Diversity and distribution of avian haematozoan parasites in the western Indian Ocean region: a molecular survey

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SUMMARY

The genetic diversity of haematozoan parasites in island avifauna has only recently begun to be explored, despite the potential insight that these data can provide into the history of association between hosts and parasites and the possible threat posed to island endemics. We used mitochondrial DNA sequencing to characterize the diversity of 2 genera of vector-mediated parasites (*Plasmodium* and *Haemoproteus*) in avian blood samples from the western Indian Ocean region and explored their relationship with parasites from continental Africa. We detected infections in 68 out of 150 (45·3%) individuals and cytochrome *b* sequences identified 9 genetically distinct lineages of *Plasmodium* spp. and 7 lineages of *Haemoproteus* spp. We found considerable heterogeneity in parasite lineage composition across islands, although limited sampling may, in part, be responsible for perceived differences. Two lineages of *Plasmodium* spp. and 2 lineages of *Haemoproteus* spp. were shared by hosts in the Indian Ocean and also on mainland Africa, suggesting that these lineages may have arrived relatively recently. Polyphyly of island parasites indicated that these parasites were unlikely to constitute an endemic radiation and instead probably represent multiple colonization events. This study represents the first molecular survey of vector-mediated parasites in the western Indian Ocean, and has uncovered a diversity of parasites. Full understanding of parasite community composition and possible threats to endemic avian hosts will require comprehensive surveys across the avifauna of this region.

Key words: Africa, *Haemoproteus*, haematozoan parasites, western Indian Ocean, *Plasmodium*.

INTRODUCTION

Geographical patterns in the distribution of parasitic organisms can provide insights into the ecological and evolutionary forces that determine host-parasite associations. Regional parasite fauna are shaped by local colonization and extinction events, which in turn may be driven by a combination of host immune responses, competition between parasites, and stochastic changes in the environment (Apanius *et al.* 2000; Fallon *et al.* 2003, 2005; Ishtiaq *et al.* 2010). Parasites with a direct life cycle are often widespread in their geographical distribution. Their presence is dependent primarily on the occurrence of a definitive host. Vector-mediated parasites such as *Plasmodium* spp. and *Haemoproteus* spp. rely on the presence of both an appropriate host and a competent vector (Bennett *et al.* 1974; Apanius *et al.* 2000). The primary vectors for *Haemoproteus* spp. are known to be biting midges of the genus *Culicoides* (Diptera: Ceratopogonidae) and louse flies (Diptera: Hippoboscidae) (Atkinson and van Riper, 1991). Species of avian *Plasmodium* are most commonly transmitted by mosquitoes (Valkiũnas, 2005). Parasites with specialized vector associations tend to have a restricted range of hosts (Killick-Kendrick, 1978) and consequently have restricted geographical distributions (Lajeuness and Forbes, 2002). In contrast, the use of a broad spectrum of blood-feeding vectors may facilitate host switching in generalist parasites (Githeko *et al.* 1994), enlarging the geographical range of those parasites. Furthermore, the spatial distribution of parasites is closely linked to the environmental, ecological, climatic and geographical connectivity which governs the faunistic exchange between populations.

Oceanic archipelagos have been used as natural laboratories for understanding the evolutionary processes of speciation and divergence (Mayr, 1942; MacArthur and Wilson, 1967) and provide a unique opportunity to explore community assemblage in similar yet geographically discrete units. The western Indian Ocean region has been used in recent years as a model area to distinguish between patterns of vicariance and dispersal in various taxonomic groups (Bossuyt and Milinkovitch, 2001; Biju and Bossuyt, 2003; Renner, 2004*a*,*b*), and to understand patterns of island colonization from neighbouring continents (e.g. Warren *et al.* 2003, 2005; Fig. 1). The western

