Coevolutionary patterns and diversification of avian malaria parasites in African sunbirds (Family Nectariniidae)

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SUMMARY

The coevolutionary relationships between avian malaria parasites and their hosts influence the host specificity, geographical distribution and pathogenicity of these parasites. However, to understand fine scale coevolutionary host–parasite relationships, robust and widespread sampling from closely related hosts is needed. We thus sought to explore the coevolutionary history of avian *Plasmodium* and the widespread African sunbirds, family Nectariniidae. These birds are distributed throughout Africa and occupy a variety of habitats. Considering the role that habitat plays in influencing host-specificity and the role that host-specificity plays in coevolutionary relationships, African sunbirds provide an exceptional model system to study the processes that govern the distribution and diversity of avian malaria. Here we evaluated the coevolutionary histories using a multi-gene phylogeny for Nectariniidae and avian *Plasmodium* found in Nectariniidae. We then assessed the host–parasite biogeography and the structuring of parasite assemblages. We recovered *Plasmodium* lineages concurrently in East, West, South and Island regions of Africa. However, several *Plasmodium* lineages were recovered exclusively within one respective region, despite being found in widely distributed hosts. In addition, we inferred the biogeographic history of these parasites and provide evidence supporting a model of biotic diversification in avian *Plasmodium* of African sunbirds.

Key words: *Plasmodium*, nectariniidae, cospeciation, African sunbird, avian malaria, host switching.

INTRODUCTION

Coevolution and natural selection may shape the interactions among species by presenting an evolutionary trade-off between specializing to perform a few activities well, and generalizing to perform many activities fairly (Levins, 1968; Brodie *et al*. 1999). Thus, the ability for parasites to specialize or generalize and to infect hosts with varying efficacy poses compelling questions: what factors determine parasite host-specificity, and how does the degree of host-specificity impact parasite distribution and diversification? These factors may ultimately influence the virulence, the geographical distributions, and the potential for parasites to emerge into novel hosts (Garamszegi, 2006; Hellgren *et al*. 2009; Cooper *et al*. 2012).

The host range of parasites primarily depends on their compatibility with the host, which is limited by a coevolutionary arms race between hosts and parasites (Kawecki, 1998). In fact, the study of host–parasite coevolutionary relationships through molecular approaches allows researchers to elucidate the phenomena of cospeciation, the hallmark of coevolution (Demastes and Hafner, 1993; Clark *et al*. 2000). For example, studies of mites and their avian hosts show significant evidence of cospeciation (Ehrnsberger, 2001; Morelli and Spicer, 2007; Hendricks *et al*. 2013). In addition, mammalian and avian hosts also show evidence of cospeciation with haemosporidian blood parasites, including the causative agents of leucocytozoonosis and malaria (Ricklefs and Fallon, 2002; Garamszegi, 2009; Hughes and Verra, 2010; Jenkins and Owens, 2011).

Infections of malaria occur in multiple vertebrate hosts including reptiles, mammals and birds (Levine, 1988). Malaria parasites of birds are found on all continents of the world, except Antarctica (Valkiunas, 2005), and have been studied intensively for over 100 years. Avian malaria has therefore proven to be an important model system for pursuing evolutionary and ecological issues such as speciation (Ricklefs and Fallon, 2002; Ricklefs *et al*. 2004; Pérez-Tris *et al*. 2007), life-history trade-offs (Garamszegi, 2006; Hellgren *et al*. 2009), and competition and community structure in a changing environment (Read and Taylor, 2001; Mideo *et al*. 2008; Mideo, 2009; Palinauskas *et al*. 2011; Loiseau *et al*. 2012;

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Svensson-Coelho *et al*. 2013). Extensive host switching of some avian *Plasmodium* lineages has been shown in birds belonging to different families (Ricklefs and Fallon, 2002; Waldenström *et al*. 2002; Fallon *et al*. 2003, 2005; Szymanski and Lovette, 2005) and the potential for these widely distributed avian *Plasmodium* parasites to switch into new hosts can have calamitous effects, as was seen in the endemic bird populations of Hawaii (van Riper, 1986). Thus, it is crucial to understand the factors that influence parasite host specificity and the longstanding host relationships among parasites.

Considering host–parasite relationships, Ricklefs and Fallon (2002) used tree-based methods to assess cospeciation and host switching between the avian haemosporidian genera *Plasmodium* and *Haemoproteus* and their hosts. Their study included 20 avian families distributed throughout six countries, in which they reported significant cospeciation and host switching. In addition, Ricklefs *et al*. (2004) conducted a more focused analysis by re-examining cospeciation of avian malaria parasites and forest dwelling songbirds within North America and the West Indies. Consistent with their previous findings, cospeciation and host switching were prominent events recovered in their study. Here, we assessed the coevolutionary host–parasite relationships of avian *Plasmodium* found in the Old-World bird family Nectariniidae. We sought to increase the sensitivity for the detection of coevolutionary relationships by focusing on the large assemblage of Africa sunbirds. This assemblage comprises 95 species that are primarily restricted to continental Africa and its neighbouring islands including Madagascar, but which includes five Asian species that represent a recent back-colonization from Africa (Bowie, 2003, Bowie *et al*. unpublished data).

Sunbirds utilize virtually all forms of habitat, including moist forests, swamps, grasslands, primary rainforests, open woodlands, and semi-arid regions (Cheke *et al*. 2001). Most species are sedentary or short-distance seasonal migrants, and can be either ecological generalists or specialists (i.e. restricted to a very small area, e.g. highlands in East Africa; Bowie *et al*. 2004). A recent study established that Nectariniidae and other bird families in Africa (Muscicapidae, Pycnonotidae, Timaliidae and Turdidae) also harbour both generalist and specialist *Plasmodium* parasites (Loiseau *et al*. 2012). The detection of multiple specialist parasite lineages and the tight host specificity that specialist parasites exhibit suggests that some degree of cospeciation may occur among avian *Plasmodium* parasites and their African sunbird hosts.

The coevolutionary relationships of avian *Plasmodium* parasites and their hosts are further complicated by vector–parasite interactions and habitat change (Chasar *et al*. 2009). Environmental changes over time may have led to changes in both host and *Plasmodium* vector distribution, which may consequently affect the transmission and prevalence of avian malaria (Kamdem *et al*. 2012; Pavlacky *et al*. 2012). Because avian *Plasmodium* are globally distributed among many bird species, the coevolution of avian *Plasmodium* parasites are likely driven at the global-scale by environmental changes with periodic cycling of geographic expansion and isolation, a process described as taxon pulses (Hoberg and Brooks, 2008; Agosta *et al*. 2010). Here we assess the cross-continental patterns and coevolutionary histories of a host–parasite system in Africa and provide empirical evidence that supports a model for biotic diversification in avian *Plasmodium*.

MATERIALS AND METHODS

Sample collection

Blood samples from birds were collected in 12 countries of Africa and in three island countries covering five biogeographic regions: West Africa (Gabon, Ghana, and Cameroon; $N = 935$), East Africa (Burundi, Kenya, Democratic Republic of the Congo, Malawi, Mozambique, Rwanda, Uganda, Tanzania and Zambia; $N = 392$), Southern Africa (The Republic of South Africa; $N = 252$), Madagascar ($N = 18$) and two small oceanic archipelagos (São Tomé and Principe; $N = 21$, and the Comoros; $N = 18$) during the period 1999 to 2009. All birds were caught with mist-nets and blood samples were collected from the brachial vein. Blood samples were stored in lysis buffer (10 mM Tris-HCL pH 8·0, 100 mM EDTA, 2% SDS) or in absolute ethanol at −80 °C or in liquid Nitrogen cryo-tanks.

PCR amplification and DNA sequencing

In total, 1636 individual sunbirds (N species = 32) were screened for *Plasmodium* parasites (see Table 1). DNA was extracted from whole blood following a DNeasy kit protocol (Qiagen, Valencia, California). Success of each DNA extraction was verified with primers that amplify the brain-derived neurotrophic factor (BDNF) (Sehgal and Lovette, 2003).

Since mitochondrial lineages have been shown to be reproductively isolated (Bensch *et al*. 2004), *Plasmodium* spp. mitochondrial haplotypes are often defined as unique *cytochrome b* lineages (Hellgren *et al*. 2004; Waldenström *et al*. 2004; Bensch *et al*. 2009). Therefore, each lineage (i.e. haplotype) differing by at least 1 bp was analysed as a separate entity (i.e. OTU). In some cases, identical *cyt b* lineages exhibited differences (1 bp or greater) in *asl* or *clpc* and were also treated as separate entities. To detect *Plasmodium* spp. lineages, we used nested PCR to amplify the *cyt b* gene (340 bp) of the mtDNA with the primers HAEMF/HAEMR2 – HAEMNF/HAEMNR2 following the protocols of Waldenström *et al*. (2004). For positive controls, we used DNA samples from

infected birds, in which infections were verified by microscopy. Purified water was used in place of DNA template as negative controls.

