International Journal for Parasitology 44 (2014) 329–338

Contents lists available at ScienceDirect

International Journal for Parasitology

journal homepage: www.elsevier.com/locate/ijpara

A review of global diversity in avian haemosporidians (*Plasmodium* and *Haemoproteus*: Haemosporida): new insights from molecular data

Nicholas J. Clark [a,b,*], Sonya M. Clegg [a], Marcos R. Lima [c]

[a] Environmental Futures Centre, School of Environment, Griffith University, Gold Coast Campus, Queensland 4222, Australia
[b] Natural Environments Program, Queensland Museum, PO Box 3300, South Brisbane, Queensland 4101, Australia
[c] Departamento de Ciências Fisiológicas – IB, Pós-Graduação em Biologia Animal, Universidade de Brasília, Brazil

ARTICLE INFO

Article history:
Received 8 December 2013
Received in revised form 21 January 2014
Accepted 23 January 2014
Available online 18 February 2014

Keywords:
Plasmodium
Haemoproteus
Avian malaria
Parasite diversity
PCR
Microscopy

ABSTRACT

Biogeographic patterns of parasite diversity are useful for determining how host–parasite interactions can influence speciation. However, variation in methodologies and sampling effort can skew diversity estimates. Avian haemosporidians are vector-transmitted blood parasites represented by over 1300 unique genetic lineages spread across over 40 countries. We used a global database of lineage distributions for two avian haemosporidian genera, *Plasmodium* and *Haemoproteus*, to test for congruence of diversity among haemosporidians and their avian hosts across 13 geographic regions. We demonstrated that avian haemosporidians exhibit similar diversity patterns to their avian hosts; however, specific patterns differ between genera. *Haemoproteus* spp. diversity estimates were significantly higher than those of *Plasmodium* spp. in all areas where the genera co-occurred, apart from the *Plasmodium* spp.-rich region of South America. The geographic distributions of parasite genera also differed, with *Haemoproteus* spp. absent from the majority of oceanic regions while *Plasmodium* spp. were cosmopolitan. These findings suggest fundamental differences in the way avian haemosporidians diverge and colonise new communities. Nevertheless, a review of the literature suggests that accurate estimates of avian haemosporidian diversity patterns are limited by (i) a concentration of sampling towards passerines from Europe and North America, (ii) a frequent failure to include microscopic techniques together with molecular screening and (iii) a paucity of studies investigating distributions across vector hosts.

1. Introduction

Global patterns of species diversity can yield important insights into the ecological and environmental mechanisms that promote diversification (Orme et al., 2005; Grenyer et al., 2006). For many taxa, high levels of diversity typically occur in biogeographic 'hotspots', which are generally centred on tropical regions in low latitudes (Gaston and Blackburn, 2000). In addition to climate and latitude, landmass also appears to play a role in regulating species diversity, with island communities often exhibiting reduced diversity compared with continental regions (MacArthur and Wilson, 1967; Diamond, 1975). However, these patterns are not always congruent across taxa, suggesting that different mechanisms may be important for maintaining different facets of biodiversity (Orme et al., 2005). For parasitic organisms, biogeographic patterns may be useful for understanding the evolutionary and ecological implications of host–parasite interactions and how they can influence parasite diversity (Krasnov et al., 2007; Ishtiaq et al., 2010; Jenkins and Owens, 2011). For instance, vector-borne protozoan parasites of primates exhibit higher richness towards tropical regions, which may be the result of higher abundance and diversity of biting arthropods that act as vectors (Nunn et al., 2005). This diversity of resources (i.e. hosts) may promote parasite diversification through co-speciation (Poulin, 2011) and permit the coexistence of a larger range of parasite species (Krasnov et al., 2007). However, despite their utility for interpreting mechanisms of speciation, global analyses of pathogen diversity are scarce (Brooks and Hoberg, 2001; Bordes et al., 2010). In this study we describe global diversity patterns for two commonly studied genera of avian blood parasites and investigate whether avian host diversity patterns can predict parasite diversity. We also conducted a literature review in order to identify emerging methodological patterns from a rapidly expanding field. In doing so, we identify geographical sampling gaps and methodological points that need to be considered as empirical data is amassed to allow global perspectives on haemosporidian diversity to be refined and tested.

Descriptions of large-scale biogeographic patterns for wildlife pathogens require sensitive methodologies and adequate sampling

* Corresponding author at: Environmental Futures Centre, School of Environment, Griffith University, Gold Coast Campus, Queensland 4222, Australia. Tel.: +61 432420979.
E-mail address: n.clark@griffith.edu.au (N.J. Clark).

http://dx.doi.org/10.1016/j.ijpara.2014.01.004
0020-7519/

regimes. However, due to the small size and often low prevalences of some pathogens, their diversity is intrinsically difficult to determine, particularly across large geographic scales or in diverse host communities (Brooks and Hoberg, 2001; Rohde, 2002). Moreover, variations in methodologies and inadequate sampling in potentially diverse geographic areas can skew estimates of pathogen diversity (Poulin, 2004). To put global patterns into context, we need to consider geographic and host-species sampling coverage as well as the contributions and limitations of different sampling methodologies. Methodical literature reviews can be useful for highlighting such gaps in sampling (Pickering and Byrne, 2013).

Avian malaria (*Plasmodium* spp.) and other haemosporidians (*Haemoproteus* and *Leucocytozoon* spp.) are a diverse group of vector-transmitted blood parasites that are abundant in most avian families (Valkiūnas, 2005). Traditionally the life-history, morphology and classification of avian haemosporidians have been studied using light microscopy (Valkiūnas, 2005). However, the recent adoption of molecular methods using PCRs to identify infections has led to a surge in research, revealing fascinating insights into the parasites' genetic diversity and host-specificity (Hellgren et al., 2009; Ventim et al., 2012). Additionally, the design of nested PCR primers to screen for infections has led to improved detection efficiency, particularly for chronic infections that typically have low levels of parasitaemia (Waldenström et al., 2004). Molecular studies have identified over 1300 unique avian haemosporidian lineages, prompting the creation of a coordinated database (MalAvi, http://mbio-serv2.mbioekol.lu.se/Malavi) to record the distributions of lineages and facilitate the investigation of global patterns (Bensch et al., 2009). For instance, MalAvi submissions have revealed that the dominant avian *Plasmodium* lineage found in Hawaii, GRW4, has been recorded in hosts spanning multiple avian families and across geographic regions from mainland USA to French Polynesia (Beadell et al., 2006; Ishtiaq et al., 2006; Marzal et al., 2011). While recent reviews of this rapidly emerging field have been conducted, they have focussed primarily on the advantages and disadvantages of molecular and microscopic methods (Braga et al., 2011; Marzal, 2012). For example, recent findings indicate a vast underestimation of mixed species haemosporidian infections using common PCR methods, highlighting limitations in the current methodologies used to identify and describe mixed infections (Valkiūnas et al., 2006; Martinez et al., 2009). However, since the advent of PCR detection protocols and the creation of the MalAvi database, no large-scale published analyses of avian haemosporidian diversity have been carried out. The objective of this study is to systematically document the results from molecular-based avian haemosporidian research in order to (i) describe biogeographic patterns of diversity for avian haemosporidians (*Plasmodium* and *Haemoproteus* spp.), (ii) determine how representative sampling for avian haemosporidians has been with respect to geography and host-species assemblages and (iii) document trends in methodology to identify potential biases that may influence interpretations of avian haemosporidian biogeography.

2. Materials and methods

2.1. Observed and estimated lineage richness

To describe patterns in the diversity and distribution of avian haemosporidians, we extracted observed lineage diversity information from the MalAvi database (Bensch et al., 2009). In this database, unique haemosporidian lineages are identified based on sequence data from a fragment of the cytochrome-*b* (cyt-*b*) gene that is commonly targeted in molecular studies of avian haemosporidians (Waldenström et al., 2004), reducing the risk of using multiple names for identical lineages. We recorded lineage occurrence information using the 'Hosts and Sites' query in the MalAvi database to record the country where sampling was conducted and the academic reference for the occurrence of each lineage (Bensch et al., 2009). To maximise the dataset, cyt-*b* lineages that were amplified from a different region of the parasite cyt-*b* gene were included (Fallon et al., 2003, 2004, 2005; Belo et al., 2011, 2012; Fecchio et al., 2013; Svensson-Coelho et al., 2013). Finally, unpublished lineage occurrence information was gathered directly from researchers (A. Marzal, Universidad de Extremadura, Spain; S. Olsson-Pons, Griffith University, Australia; and F. Ishtiaq, Indian Institute of Science, India). Due to the lack of lineages recorded within a number of countries, we grouped sampling locations into broader geographic regions in order to describe large-scale patterns in lineage diversity (Table 1). Observed lineage recordings were used to generate an estimate of undiscovered lineage diversity for *Plasmodium* and *Haemoproteus* spp. in each geographic region using the non-parametric Chao2 estimator, which takes into account the frequency of each observed lineage (Chao et al., 2005). Chao2 diversity estimates were generated using 1,000 randomisations in EstimateS v. 8.2 (http://viceroy.eeb.uconn.edu/estimates).