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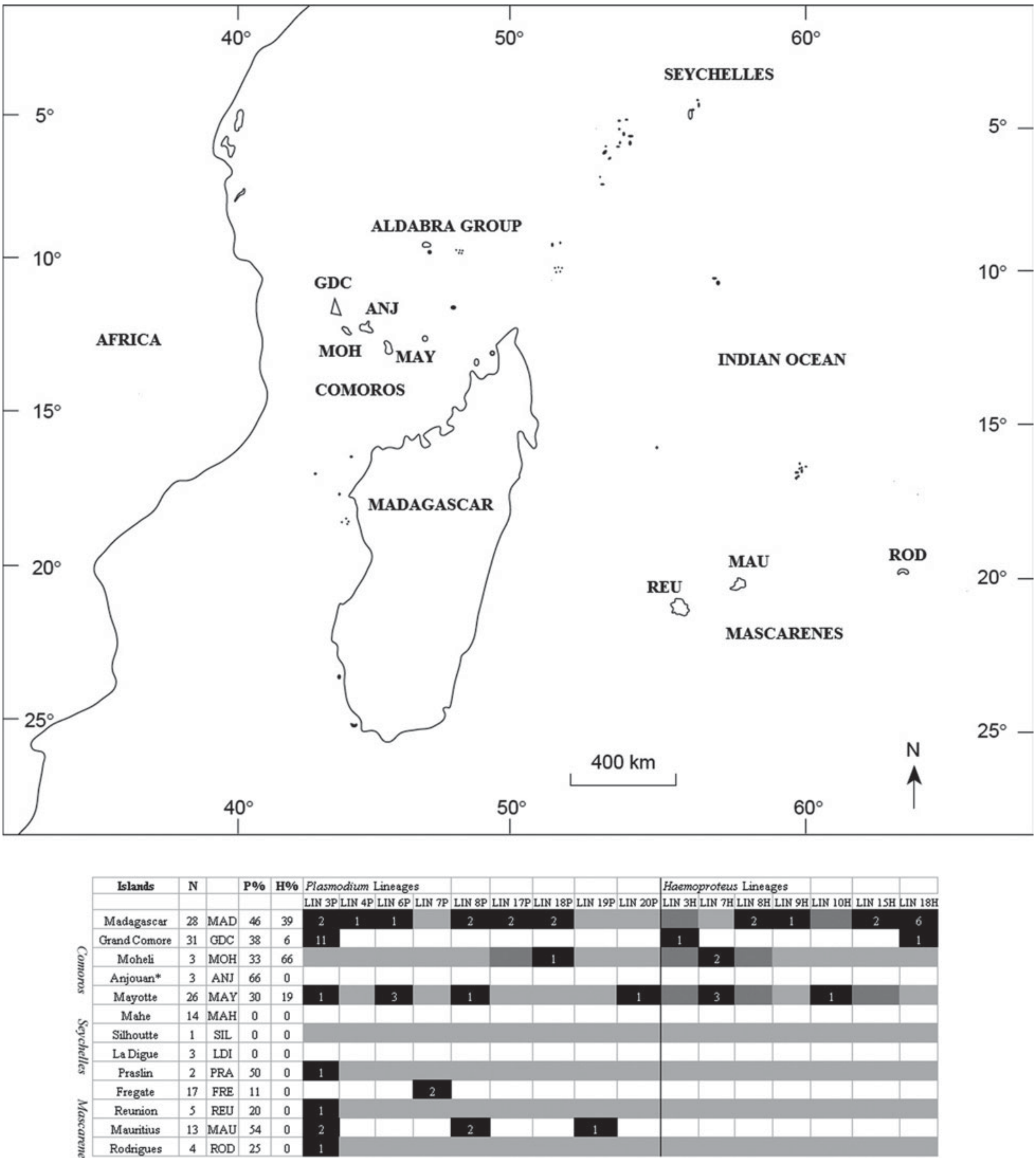

	Islands	N		P%	H%	*Plasmodium* Lineages									*Haemoproteus* Lineages						
						LIN 3P	LIN 4P	LIN 6P	LIN 7P	LIN 8P	LIN 17P	LIN 18P	LIN 19P	LIN 20P	LIN 3H	LIN 7H	LIN 8H	LIN 9H	LIN 10H	LIN 15H	LIN 18H
	Madagascar	28	MAD	46	39	2	1	1		2	2	2					2	1		2	6
Comoros	Grand Comore	31	GDC	38	6	11									1						1
	Moheli	3	MOH	33	66							1				2					
	Anjouan*	3	ANJ	66	0																
	Mayotte	26	MAY	30	19	1		3		1				1		3			1		
Seychelles	Mahe	14	MAH	0	0																
	Silhoutte	1	SIL	0	0																
	La Digue	3	LDI	0	0																
	Praslin	2	PRA	50	0	1															
	Fregate	17	FRE	11	0				2												
Mascarene	Reunion	5	REU	20	0	1															
	Mauritius	13	MAU	54	0	2				2			1								
	Rodrigues	4	ROD	25	0	1															

Fig. 1. Distribution of unique *Plasmodium* and *Haemoproteus* lineages by island. MAD = Madagascar; GDC = Grand Comore; MOH = Moheli; ANJ* = Anjouan (no lineage information available); MAY = Mayotte; MAH = Mahe; SIL = Silhoutte; LDI = La Digue; PRA = Praslin; FRE = Fregate; REU = Reunion; MAU = Mauritius; ROD: Rodrigues. P% = *Plasmodium* prevalence, H% = *Haemoproteus* prevalence. Total parasite prevalence includes 91 bp sequences (not used in phylogenetic analysis).

