Journal of Parasitology 2024 110(5) 445–454

Published 17 September 2024

DOI: 10.1645/23-109

Contents and archives available through www.bioone.org or www.jstor.org

Journal of Parasitology

journal homepage: www.journalofparasitology.org

AVIAN HAEMOSPORIDIANS IN GREATER SCAUP (*AYTHYA MARILA*) AND LESSER SCAUP (*AYTHYA AFFINIS*) FROM WISCONSIN

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KEY WORDS

Anseriformes
Blood
Haemoproteus
Leucocytozoon
Phylogeny
Plasmodium

ABSTRACT

Avian haemosporidians are a diverse group of protozoan parasites that infect a wide range of host species. Waterfowl are an ecologically and economically important group of hosts that have been underrepresented in studies of haemosporidians. Diving ducks have unique life history traits, and morphological, behavioral, and dietary differences separate them from more common dabbling ducks. Greater scaup (*Aythya marila*) and lesser scaup (*Aythya affinis*) are closely related diving ducks with declining population trends in North America. To better understand the diversity of haemosporidians within diving ducks and factors related to host infections in scaup, we surveyed 82 hunter-donated waterfowl from 8 species of divers, sea ducks, and dabblers from Green Bay, Wisconsin from 2019 to 2021. We used molecular detection methods and phylogenetic and statistical analyses to describe the diversity, host associations, and prevalence of haemosporidians. We detected 14 unique genetic lineages of haemosporidians, including 4 novel lineages. We identified at least 1 lineage of haemosporidian in each of the 8 host species of divers, sea ducks, and dabblers examined. Lesser scaup had more diverse haemosporidian communities than did greater scaup, but lineages showed no clustering among these hosts when incorporated in phylogenetic analyses with lineages from other Nearctic waterfowl. Female lesser scaup had the highest infection prevalence, but there was no effect of host age or year of sampling. Our findings underscore the importance of species and sex differences that could lead to a higher risk of infections. Our results also fill an important geographical sampling gap for haemosporidians along a key migratory route. Increased monitoring of haemosporidians in waterfowl could contribute to insights into parasite evolution and ecology and the conservation and management of host populations.

Avian haemosporidians of the genera *Haemoproteus, Leucocytozoon, Parahaemoproteus,* and *Plasmodium* are protozoan parasites that are transmitted by hematophagous dipteran vectors (Valkiūnas, 2005; Santiago-Alarcon et al., 2012; Galen et al., 2018; Fecchio et al., 2020). The development of standard molecular methods (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 distributed across all avian clades and zoogeographical regions (Bensch et al., 2009; Fecchio et al., 2020, 2021). However, the majority of avian haemosporidian research has been focused on a single avian order, Passeriformes, with limited data from the remaining 39 avian orders. For example, of the thousands of genetic lineages in the MalAvi database (http://mbio-serv2.mbioekol.lu.se/Malavi/; Bensch et al., 2009), the largest dedicated database of avian haemosporidian genetic data, more than 75% are identified only from passeriform hosts. Recent molecular data support haemosporidian research on a greater diversity of avian hosts (Smith et al., 2015; Bell et al., 2020) because undersampled avian host groups likely harbor unique haemosporidian parasites (Bertram et al., 2017; Yabsley et al., 2018; Bell et al., 2020). The addition of new hosts may aid in resolving the overall phylogeny and improving our understanding of the evolution of this parasite group (Galen et al., 2018).

North American waterfowl are a diverse and geographically widespread group of birds, yet many are underrepresented in haemosporidian studies (Bensch et al., 2009; Fleskes et al., 2017; Bell et al., 2020). Traits such as larger body size and association with aquatic habitats may lead to more encounters with vectors that are also frequently associated with aquatic habitats (Meixell et al., 2016; Bell et al., 2020). Waterfowl can form large flocks that may provide vectors the opportunity to feed on multiple hosts, leading to transmission (Matta et al., 2014). Many waterfowl are long-distance migrants and can transmit parasites across large geographic distances (Levin et al., 2013; Smith and Ramey, 2015). Recent surveys of waterfowl haemosporidians have revealed infection with these parasites from Arctic and sub-Arctic locations to the southwestern

and southeastern United States, with evidence for transmission following migration routes (Ramey et al., 2015, 2016; Reeves et al., 2015; Garvon et al., 2016; Meixell et al., 2016; Fleskes et al., 2017; Reed et al., 2018). One of the key life history differences among waterfowl is feeding guild, which is based on morphological, behavioral, and dietary differences between dabblers and divers (Pöysä, 1983). Dabblers swim along the top of the water, skimming the surface for food such as seeds, plants, and insects, and can walk easily on land. In contrast, divers walk with difficulty on land, swim deeper in more open water, and dive to feed on fish, snails, and other invertebrates. Dabblers are more common than divers in many geographic areas, which could be 1 reason haemosporidian research has focused on dabblers even when divers are included in large survey efforts (Bensch et al., 2009; Meixell et al., 2016; Fleskes et al., 2017).

