we explore the evolutionary relationships of these parasites within North American raptors.

MATERIALS AND METHODS

Sample areas and sample collection

Blood samples were collected from 15 raptor species from areas surrounding Crookston, MN (47°46′29″N, 96°36′23″W), East Grand Forks, MN (47°55′22″N, 97°00′20″W), Fargo, ND (46°52′24″N, 96°49′38″W), Grand Forks, ND (47°55′16″N, 97°05′18″W), and Roseau, MN (48°50′48″N, 95°45′39″W) from 2011 to 2017. Blood samples from Cooper's hawks (*Accipiter cooperii*) were collected from a long-studied population within East Grand Forks, MN, and Grand Forks, ND (Rosenfield et al., 2007, 2010, 2024; Sonsthagen et al., 2012; Driscoll and Rosenfield, 2015) following the protocol described by Rosenfield et al. (2015). In short, during the breeding season, adults were captured by mist nets, and nestlings were captured by hand from monitored nests. All birds were banded, and blood was collected from the brachial/ulnar vein and stored in buffer (Longmire et al., 1988) for later molecular screening. Age and sex were determined as described by Rosenfield et al. (2015). Red-tailed hawks (*Buteo jamaicensis*) were captured during fall migrations from areas surrounding Crookston, MN, East Grand Forks, MN, and Grand Forks, ND using baited bal-chatri traps (Bloom, 1987). Peregrine falcon (*Falco peregrinus*) nestlings were captured by hand from nest boxes located on the campus of the University of North Dakota in Grand Forks, ND, and the roof of the Bank of the West building in Fargo, ND, in collaboration with the Midwest Peregrine Falcon Society. Each nest box contained only a single mated pair. Additional raptor species were captured opportunistically for the University of Minnesota Crookston raptor ecology course from areas surrounding Crookston, East Grand Forks, and Roseau, MN, using a variety of methods, including baited bal-chatri traps (Bloom, 1987). For red-tailed hawks, peregrine falcons, and other raptor species captured, birds were banded, blood samples were collected as previously described, and age and sex were determined morphometrically if possible.

Molecular identification

DNA was extracted from blood samples using the Qiagen Blood and Tissue Minikit (Qiagen, Valencia, California) following the manufacturer's protocol. DNA extractions were screened by real-time polymerase chain reaction (PCR) to detect haemosporidian DNA, following the protocol of Bell et al. (2015). All positives determined by real-time analysis were amplified by nested PCR to amplify a 477-base pair (bp) region of the cytochrome b (cyt-b) gene (Bell et al., 2015). All nested PCRs were run using OneTaq master mix (New England Biolabs, Ipswich, Massachusetts) following the manufacturer's protocols. Because of the high sensitivity of nested PCR, negative controls were included in runs to check against possible contamination, although none was found in any PCR runs.

Products from nested PCR amplifications were run on 1.25% agarose gels, stained with ethidium bromide, and visualized under ultraviolet light. Positive PCR products were purified using ExoSAP-IT (Affymetrix, Santa Clara, California) and sequenced using BigDye terminator v. 3.1 cycle sequencing kit (Applied Biosystems, Foster City, California) with nested PCR primers (Bell et al., 2015). Forward and reverse sequences were visualized and assembled using Sequencher v. 5.0.1 (Gene Codes Corp., Ann Arbor, Michigan). Chromatograms that showed the presence of

multiple infections were scored as co-infections. Co-infections were resolved following the protocol described in Orlofske et al. (2024). Assembled sequences for haemosporidians were aligned using BioEdit v. 7.2.0 (Hall, 1999). A local BLAST (basic local alignment search tool) against the MalAvi database using BioEdit was conducted for all unique haplotypes to identify lineages. As evidence indicates that avian haemosporidian haplotypes differing by 1 cyt-b nucleotide may be reproductively isolated entities (Bensch et al., 2004), we used the conventional practice of referring to each unique cyt-b haplotype as a unique parasite lineage following the standard naming protocol for this group of parasites (Bensch et al., 2009). Sequences were deposited in GenBank (PQ562381–PQ562407) and the MalAvi database. The data set used in this study is provided in Suppl. Data, Table S1.

Phylogenetic and statistical analysis

To examine phylogenetic relationships of haemosporidian parasites of North American raptors (Accipitriformes, Falconiformes, Strigiformes), our newly generated sequences were combined with lineages identified in North American raptors from the MalAvi database (Bensch et al., 2009) and 2 recent works on haemosporidians in Accipitriformes (Harl et al., 2022, 2024). Only full-length, 477-bp, sequences were used for phylogenetic analysis. Four separate alignments were constructed, 1 for all lineages and 1 each for *Leucocytozoon*, *Haemoproteus*, and *Parahaemoproteus* combined, and *Plasmodium* lineages. *Theileria annulata* (GenBank accession number KP731977) served as the outgroup in all 4 analyses based on its basal position for this group (Galen et al., 2018). For each lineage, we obtained raptor host distribution within North America from the MalAvi database, and these data were added to each phylogenetic reconstruction.

The GTR+I+G model for base substitution was used for Bayesian inference (BI) phylogenetic reconstruction for each of the 4 alignments as determined by jModelTest (Guindon and Gascuel, 2003; Darriba et al., 2012). Phylogenetic reconstruction was conducted in MrBayes v.3.2.6 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) with the analysis run until the standard deviation of split frequencies stabilized below 0.01. Twenty-five percent of the resulting trees were discarded as burn-in. Trees were visualized in Figtree (Rambaut, 2009). The phylogenetic trees for each genus produced in MrBayes are shown.

All analyses were conducted in program R version 4.4.2 (R Core Team, 2024). Chi-square contingency tables were constructed to compare parasite prevalence between groups implementing Yates continuity correction. To determine whether raptor order (Accipitriformes, Falconiformes, Strigiformes) had a significant phylogenetic signal within the evolutionary history of haemosporidian parasites in North America, we performed a randomization test (Maddison and Slatkin, 1991) as described by Bush et al. (2016) and conducted in R. A significant result would indicate that host order distribution within the tree topology is more conserved than expected by chance, showing a significant host taxonomic constraint within the phylogeny. Analyses were conducted for each of the 4 alignments indicated in the foregoing. Binary trees are required for the randomization analysis, which were reconstructed in BEAST X v.10.5.0 (Suchard et al., 2018) using the Bayesian relaxed clock model (Drummond et al., 2006) and the GTR+I+G model for base substitution. For each of the 4 phylogenetic reconstructions, we generated 2 independent runs with the following parameters: an uncorrelated lognormal relaxed clock, Yule process, 100 million generations with parameters sampled every 5,000 generations, and 10% of generations discarded as burn-in. Runs were inspected using Tracer v.1.7.2 (Rambaut et al., 2018) to ensure that ESS (effective sample size) values exceeded 200. Maximum clade credibility trees for each alignment were generated using TreeAnnotator and visualized in Figtree (Rambaut, 2009). Only the phylogenetic tree produced in BEAST for all lineages is shown.

RESULTS

Five hundred ninety-five individuals from 15 raptor species were successfully screened for haemosporidian parasites, 5 species of hawks (Accipitriformes), 3 species of falcons (Falconiformes), and 7 species of owls (Strigiformes) (Table I). Cooper's hawks (n = 391) and red-tailed hawks (n = 146) represented 90% of all raptors sampled. Overall, 247 of 595 (41.5%) raptors were infected with haemosporidian parasites (Table I). The overall prevalence of infection was statistically higher in owls (85.7%) than in falcons (31.6%) and hawks (41.1%) ($\chi^2 = 12.85$, df = 2, $P = 0.002$). Infection prevalence between falcons and hawks did not differ ($\chi^2 = 0.96$, df = 1, $P = 0.3265$).