Indian Ocean region is an avian diversity hotspot with nearly 300 bird species, of which nearly 60% are endemic species. Over 55 endemic species are endangered or threatened by their restricted range. Given the documented and suspected threats posed by avian blood parasites in other island birds (Beadell *et al*. 2006; Matson and Beadell, 2010), haematozoa remain surprisingly understudied in the Indian Ocean region.

Whilst early microscopy-based surveys of Indian Ocean avifauna revealed a diverse array of blood parasites (Lowery, 1971; Peirce *et al*. 1977; Bennett and Blancou, 1974; Savage, 2003; Savage *et al*. 2009), they have so far been little-explored using molecular techniques, and their diversity and distribution is poorly known relative to that of the neighbouring African continent (e.g. Bensch *et al*. 2000; Beadell *et al*. 2009). Given the long-term geographical isolation and concurrent evolution of a unique avifauna with few migrants, the evolution of indigenous parasite fauna with endemic hosts could be considered as a possibility. With the recent advent of molecular techniques, a wealth of genetic diversity in parasite lineages has been revealed (Bensch *et al.* 2000; Ricklefs and Fallon, 2002; Waldenström *et al.* 2002; Beadell *et al.* 2004; Ishtiaq *et al.* 2007), much of which was not evident from morphology alone (Hellgren *et al.* 2007). In addition, indications from the associations with corresponding nuclear sequences suggest that some cytochrome *b* lineages may represent reproductively isolated biological entities (Bensch *et al.* 2004; Beadell *et al.* 2009). Employing a molecular approach gives us the opportunity to gain a deeper understanding of the diversity of avian haematozoa present and, by resolving the history of association, to gain insight into the threat posed to the endemic avifauna by novel parasite introductions.

In this study, using mitochondrial DNA sequences (cytochrome *b* gene), we investigated geographical structuring of *Plasmodium* and *Haemoproteus* across avian hosts from the following islands or archipelagos in the Indian Ocean region (Fig. 1): Madagascar, Mascarenes (Mauritius, Reunion and Rodrigues), Comoros (Grand Comore, Moheli, Anjouan and Mayotte) and granitic Seychelles (of which the principal islands are Mahe, Silhoutte, La Digue and Praslin). Specifically, we aimed to characterize (a) haematozoan parasite diversity; (b) the phylogenetic relationships of haematozoan parasites found in these islands and (c) the evolutionary relationships between mainland and island parasite lineages.

MATERIALS AND METHODS

Avian sampling

A total of 150 avian blood samples from western Indian Ocean islands were collected during expeditions to Madagascar, the Mascarenes, Comoros and granitic Seychelles between July 1999 and February 2002 (Table 1 for species and sample size). All samples were taken non-destructively from mist-netted individuals and stored in Queen's lysis buffer (Seutin *et al.* 1991, 1993). In addition, 145 blood samples from mainland Africa were collected from 1991 to 2005 (see Appendix 1).

Molecular analysis

Host and parasite DNA was extracted from blood samples using the Qiagen DNeasy kits and protocols. Samples were screened for haematozoan infection with primers F2/R2 (91 bp) and 850F/1024R (167 bp; Beadell *et al.* 2004) designed to amplify small fragments of cytochrome *b* gene and COIII gene, respectively. For those samples that were positive for parasites based on these tests, we attempted to amplify a larger fragment (256–533 bp) of cytochrome *b* using primers (F2/4292RW2-256 bp, FIFI/4292RW2-351 bp, F3/4292RW2-295 bp, or 3760/4292RW2-533 bp) designed from sequence that was relatively conserved between *Haemoproteus* and *Plasmodium*. Details of the polymerase chain reaction (PCR) were as reported by Beadell *et al.* (2004) and Ishtiaq *et al.* (2006). Negative and positive controls were included in all PCR reactions. In addition, to help ensure that any negative results from parasite screens were not attributable to poor quality of extractions, we amplified a small fragment (347 bp) of avian cytochrome *b* DNA using primers cytb-1/cytb2 (Kocher *et al.* 1989). These PCRs were successful in all cases.

