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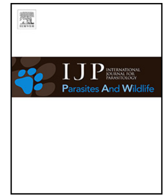

Prevalence and genetic diversity of haematozoa in South American waterfowl and evidence for intercontinental redistribution of parasites by migratory birds

Matthew M. Smith [a,b,*], Andrew M. Ramey [b]

[a] Institute of Arctic Biology, Biology and Wildlife Department, University of Alaska Fairbanks, 211 Irving I, Fairbanks, AK 99775, USA
[b] US Geological Survey, Alaska Science Center, 4210 University Drive, Anchorage, AK 99508, USA

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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.

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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

* Corresponding author. USGS Alaska Science Center, 4210 University Drive, Anchorage, Alaska 99508, USA. Tel.: +1 907 598 1200; fax: +1 907 786 7021.
E-mail address: msmit352@alaska.edu (M.M. Smith).

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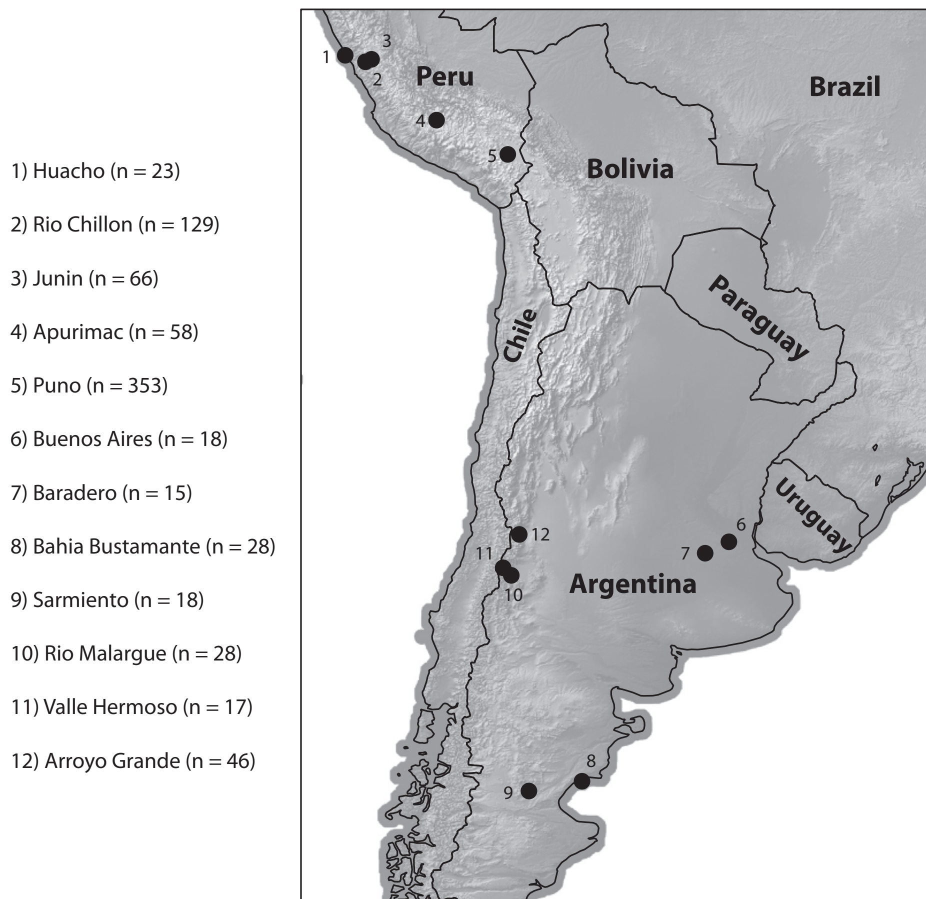

Fig. 1. Map of sampling locations in Peru and Argentina. The number of waterfowl blood samples collected at each site is provided in parentheses.