Larger sampling of Cooper's hawks and red-tailed hawks allowed for comparisons of infection status between ages, in both species, and between sexes in Cooper's hawks only. Overall infection prevalence in red-tailed hawks (63.7%) was significantly higher than in Cooper's hawks (32.7%) ($\chi^2 = 40.81$, df = 1, $P < 0.0001$) (Table I). We were able to determine age and sex for 384 of 391 (98.2%) Cooper's hawks and age only for all 146 red-tailed hawks as either adults (79 Cooper's hawks and 64 red-tailed hawks), hatch-year birds (4 Cooper's hawks and 82 red-tailed hawks), or nestlings (301 Cooper's hawks) (Table II). Adult Cooper's hawks had a significantly higher overall prevalence of haemosporidian infection (45.6%) than immature (hatch-year and nestling) hawks (30.5%) ($\chi^2 = 5.74$, df = 1, $P = 0.0166$) (Table II). This was true for both *Leucocytozoon* infections ($\chi^2 = 4.43$, df = 1, $P = 0.0353$) and *Plasmodium* infections ($\chi^2 = 5.24$, df = 1, $P = 0.0221$). *Parahaemoproteus* infections in Cooper's hawks were too low to produce a meaningful comparison (Table I). In red-tailed hawks, the overall infection prevalence did not differ statistically between adult (71.9%) and hatch-year birds (57.3%) ($\chi^2 = 2.69$, df = 1, $P = 0.101$). Overall infection prevalence did not differ significantly between sexes in Cooper's hawks ($\chi^2 = 2.74$, df = 1, $P = 0.0979$) (Table II).

Leucocytozoon was by far the most common parasite genus found to infect raptors. Of the 247 infected raptors, 180 infections contained *Leucocytozoon* (72.9%), 83 infections contained *Plasmodium* (33.6%), and 15 infections contained *Parahaemoproteus* (6.1%). *Haemoproteus* parasites were not identified in any of the raptors surveyed (Table I). Thirty-one of the 247 infected raptors (12.6%) were concurrently infected with 2 or more parasite genera. Of the infected raptors, 24 (9.7%) were co-infected with *Leucocytozoon* and *Plasmodium*, 6 (2.4%) were co-infected with *Leucocytozoon* and *Parahaemoproteus*, and 1 raptor (= Cooper's hawk) was co-infected with all 3 genera of parasites (Table I). The highest prevalence of co-infection was in owls, where 5 of the 12 infected (41.7%) were co-infected with *Leucocytozoon* and either *Parahaemoproteus* or *Plasmodium* (Table I).

Table I. Prevalence of *Leucocytozoon* (LE), *Parahaemoproteus* (PA), and *Plasmodium* (PL) in raptors sampled from Minnesota and North Dakota, 2011–2017.

Host species by avian order	Total	Number infected	Percent infected per parasite genus			
			LE	PA	PL	Co-infected
Accipitriformes (5 species)						
Cooper's hawk (*Accipiter cooperii*)	391	128	24.0%	0.5%	11.0%	2.6%*
Red-tailed hawk (*Buteo jamaicensis*)	146	93	48.6%	2.7%	22.6%	11.0%†
Sharp-shinned hawk (*Accipiter striatus*)	3	0	0%	0%	0%	0%
Rough-legged hawk (*Buteo lagopus*)	2	1	50%	0%	0%	0%
Broad-winged hawk (*Buteo platypterus*)	1	1	100%	0%	0%	0%
Total	543	223 (41.1%)	30.8%	1.1%	14.0%	4.8%‡
Falconiformes (3 species)						
Peregrine falcon (*Falco peregrinus*)	25	7	8.0%	4.0%	16.0%	0%
American kestrel (*Falco sparverius*)	10	4	0%	40.0%	0%	0%
Merlin (*Falco columbarius*)	3	1	0%	33.3%	0%	0%
Total	38	12 (31.6%)	5.3%	15.8%	10.5%	0%
Strigiformes (7 species)						
Great grey owl (*Strix nebulosa*)	5	5	80.0%	20.0%	40.0%	40%§
Great horned owl (*Bubo virginianus*)	2	1	50.0%	50.0%	0%	50.0%‖
Barred owl (*Strix varia*)	2	2	100%	0%	50.0%	50.0%#
Northern hawk-owl (*Surnia ulula*)	2	2	100%	0%	0%	0%
Northern saw-whet owl (*Aegolius acadicus*)	1	0	0%	0%	0%	0%
Long-eared owl (*Asio otus*)	1	1	100%	100%	0%	100%‖
Snowy owl (*Bubo scandiacus*)	1	1	100%	0%	0%	0%
Total	14	12 (85.7%)	78.6%	21.4%	21.4%	35.7%¶
Grand total (15 species)	595	247 (41.5%)	30.3%	2.5%	13.9%	5.2%**

* Nine hosts with *Leucocytozoon* and *Plasmodium;* 1 host with *Leucocytozoon, Parahaemoproteus*, and *Plasmodium*.
† Thirteen hosts with *Leucocytozoon* and *Plasmodium*; 3 hosts with a *Leucocytozoon* and *Parahaemoproteus*.
‡ Twenty-two hosts with *Leucocytozoon* and *Plasmodium*; 3 hosts with *Leucocytozoon* and *Parahaemoproteus*; and 1 host with *Leucocytozoon*, *Parahaemoproteus*, and *Plasmodium*.
§ One host with *Leucocytozoon* and *Parahaemoproteus*; 1 host with *Leucocytozoon* and *Plasmodium*.
‖ One host with Leucocytozoon and Parahaemoproteus.
One host with *Leucocytozoon* and *Plasmodium*.
¶ Three hosts with *Leucocytozoon* and *Parahaemoproteus*; 2 hosts with *Leucocytozoon* and *Plasmodium*.
** Twenty-four hosts with *Leucocytozoon* and *Plasmodium,* 6 hosts with *Leucocytozoon* and *Parahaemoproteus*, and 1 host with *Leucocytozoon*, *Parahaemoproteus*, and *Plasmodium*.

A total of 27 haemosporidian lineages were identified, composed of 8 *Leucocytozoon*, 6 *Parahaemoproteus*, and 13 *Plasmodium* lineages (Table III). Twelve of these lineages were identified for the first time in this study, including 4 novel *Leucocytozoon*, 3 novel *Parahaemoproteus*, and 5 novel *Plasmodium* lineages (Table III). This includes 5 new lineages for peregrine falcon nestlings, 3 new lineages for Cooper's hawks, and 1 new lineage each for barred owls (*Strix varia*), merlins (*Falco columbarius*), northern hawk-owls (*Surnia ulula*), and red-tailed hawks (Table III). Eighteen lineages (half of which were novel lineages) were identified in immature birds—10 in nestlings, 2 in hatch-year birds, and 6 in both nestlings and hatch-year birds (Table III).

Table II. Comparative prevalence of haemosporidian parasites between age classes in Cooper's hawks (*Accipiter cooperii*) and red-tailed hawks (*Buteo jamaicensis*) and between sexes in Cooper's hawks sampled from Minnesota and North Dakota, 2011–2017.