We identified infections to genus by comparing the relationship of sequences obtained from this study to sequences in GenBank with known taxonomy (see Phylogenetic analysis below). In general, we sequenced the longest fragment that successfully amplified for any given sample. Samples were purified using Qiaquick (Qiagen) and sequenced bidirectionally on an ABI 3100 Sequencer (Applied Biosystems, Inc.). Sequences were edited and aligned using the program SEQUENCHER version 4.1 and are available through GenBank (Accession numbers JN661907–JN662002 in Appendix 1). In a few cases, we were only able to obtain sequence from the small fragments F2/R2 or 850F/1024R. In these cases, we identified the infection as *Plasmodium* or *Haemoproteus*, but we did not include these sequences in lineage-specific analyses. The adjusted Wald method was used to estimate 95% confidence intervals (CIs) for the estimates of parasite prevalence (Agresti and Coull, 1998).

Phylogenetic analysis

In order to estimate the relationships of western Indian Ocean parasite lineages to each other as well as to mainland lineages, we combined sequences obtained from western Indian Ocean samples with sequences obtained from additional PCR screening of mainland African hosts, as well as GenBank sequences derived from mainland African (Bensch *et al.* 2000; Waldenström *et al.* 2002; Hellgren *et al.* 2007; Durrant *et al.* 2007; Beadell *et al.* 2009) and Asian hosts (Ishtiaq *et al.* 2007) in a single phylogenetic analysis. In order to reduce the size of the tree for final analysis, we retained the single mainland lineage that was most closely related to each island-derived sequence, plus a random collection of other parasite lineages. We estimated the parasite phylogenetic relationships using samples for which we had at least 256 base pairs of cytochrome *b* sequence. We used MODELTEST version 3.6 (Posada and Crandall, 1998), to determine the most appropriate substitution model for our data. The

Table 1. Prevalence and molecular ID of haematozoan parasites in avian hosts sampled across islands of the western Indian Ocean

Island	Family	Host species	*N*	*Plasmodium positive N* (%)	*Haemoproteus positive N* (%)	Lineages detected
Madagascar (MAD)						
	Pycnonotidae	Madagascar Bulbul (*Hypsipetes madagascariensis*)	4	4 (100)	0	17P, 18P
	Zosteropidae	Madagascar White-eye (*Zosterops maderaspatanus*)	9	2 (22)	6 (66)	3P, 18H
	Nectariniidae	Souimanga Sunbird (*Nectarinia souimanga*)	4	2 (50)	0	6P
	Ploceidae	Red Fody (*Foudia madagascariensis*)	9	5 (55)	3 (33)	3P, 4P, 8P, 9H, 15H
		Sakalava Weaver (*Ploceus sakalava*)	2	0	2 (100)	8H
COMOROS ARCHIPELAGO						
Grand Comore (GDC)	Pycnonotidae	Madagascar Bulbul (*Hypsipetes madagascariensis*)	2	0	1 (50)	3H
		Comoro Bulbul (*Hypsipetes parvirostris*)	2	0	0	–
	Zosteropidae	Comoro White-eye (*Zosterops mouroniensis*)	4	2 (50)	0	3P
		Kirk's Madagascar White-eye (*Z. d. kirki*)	4	3 (75)	1 (25)	3P, 18H
	Nectariniidae	Humblot's Sunbird (*Nectarinia humbloti*)	3	1 (33)	0	3P
		Long-billed Green Sunbird (*N. notata*)	2	1 (50)	0	3P
	Ploceidae	Red Fody (*Foudia madagascariensis*)	9	2 (22)	0	3P
		Red-headed Fody (*Foudia eminentissima*)	4	2 (50)	0	3P
	Muscicapidae	Grand Comoro Flycatcher (*Humblotia flavirostris*)	1	1 (100)	0	3P
Moheli (MOH)	Zosteropidae	Madagascar White-eye (*Zosterops maderaspatanus*)	1	1 (100)	0	18P
	Ploceidae	Red Fody (*Foudia madagascariensis*)	2	0	2 (100)	7H
Anjouan (ANJ)	Ploceidae	Red Fody (*Foudia madagascariensis*)	3	2 (66)	0	not identified
Mayotte (MAY)	Pycnonotidae	Madagascar Bulbul (*Hypsipetes madagascariensis*)	5	2 (40)	0	20P
	Zosteropidae	Madagascar White-eye (*Zosterops maderaspatanus*)	5	1 (20)	0	3P
	Nectariniidae	Mayotte Sunbird (*N. coquereli*)	5	4 (80)	0	6P
	Ploceidae	Red Fody (*Foudia madagascariensis*)	7	1 (14)	2 (29)	8P, 7H
		Red-headed Fody (*Foudia eminentissima*)	4	0	3 (75)	7H, 10H
SEYCHELLES ARCHIPELAGO						
Mahe (MAH)	Pycnonotidae	Seychelles Bulbul (*Hypsipetes crassirostris*)	5	0	0	–
	Nectariniidae	Seychelles Sunbird (*Nectarinia dussumeiri*)	4	0	0	–
	Ploceidae	Red Fody (*Foudia madagascariensis*)	5	0	0	–
Silhouette (SIL)	Nectariniidae	Seychelles Sunbird (*Nectarinia dussumeiri*)	1	0	0	–
La Digue (LDI)	Pycnonotidae	Seychelles Bulbul (*Hypsipetes crassirostris*)	2	0	0	–
	Ploceidae	Red Fody (*Foudia madagascariensis*)	1	0	0	–
Praslin (PRA)	Ploceidae	Red Fody (*Foudia madagascariensis*)	2	1 (50)	0	3P
Fregate (FRE)	Nectariniidae	Seychelles Sunbird (*Nectarinia dussumieri*)	4	1 (25)	0	7P
	Ploceidae	Seychelles Fody (*Foudia sechellarum*)	9	1 (11)	0	7P
		Red Fody (*Foudia madagascariensis*)	4	0	0	–
MASCARENES ARCHIPELAGO						
Reunion (REU)	Zosteropidae	Bourbon White-eye (*Zosterops borbonicus*)	4	1 (25)	0	3P
		Olive White-eye (*Zosterops olivaceus*)	1	0	0	–
Mauritius (MAU)	Pycnonotidae	Mauritius Bulbul (*Hypsipetes olivaceus*)	1	0	0	–
	Zosteropidae	Bourbon White-eye (*Zosterops borbonicus*)	4	3 (75)	0	3P, 19P
		Olive White-eye (*Zosterops olivaceus*)	2	1 (50)	0	not identified
	Ploceidae	Red Fody (*Foudia madagascariensis*)	2	2 (100)	0	8P
	Estrildidae	Common Waxbill (*Estrilda astrild*)	4	1 (25)	0	not identified
Rodrigues (ROD)	Ploceidae	Yellow Fody (*Foudia flavicans*)	4	1	0	3P

