



Low occurrence of hemosporidian parasites in the Neotropic cormorant (*Phalacrocorax brasilianus*) in Chile

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Abstract

Hemosporidian parasites rarely infect aquatic birds. Few studies have been conducted in South America identifying some lineages of the genera *Plasmodium*, *Leucocytozoon*, and *Haemoproteus*, but none has been done in the Neotropic cormorant (*Phalacrocorax brasilianus*). This species is widely distributed through the American continent, from Southern USA to Tierra del Fuego, using a wide range of aquatic habitats. We molecularly studied the occurrence and diversity of hemosporidian lineages infecting individuals of Neotropic cormorant across a broad latitudinal gradient in Chile (Arica to Tierra del Fuego). As expected, a very low occurrence of individuals infected by *Plasmodium* sp. (4/123, 3.3%) and *Leucocytozoon* sp. (2/123, 1.6%) was detected. We found no evidence of *Haemoproteus* sp. We identified one lineage of *Plasmodium* (ZEMAC01) and one new lineage of *Leucocytozoon* (PHABRA01) infecting cormorants. Individuals infected by *Plasmodium* sp. were birds from only one site (i.e., Chillán), whereas *Leucocytozoon* sp. was found in one bird from Valdivia and another one from Tierra del Fuego. Our results expand the known range of hemosporidian parasite lineages in aquatic birds providing an essential baseline data that contribute to a better understanding of the geographic range of hemosporidian parasites infecting Phalacrocoracidae in South America.

Keywords *Phalacrocorax brasilianus* · Hemoparasites · *Plasmodium* · *Leucocytozoon* · Pathogens · Parasite

Introduction

Avian hemosporidians are vector-transmitted protozoans that infect most of bird species (Bennett et al. 1993, 1994; Valkiunas 2005) and have the potential to cause disease, cause a reduction of the reproductive success and, ultimately, premature death (Atkinson et al. 2000; Dawson and Bortolotti 2000; Merino et al. 2000; LaPointe et al. 2005; Asghar et al. 2015). Over the past decades, there has been a significant

increase in the number of studies identifying hemosporidians infecting wild birds with the description of more than 1300 lineages of over 200 species among 4,000 bird species investigated worldwide (Valkiunas 2005; Clark et al. 2014; Marzal 2012). The recent use of DNA amplification has provided a higher sensitivity for hemoparasite detection than microscopy alone (Garamszegi 2010), allowing more accurate studies on parasite diversity, host specificity, and geographic distribution (Bensch et al. 2000; Pérez-Rodríguez et al. 2013; Soares et al. 2016).

Hemosporidian infections vary significantly among bird orders (Bennett et al. 1993; Bennett et al. 1994; Valkiunas 2005). Interestingly, some avian groups, such as aquatic birds using saline environments, are reported to be rarely infected (Peirce and Brooke 1993; Merino et al. 1997; Piersma 1997; Merino and Minguéz 1998; Figuerola 1999; Engström et al. 2000; Jovani et al. 2001; Quillfeldt et al. 2010; Soares et al. 2016). This interspecific variation has been hypothesized to be related to host immunological competence, parasite-host specificity, and the lack of appropriate vectors (Piersma 1997; Figuerola 1999; Martínez-Abraín et al. 2004), although latitudinal gradients have a strong influence on avian and vector

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diversity which may have an impact in the parasite diversity (Clark et al. 2014; Quillfeldt et al. 2010).

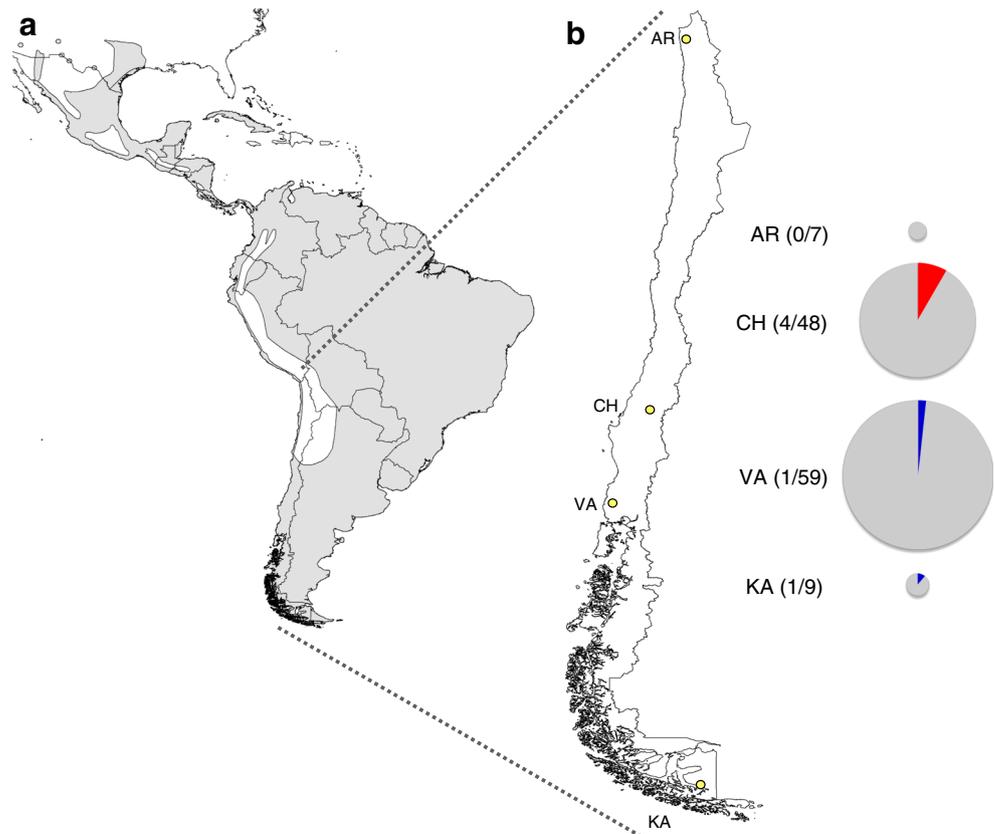
Few studies have been conducted in Chile to identify avian hematozoa and their lineage diversity (Forrester et al. 1977; Forrester et al. 2001; Merino et al. 2008; Martínez et al. 2009; Martínez et al. 2015; Sallaberry-Pincheira et al. 2015), and not much has been done in aquatic birds (but see Sallaberry-Pincheira et al. 2015). The Neotropic cormorant, *Phalacrocorax brasilianus* (Fam. Phalacrocoracidae, O. Suliformes), is one of the most numerous and widespread cormorant species of the American continent. This species is distributed from Texas in the USA to Tierra del Fuego in South America using areas of fresh, brackish, and salt water from the sea level up to 5000 m of altitude in the Andes (Stotz et al. 1996) and an overall population estimated in 2,000,000 individuals (BirdLife International 2016). The Neotropic cormorant breeds during the austral spring in late-September to mid-December. Birds tend to form large colonies that may vary from few dozens to several thousand (e.g., ca. 7000) breeding pairs (Kalmbach et al. 2001; Quintana et al. 2002) which occasionally share with other bird species such as gulls, terns, egrets, herons, and other cormorant species (Yorio et al. 1994; Quintana et al. 2002). The nesting is usually with sticks placed mostly on the tops of trees and bushes, but also cliffs and rocks, of inland wetlands, rivers, lakes, and marine shores (Kalmbach et al. 2001; Quintana et al. 2002). This species can shift the diet composition and feeding behavior according to temporal or local changes in the environment revealing a high ecological plasticity (Barquete et al. 2008). However, the Neotropic cormorant is mostly sedentary throughout its breeding range, using almost invariably the same foraging areas closest to the colonies or roosting areas in both marine and freshwater environments (Casaux et al. 2009; Quintana et al. 2002). In Chile, *P. brasilianus* is one the most abundant aquatic birds, which breeds all along the Chilean coast and inland freshwaters and roosts in areas highly associated with humans. As with other cormorant species elsewhere (e.g., Frederiksen et al. 2001), this species is considered a pest due to damaging effects of feces on buildings and public lighting. Neotropic cormorants are exposed to a diverse range of micro- and macro-parasites such as feather lice, ticks (Sepulveda et al. 1997; González-Acuña and Guglielmone 2005), nematodes and acanthocephalans (Torres et al. 1991, 1993, 2000, 2005), and several viruses (Verdugo et al. 2019). The present study aimed to molecularly determine the occurrence of hemosporidian lineages from the genera *Plasmodium*, *Haemoproteus*, and *Leucocytozoon* in individuals of Neotropic cormorant at different stages of their life cycle (i.e., fledgling, juveniles, and adults) using different habitats in a wide latitudinal gradient.