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*,

Haemoproteus, and *Plasmodium* parasites using a nested PCR protocol described by Hellgren et al. (2004). One negative control was incorporated into each 24-well PCR reaction to ensure the absence of contamination, and each sample was screened twice to account for imperfect detection of parasite DNA in host tissue (Ramey et al., 2012). Amplified PCR products were then visualized on 0.8% agarose gels as described previously.

A target fragment of 479 bp of parasite mtDNA cytochrome *b* gene was bi-directionally sequenced for all samples that were identified as positive for parasite infection to identify parasites by genera and prevent misidentification due to co-amplification (Cosgrove et al., 2006). All samples were treated with ExoSap-it (USB Inc., Cleveland, OH) according to the manufacturer's protocol and were not otherwise purified prior to sequencing. Sequencing was conducted using identical primers used for PCR, and BigDye Terminator version 3.1 mix (Applied Biosystems, Foster City, CA) and subsequently analyzed on an Applied Biosystems 3730xl automated DNA sequencer (Applied Biosystems, Foster City, CA). Raw sequence data were cleaned and edited using Sequencher 5.0.1 software (Gene Codes Corp., Ann Arbor, MI). Parasitic infections were then assigned to one of three genera (*Leucocytozoon, Haemoproteus*, or *Plasmodium*) using the nucleotide BLAST function available on the National Center for Biotechnology Information (NCBI) website. Assignment of infections was based on the top BLAST score for each sequence that produced a maximum identity score greater than 90% (Ramey et al., 2013). Samples for which double stranded sequences were not produced from sequencing protocols or could not be assigned via BLAST were considered negative.

2.3. Phylogenetic assignment and assessment of haplotype diversity

Diversity of haematozoa mtDNA haplotypes was assessed to determine the relative frequency of parasite haplotypes infecting South American waterfowl, and to establish whether or not there are any shared haplotypes between North America and South America. A median-joining minimum spanning network of parasite cytochrome *b* haplotypes was created using Network 4.6.1 (Bandelt et al., 1999). Additionally, haematozoa haplotypes infecting South American waterfowl were compared to lineages available on the MalAvi and GenBank public databases (Bensch et al., 2009) as of 27 January 2014 to determine whether haplotypes closely matched lineages previously identified infecting other avian hosts.

Phylogenetic analyses were performed on haematozoa mtDNA sequence data to verify taxonomic assignment of infections, and to assess evidence for the redistribution of parasites among species and between continents. Phylogenies were constructed by comparing haematozoa mtDNA cytochrome *b* sequence data from South American waterfowl to reference sequences. Reference samples were obtained from NCBI for haematozoa previously reported from passerines in South America, as well as North American waterfowl. *Haemoproteus* and *Plasmodium* mtDNA cytochrome *b* haplotypes identified by Durrant et al. (2006) infecting passerines in Uruguay and Guyana, and *Leucocytozoon* sequences detected in passerines in Peru and Chile obtained from the MalAvi database (Bensch et al., 2009) were included as reference sequences from South America. *Haemoproteus, Plasmodium*, and *Leucocytozoon* lineages from ducks and swans sampled in California and Alaska were included as representative sequences from North American waterfowl (Ramey et al., 2012, 2013). Parasite mtDNA haplotype sequences from South American waterfowl samples identified in this study were included in phylogenetic analyses only if they contained less than three ambiguous bases. All sequences were aligned and cropped to a final length of 358 bp, and haplotypes shorter than this were omitted from phylogenetic analyses. Phylogenies were built using MrBayes 3.2.1 (Ronquist et al., 2012) with a general time reversible model (GTR) and a gamma distribution among site variation. Four heated chains were used in this analysis, and we discarded the first 25,000 sampled generations as burn-in. The analysis was replicated multiple times to ensure similar results and each was run for a minimum of 2,000,000 generations or until the split frequencies were less than 0.01, with samples being collected every 1000 generations. Trees were rooted with mammalian *Plasmodium* cytochrome *b* haplotypes (Genbank accession numbers **AF069610**, **AF069624**, **AF055587**, **AY099051**, and **AY283019**) based on methods by Perkins and Schall (2002). Haematozoa mtDNA cytochrome *b* haplotypes generated from this study were submitted to GenBank with accession numbers **KJ527070–KJ527081**.

