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Prevalence and genetic characterization of haemosporidian blood parasites in wild turkeys (*Meleagris gallopavo*) across the Eastern United States and in a tropical congener

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ABSTRACT

Wild turkeys (*Meleagris gallopavo*) are hosts to several vector-borne haemosporidian blood parasites that can cause morbidity and mortality in wild and domestic turkeys. This study investigated the prevalence, geographical distribution, and genetic diversity of haemosporidians in 942 wild turkeys from the USA and 12 ocellated turkeys (*Meleagris ocellata*) from Belize. *Haemoproteus* was detected in 69% (650/942) of USA turkeys. Sequencing revealed six unique haplotypes in two lineages (1a-1c and 2a-2c). Haplotypes 1a-1c were 99.4–99.8% similar, and Haplotype 1a was identical to a *Haemoproteus* reported in northern bobwhite (*Colinus virginianus*). Haplotypes 2a-2c were 98.2–99.2% similar, and Haplotype 2a was most similar (99.1%) to *Haemoproteus* reported from galliforms in Austria and Thailand. Lineage 1 sequences were only 96.9–98.2% similar to Lineage 2. Lineage 1 (1a-1c) was detected in 84.6% (523/618) of *Haemoproteus*-positive USA turkeys, with Haplotype 1a being most common (81.9%, 506/618). Lineage 2 (2a-2c) was detected in 43.4% (268/618) of USA turkeys. *Leucocytozoon* was detected in 9.7% (91/942) of USA wild turkeys, and all were identical to a *Leucocytozoon* from northern bobwhite. *Haemoproteus* prevalence was higher in males *vs* females and adults *vs* juveniles, and *Leucocytozoon* prevalence was higher in adults *vs* juveniles. No *Plasmodium* infections were detected. *Haemoproteus* was the only parasite detected in ocellated turkeys; 12 (100%) were infected with Lineage 1a, and four (33.3%) were co-infected with Lineage 2a. These findings provide new insights into the prevalence and genetic diversity of haemosporidians in turkeys.

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1. Introduction

Wild turkeys (*Meleagris gallopavo*) have significant ecological, recreational, and economic importance in the USA. As a native game bird, wild turkeys support extensive hunting industries while playing crucial roles in seed dispersal and invertebrate population regulation where they occur (Groepper et al., 2013; Chamberlain et al., 2020). In recent years, wild turkey populations have experienced marked declines in portions of their range in eastern and midwestern North America (Chamberlain et al., 2020). Numerous factors have been proposed to explain these declines, which may be related to contemporary decreases in productivity (Byrne et al., 2015) and adult female survival (Lashley et al., 2025), resulting from habitat loss, predation pressure, and disease (Crawford et al., 2021; Shea et al., 2022, 2026; Londe et al., 2023; Stoakley et al., 2025).

Avian haemosporidians (*Plasmodium* spp., *Haemoproteus* spp., and *Leucocytozoon* spp.) are arthropod-vectored parasites that can cause disease in a diversity of wild and captive avifauna globally (Valkiūnas, 2005; Astudillo et al., 2013; Banda et al., 2013; Hernandez et al., 2013; Kistler et al., 2013; Ramey et al., 2014; Jones et al., 2015; Vanstreels et al., 2015; Coker et al., 2017; Niedringhaus et al., 2018; Yabsley et al., 2018, 2023; Dadam et al., 2019; Hernandez-Colina et al., 2021; Miranda Paez et al., 2022; Chong et al., 2023). The haemosporidians are transmitted by dipteran vectors: *Plasmodium* spp. by mosquitoes (Culicidae), *Haemoproteus* spp. by louse flies (Hippoboscidae) and biting midges (*Culicoides* spp.), and *Leucocytozoon* spp. by black flies (Simuliidae) and biting midges (Noblet et al., 1975; Valkiūnas et al., 2002; Valkiūnas, 2005; Hellgren et al., 2008; Kimura et al., 2010; Valkiūnas and Iezhova, 2023). Sexual reproduction of these parasites occurs in their respective insect vectors, while birds serve as intermediate hosts for asexual amplification (Valkiūnas, 2005).

Wild turkeys are hosts for several haemosporidian blood parasites including *Haemoproteus meleagridis*, *Leucocytozoon smithi,* and several *Plasmodium* spp. (Stacey et al., 1990; Valkiūnas, 2005). In many previous studies, *H. meleagridis* was the most common blood parasite in wild turkeys, with prevalence rates sometimes exceeding 80% (Cook et al., 1966; Atkinson and Forrester, 1987; Castle et al., 1988; Fedynich and Rhodes, 1995). *Leucocytozoon smithi* has also been documented in many wild turkey populations in the southeastern USA, although prevalence rates are more variable (Travis et al., 1939; Byrd, 1959; Hammond et al., 1972; Forrester et al., 1974; Noblet and Moore, 1975; Hopkins et al., 1990; Fedynich and Rhodes, 1995). While less common, sporadic *Plasmodium* infections have been reported in wild turkeys from several eastern USA states (Castle and Christensen, 1990; Stacey et al., 1990; Fedynich and Rhodes, 1995).

Haemoproteus meleagridis and *L. smithi* are well-documented causes of morbidity and mortality in both wild and domestic turkeys. Experimental infections of domestic turkey poults with wild turkey-derived *H. meleagridis* resulted in clinical signs including lethargy, anorexia, diarrhea, and lameness as early as 7 days post-infection (Atkinson et al., 1988). Clinical signs of *L. smithi* infection in domestic and wild turkeys include anorexia, ataxia, anemia, and dyspnea (Savage and Isa, 1945; Byrd, 1959; Jones et al., 1972, 2015). Experimental inoculations of domestic turkeys with *L. smithi* produced megaloschizonts, hemorrhages, and organomegaly within 6–18 days post-infection (Siccardi et al., 1974). Adult birds can often survive acute *Leucocytozoon* spp. infection, but exo-erythrocytic parasites may remain dormant in host tissues with seasonal relapses and disease emergence (Alverson and Noblet, 1977; Valkiūnas and Iezhova, 2017).

Despite their importance to the health of wild and domestic turkeys, few comparative studies have been conducted on haemosporidian parasites, and genetic characterization is lacking (Haynes et al., 2024; Edge et al., 2026). The goal of this study was to investigate the prevalence, geographical distribution, and genetic diversity of haemosporidian blood parasites in wild turkeys across the eastern USA. Additionally, we tested ocellated turkeys (*Meleagris ocellata*) from Belize to compare parasite lineages present in this population.