The amplicons were run out on a 1·8% agarose gel using 1 × TBE, and visualized by ethidium bromide staining under ultraviolet light. Amplicons from DNA samples of birds infected with *Plasmodium* spp. were purified using ExoSap (following manufacture's instructions, USB Corporation, Cleveland, Ohio). We identified lineages by sequencing the fragments (BigDye [R] version 3.1 sequencing kit, Applied Biosystems) on an ABI PRISM 3100 (TM) automated sequencer (Applied Biosystems). All unique sequences were sequenced twice for verification. DNA chromatographs containing double peaks, indicating multiple infections of parasites within a single host, were excluded from the analyses. We compared the lineages with all sequences from blood parasites already deposited in GenBank. In addition to using sequence data from the mitochondria genome, previous studies have used sequence data from the apicoplast and nuclear genomes to analyse phylogenetic relationships of *Plasmodium* species, including *clpc* and *asl* (Rathore *et al*. 2001; Hagner *et al*. 2007; Martinsen *et al*. 2008) Subsequently, we amplified the apicoplast gene *clpc* (416 bp) and the nuclear gene *asl* (166 bp) from 33 *cyt b Plasmodium* lineages following Martinsen *et al*. (2008).

For the host phylogeny, the mtDNA gene ATPase 6 (364 bp) and nuclear genes *RDPSN* (720 bp) and *TGFB2* (488 bp) were PCR amplified from *Plasmodium* positive Nectariniidae species, 18 in total, using the protocols described in Hunt *et al*. (2001) and Primmer *et al*. (2002). All new sequences were deposited into GenBank (online Table S1) and MalAvi (Bensch *et al*. 2009).

Prevalence and statistical analyses

Plasmodium parasite infection prevalence and 95% Bayesian credible interval for infection prevalence were calculated for all respective regions. We calculated credible intervals using the inverse of the cumulative distribution function of the beta distribution in R as described in Swei *et al*. (2011). The *Plasmodium* lineages were divided by region, according to the presence of one or more lineages, into the following regions: West Africa, East Africa, Southern Africa, Madagascar, and oceanic archipelagos. Oceanic archipelagos were treated as a single unit in our analyses because of low sample sizes in these regions. Analysis of Similarities (ANOSIM) was used to compare parasite assemblage data among regions. The ANOSIM R-statistic value ranges from −1 to +1 and indicates the extent to which groups are separated; an R value of 0 indicates random grouping, $R > 0{\cdot}75$ indicates strong separation, and $R < 0{\cdot}25$ indicates little separation among groups. Intermediate values, $0{\cdot}25 < R < 0{\cdot}75$, indicate separation with varying overlap. All analyses were executed in R (R Core Team, 2012). The null hypothesis of panmixia was tested in Arlequin 3.5.1.3 software (Excoffier and Lischer, 2010) using an exact test of the differentiation among parasite communities. This test is analogous to Fisher's exact test, but extends the test from a two-by-two contingency table to a contingency table of arbitrary size.

Phylogenetic analyses

The sequences were edited using Sequencher 4.8 (GeneCodes, Ann Arbor, MI). SEAVIEW software (Galtier *et al*. 1996) was used to align the sequences. Modeltest Version 3.7 (Posada and Crandall, 1998) was used to determine the most appropriate nucleotide substitution model for the Akaike Information Criterion. All genes were initially analysed separately and phylogenetic relationships were inferred using maximum likelihood (ML) implemented in RaxML. A Bayesian approach, as implemented in MrBayes v3.1.2 (Ronquist and Huelsenbeck, 2003) and described below, was used to estimate posterior probabilities of the tree branches. There was no significant conflict among individual gene trees, with any conflict being restricted to nodes that had a bootstrap of >70% and a PP of >0·95. Therefore, for our final tree, all sequences were concatenated.

For the Bayesian inference, the metropolis-coupled MCMC (MCMCMC) methods were implemented using the GTR + G model. Two Markov chains were run simultaneously for 10 million generations and sampled every 200 generations, generating 50 000 trees; 25% of the trees were discarded and the remaining 37 500 trees were used to construct a majority consensus tree. The Bayesian Posterior Probabilities (BBP) was then calculated using these remaining trees.

Host phylogeny. The phylogenetic relationships of the 18 infected Nectariniidae species were inferred using ML, as implemented in PAUP*Ver.4.0b10 (Swofford, 2001). ML methods were implemented using the TVM + G model. Four species (*Acridotheres tristis, Sturnus roseus, Sturnus unicolor*, and *Sturnus vulgaris*) from the Sturnidae family were selected as outgroup taxa to root the Nectariniidae tree.

Parasite phylogeny. A ML tree based on three genes for *Plasmodium* was generated using RAxML (Stamatakis, 2006), under the GTR + I + G model. A thorough ML search was performed along with 1000 rapid bootstrap inferences. In addition, we performed a Bayesian analyses as described above. Two *Leucocytozoon* lineages (Leuco. sp. 2208 and P157) were selected as outgroup to root the *Plasmodium* phylogeny.

Biogeographical analysis

The distribution ranges of *Plasmodium* lineages were designated as follows: Southern Africa, East Africa,

Table 1. The number of *Plasmodium* lineages (N), host species and sampling locality

Plasmodium lineage	Host species	*N*	Region	Country
P31	Newton sunbird, *Anabathmis newtonii*	1	Island	São Tomé and Príncipe
	Whyte's sunbird, *Cinnyris whytei*	1	East	Malawi
	Collared sunbird, *Hedydipna collaris*	1	East	Malawi
	Greater double-collared sunbird, *Nectarinia afra*	1	South	South Africa
PV25aL	Newton sunbird, *Anabathmis newtonii*	1	Island	São Tomé and Príncipe
	Amethyst sunbird, *Chalcomitra amethystine*	1	South	South Africa
	Southern double-collared sunbird, *Cinnyris chalybeus*	1	South	South Africa
	Double collared sunbird, *Cinnyris mediocris fuelleborni*	1	East	Malawi
	Olive sunbird, *Cyanomitra olivacea*	9	East, West	Cameroon, Kenya, Mozambique, Rwanda, Tanzania, Uganda
	Green headed sunbird, *Cyanomitra verticalis*	1	West	Cameroon
	Collared sunbird, *Hedydipna collaris*	1	East	Malawi
	Greater double-collared sunbird, *Nectarinia afra*	1	South	South Africa
P16	Orange-breasted sunbird, *Anthobaphes violacea*	5	South	South Africa
	Southern double-collared sunbird, *Cinnyris chalybeus*	1	South	South Africa
	Greater double-collared sunbird, *Nectarinia afra*	2	South	South Africa
	Malachite sunbird, *Nectarinia famosa*	10	South	South Africa
PV13	Orange-breasted sunbird, *Anthobaphes violacea*	2	South	South Africa
	Amethyst sunbird, *Chalcomitra amethystine*	3	East	Kenya
	Olive sunbird, *Cyanomitra olivacea*	35	East, West	Cameroon, Ghana, Tanzania
	Collared sunbird, *Hedydipna collaris*	2	East	Democratic Republic of the Congo, Malawi
	Greater double-collared sunbird, *Nectarinia afra*	3	South	South Africa
PV41	Orange-breasted sunbird, *Anthobaphes violacea*	2	South	South Africa
	Southern double-collared sunbird, *Cinnyris chalybeus*	5	South	South Africa
	Greater double-collared sunbird, *Nectarinia afra*	2	South	South Africa
PV45	Amethyst sunbird, *Chalcomitra amethystine*	3	East	Kenya
PV45a	Amethyst sunbird, *Chalcomitra amethystine*	1	East	Kenya
PV25	Orange-tufted sunbird, *Cinnyris bouvieri*	1	West	Cameroon
	Northern double-collared sunbird, *Cinnyris reichenowi preussi*	3	West	Cameroon
	Variable sunbird, *Cinnyris venustus*	1	West	Cameroon
	Olive sunbird, *Cyanomitra olivacea*	7	East	Uganda, Zambia
	Green headed sunbird, *Cyanomitra verticalis*	8	West	Cameroon
	Collared sunbird, *Hedydipna collaris*	5	East, West	Cameroon, Ghana, Malawi
PV25bL	Southern double-collared sunbird, *Cinnyris chalybeus*	1	South	South Africa
PV41a	Southern double-collared sunbird, *Cinnyris chalybeus*	1	South	South Africa
P36	Olive-bellied sunbird, *Cinnyris chloropygius*	3	West	Cameroon
	Cameroon sunbird, *Cyanomitra oritis*	1	West	Cameroon
PV12	Olive-bellied sunbird, *Cinnyris chloropygius*	4	West	Cameroon
	Double collared sunbird, *Cinnyris mediocris fuelleborni*	1	East	Malawi
	Olive sunbird, *Cyanomitra olivacea*	6	East, West	Cameroon, Kenya, Tanzania
	Collared sunbird, *Hedydipna collaris*	4	East, West	Ghana, Malawi, Tanzania, Uganda
PV15	Olive-bellied sunbird, *Cinnyris chloropygius*	7	West	Cameroon
	Northern double-collared sunbird, *Cinnyris reichenowi preussi*	1	West	Cameroon
	Olive sunbird, *Cyanomitra olivacea*	4	East	Rwanda, Tanzania
	Olive sunbird, *Cyanomitra olivacea*	48	West	Cameroon, Ghana
	Green headed sunbird, *Cyanomitra verticalis*	1	West	Cameroon
	Collared sunbird, *Hedydipna collaris*	1	East	Tanzania
	Malachite sunbird, *Nectarinia famosa*	1	South	South Africa
	Souimanga sunbird, *Nectarinia souimanga*	1	Island	Madagascar
PV47	Northern double-collared sunbird, *Cinnyris reichenowi preussi*	1	West	Cameroon
PV43	Variable sunbird, *Cinnyris venustus*	1	East	Malawi
PV44	Variable sunbird, *Cinnyris venustus*	1	East	Malawi