Table 1
Number of blood samples from South American waterfowl detected as positive for *Leucocytozoon, Haemoproteus*, and *Plasmodium* parasites by country and species.

Sample origin (country and species)	Samples tested	*Leucocytozoon* positive	*Haemoproteus* positive	*Plasmodium* positive
Peru				
Anas bahamensis	3	0	0	0
Anas cyanoptera	77	3	2	1
Anas flavirostris	65	1	0	0
Anas georgica	49	0	1	0
Anas puna	92	0	0	0
Chloephaga melanoptera	30	0	0	0
Lophonetta specularioides	40	0	0	0
Merganetta armata	177	1	0	12
Oxyura jamaicensis	96	3	2	0
Total	**629**	**8**	**5**	**13**
Argentina				
Anas bahamensis	2	0	0	0
Anas cyanoptera	6	0	0	0
Anas flavirostris	15	0	0	0
Anas georgica	26	0	0	0
Anas versicolor	4	0	0	0
Dendrocygna bicolor	1	0	0	1
Lohponetta specularioides	50	0	0	0
Merganetta armata	66	0	0	0
Total	**170**	**0**	**0**	**1**

3. Results

3.1. Haematozoa detection and prevalence

Of the 804 waterfowl blood samples screened for haematozoa infection, five samples failed to amplify the region of the COI gene used as a positive control. These samples were dropped from further analyses since we were unable to verify the presence of DNA in each extraction. Of the remaining 799 samples, 25 were positive for haematozoa infection (3.1% prevalence). Two samples were co-infected with parasites of multiple genera for a total of 27 haematozoa infections (Table 1). Eight, five, and fourteen samples were identified as positive for *Leucocytozoon, Haemoproteus*, and *Plasmodium* parasites, respectively (Table 1). Of the 25 parasite-positive blood samples, 24 came from hosts collected at field sites in Peru, with only a single positive sample originating from Argentina (Table 1). Infections by *haematozoan* parasites were confined to only six of eleven waterfowl species screened, with the majority of infections identified in Torrent Ducks (*Merganetta armata*; Table 1).

Table 2
Haematozoa mitochondrial DNA cytochrome *b* haplotypes detected in South American waterfowl species and results of comparison to previously identified parasite lineages as listed on the MalAvi and GenBank databases.

Haplotype	Host species	MalAvi/GenBank lineage	Identity score MalAvi/GenBank
Haem 1	*A. cyanoptera*, *O. jamaicensis*	Cygnus01/TUSW07	100%/100%
Haem 2	*O. jamaicensis*	Cygnus01/TUSW07	99%/99%
Haem 3	*A. georgica*	Cygnus01/TUSW07	99%/99%
Leuc 1	*A. cyanoptera*, *A. flavirostris*, *O. jamaicensis*, *M. armata*	TUSW04/TUSW04	100%/100%
Leuc 2	*O. jamaicensis*	HELLI02/NOPI04	97%/100%
Leuc 3	*O. jamaicensis*	HELLI02/NOPI04	96%/98%
Leuc 4	*A. cyanoptera*	TUSW05/TUSW05	100%/100%
Plas 1	*A. cyanoptera*	BT7	99%/99%
Plas 2	*M. armata*	MILANS05	100%/100%
Plas 3	*M. armata*	TFUS05	100%/100%
Plas 4	*D. bicolor*	PESA01	100%/100%
Plas 5	*M. armata*	PADOM11	100%/100%

3.2. Haplotype diversity

Analysis of haematozoa mtDNA cytochrome *b* sequences revealed a total of twelve unique haplotypes among South American waterfowl samples (Table 2; Fig. 2). Haplotypes were identified as *Plasmodium* (n = 5), *Leucocytozoon* (n = 4), and *Haemoproteus* (n = 3). Only three of the twelve haplotypes detected in our samples occurred in multiple individuals (Haem 1, Plas 3, and Leuc 1; Fig. 2). *Haemoproteus* haplotype Haem 1 was identified from two birds of separate species, while *Plasmodium* haplotype Plas 3 was found in six different individuals, all of which were Torrent Ducks. *Leucocytozoon* haplotype Leuc 1 was observed in five different individuals of four species (Table 2).