Materials and methods

Sample collection

Blood samples (1 ml) were collected from 123 Neotropic cormorants (*Phalacrocorax brasilianus*) (1.384 kg mean weight) and preserved in 2-ml tubes with 96% ethanol for further analysis. Samples were obtained from different latitudes in Chile from 2015 to 2017 (Fig. 1): from surrounding areas of Arica ($n = 7$), Ñuble river (Chillán, $n = 48$), Cruces and Valdivia rivers (Valdivia, $n = 59$), and Karukinka (Tierra del Fuego, $n = 9$). Blood samples of live animals were collected from the right jugular vein, whereas blood from dead animals was collected from the heart immediately after individuals were shot by hunters during hunting season or population control management. Arica ($18^{\circ}28'24.1''S$; $70^{\circ}18'59.5''W$) is the northernmost city located on the coast next to the border with Peru. This city is known as one of the driest inhabited places on earth featuring a mild desert climate (Cereceda et al. 2008), with an annual mean temperature variation and precipitation of 15.5 to 26.9 °C and 0 to 3.4 mm, respectively. Here, the Neotropic cormorant is considered a pest species by the Chilean law (N° 19.473, article 6°, Servicio Agrícola y Ganadero), and an eradication program has been implemented to control the urban population. Samples from Arica were obtained from adult birds hunted in October of 2015. Samples from Chillán were collected at the Ñuble river ($36^{\circ}32'58.8''S$; $72^{\circ}05'00.4''W$; 125 m.a.s.l.) in adult and juvenile birds during the months of April and May 2016 by hunters during the hunting season (April to August). This area is located in Central Chile and is characterized by a dry Mediterranean climate, sclerophyllous vegetative elements, and an annual mean temperature and precipitation variation of 4.4 to 29.1 °C and 884 to 982 mm, respectively. Samples from Valdivia were obtained in two places: 29 adult individuals sampled by hunters along the Valdivia river ($39^{\circ}51'42.9''S$; $73^{\circ}17'26.2''W$) during the months of April–August of 2016, and 30 fledgling individuals swimming nearby a breeding colony in the Cruces river ($39^{\circ}41'29.5''S$; $73^{\circ}11'34.9''W$; 5 m.a.s.l.), during January and February 2016. The Cruces and Valdivia rivers (Valdivia) correspond to a 6000-ha wetland and marshlands system in Southern Chile. Jiménez (2001) estimated around 1000 *P. brasilianus* breeding pairs in the entire area, although we estimated 150 breeding pairs in the colony when sampled. The region is characterized by a temperate rainy climate with the Mediterranean and oceanic influences with an annual mean temperature and precipitation variation of 3.3 to 25.3 °C and 1618 to 1925 mm, respectively, allowing a particular evergreen rainforest type of vegetation. Birds from Tierra del Fuego Island, the southernmost area of Chile, were fledglings captured by hand in a colony in the Karukinka reserve ($54^{\circ}11'43.9''S$; $68^{\circ}44'1''W$; 150 m.a.s.l.) during December 2017. This reserve corresponds to a

Fig. 1 **a** Map of the distribution of *Phalacrocorax brasilianus* in America (gray). **b** Sample sites and number of infected host individuals in Chile. The size of each circle is proportional to the sample size for each location. AR – Arica, CH – Chillán, VA – Valdivia, Ka – Karukinka. *Plasmodium* sp. is represented in red and *Leucocytozoon* sp. in blue. For further environmental and capture details, please refer to text



300,000-ha private, protected area in Tierra del Fuego. This area is characterized by an Andean Patagonia forest dominated by *Nothofagus pumilio* which is used by introduced beavers to build dams in small creeks, presenting an annual mean temperature and precipitation variation of -1 to 13 °C and 281 to 640 mm, respectively. Breeding colonies are scarce in the area, and this colony represents the only known to Tierra del Fuego. At the time of sampling, there were only six breeding pairs and 13 fledglings.