Table 1. (*Cont.*)

Plasmodium lineage	Host species	*N*	Region	Country
PV16L	Blue-throated brown sunbird, *Cyanomitra cyanolaema*	1	West	Cameroon
	Olive sunbird, *Cyanomitra olivacea*	24	West	Cameroon
	Cameroon sunbird, *Cyanomitra oritis*	1	West	Cameroon
PV17b	Olive sunbird, *Cyanomitra olivacea*	1	East	Kenya
PlasmP35gabona	Olive sunbird, *Cyanomitra olivacea*	1	East	Kenya
PlasmP35gabon	Olive sunbird, *Cyanomitra olivacea*	2	East	Uganda
	Greater double-collared sunbird, *Nectarinia afra*	2	South	South Africa
PlasmGBCAM1	Olive sunbird, *Cyanomitra olivacea*	2	East	Kenya
PV38	Olive sunbird, *Cyanomitra olivacea*	1	East	Malawi
PV17L	Olive sunbird, *Cyanomitra olivacea*	94	East, West	Cameroon, Ghana, Tanzania
PV40	Olive sunbird, *Cyanomitra olivacea*	8	East	Tanzania
PV1	Olive sunbird, *Cyanomitra olivacea*	1	West	Cameroon
PV1a	Olive sunbird, *Cyanomitra olivacea*	1	West	Cameroon
PV19L	Olive sunbird, *Cyanomitra olivacea*	2	West	Ghana
PV27L	Olive sunbird, *Cyanomitra olivacea*	1	West	Ghana
PV16ac	Olive sunbird, *Cyanomitra olivacea*	1	West	Cameroon
PV23L	Olive sunbird, *Cyanomitra olivacea*	1	West	Cameroon
PV30	Olive sunbird, *Cyanomitra olivacea*	2	West	Cameroon
PV49	Cameroon sunbird, *Cyanomitra oritis*	1	West	Cameroon
P27	Cameroon sunbird, *Cyanomitra oritis*	2	West	Cameroon
P27a	Cameroon sunbird, *Cyanomitra oritis*	1	West	Cameroon
PV48	Green headed sunbird, *Cyanomitra verticalis*	1	West	Cameroon
PV54	Collared sunbird, *Hedydipna collaris*	1	East	Kenya
PV54a	Collared sunbird, *Hedydipna collaris*	1	East	Kenya
AEMOO1	Collared sunbird, *Hedydipna collaris*	2	East	Kenya
PV39	Collared sunbird, *Hedydipna collaris*	1	East	Malawi
PV39a	Collared sunbird, *Hedydipna collaris*	1	East	Malawi
PV15a	Collared sunbird, *Hedydipna collaris*	1	East	Malawi

West Africa, Madagascar, Oceanic archipelagos. A Bayesian analysis was conducted in Beast v1.8.0 (Drummond *et al*. 2012). The MCMC chains were run simultaneously for 1 million generations and trees were sampled every 100 generations. The burn-in was set to 1000 and 9000 trees from the MCMC output were then used to reconstruct the ancestral distributions of the *Plasmodium* lineage phylogeny in RASP v2.1b (Yu *et al*. 2010, 2013) using the S-DIVA method (Statistical Dispersal-Vicariance Analysis), as outlined by Yu *et al*. 2010 and Ali *et al*. 2012. This method uses all trees from the MCMC output and calculates the average frequency of an ancestral range at a node in ancestral reconstructions. The inferred ancestral ranges for each node on a post burn-in tree were obtained.

Host–parasite cophylogeny

TreeMap 1.0b (Page, 1994) was used to produce a tanglegram, which shows the *Plasmodium* phylogeny relative to the bird phylogeny. Distance-based methods were used to test the extent of a global hypothesis of coevolution between hosts and their parasites with ParaFit (Legendre *et al*. 2002) and Procrustean Approach to Cophylogeny (PACo) (Balbuena *et al*. 2013). These approaches use phylogenetic distance matrices and host–parasite associations to test for overall fit, which can be interpreted as congruence between host and parasite phylogenies. Similar to the ParaFitGlobal statistic, PACo produces a goodness-of-fit statistic and also assesses the significance by randomization of the host–parasite association data. We used 1000 permutations for our distance-based analyses. Although distance-based methods are useful for dealing with large phylogenies or phylogenetic polytomies and uncertainties, such methods do not evaluate all coevolutionary scenarios. Therefore, tree reconciliation methods were implemented in JANE 4.0 (Conow *et al*. 2010) to map cospeciation, duplication, duplication with host switching, loss and failure to diverge events onto the host–parasite cophylogenies. Each event type is assigned an associated cost and a search for a

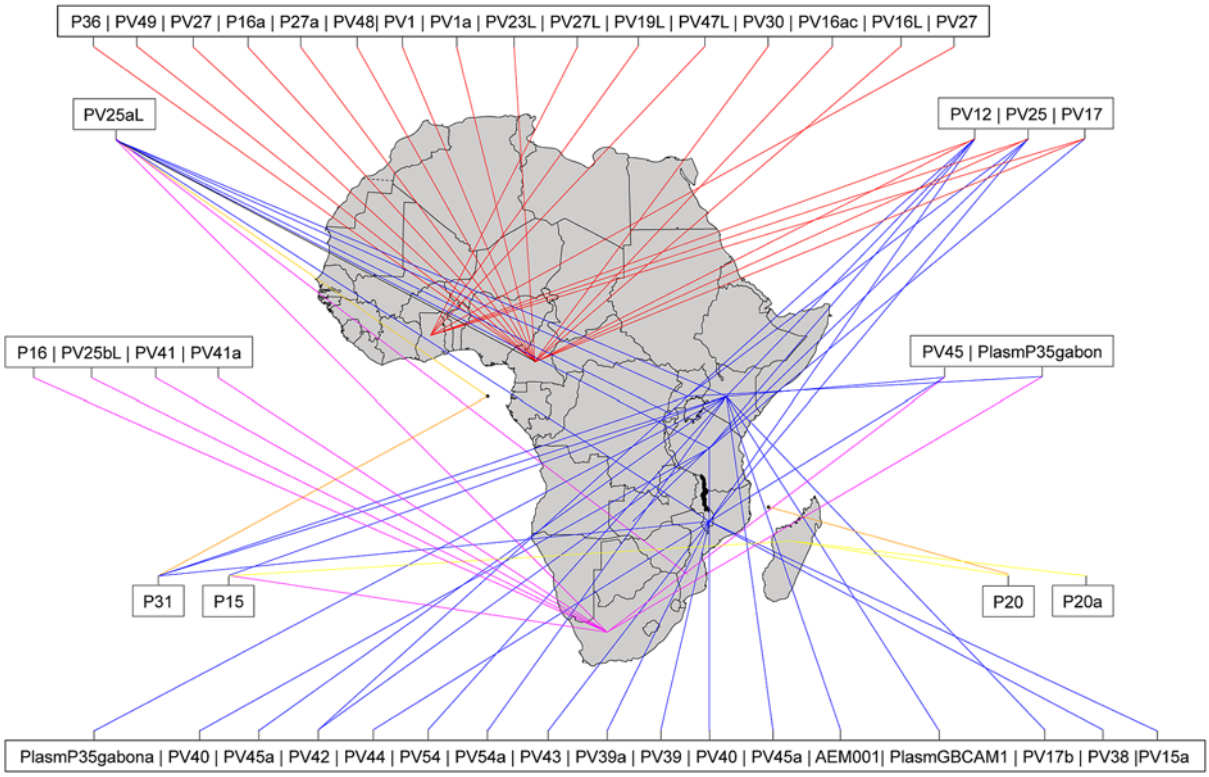

Fig. 1. Map showing sampling origins for each *Plasmodium* lineage. *Plasmodium* lineages are grouped and coloured according to regions/s that they are recovered from: Red = West, Blue = East, Pink = South, Yellow = Madagascar, Orange=Archipelagos).

cophylogeny with a minimum total cost (most parsimonious scenario) is performed. A search for the most parsimonious scenario was performed with and without host switching allowed. The event cost values were individually downweighted in separate analyses (e.g. for downweighting cospeciation the cost values used were: cospeciation = −1, duplication = 1, duplication and host switch = 2, loss = 1, failure to diverge = 1) or were set to even event cost values (cospeciation = 1, duplication = 1, duplication and host switch = 2, loss = 1, failure to diverge = 1). A second search was performed with event cost values intended to maximize cospeciation events (cospeciation = −1, duplication = 0, duplication and host switch = 0, loss = 0, failure to diverge = 0). Our analyses included 46 parasite lineages, 18 host species and 86 host–parasite links. We performed a second analysis in which we only included parasite lineages with ⩾2% sequence divergence, resulting in 23 parasite lineages, 18 hosts and 45 host–parasite links. Random tip mapping methods were implemented with a total of 1000 random replicates to estimate the likelihood of obtaining optimal reconciliations by chance. In addition, we used Core-PA (Merkle *et al.* 2010) to further assess the correspondence between parasite and host phylogenetic trees. This programme allows for the frequency of events to be evaluated without the need to individually downweigh event cost values and is useful when it is difficult to assign appropriate cost values (Merkle *et al.* 2010). Scenarios were reconstructed with automatically calculated event cost values using a simplex optimization algorithm method. Randomization testing was performed with 1000 random cycles using standard parameters and the above cost values.

