GENETIC DIVERSITY AND HOST SPECIFICITY OF BLOOD PARASITES OF WADING BIRDS IN SOUTHERN FLORIDA

by

SARAH M. COKER

(Under the Direction of Michael J. Yabsley)

ABSTRACT

White ibis (*Eudocimus albus*) have become increasingly urbanized, many now relying heavily on urban/suburban habitats. Avian haemosporidia can cause acute disease and reduced fitness. We hypothesized that prevalence of infection differs by site and has high genetic parasite diversity, with some parasites more host species specific than others. Blood from white ibis from Palm Beach, Lee, and Broward Counties in Florida were tested for haemoparasites by analyzing Giemsa-stained thin blood smears and PCR. In Palm Beach, Lee, and Broward Counties, 68%, 61%, and 27% were positive, respectively. Sequences of 139 positives revealed a novel haplotype of *Haemoproteus* (hWHIB01). Morphologically, parasites were identified as *H. plataleae.* Parasitemias of 66 positive birds were very low (average 0.085%, range <0.001%-0.890%). An infection with hWHIB01 was detected in a green heron (*Butorides virescens*). No *Plasmodium* infections were detected in white ibis, despite sympatric Pelicaniformes from Lee and Broward Counties with *Haemoproteus* and *Plasmodium* infections.

INDEX WORDS: Avian malaria, Hematozoa, White ibis, *Eudocimus albus,* Pelicaniformes, *Haemoproteus plataleae*

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Introduction

Avian malaria is caused by vector-borne, protozoan parasites in the order Hemosporidia (genera *Plasmodium, Haemoproteus,* and *Leucocytozoon*). Transmission of avian malarial parasites has been documented on every continent except Antarctica; worldwide ~68% of examined bird species are known hosts for at least one haemosporidian species (Friend and Fransen, 1999). Haemosporidia infections are often well tolerated by their natural bird hosts; however, natural hosts may develop clinical disease on rare occasions (Friend and Fransen 1999; Dawson and Bortolotti, 2000). In their natural hosts, avian malarial parasites generally establish long-term infections and the long-term consequences of these infections have been extensively studied. While most studies find little impact, a few studies have demonstrated reduced reproductive success, shortened lifespan, and increased stress and disease susceptibility (Snounou et al., 2000, Lachish et al., 2011; Arriero et al., 2008, Atkinson, 2008). Asghar et al., (2015) recently found that great reed warblers (*Acrocephalus arundinaceus*) infected with *Plasmodium ashfordi* lost telomere length, which ultimately resulted in decreased life spans. These data highlighted the potential for "non-pathogenic" parasites to exert a significantly population level impact on a host species.

Severe disease and mortality often occur in bird species that did not evolve with any species of avian malaria parasites (e.g., Hawaiian native avifauna) or birds that are introduced into areas where they are exposed to exotic parasites (e.g., penguins in zoos in temperate or

tropical locations). The most severe of these examples involved the introduction of avian malaria (*Plasmodium relictum*) to Hawaii in the early 1900s, which has caused an ecosystem disruption with numerous species of native avifauna having gone extinct and many others now threatened (van Riper et al., 1986; Warner, 1968). In addition to having severe population level effects on numerous species in Hawaii, altered distributions of some host avian species have been noted, presumably in response to avian malaria (Foster et al., 2007).

Although the White Ibis (*Eudocimus albus)* is currently listed as a species of least concern on the IUCN Red List of Threatened Species, Frederick et al. (1996) and Ogden (1994) have documented a decline in overall numbers in North America. While these declines correlate with losses in natural habitat (Caffey et al., 2003; Odgen, 1994), other factors could be involved. Interestingly, during the past few decades, increased numbers of white ibis have been observed in urban habitats. Becoming urbanized would appear to be an advantage given the loss of historically preferred habitat, but living in urban environments may come with additional risks, such as poor food quality, decreased nesting sites, and possibly exposing white ibis to unique pathogens that could have negative effects on population size and health.

Only two species of avian malarial parasites have been reported from white ibis. Nearly all reported infections have been based on blood smear analysis and infected ibis had infections with *Haemoproteus* (either identified as *Haemoproteus platalae* or simply *Haemoproteus* sp.) (Forrester, 1980). Only recently has a molecular-based survey been conducted and a single white ibis from Palm Beach County, Florida was PCR positive for a *Plasmodium* sp. (Bryan et al., 2015). Prevalence of *Haemoproteus plataleae* infections has been high (55% overall, n=98 and 90% in adults, n=39) which suggests that white ibis develop chronic infections and are a natural host for *H. plataleae* (Forrester, 1980) (Stevenson and Anderson 1994; Dorn et al. 2011).

Similarly, high prevalence rates (25-38%) for *H. plataleae* have also been noted in Australian White Ibis (*Threskiornis moluccus*) (Epstein et al., 2006; Lederer, 2000). In natural hosts that are long lived, such as ibis and some Anseriformes (e.g., mute swans (*Cygnus olor*) and northern pintails (*Ana acuta*)) high prevalence rates can occur due to exposure over numerous years, even when some seasons do not favor active transmission (Wood et al., 2013; Snounou et al., 2000; Ramey et al., 2013).

The majority of the North American white ibis population is found in Florida, which has experienced a high rate of habitat change with a loss of approximately 31% of the Everglades in the past century (Caffey et al., 2003). Since 1980, the human population of Florida has more than doubled (US Census Bureau, 1995; US Census Bureau, 2013). These changes can alter how wild animals interact with each other as well as their foraging habits, thereby changing the epidemiology of diseases in populations that live in these developing areas. The observed trend of declining population numbers is likely to continue as Florida wetlands, important breeding sites for white ibis, are continually degraded (Vuilleumier, 2011).

To date, few studies have been conducted on avian malarial parasites of white ibis and sympatric wading birds in Florida. Importantly, all but one of these studies were based only on blood-smear analysis, thus we aimed to conduct a large-scale molecular based study on the prevalence and diversity of avian malaria parasites in white ibis and sympatric wading birds. These data should aid in conservation efforts of white ibis and other species because it will provide data on the transmission of specific blood parasites in various sampled populations of birds.

White ibis

The white ibis is in the order Pelicaniformes and the family Threskiornithidae (although historically this species was in the order Ciconiiformes). They are easily distinguished from other wading birds by their long, decurved, reddish bills, and long reddish legs. Adults (>3 yrs old) have all-white plumage with black wing tips. The bodies of hatch year juveniles are brown and white, with an almost entirely brown head and neck, with second year immature birds losing the brown colors for white plumage (Figure 1.1). White Ibis are relatively large birds and have an average wingspan of 96 centimeters and can live up to 16 years in the wild (Vuilleumier, 2011). White ibis breed and are residents of the eastern coastline from as far north as Virginia and as far south as northeastern Columbia (Frederick et al., 1996) (Figure 1.2).

Figure 1.1. Plumage change of white ibis: A) 1st year juvenile. B) 2nd year juvenile and C) 3+ year mature adult.

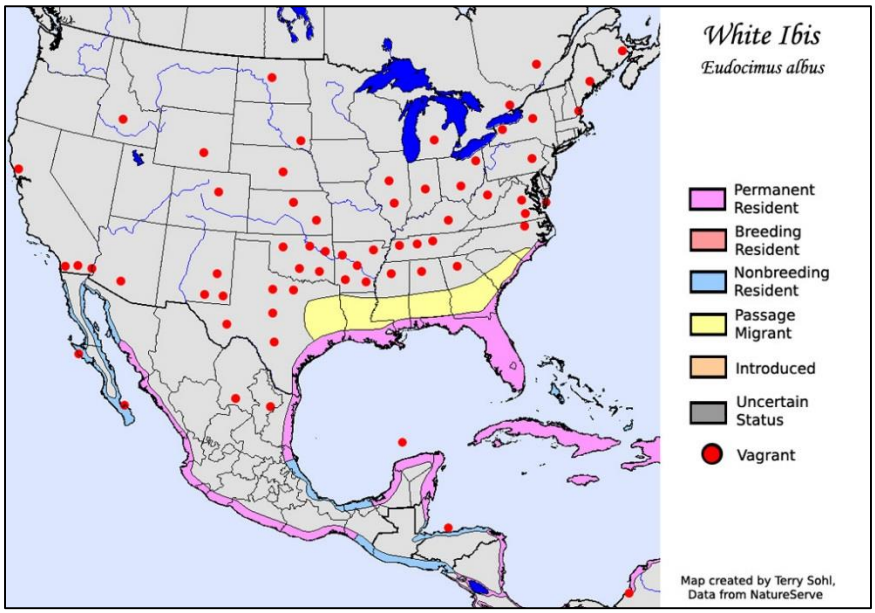

Figure 1.2. Range of *Eudocimus albus*. Created with data from NatureServe. Retrieved from: http://www.sdakotabirds.com/species/white_ibis_info.htm

Their breeding season relies on the suitability of the habitat and, thus, varies based on the location, precipitation, and hydrological cycles (Kushlan, 1976). In southern Florida, breeding usually starts in March and can extend into October, accounting for a 21-day incubation period and 6 weeks to fledge (Ogden, 1994; Heath et al., 2009; De Santo et al., 1990). White ibis are gregarious and often found in large flocks in wetlands and estuaries during foraging and roosting as well as in breeding season (Vuilleumier, 2011).

