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Diversity and prevalence of hemoparasites of wading birds in southern Florida, USA

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ABSTRACT

Relatively few studies on hemoparasites have been conducted on wading birds in the families Ardeidae and Threskiornithidae (order Pelecaniformes), especially in the United States. In this study, we obtained baseline data on the prevalence and genetic diversity of haemosporidian parasites in wading birds opportunistically sampled from southern Florida, USA. We detected blood parasites in White Ibis (*Eudocimus albus*), Glossy Ibis (*Plegadis falcinellus*), Green Heron (*Butorides virescens*), and Roseate Spoonbill (*Platalea ajaja*) with several novel host-parasite relationships. Infected birds had low parasitemias (average 0.77%, range 0–4%) suggesting that infections were chronic. Despite the low sample sizes for several of our sampled species, these data highlight the diversity of parasites in this understudied group of birds and suggest that additional studies are needed to investigate the potential impacts of these parasites on their health, especially since southern Florida is becoming increasingly urbanized which can alter parasite transmission or host susceptibility.

1. Introduction

Vector-borne, protozoan parasites in the order Haemosporida (genera *Plasmodium, Haemoproteus, Leucocytozoon*, and *Fallisia*) can cause significant morbidity and mortality in some species of birds. Generally, haemosporidian infections often are well tolerated by their natural bird hosts; however, young birds, birds that are outside their normal range, or birds in areas where parasites are introduced are more likely to develop clinical disease (Dawson and Bortolotti, 2000; Valkiūnas, 2005). Common examples include mortality of captive penguins due to *Plasmodium* spp. circulating in native bird populations around the zoo and the significant impact of *P. relictum* after it was introduced to avifauna in Hawaii (Herman et al., 1968; Vanstreels et al., 2014; Samuel et al., 2015). In their natural hosts, haemosporidian parasites generally establish long-term infections and the long-term consequences of these infections have been extensively studied. Field and experimental studies have detected significant impacts of some haemosporidian chronic infections on birds including reduced reproductive success, host fitness, increased stress and disease susceptibility (Arriero et al., 2008; Knowles et al., 2010; Lachish et al., 2011; Dhondt et al., 2017). In addition, Asghar et al. (2015) recently reported that Great Reed Warblers (*Acrocephalus arundinaceus*) infected with *Plasmodium ashfordi* had shorter telomere lengths, which ultimately was correlated with decreased life spans. Collectively, these data highlighted the potential for these parasites to exert a significant population level impact on a host species, even in cases where they might not cause acute mortality.

Although numerous studies have investigated hemoparasites of Passeriformes and Anseriformes, relatively few studies on hemoparasites have been conducted on birds in the families Ardeidae and Threskiornithidae (currently in the order Pelecaniformes, but

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historically in the order Ciconiiformes). Most surveys of these families have been outside of North America where *Haemoproteus plataleae* has been reported from Eurasian Spoonbill (*Platalea leucorodia*), Glossy Ibis (*Plegadis falcinellus*), Australian White Ibis (*Threskiornis molucca*) and Red-naped Ibis (*Pseudibis papillosa*), *Haemoproteus pelouroi* from Hadada Ibis (*Bostrychia hagedas*) and African Sacred Ibis (*T. aethiopicus*), *Leucocytozoon leboeufi* (=*L. ardeae*) from Australian White Ibis, and *Fallisia neotropicalis* from Green Ibis (*Mesembrinibis cayennensis*) and Scarlet Ibis (*Eudocimus ruber*) (Valkiūnas, 2005).

In the United States, the White Ibis (*Eudocimus albus*) is the only member of the Threskiornithidae that has been sampled for blood parasites. Based on blood smear analysis, *H. plataleae* occurs in high prevalence in adult White Ibises and this parasite has been reported in five counties in Florida (Forrester, 1980). Recently, an uncharacterized *Plasmodium* sp. was detected in a single White Ibis from southern Florida by polymerase chain reaction (PCR) testing (Bryan et al., 2015). In contrast, despite limited testing, several hemoparasites have been reported from sympatric North American species of egrets and herons (family Ardeidae) including two species of *Plasmodium* (*P. relictum* and *P. elongatum*), two species of *Haemoproteus* (*H. herodiadis* and an unnamed *Haemoproteus* sp.), and one species of *Leucocytozoon* (*L. leboeufi*) (Telford et al., 1992; Beadell et al., 2006). None of these studies used a combination of morphological data and molecular analysis for identification which can lead to an underestimation of diversity.

Although few studies have examined members of the Ardeidae for blood parasites, these studies have focused on herons and egrets which were infected with multiple genera of parasites. Therefore, ibises and their close relatives likely have an unrecognized diversity of blood parasites. Therefore, our goal was to determine the prevalence, parasitemias, and diversity of hemoparasites in opportunistically sampled Pelecaniformes of southern Florida.