Eight haplotypes detected in our samples from South American waterfowl were identical to parasite lineages reported on the MalAvi and GenBank databases. *Leucocytozoon* haplotypes Leuc 1 and Leuc 4 were identical to lineages TUSW04 and TUSW05 respectively, which were detected in Alaska Tundra Swans and California Northern Pintails (Ramey et al., 2012, 2013; Table 2). South American haplotype Leuc 2 was identical to lineage NOPI04 detected in Northern Pintails (*Anas acuta*) in California (Ramey et al., 2013). *Haemoproteus* haplotype Haem 1 was identical to CYGNUS01, which has been previously identified in Tundra Swans in Minnesota and Alaska, Northern Pintails in California, as well as a Mallard (*Anas platyrhynchos*) from Minnesota (Ricklefs and Fallon, 2002; Ramey et al., 2012, 2013). Four *Plasmodium* mtDNA cytochrome *b* haplotypes from our samples, Plas 2, Plas 3, Plas 4, and Plas 5, were identical to MILANS05, TFUS05, PESA01, and PADOM11 lineages, respectively. MILANS05 was documented infecting Black Kites (*Milvus migrans*) in Europe (Pérez-Rodríguez et al., 2013), as well as Ruffs (*Philomachus pugnax*) in Africa (Mendes et al., 2013). Lineage TFUS05 was detected infecting Great Thrushes (*Turdus fuscater*) in South America (Lotta et al., 2013). PESA01 was observed in three separate host species: a Pectoral Sandpiper (*Calidris melanotos*) on the Arctic coast of Alaska (Yohannes et al., 2008), a White-tipped Dove (*Leptotila verreauxi*) in Uruguay, and a Mouse-colored Tyrannulet (*Phaeomyias murina*) in Brazil (Durrant et al., 2006; Lacorte et al., 2013). Lastly, the lineage PADOM11 is a common lineage of *Plasmodium*, documented infecting over 20 host species in North America, South America, and Asia (e.g. Durrant et al., 2006; Martinsen et al., 2006).

3.3. Phylogenetic analysis

Phylogenetic analysis supported structuring of avian parasite mtDNA haplotypes by genera. For *Haemoproteus* and *Leucocytozoon* parasites, phylogenetic analysis provided strong support for clades