Because white ibis serve important ecological roles and can be used as indicators of wetland health and restoration (Frederick et al. 1996, Frederick et al. 2009), it is important to understand the disease dynamics within the populations and the possible implications of haemoparasite infections. Kushlan (1977b) showed that white plumage attracts other white ibis

as well as sympatric species, to a foraging site, thereby initiating mixed-species aggregation. Additionally, white ibis play a key role in commensal foraging with other wading birds (e.g. Little Blue Heron, Kushlan, 1978). Furthermore, white ibis population movement throughout the directly responds to drying/flooding of foraging habitat, thus allowing for a highly visible assessment of wetland ecosystems (Kushlan, 1979; Kushlan, 1985). Finally white ibis, though generalist feeders, prey heavily on invertebrates such as crayfish and other crustaceans (unlike other wading birds), and only consume larger prey such as fish when water levels are low (Kushlan and Kushlan, 1975; Ogden, 1994).

Haemosporida (avian malarial parasites)

The parasite life cycle begins with an infected vector biting a bird (Figure 1.3). Sporozoites are released in saliva during feeding and they invade various tissues of the bird where they develop into schizonts, which reproduce asexually. This reproduction results in merozoites, which penetrate circulating blood cells, where they develop into gametocytes. After approximately eight weeks, the parasite reaches patency and is available to a competent vector during a blood meal. In the midgut of the vector, the gametocytes mature and sexually reproduce to form an oocyst, which rupture and release sporozoites. These sporozoites then migrate out of the midgut and invade the salivary glands. Once in the salivary glands, the sporozoites can be transmitted to another host during the next vector blood meal (Valkiunas, 2005).

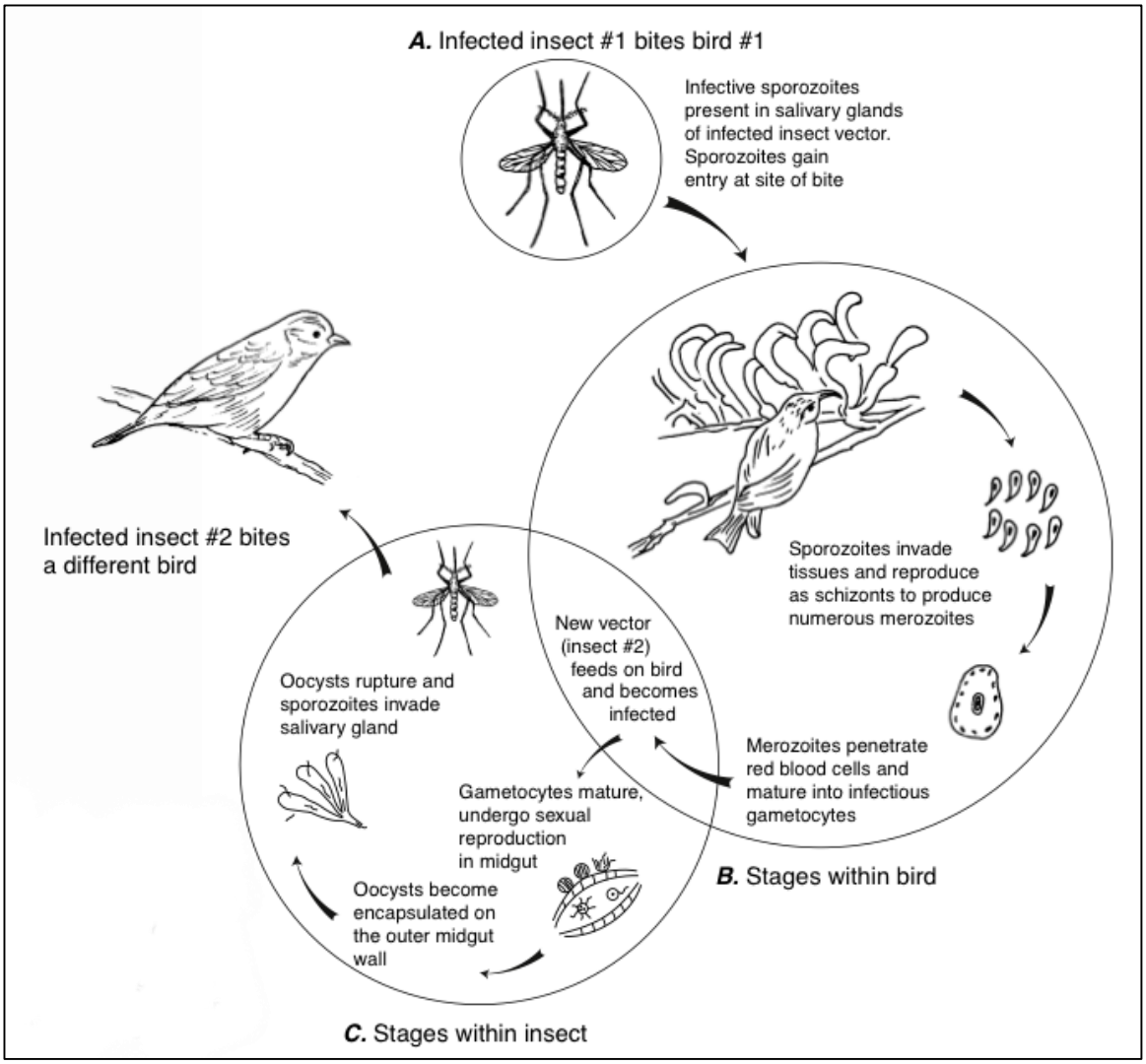

Figure 1.3. General hemosporidian parasite life cycle (Friend and Franson, 1999).

Blood parasites are traditionally detected using blood smear analysis, which not only provides the ability to calculate infection status, as well as morphological characteristics that can be used for species identification (Richard et al. 2002; Valkiunas, 2005). However, this method is generally less sensitive than polymerase chain reaction (PCR) because chronic infections are generally characterized as having low parasitemias, which are often undetectablef using blood smears (Jarvi, et al. 2003). Furthermore, morphologically similar parasites have been

consequently confirmed as distinct species based on sequencing (Martinsen et al., 2006; Hellgren et al., 2007).

Impacts of chronic haemoparasites on avian hosts

In natural hosts, most haemoprasite infections in birds are chronic. These chronic infections have historically been considered asymptomatic but an increasing number of studies have associated blood parasites with decreased survival and fecundity, poorer post-breeding body condition, and hindered mate selection (Dawson and Bortolotti, 2000; Sanz et al., 2001; Wiehn et al., 1997). For example, (Wright et al., 2005) experimentally showed *Plasmodium hermani* can cause depressed growth and death in wild turkey poults (*Meleagris gallopavo*). Furthermore, a decrease in reproductive success has been noted in some birds (e.g., blue tits (*Parus caeruleus*) (Merino et al., 2000) and house martins (*Delichon urbica*) (Marzal et al., 2013)). With a chronic infection, an individual remains infected and infectious allowing the parasite to be acquired by another vector and transmitted to other individuals, whereas, an acute fatal infection results in the removal of an infected individual from the population. Over a 25 year study of reed warblers, avian malaria parasite infections significantly decreased both lifespan and fecundity (Asghar et al., 2015).

Blood parasites from the Order Threskiornithidae

Only two species of *Haemoproteus* has been reported from the Threskiornithidae, *H. plataleae* and *H. pelouroi* (Figure 1.4). Recently, an uncharacterized Plasmodium sp. has been reported in a single white ibis from Florida (Bryan et al., 2015). To date, no *Leucocytozoon* spp. have been reported from the Threskiornithidae, but infections have been noted in Ardeidae.