2. Materials and methods

2.1. Sample collection

Samples from 656 live-captured or hunter-harvested wild turkeys from 19 USA states were obtained through collaboration with various researchers and organizations from 2018 to 2025. Blood samples (< 3 ml) were collected from the brachial vein, placed in EDTA tubes, and frozen at −20 °C until testing. An additional 266 samples were collected from diagnostic cases submitted to the Southeastern Cooperative Wildlife Disease Study (SCWDS) Research and Diagnostic Service at the University of Georgia (Athens, GA, USA) for routine mortality evaluation during 2020–2025. Most samples were whole blood that had either been frozen ($n = 886$) or fixed in ethanol ($n = 36$) before testing. However, 20 additional samples from a West Nile virus study consisted of Nobuto strips eluted in PBS and samples from SCWDS diagnostic cases were typically spleen or liver. Finally, we included ethanol-fixed whole blood samples from 12 ocellated turkeys from northwest Belize (Martin and Buchholz, 2018).

2.2. PCR methods and phylogenetic analyses

Genomic DNA was extracted from whole blood samples (10 μl), ethanol-fixed blood pellets, and Nobuto eluates (20 μl) using the DNeasy® Blood and Tissue Extraction Kit (Qiagen, Hilden, Germany). Before DNA was extracted from ethanol-fixed blood, samples were vortexed to resuspend the contents and 20–30 μl of solution was transferred to a new 1.5-ml microcentrifuge tube. The sub-sample was pelleted by centrifuging for 1 min at 14,000 *rpm*, the supernatant was pipetted off and discarded, and the tubes were left open for 2 h in a fume hood to evaporate off any remaining ethanol. One drop of molecular-grade water was added to the sample prior to evaporation to prevent complete desiccation. Tubes with Nobuto strip eluates were vortexed prior to DNA extraction to ensure the sample was thoroughly mixed.

All DNA extracts were tested for blood parasites using two nested PCR protocols targeting the cytochrome *b* gene using primary primers NF1 and NR3 and secondary primers F and R2 (for *Plasmodium* and *Haemoproteus*) and FL and R2L (*Leucocytozoon*) as described by Hellgren et al. (2004). Amplicons were visualized in 2% agarose gels stained with GelRed Stain (Biotium, Hayward, California). Positive amplicons were cut from the agarose gels and purified using the QIAquick gel extraction kit (Qiagen). Bidirectional Sanger sequencing was conducted by Genewiz (Azenta Life Sciences, South Plainfield, New Jersey, USA), and the sequences were edited and assembled using Geneious v.10.2.6 (Biomatters Limited, Auckland, New Zealand). Co-infections with two *Haemoproteus* lineages were identified based on the presence of double peaks for specific nucleotides that aligned with sequences from apparent single infections. The consensus sequences were subsequently used as queries for BLASTN searches against the National Center for Biotechnology Information (NCBI) GenBank nucleotide sequence database. Alignments were made in Geneious Prime v.2025.2.2. Phylogenetic analyses were conducted using the FastTree approximate maximum likelihood method in Geneious, and a Bayesian Inference tree was calculated with MrBayes v.3.2.6 (Huelsenbeck and Ronquist, 2001). The analysis was run for 5 million generations, and the subsampling frequency was set to 200 and the heated chain temperature to 0.2. The first 10% of trees were discarded as "burn-in", and a 50% majority rule consensus tree was calculated from the remaining trees. Unique sequences were submitted to the GenBank database under the accession numbers PZ554414-PZ554419.

DNA from a blood sample of a duck infected with *Haemoproteus nettionis* and *Leucocytozoon simondi* was used as a positive control in each set of PCR reactions. DNA extraction, primary and secondary amplification, and product analysis were completed in separate dedicated areas

to avoid and detect contamination. A negative molecular grade water control was included in each set of extractions and every 10–12 extractions within larger sets. Additional negative water controls were included in each set of primary and secondary PCR reaction sets.

2.3. Statistical analysis

The association between *Haemoproteus* or *Leucocytozoon* infection status and potential predictor variables (age, sex, region, and source) was evaluated using binomial generalized linear models (GLMs) with logit link in R (R Core Team, 2023). In addition, separate models were constructed for both lineages of *Haemoproteus* (L1 and L2, each model includes the total number of turkeys positive for single- and co-infections). Fixed predictor variables included: source (SCWDS diagnostic case submissions (DC) or live capture (Live); reference = DC); geographical region: South (Arkansas, Oklahoma, Mississippi, and Louisiana), Southeast (Alabama, Georgia, Florida, South Carolina, North Carolina, and Virginia), Ohio Valley (Tennessee, Kentucky, Missouri, and West Virginia), Northeast (Maryland, Pennsylvania, and Connecticut), and Plains (Kansas and Nebraska); reference = South); sex (female, male; reference = female); and age class (adult, juvenile; reference = adult). Birds with unknown sex or age were excluded from all analyses. Statistical significance was assessed at $\alpha = 0.05$.

3. Results

3.1. Haemoproteus spp. infections

Of 942 wild turkeys from the USA tested, 650 (69%) were positive on the *Haemoproteus/Plasmodium* screening PCR assay (Tables 1 and 2, Fig. 1). Sequence analysis confirmed that all PCR-positive samples were *Haemoproteus* spp.; no *Plasmodium* spp. infections were detected. Prevalence of *Haemoproteus* spp. was generally high across sampled states (40.4–100%) (Table 1). All 12 ocellated turkeys from Belize (100%) were infected with *Haemoproteus* spp.

Sequence analysis indicated there were six unique sequences. The six sequences phylogenetically grouped into two different clades (we have defined these as Lineages 1 and 2). Within each of these two lineages, there was additional minor genetic variation, and we have defined these as different haplotypes (1a, 1b, 1c, 2a, 2b, and 2c) (Fig. 2, Supplementary Fig. S1). During two previous studies (Haynes et al., 2024; Edge et al., 2026), it was found that many *Haemoproteus* sequences contained a low number of polymorphic bases. A total of 100 samples were re-sequenced to confirm the presence of polymorphic bases and to rule out sequence artifacts. Haplotypes 1a-1c were 99.4–99.8% similar to each other, and Haplotype 1a was identical to a *Haemoproteus* sp. previously reported in northern bobwhite (*Colinus virginianus*) in Oklahoma, USA (GenBank: OR102783) (Supplementary Table S1). The most similar lineage in the MalAvi database was hAFR151, which was 98.5% (457/464 bp) similar. Haplotypes 2a-2c were 98.2–99.2% similar to each other, and Haplotype 2a was most similar (99.1%) to two *Haemoproteus* sp. reported from other galliform birds in Austria (western capercaillie, *Tetrao urogallus*, GenBank: MK330141) and Thailand (grey peacock-pheasant, *Polyplectron bicalcaratum*, GenBank: PV520641). The most similar lineage in the MalAvi database was hTETURO01, which was 99.1% (460/464 bp) similar (this lineage is identical to MK330141 in GenBank). Haplotypes 2a-2c were only 96.9–98.2% similar to Haplotypes 1a-1c (Supplementary Table S1). Phylogenetically, the two haplotypes from turkeys grouped with other *Haemoproteus* (*Parahaemoproteus*) spp. (Fig. 2, Supplementary Fig. S1). Specifically, Haplotype 1a-1c sequences grouped with the *Haemoproteus* sp. from a bobwhite, and the Haplotype 2a-2c sequences grouped with the *Haemoproteus* sp. from a western capercaillie from Austria (Fig. 2, Supplementary Fig. S1).