For avian haemosporidians, global diversity patterns could reflect higher diversity of potential avian and vector hosts, both of which exhibit increased richness in biogeographical hotspot regions around low latitudes (Grenyer et al., 2006; Foley et al., 2007). An ideal test to examine whether avian haemosporidians follow a similar pattern would be to map the latitude and longitude of infection occurrences for each parasite lineage. Unfortunately, adequate location records are not available for the majority of haemosporidian lineages. Therefore, to facilitate statistical comparisons among biogeographic regions, we utilised a global database of the distributions of breeding avian species (sourced from Orme et al., 2005) to delineate geographic regions into three categories of avian diversity (continental avian hotspot, continental avian non-hotspot and oceanic; Table 1). The continental avian hotspot and continental avian non-hotspot regions naturally fell into categories of 300 – 1,000+ avian species and 60–299 avian species, respectively. One-way analyses of variance (ANOVAs) were then used to test for differences in the total observed lineage diversity among the three avian diversity categories. ANOVAs were also conducted to test for differences in estimated lineage diversity among categories using mean Chao2 diversity estimates for each sampling region. Significant differences among categories were determined using Tukey's post-hoc Honest Significant Difference (HSD) tests and differences were considered to be significant when $P < 0.05$. Each ANOVA was carried out for *Plasmodium* and *Haemoproteus* spp. separately in Statistica 10.0 (www.statsoft.com).

2.2. Literature review

To assess bias and knowledge gaps arising from research methodologies, peer-reviewed research articles that used PCR to investigate avian haemosporidians were identified and examined. First, we searched the electronic databases Web of Science, ProQuest, Science Direct, PubMed and Google Scholar for articles published between 1995 and November 2012 using combinations of the following key words: 'avian', 'bird*', 'malaria', '*Haemoproteus*', '*Plasmodium*', '*Leucocytozoon*', 'disease', 'vector*', 'phylogenetic*', 'haematozoa*', 'parasite*', 'PCR' and 'blood'. The asterisk (*) operator was used as a wildcard to search for all possible variations of keywords. Reference lists of all articles were then checked for additional references that were not found in the primary searches. Studies using experimentally infected or captive birds were excluded, as we were interested in patterns of natural infections in wild hosts. A flowchart showing the article selection process is presented in Supplementary Fig. S1, while a list of the references included in the literature review is provided in Supplementary Data S1.

Table 1
Avian sample sizes and lineage diversity estimates for *Plasmodium* and *Haemoproteus* spp. among geographic regions. Regions were pooled into biogeographic groups based on avian richness hotspots: continental avian hotspot (300–1,000+ spp.), continental avian non-hotspot (60–299 spp.) and oceanic (Orme et al., 2005). The total number of avian samples screened and the number of observed lineages in each region were recorded from the MalAvi database (http://mbio-serv2.mbioekol.lu.se/Malavi) and a review of the literature. Estimates of lineage diversity were generated using the Chao2 diversity estimator.

Region	Avian richness group	Countries/territories sampled	# Samples[a]	Lineage diversity			
				Observed[b]		Estimated (S.D.)	
				Plas[c]	*Haem*[d]	*Plas*	*Haem*
Sub-Sahara	Hotspot	Nigeria, Cameroon, Gabon, Kenya, Zimbabwe, Ghana, South Africa	4501	96	122	171.36 (26.15)	286.95 (96.37)
India/SE. Asia	Hotspot	India, Burma (Myanmar), Phillipines	1468	34	39	79.00 (23.41)	250.47 (75.01)
S. America	Hotspot	Brazil, Ecuador, Guyana, Venezuela, Uraguay, Peru	8672	159	86	426.54 (48.24)	304.26 (82.25)
AUS/PNG	Hotspot	Australia, Papua New Guinea	857	30	87	97.00 (47.70)	202.30 (32.27)
E. Europe	Non-hotspot	Russia, Lithuania, Ukraine, Bulgaria, Romania, Poland, Czech Republic, Hungary	4651	31	83	59.50 (17.67)	152.03 (25.81)
W. Europe	Non-hotspot	UK, Spain, France, Germany, Italy, Norway, Sweden, Belgium	11,193	60	87	147.99 (41.83)	184.65 (34.47)
N. America	Non-hotspot	Mainland USA, Canada, Mexico	3245	49	38	89.00 (19.03)	168.15 (72.80)
Melanesia	Oceanic	New Caledonia, Vanuatu	2314	24	33	49.65 (18.36)	74.34 (25.63)
Caribbean	Oceanic	Lesser Antilles, Bermuda, Puerto Rico	2780	14	15	25.09 (11.42)	33.25 (15.27)
NZ	Oceanic	New Zealand	1102	8	0	11.72 (2.82)	0
N. Atlantic	Oceanic	Bioko, Madeira, Azores Islands	1661	4	1	4.37 (0.76)	1.27 (0.39)
Hawaii	Oceanic	Hawaiian Islands	320	2	0	1.48 (0.45)	0
French Polynesia	Oceanic	Society Islands, Marquesas	174	1	0	1.14 (0.38)	0

N, north; S, south; E, east; W, west; SE, southeast; AUS, Australia; PNG, Papua New Guinea.
[a] Some samples were screened for only one genus of parasite.
[b] Some lineages were shared among regions. Note *Haemoproteus columbae* and *Haemoproteus iwa* have been previously described from blood films in Hawaii (Valkiūnas, 2005).
[c] *Plasmodium.*
[d] *Haemoproteus.*

For each article, we recorded the following information: year of publication, host group investigated (i.e. avian, vector or both), haemosporidian genera screened and the methods used to describe infections. Methods were categorised based on the type of PCR carried out, the molecular marker(s) targeted and whether microscopy of blood films was used in addition to PCR. We also recorded whether mixed infections were separately characterised and the methods utilised. Although double peaks on chromatograms have been interpreted as evidence of mixed infections (Ricklefs et al., 2005; Kimura et al., 2006), such observations do not specifically describe the infection and these cases were therefore not recorded as an investigation of mixed infection. Finally, country, latitude and longitude of each sampling site were recorded and for avian host studies, the taxonomic orders and the total number of avian species sampled were also recorded.

3. Results

3.1. Biogeography of Plasmodium and Haemoproteus

We recorded the geographic occurrences for 599 unique cyt-*b* lineages of avian *Haemoproteus* and 488 lineages of avian *Plasmodium*. Lineages of *Plasmodium* have been recorded in every region analysed (Table 1; Fig. 1). In contrast, *Haemoproteus* lineages have not been detected from three of the six oceanic regions (New Zealand, Hawaii and French Polynesia). It should be noted that *Haemoproteus columbae* and *Haemoproteus iwa* have been previously described from blood films taken from Hawaiian birds (Valkiūnas, 2005); however, molecular screening of Hawaiian passerines has not established the presence or identity of any *Haemoproteus* lineages (Beadell et al., 2006; Bensch et al., 2009). For *Haemoproteus*, total observed lineages ranged from zero to 122 across regions and varied significantly among geographic categories of avian diversity (ANOVA; degrees of freedom (df) = 2, 10; $F = 10.86$; $P < 0.01$; Table 1). Tukey's post-hoc HSD tests revealed that continental avian hotspots and continental avian non-hotspots did not differ significantly; however, oceanic regions exhibited significantly fewer observed lineages than the other two categories (Fig. 2A). In contrast, Chao2 diversity estimates for *Haemoproteus* spp. varied significantly among all three categories (ANOVA; df = 2, 10; $F = 91.49$; $P < 0.001$), with continental avian hotspot regions exhibiting the highest *Haemoproteus* spp. estimates while oceanic regions exhibited the lowest estimates (Fig. 2B).

For *Plasmodium* spp., total observed lineages ranged from one to 159 across regions and varied significantly among geographic categories of avian diversity (ANOVA; df = 2, 10; $F = 6.01$; $P = 0.02$; Table 1), with significantly fewer observed lineages in oceanic regions than in continental avian hotspot and continental avian non-hotspot regions (Fig. 2C). Chao2 diversity estimates for *Plasmodium* spp. exhibited the same pattern as observed lineages, with significantly lower estimates in oceanic regions than in continental avian hotspot and continental avian non-hotspot regions (ANOVA; df = 2, 9; $F = 4.53$; $P < 0.04$; Fig. 2D).