hierarchical likelihood ratio test selected the general time reversible model (GTR + I + G). Phylogenetic reconstruction was implemented in BEAST version 1.4.7 (Drummond and Rambaut, 2007) using a MCMC (Markov Chain Monte Carlo) algorithm and assuming a relaxed clock model (Drummond *et al.* 2006) of nucleotide substitution with a mean substitution rate of 1% per million years (and rates of evolution uncorrelated and log normal distribution among branches) and a Yule prior on branching. We conducted 2 runs for 20 million generations, each with sampling conducted every 1000 generations. *Tracer* (Rambaut and Drummond, 2003) was used to assess convergence, whether 2 chains were mixing, and whether the estimated sample (ESS) for each parameter was of sufficient size (ESS > 200) to obtain robust parameter estimates. Four million generations were discarded as burn-in from each run, leaving a posterior distribution of 32 000 trees. We used 2 published sequences of mammalian *Plasmodium* parasites (see Perkins and Schall, 2002) as outgroups. The generic identity of the parasite lineages (*Haemoproteus* [H] *vs Plasmodium* [P]) was determined by the placement of cytochrome *b* sequences within a phylogenetic tree following Perkins and Schall (2002), Beadell *et al.* (2004) and Ishtiaq *et al.* (2006, 2007).

RESULTS

Parasite prevalence

Using a PCR-based approach, a total of 150 avian samples of 21 species was screened from the Indian Ocean region, of which 68 individuals (46·3%, 95% CI 38·3 – 54·3%) representing 16 species that tested positive for *Plasmodium* spp. or *Haemoproteus* spp. Of the samples testing positive for infection, 48 harboured *Plasmodium*, and 20 harboured *Haemoproteus* spp. infections. We detected *Plasmodium* spp. in samples from all 4 of the archipelagos; this parasite exhibited relatively high prevalence in Madagascar (46·4%; 95% CI 27·9 – 64·9%; $n = 28$), the Comoros (50·8%; 95% CI 38·5 – 63·1%; $n = 63$) and Mascarenes (40·9%; 95% CI 20·3 – 61·5%; $n = 22$), compared to the Seychelles (8·1%; 95% CI 2·4 – 18·4%; $n = 37$). We detected *Haemoproteus* spp. less frequently. While prevalence of *Haemoproteus* spp. was comparable to *Plasmodium* in Madagascar (39·3%; 95% CI 21·1 – 57·5%, $n = 28$), prevalence was lower in the Comoros (14·3%; 95% CI 5·2 – 23·4%; $n = 63$), Mascarenes (0%; $n = 22$) and Seychelles (0%; $n = 37$).