Waterfowl are also economically important, and considerable time and effort have been devoted to assessing and monitoring waterfowl populations and conducting research on potential causes for population declines so that management approaches can be adapted to stabilize or reverse negative population trends (Nichols, 1991; Nichols et al., 2007; Soulliere et al., 2013). Studies of waterfowl haemosporidians have addressed questions related to pathology through experimental infections of native and domestic hosts or attempted to link patterns of infection with consequences for wild populations (Khan and Fallis, 1968; Herman et al., 1975; Fleskes et al., 2017; Merrill et al., 2018).

Among North American waterfowl, greater scaup (*Aythya marila*) and in particular lesser scaup (*Aythya affinis*) have experienced significant population declines, and more research has focused on disease and health concerns (England et al., 2018; Merrill et al., 2018). In the past 50 yr, the breeding population of lesser scaup has declined precipitously from an estimated 8 million birds in 1972 to 3.2 million in 2006 (Merrill et al., 2018). Some of the potential factors investigated to explain these declines include reduced quantity and quality of spring stopover sites (Anteau and Afton, 2004), increased contaminants (Custer et al., 2003; Anteau et al., 2007), and elevated helminth burdens (England et al., 2017). Female lesser scaup were screened for haemosporidian infection (e.g., *Plasmodium* spp. and *Haemoproteus* spp.) to examine the relationship with body condition during the spring migration that could lead to reduced reproductive output (Merrill et al., 2018). Infection prevalence was negatively associated with percentage of body fat and suggests that birds in poor condition could be more susceptible to infection or recrudescence (Merrill et al., 2018). Similar investigations of haemosporidian infection in greater scaup are lacking even in geographic areas where the species overlap, with only 1 published study reporting haemosporidians from this host species (Inumaru et al., 2017).

In the midwestern United States, the Great Lakes area is a critical stopover region for migrating waterfowl, including scaup (England, 2016). This region is within the Mississippi Flyway, which supports on average 40% of the continental scaup population (Afton and Anderson, 2001). The Bay of Green Bay is connected to Lake Michigan on the northern end and holds 50,000–100,000 migrating divers during October–December each year (Prince et al., 1992). Other common ducks in this region include the divers redhead (*Aythya americana*), bufflehead (*Bucephala albeoia*), and goldeneye (*Bucephala clangula*) and the sea ducks black scoter (*Melanitta americana*) and long-tailed ducks (*Clangula hyemalis*). Many of these species of waterfowl have received only limited attention in haemosporidian research represented by historical surveys and none from the Great Lakes region (Nelson and Gashwiler, 1941; Herman, 1951; Bennett et al., 1982; Michot et al., 1995; Dronen and Blend, 2007). Coastal wetlands support dabblers including mallards (*Anas platyrhynchos*) and wood ducks (*Aix sponsa*) (Prince et al., 1992).

We used molecular detection tools, molecular phylogenetics, and statistical analysis to describe the diversity, host associations, and prevalence of haemosporidians in greater and lesser scaup from Green Bay, Wisconsin. We collected hosts during the fall hunting season over 3 yr (2019–2021) and investigated how host sex and age could influence infection dynamics (Grieves et al., 2023). By sampling both greater and lesser scaup from the same habitats and seasons we could compare parasite diversity and prevalence across species and provide a substantial assessment of haemosporidians of greater scaup. Fall-collected birds provided the opportunity to examine both juveniles on their first migration and adults after exposure to vectors and haemosporidian transmission on the northern breeding grounds (Greiner et al., 1975; Valkiūnas, 2005; Vest et al., 2006; Ramey et al., 2016; Fleskes et al., 2017). Opportunistic sampling of other co-occurring divers, sea ducks, and dabblers permitted a more complete exploration of phylogenetic diversity and host associations in this region. Monitoring haemosporidians in this geographic area is important to fill in gaps in our understanding of haemosporidian biogeography and to describe the distribution of potentially pathogenic species.