For regions where *Haemoproteus* lineages were recorded, Chao2 diversity estimates tended to be higher for *Haemoproteus* spp. than for *Plasmodium* spp., apart from South America (Fig. 1), although this trend was not statistically significant (*t*-test, $t = 1.13$, df = 8, $P = 0.28$). However, when excluding South America, estimates for

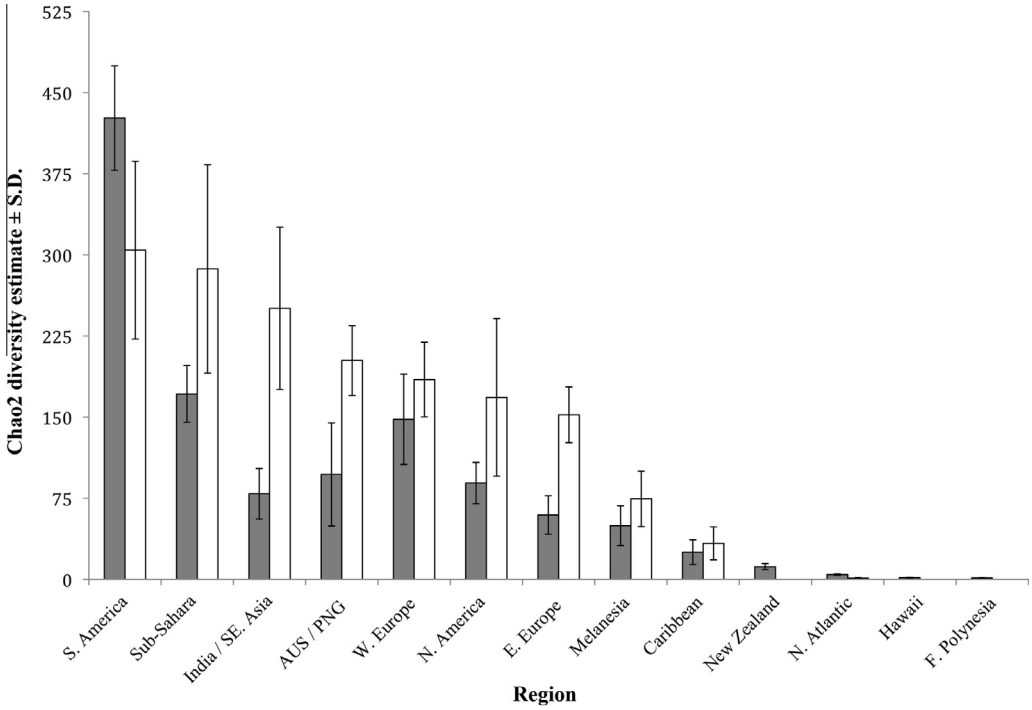

Fig. 1. Chao2 diversity estimates of avian malaria lineages among sampling regions for avian *Plasmodium* (grey bars) and *Haemoproteus* (white bars). Error bars indicate S.D.s. N, north; S, south; E, east; W, west; SE, southeast; AUS, Australia; PNG, Papua New Guinea; F, French.

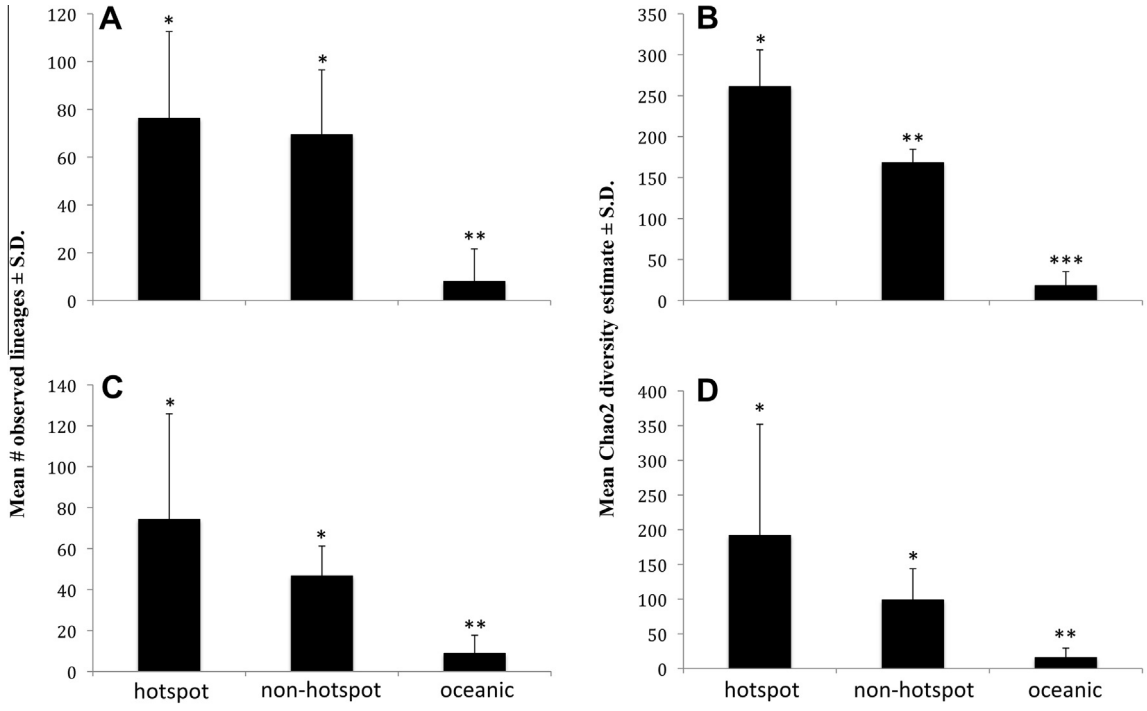

Fig. 2. Mean observed and estimated lineage diversity among avian diversity categories. (A) Observed *Haemoproteus* diversity; (B) estimated *Haemoproteus* diversity; (C) observed *Plasmodium* diversity; (D) estimated *Plasmodium* diversity. Asterisks represent significantly different groups determined from Tukey's post-hoc Honest Significant Difference tests.

Haemoproteus spp. diversity were significantly higher than *Plasmodium* spp. (df = 7, $t = 2.23$, $P = 0.04$).

3.2. Adequacy of geographic and host species sampling

We identified a total of 162 papers that used PCR to investigate avian haemosporidian infections. There has been a rapid rise in molecular avian malaria studies since the first publications in the mid-1990s, with most (98%) published after the year 2000 when cyt-*b* primers for avian *Plasmodium* and *Haemoproteus* spp. were designed. The majority of these publications (86.4%) examined avian host infection, while far fewer publications (16%) examined vector host infection (Table 2). Sampling locations for molecular studies of avian haemosporidians represent every continent and

over 100 countries (Fig. 3). However, there is a substantial geographical bias in the distribution of field collection sites, with half of all sampling sites located in continental avian non-hotspot regions in Europe and North America (170 out of 340 total; Fig. 3). Conversely, continental avian hotspot regions had a total of 88 sampling sites (22.9% of total; Fig. 3). A wide range of avian hosts have been studied, however the number of publications screening passerines (Order Passeriformes) was significantly higher than those screening other avian orders (χ^2 test; df = 19, χ^2 = 1033, $P < 0.01$; Table 1). The number of avian host species ranged from one to over 900 (mean 22.1 ± 7.5), with 41.5% of avian studies investigating a single host species and 18.5% of avian publications investigating 25 or more host species.

3.3. Trends in methodology

Nested PCR targeting cyt-*b* has been the dominant molecular screening method since 2004, used in 61.7% of all publications (Table 2). In contrast to mitochondrial screening, only 8.9% of publications used nuclear markers (Table 2). A total of six different nuclear markers have been used, however, only two of these markers (DHFR-TS and SSUrRNA) have been targeted by more than one avian host publication. To date, a single vector host study has used a nuclear marker (Table 2). Specific studies that targeted parasite genes other than cyt-*b* are highlighted in Supplementary Data S1. For both avian and vector hosts, far fewer studies screened for *Leucocytozoon* spp. than for either *Plasmodium* or *Haemoproteus* spp. (Table 2).

Less than half of all avian host publications (47.4%) included microscopy of blood films in conjunction with PCR (Table 2). A total of 16.3% of avian host publications have used multiple genetic markers to characterise infections while no vector host publications have used multiple markers (Table 2). Mixed species infections were specifically investigated in 30.7% of avian host publications, with microscopy the most frequently used method (Table 2). The single vector host publication that identified mixed infections used restriction enzyme digestion (Table 2).