DNA extraction and molecular detection of hemosporidians

Genomic DNA was extracted from blood samples using the EZNA™ Blood DNA Mini Kit (Omega Bio-tek, Norcross, GA, USA). For each sample, a 350-bp segment of the cytochrome *b* (*cytb*) gene was amplified using universal primers PALU-F and PALU-R (Martínez et al. 2009) for detecting *Plasmodium* and *Haemoproteus* species, and Leunew1F and LDRd (Merino et al. 2008; Martínez et al. 2015) for *Leucocytozoon* species. The *cytb* gene has several conserved regions between polymorphic sections of DNA which makes it suitable for detection and identification of hemosporidian lineages (Waldenström et al. 2004). Amplicons obtained from these primers had a 68% of query cover with sequences

obtained with primers used in the MalAvi database (Bensch et al. 2009). The PCR conditions started with an initial denaturation of 94 °C for 5 min followed by 30 cycles of 95 °C for 30 s, $54/58$ °C for *Plasmodium*/*Haemoproteus* and *Leucocytozoon*, respectively, for 30 s, and 72 °C for 30 s, and a final extension of 72 °C for 2 min. The PCR products from the individual positive samples were purified (QIAquick PCR Product Purification kit, QIAGEN, Hilden, Germany) and sequenced in both directions using an ABI 3730XL Genetic Analyzer (AustralOmics, Universidad Austral de Chile, Valdivia, Chile). Sequences were edited and assembled in Geneious 9.1.8 (Biomatters Ltd.). All sequences obtained were submitted to GenBank. All individuals were molecularly sexed (following Rodrigues et al. *in press*), and a χ^2 test was used to evaluate differences on the overall infection rate according to sex, age (i.e., fledgling, juvenile, adult), geographic location (i.e., Arica, Chillan, Valdivia, Karukinka), and sampling year (i.e., 2015–2017).

Phylogenetic analysis

A midpoint rooting phylogenetic tree (following Outlaw and Ricklefs 2011) was inferred using a dataset with previously published sequences of hemosporidians of the genera *Plasmodium*, *Haemoproteus*, and *Leucocytozoon* available

from MalAvi database and Genbank (Table A1). These sequences were chosen based in the diversity of hemosporidian studies in Suliformes and in studies across Chile (Merino et al. 2008; Quillfeldt et al. 2011; Martínez et al. 2015; Seimon et al. 2016). The nucleotide substitution model (GTR+I+G) was selected in jModeltest 2.1.7 (Darriba et al. 2012). A Bayesian phylogenetic analysis was inferred using MrBayes 3.2.2 (Ronquist et al. 2012) after 10 million generations in two simultaneous Markov Chain Monte Carlo simulation runs.

Results

Of the 123 cormorants, 58 were females and 59 males (six individuals did not amplify correctly), 61 non-breeding adults, 24 juveniles, and 38 fledglings. Six of the 123 (overall mean 0.049, 95% C.I. = 0.021–0.104) cormorants sampled on this study were positive for hemosporidian infections of the genera *Plasmodium* ($n = 4$) and *Leucocytozoon* ($n = 2$). Three females (two adults and one juvenile) and one adult male from Chillán were positive to *Plasmodium* sp., whereas an adult male from Valdivia and a fledgling female from Karukinka were positive to *Leucocytozoon* sp. *Haemoproteus* sp. was not detected in any sample. There were no differences on the overall infection rate among individuals of different sex ($\chi^2 = 0.74$, $df = 1$, $p = 0.38$), age ($\chi^2 = 0.84$, $df = 2$, $p = 0.65$), geographic location ($\chi^2 = 3.63$, $df = 3$, $p = 0.3$), or sampling year ($\chi^2 = 2.07$, $df = 2$, $p = 0.35$). However, *Plasmodium* sp. was present only in Chillán area (4/48), whereas *Leucocytozoon* sp. was present in Valdivia (1/59) and Karukinka (1/9) areas. All samples from Arica were negative (Fig. 1).

All four *Plasmodium* sequences showed 100% identity (accession number MG835709) and were identified as a ZEMAC01 lineage, a cosmopolitan lineage found in several bird host orders (Perkins and Schall 2002; Beadell et al. 2004; Ishtiaq et al. 2007; Martinsen et al. 2008; Ham-Dueñas et al. 2017) (Fig. 2). The two *Leucocytozoon* sequences were recovered from an adult male cormorant in Valdivia river and a juvenile female in Karukinka. Both sequences showed 100% identity (accession number MG835710) to each other but did not match any known sequence in the database. Thus, we submitted this haplotype to the MalAvi database as a new lineage: PHABRA01 (Fig. 2).

Discussion

This study represents the first reporting of the occurrence of hemosporidians infecting individuals of *Phalacrocorax brasilianus* (Fam. Phalacrocoracidae, O. Suliformes) despite its wide distribution across the American continent. Across a latitudinal gradient and different stages of their life cycle, we found a low occurrence (4.9%) of hemosporidians in the

Neotropic cormorant, similar to other aquatic birds on which hemosporidian infections are rarely detected (Valkiunas 2005; Soares et al. 2016; Campioni et al. 2018). Further, several studies have reported no infection at all by either *Plasmodium*, *Haemoproteus*, and/or *Leucocytozoon* in birds of the order Suliformes (Levin et al. 2014; Quillfeldt et al. 2014; Lee-Cruz et al. 2016; Adlard et al. 2004) and, in particular, cormorants (Jovani et al. 2001; Quillfeldt et al. 2010; Chagas et al. 2016; Inumaru et al. 2017). The scarcity of hemosporidian infections in aquatic birds has been related to intrinsic and extrinsic factors including the immunological capacity of the host, the host-parasite assemblage, and environmental filters such as the absence of appropriate vectors, lack of suitable vectors, type of nests, and duration of the nestling period (Jovani et al. 2001; Valera et al. 2003; Martínez-Abraín et al. 2004; Valkiunas 2005; Arriero and Møller 2008; Quillfeldt et al. 2011; Campioni et al. 2018).

We were unable to detect differences in the infection rates between host sex, age, year, and location, although the number of infected individuals was too small for further analyses. Sex differences in parasitic infections are well documented in a wide range of host taxa due to ecological and physiological factors, such as behavior, morphology, hormones, and immunocompetence (Zuk and McKean 1996). In the same way, age has been implicated as the primary factor explaining the variability of infection to hemoparasites as a time-dependent exposure to vectors (Arriero and Moller 2008). For instance, adults could have higher probability of infection by a cumulative exposure (Mendes et al. 2005), although fledglings could have higher intensity of infection due to yet restricted immunological competence (Apanius 1998; Knowles et al. 2010) and higher exposure to vectors during the nestling period (Merino and Potti 1995). Nevertheless, we did not find differences in the infection rate across animals at a different stage of their life cycle. The variation on the infection of hemosporidians in cormorants, thus, is likely to be dependent on the opportunistic exposure of hosts to the parasites, according to the availability and habitat preferences of vectors.