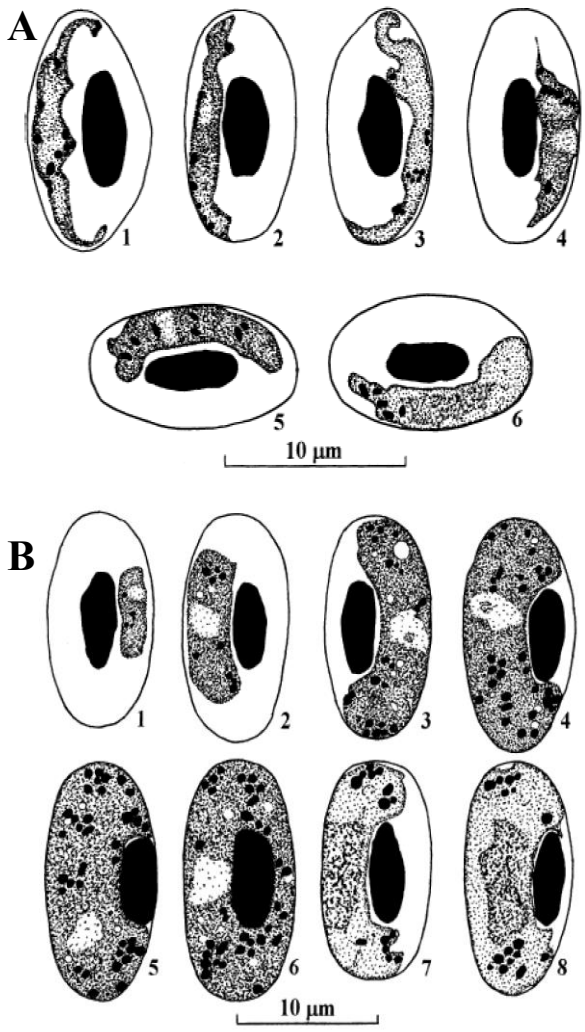

Figure 1.4. Gametocytes of A) *Haemoproteus pelouroi* from Hadada Ibis and B) *Haemoproteus plataleae* from Eurasian Spoonbill.

Haemoproteus pelouroi was first reported from the Hadada Ibis (*Bostrychia hagedash*) and the African Sacred Ibis (*Threskiornis aethiopicus*) in 1946 from West Africa. Numerous morphologic characteristics of the gametocytes, even immature ones, can be used to distinguish *H. pelouroi* from *H. plataleae,* which was first reported from India in a Eurasian Spoonbill (*Platalea leucorodia leucorodia*) (de Mello, 1935). Although this parasite has been reported from numerous hosts around the world, Bennet et al. (1975) formally re-described the species

from the white ibis. Other hosts include the Scarlet Ibis (*E. ruber*), Saddle-billed stork (*Ephippiorhynchus senegalensis*), Marabou Stork (*Leptoptilos crumeniferus*), Yellow-billed Stork (*Mycteria ibis*), Glossy Ibis (*Plegadis falcinellus*), Red-naped Ibis (*Pseudibis papillosa*), and Australian White Ibis (*Threskiornis molucca*) (Valkiunas, 2005). Thus, this *H. plataleae*, based on morphology, has been reported from five continents, which is one of the widest distributions of any known blood parasite (Figure 1.5). However, all of these reports are based only on morphologic identification of parasites of stained thin blood smears and some reports are lacking any data to indicate that proper morphometric measurements and other morphometric were examined. In recent years, many parasite species have been shown to be species complexes once sequences of various samples are obtained (Perkins, 2000; Ricklefs and Fallon, 2002). Thus, genetic characterization of *H. plataleae* from hosts in the Americas, Europe, Africa, Asia, and Australia is needed to determine if it is a species complex in this wide range of hosts.

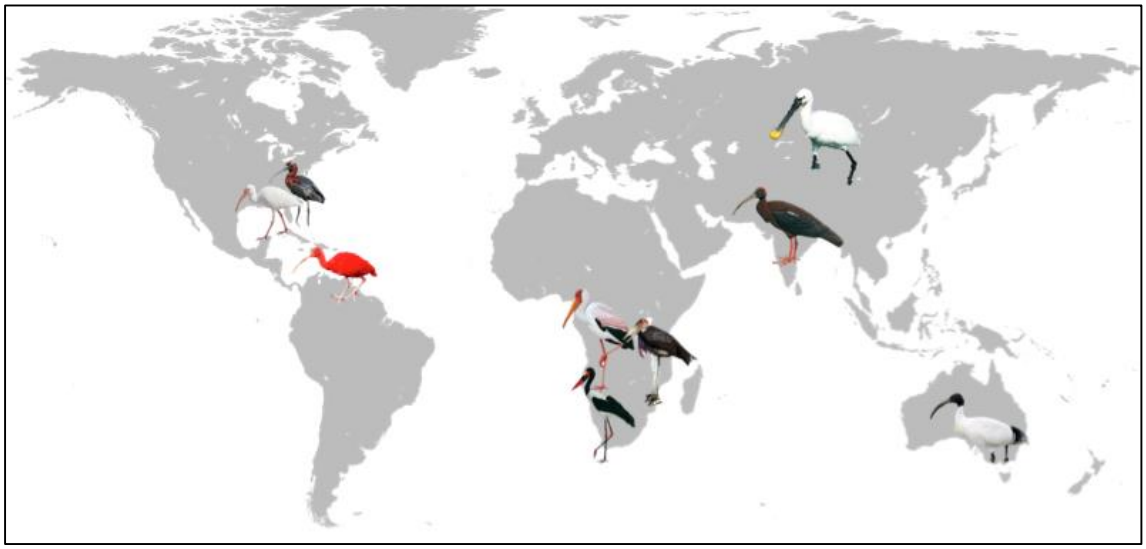

Figure 1.5. General distribution of *H. plataleae* in nine Threskiornithidae species as reported by Valkiunas (2005). Locations of bird species icons does not represent location of infected bird reports, but is just a graphic representation of the general distribution of the host bird species.

Haemosporida in white ibis

To date, few studies have been conducted on haemoparasites of white ibis. The most comprehensive of these studies (Forrester, 1980) found a 55% (n=98) prevalence of *Haemoproteus* infections in birds sampled at four sites in Florida from 1970-1975 but prevalence in adults (90%, n=39) was significantly higher than in nestlings (10%, n=40). The presence of *Haemoproteus* in nestlings was variable by site with all of the nestlings (n=20) sampled at one site being negative, but infections were noted at one other site. Additionally, Telford et al. (1992) documented *Haemoproteus* parasites in two adult white ibis from Florida (n=2), and no infected nestlings (n=45). At the time of these studies, the only available method for detection of blood parasites was blood smear analysis. Based on morphologic characteristics, all of the white ibis were infected with a single parasite species, *H. plataleae*.

Bryan et al. (2015) attempted to link mercury (Hg) levels in birds with infections (7% prevalence, n=15) of avian malaria. Sampling efforts spanned a wide range of wading birds, and only 11 nestling and 4 adult white ibis were sampled. Of these, all nestlings were negative and only a single adult was positive. Sequence analysis indicated that it was a *Plasmodium* sp. Because no accession number was provided and because blood smears were not examined, the species of *Plasmodium* is unknown. No support for an association between elevated Hg levels and haemoparasite infections was noted.

The lack of *Plasmodium* infection in white ibis by Forrester (1980) and Telford et al. 1992 is unexpected since many hosts and vectors of *Plasmodium* spp. are found in southern Florida (New Zealand BioSecure Entomology Laboratory, 2008; Savage and Miller, 1995). Importantly, three species of *Plasmodium*, *P. relictum*, *P. circumflexum,* and *P. elongatum*, in Florida have wide avian host ranges and *Plasmodium* spp. have been reported in related and sympatric waterbirds (e.g., wood storks (*Mycteria americana*) (Galindo and Sousa, 1966), cattle egrets (*Bubulcus ibis*) (Levin et al., 2013), great blue herons (*Ardea herodias herodias*), great white herons (*Ardea herodias occidentalis*) (Telford et al. 1992; Spalding and Forrester, 1991)). Use of more sensitive techniques, such as PCR, may be necessary to detect *Plasmodium* infections in white ibis. Alternatively, white ibis may be able to clear *Plasmodium* infections, which results in a short detection window.

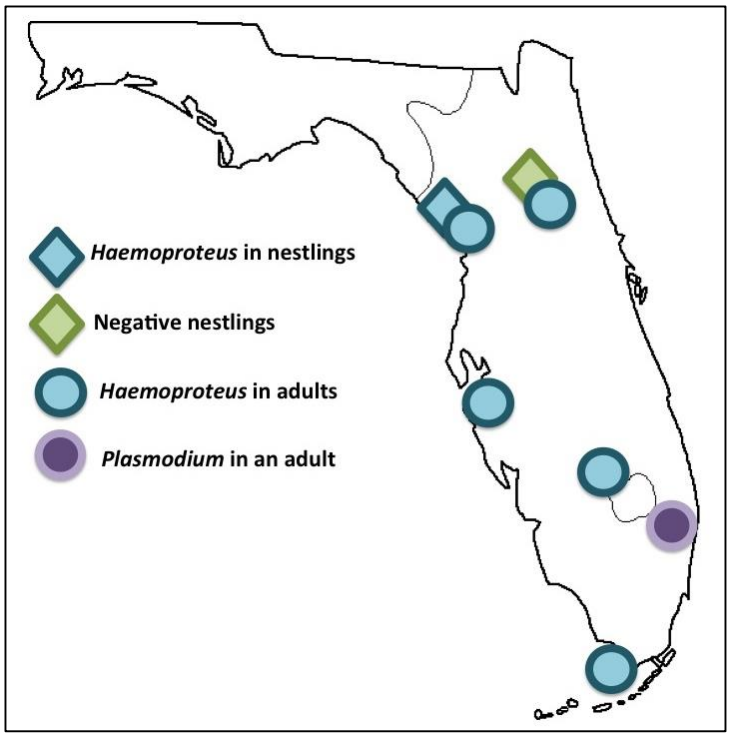

Figure 1.6. Distribution and results of white ibis sampling for *Haemoproteus* and *Plasmodium* parasites.