MATERIALS AND METHODS

Sample areas and sample collection

A total of 82 hunter-donated waterfowl from 8 species of divers, sea ducks, and dabblers were examined (Table I). Waterfowl were collected during the open hunting season from 1 October through mid-December in northeastern Wisconsin in 2019, 2020, and 2021. Most waterfowl were collected from along the west shore of the Bay of Green Bay, 1–2 miles offshore in 10–20 ft of water. This area is adjacent to the town of Pensaukee, Oconto County, between Tibbet Creek to the south and Kunzer Beach Lane to the north (Fig. 1). Two wood ducks were harvested from a wildlife scrape in a farmed wetland from the town of Chase, Oconto County (Fig. 1). Frozen breasted-out carcasses were thawed for approximately 18 hr in a refrigerator before dissections. We determined species, sex, and approximate age (juvenile or adult) based on the plumage coloration and beak size (LeMaster, 1986; Carney, 1992). We confirmed identifications with internal anatomy during dissection. Liver samples were preserved in 95% ethanol and frozen until used for molecular analysis. Liver sample vouchers were deposited in the University of Wisconsin–Stevens Point Animal Parasitology Museum Collection (UWSP-P-14995–15075). Blood samples were not obtained at the time of specimen collection, and all carcasses were frozen before dissection, precluding the creation of blood smears for morphological identification of haemosporidian parasites.

Molecular identification

DNA was extracted from liver samples using the Qiagen blood and tissue Minikit (Qiagen, Valencia, California). DNA extractions were screened by real-time polymerase chain reaction (PCR) to detect haemosporidian DNA following the protocol of Bell et al. (2015). All positive samples as determined by real-time PCR were subjected to 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

Table I. Distribution of haemosporidian genera *Haemoproteus* (H), *Leucocytozoon* (L), *Parahaemoproteus* (Pa), and *Plasmodium* (Pl), dual infections with 2 haemosporidian lineages, and total haemosporidian infections from waterfowl collected in Green Bay, Wisconsin.

Host species by feeding group and location	No.					No. (%) with dual infection			Total no. (%) hosts infected
	samples	H (%)	L (%)	Pa (%)	Pl (%)	L + L	L + Pa	L + Pl	
Bay of Green Bay									
Divers									
Greater Scaup (*Aythya marila*)	38	0 (0)	3 (7.9)	0 (0)	0 (0)	2 (5.3)	1 (2.6)	0 (0)	6 (15.8)
Lesser Scaup (*Aythya affinis*)	29	0 (0)	4 (13.8)	1 (3.4)	2 (6.9)	1 (3.4)	0 (0)	0 (0)	8 (27.6)
Redhead (*Aythya americana*)	3	1 (33.0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (33.0)
Bufflehead (*Bucephala albeola*)	1	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)
Goldeneye (*Bucephala clangula*)	5	0 (0)	0 (0)	1 (20.0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (20.0)
Total	76	1 (1.3)	8 (10.5)	2 (2.6)	2 (2.6)	3 (3.9)	1 (1.3)	0 (0)	17 (22.4)
Sea ducks									
Long-tailed Duck (*Clangula hyemalis*)	3	0 (0)	2 (66.6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (66.6)
Black Scoter (*Melanitta americana*)	1	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)
Total	4	0 (0)	3 (75.0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (75.0)
Wildlife scrape									
Dabblers									
Wood Duck (*Aix sponsa*)	2	0 (0)	0 (0)	1 (50.0)	0 (0)	0 (0)	0 (0)	1 (50.0)	2 (100)
Grand Total	82	1 (1.2)	11 (13.4)	3 (3.7)	2 (2.4)	3 (3.7)	1 (1.2)	1 (1.2)	22 (26.8)

OneTaq master mix (New England Biolabs, Ipswich, Massachusetts) following the manufacturer's protocols. Because of the high sensitivity of the nested PCR, negative controls were included in runs to check for possible contamination; none was found in any PCR runs.

Products from nested PCR amplifications were visualized on agarose gels. PCR products were purified using ExoSAP-IT (Affymetrix, Santa Clara, California) and sequenced using the BigDye terminator v. 3.1 cycle sequencing kit (Applied Biosystems, Foster City,

Figure 1. Waterfowl sampling locations in northeastern Wisconsin (QGIS 3.32.1-Lima, 2021). Color version available online.

Table II. Haemosporidian genetic lineages recovered from waterfowl hosts examined in this study.