Host group	Percent infected (number examined)	
	Cooper's hawk	Red-tailed hawk
Age		
Adult	45.6% (79)	71.9% (64)
Hatch year	25.0% (4)	57.3% (82)
Nestling	30.6% (301)	—
Sex		
Female	37.8% (196)	—
Male	29.3% (188)	—

Most (i.e., 22 of 27) of the haemosporidian lineages identified were recovered from a single raptor order. Eleven lineages were restricted to Accipitriformes (2 *Leucocytozoon*, 1 *Parahaemoproteus*, and 8 *Plasmodium*), 7 lineages were restricted to Falconiformes (1 *Leucocytozoon*, 3 *Parahaemoproteus*, and 3 *Plasmodium*), and 4 lineages were restricted to Strigiformes (3 *Leucocytozoon* and 1 *Plasmodium*). Some lineages parasitized multiple raptor orders. For example, 1 *Leucocytozoon* lineage (PHARUB01) parasitized all 3 raptor orders. Three lineages (*Leucocytozoon* lineage BUVIR04, *Parahaemoproteus* lineage STRURA02, and *Plasmodium* lineage RWB01) parasitized both Accipitriformes and Strigiformes. *Parahaemoproteus* lineage (BNOW02) parasitized both Accipitriformes and Falconiformes (Table III). Of the 27 lineages identified, 15 were previously known. Twelve of these 15 lineages infect other avian orders. This includes 3 of the 4 *Leucocytozoon* lineages (BUVIR04, CIAE02, PHARUB01), 1 of the 3 *Parahaemoproteus* lineages (STRURA02), and all 8 *Plasmodium* lineages (BT7, MYCAME02, NYCNYC01, PADOM11, RWB01, SEIAUR01, SW5, ZEMAC01).

Phylogenetic reconstruction was based on 93 haemosporidian lineages known to infect North American raptors (Fig. 1). The tree topology revealed a significant phylogenetic signal among raptor host orders ($P < 0.001$). Half of the haemosporidian lineages in the tree were composed of *Leucocytozoon*, where pronounced host

Table III. Haemosporidian lineages identified in raptors sampled from Minnesota and North Dakota, 2011–2017.

Haemosporidian genus and lineages	Raptor species	Avian order*	Accession number
Leucocytozoon			
ACCCOO03†‡	*Accipiter cooperii*	A	PQ562383
BUVIR04‡	*Accipiter cooperii*, *Buteo jamaicensis*, *Strix nebulosa*	A, S	PQ562388
BUVIR06	*Bubo virginianus*	S	PQ562389
CIAE02	*Buteo jamaicensis*	A	PQ562390
PHARUB01‡§	*Accipiter cooperii*, *Bubo scandiacus*, *Buteo jamaicensis*, *Buteo lagopus*, *Buteo platypterus*, *Falco peregrinus*	A, F, S	PQ562400
FALPER06†‡	*Falco peregrinus*	F	PQ562396
STRVAR01†§	*Strix varia*	S	PQ562404
SURULU01†	*Surnia ulula*	S	PQ562405
Parahaemoproteus			
BNOW02‡§	*Buteo jamaicensis, Falco sparverius*	A, F	PQ562384
BNOW03‡	*Falco sparverius*	F	PQ562385
BUTJAM19†§	*Buteo jamaicensis*	A	PQ562387
FALCOL01†	*Falco columbarius*	F	PQ562391
FALPER03†‡	*Falco peregrinus*	F	PQ562393
STRURA02	*Accipiter cooperii, Strix nebulosa*	A, S	PQ562403
Plasmodium			
ACCCOO01†‡	*Accipiter cooperii*	A	PQ562381
ACCCOO02†	*Accipiter cooperii*	A	PQ562382
BT7‡§	*Accipiter cooperii*, *Buteo jamaicensis*	A	PQ562386
FALPER02†‡	*Falco peregrinus*	F	PQ562392
FALPER04†‡	*Falco peregrinus*	F	PQ562394
FALPER05†‡	*Falco peregrinus*	F	PQ562395
MYCAME02	*Accipiter cooperii*	A	PQ562397
NYCNYC01	*Accipiter cooperii*	A	PQ562398
PADOM11‡§	*Accipiter cooperii*, *Buteo jamaicensis*	A	PQ562399
RWB01‡§	*Accipiter cooperii*, *Buteo jamaicensis*, *Strix nebulosa*, *Strix varia*	A, S	PQ562401
SEIAUR01‡	*Accipiter cooperii*	A	PQ562402
SW5	*Strix nebulosa*	S	PQ562406
ZEMAC01‡§	*Accipiter cooperii*, *Buteo jamaicensis*	A	PQ562407

* A = Accipitriformes, F = Falconiformes, S = Strigiformes.
† Denotes novel lineages identified in this study.
‡ Identified in nestlings.
§ Identified in hatch-year birds.

specificity was evident. For example, *Leucocytozoon* contained 1 large clade composed of 16 lineages that have only been isolated from Accipitriformes (Fig. 2, clade E). In 2 other clades (Fig. 2, clades A and B), lineages restricted from Strigiformes predominated. To date, only 3 of the 47 *Leucocytozoon* lineages recovered from North American raptors are known to infect more than 1 raptor order (Figs. 1, 2). The phylogenies for the haemosporidian genera displayed differing effects of host order on tree topology, with significant signals for *Leucocytozoon* ($P < 0.001$) and *Haemoproteus* and *Parahaemoproteus* ($P = 0.001$), but not for *Plasmodium* ($P = 0.476$) (Figs. 2–4).

The *Leucocytozoon* phylogeny is dominated by large clades composed of lineages that infect Strigiformes (Fig. 2, clades A–C) and Accipitriformes (Fig. 2, clade E). The accipitriform clade is composed of lineages from the *Leucocytozoon toddi* group. Lineages identified herein are found throughout the phylogeny, generally clustered together, including a highly supported clade composed of 3 lineages, ACCOOO03, FALPER06, PHARUB01, the first 2 identified here for the first time (Fig. 3, clade D). A lineage of the *Leucocytozoon californicus* group, CIAE02 (Fig. 2, clade C) was identified in this study, however, we did not identify any lineages from the *L. toddi* group (Fig. 2, clade E). Overall, only 4 *Leucocytozoon* lineages were identified from North American Falconiformes (Fig. 2).

Parahaemoproteus lineages were more common in Falconiformes, with 6 lineages specific to this order, including a large clade composed of 2 main smaller clades, containing lineages primarily identified from Falconiformes (Fig. 3, clades A–C). Lineages from Strigiformes were also well represented, including a large well-supported clade that except for the lineage STRURA02 was restricted wholly to Strigiformes (Fig. 3, clade D). Only 4 *Parahaemoproteus* lineages were known to infect North American Accipitriformes, including the newly identified lineage, BUTJAM19 (Fig. 3, clade B; Table III). *Parahaemoproteus* lineages identified herein were distributed throughout the phylogeny. *Haemoproteus nisi* and *Haemoproteus multivacuolatus* (Fig. 3, clade E) formed a well-supported clade distinct from *Parahaemoproteus* clades.

For *Plasmodium*, the 13 lineages identified were distributed throughout the entire phylogeny (Fig. 4). Raptor host classification shows little congruence with the *Plasmodium* phylogeny. Several well-supported clades were composed of lineages infecting multiple raptor orders with multiple lineages recovered from 2 or 3 different raptor orders (Fig. 4).