RESULTS

Origins and biogeography of malaria parasites in African sunbirds

We acquired and analysed a comprehensive set of blood samples from sunbirds collected across Africa. Fifteen parasite lineages were found exclusively in West Africa. Similarly, 15 parasite lineages were found exclusively in East Africa. Only four parasite lineages were found exclusively in South Africa (Fig. 1). These parasite lineages were found at low prevalence (0·1–8%); and our 95% credible Bayesian intervals were consistent with prevalence being low for the majority of parasites restricted to East and West Africa (See online Table S2, supporting information). Two parasite lineages, PV20 and PV20a, were recovered from the endemic Souimanga Sunbird (*Cinnyris sovimanga*) of Madagascar and the Comoros (Cheke *et al.* 2001). We also recovered *Plasmodium* lineages P15 in the Souimanga Sunbird. However, P15 is not restricted to Madagascar and was also found in the Collared Sunbird (*Hedydipna collaris*) and the Malachite Sunbird (*Nectarinia famosa*) from East and South Africa, respectively. Several parasite lineages

were found in either two, three, or in all four regions of our study (Fig. 1). ANOSIM revealed that the parasite assemblages did not vary significantly among biogeographic regions (*R*-value 0·21; $P = 0·03$). Furthermore, the *Plasmodium* lineages are generally not grouped into distinct clades according to biogeographic origin (online Fig. S2). However, the non-differentiation exact *P* value was significant ($P < 0·00001$) among all biogeographic regions sampled, suggesting non-random structure likely indicative of differences in frequency of *Plasmodium* parasite communities among biogeographic regions.

Phylogeny of African sunbirds-hosts

Using the combined dataset (ATPase + *RDPSN* + *TGFB2*), the ML analyses produced one tree with a score of 7583·538 (online Fig. S1). Our preliminary phylogeny shows no monophyletic relationships among all genera of the family Nectariniidae. This topology and lack of monophyly among sunbird genera is consistent with a study that encompasses 95% of African species (Bowie Unpublished data). However, the family Nectariniidae itself is monophyletic and sister to the family Dicaeidae (Flowerpeckers) (Bowie Unpublished data).

Phylogeny of the Plasmodium-parasites

We obtained 396 *Plasmodium cyt b* sequences sourced from 18 host species. Of these sequences, 33 represent distinct parasite mitochondrial DNA lineages. Five previously identified generalists *Plasmodium* lineages (PV12L, P15, P31 and PV13L) were recovered along with nine reported specialist lineages (P16, PV40, PV41, PV38, P36, PV19L, PV16L, PV25aL and PV1L) (Loiseau *et al*. 2012). These lineages were dispersed throughout the phylogeny and did not form distinct clades based on specializing or generalizing strategies. However, the specialists reported by Loiseau *et al*. (2012) may be specialists at the family, but not the species level.

We recovered 12 parasite lineages with identical *cyt b* sequences that exhibit sequence divergence in either the nuclear *asl* ($N = 4$; 0·3–28%) or the apicoplast *clpc* ($N = 8$; 0·2–11%) gene. Two *cyt b* parasite lineages exhibited diversity in both *asl* and *clpc* (1·2%). Conversely, two different *cyt b* parasite lineages, PV47 and PV19L, share the same *asl* sequence. Overall, the phylogeny reveals the tremendous diversity of *Plasmodium* parasites in this group of birds (online Fig. S2).

Historical biogeography of malaria parasites in African sunbirds

We applied the S-DIVA method to determine whether historical processes of vicariance and dispersal may have contributed to the evolution and assembly of *Plasmodium* parasite communities. The S-DIVA results reveal 16 vicariance events and 39 dispersals, suggesting an important role for dispersal in influencing the observed *Plasmodium* parasite distribution patterns (online Table S3). Node 94 suggests an East African origin (frequency of occurrence is 64·09%) of the current *Plasmodium* lineages recovered (Fig. 2). An early dispersal is predicted at node 64. The possible ancestral ranges at this node are East Africa, West Africa, East + West Africa with the frequency of occurrence being 51·48, 27·45 and 21·45%, respectively. Vicariance at this node is also suggested, resulting in East and West African *Plasmodium* lineages.

The remaining lineages of East and West Africa most likely arose from an early dispersal event at node 92. The most favoured ancestral range at this node is East Africa. Interestingly, the majority of Southern African lineages were predicted to arise from dispersal events, with the exception of a vicariance event evident in node 91. The possible ancestral range at node 91 is Southern + East Africa with 100% marginal probability. Dispersal events are also postulated to contribute to the origin of *Plasmodium* lineages of Madagascar and oceanic archipelagos (online Table S3).

With respect to the host biogeography, JANE predicted 19 parasite lineages of putative duplication with host switching events occurring in hosts that have overlapping distribution regions. Duplication with host switching events occur when a *Plasmodium* parasite speciates and one of the new species switches into a different host. Fourteen host taxa harboured two or more parasite lineages and thus host switching was expected. Interestingly, two parasite lineages that are considered specialists, PV1L and PV41, arose from putative duplications with host switching events.

Host–parasite cophylogeny

The tanglegram produced by TreeMap (Fig. 3) shows no obvious congruence between the *Plasmodium* and the Nectariniidae topologies. We found no significant fit between the host and parasite phylogenies with the observed ParaFitGlobal statistic ($P = 0·66$) and PACo global goodness-of-fit statistic ($P = 0·84$). Only 6% of individual host–parasite associations contributed significantly to the ParaFitGlobal statistic (online Fig. S3). The JANE optimal reconstruction trees recovered when setting the event cost values to maximize cospeciation also show that cospeciation was not significantly more frequent than random ($P = 0·72$). When using even event cost values or cost values with individual events downweighted, the JANE optimal reconstruction trees revealed significant cophylogeny ($P < 0·05$), except when individually downweighting loss/sorting events. These results suggest that all individual