2. Materials and methods

Sample collection. From 2013 to 2016, blood samples were collected throughout the year from wading birds admitted to rehabilitation centers in urbanized areas of Lee and Broward Counties (26° 26′37.6″ N 82° 06′55.52″ W and 26° 05′04.20″ N 80° 08′46.53″ W, respectively). Birds exhibited a variety of conditions including lacerations, fractures, and dehydration. In May 2014, wading bird chicks were hand captured from two nesting sites from a natural area in Broward County (26°11′41.02″N 80°31′28.29″W and 26° 7′19.35″N 80°32′29.09″W) as described by Hernandez et al. (2016). Blood samples were collected from the jugular or medial metatarsal vein into heparinized microtainer® tubes (Beckton Dickinson, Franklin Lakes, New Jersey) and two thin blood smears were immediately prepared, dried, and fixed in methanol. Remaining blood was frozen at −20C until PCR testing. In some cases, birds admitted to rehabilitation centers died prior to blood collection; therefore, only clotted blood was available for PCR testing. All capture and sampling techniques were reviewed and approved by the University of Georgia's IACUC (#A2013- 10–016).

Genetic characterization. DNA was extracted from whole blood samples (10 μl) using a Qiagen DNeasy blood extraction kit per the manufacturer's instructions (Qiagen, Valencia, California). Nested PCR was used to target a 480 base pair (bp) fragment of the mitochondrial cytochrome *b* gene of *Haemoproteus* and *Plasmodium* and a 478 bp fragment of *Leucocytozoon*, as described by (Hellgren et al., 2004; Waldenström et al., 2004). Secondary PCR products were electrophoresed in 2% agarose gels stained with ethidium bromide. Amplicons were excised from the gel and purified using the Qiagen QIAquick gel extraction kit (Qiagen) and sequenced at the Georgia Genomics Facility in Athens, GA using the Sanger method. Sequences were analyzed and aligned using Sequencher (v5.0) and then compared with related sequences in the GenBank and the MalAvi databases to determine related haplotypes. The GenBank accession number for the novel sequence of *H. plataleae* is MF536976.

DNA from blood samples from ducks infected with *H. nettionis* or *L. simondi* were used as a positive control in each set of PCR reactions. To prevent and detect contamination, DNA extraction, primary and secondary amplification, and product analysis were done in separate dedicated areas. A negative water control was included before and after each set of 10–12 extractions. Additional negative water controls were included in each set of primary and secondary PCR reaction sets.

Blood Smear Analyses. Thin blood smears were stained with a modified Giemsa stain (Dipquick, Jorgensen Laboratories, Inc., Loveland, CO). To estimate parasitemias, approximately 20,000 erythrocytes were examined for blood smears determined to be positive as suggested by Godfrey et al. (1987). If no parasites were observed during this initial scan, the smear was examined for another 5 min (generally this would result in another 20,000 erythrocytes or more examined). The parasites were morphologically identified using a published key (Valkiūnas, 2005) or by examination of specific parasite descriptions (e.g., Tostes et al., 2017).

3. Results

Samples were collected from six species of wading birds: White Ibis, Glossy Ibis, Roseate Spoonbill (*Platalea ajaja)*, Green Heron (*Butorides virescens*), Tricolored Heron (*Egretta tricolor*), and Great Blue Heron (*Ardea herodias*) (Table 1). In Lee County, three species (White Ibis, Glossy Ibis, and Green Herons) were positive using the *Plasmodium/Haemoproteus* PCR assay (Table 1). At the Broward County rehabilitation center, 5/14 (36%) White Ibises were PCR positive with the *Plasmodium/Haemoproteus* PCR assay. Among the chicks sampled in Broward County, only one Roseate Spoonbill chick estimated to be 20–30 days old was PCR positive (Table 1). The remaining chicks were negative; the White Ibises were estimated to be either 8–12 days (n = 2) or 29–33 days old (n = 2) and the Glossy Ibis chick was 8–12 days old.

Overall, based on sequence analysis, two *Plasmodium* spp. and one *Haemoproteus* sp. were detected (Table 2). One Green Heron and the Roseate Spoonbill were infected with *Plasmodium elongatum* (haplotype pGRW06/MD-2011) (Table 2). The Glossy Ibis *Plasmodium* sp. sequence was identical to lineage pMYCAME02 (also called CMV-2012). All of the positive White Ibises and one Green Heron were infected with a novel *Haemoproteus* haplotype (designated hWHIB01) that was 98.6% similar (477/484bp) to a *Haemoproteus* sp. from West Africa (hCELEC01/haplotype WAH8).