DISCUSSION

High haemosporidian infection prevalence and parasite diversity are known for raptors (Krone et al., 2001; Ishak et al., 2008; Ortego and Cordero, 2009; Synek et al., 2016; Walther et al., 2016; Inumaru et al., 2017; Barino et al., 2021; Gao et al., 2021; Wiegmann et al., 2021; Harl et al., 2022, 2024; Martín-Maldonado et al., 2023; Svobodová et al., 2023). Previous works have focused mainly on European raptor species (see Harl et al., 2022, 2024), with our results adding to the known diversity of haemosporidian parasites of North American raptors, showing an overall prevalence of 41.5% and 27 distinct haemosporidian lineages (Tables I, III). We identified 12 new haemosporidian lineages, including 5 new lineages for peregrine falcons (Table III). Additionally, we add the first report of a genetic lineage infecting the northern hawk-owl, the second report for the broad-winged hawk (*Buteo platypterus*) (Outlaw and Ricklefs, 2009), and the third report for the merlin (Nardoni et al., 2020). Thus, there appears to be a rich diversity of haemosporidian parasites within North American raptors. Nevertheless, continued sampling within North America is warranted because to date less than 14% of North American raptor species have been examined for haemosporidian parasites (MalAvi database; Bensch et al., 2009).

Strigiformes (owls) had higher overall infection prevalence (85.7%) than either Accipitriformes (41.1%) or Falconiformes (31.6%) (Table I). This agrees with other works showing a high prevalence of haemosporidian infection in owls (Ishak et al., 2008; Ortego and Cordero, 2009; Synek et al., 2016; Inumaru et al., 2017; Barino et al., 2021; Gao et al., 2021; Martín-Maldonado et al., 2023). Overall, *Leucocytozoon* infections were more prevalent (30%) among raptors than either *Parahaemoproteus* (2%) or *Plasmodium* (14%) infections. *Leucocytozoon* prevalence was particularly high in owls (79%)—3 times higher than either *Parahaemoproteus* (21%) or *Plasmodium* (21.4%), and more than twice that of Accipitriformes (31%) or

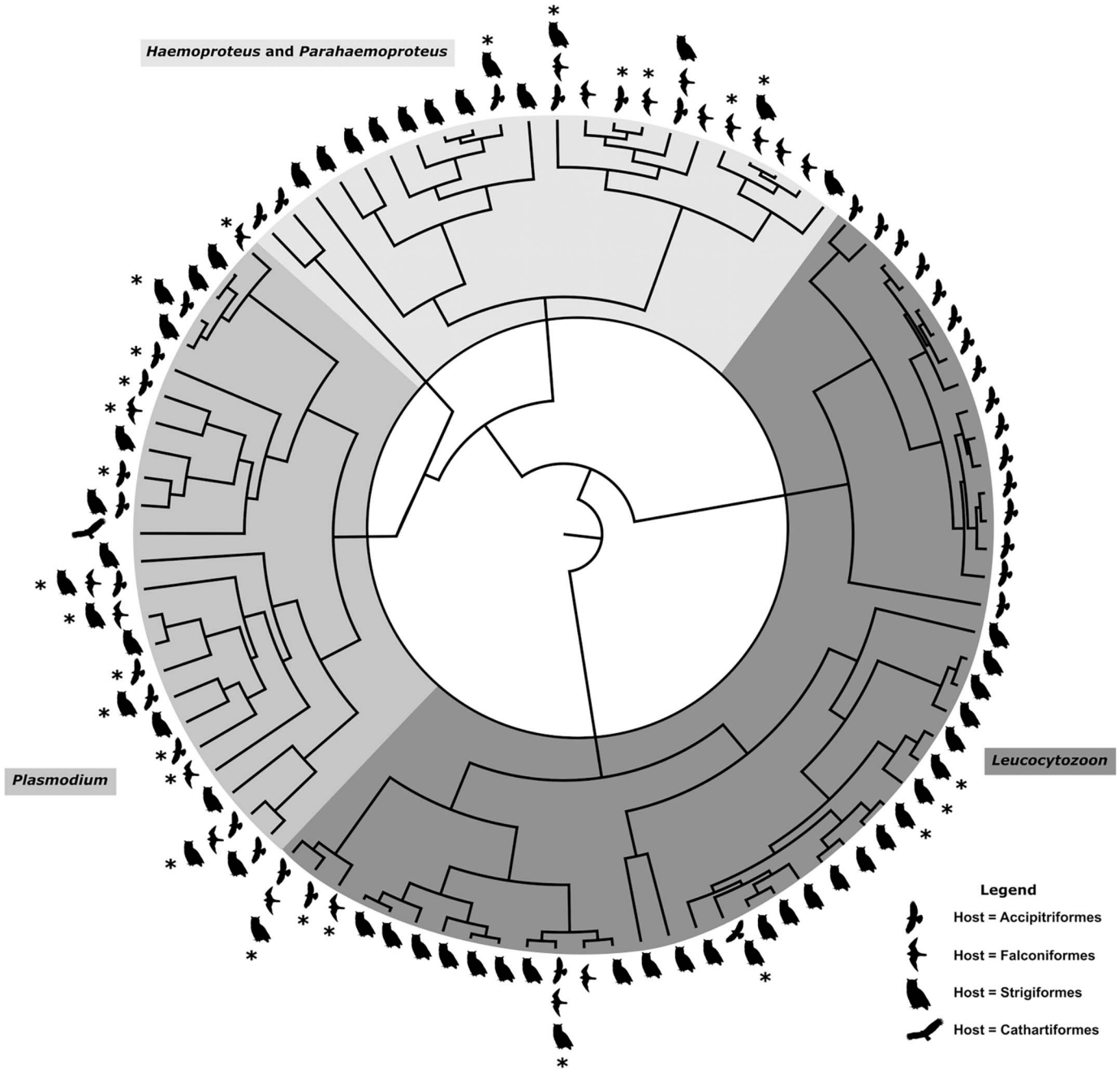

Figure 1. Bayesian inference phylogenetic reconstruction of haemosporidian lineages from North American raptors. Host order and parasite genus are indicated. Maddison-Slatkin analysis showed a phylogenetic signal for host order within the tree topology ($P < 0.001$). For clarity lineage names and posterior probability internodal support are not shown. All lineages identified in this study are indicated (*).

Falconiformes (5%) (Table 1). One reason for this may involve differences in the pattern of circadian behaviors between vector and host. Host defensive behavior against biting mosquitoes is more ineffective when hosts are at rest than when hosts are alert and fully awake (Day and Edman, 1984). Thus, it stands to reason that the transmission of haemosporidian parasites by nematoceran vectors (e.g., mosquitoes, midges, black flies) would be more efficient when the circadian patterns of the vector and the host are inverted. For example, *Plasmodium* and *Parahaemoproteus* parasites are transmitted by mosquitoes and *Culicoides*, which feed primarily at dusk and night. Crepuscular and/or nocturnal feeding, and subsequent transmission of *Plasmodium* and *Parahaemoproteus*, may be more successful against diurnally active raptor species roosting at night (i.e., hawks and falcons). Conversely, *Leucocytozoon* parasites are transmitted by black flies, which feed during the day. Daytime transmission of *Leucocytozoon* may be more efficient among owls than hawks or falcons because owls typically rest during the day. High *Leucocytozoon* prevalence in Accipitriformes is harder to explain but may be an aspect of infection chronicity, as birds can eliminate *Plasmodium* and *Parahaemoproteus* infections (Bensch et al., 2007; Knowles et al., 2011; Wood et al., 2013;