Human alterations of habitat

Unfortunately, despite conservation efforts, the Florida Everglades continues to decline. From the mid-1980s to mid-1990s, an estimated 0.2% (11million acres) of Florida wetlands were lost, indicating about a 0.02% annual reduction. This rate is in contrast to the estimated 46% reduction from ~1780 to ~1980, or a 0.23% annual reduction (Caffey et al., 2003). Ogden (1994) documented a 75-80% decline of nesting wading birds in the Everglades from 1934-1976; the estimations of white ibis populations dropped most severely. This sharp decline correlated with a decline in marshes and wetlands caused by flood control projects and a decline in prey availability (Ogden 1994). Kushlan (1977a) notes white ibis rarely rely on visual prey items, favoring instead probing through the sand in shallow waters. However, in urban settings, humans

interrupt this natural behavior by providing alternate, lower-quality food items that white ibis easily consume, such as white bread.

Thus, the rapid urbanization of Florida may change how white ibis are able to cope with pathogens, as is seen in other avifauna. For example, Boal and Manna (1999) found significantly higher trichomoniasis-related deaths in Cooper's hawk nestlings in urban areas compared to natural landscapes, which can be attributed to an increase in consumption of Columbiformes, which are not a large prey source outside of urban landscapes. Also, Zylberberg et al. (2013) found that finches in the Galapagos that foraged in agricultural areas had a significantly higher prevalence of avian pox virus than those that foraged in urban or undisturbed areas. They suggest that the agricultural foragers may be more stressed due to a combination of exposure to chemicals, contact with domestic animals and humans, and relying on seeds that lack nutrients. Similarly, urbanization potentially increases risks of wildlife to diseases by diminishing host immune responses through stress or lower nutrient quality or exposure to novel pathogens or animals (Delahay et al., 2009; Cornet et al., 2014). Relevant to our study, Evans et al. (2009) found that blackbirds (*Turdus merula*) sampled in urban sites with large bodies of water had a higher prevalence of avian malaria (*Plasmodium and Haemoproteus*) compared with those from rural sites.

Summary

Because all but one source of data for haemosporidia in white ibis are more than 25 years old, a revisit of this parasite-host system is warranted to determine if the increasing human densities/disturbance and changing Florida landscape (e.g., habitat type/quality, exotic flora and fauna and possible vectors) has affected haemosporidia in Florida populations of white ibis. The

objectives of this study were to determine the prevalence and parasitemia levels of blood parasites in white ibis from Southern Florida and determine the genetic diversity of these malarial parasites. A recent study (Bryan et al. 2015), which was based only on PCR analysis, detected the first report of *Plasmodium* in ibis; however, the sample size was extremely low (n=15) and only a single bird was infected. Thus, this study intends to examine the genetic diversity of haemoparasites from white ibis across a larger geographic scale. Because of the recent detection of a *Plasmodium* infection (Bryan et al., 2015), despite Forrester (1980) and Telford et al. (1992) only documenting *H. plataleae*, we hypothesize that a larger diversity of parasites occurs in this host species and that analysis of a larger sample size will reveal new parasite species.

CHAPTER 2

HAEMOPARASITES OF WADING BIRDS IN SOUTHERN FLORIDA

Introduction

In the United States, few studies of haemoparasites have been conducted on birds in the families Ardeidae and Threskiornithidae (currently in Order Pelecaniformes, but historically in the Order Ciconiiformes). In the United States, the white ibis 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 birds and this parasite has been found in at least five counties. Recently, an uncharacterized *Plasmodium* sp. was detected in a single bird by PCR (Forrester 1980; Bryan et al. 2015). In contrast, among sympatric North American species of egrets and herons (Family Ardeidae), 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 (=L. ardeae)*) have been reported (Beadell et al., 2006; Telford et al. 1992).

While the literature provides insight into the diversity of hemosporida in wading birds of North America, no study has used both sequencing and morphological data for analysis. Beadell et al. (2006) use molecular techniques to characterize haemosporida infections; however their main interest was *Plasmodium*, rather than surveying wading birds for parasite genetic diversity. Telford et al. (1992) and Forrester (1980) both used only blood smear analysis, and Bryan et al. (2015) used only PCR.

Compared with passerines (perching birds) and anseriforms, (ducks and geese), relatively few studies have surveyed members of the Ardeidae for blood parasites, but the high diversity of parasites that have been reported suggests that ibis and their relatives have an unknown diversity of blood parasites. Therefore, our goal was to genetically characterize, determine the prevalence, and estimate parasitemias of haemoparasites in opportunistically sampled Pelecaniformes of southern Florida. Overall, we expected high prevalence and diversity of blood parasites in the Threskiornithidae because of their phylogenetic relationship to the Ardeidae and the sharing of habitat.

Sampling Methods

From 2013-2014, blood samples were collected from wading birds admitted to rehabilitation centers in urbanized areas of Lee and Broward Counties (Figure 2.1). Birds exhibited a variety of conditions including lacerations, fractures, and dehydration. Blood samples were collected from the jugular or metatarsal vein into heparinized microtainer® tubes (Beckton Dickinson, Franklin Lakes, New Jersey) and two thin blood smears were prepared, dried, and fixed in methanol. Remaining blood was frozen at -20C until PCR testing. In some cases, birds died prior to blood collection; therefore, only clotted blood was available for testing.

In 2014, wading bird nestlings were hand captured from nesting areas from a natural area in Broward County. Blood samples were collected from the brachial or metatarsal vein into heparinized microtainer® tubes (Becton Dickinson, Franklin Lanes, NJ) and stored on ice while in the field. Two thin blood smears were made within three hours, dried and fixed in methanol. Remaining blood was frozen until testing.

All capture and sampling techniques were reviewed and approved by the University of Georgia's IACUC (#A2013- 10-016).

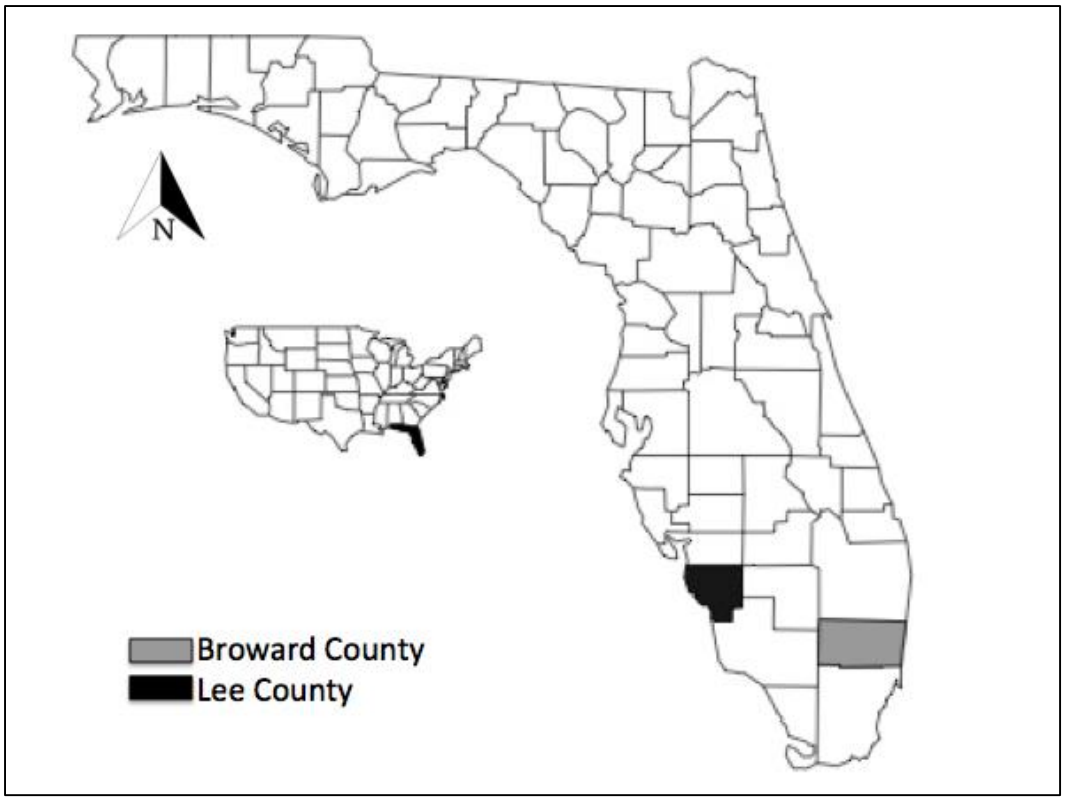

Figure 2.1. Sampling locations Lee and Broward Counties in Southern Florida.