Although the overall occurrence of infection did not change across sampling locations, there was a clear geographic pattern of hemosporidian infections. *Plasmodium* sp. was found only in the Chillán area, while *Leucocytozoon* sp. was present in Valdivia and Karukinka areas. The vectors of *Plasmodium* are known to be mosquitoes of the Culicidae family, whereas the vectors of *Leucocytozoon* are dipterans of the Simuliidae family, and for *Haemoproteus* are biting midges and louse flies of the Ceratopogonidae and Hippoboscidae families, respectively (Valkiunas 2005; Atkinson 2008). The Culicidae larvae are aquatic, developing mainly in standing water that is sheltered from the wind, such as temporary pools, discarded containers, saltmarshes, and pounds (Adler et al. 2004; Manimegalai and Sukanya 2014). In Chile, 13 species of Culicidae grouped in two subfamilies and four genera have been described, but none

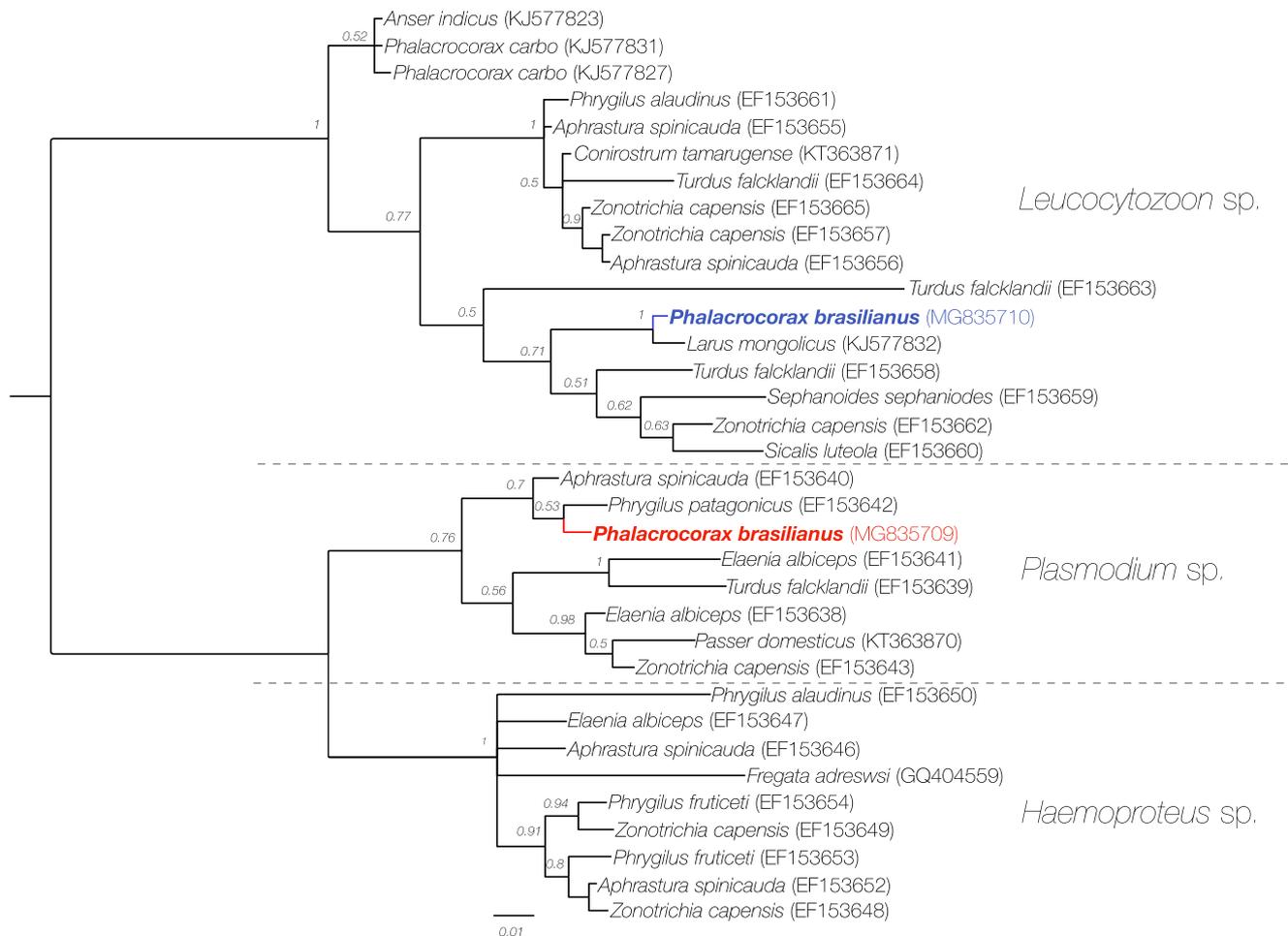


Fig. 2 Mid-root phylogenetic relationship among *Leucocytozoon* sp., *Plasmodium* sp., and *Haemoproteus* sp. found in avian blood samples. Hemoparasites found in this study are in color. Posterior probabilities higher than 0.5 are shown for each clade

of them was registered in the southern regions of Chile (González et al. 2005), which could explain the absence in *P. brasilianus* in Valdivia and Karukinka. The Simuliidae larvae develop in running water of all types, from the smallest leaks and streams to the largest rivers and waterfalls (Currie and Adler 2008). Seven genera and more than 40 species of Simuliidae have been found throughout Chile (Henry 1993). Seven are inhabitants of southern South America and are characterized by their high degree of endemism (Coscarón and Coscarón-Arias 1995), as also are *Simulium* (Pternaspatha) and *Simulium* “blancasi group” both in the Simuliini tribe. Further studies are needed to evaluate the potential roles of these dipterans as vectors of *Leucocytozoon* in Southern South America. Finally, Ceratopogonidae biting midges and Hippoboscidae louse flies, recognized vectors of *Haemoproteus*, are widely distributed in Chile (Cazorla and Spinelli 2007; Fuentes-Castillo et al. 2016). However, we have been unable to detect exposure of Neotropic cormorants to *Haemoproteus*. There is evidence of two lousefly, *Olfersia sordida* and *Icosta nigra*, parasitizing flightless cormorants *Phalacrocorax harrisi* (Whiteman et al. 2006; Levin and

Parker 2013) with the potential of transmitting hemosporidians to avian hosts. Thus, more research is indeed required to identify and understand the potential roles of local species of Culicidae, Simuliidae, Ceratopogonidae, and Hippoboscidae families as hemosporidian vectors.