Lineage name	Genus	Host(s)	Accession no.
ANACRE01	*Parahaemoproteus*	Greater scaup (*Aythya marila*)	OR352189
ANACRE02	*Leucocytozoon*	Lesser scaup (*Aythya affinis*)	OR352191
ANSFAB01	*Leucocytozoon*	Long-tailed duck (*Clangula hyemalis*)	OR352193
BT7	*Plasmodium*	Lesser scaup (*Aythya affinis*)	OR352194
BUVIR04	*Plasmodium*	Wood duck (*Aix sponsa*)	OR352203
BWTE19	*Leucocytozoon*	Lesser scaup (*Aythya affinis*), greater scaup (*Aythya marila*)	OR352190, OR352192
CLAHYE01	*Leucocytozoon*	Bufflehead (*Bucephala albeola*), black scoter (*Melanitta americana*)	OR352195, OR352196
GEOMON01	*Haemoproteus*	Redhead (*Aythya americana*)	OR352197
STVAR04	*Leucocytozoon*	Wood duck (*Aix sponsa*)	OR352202
TUSW04	*Leucocytozoon*	Lesser scaup (*Aythya affinis*), greater scaup (*Aythya marila*)	OR352204, OR352205
New lineage 1: AIXSPO01	*Parahaemoproteus*	Wood duck (*Aix sponsa*)	OR352198
New lineage 2: AYTAFF01	*Leucocytozoon*	Lesser scaup (*Aythya affinis*)	OR352199
New lineage 3: BUCCLA01	*Parahaemoproteus*	Goldeneye (*Bucephala clangula*)	OR352200
New lineage 4: AYTAFF02	*Parahaemoproteus*	Lesser scaup (*Aythya affinis*)	OR352201

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 coinfections. Coinfections were separated manually following the protocols of Galen et al. (2018) or by using the program PHASE 2.1.1 (Stephens et al., 2001; Stephens and Donnelly, 2003) following the protocol of Harrigan et al. (2014). Assembled sequences for haemosporidians were aligned using BioEdit v. 7.2.0 (Hall, 1999). Aligned sequences were collapsed into unique haplotypes using the FaBox haplotype collapse and converter tool (Villesen, 2007). A local BLAST (basic local alignment search tool) search against the MalAvi database (Bensch et al., 2009) using BioEdit was conducted for all unique haplotypes to identify lineages. Because 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 for this group of parasites (Bensch et al., 2009). Sequences were deposited in GenBank (OR352189-201) and the MalAvi database.

Phylogenetic and statistical analysis

To examine the evolutionary relationships of haemosporidian lineages from anseriform hosts, our newly generated sequences were combined with previously published sequences of haemosporidians from Anseriformes (Valkiūnas, 2005; Bensch et al., 2009; Matta et al., 2014; Smith and Ramey, 2015; Chagas et al., 2017; Bell et al., 2020). Only full-length 477-bp sequences were used for phylogenetic analysis. *Theileria annulata* (accession KP731977) was used as the outgroup based on its position as the basal group for haemosporidians (Galen et al., 2018).

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

Prevalence was calculated according to Bush et al. (1997). For greater and lesser scaup we tested the association of the categorical predictor variables year, species, sex, age, and their potential interactions with haemosporidian prevalence as the response variable, using generalized linear models in the R statistical program (R version 4.2.1; Crawley, 2013). We used a binomial distribution and the logit-link function (Crawley, 2013). We evaluated the models in a stepwise fashion and compared each model to successively simpler models using chi-square analyses (Crawley, 2013).

RESULTS

Fourteen haemosporidian lineages were recovered from ducks surveyed as part of this study: 7 lineages of *Leucocytozoon,* 4 lineages of *Parahaemoproteus,* 1 lineage of *Haemoproteus,* and 2 lineages of *Plasmodium* (Table II). Three *Parahaemoproteus* lineages (AIXSPO01, AYTAFF02, and BUCCLA01) and 1 *Leucocytozoon* lineage (AYTAFF01) were identified as unique. Lesser scaup were infected by haemosporidians from 3 genera, *Leucocytozoon, Parahaemoproteus,* and *Plasmodium*, whereas greater scaup were infected by only *Parahaemoproteus* and *Leucocytozoon* (Table II). Lesser scaup also supported higher lineage diversity with 6 lineages identified including 2 newly identified lineages compared with only 3 lineages for greater scaup. There was 1 lineage found only in greater scaup, whereas the other 2 lineages were also found in lesser scaup (Table II).

Phylogenetic reconstruction did not show any clustering of scaup lineages within the larger phylogeny of those parasites known to infect Nearctic waterfowl (Fig. 2). Scaup lineages are scattered throughout the phylogeny, often closely aligned with those lineages known to infect various waterfowl species and not restricted to only diving ducks (Fig. 2). Even in *Leucocytozoon* and to a lesser extent in *Parahaemoproteus* where most lineages are restricted to Nearctic Anseriformes, no phylogenetic pattern was found for scaup haemosporidian lineages and their hosts.

Our phylogenetic analysis revealed a single lineage of *Haemoproteus* in a redhead duck (GEOMON01) (Fig. 2; Table II). The 2 new lineages of *Parahaemoproteus* were identified from a goldeneye (BUCCLA01) and from 1 of the wood ducks from the wildlife scrape location (AIXSPO01) (Fig. 2; Table II).