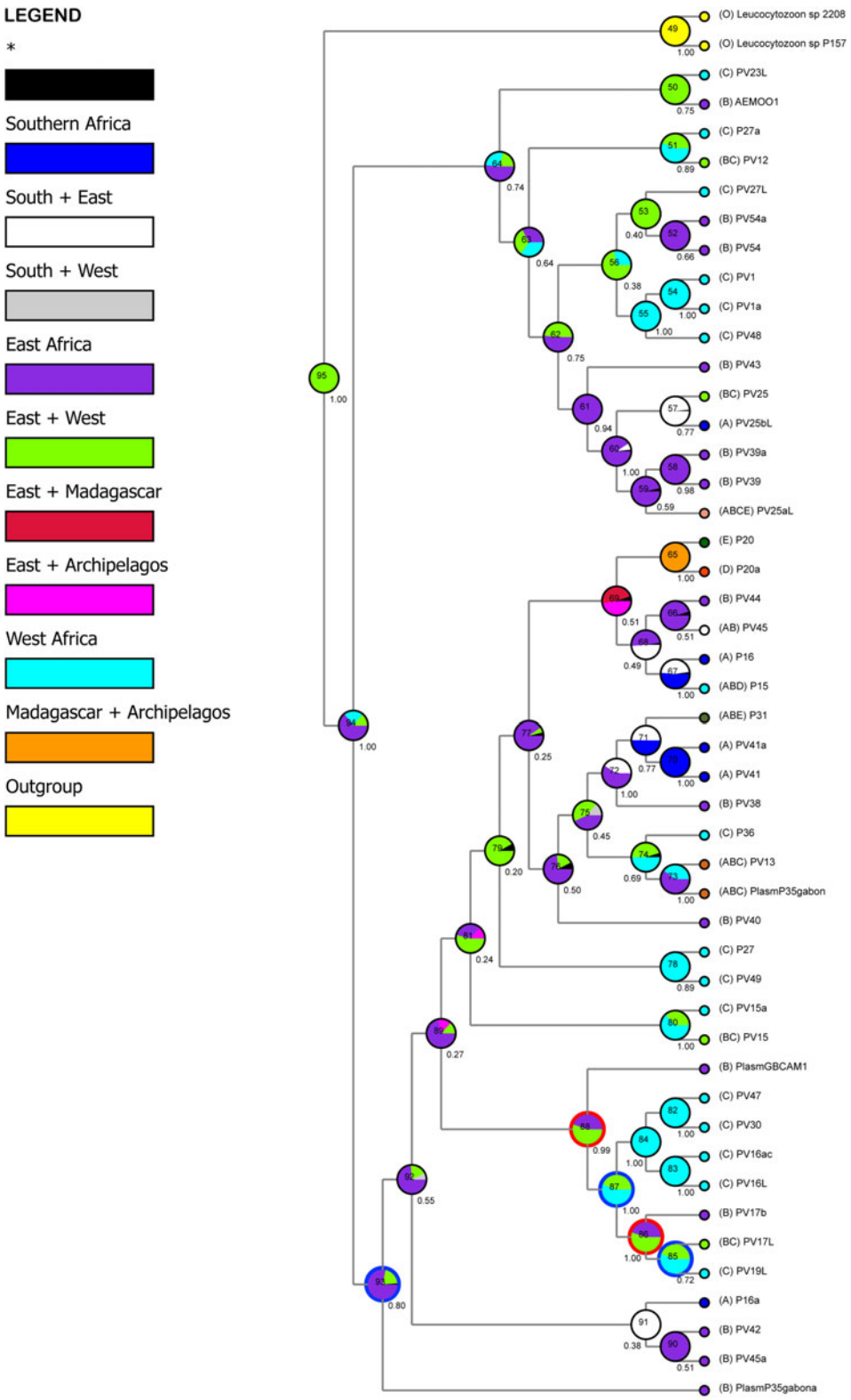

Fig. 2. Ancestral distribution and phylogeny of *Plasmodium* lineages obtained by S-DIVA (RASP). Legend key to the inferred ancestral ranges, shown in pie chart form, at different nodes; black with an asterisk represent other ancestral ranges. The probability of ancestral ranges is shown at each node of one post-burn Bayesian tree with Bayesian Posterior Probability values indicated below the pie charts. Select nodes in which dispersal (blue) and vicariant (red) events are predicted to occur are highlighted to illustrate a potential taxon pulse. Biogeographical regions: A = South Africa, B = East, C = West, D = Madagascar, E = Archipelagos.

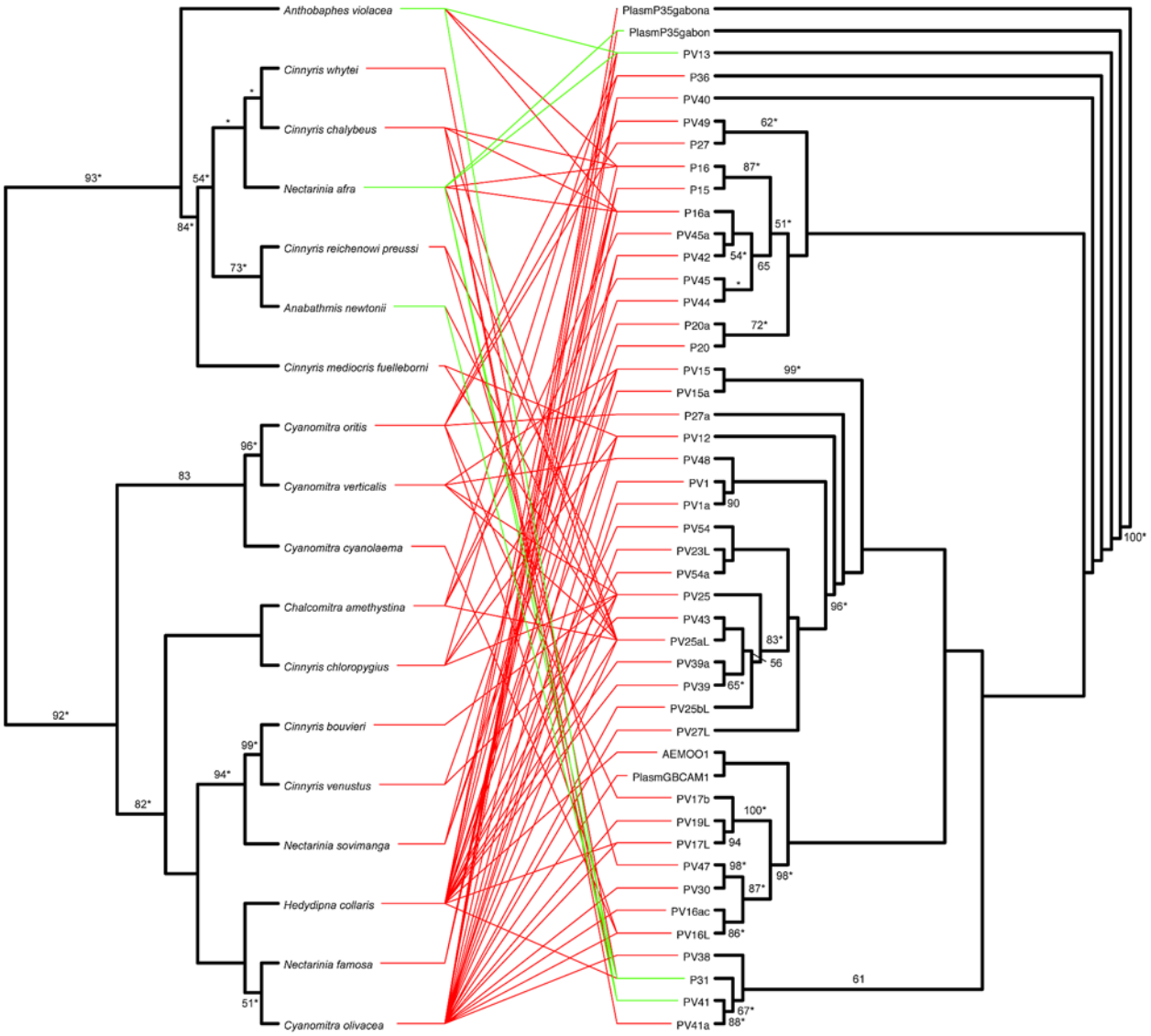

Fig. 3. A tanglegram of the Nectariniidae host species ML tree (left) compared to a ML tree of *Plasmodium* lineages (right) with lines indicating host–parasite associations. Only maximum likelihood bootstrap values above 50 and Bayesian Posterior Probability values $\geqslant 0{\cdot}95$ (indicated by asterisks) are shown. Green lines indicate host–parasite associations that contribute significantly to the ParaFitGlobal statistic, whereas red lines indicate associations that do not contribute significantly to the ParaFitGlobal statistic.

events evaluated, with the exception of loss/sorting and cospeciation events, contribute significantly to the coevolution of avian *Plasmodium* parasites.

It is possible that some *Plasmodium* lineages in the parasite phylogeny may exhibit intraspecific polymorphisms and may not represent distinct species. Taking into account errors due to excessive parasite duplications within host species, we excluded *Plasmodium* lineages with less than 2% sequence divergence and repeated our cophylogenetic analyses with the event cost values used for maximizing cospeciation. Applying ParaFit, PACo, and JANE suggested no significant cospeciation in this analysis ($P > 0{\cdot}05$).

Given that the results strongly depend on good estimations of the set of event cost values, we conducted separate analyses in Core-PA with the event cost values automatically estimated. The estimated event cost values obtained were cospeciation = 0·0029, sorting = 0·0006, duplications = 0·0029, host switching = 0·9936. When applying these event cost values, all number of events was significantly higher than expected by chance alone ($P \leqslant 0{\cdot}05$), with the exception of cospeciation events ($P = 0{\cdot}25$). In summary, we consistently detected significant sorting, duplication, and host switching events throughout our cophylogenetic analyses.

DISCUSSION

A lack of cospeciation and a significant role for host switching in the coevolution of avian malaria parasites

Our study constitutes the first investigation of coevolution between *Plasmodium* parasites and an

African bird family that includes taxa distributed across Africa, comprising a local, regional and continental scale. In our analysis, we found significant cospeciation within *Plasmodium* parasites when implementing a tree-based cophylogenetic analysis with an even cost structure and with a cost structure downweighting cospeciation events. However, no significant cospeciation was apparent when applying a cost structure intended to maximize cospeciation events or when applying the explicit statistical approaches ParaFit and PACo. These data suggest that low levels of cospeciation potentially occur between avian malaria parasites and their hosts, but have little influence on *Plasmodium* diversification.

Therefore, *Plasmodium* diversification is more likely influenced by host switching. Here, we provide additional evidence for this well-described process (Bensch *et al*. 2000; Ricklefs and Fallon, 2002; Waldenström *et al*. 2002). Several *Plasmodium* lineages of our analyses were shared across several avian families of Africa, including Muscicapidae, Nectariniidae, Pycnonotidae and Turdidae (Loiseau *et al*. 2012); suggesting that host-relatedness within avian families may not necessarily limit the evolution of *Plasmodium* parasites *via* host switching. The JANE analysis revealed two specialist parasite lineages arising from duplication and host switching events. These findings support the hypothesis of oscillation and taxon pulses (Hoberg and Brooks, 2008), and also corroborate the greater rates for *Plasmodium* parasites in Africa to transition from generalists to specialists as opposed to transitioning from specialists to generalists (Loiseau *et al*. 2012).