We identified four individuals positive for *Plasmodium* sp., all from Chillan area. Previous studies have reported the presence of *Plasmodium* infecting bird species of the order Suliformes, consistent with the fact that this hemosporidian genus is considered a generalist parasite with a broad host range (Valkiunas 2005). For instance, *Plasmodium* has been reported in great frigatebirds (Bastien et al. 2014), cape gannets (*Morus capensis*), red-footed boobies (*Sula sula*) (Parsons et al. 2010), and, in particular, cormorant species, such as bank cormorant (*P. neglectus*), cape cormorant (*P. capensis*), white-breasted cormorant (*P. lucidus*), and crowned cormorant (*Microcarbo coronatus*) (Parsons et al. 2017), all from wildlife rehabilitation centers. Interestingly, wild great cormorants (*P. carbo*), which is also a widespread cormorant species as the Neotropic cormorant, harbored three different lineages of *Plasmodium* sp. (Seimon et al. 2016). The lineage

identified in Neotropic cormorant was 100% identical to the ZEMAC01 lineage, previously found in common iora (*Aegithina tiphia*) in Birmania (EF380133), red munia (*Estrilda amandava*) in India (EF380152), black-throated sparrow (*Amphispiza bilineata*) in Mexico (KX811227), and yellow-spotted honeyeater (*Meliphaga notata*) in Australia (AY714207) (Beadell et al. 2004; Ham-Dueñas et al. 2017; Ishtiaq et al. 2007). This may be indicative of the cosmopolitan feeding habits of the vector because generalist feeders, such as mosquitoes, could systematically introduce several *Plasmodium* lineages into various hosts (Waldenström et al. 2002). Alternatively, the same lineage may be transmitted by different mosquito species in different areas generating multi-vector-parasite assemblages (Ferraguti et al. 2013).

We detected two individuals positive for *Leucocytozoon* sp., an adult male from Valdivia and a fledgling female from Karukinka. *Leucocytozoon* has been reported in several species of cormorants worldwide. For instance, *Leucocytozoon vandenbrandeni* was reported infecting Australian little-pied cormorant (*Phalacrocorax melanoleucos*) in South Africa (Mackerras and Mackerras 1960), and erythrocytic gametocytes of *Leucocytozoon ugwidii* were found in cape cormorants, crowned cormorants, and reed cormorants (*Microcarbo africanus*) (Parsons et al. 2010; Parsons et al. 2017). Two lineages were recovered from great cormorant from Mongolia, the TUSW04 found in tundra swans and a PHACAR02 found only in great cormorants (Seimon et al. 2016). The two individuals from our study were infected by the same lineage of *Leucocytozoon* sp. although they were located almost 1800 km apart. If the low occurrence of *Leucocytozoon* sp. in *P. brasiliensis* and the phylogenetic distance to the lineage infecting *P. carbo* are taken into consideration, the new lineage detected in this study could be the result of a recent host-switching followed by a co-evolution process (see Ricklefs et al. 2004). Otherwise, this lineage may be present in other cormorant species, probably from species of the same family that are present in South America, since most species of *Leucocytozoon* are family specific (Peirce 2005). Further research in other cormorant species will be necessary to determine whether *P. brasiliensis* is the sole host of this *Leucocytozoon* lineage or if there are other possible host species.

Haemoproteus has been largely described infecting frigatebirds: *H. iwa* found in the Pacific, Atlantic, and Indian oceans (Quillfeldt et al. 2011; Levin et al. 2011; Bastien et al. 2014) and *H. valkiunasi* found in Eastern Indian Ocean (Merino et al. 2012). Blue-footed and red-footed boobies from the Galápagos Islands have also been reported to be infected by *Haemoproteus* (Padilla et al. 2006; Lee-Cruz et al. 2016). Unlike *Plasmodium* lineages, *Haemoproteus* lineages have been suggested to be largely constrained by the phylogenetic relationship of their hosts where evolutionarily stable jumps between host families are likely to be rare (Beadell et al. 2004). However, this narrow host-affinity could not be the

rule, since Moens et al. (2016) find that hummingbirds act as the reservoir of *Haemoproteus* for several other Andean bird species. Further, Seimon et al. (2016) reported one lineage of *Haemoproteus* (ANSIND01) in great cormorant. The same lineage was also found infecting bar-headed goose and ruddy shelduck from the same study inferring that this lineage is shared among sympatric avian host species if compatible vectors are present. Jovani et al. (2001) found no evidence of infection in several birds from the Phalacrocoracidae family and other marine birds from colonies located in the coastline of Argentina, arguing the lack of suitable vectors in the area. Although *Haemoproteus* has been reported in several passerine birds from a wide latitudinal range in Chile (see Merino et al. 2008), the only previous study performed in marine birds (i.e., Humboldt and Magellanic penguins) in Chile reported absence of infection (Sallaberry-Pincheira et al. 2015). Thus, it is unclear whether the infection by *Haemoproteus* is restricted by the availability of vectors in marine environments, even though most of the cormorants sampled in our study were from freshwater.

A pitfall of this study that may also explain the low occurrence of hemosporidians was the opportunistic sampling we have applied as an approach. Although we did not find significant differences on the infection rates among sex, age, location, or year, the sampling method did not allow us to obtain larger numbers of individuals, particularly from Arica and Tierra del Fuego. Future studies should consider a higher sampling effort along the species distribution but also in sympatric species. Nonetheless, our results expand the known range of hemosporidian parasite lineages in aquatic birds which are often negative to the infection by hemosporidians and provide important baseline data that contribute to a better understanding of the geographic range and types of hemosporidian parasites that infect Phalacrocoracidae birds in South America.

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Compliance with ethical standards

The present study was conducted according to the animal welfare guidelines of the Bioethical Committee of the Universidad Austral de Chile and with permission 7597/2016 from the Servicio Agrícola y Ganadero (SAG) and permission 019/2015 from the Corporación Nacional Forestal (CONAF). All experiments comply with the current laws of the Republic of Chile.

Conflict of interest The authors declare that they have no conflict of interest.