Table 2
Molecular screening methods and the host–parasite systems examined in avian haemosporidian publications (1995–2012).

	Total[a]	Avian hosts	Vector hosts
	162	140	26
Genus targeted			
Plasmodium	146	127	22
Haemoproteus	126	115	15
Leucocytozoon	43	39	4
Mitochondrial marker(s)	154	133	25
Cyt-*b*	153	132	25
Cytochrome oxidase 3	5	5	0
Nuclear marker(s)	14	13	1
DHFR-TS	4	4	0
SSU-rRNA	9	8	1
DGAT	1	1	0
LSU-rRNA	1	1	0
TRAP	1	1	0
ClpC	1	1	0
Reaction methods			
Nested PCR	100	85	17
Quantitative PCR	9	7	2
PCR + 1	1	1	0
Mixed infections identified	44	43	1
Microscopy	19	19	0
Restriction enzyme digestion	11	10	1
TA cloning	14	14	0
Genus-specific primers	2	2	0
Avian orders screened			
Passeriformes	114	–	–
Columbiformes	21	–	–
Falconiformes	14	–	–
Strigiformes	11	–	–
Piciformes	10	–	–
Galliformes	9	–	–
Charadriiformes	9	–	–
Coraciiformes	5	–	–
Cuculiformes	5	–	–
Apodiformes	5	–	–
Other orders	22	–	–

[a] Some studies analysed avian and vector hosts simultaneously. Specific studies that targeted parasite genes other than cyt-*b* are highlighted in Supplementary Data S1.

4. Discussion

Our review of studies of avian haemosporidian cyt-*b* lineages indicated a high diversity of parasite lineages that are heterogeneously dispersed across biogeographic regions. Moreover, global patterns of observed and estimated lineage diversity varied between the two parasite genera, *Plasmodium* and *Haemoproteus*. There was a tendency for continental avian diversity hotspots to have higher estimates of lineage richness compared to avian non-hotspots and oceanic areas, both for *Haemoproteus* spp. and, to a lesser extent, *Plasmodium* spp. Our findings therefore reflect a biogeographic pattern of higher diversity in low latitude tropical areas, as has been widely recognised across a range of taxa, including parasites (Sherratt and Wilkinson, 2009). A number of theories have been proposed to explain the phenomenon of a latitudinal diversity gradient, most of which are probably not mutually exclusive (Pianka, 2011). For example, the relatively stable year-round climate in tropical areas has been suggested to promote ecosystem productivity (Phillips et al., 1994). For avian haemosporidians, temperature and precipitation are important abiotic variables that may be conducive to parasite diversification by promoting parasite development and vector breeding opportunities (Beier, 1998; Santiago-Alarcon et al., 2012b). However, tropical archipelagos appear to be limited in avian haemosporidian diversity, suggesting that climatic conditions alone do not drive diversity. Rather, haemosporidian diversity may be a function of avian and/or vector host diversity, both of which increase in tropical continental regions (Grenyer et al., 2006; Foley et al., 2007).

The tropics have also been suggested to have stronger biotic interactions than temperate habitats, with higher rates of parasitism or predation driving increased rates of speciation (Pitts and Roberts, 1997). This hypothesis of 'diversity begets diversity' is reliant on the principle that if new species evolve in an already hyper-diverse ecosystem, they are likely to remain in that ecosystem and maintain or even accelerate the diversity gradient (MacArthur and MacArthur, 1961; Hechinger and Lafferty, 2005; Sherratt and Wilkinson, 2009). For avian haemosporidians, many vectors feed on a variety of avian host species (Ejiri et al., 2011; Santiago-Alarcon et al., 2012a) suggesting that opportunities for a parasite to infect a naïve host (i.e. host-switching) should be higher in species-rich vector-host systems than in species-poor systems. Phylogenetic and molecular studies suggest that host switching is common in avian haemosporidians (Ricklefs et al., 2004; Krizanauskiene et al., 2006), a process that may be facilitated when potential hosts are phylogenetically close to the original host (Poulin, 2011). Therefore, high diversities of potential hosts could provide more opportunities for host switching and subsequent diversification (Hayakawa et al., 2008). Finally, the tropics may encourage faster rates of evolution either through shorter generation times or through the facilitation of sympatric speciation due to increased niche complexity (Mittelbach et al., 2007). Time-calibrated phylogenies can be useful for determining whether parasites in the tropics have diverged faster than their temperate counterparts

Fig. 3. Sampling locations used in published molecular avian haemosporidian studies from 1995 to April 2012. Geographic Information System layers for avian species richness data were sourced from Orme et al. (2005).

(Currie et al., 2004; Bordes et al., 2010). However, while molecular clocks for avian haemosporidians have been estimated (Ricklefs and Outlaw, 2010), the frequent host-shifts evident in the evolutionary history of these parasites make estimations of divergence rates particularly problematic (Bensch et al., 2013).

The higher lineage richness exhibited by *Haemoproteus* spp. compared with *Plasmodium* spp. and the different biogeographic patterns exhibited by the two genera suggest fundamental differences in the way lineages from the two genera diverge. Lineage diversity may result from high degrees of host specialisation, a life-history strategy that promotes the partitioning of resources (i.e. avian and vector hosts) and facilitates species coexistence (Lewinsohn and Roslin, 2008). Host occurrence information from a number of studies suggests that *Haemoproteus* spp. tends to be more host-specific compared with *Plasmodium* spp. (Ishtiaq et al., 2007, 2010; Beadell et al., 2009; Dimitrov et al., 2010), which may explain why estimates of *Haemoproteus* spp. diversity were consistently higher than *Plasmodium* spp. estimates. Greater partitioning of resources could also explain why *Haemoproteus* spp. diversity significantly increased in continental regions with higher avian host diversity while *Plasmodium* spp. diversity did not. With more potential avian hosts available in the tropics, the relative abundance of hosts may promote niche-specialisation, particularly for parasites that demonstrate a proclivity for host specialisation (Norton and De Lange, 1999). Alternatively, *Haemoproteus* spp. infections may simply be easier to detect using molecular techniques. For example, the duration of relapses (i.e. secondary parasitaemia in the host blood), tends to be longer in *Haemoproteus* spp. (up to several months) compared with generally shorter relapse durations in *Plasmodium* spp. (Valkiūnas, 2005). Because PCR can fail to amplify DNA when parasite intensity is very low (Waldenström et al., 2004), it is possible that the reported higher richness of *Haemoproteus* lineages is partially an artefact of the longer window of opportunity to detect secondary parasitaemias.

An exception to the pattern of higher diversity for *Haemoproteus* lineages was displayed in South America. This region exhibited considerably more lineage recordings and higher lineage diversity estimates for *Plasmodium* spp. than any other region. This high richness of *Plasmodium* lineages in South America is well exemplified by two particular studies, one in southeastern Brazil (Lacorte et al., 2013), and the other in the western Amazon of Ecuador (Svensson-Coelho et al., 2013). *Plasmodium* lineages sampled from the western Amazon of Ecuador were more specialised than *Haemoproteus* linages (Svensson-Coelho et al., 2013), while in southeastern Brazil, over 55 *Plasmodium* lineages exhibited host ranges of only one species (Lacorte et al., 2013). This suggests that *Plasmodium* spp. may have experienced an exceptional radiation alongside avian hosts in South America's ecologically diverse tropical habitats. However, even though South America contains the most speciose avian and mosquito communities in the world (Grenyer et al., 2006; Rueda, 2008), this does not explain why *Haemoproteus* lineage diversity was not similarly escalated in this region. It may be possible that *Plasmodium* lineages in South America are phylogenetically distinct and contain particular cyt-*b* sequences that increase the rate of detection over *Haemoproteus* lineages during mixed infections (see Valkiūnas et al., 2006). However, more sampling is needed in South America's diverse habitats to improve assessments of avian haemosporidian diversity and to certify the existence of these unique biogeographical patterns.

The lack of *Haemoproteus* lineages on some archipelagoes (e.g. Hawaii, French Polynesia) may be due to the absence of appropriate vectors or to founder events where low numbers of colonisers are less likely to carry infections (Peirce and Adlard, 2004; Krasnov et al., 2007; Hellgren et al., 2011). For *Plasmodium* spp., the occurrence of lineages on south Pacific islands such as Hawaii, French Polynesia and New Zealand are thought to be in large part due to anthropogenic introductions of infected hosts and suitable vectors (Beadell et al., 2006; Tompkins and Gleeson, 2006; Ewen et al., 2012). However for *Haemoproteus* spp., the complete life cycles, specifically transmission dynamics between avian and vector hosts, have been poorly studied (but see Ishtiaq et al., 2008; Valkiūnas et al., 2010; Martínez-de la Puente et al., 2011). Further

investigation into vector transmission will help to determine whether a lack of suitable vectors has led to the absence of *Haemoproteus* spp. on certain islands.