Haemoproteus Lineage 1 (1a, 1b, and 1c) was detected in 84.6% (523/618) of 618 *Haemoproteus*-positive USA wild turkeys with high-quality full-length sequences (Tables 1 and 2); Haplotype 1a was the most common haplotype detected (81.9%, 506/618). Lineage 2 (2a, 2b, 2c) was detected in 43.4% (268/618) of these turkeys. The ocellated turkeys sampled from Belize were infected with *Haemoproteus* Haplotype 1a (12/12), with four of these (33.3%) co-infected with Haplotype 2a. Co-infections involving two or three *Haemoproteus* haplotypes were identified in 183 of 618 (29.6%) wild turkeys and 4 of 12 (33.3%) ocellated turkeys (Table 2). Of the 183 USA co-infections, 173 involved haplotypes from both lineages (Lineage 1 + Lineage 2 co-infection), while the remaining 10 involved haplotypes within a single lineage. Five wild turkeys harbored three haplotypes (Table 2). All four Belize co-infections were with two haplotypes (1a + 2a).

3.2. Leucocytozoon spp. infections

Leucocytozoon was detected in 91/942 (9.7%) wild turkeys and in 14 USA states, with prevalence varying widely by state (0–43.8%) (Tables 1 and 2, Fig. 1). No *Leucocytozoon* infections were detected in the Belize

Table 1
Prevalence of *Haemoproteus* and *Leucocytozoon* in 942 wild turkeys (*Meleagris gallopavo*) from the USA by sampling source, geographical region, sex, and age classes.

Variable	*Haemoproteus*			*Leucocytozoon*
	Total	Lineage 1	Lineage 2	
Source				
SCWDS diagnostic case	191/266 (71.8%)	117/165 (70.9%)	102/165 (61.8%)	52/266 (19.5%)
Live capture	459/676 (67.9%)	406 (89.6%)	166 (36.6%)	39/676 (5.8%)
Region				
South (AR, OK, MS, LA)	151/330 (45.8%)	133/147 (90.5%)	73/147 (49.7%)	28/330 (8.5%)
Southeast (AL, GA, FL, SC, NC, VA)	229/253 (90.5%)	211/224 (94.2%)	82/224 (36.6%)	42/253 (16.6%)
Ohio Valley (TN, KY, MO, WV)	252/319 (79.0%)	166/230 (72.2%)	106/230 (46.1%)	18/319 (5.6%)
Northeast (MD, PA, CT)	12/28 (42.9%)	10/12 (83.3%)	5/12 (41.7%)	3/28 (10.7%)
Plains (KS, NE)	6/12 (50.0%)	3/5 (60.0%)	2/5 (40.0%)	0/12 (0%)
Sex				
Female	274/438 (62.6%)	240/261 (91.9%)	103/261 (39.5%)	46/438 (10.5%)
Male	295/383 (77.0%)	232/280 (82.9%)	128/280 (45.7%)	44/383 (11.5%)
Age				
Adult	433/576 (75.2%)	349/408 (85.5%)	174/408 (42.6%)	77/576 (13.4%)
Juvenile	81/153 (52.9%)	72/79 (91.1%)	31/79 (39.2%)	5/153 (3.3%)
Total	650 (69.0%)	523 (84.6%)	268 (43.4%)	91/942 (9.7%)

Note: The *Haemoproteus* lineage was not determined for 32 wild turkeys, so the denominator for Lineage 1 (L1) and Lineage 2 (L2) prevalences is not the total number of birds positive for *Haemoproteus*. Also, the percentages of L1 plus L2 are more than 100% due to the high number of co-infections.
Abbreviations: AL, Alabama; AR, Arkansas; CT, Connecticut; FL, Florida; GA, Georgia; KS, Kansas; KY, Kentucky; LA, Louisiana; MD, Maryland; MS, Mississippi; MO, Missouri; NE, Nebraska; NC, North Carolina; OK, Oklahoma; PA, Pennsylvania; SC, South Carolina; TN, Tennessee; VA, Virginia; WV, West Virginia.

Table 2
Prevalence of *Haemoproteus* spp. and lineages and *Leucocytozoon* sp. in wild turkeys (*Meleagris gallopavo*) from the USA. Samples sizes for each state are given under the two-letter state abbreviation in parentheses. The number of birds and percent positive (%) are provided for the genera *Haemoproteus* and *Leucocytozoon*, as well as for both *Haemoproteus* lineages and the six unique *Haemoproteus* haplotypes. Haplotype data do not always add up to the total number of birds infected with *Haemoproteus* because of co-infections.

Parasite	State (sample size) and No. positive (%)																			Total (942)
	AL (13)	AR (6)	CT (1)	FL (41)	GA (16)	KS (6)	KY (265)	LA (277)	MD (1)	MO (9)	MS (38)	NC (174)	NE (6)	OK (9)	PA (26)	SC (6)	TN (22)	VA (3)	WV (23)	
***Haemoproteus* spp.-positive**	11 (84.6)	4 (66.7)	1 (100)	30 (73.2)	12 (75.0)	3 (50.0)	212 (80.0)	112 (40.4)	0	6 (66.7)	30 (78.9)	168 (96.6)	3 (50.0)	5 (55.6)	11 (42.3)	5 (83.3)	18 (81.8)	3 (100)	16 (69.6)	650 (69)[c]
Lineage 1	9 (69.2)	3 (50.0)	1 (100)	28 (68.3)	9 (56.3)	1 (16.7)	139 (52.5)	99 (35.7)[a]	0	2 (22.2)	27 (71.0)	160 (91.9)[b]	2 (33.3)	4 (44.4)	9 (34.6)	5 (83.3)	15 (68.2)	0	10 (43.5)	523/618 (84.6)
Haplotype 1a	9	3	1	28	9	1	138	93		2	26	151	2	4	9	5	15		10	506 (81.9)
Haplotype 1b							1	8			1	5				1				16 (2.6)
Haplotype 1c												6								6 (0.9)
Lineage 2	7 (53.8)	1 (16.7)	1 (100)	8 (19.5)	4 (25)	2 (33.3)	92 (34.7)[d]	55 (19.9)	0	4 (44.4)	15 (39.5)[e]	59 (33.9)	0	2 (22.2)	4 (15.4)	1 (16.7)	6 (27.3)	3 (100)	4 (17.4)	268/618 (43.4)
Haplotype 2a	7	1	1	8	4	2	64	49		4	15	52		2	4	1	6	3	4	227 (36.7)
Haplotype 2b							1				1	3								5 (0.8)
Haplotype 2c							34	6			2	4								46 (7.4)
***Haemoproteus* spp. (undetermined lineage)**	1			1	2		13	1		2	2	1	1	1			2		5	32
***Leucocytozoon* sp.-positive**	2 (15.4)	2 (33.3)	0	12 (29.3)	7 (43.8)	0	10 (3.8)	16 (5.8)	0	1 (11.1)	9 (23.7)	17 (9.8)	0	1 (11.1)	3 (11.5)	3 (50)	0	1 (33.3)	7 (30.4)	91 (9.7)