Based on our molecular identification of haemosporidian lineages, the overall infection prevalence was 26.8% (Table I). However, prevalence varied widely across the hosts and genera of haemosporidians. *Leucocytozoon* was the most common genus detected in a total of 16 hosts with a prevalence of 19.5% (Table I). *Plasmodium* and *Parahaemoproteus* had similar infection levels with 3 and 4 hosts infected for

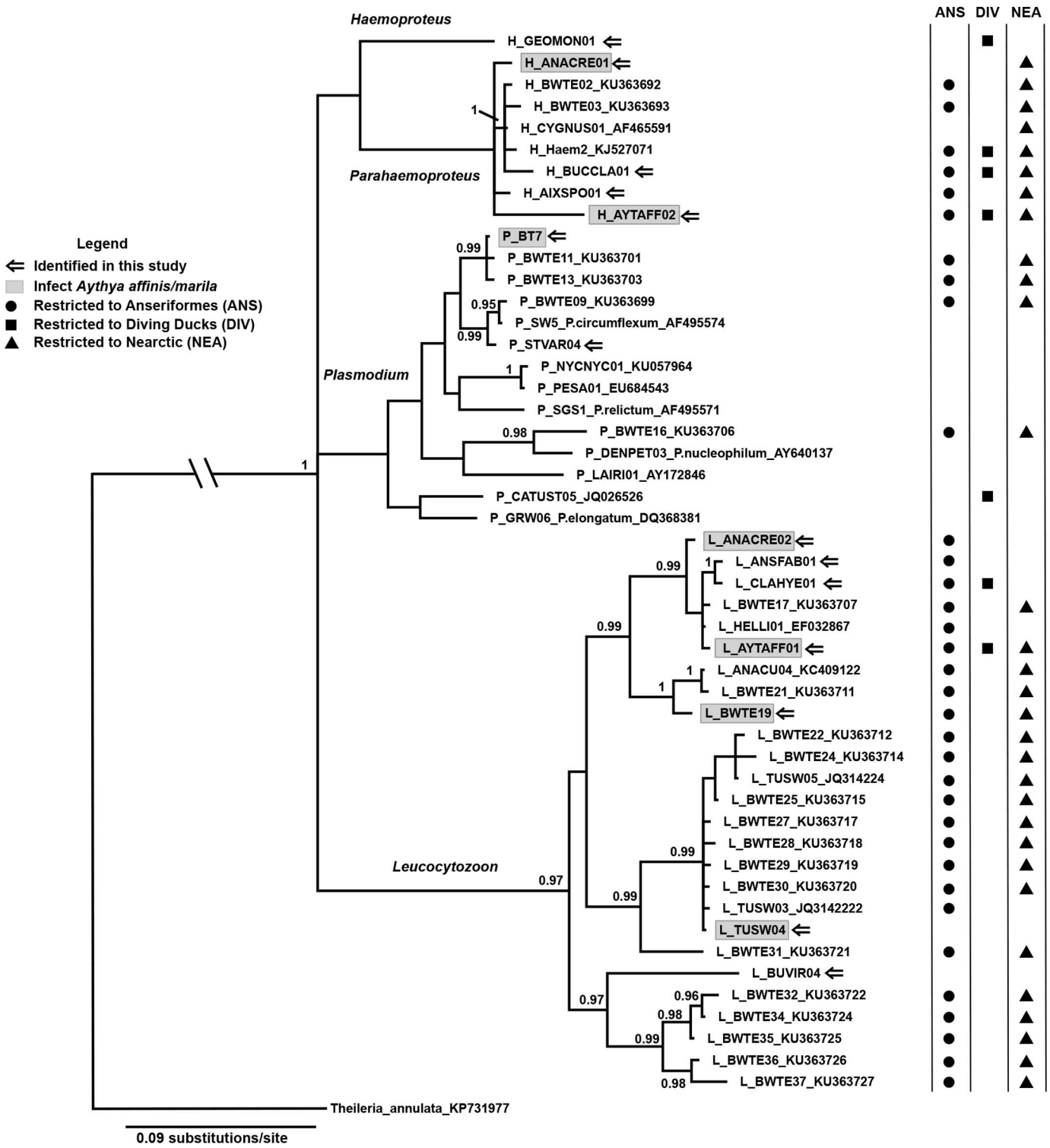

Figure 2. Bayesian inference reconstruction of haemosporidian lineages known to infect anseriform hosts from the Nearctic. Lineages identified from this study are indicated, with lineages found in scaup highlighted. Host, biogeographic, and host feeding guild distribution of lineages are indicated. The numbers above nodes represent posterior probability nodal support; support values below 0.9 are not shown.

a total prevalence of 3.6% and 4.8%, respectively, across the entire study (Table I). A single host was infected with *Haemoproteus,* which had the lowest overall prevalence of 1.2% (Table I). Of the 22 total infections, 17 were single infections and 5 were dual infections. Each of the infections included 1 lineage of *Leucocytozoon*: 3 dual infections consisting of 2 distinct lineages of this genus, 1 dual infection consisting of *Parahaemoproteus* plus *Leucocytozoon,* and 1 consisted of *Plasmodium* plus *Leucocytozoon*.