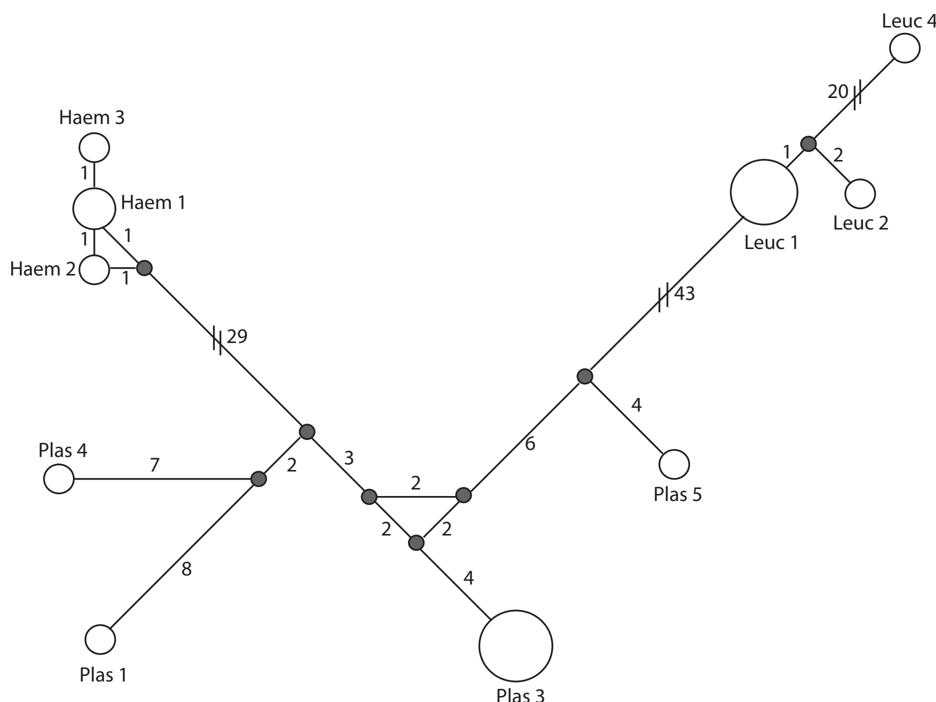

Fig. 2. Minimum spanning network for haematozoa mitochondrial DNA cytochrome *b* haplotypes detected in South American waterfowl. Shaded circles represent unsampled nodes. All circles are drawn proportional to the frequency at which haplotypes were observed. Lines separating nodes are drawn to scale based on the number of nucleotide mutations, unless otherwise indicated by hash marks. Only haplotypes with a length of 358 bp or greater were included. Haplotype name abbreviations are as follows: Haem = *Haemoproteus*, Leuc = *Leucocytozoon*, and Plas = *Plasmodium*.