Abbreviations: AL, Alabama; AR, Arkansas; CT, Connecticut; FL, Florida; GA, Georgia; KS, Kansas; KY, Kentucky; LA, Louisiana; MD, Maryland; MS, Mississippi; MO, Missouri; NE, Nebraska; NC, North Carolina; OK, Oklahoma; PA, Pennsylvania; SC, South Carolina; TN, Tennessee; VA, Virginia; WV, West Virginia.

[a] Includes two birds coinfected with haplotypes 1a + 1b.

[b] Includes one bird coinfected with haplotype 1a + 1b and another with 1a + 1c.

[c] Includes 173 birds co-infected with Lineage 1 and Lineage 2.

[d] Includes one bird coinfected with haplotypes 1a + 2a + 2b, one with 1a + 2a + 2c, and five birds with 2a + 2c.

[e] Includes one bird coinfected with haplotypes 1a + 2a + 2b and two birds with 1a + 2a + 2c.

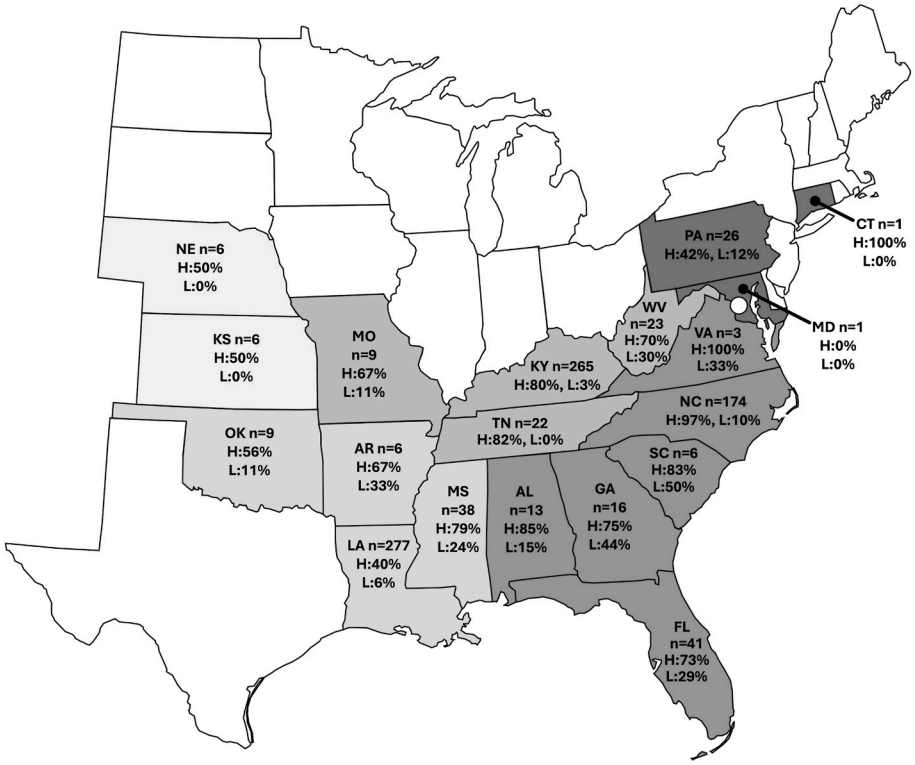

Fig. 1. Map of the 19 USA states sampled in the present study. Sample sizes are given as well as prevalence of *Haemoproteus* (H) and *Leucocytozoon* (L). The different regions of the USA are colored in different shades.

ocellated turkeys. *Leucocytozoon* sequences from positive samples were all identical to one another and were a 100% match to sequences from northern bobwhite from Oklahoma, USA (GenBank: OR102784) (Fig. 2, Supplementary Fig. S1). The *Leucocytozoon* sequence from wild turkeys was only 95% similar to lineage lPHICAS01, which was the closest match in the MalAvi database. Of the 90 *Leucocytozoon*-positive wild turkeys, 86 (95%) were co-infected with *Haemoproteus*. Among the 78 birds that were positive for both genera and had *Haemoproteus* haplotypes determined, the most common co-occurring *Haemoproteus* haplotypes were 1a alone ($n = 40$), 1a + 2a ($n = 27$), 2a alone ($n = 7$), 1a + 1b ($n = 2$), 1b alone ($n = 1$), and 1b + 2a ($n = 1$).

3.3. Demographic factors

Demographic data (age and/or sex) were available for a subset of USA wild turkeys (Table 1). Limited data were available for the ocellated turkeys from Belize, so demographic comparisons were not conducted for this species.

For the *Haemoproteus* infection model, the sample size was 729 and region was the strongest predictor with turkeys from the Southeast having 16.0 times the odds of being positive compared to birds from the South region (OR = 16.046, 95% CI: 9.47–27.20, $P < 0.001$), and birds from the Ohio Valley having 8.7 times higher odds (OR = 8.660, 95% CI: 4.96–15.12, $P < 0.001$) (Table 3, Fig. 1). Sampling source was not a significant predictor of overall *Haemoproteus* infection (OR = 1.594, 95% CI: 0.98–2.59, $P = 0.061$). Males had nearly three times the odds of infection compared to females (OR = 2.797, 95% CI: 1.87–4.18, $P < 0.001$), and juveniles had approximately 68% lower odds than adults (OR = 0.319, 95% CI: 0.21–0.50, $P < 0.001$) (Table 3).