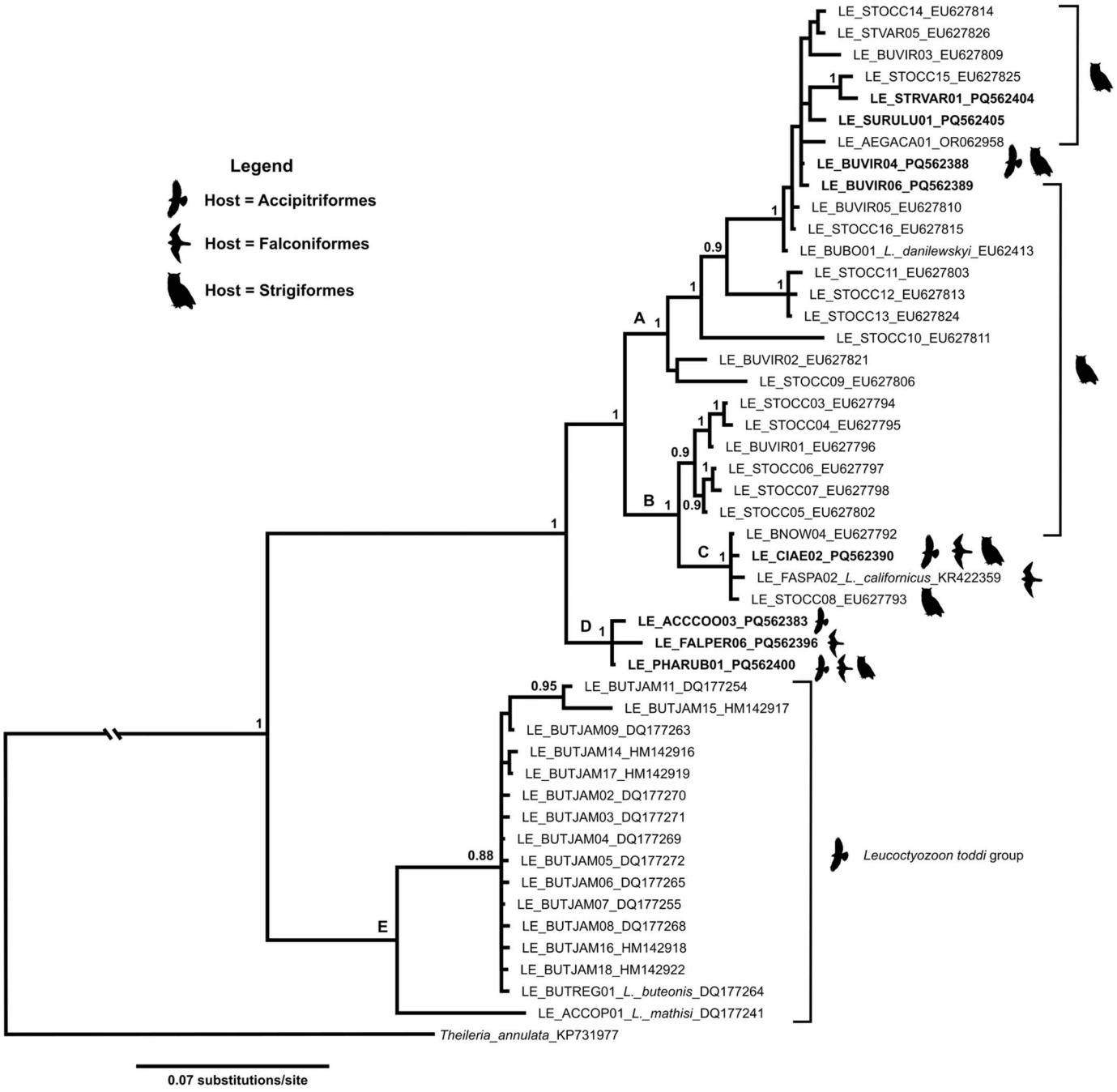

Figure 2. Bayesian inference phylogenetic reconstruction of *Leucocytozoon* (LE) lineages from North American raptors. Host order is indicated and all lineages identified from this study are in bold font. Maddison-Slatkin analysis showed a phylogenetic signal for host order within the tree topology ($P < 0.001$). For clarity of discussion, specific clades are identified (A–E). Numbers above internodes indicate posterior probability nodal support, with support values lower than 0.9 posterior probability not shown.

Hammers et al., 2016), whereas *Leucocytozoon* infections are more persistent (Ashford et al., 1990; Appleby et al., 1999; Leppert et al., 2008; Hanel et al., 2016).

Compared to hawks and owls, falcons had remarkedly lower *Leucocytozoon* prevalence (5%; Table I). However, *Parahaemoproteus* was higher in Falconiformes (16%) than Accipitriformes (6%; Table I). Similar patterns of lower *Leucocytozoon* versus higher *Parahaemoproteus* prevalence have been documented previously in several studies involving North American and European falcons (Korpimäki et al., 1995; Tella et al., 1996; Dawson and Bortolotti, 1999, 2001). However, Walther et al. (2016) reported high *Leucocytozoon* prevalence (37%) in American kestrels from California. These conflicting reports warrant additional studies on the haemosporidian parasites of falcons.

Adult Cooper's hawks had higher infection prevalence than immature birds (Table II). Previous reports of age-class differences in haemosporidian infections among birds have shown conflicting results. Some surveys reported higher prevalence in