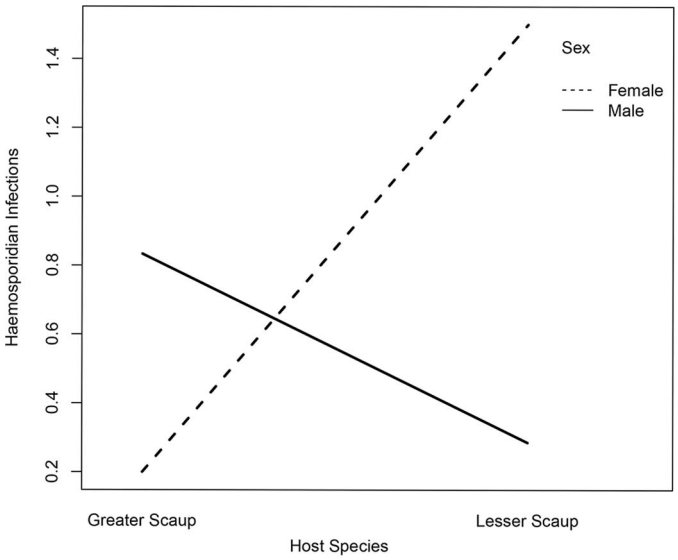

Figure 3. Interaction plot showing the relationship of waterfowl species and sex on the average number of hosts infected by haemosporidians ($P = 0.0205$).

Based on all haemosporidian infections in scaup, 6 of the 38 examined greater scaup were infected (15.8%), whereas 8 of the 29 lesser scaup were infected (27.6%). Because of our small overall sample size and smaller sample sizes for subsets of the data, our statistical results should be interpreted with caution. In the most well-supported statistical model, we found a significant interaction between host species and sex because female lesser scaup had a higher infection prevalence (6 of 13, 46.2%) than did males (2 of 16, 12.5%) and female greater scaup had a lower infection prevalence (1 of 17, 5.9%) than did males (5 of 21, 23.8%) (GLM: $z = -2.316$, $P = 0.0205$; Fig. 3). Overall species differences in prevalence between lesser and greater scaup were also significant in this model but cannot be interpreted separately from the significant interaction effect (GLM: $z = 2.237$, $P = 0.0253$). However, independent of the interaction, the main effect of sex was not significant (GLM: $z = 1.349$, $P = 0.1772$). Prevalence differed from a low of 16% (4 of 24 infected) in 2019, a high of 33% (5 of 20 infected) in 2020, and an intermediate prevalence of 27.8% (5 of 18 infected) in 2021, with no significant differences in prevalence across years (GLM: $z = 0.748$, $P = 0.4547$). Likewise, prevalence did not differ based on bird age groups (GLM: $z = 0.587$, $P = 0.5572$ and $z = -0.003$, $P = 0.9973$). In 2019, 5 individual hosts could not be accurately aged, but we included them in the analysis of the full dataset as being of unknown age because the statistical modeling results did not differ regardless of whether they were included (Suppl. Data, Table S1).

Although our sample sizes of other waterfowl species are too small for statistical analysis of factors related to prevalence, we detected at least 1 infection in each of the other host species and sampling locations in our study (Table I). Among the other divers, we found a single infected host for redhead, bufflehead, and goldeneye, so the differences in prevalence were likely related to the number of uninfected hosts sampled for each of these species. Likewise, our only sampled black scoter was infected as were 2 of our 3 long-tailed ducks (Table I). Both wood ducks collected from the wildlife scape habitat were infected with a total of 3 lineages between the 2 ducks because of dual infection with *Leucocytozoon* and *Plasmodium* in 1 duck (Table I).

DISCUSSION

Greater and lesser scaup in the study area supported a diverse and distinctive haemosporidian community (Table I). Molecular detection methods and phylogenetic analyses revealed higher haemosporidian diversity in lesser scaup, including 2 newly identified lineages (Fig. 2). There was an overlap of 2 lineages between greater and lesser scaup, with only 1 unique lineage in greater scaup. The overall infection prevalence in our study of 26.8% was lower than the 35.4% average prevalence reported in another study conducted in Wisconsin on female lesser scaup during the spring migration (Merrill et al., 2018). However, Merrill et al. (2018) examined only *Haemoproteus* and *Plasmodium,* so their infection levels could have been higher if *Leucocytozoon* had been included. In our study, *Leucocytozoon* was the most prevalent haemosporidian genus detected in both scaup species and all but 2 of our sampled host taxa (Table I). This finding is consistent with those in other waterfowl studies, indicating that *Leucocytozoon* spp. are the most common and widely distributed haemosporidians at higher latitudes (Reeves et al., 2015; Meixell et al., 2016; Fleskes et al., 2017). *Leucocytozoon* spp. are transmitted by black flies (Simulidae), which are more diverse and prevalent at higher latitudes (Fecchio et al., 2020; Starkloff and Galen, 2023). Infection by *Leucocytozoon* has been associated with lower duck body mass, which suggests that this genus could be the most significant in terms of negative health impacts (Fleskes et al., 2017). However, research on *Leucocytozoon* is more limited than that on the other genera, so more investigations, particularly on pathology, are needed (Fecchio et al., 2020).