Another hindrance to colonisation of *Haemoproteus* spp. could be higher degrees of avian host specialisation for *Haemoproteus* lineages compared with *Plasmodium* lineages and, therefore, *Haemoproteus* spp. may have difficulty switching to new avian hosts in isolated island communities. Nevertheless, there are numerous cases of generalist *Haemoproteus* lineages recorded from avian hosts across broad geographic ranges including islands (Fallon et al., 2005; Beadell et al., 2009). It is more plausible that *Haemoproteus* spp. are absent on islands that are simply too isolated for natural colonisation of these parasites to occur without anthropogenic introductions of infected hosts and / or suitable vectors (e.g. Pérez-Rodríguez et al., 2013). Regardless of the mechanisms limiting *Haemoproteus* spp. distributions on islands, continued monitoring for introduced haemosporidian parasites will be valuable as the threat of emerging wildlife disease accelerates with the increasing mobility of humans (Huijben et al., 2007).

Diversity patterns from molecular studies are supported by studies using only microscopic data, with higher *Haemoproteus* spp. richness for the majority of zoogeographical regions (Valkiūnas, 2005). For example, Bennett et al. (1992) found that sub-Saharan birds have a higher *Haemoproteus* spp. richness (63 species) compared with *Plasmodium* (16 species). The same pattern was found in western Europe, with the occurrence of 28 *Haemoproteus* spp. and 12 *Plasmodium* spp. (Peirce, 1981). In addition, some microscopic evidence from the Neotropics suggests that avian hosts in both the Amazon basin and central America have a higher prevalence of *Plasmodium* spp. than of *Haemoproteus* spp. (White et al., 1978). However, our analysis of cyt-*b* lineages suggests variations across biogeographic regions that were not previously noted using microscopy alone (Valkiūnas, 2005), such as the high diversity of *Plasmodium* spp. in the neotropical region and the higher diversity of haemosporidians in the tropics. These differences may therefore have arisen from infections overlooked due to the often low parasitaemias in natural infections.

The high estimates of undiscovered richness coupled with relatively limited sampling in many regions with high avian diversity indicate a need for further molecular studies, which should ideally be complemented with microscopic methods. However, the spread of current research suggests a bias towards European and North American passeriform communities. While a wealth of information regarding the prevalence, seasonality, diversity and host distribution of avian haemosporidians has been generated in these host–parasite communities (Krizanauskiene et al., 2006; Bensch et al., 2007; Shurulinkov and Ilieva, 2009), such knowledge is currently limited elsewhere. In Australia, for example, morphological descriptions previously indicated a paucity of haemosporidian species, interpreted as a result of limited colonisation and restricted diversification as well as the limited number of microscopic studies in the region (Valkiūnas, 2005). However, it is becoming increasingly apparent that Australia harbours high haemosporidian lineage diversity, particularly for *Haemoproteus* spp., even though Australian studies have been restricted to two major community studies in the north (Beadell et al., 2004; Zamora-Vilchis et al., 2012) and one study in the south (Balasubramaniam et al., 2013). Additionally, while Passeriformes is the most diverse of the avian orders (Hackett et al., 2008) and likely contains the most diverse haemosporidian fauna (Valkiūnas, 2005), sampling from other orders, such as Columbiformes, Falconiformes and Strigiformes, has revealed high diversity and cryptic speciation of haemosporidians (Sehgal et al., 2006; Santiago-Alarcon et al., 2010; Valkiūnas et al., 2010; Yildirim et al., 2013). The limited sampling in species-rich habitats and among a range of avian host orders likely hampers descriptions of the overall diversity and the phylogenetic relationships of haemosporidians.

The creation of the MalAvi database has encouraged the widespread use of a single nested PCR protocol, leading to methodological conformity among the majority of molecular studies and aiding our understanding of haemosporidian diversity. While it may be suggested that the dearth of nuclear investigations presents a hindrance to true estimates of parasite diversity, evidence from simultaneous nuclear and mitochondrial studies suggests that most cyt-*b* lineages probably represent reproductively isolated entities (Bensch et al., 2004; Beadell et al., 2009). However, this rapid increase in molecular studies may be leading a shift away from more traditional parasitological methods, such as rigorous microscopic examinations (Braga et al., 2011). It is therefore important to emphasise that our conclusions are based on lineage detection using PCR methods. This method has a very low detection threshold and is capable of amplifying DNA from different life stages of haemosporidians (Valkiūnas et al., 2009), even when parasites are present in non-competent hosts. This can be a problem because sporozoites, which are injected by dipteran vectors into the avian hosts' blood stream, can persist for some time in the peripheral bloodstream without developing into a full infection (Valkiūnas, 2005). Although such abortive infections can limit our understanding of parasite–host transmission dynamics, inference of biogeographical patterns is less affected as these types of infections still inform the number of existing lineages per region.

In addition to confirming vector and host competence, microscopy together with PCR can link genetic and morphological descriptions of parasite diversity, which is crucial for phylogeographic studies and should lead to a better understanding of the evolutionary histories of avian haemosporidians (Martinsen et al., 2006; Palinauskas et al., 2007). However, such studies are lacking in many host–parasite communities, leading to a poor understanding of the true diversity of these parasites (Braga et al., 2011). The dynamics and frequency of mixed infections may also be overlooked without microscopic analysis (Valkiūnas et al., 2006, 2008), which may also limit our knowledge of haemosporidian diversity. Examination of blood films together with PCR remains the ideal approach when characterising infections from avian hosts.

The paucity of studies analysing haemosporidian infections in vectors may lead to a limited understanding of the true diversity of lineages as well as the dynamics of haemosporidian transmission (Santiago-Alarcon et al., 2012b). For instance, recent molecular surveys in southern Melanesia recorded numerous lineages of *Plasmodium* and *Haemoproteus* spp. in vectors that have not yet been recovered from avian hosts in the region (Ishtiaq et al., 2008, 2010). Because sexual reproduction occurs in the vector host (Valkiūnas, 2005), vectors are vital for the reproductive isolation of haemosporidian species (Gager et al., 2008). Therefore, it is fundamental to establish vector competence, which at the moment is only possible with the use of microscopy that allows the observation of sporozoites. Studies of vector host-specificity may therefore help to determine the role of vectors in driving lineage distributions among avian hosts (Medeiros et al., 2013). However, vector feeding patterns can be complex, particularly since some species seem to adjust their feeding preferences according to host availability (Santiago-Alarcon et al., 2012b). Some studies have recorded a tendency for vector host-specialisation among lineages, suggesting that vectors can act as ecological barriers by restricting lineages to avian hosts that belong to the vector's diet (Ejiri et al., 2008; Hellgren et al., 2008). This suggests that differences in parasite prevalence among different habitats may be strongly related to the presence or absence of suitable vectors (Mendes et al., 2005; Svensson and Ricklefs, 2009; Yohannes et al., 2009). It is clear that

a great deal more remains to be discovered about vector host-specialisation and its role in driving parasite diversification (Santiago-Alarcon et al., 2012b).

While nested PCR targeting of cyt-*b* has been instrumental to uncovering avian haemosporidian diversity across the globe, future studies may benefit from the use of variable nuclear markers that are already widely used in human malaria studies, such as merozoite surface protein-1 (Silva et al., 2000; Hellgren et al., 2013). The development of such markers for avian haemosporidian studies could lead to a better understanding of host-specificity and geographic limitations for parasite lineages as well as more sophisticated taxonomic revisions (e.g. Pick et al., 2011; Tachibana et al., 2012). However, no complete genomes for avian haemosporidians are currently available, (but see incomplete genome for *Plasmodium gallinaceum*; Wellcome Trust Sanger Institute, UK), owing primarily to the difficulty of obtaining adequate concentrations of parasite DNA from avian blood samples (Palinauskas et al., 2010). As methods for collection of pure parasite template improve, we can expect avian haemosporidian studies to branch into more fine-scale population genetics as well as more informative phylogenetic analyses of relatedness among haemosporidians of vertebrates (Bensch et al., 2013). However, the identification of competent vector and avian hosts relies on widespread utilisation of traditional parasitological methods, particularly as PCR detection of haemosporidian DNA can occur despite abortive development in the avian host. Moreover, because remnants of infected blood cells and the presence of oocysts and ookinetes in the thorax of vectors can also provide parasite DNA template, positive parasite detection from the thorax of vectors species is inconclusive evidence for parasite transmission (Valkiūnas et al., 2013). Therefore, microscopic methods are still an essential tool, both for determining the vector species responsible for parasite transmission and for generating a more complete understanding of haemosporidian diversity.