Parasite diversity

We detected a total of 16 distinct haematozoan lineages in the samples surveyed from the Indian Ocean region (Fig. 1). Madagascar and the Comoros exhibited the highest diversity of parasite lineages (10 and 9 lineages, respectively), while lineage diversity was lower in the Mascarenes (3 lineages) and the Seychelles (1 lineage). Lineage 3P accounted for 49% of all *Plasmodium* infections which were successfully sequenced. This lineage was detected in each of the 4 archipelagos and in 9 different host species. Lineage 8P was also relatively widely distributed, occurring in 3 of the 4 archipelagos, but only in *Foudia madagascariensis*.

Genetic relationships among parasite lineages

Of 145 mainland samples that were positive for haematozoa, 125 were successfully sequenced for parasite mitochondrial DNA. In total we identified 20 genetically distinct *Plasmodium* lineages (1P to 20P) and 21 *Haemoproteus* lineages (1H to 21H; Appendix 1). A comparison of these lineages with lineages detected in the Indian Ocean region, plus sequences available on GenBank, revealed only 4 cases of identical matches between island and mainland parasites. Among *Plasmodium* lineages, lineage 3P, which was widely distributed in the Indian Ocean, has also been detected in dozens of host species across the globe and, notably, on several remote island groups (Beadell *et al.* 2006). Lineage 19P, which was found in *Zosterops borbonicus* from Mauritius matched GenBank sequence DQ368373, derived from *Anthus trivialis* (geographical origin unknown). Among lineages of *Haemoproteus*, lineage 3H retrieved from *Hypsipetes madagascarensis* matched identically to sequence retrieved from *Pycnonotus nigricans* in Botswana. Also, lineage 18H from *Zosterops maderaspatanus* and *Z. m. kirki* from Madagascar and Grand Comore, exhibited a sequence identical to parasites recovered from Tanzanian specimens of *Zosterops senegalensis, Z. virens*, and *Z. poliogastra*.

All parasite lineages fell into 2 distinct well-supported clades consistent with the genera *Plasmodium* and *Haemoproteus* (Fig. 2). Within each genus, lineages derived from the Indian Ocean islands did not form monophyletic groups, often exhibiting closer relationships to mainland-derived parasite lineages than other island lineages.

DISCUSSION

In this study, which represents the first molecular survey of avian haematozoan parasites in the islands of the western Indian Ocean, we have identified multiple lineages of both *Plasmodium* spp. and *Haemoproteus* spp. in many of the endemic avian hosts in the region. Importantly, preliminary phylogenetic analysis of this parasite diversity suggests that these parasites do not constitute an endemic radiation and that at least certain lineages have likely colonized the islands relatively recently. We elaborate on these findings below.