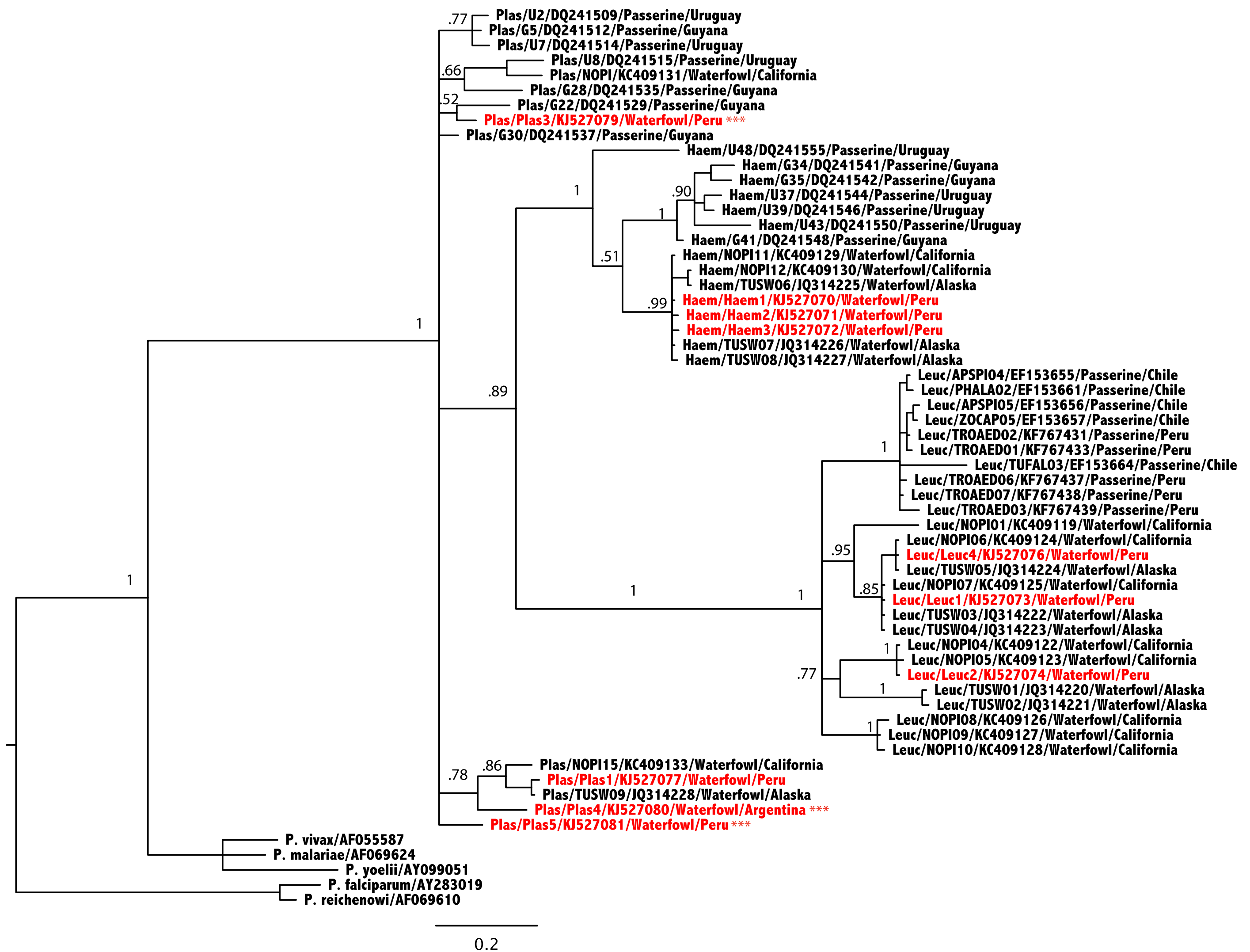

Fig. 3. Bayesian phylogenetic tree of haematozoa mitochondrial DNA cytochrome *b* haplotypes obtained from infected waterfowl. Trees were rooted with mammalian *Plasmodium* outgroups. Node tips are labeled with parasite genus (Haem = *Haemoproteus*, Leuc = *Leucocytozoon*, and Plas = *Plasmodium*), followed by the lineage name, GenBank accession number for each sequence, host order (passerine/waterfowl), and the country/state from which the samples were collected. All haplotypes identified in this study are highlighted in red. Numbers on branches represent posterior probabilities from the analysis. Asterisks after node tip labels indicate sequences from our study that were identical to lineages previously found in non-waterfowl hosts. All reference sequences were obtained from the National Center for Biotechnology Information website.

comprised of lineages originating from waterfowl sampled in both North and South America, and other clades comprised entirely of parasite mtDNA sequences originating from passerines sampled in South America (Fig. 3). Sub-structuring of *Haemoproteus* and *Leucocytozoon* clades by continent of origin was not well supported for lineages detected in waterfowl. Avian *Plasmodium* lineages clustered into two groups of closely related sequences. Both groups of closely related *Plasmodium* lineages contained sequences originating from both North America and South America.

4. Discussion

In our survey of blood parasites in endemic South American waterfowl, we detected a relatively low level of haematozoa infections among the eleven species sampled (3.1%). The genetic diversity of the parasites we detected included a total of twelve haplotypes infecting six different species of waterfowl. Nucleotide identity comparisons with haematozoa lineages on public databases and phylogenetic analysis of parasite haplotypes provided support for exchange of *Haemoproteus* and *Leucocytozoon* parasites among waterfowl species, and a wider taxonomic range of parasite exchange for *Plasmodium* lineages. Furthermore, we were able to identify identical parasite haplotypes infecting both South American and North American waterfowl, a pattern that suggests intercontinental exchange of haematozoa by migratory birds.