References

- Adlard RA, Peirce MA, Lederer R (2004) Blood parasites of birds from south-east Queensland. *EMU* 104:191–196
- Adler PH, Currie DC, Wood DM (2004) *The black flies (Simuliidae) of North America*. Cornell University Press, New York
- Apanius V (1998) Stress and immune response. In: Møller AP, Milinski M, Slater PJB (eds) *Stress and behavior*. Academic Press, New York, pp 133–154
- Ariero E, Møller AP (2008) Host ecology and life-history traits associated with blood parasite species richness in birds. *J Evol Biol* 21:1504–1513
- Asghar M, Hasselquist D, Hansson B, Zehndjiev P, Westerdahl H, Bensch S (2015) Hidden costs of infection: chronic malaria accelerates telomere degradation and senescence in wild birds. *Science* 347:436–438
- Atkinson CT (2008) *Haemoproteus*. In: Atkinson CT, Thomas NJ, Hunter BC (eds) *Parasitic diseases of wild birds*. Wiley-Blackwell, Ames, pp 13–35
- Atkinson CT, Dusek RJ, Woods KL, Iko WM (2000) Pathogenicity of avian malaria in experimentally-infected Hawaii Amakihi. *J Wildl Dis* 36:197–204
- Barquete V, Bugoni L, Vooren CM (2008) Diet of Neotropical cormorant (*Phalacrocorax brasilianus*) in an estuarine environment. *Mar Biol* 153:431–443
- Bastien M, Jaeger A, Le Corre M, Tortosa P, Lebarbenchon C (2014) *Haemoproteus iwa* in great frigatebirds (*Fregata minor*) in the islands of the Western Indian Ocean. *PLoS One* 9:e97185
- Beadell JS, Gering E, Austin J, Dumbacher JP, Peirce MA, Pratt TK, Atkinson CT, Fleischer RC (2004) Prevalence and differential host-specificity of two avian blood parasite genera in the Australo-Papuan region. *Mol Ecol* 13:3829–3844
- Bennett GF, Bishop MA, Peirce MA (1993) Checklist of the avian species of *Plasmodium* Marchiafava and Celli, 1885 (Apicomplexa) and their distribution by avian family and Wallacean life zones. *Syst Parasitol* 26:171–179
- Bennett GF, Pierce MA, Earlé RA (1994) An annotated checklist of the valid avian species of *Haemoproteus*, *Leucocytozoon* (Apicomplexa: Haemosporida) and *Hepatozoon* (Apicomplexa: Haemogregarinidae). *Syst Parasitol* 29:61–73
- Bensch S, Stjernman M, Hasselquist D, Ostman O, Hansson B, Westerdahl H, Pinheiro RT (2000) Host specificity in avian blood parasites: a study of *Plasmodium* and *Haemoproteus* mitochondrial DNA amplified from birds. *Proc Biol Sci* 267:1583–1589
- Bensch S, Hellgren O, Pérez-Tris J (2009) MalAvi: a public database of malaria parasites and related haemosporidians in avian hosts based on mitochondrial cytochrome b lineages. *Mol Ecol Resour* 9:1353–1358
- BirdLife International (2016) *Phalacrocorax brasilianus*. The IUCN Red List of Threatened Species 2016: e.T22696773A93585558
- Campioni L, Martínez-de la Puente J, Figuerola J, Granadeiro JP, Silva MC, Cstry P (2018) Absence of haemosporidian parasite infections in the long-lived Cory's shearwater: evidence from molecular analyses and review of the literature. *Parasitol Res* 117:323–329
- Casaux RJ, di Prinzio CY, Bertolin ML, Tartara MA (2009) Diet of the Neotropical cormorant *Phalacrocorax olivaceus* at West Chubut, Patagonia, Argentina. *Waterbirds* 32:444–449
- Cazorla CG, Spinelli GR (2007) A new species of Patagonian *Stilobezzia* (Acanthohelea) and a redescription of *S. (A.) nigerrima* Ingram and Macfie (Diptera: Ceratopogonidae). *Trans Am Entomol Soc* 133:181–187
- Cereceda P, Larrain H, Osses P, Fariás M, Egaña I (2008) The climate of the coast and fog zone in the Tarapacá Region, Atacama Desert, Chile. *Atmos Res* 87:301–311
- Chagas CR, Guimarães LO, Monteiro EF et al (2016) Hemosporidian parasites of free-living birds in the São Paulo Zoo, Brazil. *Parasitol Res* 115:1443–1452
- Clark NJ, Clegg SM, Lima MR (2014) A review of global diversity in avian haemosporidians (*Plasmodium* and *Haemoproteus*: *Haemosporida*): new insights from molecular data. *Int J Parasitol* 44:329–338
- Coscarón S, Coscarón-Arias CL (1995) Distribution of Neotropical Simuliidae (Insecta-Diptera) and its areas of endemism. *Rev Acad Colomb Cienc* 19:717–732
- Currie DC, Adler PH (2008) Global diversity of black flies (Diptera: Simuliidae) in freshwater. *Hydrobiol* 595:469–475
- Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nat Methods* 9:772–772
- Dawson RD, Bortolotti GR (2000) Effects of hematozoan parasites on condition and return rates of American kestrels. *Auk* 117:373–380
- Engström H, Dufva R, Olsson G (2000) Absence of haematozoa and ectoparasites in a highly sexually ornamented species, the crested auklet. *Waterbirds* 23:486–488
- Ferraguti M, Martínez-de la Puente J, Muñoz J, Roiz D, Ruiz S, Soriguer R, Figuerola J (2013) Avian *Plasmodium* in *Culex* and *Ochlerotatus* mosquitoes from southern Spain: effects of season and host-feeding source on parasite dynamics. *PLoS One* 8:e66238
- Figuerola J (1999) Effects of salinity on rates of infestation of waterbirds by haematozoa. *Ecography* 22:681–685
- Forrester DJ, Greiner EC, McFarlane RW (1977) Blood parasites of some columbiform and passeriform birds from Chile. *J Wildl Dis* 13:94–96
- Forrester DJ, Foster GW, Morrison JL (2001) *Leucocytozoon toddi* and *Haemoproteus tinnunculi* (Protozoa: Haemosporina) in the Chimango caracara (*Milvago chimango*) in southern Chile. *Mem Inst Osvaldo Cruz* 96:1023–1024
- Frederiksen M, Lebreton J, Bregnballe T (2001) The interplay between culling and density-dependence in the great cormorant: a modeling approach. *J Appl Ecol* 38:617–627
- Fuentes-Castillo D, Cicchino A, Mironov S, Moreno L, Landaeta-Aqueveque C, Barrientos C, González-Acuña D (2016) Ectoparasites of the black-chinned siskin *Spinus barbatus* (Passeriformes: Fringillidae) in Chile. *Rev Bras Parasitol Vet* 25:476–483
- Garamszegi LZ (2010) The sensitivity of microscopy and PCR-based detection methods affecting estimates of prevalence of blood parasites in birds. *J Parasitol* 96:1197–1203
- González CR, Jercic MI, Muñoz L (2005) The mosquitoes from Chile (Diptera: Culicidae). *Acta Ent Chilena* 29:31–35
- González-Acuña D, Guglielmo A (2005) Ticks (Acari: Ixodoidea: Argasidae, Ixodidae) of Chile. *Exp Appl Acarol* 35:147–163
- Ham-Dueñas JG, Chapa-Vargas L, Stracey CM, Huber-Sannwald (2017) Haemosporidian prevalence and parasitaemia in the black-throated sparrow (*Amphispiza bilineata*) in central-Mexican dryland habitats. *Parasitol Res* 116:2527–2537
- Henry A (1993) Simuliidae de Chile: Lista de especies y su distribución geográfica (Diptera: Simuliidae). *Acta Ent Chilena* 18:89–96
- Inumaru M, Murata K, Sato Y (2017) Prevalence of avian haemosporidian among injured wild birds in Tokyo and environs, Japan. *Int J Parasitol Parasites Wildl* 6:299–309
- Ishtiaq F, Gering E, Rappole JH, Rahmani AR, Jhala YV, Dove CJ, Milensky C, Olson SL, Peirce MA, Fleischer RC (2007) Prevalence and diversity of avian hematozoan parasites in Asia: a regional survey. *J Wildl Dis* 43:382–398
- Jiménez E (2001) *Biología reproductiva y alimentaria del cormorán yeco (Phalacrocorax brasilianus, Gmelin; 1789) en la colonia del Santuario de la Naturaleza del Ríos Cruces, Valdivia*. Dissertation, Universidad Austral de Chile