Host switching may affect the coevolutionary cycles between host–parasite histories, and can be linked to the heterogeneous mosquito feeding tendencies across avian hosts (Hamer *et al*. 2009). These feeding patterns contribute to the distribution of avian malaria parasites and may facilitate host switching (Kim and Tsuda, 2012). Such factors will in turn weaken the association between *Plasmodium* parasite and host phylogenies. However, in some cases vectors do not play a major role in the distribution patterns of *Plasmodium* parasites across hosts (Njabo *et al*. 2011; Medeiros *et al*. 2013). Thus host switching may depend more on inherent features of *Plasmodium* or more likely their hosts (Agosta *et al*. 2010). Some hosts may harbour a community of parasites, which can force parasites to compete for host resources (de Roode *et al*. 2005; Graham, 2008). This may be a determining factor for *Plasmodium* parasites to colonize new hosts and possibly explains the prominence of sorting events recovered in our cophylogenetic analyses. Our data confirm the remarkable complexity of the host parasite interactions in the malaria system, and that host and vector ecology likely play important roles in parasite diversification.

Evidence for biotic diversification in avian malaria parasites of African sunbirds

The diversity and structure of parasite communities are likely determined by the distribution of hosts. Hosts with wide distributions, particularly migratory birds, can encounter more parasites and often harbour a greater diversity of parasites in comparison to hosts with restricted distributions (Figuerola and Green, 2000; Hubálek, 2004; Pérez-Tris and Bensch, 2005; Jenkins *et al*. 2012). Migratory birds may also facilitate shifting of *Plasmodium* transmission areas (Hellgren *et al*. 2007*b*). Such a system could result in a lack of phylogeographic structuring of parasite communities, as shown in avian malaria parasite communities of the Black-throated Blue Warbler (*Dendroica caerulescens*) (Fallon *et al*. 2006) and the Common Yellowthroat (*Geothylpis trichas*) (Pagenkopp *et al*. 2008). However, in systems with restricted host distributions, some parasite communities are phylogeographically structured (Fallon *et al*. 2003, 2005). African sunbirds, although not migratory, can have both wide and restricted ranges and thus provided an interesting model for assessing the biogeographic patterns of *Plasmodium* parasite communities. We found that current *Plasmodium* lineage assemblages likely originated in East Africa and that dispersal appears to have played an important role in shaping the observed *Plasmodium* communities. Our results indicate that early and late vicariance events may have also played a role in *Plasmodium* diversification, suggesting that *Plasmodium* parasites may diversify by taxon pulses (Fig. 2), episodes of vicariant events alternating with episodes of dispersal events (Hoberg and Brooks, 2008).

Dispersal events may occur as a result of host switching, which may be followed by isolation or specialization in a particular host (Zarlenga *et al*. 2006; Janz and Nylin, 2007; Waltari *et al*. 2007; Hoberg and Brooks, 2008; Loiseau *et al*. 2012). It is possible that related hosts provide an ecological fit through similar physiological and biochemical environments that allow parasites to persist in novel hosts. Alternatively, parasites may possess phenotypes that are pre-adapted to colonize and survive (i.e. possessing untapped potential fitness) in novel conditions provided through different host (Agosta *et al*. 2010). Our results suggest that host switching is a significant factor that likely leads to a continuum of biotic expansion and biotic isolation over evolutionary and ecological time, which may explain the lack of phylogeographic structuring in *Plasmodium* parasites of African sunbirds.

Although we did not find evidence to suggest strong phylogeographic and biogeographic structuring in the current *Plasmodium* communities of African sunbirds, we observed several interesting *Plasmodium* biogeographic patterns. Some *Plasmodium* lineages were recovered exclusively in

Madagascar and the Comoros. This may in part be due to the host distribution since the *Plasmodium* lineages recovered exclusively in Madagascar and Comoros were found in an endemic host. Conversely, 22% ($N = 10$) of *Plasmodium* lineages were recovered in either two or three of the regions sampled throughout Africa; the majority of these lineages were present in both West and East Africa. These results are not surprising considering that all *Plasmodium* lineages recovered concurrently in West and East Africa were found in a single species, the Olive Sunbird (*Cyanomitra olivacea*). This species is considered to be an ecological generalist and lives in various habitats spanning entirely across West and East Africa (Cheke *et al*. 2001; Bowie *et al*. 2004; Smith *et al*. 2011).

In addition, we find that the majority of *Plasmodium* lineages recovered are restricted to either West Africa or East Africa. Interestingly, 14 *Plasmodium* lineages recovered exclusively in East Africa ($N = 7$) and West Africa ($N = 7$) were also found in the Olive Sunbird. Moreover, these *Plasmodium* lineages are separated not only by region, but by habitat type as well (Loiseau *et al*. 2012). The *Plasmodium* lineages of the Olive Sunbird recovered exclusively in East Africa were found in montane habitats, whereas *Plasmodium* lineages recovered exclusively in West Africa were found in rainforest habitats. As was recently described, it is possible that the differences in habitat or climatic conditions affect the geographical distribution and transmission of *Plasmodium* parasites (Hellgren *et al*. 2007*a*, *b*; Sehgal *et al*. 2011; Loiseau *et al*. 2012), resulting in the observed parasite biogeographic patterns. For instance, temperature and altitudinal variables were shown to impact both parasite diversity and abundance, which in turn can alter the parasite community structure (Paaijmans *et al*. 2010; Van Rooyen *et al*. 2013).

Climate variability also affects the development of parasites and the survival of the vector populations that transmit them (Koenraadt *et al*. 2006; Minakawa *et al*. 2006; Afrane *et al*. 2008; Paaijmans *et al*. 2009, 2010; Chaves and Koenraadt, 2010; LaPointe *et al*. 2010). In theory, parasite and host population structures drive host–parasite coevolution and ultimately leads to a geographic mosaic of coevolutionary hot and cold spots (Thompson, 1994; Lively, 1999; King *et al*. 2009). Taken together, our findings are important in understanding host–parasite coevolutionary dynamics and provide a basis for future studies on the structuring of host–parasite systems.

SUPPLEMENTARY MATERIAL

To view supplementary material for this article, please visit http://dx.doi.org/S0031182014001681.

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REFERENCES

Afrane, Y. A., Little, T. J., Lawson, B. W., Githeko, A. K. and Yan, G. (2008). Deforestation and vectorial capacity of *Anopheles gambiae* Giles mosquitoes in malaria transmission, Kenya. *Emerging Infectious Diseases* **14**, 1533–1538.

Agosta, S. J., Janz, N. and Brooks, D. R. (2010). How specialists can be generalists: resolving the 'parasite paradox' and implications for emerging infectious disease. *Zoologia* **27**, 151–162.

Ali, S. S., Yu, Y., Pfosser, M. and Wetschnig, W. (2011). Inferences of biogeographical histories within subfamily Hyacinthoideae using S-DIVA and Bayesian binary MCMC analysis implemented in RASP (Reconstruct Ancestral State in Phylogenies). *Annals of Botany* **109**, 95–107.

Balbuena, J. A., Míguez-Lozano, R. and Blasco-Costa, I. (2013). PACo: a novel procrustes application to cophylogenetic analysis. *PLoS ONE* **8**, e61048.

Bensch, S., Stjernman, M., Hasselquist, D., Ostman, O., Hansson, B., Westerdahl, H. and Pinheiro, R. T. (2000). Host specificity in avian blood parasites: a study of *Plasmodium* and *Haemoproteus* mitochondrial DNA amplified from birds. *Proceedings of the Royal Society B: Biological Sciences* **267**, 1583–1589.

Bensch, S., Hellgren, O. and Pérez-Tris, J. (2009). MalAvi: a public database of malaria parasites and related haemosporidians in avian hosts based on mitochondrial cytochrome b lineages. *Molecular Ecology Resource* **9**, 1353–1358.

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

Bowie, R. C. K. (2003). Birds, molecules, and evolutionary patterns among Africa's islands in the sky. Ph.D. Thesis. University of Cape Town, South Africa.

Bowie, R. C. K., Fjeldså, J., Hackett, S. J. and Crowe, T. M. (2004). Molecular evolution in space and through time: mtDNA phylogeography of the Olive Sunbird (*Nectarinia olivacea/obscura*) throughout continental Africa. *Molecular Phylogenetics and Evolution* **33**, 56–74.

Brodie, E. D., III and Brodie, E. D., Jr. (1999). Predator–prey arms race and dangerous prey. *Biosciences* **49**, 557–568.

Clark, M. A., Moran, N. A., Baumann, P. and Wernegreen, J. J. (2000). Cospeciation between bacterial endosymbionts (*Buchnera*) and a recent radiation of aphids (*Uroleucon*) and pitfalls of testing for phylogenetic congruence. *Evolution* **54**, 517–525.

Chasar, A., Loiseau, C., Valkiūnas, G., Iezhova, T., Smith, T. B. and Sehgal, R N M. (2009). Prevalence and diversity patterns of avian blood

parasites in degraded African rainforest habitats. *Molecular Ecology* **18**, 4121–4133.