Acknowledgements

We thank two anonymous reviewers for comments that improved the manuscript. We are grateful to D. Orme for providing global avian richness data and to S. Bensch, B Canbäck and M. Egerhill for maintaining the MalAvi Database. We also thank A. Marzal, S. Olsson-Pons and F. Ishtiaq for providing additional lineage distribution information. Finally, we would like to thank the Malaria Research Coordination Network (supported by the US National Science Foundation) for their facilitation of collaborative meetings on haemosporidian research, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for a PNPD fellowship to MRL and the Griffith University School of Environment and Environmental Futures Centre, Australia, for funding to NC to attend the 2012 Malaria RCN meeting.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijpara.2014.01.004.

References

Balasubramaniam, S., Mulder, R.A., Sunnucks, P., Pavlova, A., Amos, J.N., Melville, J., 2013. Prevalence and diversity of avian haematozoa in three species of Australian passerine. Emu 113, 353–358.

Beadell, J.S., Covas, R., Gebhard, C., Ishtiaq, F., Melo, M., Schmidt, B.K., Perkins, S.L., Graves, G.R., Fleischer, R.C., 2009. Host associations and evolutionary relationships of avian blood parasites from West Africa. Int. J. Parasitol. 39, 257–266.

Beadell, J.S., Gering, E., Austin, J., Dumbacher, J.P., Peirce, M.A., Pratt, T.K., Atkinson, C.T., Fleischer, R.C., 2004. Prevalence and differential host-specificity of two avian blood parasite genera in the Australo-Papuan region. Mol. Ecol. 13, 3829–3844.

Beadell, J.S., Ishtiaq, F., Covas, R., Melo, M., Warren, B.H., Atkinson, C.T., Bensch, S., Graves, G.R., Jhala, Y.V., Peirce, M.A., Rahmani, A.R., Fonseca, D.M., Fleischer, R.C., 2006. Global phylogeographic limits of Hawaii's avian malaria. Proc. R. Soc. Lond. B Biol. Sci. 273, 2935–2944.

Beier, J.C., 1998. Malaria parasite development in mosquitoes. Ann. Rev. Entomol. 43, 519–543.

Belo, N.O., Pinheiro, R.T., Reis, E.S., Ricklefs, R.E., Braga, E.M., 2011. Prevalence and lineage diversity of avian aaemosporidians from three distinct cerrado habitats in Brazil. PLoS One 6 (3), e17654.

Belo, N.O., Rodriguez-Ferraro, A., Braga, E.M., Ricklefs, R.E., 2012. Diversity of avian haemosporidians in arid zones of northern Venezuela. Parasitology 139, 1021–1028.

Bennett, G.F., Earle, R.A., Dutoit, H., Huchzermeyer, F.W., 1992. A host-parasite catalog of the hematozoa of the sub-Saharan birds. Onderstepoort J. Vet. 59, 1–73.

Bensch, S., Hellgren, O., Krizanaskiene, A., Palinauskas, V., Valkiūnas, G., Outlaw, D., Ricklefs, R.E., 2013. How can we determine the molecular clock of malaria parasites? Trends Parasitol. 29, 363–369.

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.

Bensch, S., Pérez-Tris, J., Waldenström, J., Hellgren, O., 2004. Linkage between nuclear and mitochondrial DNA sequences in avian malaria parasites: multiple cases of cryptic speciation? Evolution 58, 1617–1621.

Bensch, S., Waldenstrom, J., Jonzen, N., Westerdahl, H., Hansson, B., Sejberg, D., Hasselquist, D., 2007. Temporal dynamics and diversity of avian malaria parasites in a single host species. J. Anim. Ecol. 76, 112–122.

Bordes, F., Morand, S., Krasnov, B.R., Poulin, R., 2010. Parasite diversity and latitudinal gradients in terrestrial mammals. In: Morand, S., Krasnov, B.R. (Eds.), The Biogeography of Host–Parasite Interactions. Oxford University Press, New York, pp. 89–98.

Braga, E.M., Silveira, P., Belo, N.O., Valkiūnas, G., 2011. Recent advances in the study of avian malaria: an overview with an emphasis on the distribution of *Plasmodium* spp in Brazil. Mem. Inst. Oswaldo Cruz 106, 3–11.

Brooks, D.R., Hoberg, E.P., 2001. Parasite systematics in the 21st century: opportunities and obstacles. Trends Parasitol. 17, 273–275.

Chao, A., Chazdon, R.L., Colwell, R.K., Shen, T.Ä., 2005. A new statistical approach for assessing similarity of species composition with incidence and abundance data. Ecol. Lett. 8, 148–159.

Currie, D.J., Mittelbach, G.G., Cornell, H.V., Field, R., Guegan, J.F., Hawkins, B.A., Kaufman, D.M., Kerr, J.T., Oberdorff, T., O'Brien, E., 2004. Predictions and tests of climate-based hypotheses of broad-scale variation in taxonomic richness. Ecol. Lett. 7, 1121–1134.

Diamond, J.M., 1975. The island dilemma: lessons of modern biogeographic studies for the design of natural reserves. Biol. Conserv. 7, 129–146.

Dimitrov, D., Zehtindjiev, P., Bensch, S., 2010. Genetic diversity of avian blood parasites in SE Europe: cytochrome *b* lineages of the genera *Plasmodium* and *Haemoproteus* (Haemosporida) from Bulgaria. Acta Parasitol. 55, 201–209.

Ejiri, H., Sato, Y., Kim, K.S., Tsuda, Y., Murata, K., Saito, K., Watanabe, Y., Shimura, Y., Yukawa, M., 2011. Blood meal identification and prevalence of avian malaria parasite in mosquitoes collected at Kushiro Wetland, a subarctic zone of Japan. J. Med. Entomol. 48, 904–908.

Ejiri, H., Sato, Y., Sasaki, E., Sumiyama, D., Tsuda, Y., Sawabe, K., Matsui, S., Horie, S., Akatani, K., Takagi, M., Omori, S., Murata, K., Yukawa, M., 2008. Detection of avian *Plasmodium* spp. DNA sequences from mosquitoes captured in Minami Daito Island of Japan. J. Vet. Med. Sci. 70, 1205–1210.

Ewen, J.G., Bensch, S., Blackburn, T.M., Bonneaud, C., Brown, R., Cassey, P., Clarke, R.H., Pérez-Tris, J., 2012. Establishment of exotic parasites: the origins and characteristics of an avian malaria community in an isolated island avifauna. Ecol. Lett. 15, 1112–1119.

Fallon, S.M., Bermingham, E., Ricklefs, R.E., 2003. Island and taxon effects in parasitism revisited: avian malaria in the Lesser Antilles. Evolution 57, 606–615.

Fallon, S.M., Bermingham, E., Ricklefs, R.E., 2005. Host specialization and geographic localization of avian malaria parasites: a regional analysis in the Lesser Antilles. Am. Nat. 165, 466–480.

Fallon, S.M., Ricklefs, R.E., Latta, S.C., Bermingham, E., 2004. Temporal stability of insular avian malarial parasite communities. Proc. R. Soc. Lond. B Biol. Sci. 271, 493–500.

Fecchio, A., Lima, M., Svensson, L.M.E., Marini, M.A., Ricklefs, R.E., 2013. Structure and organization of an avian haemosporidian assemblage in a Neotropical savanna in Brazil. Parasitology 140, 181–192.

Foley, D.H., Rueda, L.M., Wilkerson, R.C., 2007. Insight into global mosquito biogeography from country species records. J. Med. Entomol. 44, 554–567.

Gager, A.B., Loaiza, J.D.R., Dearborn, D.C., Bermingham, E., 2008. Do mosquitoes filter the access of *Plasmodium* cytochrome b lineages to an avian host? Mol. Ecol. 17, 2552–2561.

Gaston, K.J., Blackburn, T.M., 2000. Pattern and Process in Macroecology. Blackwell Publishing, Oxford.

Grenyer, R., Orme, C.D.L., Jackson, S.F., Thomas, G.H., Davies, R.G., Davies, T.J., Jones, K.E., Olson, V.A., Ridgely, R.S., Rasmussen, P.C., 2006. Global distribution and conservation of rare and threatened vertebrates. Nature 444, 93–96.

Hackett, S.J., Kimball, R.T., Reddy, S., Bowie, R.C., Braun, E.L., Braun, M.J., Chojnowski, J.L., Cox, W.A., Han, K.-L., Harshman, J., 2008. A phylogenomic study of birds reveals their evolutionary history. Science 320, 1763–1768.