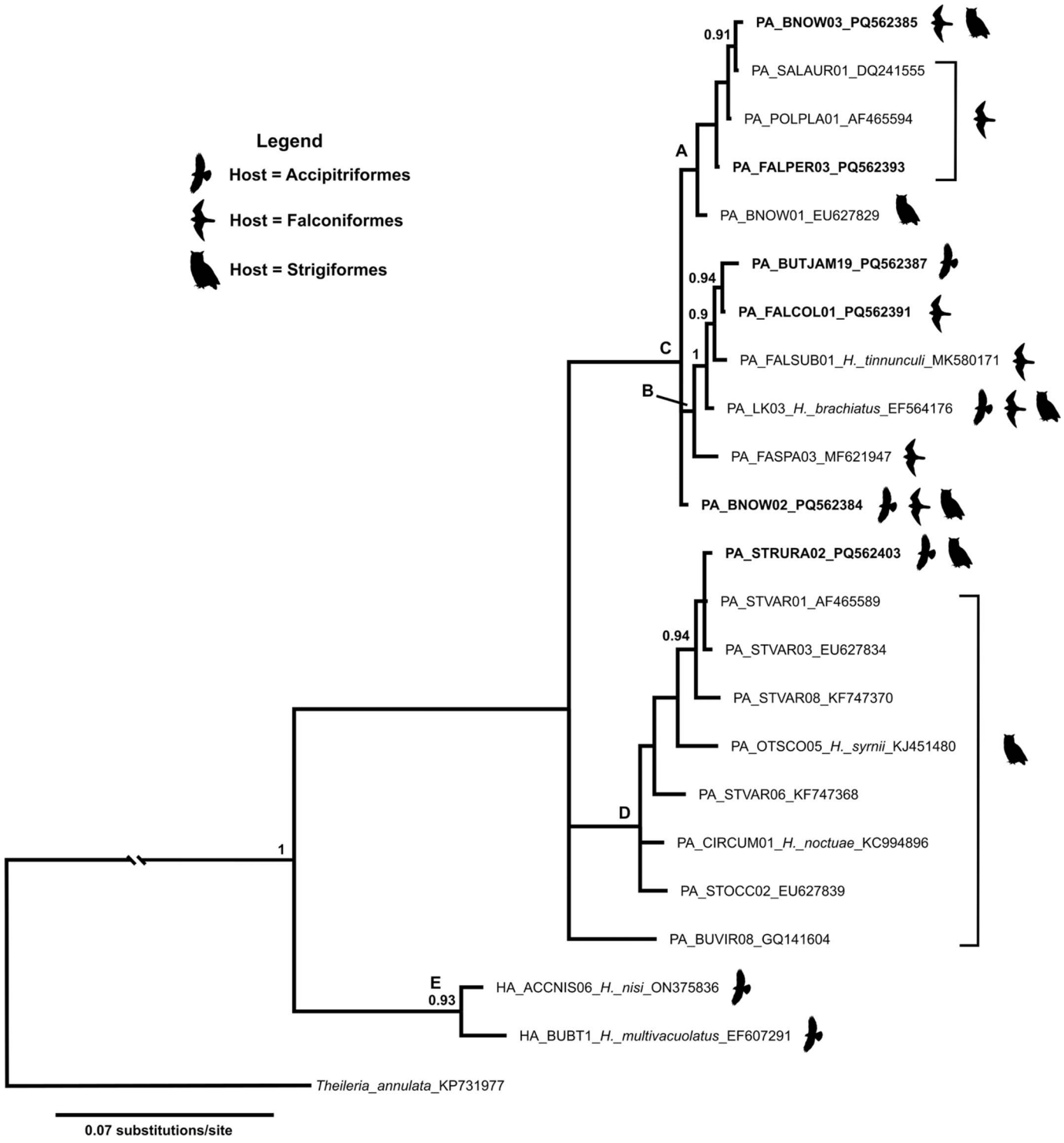

Figure 3. Bayesian inference phylogenetic reconstruction of *Haemoproteus* (HA) and *Parahaemoproteus* (PA) lineages from North American raptors. Host order is indicated and all lineages identified from this study are in bold font. Maddison-Slatkin analysis showed a phylogenetic signal for host order within the tree topology ($P = 0.001$). For clarity of discussion specific clades are identified (A–E). Numbers above internodes indicate posterior probability nodal support, with support values lower than 0.9 posterior probability not shown.

adults (Krone et al., 2001; Meixell et al., 2016; Synek et al., 2016; Carlson et al., 2018; Martín-Moldonado et al., 2023), whereas others reported the opposite (Murdock et al., 2013; Ramey et al., 2014; Lutz et al., 2015; Hammers et al., 2016; Meixell et al., 2016; Carlson et al., 2018). In explaining variation in haemosporidian infection between age classes, Sol et al. (2003) formulated 3 nonmutually exclusive hypotheses. The 'selection hypothesis' postulates that heavily infected young

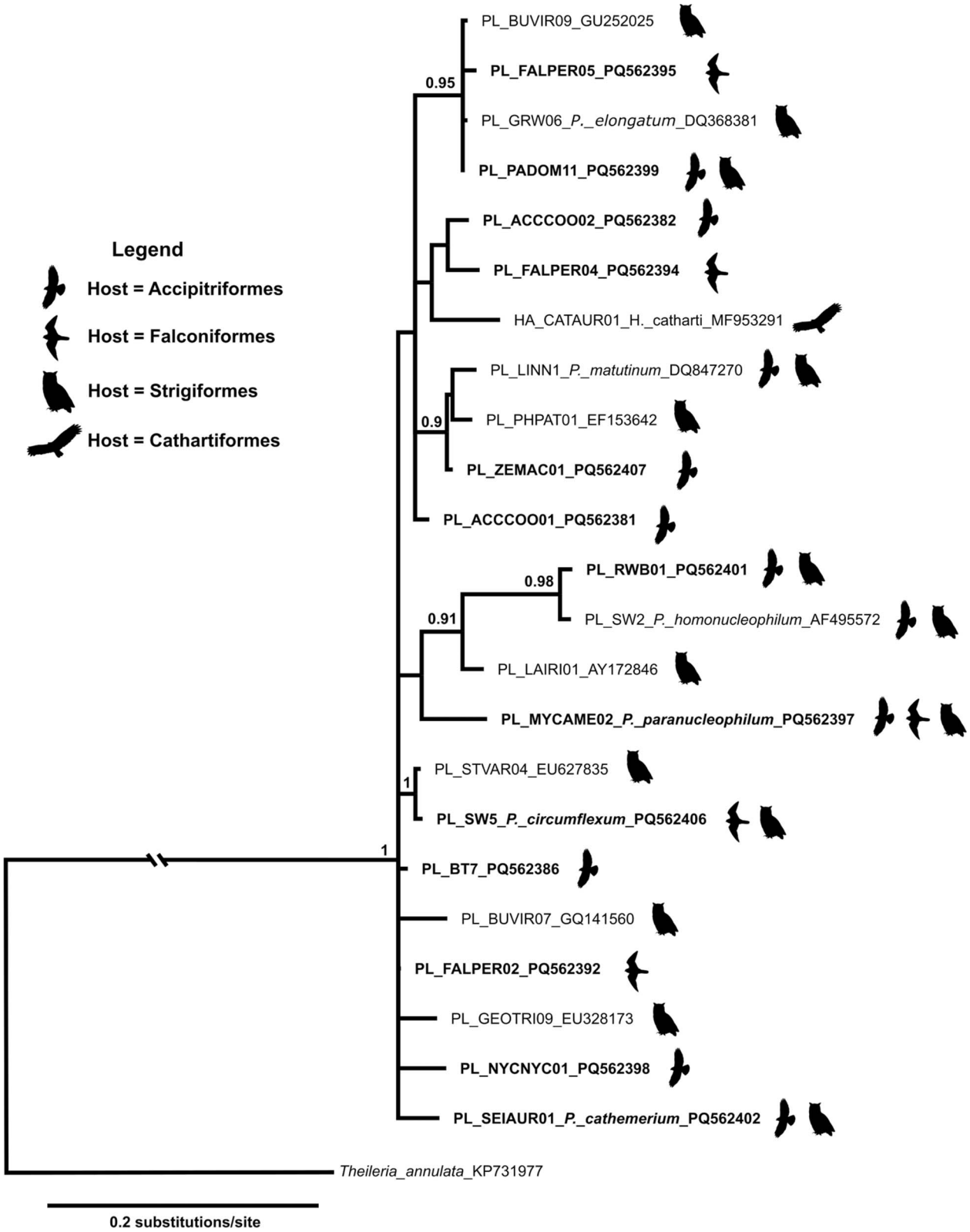

Figure 4. Bayesian inference phylogenetic reconstruction of *Plasmodium* (PL) lineages from North American raptors. *Haemoproteus catharti* (HA_CATAUR01) is included in this tree, as the lineage reported for this parasite is a *Plasmodium* lineage; see text for explanation. Host order is indicated and all lineages identified from this study are in bold font. Maddison-Slatkin analysis did not find a phylogenetic signal for host order within the tree topology ($P = 0.476$). Numbers above internodes indicate posterior probability nodal support, with support values lower than 0.9 posterior probability not shown.