Genetic Characterization

DNA was extracted from whole blood samples (10µl) 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 fragment of the mitochondrial cytochrome *b* (*cyt b*) gene of *Haemoproteus* and *Plasmodium* and a 478 bp fragment of *Leucocytozoon*, as described by (Waldenström et al., 2004; Hellgren 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 compared with related sequences in the GenBank and the MalAvi databases to determine related haplotypes. Sequences obtained from this study and related sequences were aligned using the multisequence alignment tool in MEGA (Molecular Evolutionary Genetics Analysis) version 3.1 program (Kumar et al. 1993). Phylogenetic analysis using the neighbor-joining algorithm using the Kimura 2-parameter model and maximum parsimony using a heuristic search was conducted in MEGA. The GenBank accession number for gene sequences reported in this study are xxxxxx (to be added when available).

DNA from a blood sample of a duck infected with *H. nettionis* and *L. simondi* was used as 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 each set of extractions and after each 10-12 extractions. Additional negative water controls were included in each set of primary and secondary PCR reaction sets.

Analyses

Only PCR positive samples were examined for parasites since the sensitivity of PCR analysis is similar or higher than analysis of blood smears, (Valkiunas et al. 2008, Waldenström et al. 2004). Thin blood smears were stained with Geimsa stain (Dipquick, Jorgensen Laboratories, Inc., Loveland, CO). To estimate parasitemias, approximately 20,000 erythrocytes were examined for parasites as suggested by Godfrey et al. (1987) when extremely low parasite loads is expected. If no parasites were observed during this initial scan, the smear was examined

for another five minutes (Valkiunas et al. 2008). The parasites were morphologically identified using a published key (Valkiunas 2005).

Chi-square tests were performed in R Studio (v0.98.1103). The variables of interest were prevalence, county, and year. Age was not included due to missing data from many individuals.

Results

Samples were collected from six species of wading birds including 20 white ibis, three glossy ibis (*Plegadis falcinellus*), one roseate spoonbill (*Platalea ajaja*), three green herons (*Butorides virescens*), two tricolor herons (*Egretta tricolor*), and two great blue herons (*Ardea herodias*) (Table 2.1). In Lee County, three species (white ibis, glossy ibis, and green herons) were positive on the *Plasmodium/Haemoproteus* PCR assay (Table 2.1). At the Broward County rehabilitation center, only white ibis 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 2.1). The remaining chicks were negative; the white ibis 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. The prevalence of haemoparasites was significantly higher in 2014 compared with 2013 (GLM, df = 1, $P<0.05$), but no difference was noted between the two counties (GLM, df=1, $P<0.1$). All birds sampled in the study were negative for *Leucocytozoon* based on PCR testing and *Plasmodium* based on sequence analysis.

Overall, based on sequence analysis, two species of *Plasmodium* and one *Haemoproteus* were detected (Table 2.2). One green heron (485 bp) and the Roseate spoonbill (462 bp) were infected with *Plasmodium elongatum* (haplotype GRW06/MD-2011) (Table 2). The glossy ibis (478 bp) was infected with a *Plasmodium* sp. that was 100% identical to haplotype

MYCAMP2/CMV-2012 H2 that was previously only reported in wood storks (*Mycteria americana*) from Brazil (Villar et al. 2013). All of the positive white ibis and one green heron were infected with a novel *Haemoproteus* haplotype (designated hWHIB01) that was 99% similar to a *Haemoproteus* sp. from West Africa (CELEC01/haplotype WAH8).

Blood smears were only available for nine of the 20 PCR-positive birds. In general, the parasitemias were low and ranged from 0 to 3.98% (average of 0.766%). The *Haemoproteus* haplotype found in the white ibis was morphologically identified as *H. plataleae* (Table 2.2).

Discussion

The Order Pelecaniformes has recently been reorganized and now contains five extant families, with the Ardeidae and Threskiornithidase as the most species rich. Despite the worldwide distribution of birds in these two families, relatively few studies have been conducted on the prevalence and diversity of blood parasites. In addition, most studies have been based solely on analysis of blood smears. In the current study, we used molecular methods to characterize identify new host-parasite relationships for *Plasmodium* and *Haemproteus* in several species of herons, ibis, and spoonbills.

This is the first report of blood parasites from the glossy ibis or roseate spoonbill. The single infected glossy ibis was infected with a *Plasmodium* sp. that has only previously been detected in wood storks from Brazil (Villar et al. 2003). This haplotype (MYCAMP2) has now been reported in two Orders (Ciconiiformes and Pelecaniformes) from North and South America. Interestingly, this glossy ibis had the highest parasitemia (3.982%) we detected in this study. The bird was originally submitted to the rehabilitation center with a broken leg, which required euthanasia, but it is unknown in the *Plasmodium* infection contributed to its injury. The roseate

spoonbill was infected with *P. elongatum,* which has a wide host range including the Orders Apterygiformes, Ciconiiformes, Columbiformes, Coraciiformes, Galbuliformes, Gruiformes, Passeriformes, and Strigiformes (Valkiunas, 2005). Within the Ciconiiformes, *P. elongatum* has been reported from Great Blue Herons from the USA and cattle egrets in Spain (Beadell et al., 2006; Ferraguti et al., 2013). Disease associated with *P. elongatum* has been reported in a number of bird species, but especially in penguins (Vanstreels et al., 2015; Valkiunas et al., 2008; Dinhopl et al., 2015; Fleischman et al., 1968; Herman et al., 1968).

Statistically, birds sampled in 2013 had a significantly lower prevalence than 2014, indicating possible shifts in annual infection rates. While there was no significant difference between counties, a p-value of 0.1 may suggest biological significance and may increase given a larger sampling size. Spatial variation in hemosporidia infections in birds is expected with the complex relationships among hosts, vectors, and environmental conditions, thus further testing is encouraged (Loiseau et al., 2010; Ishtiaq et al., 2007; Bensch and Akesson, 2003; Wood et al., 2007).

All three genera of hematozoa (*Plasmodium, Haemoproteus,* and *Leucocytozoon)* have been reported from birds in the Order Ardeidae. We did not detect blood parasites in the great blue herons sampled, despite *P. elongatum* and *P. relictum* being reported in great blue herons (Telford et al. 1992; Beadell et al., 2006). However, this is not surprising considering the low prevalence (7%) reported (Telford et al., 1992). Similarly, the two sampled tricolor herons were negative; however, our sample sizes were small. 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 species identification and it was a *P. elongatum* infection in

the Anthony 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 passerines (Galindo and Sousa, 1966; Mehus and Vaughan, 2013).

Overall, a relatively high prevalence of *Haemoproteus* was detected in white ibis from southern Florida, similar to data from previous studies (Forrester, 1980; Telford et al. 1994) suggesting that *H. plataleae* may cause chronic infections in ibis or reinfections are common. We failed to detect infections in chicks at the Broward nesting site; however, sample sizes were low and two of them may have been too young to have patent parasitemias for *Haemoproteus*, although the infection dynamics of *H. plataleae* are unknown (Dusek et al., 2004). We only detected one *Haemoproteus* haplotype in white ibis, 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 ibis, although Bryan et al. (2015) recently detected a *Plasmodium* sp. in 1 of 4 sampled adult white ibis. Similarly, *Leucocytozoon* was not found, despite this genus being reported from numerous sympatric Ardeidae (Coatney, 1938; Wood and Herman, 1943; Galindo and Sousa, 1966). However, we only sampled white ibis from two sites, so additional studies are needed on ibis 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, great egret, cattle egret, and snowy egret (Valkiunas, 2005; Coatney, 1938). In general, the prevalence is low so our failure to detect infections is likely due to low sample size. However, we encourage future work on the genetic

characterization of *Leucocytozoon* from the Pelecaniformes because *L. leboeufi* has a cosmopolitan distribution among numerous bird species from North America, Africa, Europe, Asia, and Australia and because recent molecular work on *L. simondi* from ducks indicates that one or more cryptic species exists, it is possible that herons, egrets, and their relatives are infected with more than one *Leucocytozoon* species (Reeves et al., 2015).