- Jovani R, Tella JL, Forero MG, Bertellotti M, Blanco G, Ceballos O, Donazar JA (2001) Apparent absence of blood parasites in the Patagonian seabird community: is it related to marine environment? *Waterbirds* 24:430–433
- Kalmbach E, Ramsay SC, Wendeln H, Becker PH (2001) A study of Neotropical cormorants in Central Chile: possible effects of El Niño. *Waterbirds* 24:345–351
- Knowles SCL, Wood MJ, Sheldon BC (2010) Context-dependent effects of parental effort on malaria infection in a wild bird population, and their role in reproductive trade-offs. *Oecologia* 164:87–97
- LaPointe DA, Goff ML, Atkinson CT (2005) Comparative susceptibility of introduced forest-dwelling mosquitoes in Hawaii to avian malaria, *Plasmodium relictum*. *J Parasitol* 91:843–849
- Lee-Cruz L, Cunningham A, Martínez P, Cruz M, Goodman S, Hamer K (2016) Prevalence of *Haemoproteus* sp. in Galápagos blue-footed boobies: effects on health and reproduction. *Parasitol Open* 2:1–10. <https://doi.org/10.1017/pao.2015.6>
- Levin II, Parker PG (2013) Comparative host–parasite population genetic structures: obligate fly ectoparasites on Galapagos seabirds. *Parasitology* 140:1061–1010
- Levin II, Valkiunas G, Santiago-Alarcon D, Cruz LL, Iezhova TA et al (2011) Hippoboscids-transmitted *Haemoproteus* parasites (Haemosporida) infect Galapagos Pelecaniform birds: evidence from molecular and morphological studies, with a description of *Haemoproteus iwa*. *Int J Parasitol* 41:1019–1027
- Levin II, Adkesson MJ, Evans M, Rettke CK, Parker PG (2014) No evidence for Galapagos *Plasmodium* lineage arriving via Humboldt Current seabirds. *Pac Conserv Biol* 20:37–40
- Mackerras MJ, Mackerras IM (1960) The haematozoa of Australian birds. *Aust J Zool* 8:226–260
- Manimegalai K, Sukanya S (2014) Biology of the filarial vector, *Culex quinquefasciatus* (Diptera: Culicidae). *Int J Curr Microbiol App Sci* 3:718–724
- Martínez J, Martínez-de la Puente J, Herrero J, del Cerro S, Lobato E, Rivero-de Aguilar J, Vásquez RA, Merino S (2009) A restriction site to differentiate *Plasmodium* and *Haemoproteus* infections in birds: on the inefficiency of general primers for detection of mixed infections. *Parasitology* 136:713–722
- Martínez J, Vásquez RA, Venegas C, Merino S (2015) Molecular characterisation of haemoparasites in forest birds from Robison Crusoe Island: is the austral thrush a potential threat to endemic birds? *Bird Conserv Int* 25:139–152
- Martínez-Abraín A, Esparza B, Oro D (2004) Lack of blood parasites in bird species: does absence of blood parasite vectors explain it all? *Ardeola* 5:225–232
- Marzal A (2012) Recent advances in studies on avian malaria parasites. In: Okwa O (ed) *Malaria parasites*. InTech Open, London, pp 135–158
- Mendes L, Piersma T, Lecoq M, Spaans B, Ricklefs RE (2005) Disease-limited distributions? Contrasts in the prevalence of avian malaria in shorebird species using marine and freshwater habitats. *Oikos* 109:396–404
- Merino S, Mínguez E (1998) Absence of hematozoa in a breeding colony of the storm petrel *Hydrobates pelagicus*. *IBIS* 140:180–181
- Merino S, Potti J (1995) High prevalence of hematozoa in nestlings of a passerine species, the pied flycatcher (*Ficedula hypoleuca*). *Auk* 112:1041–1043
- Merino S, Barbosa A, Moreno J, Potti J (1997) Absence of haematozoa in a wild chinstrap penguin *Pygoscelis* Antarctica population. *Polar Biol* 18:227–228
- Merino S, Moreno J, Sanz JJ, Arriero E (2000) Are avian blood parasites pathogenic in the wild? A medication experiment in blue tits (*Parus caeruleus*). *Proc Biol Sci* 267:2507–2510
- Merino S, Moreno J, Vásquez RA, Martínez J, Sánchez-Monsálvez I, Estades CF, Ippi S, Sabat P, Rozzi R, McGehee S (2008) Haematozoa in forest birds from southern Chile: latitudinal gradients in prevalence and parasite lineage richness. *Austral Ecol* 33:329–340
- Merino S, Hennicke J, Martínez J, Ludynia K, Torres R, Work TM, Stroud S, Masello JF, Quillfeldt P (2012) Infection by *Haemoproteus* parasites in four species of frigatebirds and the description of a new species of *Haemoproteus* (Haemosporida: Haemoproteidae). *J Parasitol* 98:388–397
- Moens MAJ, Valkiunas G, Paca A, Bonaccorso E, Aguirre N, Pérez-Tris J (2016) Parasite specialization in a unique habitat: hummingbirds as reservoirs of generalist blood parasites of Andean birds. *J Anim Ecol* 85:1234–1245
- Outlaw DC, Ricklefs RE (2011) Rerooting the evolutionary tree of malaria parasites. *PNAS* 108:13183–13187
- Padilla LR, Whiteman NK, Merkel J, Huyvaert KP, Parker PG (2006) Health assessment of seabirds on Isla Genovesa, Galápagos Island. *Ornithol Monogr* 60:86–97
- Parsons NJ, Peirce MA, Strauss V (2010) New species of haematozoa in Phalacrocoracidae and Stercorariidae in South Africa. *Ostrich* 81:103–108
- Parsons NJ, Voogt NM, Schaefer AM, Peirce MA, Vanstreels RET (2017) Occurrence of blood parasites in seabirds for rehabilitation in the Western Cape, South Africa, 2001–2013. *Vet Parasitol* 233:52–61
- Peirce MA (2005) A checklist of the valid avian species of *Babesia* (Apicomplexa: Piroplasmorida), *Haemoproteus*, *Leucocytozoon* (Apicomplexa: Haemosporida), and *Hepatozoon* (Apicomplexa: Haemogregarinidae). *J Nat Hist* 39:3621–3632
- Peirce MA, Brooke M (1993) Failure to detect blood parasites in seabirds from the Pitcairn Islands. *Seabird* 15:72–74
- Pérez-Rodríguez A, Ramírez Á, Richardson DS, Pérez-Tris J (2013) Evolution of parasite island syndromes without long-term host population isolation: parasite dynamics in Macaronesian blackcaps *Sylvia atricapilla*. *Glob Ecol Biogeogr* 22:1272–1281
- Perkins SL, Schall J (2002) A molecular phylogeny of malarial parasites recovered from cytochrome b gene sequences. *J Parasitol* 88:972–978
- Piersma T (1997) Do global patterns of habitat use and migration strategies co-evolve with relative investment in immunocompetence due to spatial variation in parasites pressure? *Oikos* 80:623–631
- Quillfeldt P, Martínez J, Hennicke J, Ludynia K, Gladbach A, Masello JF, Riou S, Merino S (2010) Hemosporidian blood parasites in seabirds—a comparative genetic study of species from Antarctic to tropical habitats. *Naturwissenschaften* 97:809–817
- Quillfeldt P, Arriero E, Martínez J, Masello JF, Merino S (2011) Prevalence of blood parasites in seabirds—a review. *Front Zool* 8:26
- Quillfeldt P, Martínez J, Bugoni L, Mancini P, Merino S (2014) Blood parasites in noddies and boobies from Brazilian offshore islands—differences between species and influence of nesting habitat. *Parasitology* 141:399–410
- Quintana F, Yorío P, Borboroglu PG (2002) Aspects of the breeding biology of the Neotropical cormorant *Phalacrocorax olivaceus* at Golfo San Jorge, Argentina. *Mar Ornithol* 30:25–29
- Ricklefs RE, Fallon SM, Bermingham E (2004) Evolutionary relationships, cospeciation, and host switching in avian malaria parasites. *Syst Biol* 53:111–119
- Rodrigues P, Campos E, Micael J, Verdugo C (in press) Sex determination of Neotropical cormorant (*Phalacrocorax brasilianus*) by molecular sexing. *Avian Biol Res*
- Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, M a S, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst Biol* 61:539–542
- Sallaberry-Pincheira N, Gonzalez-Acuña D, Herrera-Tello Y, Dantas GPM, Luna-Jorquera G, Frere E, Valdés-Velasquez A, Simeone A, Vianna J (2015) Molecular epidemiology of avian malaria in wild breeding colonies of Humboldt and Magellanic penguins in South America. *EcoHealth* 12:267–277