Leucocytozoon was also found in all 5 ducks with dual infections: 3 infections were with 2 distinct lineages of this genus and each of the other dual infections had this genus with *Parahaemoproteus* or *Plasmodium* (Table I). Co-infection among multiple genera of haemosporidians is common; however, infection with 1 haemosporidian lineage may increase or decrease susceptibility to others (Meixell et al., 2016). Co-infections could be mediated by the host immune response either from immunosuppression by an existing infection or a from a protective immune response that limits further infection (Meixell et al., 2016). Co-infection may also result from encounters with vectors; however, information on which vectors carry these lineages is lacking (Meixell et al., 2016). These co-infections may have resulted from exposure on northern breeding grounds because co-infection may be more common at higher latitudes (Starkloff and Galen, 2023).

The prevalence of haemosporidians in greater and lesser scaup revealed a significant interaction between host species and sex (Fig. 3). This relationship was driven by the highest infection prevalence in female lesser scaup and the lowest infection prevalence in female greater scaup (Fig. 3). This result is similar to data from recent research on haemosporidians in female lesser scaup in which an association was found between infection and reduced body condition (Merrill et al., 2018). However, that study was conducted with only female lesser scaup, so an analogous comparison of host species and sexes was not possible (Merrill et al., 2018). Higher infection levels in female lesser scaup could be related to behavioral or physiological differences between males and females (e.g., feeding rates, pairing behavior, molt metabolism, and body size) (Anteau and Afton, 2004). In other studies of waterfowl haemosporidian infection,

patterns of prevalence between sexes across seasons were inconsistent (Meixell et al., 2016).

Species-level differences in haemosporidian prevalence between greater and lesser scaup should be interpreted with caution because of the significant interaction with sex but are worthy of further investigation. Variation in haemosporidian prevalence between species could be related to differences in exposure to vectors, the timing of nest initiation, habitat selection, migration patterns and timing, or species-specific immune responses (Meixell et al., 2016). Other factors such as body size or plumage characteristics are unlikely to play a role in our study because of the close morphological similarities between these 2 species and the higher prevalence in the species with the smaller body size even though larger body size may result in greater exposure to vectors (Meixell et al., 2016). In contrast to our study, there was little evidence of species-level differences in haemosporidian co-infection in 2 closely related species of thrushes (Starkloff and Galen, 2023).

We found that across both species adults and juveniles had a similar haemosporidian prevalence and that there were no differences among collection years. Previous research was conducted on spring migrating lesser scaup, so juveniles in those studies would be birds returning from their first winter, whereas our study was done on infections that birds obtained directly during the summer breeding season (Meixell et al., 2016; Merrill et al., 2018). However, infections may persist for a long time within the host and have been detected throughout the year, which could lead to the expectation that adults would have a higher prevalence (Valkiūnas, 2005; Meixell et al., 2016; Ramey et al., 2016; Fleskes et al., 2017). Our study differs from previous research on female lesser scaup in which the authors found no differences in age classes but did find differences in years for spring migrating birds (Merrill et al., 2018). Across other species of waterfowl haemosporidian prevalence varied by sample year and season (Ramey et al., 2013; Smith and Ramey, 2015). Haemosporidian prevalence in scaup and other divers was much lower than that reported from large surveys of dabbling ducks (Ramey et al., 2015, 2016). It is possible that differences in life history traits between these 2 feeding guilds, specifically habitat usage for breeding, alter their exposure to haemosporidian vectors. However, additional work is warranted to determine whether differential vector exposure alone can account for differences in parasite prevalence or whether other characteristics, such as immune response, also play a role.