birds die before reaching adulthood, leading to lower infection prevalence in adults. Our data on Cooper's hawks do not support this hypothesis. The 'vector exposure hypothesis' suggests that cumulative exposure to vectors experienced over time by older birds tends to increase haemosporidian prevalence in adults (Valkiūnas, 2005; Karadjian et al., 2013; Synek et al., 2016; van Hemert et al., 2019; Martín-Maldonado et al., 2023). In the upper Midwest, Cooper's hawks in the wild often live several years (i.e., several transmission seasons), but thereafter their probability of survival markedly decreases (Rosenfield et al., 2009). Thus, in the case of Cooper's hawks, the vector exposure hypothesis may partially explain the age-class differences in haemosporidian infection prevalence. Finally, the 'immunity hypothesis' postulates that differences in immune function between immature and adult birds account for differences in infection prevalence. As the immune response to infection develops with age (Ziman et al., 2004; Leppert et al., 2008: Dehnard et al., 2011; Jakubas et al., 2015), 2 outcomes are possible. First, older birds that survive infection develop sterile immunity, may clear the infection, and prevalence declines in older age classes. Alternatively, a state of 'semi-immunity' may develop in older birds (analogous to human malaria in parts of Africa). The truly detrimental effects of infection are kept in check, but the parasite survives, keeping older infected individuals within the population. Our results with Cooper's hawks support this second scenario and, together with cumulative infections (vector exposure hypothesis), may be the most probable explanation for our observations.

The nesting period is a key time for haemosporidian transmission. During this time nestlings and attending adults provide sedentary targets and emit volatile attractants for blood-feeding vectors (McCurdy et al., 1998; Gibson and Torr, 1999; Valkiūnas, 2005; Bishop et al., 2008; Logan et al., 2010; Weinandt et al., 2012; Caillouët et al., 2013; McMeniman et al., 2014; Lutz et al., 2015; Dubiec et al., 2016; Castaño-Vázquez et al., 2020; Jones et al., 2024). In addition, infected adults at the nest site can serve as the source of transmission to nestlings via interrupted feeding of infectious vectors within the nest (Ashford et al., 1990, 1991; Appleby et al., 1999; Chakarov et al., 2015; Svobodová et al., 2015; Bukauskaitė et al., 2024). For Cooper's hawks, we collected blood concurrently from nestlings and their parents. Of the 9 lineages found in nestling Cooper's hawks, 5 were shared with adults within the population. This strongly supports that lineage transmission within the nest from infected parent to offspring occurs with Cooper's hawks. If so, then the detection of blood parasitemia within nestlings further suggests that parasite transmission to nestlings probably occurred soon after hatching because sporozoite-induced infections require several days to develop into fulminant blood infections (Valkiūnas, 2005). Any differences in lineage composition between adults and nestlings most likely result from adults acquiring lineages outside the nesting period.

We also sampled nestling peregrine falcons but unfortunately did not sample adults. The nesting boxes were situated on tall (>45 m) structures within urban areas that maintained active mosquito control ground spraying programs during the summer. Even so, 7 of the 25 falcon chicks were infected, demonstrating the vertical height to which ornithophilic vectors can feed and transmit haemosporidian infections. For red-tailed hawks, we sampled adults and hatch-year birds during the fall migration. Hatch-year birds shared 7 of 9 lineages with adult red-tailed hawks (Table III). This suggests that both adults and immatures had acquired common lineages at breeding sites and perhaps even in the nest.

Distinct parasite clades from raptor hosts are known for *Leucocytozoon*, *Haemoproteus*, and *Parahaemoproteus* (Sehgal et al., 2006; Krone et al., 2008; Outlaw and Ricklefs, 2009; Valkiūnas et al., 2010; Walther et al., 2016; Barino et al., 2021; Harl et al., 2022, 2024; Svobodová et al., 2023) and here we show that host order has a significant constraint on the phylogeny of these haemosporidian genera in North American raptors (Figs. 1–3). Walther et al. (2016) found similar results in California, showing that Falconiformes and Accipitriformes harbor distinct *Leucocytozoon* parasites, with lineages from Falconiformes more closely aligned with lineage from Strigiformes and Passeriformes. Harl et al. (2022) found that the majority of *Leucocytozoon* and *Parahaemoproteus* lineages were specific to Accipitriformes, including the diverse *L. toddi* species group (Fig. 2, clade E). In addition, the *Haemoproteus* parasites of Accipitriformes, *H. multivacuolatus* and *H. nisi*, are distinct to this host order and likely represent a distinct subgenus or genus (Svobodová et al., 2023; Harl et al., 2024) (Fig. 3, clade E). The inability to detect lineages within either the *L. toddi* group or related to *H. multivacuolatus* or *H. nisi* is likely a consequence of the primers used in this study, which are not a close match to *Leucocytozoon* and *Haemoproteus* parasites that infect nonpasserines (Hanel et al., 2016; Valkiūnas et al., 2016; Himmel et al., 2019; Harl et al., 2022, 2024; Vanstreels et al., 2022). Rescreening extractions with the nested primers sets Cytb_L2_F/CytB_L2_R, CytB_L2_nF/Cyt_L2_nR for the *L. toddi* group (Himmel et al., 2019) and CytB_Hnis_F1/CytB_Hnis_R1, Cytb_Hnis_F2/Cytb_Hnis_R2 for *Haemoproteus* (Harl et al., 2024) is warranted to determine the true distribution of these parasites within local raptor populations.

We identified large strigiform-specific parasite clades within *Leucocytozoon* (Fig. 2, clades A, B) and *Parahaemoproteus* (Fig. 3, clade D), which include the species *Haemoproteus noctuae, Haemoproteus syrnii,* and *Leucocytozoon danilewskyi* that are restricted to Strigiformes (Remple, 2004; Valkiūnas, 2005; Krone et al., 2008; Ortego and Cordero, 2009; Barino et al., 2021). The limited sharing of lineages of *Leucocytozoon*, *Haemoproteus*, and *Parahaemoproteus* between diurnal raptor orders, Accipitriformes and Falconiformes, may be a consequence of host phylogeny, as Falconiformes are most closely related to Passeriformes and Psittaciformes than Accipitriformes (Hackett et al., 2008; McCormack et al., 2013; Jarvis et al., 2014) (Figs. 1–3).

The impact of host order is evident within the phylogeny of *Leucocytozoon*, with large clades composed of lineages infecting Accipitriformes (Fig. 2, clade E) and Strigiformes (Fig. 2, clades A, B). The few lineages from Falconiformes are restricted to 2 clades, 1 composed of lineages within the *L. californicus* group, and the other composed of mainly new lineages described here (Fig. 2, clades C, E). *Leucocytozoon californicus* was first described from American kestrels in California (Walther et al., 2016) and later Harl et al. (2022) identified it as a cryptic species complex, including lineage CIAE02 we identified here from red-tailed hawks (Table III). Walther et al. (2016) suggested that the clustering of *L. californicus* with lineages from Strigiformes was due to host switching between falcon and owl hosts, which is supported by our phylogenetic reconstruction. We identify a highly supported clade of *Leucocytozoon* lineages composed of 2 new lineages ACCCOO03 and FALPER06, and the previously described lineage PHARUB01 (Fig. 2, clade D). This may represent a new and

unique clade of *Leucocytozoon* parasites within North American raptors, which awaits further sampling to determine if this is indeed the case. Our phylogenetic reconstruction supports lineage PHARUB01 as a parasite of raptors, even though it was previously described in Brazil from nonraptor hosts (Fecchio et al., 2020a). Its initial identification is likely the result of an abortive infection with the parasite unable to complete its life cycle in hosts outside of raptors (Valkiūnas et al., 2013, 2014; Harl et al., 2022).