Blood smears were only available for 17 birds, nine of which were PCR-positive birds. No parasites were observed in PCR negative birds but they were observed in eight of nine PCR-positive birds. In general, the parasitemias were low and ranged from 0 to 4% (average of 0.77%). The *Haemoproteus* haplotype found in the White Ibis was morphologically identified as *H. plataleae* (Table 2). The parasites in the *Plasmodium*-positive Green Heron were morphologically consistent with *P. elongatum* with gametocytes that measured approximately 16 μm × 2.5 μm and did not displace the host cell nucleus. The Glossy Ibis infected with *Plasmodium* had the highest parasitemia detected in the study (4%); ~10% of infected erythrocytes contained 2–4 parasites (Fig. 1). The parasites were identified as a *Plasmodium* (*Novyella*) sp. and shared some morphological characteristics reported for *P. paranucleophilum* as described by Tostes et al. (2017). However, because no phanerozoites were observed in circulating blood cells, few gametocytes were observed, and the parasite nuclei stained poorly, we were

Table 1
Prevalence of hemoparasites in wading birds from southern Florida, USA.

County	Site	Species	No. PCR positive/No. tested (%)
Lee	Urban rehabilitation center	White Ibis	11/17 (65)
		Glossy Ibis	1/2 (50)
		Green Heron	2/3 (67)
		Tricolored Heron	0/2
		Great Blue Heron	0/2
Broward	Urban rehabilitation center	White Ibis	5/14 (36)
	Natural breeding sites	White Ibis	0/4
		Glossy Ibis	0/1
		Roseate Spoonbill	1/1 (100)
Total			20/46 (43)

Table 2
Parasitemias, morphological identification, and haplotype of *Plasmodium* and *Haemoproteus* spp. from wading birds from southern Florida, USA.

Sample ID	Species	Haplotype	Parasitemia (%)	Morphological identification
13–3084	Green Heron	hWHIB01 (*H. plataleae*)	N/A	N/A
13–2774	Green Heron	pGRW06 (*P. elongatum*)	0.018	*P. elongatum*
14–1312	Glossy Ibis	pMYCAME02	3.982	*Plasmodium* (*Novyella*) sp.
SB1	Roseate Spoonbill	pGRW06 (*P. elongatum*)	0	N/A
13–2738	White Ibis	hWHIB01	0.01	*H. plataleae*
14–2548	White Ibis	hWHIB01	0.464	*H. plataleae*
14–2207	White Ibis	hWHIB01	0.575	*H. plataleae*
14–1831	White Ibis	hWHIB01	0.298	*H. plataleae*
14–1756	White Ibis	hWHIB01	0.01	*H. plataleae*
14–2523	White Ibis	hWHIB01	0.004	*H. plataleae*
14–2711	White Ibis	hWHIB01	N/A	N/A
14–2916	White Ibis	hWHIB01	N/A	N/A
14–1168	White Ibis	hWHIB01	N/A	N/A
14–2813	White Ibis	hWHIB01	N/A	N/A