For the *Haemoproteus* lineage models, the sample size was 702 because not all birds had resolved lineages. For L1, live-captured turkeys had 3.4 times the odds of being positive compared to diagnostic case submissions (OR = 3.423, 95% CI: 2.19–5.35, $P < 0.001$) (Table 3). Turkeys from the Southeast had 12.4 times higher odds of L1 infection relative to the South (OR = 12.398, 95% CI: 7.73–19.87, $P < 0.001$), and Ohio Valley birds had 4.5 times higher odds compared to the South (OR = 4.534, 95% CI: 2.77–7.42, $P < 0.001$). Turkeys from the Northeast had significantly higher L1 odds than turkeys from the South (OR = 2.753, 95% CI: 1.09–6.95, $P = 0.032$). Males had 72% higher odds of having L1 infection compared with females (OR = 1.719, 95% CI: 1.20–2.46, $P = 0.003$), and juveniles had approximately 46% lower odds of L1 infection than adults (OR = 0.545, 95% CI: 0.36–0.83, $P = 0.004$). For L2 infections, live-captured turkeys had approximately 66% lower odds of being positive compared to diagnostic case submissions (OR = 0.337, 95% CI: 0.23–0.50, $P < 0.001$) (Table 3). The odds of L2 infection were higher in the Southeast compared to the South (OR = 1.660, 95% CI: 1.09–2.53, $P = 0.018$), while the Northeast had significantly lower odds (OR = 0.329, 95% CI: 0.11–0.96, $P = 0.042$) compared to the South. Males had 44% higher odds of being positive for L2 than females (OR = 1.444, 95% CI: 1.02–2.05, $P = 0.041$), and juveniles had approximately 45% lower odds than adults (OR = 0.554, 95% CI: 0.35–0.87, $P = 0.010$).

For the *Leucocytozoon* model ($n = 729$), live-captured turkeys had approximately 84% lower odds of infection compared to diagnostic case submissions (OR = 0.157, 95% CI: 0.089–0.276, $P < 0.001$). Turkeys from the Southeast had higher odds of infection relative to the South region (OR = 1.932, 95% CI: 1.06–3.52, $P = 0.031$), while Ohio Valley birds had significantly lower odds relative to the South (OR = 0.401, 95% CI: 0.19–0.85, $P = 0.017$) (Table 3, Fig. 1). Juveniles had approximately 80% lower odds of *Leucocytozoon* infection compared to adults (OR = 0.197, 95% CI: 0.076–0.512, $P < 0.001$) (Table 3).

4. Discussion

This study represents the most geographically extensive analysis of haemosporidian blood parasite prevalence, genetic diversity, and distribution in wild turkeys across the eastern USA. Most wild turkeys in our study were infected with *Haemoproteus*, and sequence analysis

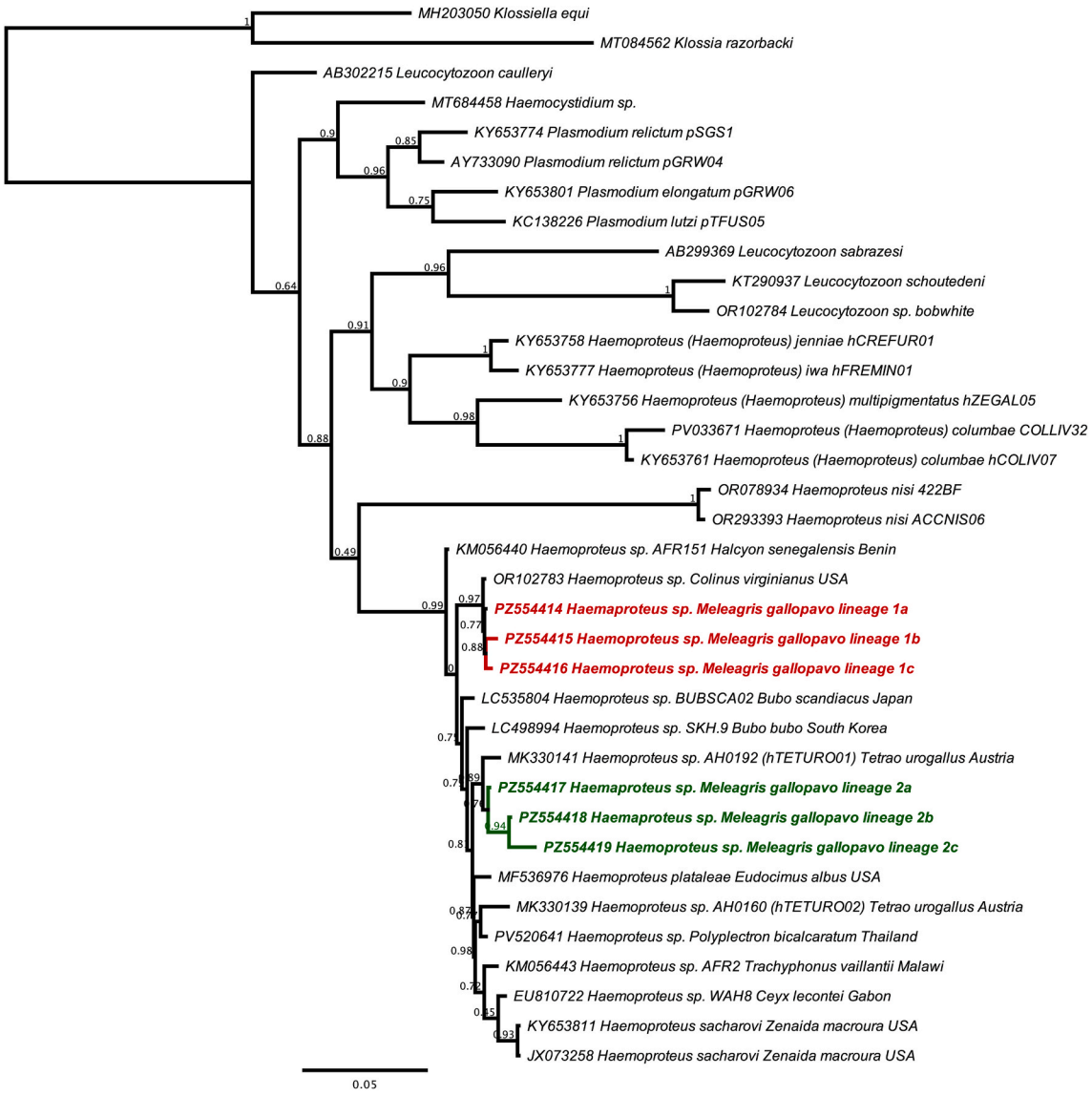

Fig. 2. Phylogenetic relationships of *Haemoproteus* sequences from wild turkeys (*Meleagris gallopavo*) from the USA and ocellated turkeys (*Meleagris ocellata*) from Belize compared with related *Haemoproteus* spp. based on cytochrome *b* gene sequences. The entries in bold text represent new sequences from this study. GenBank accession numbers are listed before species names and, when applicable, codes of lineages according to the MalAvi database are given.