The *Leucocytozoon* parasites of raptors are diverse with high levels of divergence and contain at least 3 cryptic species complexes, *L. californicus* in Falconiformes (Walther et al., 2016; Harl et al., 2022), *L. danilewskyi* in Strigiformes (Krone et al., 2008; Ortego and Cordero, 2009), and *L. toddi* in Accipitriformes (Sehgal et al., 2006; Outlaw and Ricklefs, 2009; Valkiūnas et al., 2010; Hanel et al., 2016; Harl et al., 2022). The *L. toddi* group (Fig. 2, clade E) is especially interesting as members differ from other *Leucocytozoon* lineages by up to 20% (Valkiūnas et al., 2010; Harl et al., 2022), with over 10% divergence between lineages within this group that infect species of the genera *Accipiter* and *Buteo* (Sehgal et al., 2006; Valkiūnas et al., 2010; Harl et al., 2022). For this reason, Valkiūnas et al. (2010) redescribed *Leucocytozoon buteonis,* which infects the genus *Buteo,* and *Leucocytozoon mathisi*, which infects the genus *Accipiter* as separate species within the *L. toddi* group. Additionally, Harl et al. (2022) discovered several subclades within the *L. toddi* group clustered by host genus and hypothesized that this species complex alone may contain up to 20 species. As only 7% of Accipitriformes have been sampled for *L. toddi* (Harl et al., 2022), the true diversity of this species group and *Leucocytozoon* parasites of raptors, in general, is likely extremely high; however, many species are undescribed, partially because of limited morphological characteristics for species description (Valkiūnas, 2005; Harl et al., 2022).

Parahaemoproteus lineages are more prevalent among Falconiformes (8 of 20 total lineages) than lineages of *Leucocytozoon* (4 of 47 lineages) and *Plasmodium* (5 of 23 lineages). Several instances exist of potential *Parahaemoproteus* host switching between Falconiformes and Strigiformes (Fig. 3, clades A and B). *Parahaemoproteus* contains a large clade (Fig. 3, clade D), which, except for the lineage STRURA02 (also known to infect Passeriformes), are restricted to Strigiformes. This clade includes *Haemoproteus syrnii*, which Barino et al. (2021) suggest may represent a cryptic species complex due to high intraspecific variation. Two species, *H. multivacuolatus* and *H. nisi*, from Accipitriformes form a unique clade, distinct from *Parahaemoproteus* (Fig 3, clade E). These 2 parasite species potentially represent a new taxonomic group of haemosporidian parasites (Svobodová et al., 2023; Harl et al., 2024), diverging from known haemosporidian lineages by 15% (Harl et al., 2024). *Haemoproteus nisi* likely represents a species group, similar to *H. syrnii* in Strigiformes (Barino et al., 2021). The phylogenetic diversity of *Haemoproteus* and *Parahaemoproteus* in raptors will certainly increase when traditional *Haemoproteus* species, based solely on morphology—for example, *Haemoproteus buteonis*, *Haemoproteus elani*, and *Haemoproteus janovyi*—are defined molecularly (Valkiūnas, 2005; Harl et al., 2022, 2024).

The lack of phylogenetic signal within the *Plasmodium* phylogeny (Fig. 4) is not surprising, as many of the lineages shown here infect other avian orders, most notably Passeriformes. For example, *Plasmodium* lineages BT7, GRW06, LINN1, PADOM11, RWB01, SEIAUR01, SW2, SW5, and ZEMAC01 are rather non–host specific, widely distributed among a variety of nonraptor species throughout the Americas. Many of the lineages identified from raptors in North America may represent parasites that are unable to complete their life cycles in raptors rather than true host associations (Valkiūnas et al., 2013, 2014; Harl et al., 2022). For example, lineage MYCAME02 (Table III) likely naturally infects Ciconiformes (storks) and not raptors (Harl et al., 2022). Harl et al. (2022) hypothesized that because many reports of *Plasmodium* in raptors come from rehabilitation centers, *Plasmodium* lineages are transmitted to raptors from other birds kept in close proximity and unable to develop within raptor hosts.

Lineage CATAUR01, although linked to *Haemoproteus catharti* (Yabsley et al., 2018) described from the turkey vultures (*Cathartes aur*a) (Greiner et al., 2011), is a *Plasmodium* lineage as shown here (Fig. 4) and previously (Harl et al., 2022). The description of *H. catharti* is valid (Greiner et al., 2011); however, as the original description is from blood smears containing coinfections of at least 2 different *Plasmodium* species, lineage CATAUR01 likely belongs to one of these *Plasmodium* species (Harl et al., 2022). Future work is needed to address the systematic position of *H. catharti*, a unique parasite in New World vultures (Cathartiformes).

Haemosporidian infections can be a concern for raptor populations as health impacts, survival reduction, and even infection-induced mortality are known (Ishak et al., 2008; Leppert et al., 2008; Niedringhaus et al., 2018; Giorgiadis et al., 2020; Yoshimoto et al., 2021; Briggs et al., 2022; Martín-Maldonado et al., 2023). Understanding haemosporidian infection dynamics is especially important for those species that are of special conservation concern. This includes many species of raptors. Owl species are highly susceptible to haemosporidian infection (Ishak et al., 2008; Ortego and Cordero, 2009; Synek et al., 2016; Inumaru et al., 2017; Barino et al., 2021; Gao et al., 2021; Martín-Maldonado et al., 2023), which, coupled with known parasite pathogenicity (Korpimäki et al., 1993; Hisada et al., 2004; Karadjian et al., 2013; Baker et al., 2018; Niedringhaus et al., 2018; Salakij et al., 2018; Pornpanom et al., 2019; Barino et al., 2021; Yoshimoto et al., 2021; Martín-Maldonado et al., 2023), places them at high risk of population impacts due to infection. Despite our rather sparse sampling of owl species, we found a very high prevalence of infection (Table I). For those species with low population sizes, such as great gray owls (*Strix nebulosa*), northern hawk-owls, and snowy owls (*Bubo scandiacus*), the potential for negative impacts of infection exist. However, these species remain undersampled for haemosporidian infections, so the true impact is unknown. Our results show a relatively high prevalence for peregrine falcon nestlings and, although they have been removed from the Endangered Species List, they are still a CITES Appendix I listed species and of specific conservation concern. As *Leucocytozoon* infections in peregrine falcons have been linked to neurological symptoms (Raidal et al., 1999; Raidal and Jaensch, 2000) future work is warranted to determine the potential impact of haemosporidian infection on the local peregrine falcon populations.

In summary, our results add to the growing body of evidence that raptors support distinct haemosporidian parasites generally restricted to this host group. *Leucocytozoon* parasites of raptors were found to be common and diverse, with high levels of lineage divergence, tight host associations, and multiple examples of cryptic species groups (Sehgal et al., 2006; Ishak et al., 2008;

Krone et al., 2008; Ortego and Cordero, 2009; Valkiūnas et al., 2010; Walther et al., 2016; Harl et al., 2022, 2024). Conversely, *Plasmodium* parasites of raptors appeared to be more generalists, with looser host associations. Adequately surveying raptor hosts presents unique challenges, but in partnership with bird banders, governmental agencies, and rehabilitation centers, opportunities exist to achieve a broader sampling of North American raptors to define this group of parasites more fully.

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