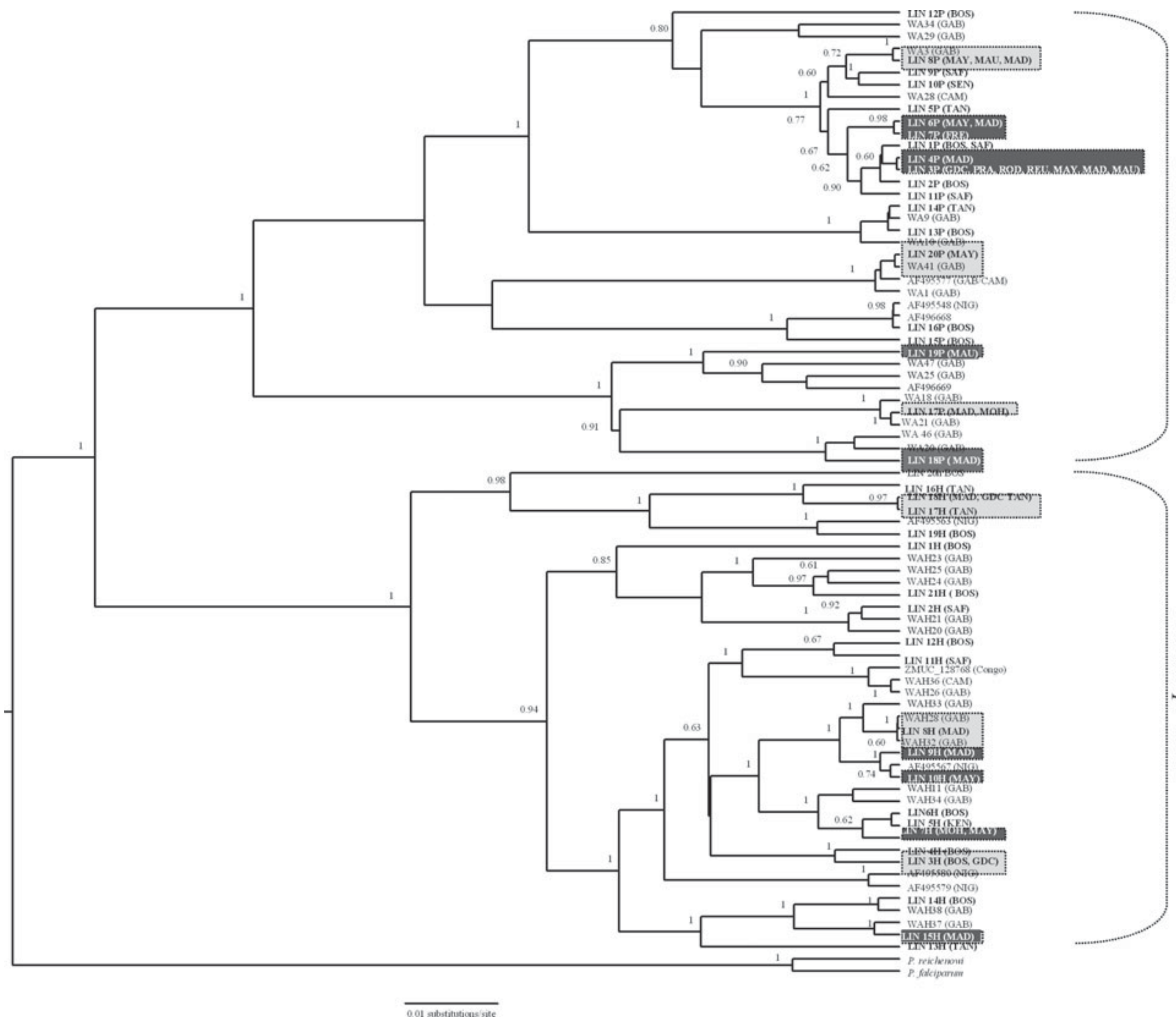

Fig. 2. Phylogenetic relationships of *Plasmodium* and *Haemoproteus* lineages based on maximum credibility clade using the Bayesian analysis. Shared lineages between mainland and island populations are in light grey squares and island shared lineages in dark grey squares. Parasite lineages from mainland Africa are labelled as BOS: Botswana; CAM: Cameroon; GAB: Gabon; KEN: Kenya; NIG: Nigeria; SEN: Senegal; SAF: South Africa; TAN: Tanzania. Lineages with prefix WA and WAH are from Beadell *et al*. (2009). Parasite lineages from islands are labelled as MAD = Madagascar; GDC = Grand Comore; MOH = Moheli; MAY = Mayotte; MAH = Mahe; SIL = Silhoutte; LDI = La Digue; PRA = Praslin; FRE = Fregate; REU = Reunion; MAU = Mauritius; ROD: Rodrigues. See Appendix 1 for details on host species.

Distribution of parasite lineages among islands

Haematozoan parasite community composition was heterogeneous across islands and archipelagos. While limited sampling may have amplified differences among islands, the observed geographical structuring of parasite lineages likely reflects the proximity of islands to mainland parasite sources, geographical area, avian host distribution, and the composition of vector communities on each island. Lineage composition on islands is also likely to be influenced by the extent of movement of vectors and hosts between islands as well as the host-specificity of parasites and their association with endemic or specialized vector species (e.g. Ishtiaq *et al*. 2008). Although genetic differentiation between populations of several avian host families suggests that there is little or no movement of resident avifauna between islands (Nectariniidae, Warren *et al*. 2003; Zosteropidae, Warren *et al*. 2006; Mila *et al*. 2010), human-mediated movements of birds has been extensive. In our survey, Madagascar and the Comoros exhibited the highest parasite lineage diversity, whereas the Seychelles and Mascarenes, more isolated archipelagos with smaller area and more limited avian diversity, exhibited lower parasite diversity. In the Indian Ocean region, we detected just 2 parasite lineages (LIN 3P, LIN 8P) that were found across 3 or more archipelagos. LIN 3P was the only lineage common across all 4 main island groups and occurred in 10 different host species. This parasite lineage (GRW4; AY099041, Bensch *et al*. 2000), which has colonized several other oceanic islands coincident with the global introduction of non-native birds over the last century (Beadell *et al*. 2006), is likely to have followed the same route to the Indian Ocean region. This lineage shares a cytochrome *b* sequence identical to the strain of avian *Plasmodium relictum* (Beadell *et al*. 2006) that decimated endemic bird populations in Hawaii (Warner 1968; van Riper *et al*. 1986), and therefore, a closer examination of its impact on the endemic avifauna of the Indian Ocean is warranted.