A review of microscopic investigations of haematozoa in Neotropical birds by White et al. (1978) reported an overall prevalence of haematozoa in avian species to be approximately 10.5%. Subsequent studies conducted in Colombia (Valkiūnas et al., 2003; Moreno et al., 2008), Guyana, and Uruguay (Durrant et al., 2006) reported apparent prevalence rates of haematozoa infections in birds of 7.8–42.1%. The low level of haematozoa infection we detected in waterfowl sampled from two regions of South America during the dry season (3.1%) was comparable to apparent prevalence rates for *Haemoproteus, Plasmodium*, and *Leucocytozoon* parasites in waterfowl sampled throughout the Neotropics (2.2%) as reported by White et al. (1978). In contrast, a recently published study by Matta et al. (2014), which included the description of a new species, *Haemoproteus macrovacuolatus*, in Black-bellied Whistling Ducks in Colombia reported a 41% apparent prevalence rate for this

parasite species. Difference in methodology, taxa sampled, year, and season complicate rigorous inference through direct comparison of apparent prevalence across these studies. For example, molecular methods used in our study may be more sensitive at detecting low-level infections than microscopic methods used in previous studies (Alexander et al., 2002), and all of our samples were collected during the dry season when the density of vectors is likely lower, which could be partially responsible for lower prevalence. Additionally, haematozoa prevalence has been shown to vary by sample year and season in Anatid waterfowl (Bennett et al., 1974; Ramey et al., 2013).

All positive samples collected from Peru were confined to two sampling locations. Twelve positive blood samples were collected from Lake Titicaca near Puno, and another twelve positive samples were collected along the Rio Chillón River, north of Lima (Fig. 1). Sampling in Puno accounted for the majority of our Peruvian samples (n = 353) and was conducted at an elevation of approximately 3824 meters. Sampling along the Rio Chillón River (n = 129) was done over an elevation gradient ranging from 1092 to just over 4000 m. Of the 147 samples that were collected at other sites, 124 were collected at locations greater than 1000 m. In previous studies in the Peruvian Andes, the majority of *Haemoproteus, Plasmodium*, and *Leucocytozoon* infections detected in passerines were from samples collected between 1000 and 4000 m above sea level (Jones et al., 2013; Galen and Witt, 2014). Therefore, most of our samples were collected at elevations at which we would expect haematozoa prevalence to be highest.

Blood samples collected from Argentina produced only one sample detected as positive for parasite infection (Table 1). The habitats in Argentina from which our samples were collected tended to be arid and very windy, which may explain the low apparent parasite prevalence. The blood parasites investigated in this study are all transmitted by haematophagous dipteran vectors that have aquatic and flighted life stages (Borror and Delong, 1954), both of which may be impeded by adverse climatic conditions at sampling locations (Martínez-Abraín et al., 2004). Previous studies on avian haematozoa conducted in Argentina have produced results showing either very low prevalence rates or a complete lack of blood parasite infection in wild birds (Masello et al., 2006; D'Amico et al., 2008).

Of all the zoogeographical regions in which avian haemosporidians have been documented infecting wild bird populations, the Neotropics have the second lowest species diversity of avian haematozoa next to the Australian region based on historical studies using microscopic screening (Valkiūnas, 2005). Of the three genera of parasites examined in this study, only *Plasmodium* has historically been observed to have similar levels of species diversity in the Neotropics compared to other zoogeographical regions. More recent molecular evidence suggests, however, that *Plasmodium* and *Haemoproteus* parasites may be highly diverse within the Neotropics (Lacorte et al., 2013). In concordance with previous studies, we found a greater number of *Plasmodium* haplotypes in South American waterfowl than for either *Haemoproteus* or *Leucocytozoon*. Therefore, migratory waterfowl may be important for maintaining geographically widespread distributions of these parasite lineages.