In summary, we morphologically and genetically characterized the blood parasites of several species of wading birds in southern Florida. Novel host-parasite relationships were noted and we provide the first morphologic data for *Plasmodium* sp. lineage MYCAMP2. We did detect a different in prevalence between the years sampled, but this difference is likely due to sampling bias. Our failure to detect a higher diversity could be due to our limited sample sizes for each species and restriction of sampling to two sites. Spatial variation in hemosporidia infections in birds is expected with the complex relationships among hosts, vectors, and environmental conditions (Loiseau et al., 2010; Ishtiaq et al., 2007; Bensch and Akesson, 2003; Wood et al., 2007). This group of birds is found worldwide, yet 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 can lead to a decline in diet quality and thus the ability to cope with disease (Delahay et al., 2009; Cornet et al., 2014; Caffey et al., 2003).

Table 2.1. Prevalence of haemoparasites in wading birds from Lee and Broward Counties Florida.

County	*Site*	*Species*	*No PCR Pos/No. Tested (%)*
Lee	Urban rehabilitation center	White ibis	11/17 (65)
		Glossy ibis	1/2 (50)
		Green heron	2/3 (67)
		Tricolor heron	0/2
		Great blue heron	0/2
Broward	Urban rehabilitation center	White ibis	5/14 (36)
	Rural breeding site	White ibis	0/4
		Glossy ibis	0/1
		Roseate spoonbill	1/1 (100)
Total			20/46 (43)

Table 2.2. Parasitemia, morphological identification, and haplotype of *Plasmodium* and *Haemoproteus* spp. from wading birds from southern Florida.

Host ID	Species	Haplotype	Parasitemia (%)	Morphological identification
13-3084	Green heron	hWHIB01 (*H. plataleae*)	N/A	N/A
13-2774	Green heron	GRW06 (*P. elongatum*)	0.018	*P. elongatum*
14-1312	Glossy ibis	MYCAMP2	3.982	*Plasmodium* sp.
SB1	Roseate spoonbill	GRW06 (*P. elongatum*)	Negative	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

CHAPTER 3

A NOVEL HAPLOTYPE OF *HAEMOPROTEUS* IS WIDEPSREAD IN WHITE IBIS (*EUDOCIMUS ALBUS*) FROM URBAN AND RURAL SITES IN SOUTHERN FLORIDA

Introduction

The American white ibis (*Eudocimus albus)* (Pelicaniformes: Threskiornithidae) is a year-round resident of Florida. This medium-sized wading bird historically breeds and forages in wetlands in southern Florida, but is now commonly also observed foraging in urbanized areas (Stevenson and Anderson 1994; Dorn et al. 2011). In southern Florida, breeding usually begins in March and can extend into October (Ogden 1994; Heath et al. 2009; De Santo et al. 1990). White ibis play important ecological roles and can be used as indicators of wetland health and restoration (Frederick et al. 1996, Frederick et al. 2009). Kushlan (1977b) showed that white plumage attracts other birds, including other white ibis as well as sympatric species (e.g. little blue heron, Kushlan, 1978), to a foraging site, thereby initiating mixed-species aggregations.

Throughout the Everglades, a 75-80% decline of nesting wading birds, including white ibis, has been documented (Ogden 1994). This marked decline corresponded to habitat loss, particularly of marshes and wetlands, as a result of flood control projects and a decline in prey availability (Ogden 1994). Rapid urbanization of Florida may alter the exposure of ibis and other wading birds to pathogens and their ability to respond to infections, as is seen in other avifauna (Boal and Manna 1999; Zylberberg et al. 2013; Delahay et al. 2009; Cornet et al. 2014; Evans et al. 2009). In addition, white ibis in urban settings are becoming habituated to artificial food

handouts from humans (e.g., bread), which can lead to chronic issues related to poor food quality.

In natural hosts, haemoparasite infections in birds are often chronic and although they are generally considered nonpathogenic, decreased survival and fecundity, poorer post-breeding body condition, and hindered mate selection have been documented (Dawson and Bortolotti, 2000; Sanz et al., 2001; Wiehn et al., 1997). Importantly, research on reed warblers (*Acrocephalus arundinaceus*) over a 25-year period showed that birds with haemoparasite infections had significantly decreased lifespans and their lifetime reproductive success (Asghar et al., 2015).

Few studies have been conducted on haemoparasites of white ibis. Forrester (1980) found a 55% (n=98) prevalence of *Haemoproteus* infections while Telford et al. (1992) documented *Haemoproteus* parasites in two adult white ibis, but not in nestlings (n=45). Both studies morphologically identified the parasites as *H. plataleae*; however, a recent molecular study detected a *Plasmodium* sp. infection in 1 of 4 white ibis from southern Florida and failed to detect *Haemoproteus* infections (Bryan et al., 2015). Therefore, the primary goals of the current study were to determine the prevalence, parasitemia level, and genetic diversity of blood parasites in white ibis in southern Florida using PCR, sequencing, and blood smear analysis. A high diversity of haemosporidia have been reported in conspecific wading birds in the Order Pelecaniformes (Family Ardeidae); therefore, we hypothesize that unrecognized diversity of blood parasites are present in white ibis. In addition, because white ibis utilize a wide range of habitats, we hypothesize that parasite exposure will vary by habitat type.

Methods

Animal Capture and Sampling

White ibis were caught using nylon leg loop traps or mist nets at 11 sites in West Palm Beach County, Florida, USA (Figure 3.1) from 2010-2014 in urban and suburban sites (Table 3.1). At several sites, birds were habituated to the presence of humans and foraged at ponds in residential parks and neighborhoods so these birds were captured with loop traps made of clear fishing line (Mehl et al. 2003). At one site, 12m mist nests were positioned in a "V" near the edge of water bodies and white, plastic flamingo decoys were used to lure in flocks (Heath and Frederick, 2003).

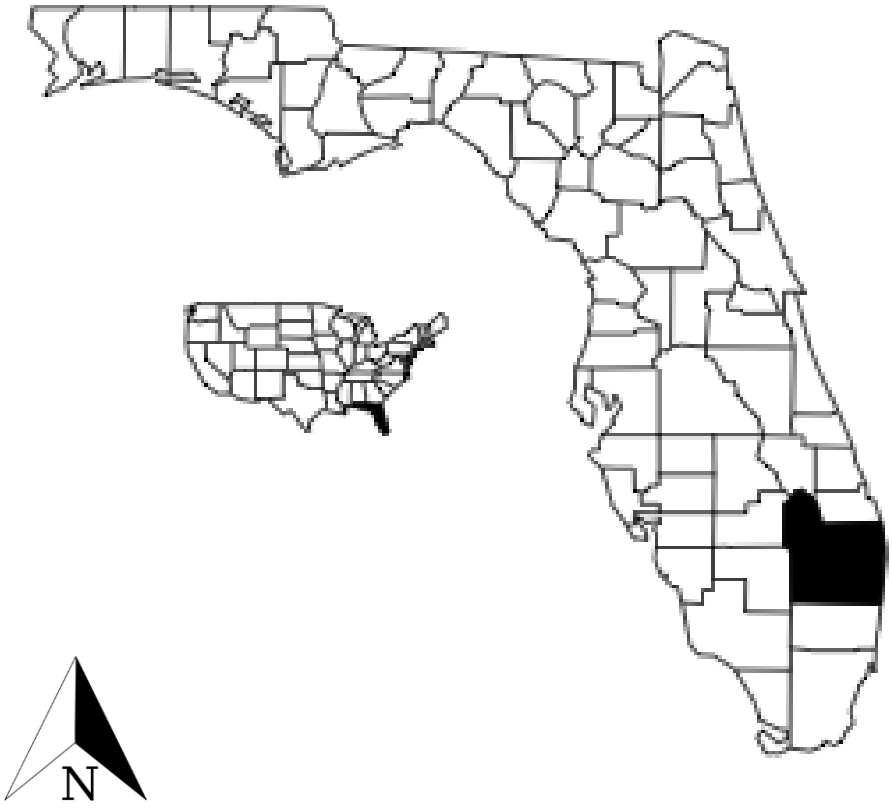

Figure 3.1. Map highlighting location of Palm Beach County, Florida and the state of Florida in USA (inset).

Table 3.1. White ibis capture sites in Palm Beach County, Florida categorized by habituation score. Numbers sampled include both juvenile and adult birds.