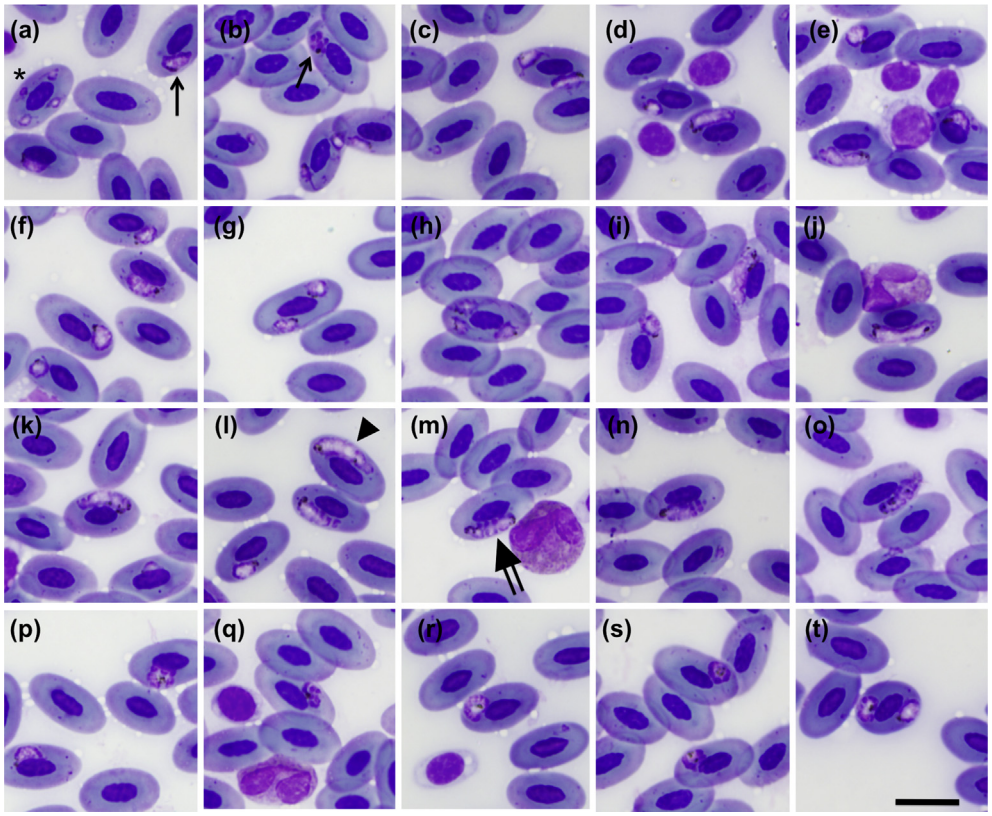

Fig. 1. *Plasmodium* (*Novyella*) sp. stages observed in blood smear of a Glossy Ibis from Florida, USA that matched haplotype pMYCAME02. Representative trophozoites (*), erythrocytic meronts (arrow), macrogametocytes (double arrow), and microgametocytes (arrowhead) are marked. Some erythrocytes were infected with multiple parasites, e.g., (a) a cell with 4 trophozoites, (h) a cell with three early meronts, and (t) a cell with two early meronts. Scale bar = 10 μm.

unable to confirm the identity of our parasite. Similar to what was reported by Tostes et al. (2017), the nuclei of erythrocytes infected with mature gametocytes were often displaced but few mature gametocytes (n = 11) were observed. Those observed measured >10 μm (average of 11.2 μm) (10.5–12 μm) which is slightly shorter than reported for *P. nucleophilum* (Tostes et al., 2017). Also, meronts did not displace the nuclei of infected erythrocytes but meronts contained dark-brown colored pigment granules that were more aggregated and larger compared to those described by Tostes et al. (2017).

4. Discussion

The order Pelecaniformes recently was reorganized and now contains five extant families, with the Ardeidae and Threskiornithidae being the most species-rich (Gill and Donsker, 2017). Despite the worldwide distribution of birds in these two families, relatively few studies have been conducted on the prevalence and diversity of their blood parasites. In addition, most studies have been based solely on analysis of blood smears. In the current study, we used molecular methods to identify new host-parasite relationships for *Plasmodium* and *Haemproteus* in several species of herons, ibises, and spoonbills.

This is the first report of a blood parasite from Glossy Ibis; the single infected bird in our study had a parasite that was genetically consistent with parasites reported to be *P. paranucleophilum* from various birds of prey (Falconiformes and Strigiformes) from South America and *Plasmodium* spp. reported from Wood Storks (*Mycteria americana*) and a Streaked Flycatcher (*Myiodynastes maculatus*) from Brazil and Blue-winged Teal (*Anas discors*) from North America (Villar et al., 2013; Ramey et al., 2016; Fecchio et al., 2017; Ferreira Jr. et al., 2017; Tostes et al., 2017). During the study on Blue-winged Teal, only adults were infected so there is currently no evidence of pMYCAME02 transmission to chicks in North America (Ramey et al., 2016). Finding this lineage in Glossy Ibis is interesting as this bird species does not have an extensive distribution in South America whereas the previous hosts of this parasite lineage has been from resident Central or South American hosts or from a host (Blue-winged Teal) that migrates between North and South America. Migration has been shown an effective mechanism to disperse avian haemosporidian parasites and has been suspected to introduce novel lineages in some regions (Levin et al., 2013; Ramey et al., 2014, 2016; Smith and Ramey, 2015). Glossy Ibis are native to the Old World but have been introduced or naturally spread to the New World where they are found along the coastal United States (primarily Southeast), throughout the Caribbean, parts of Central America and in small regions of northern South America (Hancock et al., 1992). Some individuals winter in Florida (USA) and do not migrate to South America but the migration history of our sampled bird is unknown; sampling Glossy Ibis chicks or possible vectors from North America would be of interest to determine if lineage pMYCAME02 is transmitted outside of South America. Interestingly, this Glossy Ibis had the highest parasitemia (4%) detected in the current study and although it was originally submitted to the rehabilitation center with a broken leg that required euthanasia, it is unknown if the *Plasmodium* infection contributed to morbidity leading to its injury.

Although our Glossy Ibis *Plasmodium* sp. parasite shared some morphologic characteristics as reported in the recent redescription of *P. paranucleophilum* by Tostes et al. (2017), the Glossy Ibis parasite did not fully conform. For example, the morphology of our meronts differed and no phanerozoites were observed in the Glossy Ibis blood smear. It is unknown if this parasite is a novel *Plasmodium (Novyella)* species or is a morphologic variant of *P. paranucleophilum*. A morphologically similar parasite, *P. nucleophilum*, has a broad host and geographic range but our sequence was distinct (466/478, 97.5%) from those from *P. nucleophilum* (Iezhova et al., 2005; Valkiūnas, 2005; Chagas et al., 2013; Tostes et al., 2017). Regardless, this is the first report of lineage pMYCAME02 from any species of Pelecaniformes.