The prevalence of infections by *Leucocytozoon* parasites has historically been reported to be low in wild birds sampled throughout South America (e.g. Bennett and Borrero, 1976; Bennett et al., 1991; Rodríguez and Matta, 2001; Matta et al., 2004). This may be attributed to a lack of viable vectors for transmission of *Leucocytozoon* spp., since numerous species of birds breeding in North America overwinter in Neotropical regions and may act as carriers for parasites between continents (Bennett et al., 1991; Rodríguez and Matta, 2001). The only species of *Leucocytozoon* reported to infect waterfowl is *L. simondi*, which has been reported to be endemic to Holarctic regions and is not known to be successfully transmitted below 42° North latitude (Valkiūnas, 2005). The detection of four *Leucocytozoon* haplotypes infecting four separate species of waterfowl in Peru (Table 2; Fig. 2) provides evidence that *Leucocytozoon* parasites are transmitted in South America among waterfowl species, although most likely at very low rates. Morphological examination of blood smears made from South American waterfowl is necessary to identify the morphospecies of *Leucocytozoon* parasites infecting ducks in Peru, although close genetic relationships among *Leucocytozoon* haplotypes from waterfowl sampled in both North America and South America suggest that these parasites may be *L. simondi*.

Comparison of haplotypes detected in our study to parasite lineages reported in public databases and phylogenetic analysis supports haplotype sharing for *Plasmodium* parasites among continents and diverse avian taxa. Additionally, these analyses support gene exchange of *Leucocytozoon* and *Haemoproteus* haplotypes among species of North American and South American waterfowl. Although species and sub-species of waterfowl from which *Leucocytozoon* and *Haemoproteus* parasites were detected in our study are not reported to migrate between North America and South America, other species including Blue-winged Teal (*Anas discors*) breed in North America and winter in the Neotropics (Botero and Rusch, 1988) which may facilitate the redistribution of haematozoa between continents. Of the species of endemic South American waterfowl we sampled, Cinnamon teal (*Anas cyanoptera*), Fulvous Whistling Ducks (*Dendrocygna bicolor*), Ruddy Ducks, Speckled Teal (*Anas flavirostris*), and Yellow-billed Pintail (*Anas georgica*) distributions all occur within the wintering distribution of Blue-winged Teal (Madge and Burn, 1988) and therefore provide a potential route by which parasite transmission may occur in the presence of suitable dipteran vectors.

The lack of identical *Haemoproteus* and *Leucocytozoon* lineages on public databases from non-waterfowl hosts and phylogenetic divergence between parasite haplotypes previously detected in South American passerines and those originating from waterfowl support some degree of host-specificity for *Haemoproteus* and *Leucocytozoon* lineages detected in this study. Previous research has found evidence that *Haemoproteus* parasites exhibit higher levels of host specificity when compared to *Plasmodium* species (Bensch et al., 2000; Krizanauskiene et al., 2009), but less work has been conducted to assess the specificity of *Leucocytozoon* parasites. Fallis et al. (1954) attempted to experimentally infect wild and domestic hosts with *L. simondi* including ducks, geese, grouse, chickens, turkeys, and pheasants. However, infections only developed in ducks and geese, which suggest that this species may be specific to waterfowl.

Our study found evidence of shared haematozoa lineages between waterfowl species in North America and species of waterfowl endemic to South America. Additionally, we observed a low apparent prevalence rate of haematozoa infection in our samples (3.1%) and evidence for host specificity for *Haemoproteus* and *Leucocytozoon* parasites infecting waterfowl species. While the current investigation extends the available data for haematozoa infections in South American waterfowl, additional studies may be useful to better understand the ecology of blood parasites in Neotropical birds (Braga et al., 2011; Clark et al., 2014). Sampling a range of host species including passerines, waterfowl, and other taxa at specific locations through time could provide important information for further assessment of parasite exchange and seasonality of transmission. Contrasting parasite screening results from samples collected during wet and dry seasons, on either side of geographic barriers such as the Andes, and over a well-defined elevation gradient could help inform how ecological factors affect parasite prevalence rates. Furthermore, genetic characterization of haematozoa infections, paired with microscopic examination of blood smears would provide resolution into the distribution and lineage diversity of haematozoa

parasites in South American waterfowl. Lastly, further investigation into host and regional patterns of *Haemoproteus, Plasmodium*, and *Leucocytozoon* parasite infections in the avifauna of South America may provide further inference into potential for shifts in geographic and host ranges.

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Conflict of interest

The authors declared that there is no conflict of interest.

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