Chaves, L. F. and Koenraadt, C. J. M. (2010). Climate change and highland malaria: fresh air for a hot debate. *Quarterly Review of Biology* **85**, 27–55.

Cheke, R. S., Clive, M. F. and Allen, R. (2001). *Sunbirds*. Yale University Press, New Haven and London.

Conow, C., Fielder, D., Ovadia, Y. and Libeskind-Hadas, R. (2010). Jane: a new tool for the cophylogeny reconstruction problem. *Algorithms for Molecular Biology* **5**, 16.

Cooper, N., Griffin, R., Franz, M., Omotayo, M., Nunn, C. L. and Fryxell, J. (2012). Phylogenetic host specificity and understanding parasite sharing in primates. *Ecology Letters* **15**, 1370–1377.

Demastes, J. W. and Hafner, M. S. (1993). Cospeciation of pocket gophers (*Geomys*) and their chewing lice (*Geomydoecus*). *Journal of Mammalogy* **74**, 521.

De Roode, J. C., Pansini, R., Cheesman, S. J., Helinski, M. E. H., Huijben, S., Wargo, A. R., Bell, A. S., Chan, B. H. K., Walliker, D. and Read, A. F. (2005). Virulence and competitive ability in genetically diverse malaria infections. *Proceedings of the National Academy of Sciences of the USA* **102**, 7624–7628.

Drummond, A. J., Suchard, M. A., Xie, D. and Rambaut, A. (2012). Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* **29**, 1969–1973.

Ehrnsberger, R. (2001). A preliminary analysis of phylogenetic relationships of the feather mite family Freyanidae Dubinin, 1953 (Acari: Astigmata). *Biological Bulletin of Poznań* **38**, 181–201.

Excoffier, L. and Lischer, H. E. L. (2010). Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* **10**, 564–567.

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

Fallon, S. M., Fleischer, R. C. and Graves, G. R. (2006). Malarial parasites as geographical markers in migratory birds? *Biology Letters* **2**, 213–216.

Figuerola, J. and Green, A. J. (2000). Haematozoan parasites and migratory behaviour in waterfowl. http://digital.csic.es/handle/10261/43207.

Galtier, N., Gouy, M. and Gautier, C. (1996). SEAVIEW and PHYLO_WIN: two graphic tools for sequence alignment and molecular phylogeny. *Computer Applications in the Biosciences: CABIOS* **12**, 543–548.

Garamszegi, L. Z. (2006). The evolution of virulence and host specialization in malaria parasites of primates. *Ecology Letters* **9**, 933–940.

Garamszegi, L. Z. (2009). Patterns of co-speciation and host switching in primate malaria parasites. *Malaria Journal* **8**, 110.

Graham, A. L. (2008). Ecological rules governing helminth–microparasite coinfection. *Proceedings of the National Academy of Sciences* **105**, 566–570.

Hagner, S. C., Misof, B., Maier, W. A. and Kampen, H. (2007). Bayesian analysis of new and old malaria parasite DNA sequence data demonstrates the need for more phylogenetic signal to clarify the descent of *Plasmodium falciparum*. *Parasitology Research* **101**, 493–503.

Hamer, G. L., Kitron, U. D., Goldberg, T. L., Brawn, J. D., Loss, S. R., Ruiz, M. O., Hayes, G. L. and Walker, E. D. (2009). Host selection by *Culex pipiens* mosquitoes and West Nile virus amplification. *American Journal of Tropical Medicine and Hygiene* **80**, 268–278.

Hellgren, O., Waldenström, J. and Bensch, S. (2004). A new PCR assay for simultaneous studies of Leucocytozoon, Plasmodium, and Haemoproteus from avian blood. *Journal of Parasitology* **90**, 797–802.

Hellgren, O., Krizanauskiene, A., Valkiūnas, G. and Bensch, S. (2007*a*). Diversity and phylogeny of mitochondrial cytochrome B lineages from six morphospecies of avian *Haemoproteus* (Haemosporida: Haemoproteidae). *Journal of Parasitology* **93**, 889–896.

Hellgren, O., Waldenström, J., Peréz-Tris, J., Szöll, E., Si, O., Hasselquist, D., Krizanauskiene, A., Ottosson, U. and Bensch, S. (2007*b*). Detecting shifts of transmission areas in avian blood parasites: a phylogenetic approach. *Molecular Ecology* **16**, 1281–1290.

Hellgren, O., Pérez-Tris, J. and 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.

Hendricks, S., Flannery, M. E. and Spicer, G. S. (2013). Cophylogeny of quill mites from the genus *Syringophilopsis* (Acari: Syringophilidae) and their North American passerine hosts. *Journal of Parasitology* **99**, 827–834.

Hoberg, E. P. and Brooks, D. R. (2008). A macroevolutionary mosaic: episodic host-switching, geographical colonization and diversification in complex host–parasite systems. *Journal of Biogeography* **35**, 1533–1550.

Hubálek, Z. (2004). An annotated checklist of pathogenic microorganisms associated with migratory birds. *Journal of Wildlife Diseases* **40**, 639–659.

Hughes, A. L. and Verra, F. (2010). Malaria parasite sequences from chimpanzee support the co-speciation hypothesis for the origin of virulent human malaria (*Plasmodium falciparum*). *Molecular Phylogenetics and Evolution* **57**, 135–143.

Hunt, J. S., Bermingham, E. and Ricklefs, R. E. (2001). Molecular systematics and biogeography of antillean thrashers, tremblers, and mockingbirds (Aves: Mimidae). *Auk* **118**, 35–55.

Janz, N. and Nylin, S. (2007). The oscillation hypothesis of host plant-range and speciation. In *Specialization, Speciation and Radiation: the Evolutionary Biology of Herbivorus Insects* (ed. Tilman, J. T.), pp. 203–215. University of California Press, Berkeley, CA.

Jenkins, T. and 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? *Molecular Ecology* **20**, 3910–3920.

Jenkins, T., Thomas, G. H., Hellgren, O. and Owens, I. P. F. (2012). Migratory behavior of birds affects their coevolutionary relationship with blood parasites. *Evolution* **66**, 740–751.

Kamdem, C., Tene Fossog, B., Simard, F., Etouna, J., Ndo, C., Kengne, P., Boussès, P., Etoa, F.-X., Awono-Ambene, P., Fontenille, D., Antonio-Nkondjio, C., Besansky, N. J. and Costantini, C. (2012). Anthropogenic habitat disturbance and ecological divergence between incipient species of the malaria mosquito *Anopheles gambiae*. *PLoS ONE* **7**, e39453.

Kawecki, T. J. (1998). Red queen meets Santa Rosalia: arms races and the evolution of host specialization in organisms with parasitic lifestyles. *American Naturalist* **152**, 635–651.

Kim, K. S. and Tsuda, Y. (2012). Avian *Plasmodium* lineages found in spot surveys of mosquitoes from 2007 to 2010 at Sakata wetland, Japan: do dominant lineages persist for multiple years? *Molecular Ecology* **21**, 5374–5385.

King, K. C., Delph, L. F., Jokela, J. and Lively, C. M. (2009). The geographic mosaic of sex and the Red Queen. *Current Biology* **19**, 1438–1441.

Koenraadt, C. J. M., Paaijmans, K. P., Schneider, P., Githeko, A. K. and Takken, W. (2006). Low larval vector survival explains unstable malaria in the western Kenya highlands. *Tropical Medicine and International Health* **11**, 1195–1205.

LaPointe, D. A., Goff, M. L. and Atkinson, C. T. (2010). Thermal constraints to the sporogonic development and altitudinal distribution of avian malaria *Plasmodium relictum* in Hawai'i. *Journal of Parasitology* **96**, 318–324.

Legendre, P., Desdevises, Y. and Bazin, E. (2002). A statistical test for host–parasite coevolution. *Systematic Biology* **51**, 217–234.

Levine, N. D. (1988). *The Protozoan Phylum Apicomplexa*. CRC Press, Baca Raton.

Levins, R. (1968). *Evolution in Changing Environments*. Princeton University Press, New Jersey.

Lively, C. M. (1999). Migration, virulence, and the geographic mosaic of adaptation by parasites. *American Naturalist* **153**, S34–S47.

Loiseau, C., Harrigan, R. J., Robert, A., Bowie, R. C. K., Henri, A. T., Smith, T. B. and Sehgal, R. N. M. (2012). Host and habitat specialization of avian malaria in Africa. *Molecular Ecology* **21**, 431–441.

Martinsen, E. S., Perkins, S. L. and Schall, J. J. (2008). A three-genome phylogeny of malaria parasites (*Plasmodium* and closely related genera): evolution of life-history traits and host switches. *Molecular Phylogenetics and Evolution* **47**, 261–273.

Medeiros, M. C. I., Hamer, G. L. and Ricklefs, R. E. (2013). Host compatibility rather than vector-host encounter rate determines the host range of avian *Plasmodium* parasites. *Proceedings of the Royal Society B: Biological Sciences* **280**, 20122947.

Merkle, D., Middendorf, M. and Wieseke, N. (2010). A parameter-adaptive dynamic programming approach for inferring cophylogenies. *BMC Bioinformatics* **11**, S60.

