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Prevalence and genetic diversity of haematozoa in South American waterfowl and evidence for intercontinental redistribution of parasites by migratory birds

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ABSTRACT

To understand the role of migratory birds in the movement and transmission of haematozoa within and between continental regions, we examined 804 blood samples collected from eleven endemic species of South American waterfowl in Peru and Argentina for infection by *Haemoproteus, Plasmodium*, and/or *Leucocytozoon* blood parasites. Infections were detected in 25 individuals of six species for an overall apparent prevalence rate of 3.1%. Analysis of haematozoa mitochondrial DNA revealed twelve distinct parasite haplotypes infecting South American waterfowl, four of which were identical to lineages previously observed infecting ducks and swans sampled in North America. Analysis of parasite mitochondrial DNA sequences revealed close phylogenetic relationships between lineages originating from waterfowl samples regardless of continental affiliation. In contrast, more distant phylogenetic relationships were observed between parasite lineages from waterfowl and passerines sampled in South America for *Haemoproteus* and *Leucocytozoon*, suggesting some level of host specificity for parasites of these genera. The detection of identical parasite lineages in endemic, South American waterfowl and North American ducks and swans, paired with the close phylogenetic relationships of haematozoa infecting waterfowl on both continents, provides evidence for parasite redistribution between these regions by migratory birds.

1. Introduction

Protozoan blood parasite infections have been studied in avian species for more than a century (Valkiūnas, 2005), and representative species of parasites from the genera *Haemoproteus, Plasmodium*, and *Leucocytozoon* have been detected on every continent except Antarctica (Valkiūnas, 2005; Beadell et al., 2006). Studies have shown that these haematozoa infections can have adverse fitness effects on certain avian species (Anderson et al., 1962; Van Riper et al., 1986; Valkiūnas, 2005), with host populations that are restricted to islands, or host species that have not previously been exposed to haematozoa infection being particularly vulnerable to pathogenic effects of these parasites.

Waterfowl (family Anatidae) have multiple traits that make them important host species for avian haematozoa parasites. Being gregarious in nature, they present ample opportunities for haematozoa transmission in the presence of suitable dipteran vectors (Matta et al., 2014). Furthermore, many waterfowl species migrate long distances which may provide parasites the possibility of being introduced into novel regions (Levin et al., 2013). Previous studies have identified haematozoa infections in waterfowl species around the globe, with reported prevalence rates varying upon sampling location and screening methodology (e.g. Greiner et al., 1975; Bennett et al., 1981; Cumming et al., 2012; Ramey et al., 2012). To date, there have been at least twelve morphologically described species from the genera *Plasmodium, Haemoproteus*, and *Leucocytozoon* identified in waterfowl hosts (Valkiūnas, 2005) and evidence suggests that some of these species may be specific to Anatidae (Fallis et al., 1954). Given that haematozoa infections can persist in hosts throughout long distance migrations (Bennett et al., 1991; Valkiūnas, 2005), and some waterfowl species migrate between North America and South America (Botero and Rusch, 1988), it is possible that blood parasite infections could be redistributed between these continents.

In South America, blood parasites belonging to the genera *Haemoproteus, Plasmodium*, and *Leucocytozoon* have been detected in a broad range of avian families throughout the continent (e.g. White et al., 1978; Bennett et al., 1991; Valkiūnas et al., 2003; Durrant et al., 2006); however, very little work has been conducted on waterfowl species. White et al. (1978) conducted a review of studies examining haematozoa infection in Neotropical birds and out of all waterfowl sampled (n = 449) only 2.2% of samples collected were

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Fig. 1. Map of sampling locations in Peru and Argentina. The number of waterfowl blood samples collected at each site is provided in parentheses.

positive for haematozoa infection as assessed via microscopy. Only *Haemoproteus* and *Plasmodium* parasites were detected, and infections were limited to three host species: Brazilian Teal (*Anas brasiliensis*), Black-bellied Whistling Duck (*Dendrocygna autumnalis*), and White-faced Whistling Duck (*Dendrocygna viduata*; White et al., 1978). More recently, molecular methods to detect haematozoa have been applied to samples collected from Black-bellied Whistling Ducks in Colombia, which resulted in the identification of a novel parasite species (Matta et al., 2014). This latter finding highlights the general lack of information currently available regarding the molecular detection of haematozoa in South American waterfowl and the genetic diversity of parasites inhabiting this region.

In this study, our objectives were to: (1) obtain information about the prevalence and geographic distribution of *Leucocytozoon, Haemoproteus*, and *Plasmodium* parasites in endemic South American waterfowl from Peru and Argentina; (2) assess the genetic diversity of haematozoa parasites using PCR-based molecular techniques; and (3) compare the genetic relationships among haematozoa haplotypes in South American waterfowl to those previously identified in other investigations. Results from this study will allow for the assessment of parasite exchange among species and continents, which may be useful information for understanding past and potential future shifts in parasite distribution and host range.

2. Materials and methods

2.1. Sample collection

Whole blood samples were collected from eleven species of endemic South American waterfowl (n = 804) at sites in Peru and Argentina (Fig. 1) during dry seasons of 2010–2012. Blood samples were collected either from the brachial vein of birds live-captured in mist nets or via cardiac punctures from specimens immediately after collection. Samples were immediately frozen in liquid nitrogen and subsequently stored at –80 °C until analysis. All capture methods and sampling procedures for this study were reviewed and approved by the University of Alaska Fairbanks Institutional Animal Care and Use Committee (permit #152985).

2.2. Detecting haematozoa infection

DNA was extracted from all blood samples using the DNeasy Blood and Tissue Extraction Kit (Qiagen, Valencia, CA) following the manufacturer's protocol. In order to confirm the viability of each DNA extraction, a 695 base pair (bp) fragment of the mitochondrial DNA (mtDNA) cytochrome oxidase I (COI) gene was amplified using Bird F1 and BirdR1 primers and PCR protocols from Kerr et al. (2007) for all samples except those from Ruddy Ducks (*Oxyura jamaicensis*). These primers were unsuccessful in amplifying this fragment of the COI gene in this species, possibly due to the deep divergence from the other species sampled (Gonzalez et al., 2009). Therefore, all Ruddy Duck samples were verified by amplifying a 529 bp fragment of the COI gene by primers specifically designed for this study (RUDUCOI F2: GTC AAC CAG GAA CTC TTC TAG GG and RUDUCOI R2: GAG ACC CAA TCC TGT ATC AAC AC) and the same protocol used by Kerr et al. (2007). Amplified PCR products for the COI reaction were visualized on 0.8% agarose gels stained with Gel Red Nucleic Acid Gel Stain (Biotium, Hayward, CA).

Each extracted DNA sample that was shown to be viable via our COI positive control was screened for the presence of *Leucocytozoon,*