revealed previously unrecognized genetic diversity within what has historically been considered a single species, *H. meleagridis*. The overall *Haemoproteus* prevalence of 69.4% in our study is consistent with previous reports documenting high prevalence in wild turkeys in various regions, though somewhat lower than the >80% prevalence reported in some historical studies from Florida (Forrester et al., 1974; Castle and Christensen, 1984; Atkinson and Forrester, 1987; Castle et al., 1988). We also found variable prevalence of *Leucocytozoon* in turkeys, with several states having prevalence rates near or exceeding 30% (i.e. Georgia, West Virginia, Virginia, and Florida). There were regional differences in prevalence, with turkeys from the Southeast having higher odds of infection with both *Haemoproteus* and *Leucocytozoon*, which likely corresponds to vector activity. A high prevalence of *Haemoproteus* was detected in the ocellated turkeys from Belize, and none were positive for *Leucocytozoon* or *Plasmodium*; however, we only sampled a small number of birds from a localized area, so additional testing is needed to understand the prevalence and diversity of haemosporidian blood parasites in ocellated turkeys. Although we found a high prevalence of these parasites in free-ranging turkeys, the population-level impacts of these parasites are poorly understood, and individual mortalities of wild and domestic turkeys are frequently reported, and *L. smithi* infections can decrease egg production of domestic turkeys (Wehr, 1962; Jones et al., 1972; Barnett, 1977; Atkinson et al., 1986, 1988; Atkinson and Forrester, 1987; Lynch et al., 2025).

We detected six unique *Haemoproteus* haplotypes that formed two lineages in wild turkeys from the USA and ocellated turkeys from Belize. The substantial genetic difference (1.8–2.3%) between these two lineages suggests they may be distinct species rather than intraspecific variants. This level of genetic divergence exceeds that used to distinguish several related *Haemoproteus* species. For example, genetic differences as small as 0.7% have been used to distinguish recognized *Haemoproteus* species (e.g. *H. pallidus vs H. minutus*), and differences of only 0.3% in Caribbean lineages have led to recognition of three distinct species in that group (Palinauskas et al., 2013). Historically, only one species of *Haemoproteus*, *H. meleagridis*, has been reported from wild turkeys, but because blood smears were not available to examine the morphology of the parasites from birds in our study, we could not determine which lineage corresponded to *H. meleagridis* infections. One concern with PCR-based testing of blood samples for haemosporidians is the detection of abortive infections; however, we have conducted

Table 3
Generalized linear model results for factors associated with *Haemoproteus* spp. (including both lineages) and *Leucocytozoon* infection status in wild turkeys (*Meleagris gallopavo*) from the USA.

Model	Term	Coefficient	SE	*P*-value	OR	95% CI
Haemoproteus (ref DC, South, Female, Adult)	Intercept	**−0.8499**	**0.2737**	**0.002**	**0.427**	**0.250–0.731**
	Source: Live	0.4659	0.2486	0.061	1.594	0.979–2.594
	Region: Southeast	**2.7755**	**0.2692**	**<0.001**	**16.046**	**9.468–27.195**
	Region: Ohio Valley	**2.1587**	**0.2842**	**<0.001**	**8.660**	**4.962–15.115**
	Region: Northeast	0.4308	0.4770	0.367	1.538	0.604–3.918
	Region: Plains	1.1785	0.6940	0.090	3.249	0.834–12.664
	Sex: Male	**1.0285**	**0.2049**	**<0.001**	**2.797**	**1.872–4.179**
	Age: Juvenile	**−1.1430**	**0.2261**	**<0.001**	**0.319**	**0.205–0.497**
Haemoproteus lineage 1 (L1)	Intercept	**−1.6686**	**0.2704**	**<0.001**	**0.189**	**0.111–0.320**
	Source: Live	**1.2306**	**0.2277**	**<0.001**	**3.423**	**2.191–5.349**
	Region: Southeast	**2.5176**	**0.2408**	**<0.001**	**12.398**	**7.734–19.874**
	Region: Ohio Valley	**1.5117**	**0.2513**	**<0.001**	**4.534**	**2.771–7.421**
	Region: Northeast	**1.0128**	**0.4721**	**0.032**	**2.753**	**1.091–6.946**
	Region: Plains	0.8628	0.7459	0.247	2.370	0.549–10.223
	Sex: Male	**0.5419**	**0.1832**	**0.003**	**1.719**	**1.201–2.462**
	Age: Juvenile	**−0.6074**	**0.2115**	**0.004**	**0.545**	**0.360–0.825**
Haemoproteus lineage 2 (L2)	Intercept	−0.4444	0.2514	0.077	0.641	0.392–1.049
	Source: Live	**−1.0875**	**0.2021**	**<0.001**	**0.337**	**0.227–0.501**
	Region: Southeast	**0.5069**	**0.2142**	**0.018**	**1.660**	**1.091–2.527**
	Region: Ohio Valley	0.1655	0.2486	0.506	1.180	0.725–1.921
	Region: Northeast	**−1.1126**	**0.5466**	**0.042**	**0.329**	**0.113–0.960**
	Region: Plains	−0.8739	0.8302	0.293	0.417	0.082–2.124
	Sex: Male	**0.3671**	**0.1799**	**0.041**	**1.444**	**1.015–2.054**
	Age: Juvenile	**−0.5910**	**0.2298**	**0.010**	**0.554**	**0.353–0.869**
Leucocytozoon	Intercept	**−0.8763**	**0.3361**	**0.009**	**0.416**	**0.215–0.804**
	Source: Live	**−1.8519**	**0.2887**	**<0.001**	**0.157**	**0.089–0.276**
	Region: Southeast	**0.6586**	**0.3058**	**0.031**	**1.932**	**1.061–3.518**
	Region: Ohio Valley	**−0.9135**	**0.3836**	**0.017**	**0.401**	**0.189–0.851**
	Region: Northeast	−1.0784	0.6858	0.116	0.340	0.089–1.304
	Region: Plains	ND	ND	ND	ND	ND
	Sex: Male	0.0130	0.2619	0.960	1.013	0.606–1.693
	Age: Juvenile	**−1.6233**	**0.4864**	**<0.001**	**0.197**	**0.076–0.512**

Abbreviations: DC, SCWDS diagnostic case submissions; OR, odds ratio; CI, confidence interval; ND, not determined because no positive turkeys.
Note: Bold indicates $P < 0.05$.

concurrent molecular and microscopic examination of a new set of wild turkey samples, and results were highly concordant (Yabsley, unpublished data) (Ciloglu et al., 2016, 2020). In general, a difference in the proportion of the two lineages, with Lineage 1 being more common, was evident in many sampled USA states, and this pattern could reflect differences in vector communities, historical turkey population movements and translocations, or other ecological factors that influence parasite transmission dynamics. Future studies need to incorporate both morphological and molecular approaches, including additional gene targets, to resolve the taxonomic status of these lineages. The existence of multiple *Haemoproteus* species infecting wild turkeys has important implications for understanding disease ecology, as different species may vary in pathogenicity, vector relationships, and host specificity.