Hayakawa, T., Culleton, R., Otani, H., Horii, T., Tanabe, K., 2008. Big bang in the evolution of extant malaria parasites. Mol. Biol. Evol. 25, 2233–2239.
Hechinger, R.F., Lafferty, K.D., 2005. Host diversity begets parasite diversity: bird final hosts and trematodes in snail intermediate hosts. Proc. R. Soc. Lond. B Biol. Sci. 272, 1059–1066.
Hellgren, O., Bensch, S., Malmqvist, B., 2008. Bird hosts, blood parasites and their vectors – associations uncovered by molecular analyses of blackfly blood meals. Mol. Ecol. 17, 1605–1613.
Hellgren, O., Krizanauskiene, A., Hasselquist, D., Bensch, S., 2011. Low haemosporidian diversity and one key-host species in a bird malaria community on a mid-Atlantic island (Sao Miguel, Azores). J. Wildl. Dis. 47, 849–859.
Hellgren, O., Kutzer, M., Bensch, S., Valkiūnas, G., Palinauskas, V., 2013. Identification and characterization of the merozoite surface protein 1 (msp1) gene in a host-generalist avian malaria parasite, *Plasmodium relictum* (lineages SGS1 and GRW4) with the use of blood transcriptome. Malar. J. 12, 381.
Hellgren, O., Pérez-Tris, J., Bensch, S., 2009. A jack-of-all-trades and still a master of some: prevalence and host range in avian malaria and related blood parasites. Ecology 90, 2840–2849.
Huijben, S., Schaftenaar, W., Wijsman, A., Paaijmans, K., Takken, W., 2007. Avian malaria in Europe: an emerging infectious disease? In: Takken, W.K.B.G.J. (Ed.), Emerging pests and vector-borne diseases in Europe. Wageningen Academic Publishers, Wageningen, pp. 59–74.
Ishtiaq, F., Beadell, J.S., Baker, A.J., Rahmani, A.R., Jhala, Y.V., Fleischer, R.C., 2006. Prevalence and evolutionary relationships of haematozoan parasites in native versus introduced populations of common myna *Acridotheres tristis*. Proc. R. Soc. Lond. B Biol. Sci. 273, 587–594.
Ishtiaq, F., Clegg, S.M., Phillimore, A.B., Black, R.A., Owens, I.P.F., Sheldon, B.C., 2010. Biogeographical patterns of blood parasite lineage diversity in avian hosts from southern Melanesian islands. J. Biogeogr. 37, 120–132.
Ishtiaq, F., Gering, E., Rappole, J.H., Rahmani, A.R., Jhala, Y.V., Dove, C.J., Milensky, C., Olson, S.L., Peirce, M.A., Fleischer, R.C., 2007. Prevalence and diversity of avian hematozoan parasites in Asia: a regional survey. J. Wildl. Dis. 43, 382–398.
Ishtiaq, F., Guillaumot, L., Clegg, S.M., Phillimore, A.B., Black, R.A., Owens, I.P.F., Mundy, N.I., Sheldon, B.C., 2008. Avian haematozoan parasites and their associations with mosquitoes across Southwest Pacific Islands. Mol. Ecol. 17, 4545–4555.
Jenkins, T., Owens, I.P.F., 2011. Biogeography of avian blood parasites (*Leucocytozoon* spp.) in two resident hosts across Europe: phylogeographic structuring or the abundance-occupancy relationship? Mol. Ecol. 20, 3910–3920.
Kimura, M., Dhondt, A.A., Lovette, I.J., 2006. Phylogeographic structuring of *Plasmodium* lineages across the north american range of the house finch (*Carpodacus mexicanus*). J. Parasitol. 92, 1043–1049.
Krasnov, B.R., Shenbrot, G.I., Khokhlova, I.S., Poulin, R., 2007. Geographical variation in the, bottom-up control of diversity: fleas and their small mammalian hosts. Global Ecol. Biogeogr. 16, 179–186.
Krizanauskiene, A., Hellgren, O., Kosarev, V., Sokolov, L., Bensch, S., Valkiūnas, G., 2006. Variation in host specificity between species of avian hemosporidian parasites: evidence from parasite morphology and cytochrome *B* gene sequences. J. Parasitol. 92, 1319–1324.
Lacorte, G.A., Felix, G.M., Pinheiro, R.R., Chaves, A.V., Almeida-Neto, G., Neves, F.S., Leite, L.O., Santos, F.R., Braga, E.M., 2013. Exploring the diversity and distribution of Neotropical avian malaria parasites, a molecular survey from southeast Brazil. PLoS One 8, e57770.
Lewinsohn, T.M., Roslin, T., 2008. Four ways towards tropical herbivore megadiversity. Ecol. Lett. 11, 398–416.
MacArthur, R.H., Wilson, E.O., 1967. The Theory of Island Biogeography. Princeton University Press, Princeton.
MacArthur, R.H., MacArthur, J.W., 1961. On bird species diversity. Ecology 42, 594–598.
Martinez, J., Martinez-De La Puente, J., Herrero, J., Del Cerro, S., Lobato, E., Rivero-De Aguilar, J., Vasquez, R.A., 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.
Martinez-de la Puente, J., Martinez, J., Rivero-de Aguilar, J., Herrero, J., Merino, S., 2011. On the specificity of avian blood parasites: revealing specific and generalist relationships between haemosporidians and biting midges. Mol. Ecol. 20, 3275–3287.
Martinsen, E.S., Paperna, I., Schall, J.J., 2006. Morphological versus molecular identification of avian Haemosporidia: an exploration of three species concepts. Parasitology 133, 279–288.
Marzal, A., 2012. Recent advances in studies on avian malaria parasites. In: Okwa, O.O. (Ed.), Malaria Parasites, Intech, pp. 135–158. Available from: <http://www.intechopen.com/books/malaria-parasites>.
Marzal, A., Ricklefs, R.E., Valkiūnas, G., Albayrak, T., Arriero, E., Bonneaud, C., Czirjak, G.A., Ewen, J., Hellgren, O., Horakova, D., Iezhova, T.A., Jensen, H., Krizanauskiene, A., Lima, M.R., de Lope, F., Magnussen, E., Martin, L.B., Moller, A.P., Palinauskas, V., Pap, P.L., Pérez-Tris, J., Sehgal, R.N.M., Soler, M., Eszter, S., Westerdahl, H., Zetindjiev, P., Bensch, S., 2011. Diversity, loss, and gain of malaria parasites in a globally invasive bird. PLoS One 6, e21905.
Medeiros, M.C., Hamer, G.L., Ricklefs, R.E., 2013. Host compatibility rather than vector-host-encounter rate determines the host range of avian Plasmodium parasites. Proc. R. Soc. Lond. B. 280, 20122947.
Mendes, L., Piersma, T., Lecoq, M., Spaans, B., Ricklefs, R.E., 2005. Disease-limited distributions? Contrasts in the prevalence of avian malaria in shorebird species using marine and freshwater habitats. Oikos 109, 396–404.
Mittelbach, G.G., Schemske, D.W., Cornell, H.V., Allen, A.P., Brown, J.M., Bush, M.B., Harrison, S.P., Hurlbert, A.H., Knowlton, N., Lessios, H.A., 2007. Evolution and the latitudinal diversity gradient: speciation, extinction and biogeography. Ecol. Lett. 10, 315–331.
Norton, D., De Lange, P., 1999. Host specificity in parasitic mistletoes (Loranthaceae) in New Zealand. Funct. Ecol. 13, 552–559.
Nunn, C.L., Altizer, S.M., Sechrest, W., Cunningham, A.A., 2005. Latitudinal gradients of parasite species richness in primates. Divers. Distrib. 11, 249–256.
Orme, C.D.L., Davies, R.G., Burgess, M., Eigenbrod, F., Pickup, N., Olson, V.A., Webster, A.J., Ding, T.-S., Rasmussen, P.C., Ridgely, R.S., 2005. Global hotspots of species richness are not congruent with endemism or threat. Nature 436, 1016–1019.
Palinauskas, V., Dolnik, O.V., Valkiūnas, G., Bensch, S., 2010. Laser microdissection microscopy and single cell PCR of avian haemosporidians. J. Parasitol. 96, 420–424.
Palinauskas, V., Kosarev, V., Shapoval, A., Bensch, S., Valkiūnas, G., 2007. Comparison of mitochondrial cytochrome b lineages and morphospecies of two avian malaria parasites of the subgenera *Haemamoeba* and *Giovannolaia* (Haemosporida: Plasmodiidae). Zootaxa, 39–50.
Peirce, M.A., 1981. Distribution and host–parasite check-list of the haematozoa of birds in Western Europe. J. Nat. Hist. 15, 419–458.
Peirce, M.A., Adlard, R.D., 2004. Absence of blood parasites in birds on Heron Island, Great Barrier Reef. Corella 28, 38–39.
Pérez-Rodríguez, A., Ramírez, A., Richardson, D., Pérez-Tris, J., 2013. Evolution of parasite island syndromes without a long-term host population isolation: parasite dynamics in Macaronesian blackcaps *Sylvia atricapilla*. Global Ecol. Biogeogr. 22, 1272–1281.
Phillips, O., Hall, P., Gentry, A.H., Sawyer, S., Vasquez, R., 1994. Dynamics and species richness of tropical rain forests. Proc. Natl. Acad. Sci. USA 91, 2805–2809.
Pianka, E.R., 2011. Evolutionary ecology. Eric R. Pianka. Available from: <http://books.google.com.br/books?id=giFL5bonGhQC&printsec=frontcover&source=gbs_ge_summary_r&cad=0#v=onepage&q&f=false>.
Pick, C., Ebersberger, I., Spielmann, T., Bruchhaus, I., Burmester, T., 2011. Phylogenomic analyses of malaria parasites and evolution of their exported proteins. BMC Evol. Biol. 11, 167.
Pickering, C.M., Byrne, J.A., 2013. The benefits of publishing systematic quantitative literature reviews for PhD candidates and other early career researchers. High. Educ. Res. Devel., 1–15.
Pitts, M.W., Roberts, M., 1997. Fairweather Eden: Life in Britain half a million years ago as revealed by the excavations at Boxgrove. Random House UK.
Poulin, R., 2004. Parasite species richness in New Zealand fishes: a grossly underestimated component of biodiversity? Divers. Distrib. 10, 31–37.
Poulin, R., 2011. Evolutionary Ecology of Parasites. Princeton University Press, Princeton.
Ricklefs, R.E., Fallon, S.M., Bermingham, E., 2004. Evolutionary relationships, cospeciation, and host switching in avian malaria parasites. Syst. Biol. 53, 111–119.
Ricklefs, R.E., Outlaw, D.C., 2010. A molecular clock for malaria parasites. Science 329, 226–229.
Ricklefs, R.E., Swanson, B.L., Fallon, S.M., Martinez-Abrain, A., Scheuerlein, A., Gray, J., Latta, S.C., 2005. Community relationships of avian malaria parasites in southern Missouri. Ecol. Monogr. 75, 543–559.
Rohde, K., 2002. Ecology and biogeography of marine parasites. Adv. Mar. Biol. 43, 1–83.
Rueda, L.M., 2008. Global diversity of mosquitoes (Insecta: Diptera: Culicidae) in freshwater. In: Balian, E.V., Lévêque, C., Sergers, H., Martens, K. (Eds.), Freshwater Animal Diversity Assessment. Springer, Berlin, pp. 477–487.
Santiago-Alarcon, D., Havelka, P., Schaefer, H.M., Segelbacher, G., 2012a. Bloodmeal analysis reveals avian *Plasmodium* infections and broad host preferences of *Culicoides* (Diptera: Ceratopogonidae) vectors. PLoS One 7, e31098.
Santiago-Alarcon, D., Outlaw, D.C., Ricklefs, R.E., Parker, P.G., 2010. Phylogenetic relationships of haemosporidian parasites in New World Columbiformes, with emphasis on the endemic Galapagos dove. Int. J. Parasitol. 40, 463–470.
Santiago-Alarcon, D., Palinauskas, V., Schaefer, H.M., 2012b. Diptera vectors of avian Haemosporidian parasites: untangling parasite life cycles and their taxonomy. Biol. Rev. 87, 928–964.
Sehgal, R.N.M., Hull, A.C., Anderson, N.L., Valkiūnas, G., Markovets, M.J., Kawamura, S., Tell, L.A., 2006. Evidence for cryptic speciation of *Leucocytozoon* spp. (Haemosporida, Leucocytozoidae) in diurnal raptors. J. Parasitol. 92, 375–379.
Sherratt, T.N., Wilkinson, D.M., 2009. Big Questions in Ecology and Evolution. Oxford University Press, New York.
Shurulinkov, P., Ilieva, M., 2009. Spatial and temporal differences in the blood parasite fauna of passerine birds during the spring migration in Bulgaria. Parasitol. Res. 104, 1453–1458.
Silva, N., Silveira, L., Machado, R., Povoa, M., Ferreira, M., 2000. Temporal and spatial distribution of the variants of merozoite surface protein-1 (MSP-1) in *Plasmodium falciparum* populations in Brazil. Ann. Trop. Med. Parasitol. 94, 675–688.
Svensson, L.M.E., Ricklefs, R.E., 2009. Low diversity and high intra-island variation in prevalence of avian *Haemoproteus* parasites on Barbados, Lesser Antilles. Parasitology 136, 1121–1131.