The *Plasmodium*-infected Roseate Spoonbill chick and Green Heron adult were infected with *P. elongatum*. The prepatent period of *P. elongatum* is usually 9–12 days; therefore, some of the other chicks (White Ibis and Glossy Ibis) that were sampled were only 8–12 days and may have been too young to detect infections (Valkiūnas, 2005). This is the first report of a blood parasite in the Roseate Spoonbill, but the finding of this parasite species was not surprising as it has a near cosmopolitan distribution and a broad host range including the orders Apterygiformes, Ciconiiformes, Columbiformes, Coraciiformes, Galbuliformes, Gruiformes, Passeriformes, Pelecaniformes and Strigiformes (Valkiūnas, 2005). Within the Pelecaniformes, *P. elongatum* has been reported from Great Blue Herons in the USA and Cattle Egrets (*Bubulcus ibis*) in Spain (Forrester and Spalding, 2003; Beadell et al., 2006; Ferraguti, 2013). Disease associated with *P. elongatum* has been reported in a number of bird species, but especially in captive penguins (Fleischman et al., 1968; Herman et al., 1968; Valkiūnas et al., 2008; Dinhopl et al., 2015; Vanstreels et al., 2014).

Four genera of hematozoa (*Plasmodium, Haemoproteus, Leucocytozoon* and *Fallisia*) have been reported from birds in the family Ardeidae, but we did not detect blood parasites in the Great Blue Herons or Tricolored Herons, despite *P. elongatum*, *P. relictum, H. herodiadis, and L. leboeufi* previously being reported in Great Blue Herons and an undescribed *Haemoproteus* sp. from Tricolored Herons (Telford et al., 1992; Forrester and Spalding, 2003; Beadell et al., 2006). However, this is not surprising considering the low prevalence (7%) previously reported (Telford et al., 1992) and our low sample sizes. Despite a low sample size of Green Herons, we detected infections with *H. plataleae* and *P. elongatum*. Several *Plasmodium* spp., including *P. elongatum,* have been reported from Green Herons or mosquitoes containing Green Heron blood. Only one of these reports included parasite species identification and it was a *P. elongatum* infection in the Green Heron, found in the western USA (Wood and Herman, 1943). The other two reports were a *Plasmodium* sp. ("Butorides" type) in 3 of 6 birds from Panama and a *Plasmodium* DNA sequence from a Green Heron-fed mosquito from North Dakota, USA which matched *Plasmodium* sequences from a Barred Owl (*Strix varia*) and several passerine species (Galindo and Sousa, 1966; Mehus and Vaughan, 2013).

Overall, a relatively high prevalence of *Haemoproteus* was detected in White Ibises from southern Florida, similar to data from previous studies (Forrester, 1980; Telford et al., 1992) suggesting that *H. plataleae* may cause chronic infections in White Ibises or that reinfections are common. The former is supported by the fact that we found predominately mature gametocytes in >200 White Ibis blood smears we have examined in a separate study (data not shown). We failed to detect infections in chicks at the Broward County nesting site; however, sample sizes were low and two of them may have been too young to have patent *Haemoproteus* parasitemias, although the infection dynamics of *H. plataleae* are unknown (Dusek et al., 2004). Similarly, negative results were also obtained for numerous heron and egret chicks in southern France, despite mosquitoes captured in the area having 4 lineages of *Plasmodium* (Larcombe and Gauthier-Clerc, 2015). We only detected one *Haemoproteus* haplotype in the White Ibises, supporting previous data that only a single morphospecies (*H. plataleae*) has been reported (Forrester, 1980; Telford et al., 1992). We failed to detect *Plasmodium* infections in White Ibises, although Bryan et al. (2015) recently detected a *Plasmodium* sp. in 1 of 4 sampled adult White Ibis.

Leucocytozoon was not found in any of our sampled birds, despite this parasite genus being reported from numerous sympatric Ardeidae species (Coatney, 1938; Wood and Herman, 1943; Galindo and Sousa, 1966; Forrester and Spalding, 2003). However, we only sampled White Ibises from two sites, so additional studies are needed on ibises from other locations to determine hematozoa diversity. *Leucocytozoon leboeufi* has been reported from numerous species of Pelecaniformes and hosts that are found in the United States include the Green Heron, Great Blue Heron, Grey Heron (*Ardea cinerea*), Black-crowned Night Heron (*Nycticorax nycticorax*), Great Egret (*Ardea alba*), Cattle Egret, and Snowy Egret (*Egretta thula*) (Coatney, 1938; Valkiūnas, 2005). We encourage future work on the genetic characterization of *Leucocytozoon* from the Pelecaniformes considering L. *leboeufi* has a cosmopolitan distribution among numerous bird species and recent molecular work on *L. simondi* from several duck species indicates that one or more cryptic species exist (Reeves et al., 2015). Thus it is possible that herons, egrets, and their relatives, especially from different continents, are infected with more than one *Leucocytozoon* species.