Based on phylogenetic analysis, *Haemoproteus* Haplotype 1a was identical to parasites previously reported in northern bobwhite in Oklahoma, USA, while Haplotype 2a was most similar to sequences from a galliform species from Europe, the western capercaillie in Austria (Himmel et al., 2019). The natural history of these two lineages is poorly understood because data regarding host specificity and vectors were collected on parasites, presumed at the time to be *H. meleagridis*, which predated our knowledge that there were two lineages infecting wild turkeys. Previous studies showed that *Culicoides edeni* was implicated as a primary vector of *H. meleagridis,* but two other species (*C. hinmani* and *C. arboricola*) were shown to be susceptible experimentally; thus, any of these could be vectors for one or both of the lineages we detected in our study (Atkinson et al., 1983; Atkinson, 1988, 1991). Because both *Haemoproteus* lineages were detected in ocellated turkeys in Belize, the vector(s) must be broadly distributed, or multiple species of *Culicoides* may be responsible for transmission.

Although *H. meleagridis* is the only *Haemoproteus* species that has been reported from wild turkeys in the USA, Valkiūnas (2005) synonymized this parasite with *Haemoproteus mansoni*, which has a wide host range in Europe. *Haemoproteus mansoni* was originally described from red grouse (*Lagopus scoticus*) in Scotland and subsequently was reported from numerous grouse species in Europe and North America (Valkiūnas, 2005). The argument for this combination was that parasite host-specificity was assumed within host families. Atkinson (1986) demonstrated that *H. meleagridis* from turkeys could develop in chukar partridge (*Alectoris chukar*) and common pheasant (*Phasianus colchicus*). However, this work pre-dated molecular work, and it is unknown which lineage(s) detected in our study was used in Atkinson's experiments in Florida. Interestingly, two distinct *Haemoproteus* lineages (GenBank: MK330141 and MK33039) have been detected in western capercaillie from Austria, and this species is a reported host of *H. mansoni* (Himmel et al., 2019). But, similar to our work, it is unknown which genetic lineage corresponds to *H. mansoni*. The Lineage 2 sequence from our study on wild turkeys was most similar to one of these western capercaillie lineages. In light of these new data on unique lineages infecting wild turkeys in the USA, the presence of multiple lineages in galliforms in Europe, and the limited data for known hosts of *H. mansoni*, more work is needed to understand the host-parasite relationships and the true number of *Haemoproteus* spp. infecting galliform birds.

We only detected a single *Leucocytozoon* species in wild turkeys from the USA. Our *Leucocytozoon* sequence was identical to sequences detected in bobwhite from Oklahoma, USA, which were in a large clade of *L. schoutedeni* sequences (Wyckoff et al., 2024), and similar sequences were reported in wild turkeys from Louisiana (Lynch et al., 2025). *Leucocytozoon schoutedeni* is predominantly reported from chickens in Africa and Asia but has also been reported from the USA (Sehgal et al., 2006). Previously, the only species of *Leucocytozoon* reported from wild turkeys was *L. smithi*, which is morphologically distinct from *L. schoutedeni.* However, again, the lack of available blood smears to

assess *Leucocytozoon* morphology precluded our ability to assess which morphospecies was present, and no *L. smithi* sequences are available in GenBank for comparison. Combined morphological and molecular data, and possibly experimental infection trials, are needed to determine whether *L. smithi* is a morphologically distinct variant of *L. schoutedeni* in turkey hosts or whether these two distinct species are genetically identical at this gene target.

The absence of *Plasmodium* spp. infections in our turkey samples was unexpected, given previous sporadic reports of *Plasmodium* spp. in wild turkeys from several states, including Iowa, Texas, Florida, Missouri, Oklahoma, North Carolina, Arkansas, Illinois, Louisiana and Virginia (Fedynich and Rhodes, 1995; Forrester and Spalding, 2003; Lynch et al., 2025). The reason for the lack of *Plasmodium* detection is unknown, but could be due to a bias of our nested PCR protocol to preferentially amplify *Haemoproteus* over *Plasmodium* in co-infected samples, as has been noted for some primer sets (Pacheco et al., 2018). Future studies should investigate the use of alternative protocols to determine if amplification bias is an issue with wild turkeys (Ciloglu et al., 2023). Several studies that detected *Plasmodium* spp. in wild turkeys did so by subinoculation of blood into domestic turkey poults, which may be more sensitive at detecting low parasitemias compared to blood smear analysis or PCR (Castle et al., 1988; Castle and Christensen, 1990; Forrester and Spalding, 2003). Finally, *Plasmodium* was not present in most previously published surveys of wild turkey blood parasites, and when found, prevalence was low, except in isolated areas where subinoculation of poults was conducted (Castle et al., 1988; Hopkins et al., 1990; Stacey et al., 1990; Forrester and Spalding, 2003; Lynch et al., 2025).

Our findings that adults were significantly more likely to be infected with *Haemoproteus* than juveniles, and that so few juveniles were positive for *Leucocytozoon*, likely reflect the cumulative exposure of adult birds to vectors over their lifetimes or unique age-vector contact rates. Most previous studies failed to find any differences in the prevalence of these parasites in adult *vs* juvenile birds, but some studies found higher prevalences of these parasites in juvenile birds, which may not have been found in our study because of the increased detections of lower parasitemias in adult birds compared to juveniles (Fedynich and Rhodes, 1995). Both *Haemoproteus* and *Leucocytozoon* can establish chronic infections that persist in host tissues, with seasonal relapses from dormant exo-erythrocytic stages (Valkiūnas et al., 2004; Valkiūnas and Iezhova, 2023). Adult birds have experienced multiple seasons of vector exposure and thus greater opportunities to become infected. Additionally, transmission of *L. smithi* occurs primarily before wild turkey hatching and brood rearing, making infections of poults and juveniles relatively rare (Stacey et al., 1990). However, when infections do occur among young birds, disease is significantly more severe and almost always fatal, and clinical signs are not frequently observed because these birds are likely to die before discovery (Stacey et al., 1990).