Site	Years Sampled	Habituation Level	Number Sampled	Type
DP	2012, 2013	4	16	Urban park
GL	2010	3	3	Urban/Commercial
ICP	2012, 2013	4	25	Urban park
JB	2010, 2012, 2013	4	37	Urban park
LW	2013	1	14	Urban park
LCS	2010, 2011, 2012, 2013	4	26	Rural zoo
PP	2013	4	9	Residential
PV	2012, 2013	4	21	Residential
RP	2010	4	2	Urban park
SM	2010	4	2	Urban/Commercial
SWA	2014	2	126	Rural/Commercial
WPZ	2010	4	2	Urban zoo

After capture, the birds were placed in pillowcases prior to sampling and no bird was handled for longer than 1 hour. Age (adult or juvenile), sex, standard morphometric measurements (weight, wing chord, tarsus length and width, and culmen length), body condition (scale of 1-5) and ectoparasite loads were recorded. Blood samples were collected from the jugular or metatarsal vein using a 25g needle. Two thin blood smears were prepared, dried on site, and fixed in methanol within 24 hours. Remaining blood was stored in heparinized tubes (Microtainer, Becton Dickinson, Frankin Lakes, NJ) and kept on ice in the field prior to being frozen at -20C. All capture and sampling techniques were reviewed and approved by the University of Georgia's IACUC (#A2013- 10-016).

Genetic Characterization

A Qiagen DNeasy blood extraction kit was used as per the manufacturer's instructions (Qiagen, Valencia, California) to extract DNA from nucleated whole blood samples (10µl). A 480 base pair fragment of the mitochondrial cytochrome *b* (*cyt b*) gene of *Haemoproteus* and *Plasmodium* and a 478 bp fragment of *Leucocytozoon* was targeted with a nested PCR. The primers HaemNFI (5'-CATATATTAAGAGAAITATG-GAG-3') and HaemNR3 (5'-ATAGAAAGATAAGAAATACCATTC-3') were used in the primary PCR to amplify a 617bp fragment (including primers) of all three genera (Hellgren et al., 2004) and two separate secondary PCR reactions were run using primers HaemF (5'- ATGGTG-CTTTCGATATATGCATG-3') and HaemR2 (5'- GCATTATCTGGATGTGATAATGGT-3') to amplify *Haemoproteus* and *Plasmodium* and primers HaemFL (5'- ATGGTGTTTT-AGATACTTACATT-3') and HaemR2L (5'-CATTATCTGGATGAGATAATGGIGC-3') to amplify *Leucocytozoon* (Waldenström et al., 2004; Hellgren et al., 2004). All PCR reactions were run for 20 cycles (primary reaction) or 35 cycles (for both secondary reactions) of 94°C for 30s, 50°C for 30 s, and 72°C for 45s in 25µl volumes with 1.25mM dNTPs (Promega, Madison, Wisconsin), 3.125mM $MgCl_2$, 1.25u GoTaq® Flexi DNA polymerase (Promega), 1.25µM of each primer combined with 5µl of DNA for the primary reaction or 1µl of the primary product for the secondary reactions.

Products were visualized in ethidium bromide stained 2% agarose gels. Amplicons were excised from the gel, purified using the Qiagen QIAquick gel extraction kit (Qiagen), and sequenced at the Georgia Genomics Facility in Athens, GA using the Sanger method. Sequencher (v5.0) was used to align and analyze the sequences. Related sequences in the GenBank and the MalAvi databases were obtained for comparison. Sequences obtained from

this study and related sequences were aligned using the multisequence alignment tool in MEGA (Molecular Evolutionary Genetics Analysis) version 3.1 program (Kumar et al. 1993). Phylogenetic analysis using the neighbor-joining algorithm using the Kimura 2-parameter model and maximum parsimony using a heuristic search was conducted in MEGA. The GenBank accession number for gene sequences reported in this study is xxxxxx.

DNA from a blood sample of a duck infected with *H. nettionis* and *L. simondi* was used as 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 before 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.

Blood Smear Analysis

In general, PCR is considered as sensitive as or more sensitive than blood smear analysis; therefore, parasitemias were determined only for PCR positive samples (Valkiunas et al. 2008, Waldenström et al. 2004). Thin blood smears were stained with Geimsa stain (Dipquick, Jorgensen Laboratories, Inc., Loveland, CO). Approximately 20,000 erythrocytes were examined as suggested by Godfrey et al. (1987) when anticipating extremely low parasitemias. If no parasites were observed during this initial scan, the smear was examined for another five minutes. If parasites were found during this subsequent slide, a parasitemia of <0.001% was recorded for statistical analysis (Valkiunas et al. 2008). The parasites were morphologically identified using a dichotomous key (Valkiunas 2005).

Data Analysis

A general linearized model with a binomial distribution and logit link function was used to model infection probability as a function of site, season, age, and habituation level with backward elimination from a maximal model. The best fit model was chosen based on P values. Neither year nor body condition were used as predictor variables due to differences in sampling effort or methods, respectively.

Results

Samples were collected from 263 white ibis from 12 sites in West Palm Beach County, Florida. Based on PCR testing, 179 (68%) were positive for *Plasmodium* and/or *Haemoproteus*. Infections were noted at 11 of 12 sampled sites (negative site only having two samples); prevalence was high at all positive sites (range 50-100%). Of the 179 positives, high quality sequences were obtained for 139 samples and all were identical. The sequences were identified as a novel *Haemoproteus* haplotype (hWHIB01) that was 99% similar to a *Haemoproteus* sp. from West Africa (CELEC01/haplotype WAH8). All birds were negative for *Leucocytozoon.* Blood smears from 66 PCR positive birds were examined and all parasites were identified as *H. plataleae*. Overall, parasitemias were low and ranged from 0 to 0.890% (avg 0.049%).

The only factor that was associated with prevalence was season. Birds sampled in the breeding season (n=201) were more likely to be infected with *Haemoproteus* compared with birds sampled in the nonbreeding season (n=60) ($df = 2$, $P<0.001$) (Figure 3.2).

Table 3.2. Prevalence of *Plasmodium/Haemoproteus* by site in white ibis from southern Florida, USA.

Site	No PCR Pos/No. Tested (%)
DP	12/17 (71)
GL	3/3 (100)
ICP	14/25 (56)
JB	23/37 (62)
LW	7/14 (50)
LCS	20/26 (77)
PP	6/9 (67)
PV	14/20 (70)
RP	0/2 (0)
SM	2/2 (100)
SWA	75/104 (72)
WPZ	2/2 (100)

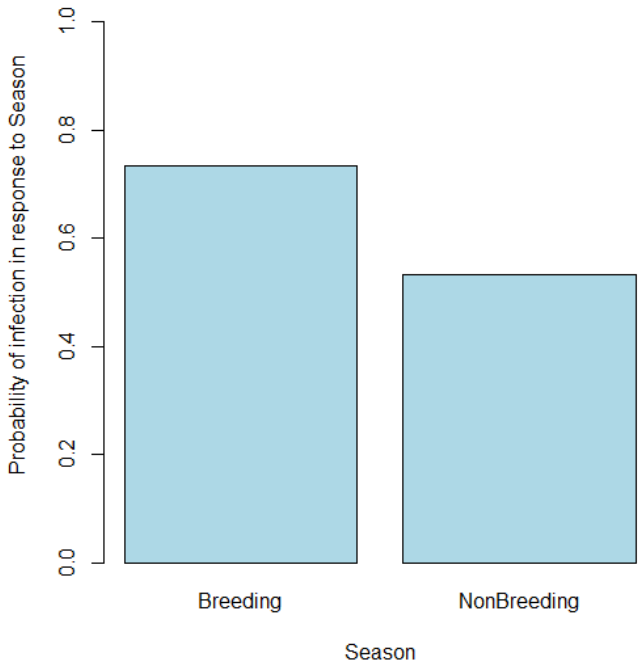

Figure 3.2. The probability of infection by hematozoa in white ibis in response to season.

Discussion

To date, this is the largest study to genetically characterize and morphologically identify haemoparasites in white ibis. Similar to previous studies, we detected a high prevalence of *H. plataleae,* which was confirmed genetically to be a single lineage. Because of the high prevalence and the low parasitemia levels we detected, white ibis likely develop chronic infections with *H. plataleae* (Forrester, 1980; Telford et al. 1992; Stevenson and Anderson, 1994; Dorn et al., 2011). No difference in prevalence among habituation levels, age, or site was noted, but higher prevalence rates were noted during the breeding season.

In natural hosts, the prevalence of *Haemoproteus* tends to be high due to the development of chronic infections (Ventim et al., 2011; Wiehn et al., 1997; Atkinson and Van Riper, 1991). The low parasitemia (avg 0.049%) we detected supports that infection with *H. plataleae* is a chronic infections or, less likely, that reinfections are common (Jarvi et al., 2003; Forrester, 1980; Valkiunas, 2005). Low level, chronic infections have historically been considered of little clinical consequence, but an increasing number of studies have associated chronic infection with blood parasites with decreased survival and fecundity, poorer post-breeding body condition, hindered mate selection, and decreased life span due to telomere shortening (Dawson and Bortolotti, 2000; Sanz et al., 2001; Wiehn et al., 1997; Asghar et al., 2015). Therefore, it is important to understand factors associated with infection of white ibis with blood parasites, even if they have not been associated with acute clinical disease, although the latter has not been evaluated in chicks.