Another parasite that we unfortunately did not detect, morphologically or genetically, was *Fallisia neotropicalis*. This parasite is unusual for numerous reasons including 1) it is the only member of the family Garniidae that has been reported in birds, 2) is found exclusively found in the Neotropics (specifically Venezuela), mostly in Pelecaniformes species, and 3) intracellular forms are predominately in thrombocytes, lymphocytes, and monocytes (Valkiūnas, 2005). Although the type host is the Rock Pigeon (*Columba livia*) (not native to Venezuela), most reported infected hosts have been Pelecaniformes species native to Venezuela, two of which were sampled in our study (i.e., Roseate Spoonbill and Great Blue Heron).

In summary, we morphologically and genetically characterized the haemosporidian parasites of several species of wading birds in southern Florida. Novel host-parasite relationships were noted. Our failure to detect a higher diversity could be due to our limited sample sizes for each species, restriction of sampling to a limited number of sites, or biases associated with our sampling groups (i.e., injured or debilitated birds from rehabilitation centers or young chicks with limited vector exposure). Spatial variation in haemosporidian infections in birds is expected with the complex relationships among hosts, vectors, and environmental conditions (including human influences such as habitat alterations and vector-control programs) (Bensch and Akesson, 2003; Ishtiaq et al., 2007; Wood et al., 2007; Loiseau et al., 2010). This group of birds is found worldwide, yet relatively few data are available on the diversity of blood parasites and impacts of these parasites on avian health. This gap in knowledge is compounded by the rapid increase in urbanization in southern Florida over the past century, which may influence both host and vector biology.

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References

Arriero, E., Moreno, J., Merino, S., Martínez, J., 2008. Habitat effects on physiological stress response in nestling blue tits are mediated through parasitism. Phys. Biochem. Zool. 81, 195–203.

Asghar, M., Hasselquist, D., Hansson, B., Zehtindjiev, P., Westerdahl, H., Bensch, S., 2015. Chronic infection. Hidden costs of infection: chronic malaria accelerates telomere degradation and senescence in wild birds. Science 347, 436–438.

Beadell, J.S., Ishtiaq, F., Covas, R., Melo, M., Warren, B.H., Atkinson, C.T., Fleischer, R.C., 2006. Global phylogeographic limits of Hawaii's avian malaria. Proc. R. Soc. Lond. B Biol. Sci. 273, 2935–2944.

Bensch, S., Akesson, S., 2003. Temporal and spatial variation of hematozoans in Scandinavian willow warblers. J. Parasit. 89, 388–391.

Bryan, A.L., Love, C.N., Mills, G.L., Borkhataria, R.R., Lance, S.L., 2015. Testing for associations between hematozoa infection and mercury in wading bird nestlings. J. Wild. Dis. 51, 222–226.

Chagas, C.R., Valkiūnas, G., Nery, C.V., Henrique, P.C., Gonzalez, I.H., Monteiro, E.F., Guimarães Lde, O., Romano, C.M., Kirchgatter, K., 2013. *Plasmodium (novyella) nucleophilum* from an egyptian goose in são paulo zoo, Brazil: microscopic confirmation and molecular characterization. Int. J. Parasitol. Parasites Wildl. 2, 286–291.

Coatney, G.R., 1938. Some blood parasites from birds of the lake Okaboji region. Am. Midl. Nat. 20, 336–340.

Dawson, R.D., Bortolotti, G.R., 2000. Effects of hematozoan parasites on condition and return rates of American kestrels. Auk 117, 373–380.

Dhondt, A.A., Dhondt, K.V., Nazeri, S., 2017. Apparent effect of chronic *Plasmodium* infections on disease severity caused by experimental infections with *Mycoplasma gallisepticum* in house finches. Int. J. Parasitol. Parasites Wildl. 6, 49–53.

Dinhopl, N., Nedorost, N., Mostegl, M.M., Weissenbacher-Lang, C., Weissenböck, H., 2015. In situ hybridization and sequence analysis reveal an association of *Plasmodium* spp. with mortalities in wild passerine birds in Austria. Parasitol. Res. 114, 1455–1462.

Dusek, R.J., Spalding, M.G., Forrester, D.J., Greiner, E.C., 2004. *Haemoproteus balearicae* and other blood parasites of free-ranging Florida sandhill crane chicks. J. Wildl. Dis. 40, 682–687.