Mideo, N. (2009). Parasite adaptations to within-host competition. *Trends in Parasitology* **25**, 261–268.

Mideo, N., Alizon, S. and Day, T. (2008). Linking within- and between-host dynamics in the evolutionary epidemiology of infectious diseases. *Trends in Ecology and Evolution* **23**, 511–517.

Minakawa, N., Omukunda, E., Zhou, G., Githeko, A. and Yan, G. (2006). Malaria vector productivity in relation to the highland environment in Kenya. *American Journal of Tropical Medicine and Hygiene* **75**, 448–453.

Morelli, M. and Spicer, G. (2007). Cospeciation between the nasal mite *Ptilonyssus sairae* (Acari: Rhinonyssidae) and its bird hosts. *Systematic and Applied Acarology* **12**, 179–188.

Njabo, K. Y., Cornel, A. J., Bonneaud, C., Toffelmier, E., Sehgal, R. N. M., Valkiūnas, G., Russell, A. F. and Smith, T. B. (2011). Nonspecific patterns of vector, host and avian malaria parasite associations in a central African rainforest. *Molecular Ecology* **20**, 1049–1061.

Paaijmans, K. P., Read, A. F. and Thomas, M. B. (2009). Understanding the link between malaria risk and climate. *PNAS* **106**, 13844–13849.

Paaijmans, K. P., Blanford, S., Bell, A. S., Blanford, J. I., Read, A. F. and Thomas, M. B. (2010). Influence of climate on malaria transmission depends on daily temperature variation. *Proceedings of the National Academy of Sciences* **107**, 15135–15139.

Page, R. D. M. (1994). Parallel phylogenies: reconstructing the history of host-parasite assemblages. *Cladistics* **10**, 155–173.

Pagenkopp, K., Klicka, J., Durrant, K., Garvin, J. and Fleischer, R. (2008). Geographic variation in malarial parasite lineages in the Common Yellowthroat (*Geothylpis trichas*). *Conservation Genetics* 1577–1588.

Palinauskas, V., Valkiūnas, G., Bolshakov, C. V. and Bensch, S. (2011). *Plasmodium relictum* (lineage SGS1) and *Plasmodium ashfordi* (lineage GRW2): the effects of the co-infection on experimentally infected passerine birds. *Experimental Parasitology* **127**, 527–533.

Pavlacky, D. C., Possingham, H. P., Lowe, A. J., Prentis, P. J., Green, D. J. and Goldizen, A. W. (2012). Anthropogenic landscape change promotes asymmetric dispersal and limits regional patch occupancy in a spatially structured bird population. *Journal of Animal Ecology* **81**, 940–952.

Pérez-Tris, J. and Bensch, S. (2005). Dispersal increases local transmission of avian malarial parasites. *Ecology Letters* **8**, 838–845.

Pérez-Tris, J., Hellgren, O., Križanauskienė, A., Waldenström, J., Secondi, J., Bonneaud, C., Fjeldså, J., Hasselquist, D. and Bensch, S. (2007). Within-host speciation of malaria parasites. *PLoS ONE* **2**, e235.

Posada, D. and Crandall, K. A. (1998). MODELTEST: testing the model of DNA substitution. *Bioinformatics* **14**, 817–818.

Primmer, C. R., Borge, T., Lindell, J. and Saetre, G.-P. (2002). Single-nucleotide polymorphism characterization in species with limited available sequence information: high nucleotide diversity revealed in the avian genome. *Molecular Ecology* **11**, 603–612.

Rathore, D., Wahl, A. M., Sullivan, M. and McCutchan, T. F. (2001). A phylogenetic comparison of gene trees constructed from plastid, mitochondrial and genomic DNA of *Plasmodium* species. *Molecular and Biochemical Parasitology* **114**, 89–94.

R Core Team. (2012). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Read, A. F. and Taylor, L. H. (2001). The ecology of genetically diverse infections. *Science* **292**, 1099–1102.

Ricklefs, R. E. and Fallon, S. M. (2002). Diversification and host switching in avian malaria parasites. *Proceedings of the Royal Society B: Biological Sciences* **269**, 885–892.

Ricklefs, R. E., Fallon, S. M. and Bermingham, E. (2004). Evolutionary relationships, cospeciation, and host switching in avian malaria parasites. *Systematic Biology* **53**, 111–119.

Ronquist, F. and Huelsenbeck, J. P. (2003). MrBayes 3: bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**, 1572–1574.

Sehgal, R. N. M. and Lovette, I. J. (2003). Molecular evolution of three avian neurotrophin genes: implications for proregion functional constraints. *Journal of Molecular Evolution* **57**, 335–342.

Sehgal, R. N. M., Buermann, W., Harrigan, R. J., Bonneaud, C., Loiseau, C., Chasar, A., Sepil, I., Valkiūnas, G., Iezhova, T., Saatchi, S. and Smith, T. B. (2011). Spatially explicit predictions of blood parasites in a widely distributed African rainforest bird. *Proceedings of the Royal Society B: Biological Sciences* **278**, 1025–1033.

Smith, T. B., Thomassen, H. A., Freedman, A. H., Sehgal, R. N. M., Buermann, W., Saatchi, S., Pollinger, J., Milá, B., Pires, D., Valkiūnas, G. and Wayne, R. K. (2011). Patterns of divergence in the olive sunbird *Cyanomitra olivacea* (Aves: Nectariniidae) across the African rainforest–savanna ecotone. *Biological Journal of the Linnean Society* **103**, 821–835.

Stamatakis, A. (2006). RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22**, 2688–2690.

Svensson-Coelho, M., Blake, J. G., Loiselle, B. A., Penrose, A. S., Parker, P. G. and Ricklefs, R. E. (2013). Diversity, prevalence, and host specificity of avian Plasmodium and Haemoproteus in a western amazon assemblage. *Ornithological Monographs No. 76* No. 76, 1–47 (American Ornithologists' Union).

Swei, A., Rowley, J. J. L., Rödder, D., Diesmos, M. L. L., Diesmos, A. C., Briggs, C. J., Brown, R., Cao, T. T., Cheng, T. L., Chong, R. A., Han, B., Hero, J., Hoang, H. D., Kusrini, M. D., Le, D. T. T., McGuire, J. A., Meegaskumbura, M., Min, M., Mulcahy, D. G., Neang, T., Phimmachak, S., Rao, D., Schoville, S. D., Sivongxay, N., Srei, N., Stöck, M., Stuart, B. L., Torres, L. S., Tran, D. T. A., Tunstall, T. S., Vieites, D. and Vredenburg, V. T. (2011). Is chytridiomycosis an emerging infectious disease in Asia? *PLoS ONE* **6**, e23179.

Swofford, D. (2001). *PAUP* 4.0*. Sinauer Associates.

Szymanski, M. M. and Lovette, I. J. (2005). High lineage diversity and host sharing of malarial parasites in a local avian assemblage. *Journal of Parasitology* **91**, 768–774.

Thompson, J. N. (1994). *The Coevolutionary Process*. The University of Chicago Press, Chicago, USA.

Valkiunas, G. (2005). *Avian Malaria Parasite and other Haemosporidia*. CRC Press, Boca Raton, FL, USA.

Van Riper, C., III, van Riper, S. G., Goff, M. L. and Laird, M. (1986). The epizootiology and ecological significance of malaria in Hawaiian land birds. *Ecological Monographs* **56**, 327.

van Rooyen, J., Lalubin, F., Glaizot, O. and Christe, P. (2013). Altitudinal variation in haemosporidian parasite distribution in great tit populations. *Parasites and Vectors* **6**, 139.

Waldenström, J., Bensch, S., Kiboi, S., Hasselquist, D. and Ottosson, U. (2002). Cross-species infection of blood parasites between resident and migratory songbirds in Africa. *Molecular Ecology* **11**, 1545–1554.

Waldenström, J., Bensch, S., Hasselquist, D. and Ostman, O. (2004). A new nested polymerase chain reaction method very efficient in detecting *Plasmodium* and *Haemoproteus* infections from avian blood. *Journal of Parasitology* **90**, 191–194.

Waltari, E., Hoberg, E. P., Lessa, E. P. and Cook, J. A. (2007). Eastward Ho: phylogeographical perspectives on colonization of hosts and parasites across the Beringian nexus. *Journal of Biogeography* **34**, 561–574.

Yu, Y., Harris, A. J. and He, X. (2010). S-DIVA (Statistical Dispersal-Vicariance Analysis): a tool for inferring biogeographic histories. *Molecular Phylogenetics and Evolution* **56**, 848–850.

Yu, Y., Harris, A. J. and He, X. (2013). RASP (Reconstruct Ancestral State in Phylogenies). 2.1 beta. Available at http://mnh.scu.edu.cn/soft/blog/RASP

Zarlenga, D. S., Rosenthal, B. M., La Rosa, G., Pozio, E. and Hoberg, E. P. (2006). Post-miocene expansion, colonization, and host switching drove speciation among extant nematodes of the archaic genus *Trichinella*. *Proceedings of the National Academy of Sciences of the USA* **103**, 7354–7359.