Although several studies have shown that the prevalence of *H. plataleae* was high in white ibis, our work confirmed genetically that only a single haplotype is found in high prevalence (68%), which agrees with work by Forrester (1980) and Telford et al. (1992) who only detected *H. plataleae* based on morphology. Interestingly, this high prevalence is in contrast to the Ardeidae, the closest relatives of the Threskiornithidae; however, sampling of birds in these groups are very limited Pelecaniformes (Telford et al., 1992). Morphologically, *H. plataleae,* has been reported from numerous Threskiornithidae species including Eurasian Spoonbill (*Platalea leucorodia leucorodia*), Scarlet Ibis (*E. ruber*), Saddle-billed stork (*Ephippiorhynchus senegalensis*), Marabou Stork (*Leptoptilos crumeniferus*), Yellow-billed Stork (*Mycteria ibis*), Glossy Ibis (*Plegadis falcinellus*), Red-naped Ibis (*Pseudibis papillosa*), and Australian White Ibis (*Threskiornis molucca*) (de Mello, 1935; Valkiunas, 2005). Thus, *H.*

plataleae, based on morphology, has been reported from five continents, which is one of the widest distributions of any known blood parasite. However, all of these reports are based on only morphologic identification of parasites on stained thin blood smears and some reports are lacking any data to indicate that proper measurements and other morphometric were examined. In recent years, many parasite species have been shown to be species complexes once sequences of various samples are obtained (Perkins, 2000; Ricklefs and Fallon, 2002). Thus, genetic characterization of *H. plataleae* from hosts in the Americas, Europe, Africa, Asia, and Australia is needed to determine if it is a species complex in this wide range of hosts.

Among the factors tested, only season was significant associated with prevalence with white ibis sampled in the breeding season being more likely to be infected. The reason for this increased prevalence is unknown, but could be due hormone fluctuations; however, increased testosterone levels have not previously been linked to increased susceptibility to haemosporida in birds or reptiles (Oppliger et al., 2004; Buttemer and Astheimer, 2000). Similarly, studies have not associated sex hormones with an increase in prevalence or parasitemia levels of blood parasites (Garin and Schoech, 2006; Applegate and Beaudoin, 1970). While in increase in vector abundance during the breeding season may cause an increase in prevalence, it could also be attributed to a decreased ability to clear infections due to the stress associated with reproduction (Tschirren et al. 2003; Garvin and Schoech, 2006; Allander, 1997). Furthermore, many species experience increases in blood parasite parasitemias during spring relapses which could result in increased detection (Valkiunas, 2005).

Genetic sequencing of blood parasites from ibis from numerous sites in Palm Beach County, Florida in the current study and two other counties from southern Florida in a previous study (Coker et al., Chapter 2) indicated that the diversity of parasites was low. To date, only a

single *Haemoproteus* haplotype (hWHIB01) has been detected in 154 positive ibis from these three counties and a single infection with a *Plasmodium* sp. in an ibis from Palm Beach County by Bryan et al. (2015). The low prevalence of *Plasmodium* infections in sampled white ibis could be due to short-term detectable parasitemia for this *Plasmodium* sp. in ibis, rare transmission of this *Plasmodium* sp. to ibis in southern Florida, or infection of this ibis could have occurred at a site not sampled. Rare transmission seems unlikely as we have documented the occurrence of *Plasmodium* spp. in sympatric wading birds and these parasite species are known to have low host specificity (Coker et al., Chapter 2, Bensch et al., 2000). Certainly, transmission of this *Plasmodium* species at the site of capture seems unlikely as we sampled 126 ibis from the same site where the *Plasmodium*-infected ibis was sampled, of which, 53 were infected with *Haemoproteus* based on sequence analysis.

Importantly, the long-term consequences of urbanization of ibis populations have only begun to be evaluated (Hernandez, unpublished). Currently, the effects of urbanization on haemoparasites in white ibis remain unclear and, although we know that infections of ibis with *H. plataleae* are common, human development, habitat changes or altered interactions with other wildlife could increase susceptibility to clinical disease due to *H. plataleae* infections. Going forward, studies on white ibis that rely solely on natural landscapes versus urban landscapes are needed to determine the long-term impacts of urbanization on the health of white ibis and other wading bird populations and one factor to include would be blood parasites due to their possible impacts on hosts with chronic infections.

CHAPTER 4

SUMMARY

The objective of this thesis was to document the blood parasites from birds in the order Pelecaniformes of southern Florida. Previous studies on this group of birds have been limited and were based solely on genetic or morphological techniques. This study was conducted to obtain contemporary information on blood parasites of selected species using both traditional morphology and molecular based phylogenetic methods. In the current study, we identified new host-parasite relationships for *Plasmodium* and *Haemoproteus* in several species of herons, glossy and white ibis, and spoonbills. The majority of samples were from white ibis, yet this species had very low parasite diversity; only a single new haplotype corresponding to *H. plataleae* was detected. Prevalence of *H. plataleae* in white ibis was high and present in all three sampled counties. Currently, the effects of urbanization on haemoparasites in white ibis and other wading birds remain unclear and, although we know that infections of white ibis with *H. plataleae* are common, human development, habitat changes, or altered interactions with other wildlife could increase susceptibility to clinical disease due to *H. plataleae* infections. Going forward, studies on white ibis that rely mainly on natural landscapes versus urban landscapes are needed to determine the long-term impacts of urbanization on the health of white ibis and other wading bird populations; one factor to include would be blood parasites due to their possible impacts on hosts with chronic infections.

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APPENDIX A

Individual characteristics of samples included in Chapter 2.

Host ID	Species	County	Site	Year	+/-
AN4	White Ibis	Broward	Rural	2014	-
AN6	White Ibis	Broward	Rural	2014	-
AN330	White Ibis	Broward	Rural	2014	-
SB396	White Ibis	Broward	Rural	2014	-
SB1	Roseate Spoonbill	Broward	Rural	2014	+
SB380	Glossy Ibis	Broward	Rural	2014	-
13073104	White Ibis	Broward	Urban	2013	-
13080831	White Ibis	Broward	Urban	2013	-
13080125	White Ibis	Broward	Urban	2013	-
13091704	White Ibis	Broward	Urban	2013	-
13083118	White Ibis	Broward	Urban	2013	-
13100912	White Ibis	Broward	Urban	2013	-
13100822	White Ibis	Broward	Urban	2013	-
13111710	White Ibis	Broward	Urban	2013	+
13101412	White Ibis	Broward	Urban	2013	+
13102809	White Ibis	Broward	Urban	2013	+
13110901	White Ibis	Broward	Urban	2013	-
13102914	White Ibis	Broward	Urban	2013	+
13101719	White Ibis	Broward	Urban	2013	-
13122917	White Ibis	Broward	Urban	2013	+
13-2051	White Ibis	Lee	Urban	2013	-
13-2738	White Ibis	Lee	Urban	2013	+
13-2386	White Ibis	Lee	Urban	2013	-
13-2341	White Ibis	Lee	Urban	2013	-
13-2841	White Ibis	Lee	Urban	2013	-
14-1831	White Ibis	Lee	Urban	2014	+
14-1756	White Ibis	Lee	Urban	2014	+
14-2207	White Ibis	Lee	Urban	2014	+
14-2548	White Ibis	Lee	Urban	2014	+
14-2711	White Ibis	Lee	Urban	2014	+
14-2523	White Ibis	Lee	Urban	2014	+
13-3389	White Ibis	Lee	Urban	2014	-
14-2916	White Ibis	Lee	Urban	2014	+
14-1168	White Ibis	Lee	Urban	2014	+
14-2813	White Ibis	Lee	Urban	2014	+
13-2646	Tricolor Heron	Lee	Urban	2013	-
13-2729	Tricolor Heron	Lee	Urban	2013	-

Host ID	Species	County	Site	Year	+/-
13-2690	Green Heron	Lee	Urban	2013	-
13-1994	Great Blue Heron	Lee	Urban	2013	-
13-3325	Great Blue Heron	Lee	Urban	2013	-
13-3084	Green Heron	Lee	Urban	2013	+
13-2774	Green Heron	Lee	Urban	2013	+
14-1312	Glossy Ibis	Lee	Urban	2014	+
14-1535	Glossy Ibis	Lee	Urban	2014	-
14-0088	White Ibis	Lee	Urban	2014	+
13-3038	White Ibis	Lee	Urban	2013	-