Fecchio, A., Pinheiro, R., Felix, G., Faria, I.P., Pinho, J.B., Lacorte, G.A., Braga, E.M., Farias, I.P., Aleixo, A., Tkach, V.V., Collins, M.D., Bell, J.A., Weckstein, J.D., 2017. Host community similarity and geography shape the diversity and distribution of haemosporidian parasites in Amazonian birds. Ecography. http://dx.doi.org/10.1111/ecog.03058.

Ferraguti, M., Martínez-de la Puente, J., Muñoz, J., Roiz, D., Ruiz, S., Soriguer, R., Figuerola, J., 2013. Avian *Plasmodium* in *Culex* and *Ochlerotatus* mosquitoes from Southern Spain: effects of season and host-feeding source on parasite dynamics. PLoS One 8, e66237.

Fleischman, R.W., Squire, R.A., Moore, W.J.L.S., 1968. Pathological confirmation of malaria (*Plasmodium elongatum*) in African penguins (*Spheniscus demersus*). J. Wildl. Dis. 4, 133–135.

Forrester, D.J., 1980. Hematozoa and mallophaga from white ibis, *Eudocimus albus* L., in Florida. J. Parasitol. 66, 58.

Forrester, D.J., Spalding, M.G., 2003. Parasites and Diseases of Wild Birds in Florida. University Press of Florida, 1132pp.

Galindo, P., Sousa, O., 1966. Blood parasites of birds from Almirante, Panama with ecological notes on the hosts. Rev. Biol. Trop. 14, 27–46.

Gill, F., Donsker, D., 2017. In: IOC World Bird List. http://dx.doi.org/10.14344/IOC.ML.7.1, v. 7.1. .

Godfrey Jr., R.D., Fedynich, A.M., Pence, D.B., 1987. Quantification of hematozoa in blood smears. J. Wildl. Dis. 23, 558–565.

Hancock, J.A., Kushlan, J.A., Kahl, M.P., 1992. Storks, Ibises, and Spoonbills of the World. Academic Press, London.

Hellgren, O., Waldenstrom, J., Bensch, S., 2004. A new PCR assay for simultaneous studies of *Leucocytozoon, Plasmodium*, and *Haemoproteus* from avian blood. J. Parasitol. 90, 797–802.

Herman, C.M., Kocan, R.M., Snyder, E.L., Knisley, J.O., 1968. *Plasmodium elongatum* from a penguin. J. Wildl. Dis. 4, 132.

Hernandez, S.M., Welch, C.N., Peters, V.E., Lipp, E.K., Curry, S., Yabsley, M.J., Sanchez, S., Presotto, A., Gerner-Smidt, P., Hise, K.B., Hammond, E., Kistler, W.M., Madden, M., Conway, A.L., Kwan, T., Maurer, J.J., 2016. Urbanized white ibises (*Eudocimus albus*) as carriers of *Salmonella enterica* of significance to public health and wildlife. PLoS One 11 (10), e0164402.

Iezhova, T.A., Valkiūnas, G., Barlein, F., 2005. Vertebrate host specificity of two avian malaria parasites of subgenus *Novyella: Plasmodium nucleophilum* and *Plasmodium vaughani*. J. Parasitol. 91, 472–474.

Ishtiaq, F., Gering, E., Rappole, J.H., Rahmani, A.R., Jhala, Y.V., Dove, C.J., Fleischer, R.C., 2007. Prevalence and diversity of avian hematozoan parasites in Asia: a regional survey. J. Wildl. Dis. 43, 382–398.

Ferreira Jr, F.C., Rodrigues, R.A., Ellis, V.A., Leite, L.O., Borges, M.A.Z., Braga, É.M., 2017. Habitat modification and seasonality influence avian haemosporidian parasite distributions in southeastern Brazil. PLoS One 12, e0178791.

Knowles, S.C., Palinauskas, V., Sheldon, B.C., 2010. Chronic malaria infections increase family inequalities and reduce parental fitness: experimental evidence from a wild bird population. J. Evol. Biol. 23, 557–569.

Lachish, S., Knowles, S.C.L., Alves, R., Wood, M.J., Sheldon, B.C., 2011. Fitness effects of endemic malaria infections in a wild bird population: the importance of ecological structure. J. Anim. Ecol. 80, 1207–1216.

Larcombe, S.D., Gauthier-Clerc, M., 2015. Avian Malaria is absent in juvenile colonial herons (*Ardeidae*) but not *Culex pipiens* mosquitoes in the Camargue, Southern France. Waterbirds 38, 387–395.

Levin, I.I., Zwiers, P., Deem, S.L., Geest, E.A., Higashiguchi, J.M., Iezhova, T.A., Jiménez-Uzcátegui, G., Kim, D.H., Morton, J.P., Perlut, N.G., Renfrew, R.B., Sari, E.H., Valkiunas, G., Parker, P.G., 2013. Multiple lineages of avian malaria parasites (*Plasmodium*) in the Galapagos Islands and evidence for arrival via migratory birds. Conserv. Biol. 27, 1366–1377.