Svensson-Coelho, M., Blake, J.G., Loiselle, B.A., Penrose, A.S., Parker, P.G., Ricklefs, R.E., 2013. Diversity, prevalence, and host specificity of avian *Plasmodium* and *Haemoproteus* in a western Amazon assemblage. Ornithol. Monogr. 2013, 1–47.

Tachibana, S.-I., Sullivan, S.A., Kawai, S., Nakamura, S., Kim, H.R., Goto, N., Arisue, N., Palacpac, N.M., Honma, H., Yagi, M., 2012. *Plasmodium cynomolgi* genome sequences provide insight into *Plasmodium vivax* and the monkey malaria clade. Nature Genet. 44, 1051–1055.

Tompkins, D.M., Gleeson, D.M., 2006. Relationship between avian malaria distribution and an exotic invasive mosquito in New Zealand. J. R. Soc. N. Z. 36, 51–62.

Valkiūnas, G., 2005. Avian Malaria Parasites and other Haemosporida. CRC Press, Boca Raton, Florida.

Valkiūnas, G., Bensch, S., Iezhova, T.A., Krizanauskiene, A., Hellgren, O., Bolshakov, C.V., 2006. Nested cytochrome *b* polymerase chain reaction diagnostics underestimate mixed infections of avian blood haemosporidian parasites: microscopy is still essential. J. Parasitol. 92, 418–422.

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

Valkiūnas, G., Iezhova, T.A., Loiseau, C., Sehgal, R.N.M., 2009. Nested cytochrome *b* polymerase chain reaction diagnostics detect sporozoites of haemosporidian parasites in peripheral blood of naturally infected birds. J. Parasitol. 95, 1512–1515.

Valkiūnas, G., Kazlauskiene, R., Bernotiene, R., Palinauskas, V., Iezhova, T.A., 2013. Abortive long-lasting sporogony of two *Haemoproteus* species (Haemosporida, Haemoproteidae) in the mosquito *Ochlerotatus cantans*, with perspectives on haemosporidian vector research. Parasitol. Res. 112, 2159–2169.

Valkiūnas, G., Santiago-Alarcon, D., Levin, I.I., Iezhova, T.A., Parker, P.G., 2010. A new *Haemoproteus* species (Haemosporida: Haemoproteidae) from the endemic Galapagos dove *Zenaida galapagoensis*, with remarks on the parasite distribution, vectors, and molecular diagnostics. J. Parasitol. 96, 783–792.

Ventim, R., Morais, J., Pardal, S., Mendes, L., Ramos, J.A., Perez-Tris, J., 2012. Host–parasite associations and host-specificity in haemoparasites of reed bed passerines. Parasitology 139, 310–316.

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.

White, E.M., Greiner, E.C., Bennett, G.F., Herman, C.M., 1978. Distribution of the hematozoa of neotropical birds. Rev. Biol. Trop. 26, 43–102.

Yildirim, A., Aysul, N., Bayramli, G., Inci, A., Eren, H., Aypak, S., Duzlu, O., Ciloglu, A., Onder, Z., 2013. Detection and molecular characterization of a *Haemoproteus* lineage in a Tawny Owl (*Strix aluco*) in Turkey. Ankara Univ. Veteriner Fakultesi Dergisi 60, 179–183.

Yohannes, E., Krizanauskiene, A., Valcu, M., Bensch, S., Kempenaers, B., 2009. Prevalence of malaria and related haemosporidian parasites in two shorebird species with different winter habitat distribution. J. Ornithol. 150, 287–291.

Zamora-Vilchis, I., Williams, S.E., Johnson, C.N., 2012. Environmental temperature affects prevalence of blood parasites of birds on an elevation gradient: implications for disease in a warming climate. PLoS One 7, e39208.