Examination of all the haemosporidian lineages within the phylogenetic framework revealed a lack of specificity to hosts or to the guild of diving ducks (Fig. 2). Of the 14 lineages identified, only 8 were specific to anseriforms; however, this group includes 4 novel lineages whose full host range is unknown. These findings contrast with those of previous studies in which an association was found between lineages originating from waterfowl samples regardless of continental affiliation, suggesting potential co-phylogenetic associations with hosts (Smith and Ramey, 2015). Our detection of 4 unique lineages and similar patterns found in previous studies suggest that waterfowl possess a high diversity of previously undiscovered haemosporidian lineages. Reflecting the overall patterns in prevalence, lesser scaup also had a higher lineage diversity, including 2 new lineages. However, the lineages found in scaup were not clustered and were found among lineages from other divers and dabblers, including those from our study. Among the other species we sampled, one redhead had one lineage of *Haemoproteus,* which is rare among waterfowl; the only other identification of this parasite genus was from 2 species of ducks in Chile (Bell et al., 2020). However, this lineage from the redhead differs in only 1 bp from another *Haemoproteus* lineage (GEOTRY01) that is generally widespread, and with the high rates of saturation known for the DNA fragment sequenced it is possible that this difference is due to random mutation rather than a specific host/parasite relationship. Only through investigation of blood films and identification of gametocytes, which was not possible in this study, can be we certain that this parasite lineage completes its lifecycle in anseriform hosts and is not simply an example of an abortive infection. Overall, the phylogeny does not support any impact of duck feeding guild on haemosporidian evolution within Anseriformes (Fig. 2).

Our results suggest that some lineages of haemosporidians are widespread with distributions that include the Great Lakes region, whereas others newly detected in our study require further investigation to determine their geographic distributions. For black scoter and long-tailed ducks, the Great Lakes are the overwintering grounds and nearly the southern extent of their range, so our results provide initial haemosporidian records for these birds during this period of their annual cycle (Prince et al., 1992). Like the scaup, these 2 species are of conservation concern, and future haemosporidian monitoring could be part of management efforts (Plumpton et al., 2020, Mineev et al., 2023). The Great Lakes ecosystem also includes important coastal wetlands and other adjacent habitats important to waterfowl, including dabblers (Prince et al., 1992). Although our study included only 2 samples of wood ducks, the information contributed to the knowledge on the diversity of haemosporidians in this geographic area, including a new lineage, suggesting that the much larger dataset on dabbling ducks still has significant gaps. For example, of the over 16,000 host records in the MalAvi database, only a small fraction are from anseriform hosts, representing only 40 host species (Bensch et al., 2009).

Our study contributes to our understanding of haemosporidian diversity, biogeography, and host associations in an ecological and economically important group of avian hosts. Sequence data and the results of phylogenetic analyses conducted in our study provide a baseline for future work. However, there are limitations to this approach without a morphological component. Future research should address the need for morphological vouchers for updating taxonomy through the formal descriptions of species-level lineages, confirmation of active infections, and distinguishing co-infecting species (Bell et al., 2020, Starkloff and Galen, 2023). Additional molecular markers would also be useful for addressing species limits (Galen et al., 2018; Starkloff and Galen, 2023). This research also addressed only the waterfowl hosts of these parasites but not the vectors. Assessment of vectors is essential for understanding haemosporidian biogeography and habitats and to determine the effects of climate change (Assel, 2005; Fecchio et al., 2017; Merrill et al., 2018; Starkloff and Galen, 2023). This study was a collaboration among parasitologists, hunters, and waterfowl organizations (Sheehan et al., 2022) designed to conduct basic research with important implications for management and conservation (Finger and Rohrer, 2020). Birds harvested by hunters may not represent a random sample and limit the health parameters that could be measured but provide an opportunity to obtain useful wildlife disease data important to multiple stakeholders (Vest et al., 2006; Fleskes et al., 2017; Sheehan et al., 2022). By working together, we can generate data to inform waterfowl managers and partners for on-the-ground decision-making and expand and explore new research, monitoring, and management approaches to investigate parasites and the conditions

that promote negative parasite impacts on hosts (Fleskes et al., 2017; Finger and Rohrer, 2020).

ACKNOWLEDGMENTS

The authors assert that all applicable state and national permits (U.S. Fish and Wildlife Service Migratory Bird SPPSDS-45314C and Wisconsin Department of Natural Resources SCPN-19-014, SCPN-21-13, and SCPR-22-19) and institutional guidelines for the care and use of animals and biological safety (UWSP IBC 2020-06-03-03) were followed. We thank Katherine Brown, Reece Mullen, Demirae Berceau, Michaela Meehl, Allison Luebke, Nicole Wagner, Kao Lee Thao, and students in the University of Wisconsin–Stevens Point BIOL 160 Introduction to Animal Biology and BIOL 362 Animal Parasitology courses for help with waterfowl dissections. We thank two anonymous reviewers for their thoughtful comments that improved the manuscript. This study was supported by the University of Wisconsin–Stevens Point through New Faculty Start-up, Pathways Internship, and University Personnel Development Committee funding to S.A.O.

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