Loiseau, C., Iezhova, T., Valkiūnas, G., Chasar, A., Hutchinson, A., Buermann, W., Sehgal, R.N.M., 2010. Spatial variation of haemosporidian parasite infection in African rainforest bird species. J. Parasitol. 96, 21–29.

Mehus, J.O., Vaughan, J.A., 2013. Molecular identification of vertebrate and hemoparasite DNA within mosquito blood meals from eastern North Dakota. Vector Borne Zoo. Dis. 13, 818–824.

Ramey, A.M., Schmutz, J.A., Reed, J.A., Fujita, G., Scotton, B.D., Casler, B., Fleskes, J.P., Konishi, K., Uchida, K., Yabsley, M.J., 2014. Evidence for intercontinental parasite exchange through molecular detection and characterization of haematozoa in northern pintails (*Anas acuta*) sampled throughout the North Pacific Basin. Int. J. Parasitol. Parasites Wildl. 4, 11–21.

Ramey, A.M., Reed, J.A., Walther, P., Link, P., Schmutz, J.A., Douglas, D.C., Stallknecht, D.E., Soos, C., 2016. Evidence for the exchange of blood parasites between North America and the Neotropics in blue-winged teal (*Anas discors*). Parasitol. Res. 115, 3923–3939.

Reeves, A.B., Smith, M.M., Meixell, B.W., Fleskes, J.P., Ramey, A.M., 2015. Genetic diversity and host specificity varies across three genera of blood parasites in ducks of the Pacific Americas Flyway. PLoS One 10, e0116661. http://dx.doi.org/10.1371/journal.pone.0116661.

Samuel, M.D., Woodworth, B.L., Atkinson, C.T., Hart, P.J., LaPointe, D.A., 2015. Avian malaria in Hawaiian forest birds: infection and population impacts across species and elevations. Ecosphere 6, 1–21.

Smith, M.M., Ramey, A.M., 2015. Prevalence and genetic diversity of haematozoa in South American waterfowl and evidence for intercontinental redistribution of parasites by migratory birds. Int. J. Parasitol. Parasites Wildl. 4, 22–28.

Telford Jr., S.R., Spalding, M.G., Forrester, D.J., 1992. Hemoparasites of wading birds (Ciconiiformes) in Florida. Can. J. Zool. 70, 1397–1408.

Tostes, R., Dias, R.J.P., Martinele, I., Senra, M.V.X., D'Agosto, M., Massard, C.L., 2017. Multidisciplinary re-description of *Plasmodium (Novyella) paranucleophilum* in Brazilian wild birds of the Atlantic Forest kept in captivity. Parasitol. Res. http://dx.doi.org/10.1007/s00436-017-5465-3.

Valkiūnas, G., 2005. Avian Malaria Parasites and Other Haemosporidia. CRC Press, 1043 Boca Raton, FL, 935 pp.

Valkiūnas, G., Iezhova, T.A., Krizanauskiene, A., Palinauskas, V., Sehgal, R.N.M., Bensch, R., 2008. A comparative analysis of microscopy and PCR-based detection methods for blood parasites. J. Parasitol. 94, 1395–1401.

Vanstreels, R.E.T., Kolesnikovas, C.K.M., Sandri, S., Silveira, P., Belo, N.O., Ferreira Jr., F.C., Epiphanio, S., Steindel, M., Braga, É.M., Catão-Dias, J.L., 2014. Outbreak of avian malaria associated to multiple species of *Plasmodium* in Magellanic penguins undergoing rehabilitation in Southern Brazil. PLoS One 9, e94994.

Villar, C.M., Bryan Jr., A.L., Lance, S.L., Braga, E.M., Congrains, C., Nassif, Del Lama, S.N., 2013. Blood parasites in nestlings of wood stork populations from three regions of the American continent. J. Parasitol. 99, 522–527.

Waldenström, J., Bensch, S., Hasselquist, D., Ostman, O., 2004. A new nested polymerase chain reaction method very efficient in detecting *Plasmodium* and *Haemoproteus* infections from avian blood. J. Parasitol. 90, 191–194.

Wood, S.F., Herman, C.M., 1943. The occurrence of blood parasites in birds from southwestern United States. J. Parasitol. 29, 187–196.

Wood, M.J., Cosgrove, C.L., Wilkin, T.A., Knowles, S.C.L., Day, K.P., Sheldon, B.C., 2007. Within-population variation in prevalence and lineage distribution of avian malaria in blue tits, *Cyanistes caeruleus*. Mol. Ecol. 16, 3263–3273.