Male turkeys were more likely to be infected with *Haemoproteus*. Previous studies either failed to find any differences in sex (Forrester et al., 1974; Hopkins et al., 1990; Stacey et al., 1990; Fedynich and Rhodes, 1995; Lynch et al., 2025), only sampled male birds (Noblet and Moore, 1975; Haynes et al., 2024), or did not report results (Castle et al., 1988; Castle and Christensen, 1990). Similarly, male turkeys were more likely than females to be infected with both lineages of *Haemoproteus* although risk was variable (72% higher odds for males with L1 *vs* 44% higher odds for males with L2). Previous studies were conducted before the recognition that there are two lineages of *Haemoproteus* in wild turkeys. The variation in risk by sex for the two Lineages of *Haemoproteus* may be related to differential exposure to vectors or some other unknown factor. The lack of sex differences in *Leucocytozoon* infection prevalence was similar to several studies (e.g. Forrester et al., 1974; Hopkins et al., 1990; Stacey et al., 1990; Fedynich and Rhodes, 1995) but contrasts with recent findings from Louisiana, where male turkeys were significantly more likely to be infected with *Leucocytozoon* than females (Lynch et al., 2025). Various reasons have been proposed for sex-biased prevalence or intensities for blood parasites in birds and could include increased testosterone in males making them more susceptible, differences in behavior between the two sexes, different habitat use, or other factors (Calero-Riestra and García, 2016; Berkel and Cacan, 2023). Wild turkeys have mating systems of harem defense or male dominance polygyny, where males form dominance hierarchies that grant access to moving female groups, and females provide sole parental care. This mating structure may differentially affect habitat preference and movement patterns, potentially leading to differential vector exposure in some populations but not others.

The probability of *Leucocytozoon* infection was higher in dead turkeys submitted to SCWDS for diagnostic evaluation compared to those that were live captured. This finding could be related to the bias of adult birds submitted to SCWDS, a bias in where birds are submitted from (states or regions with higher *Leucocytozoon* prevalence), or a true higher prevalence in sick turkeys. Member states of SCWDS are predominantly in the Southeast, which is the region with the highest prevalence of *Leucocytozoon*. Overall, the sampling source was not a significant factor in *Haemoproteus* infection; however, there were significant differences in the *Haemoproteus* lineage detection probabilities. There were higher odds of detecting L1 in live turkeys, but in contrast, L2 was much more likely to be detected in SCWDS diagnostic case submissions. This difference may be related to bias in submissions (e.g. from states with higher L2 prevalence such as Kentucky) or may suggest that L2 is more likely to cause morbidity or mortality. Previous experimental infection trials of turkeys with *H. meleagridis* resulted in morbidity (Atkinson et al., 1988), but whether L1 and L2 differ in pathogenicity has not been evaluated and needs additional investigation.

5. Conclusions

This study demonstrates that haemosporidian blood parasites are common in wild turkey populations across the eastern USA and reveals previously unrecognized genetic diversity within the genus *Haemoproteus*. The identification of two distinct *Haemoproteus* lineages, likely representing separate species, provides new insights into the parasite community affecting this important game bird. To better understand the host-parasite dynamics of these two *Haemoproteus* species, future studies should combine both molecular and morphological characterization of these parasites. Both *Haemoproteus* and *Leucocytozoon* can cause disease in wild turkeys, and factors related to development of disease are unknown, but reasons for differential prevalence could be related to stress, co-infections, or parasite lineage. Although this study focused on wild turkeys, both parasites are important pathogens of domestic turkeys (Atkinson and Forrester, 1987; Atkinson et al., 1988). There are no *Haemoproteus* sequences from domestic turkeys, so it is unknown if one or both lineages we detected can infect and/or cause disease in domestic turkeys. Similarly, there are no *Leucocytozoon* sequences from domestic turkeys in the USA, so it is unknown if *L. smithi* that has been reported from domestic turkeys is the same parasite detected in wild turkeys in our study. Genetic characterization of parasites from domestic turkeys is needed to better understand the transmission of these parasites between wild and domestic turkeys. Finally, vector surveillance and testing are needed to identify vectors of the different *Haemoproteus* lineages.

Ethical approval

No animals were specifically harmed for the purpose of this study but were used opportunistically and the testing of samples collected by state biologists, researchers or from diagnostic cases was reviewed and approved by UGA's IACUC (A2018 02-010, A2020 11-010, and A2024 02-014).

CRediT authorship contribution statement

Håkon Jones: Methodology, Formal analysis, Data Curation, Investigation, Writing - original draft, Writing - review & editing.

Stephanie Osip: Investigation, Writing - original draft, Writing - review & editing. **Marcelo H. Jorge:** Investigation, Writing - review & editing. **Kayla B. Garrett:** Investigation, Writing - review & editing. **Jillian R. Broadhurst:** Investigation, Writing - review & editing. **Nicole M. Nemeth:** Investigation, Writing - review & editing. **Melanie R. Kunkel:** Resources, Writing - review & editing. **Richard Buchholz:** Resources, Writing - review & editing. **Thomas Martin:** Resources, Writing - review & editing. **Christine Casey:** Resources, Writing - review & editing. **Christopher E. Moorman:** Resources, Writing - review & editing. **Krishna Pacifici:** Resources, Writing - review & editing. **David J. Moscicki:** Resources, Writing - review & editing. **Christopher D. Kreh:** Resources, Writing - review & editing. **Hannah M. Plumpton:** Resources, Writing - review & editing. **Bret A. Collier:** Resources, Writing - review & editing. **Bobbi G. Carpenter:** Resources, Writing - review & editing. **Juliana Ofalt:** Resources, Writing - review & editing. **Marcus A. Lashley:** Resources, Writing - review & editing. **Sonia M. Hernandez:** Resources, Writing - review & editing. **Mark G. Ruder:** Writing - review & editing, Funding acquisition. **Elizabeth Kurimo-Beechuk:** Project Administration, Writing - review & editing. **Victoria A. Andreasen:** Resources, Writing - review & editing. **Christopher A. Cleveland:** Writing - review & editing, Supervision. **Ellen Haynes:** Resources, Writing - review & editing. **Michael J. Yabsley:** Conceptualization, Methodology, Formal analysis, Investigation, Data Curation, Writing - original draft, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

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Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crpvbd.2026.100408.

Data availability

The data supporting the conclusions of this article are included within the article and its supplementary files. The unique newly generated sequences were submitted to the GenBank database under the accession numbers PZ554414-PZ554419.

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