- Seimon TA, Gilbert M, Neabore S, Hollinger C, Tomaszewicz A, Newton A, Chang T, McAloose D (2016) Avian haemosporidian parasite lineages in four species of free-ranging migratory waterbirds from Mongolia, 2008. *J Wildl Dis* 52:682–687
- Sepulveda MS, Palma RL, Ochoa-Acuna H (1997) New records of feather lice from some seabirds in Chile. *J Wildl Dis* 33:371–372
- Soares L, Escudero G, Penha V, Ricklefs RE (2016) Low prevalence of haemosporidian parasites in shorebirds. *Ardea* 104:129–141
- Stotz DF, Fitzpatrick J, Parker T, Moskovits D (1996) Neotropical birds. In: Ecology and conservation. The University of Chicago Press, Chicago
- Torres P, Ruiz E, Gesche W, Montefusco A (1991) Gastrointestinal helminths of fish-eating birds from Chiloe Island, Chile. *J Wildl Dis* 27: 178–179
- Torres P, Schlatter R, Montefusco A, Gesche W, Ruiz E, Contreras A (1993) Helminth parasites of piscivorous birds from lakes in the south of Chile. *Mem Inst Oswaldo Cruz* 88:341–343
- Torres P, Valdivieso J, Schlatter R, Montefusco A, Revenga J, Marín F, Lamilla J, Ramallo G (2000) Infection by *Contraecaecum rudolphii* (Nematoda: Anisakidae) in the Neotropical cormorant *Phalacrocorax brasilianus*, and fishes from the estuary of the Valdivia river, Chile. *Stud Neotropical Fauna Environ* 35:101–108
- Torres P, Ortega J, Schlatter R (2005) Nematode parasites of the digestive tract in Neotropical cormorant chicks (*Phalacrocorax brasilianus*) from the River Cruces Ramsar site in southern Chile. *Parasitol Res* 97:103–107
- Valera F, Carrillo CM, Barbosa A, Moreno E (2003) Low prevalence of haematozoa in trumpeter finches *Bucanetes githagineus* from South-Eastern Spain: additional support for a restricted distribution of blood parasites in arid lands. *J Arid Environ* 55:209–213
- Valkiunas G (2005) Avian malaria parasites and other haemosporidia. CRC Press, Boca Raton
- Verdugo C, Pinto A, Ariyama N, Moroni M, Hernandez C (2019) Molecular identification of avian viruses in Neotropical cormorants (*Phalacrocorax brasilianus*). *J Wildl Dis* 51. doi: <https://doi.org/10.7589/2017-10-256>
- Waldenström J, Bensch S, Kiboi S, Hasselquist D, Ottosson U (2002) Cross-species infection of blood parasites between resident and migratory songbirds in Africa. *Mol Ecol* 11:1545–1554
- Waldenström J, Bensch S, Hasselquist D, Ostman O (2004) A new nested polymerase chain reaction method very efficient in detecting *Plasmodium* and *Haemoproteus* infections from avian blood. *J Parasitol* 90:191–194
- Whiteman NK, Sánchez P, Merkel J, Klompen H, Parker PG (2006) Cryptic host specificity of an avian skin mite (Epidermoptidae) vectored by louse flies (Hippoboscidae) associated with two endemic Galápagos bird species. *J Parasitol* 92:1218–1228
- Yorio P, Quintana F, Campagna C, Harris G (1994) Diversidad, abundancia y dinámica espacio-temporal de la colonia mixta de aves marinas en Punta León, Patagonia. *Ornitol Neotrop* 5:69–77
- Zuk M, McKean KA (1996) Sex differences in parasite infections: patterns and processes. *Int J Parasitol* 26:1009–1024