The other lineage with relatively wide distribution (LIN 8P), also appears to have extended its range via human-mediated host introductions. We recovered *Plasmodium* lineage 8P from *Foudia madagascariensis* in Madagascar, as well as Mayotte (Comoros) and Mauritius (Mascarenes). *Foudia madagascariensis*, which was the only species sampled across all 4 major archipelagos, is a Madagascar endemic that was introduced to many other islands in the Indian Ocean (B.H. Warren, Ph.D. thesis). Given the apparent specificity of LIN 8P, which was not recovered from any other host species, this lineage appears to have co-colonized Mayotte and Mauritius along with its introduced host.

Unfortunately, the distribution and vector competence of mosquitoes and other arthropod vectors have been little studied in the Indian Ocean region (but see Gerberg and Arnett, 1979) despite their likely influence on parasite distribution (Ishtiaq *et al*. 2008). We did not detect *Haemoproteus* spp. in samples from the Seychelles or the Mascarenes, which might point to the absence of an appropriate vector. However, a microscopy-based survey by Lowery (1971) detected *Haemoproteus* spp. in passerines from Aldabra (Seychelles) and Peirce *et al*. (1977) reported *Haemoproteus columbae* only in samples of *Columba livia* in the Mascarenes. Our failure to detect *Haemoproteus* spp. in this survey, which did not include avian samples from Aldabra or *C. livia* from the Mascarenes, highlights the difficulty in fully capturing the structure of a diverse and complex parasite system. In many cases, the apparent absence of parasite lineages from particular hosts and islands may simply stem from limited sampling, temporary local extinctions, or infection intensities for certain lineages that frequently fall below levels of detection (e.g. Perkins, 2001; Fallon *et al*. 2003). Also, differences in the timing of avian blood sampling could have resulted in discrepancies in the prevalence and distribution of certain parasite lineages; year-to-year temporal fluctuations in avian parasite prevalence and diversity have been documented (Wood *et al*. 2007), as have seasonal effects (Bensch and Åkesson, 2003; Cosgrove *et al*. 2008). Whilst annual temporal (and spatial) variation in blood parasite prevalence is more pronounced in temperate faunas than in tropical ones (e.g. Schall and Marghoob, 1995; Schall *et al*. 2000; Freeman-Gallant *et al*. 2001; Fallon *et al*. 2003), it should be noted that our sampling has been conducted over an extended time-period (1999–2002) and across a diverse range of islands with varying climatic regimes. Therefore temporal effects could also play a role in the observed heterogeneity of prevalence and diversity across islands.

Comparison between mainland and island parasite lineages

Sparse sampling of parasite lineages from continental Africa prevents us from drawing firm conclusions regarding the geographical and evolutionary origins of parasites in the Indian Ocean avifauna. In particular, we cannot be sure that we have recovered the continental lineage that is most closely related to any particular island lineage or that the apparent geographical or host distribution of continental lineages is representative. Nonetheless, 2 observations stand out. First, based on our phylogenetic analysis, the western Indian Ocean parasite lineages are polyphyletic with respect to mainland African lineages, suggesting that the Indian Ocean parasite community reflects multiple independent colonizations as opposed to a single endemic radiation of parasites. Second, the low divergence between several island and mainland parasites suggests that these parasite lineages may have colonized the islands relatively recently. We identified several Indian Ocean parasite lineages that were identical (LIN 3P, LIN 19P, LIN 3H LIN 18H) or nearly identical (genetic divergence 0·1–0·4%; LIN 8P, LIN 20P and LIN 8H, LIN 15H) to continental lineages across the segment of cytochrome *b* sequenced (minimum of 256 bp). Assuming that parasite divergence at cytochrome *b* occurs at approximately 1·2% per million years (Ricklefs and Outlaw, 2010), then identical sequences might be retrieved from lineages that have been diverging for anywhere between 0 and 325 000 years, the time expected to pass prior to a single mutation arising to distinguish 2 sequences of 256 bp. This time of divergence is low compared to the maximum estimated dates of island colonization for the avian host genera *Nectarinia*, *Hypsipetes*, *Zosterops* and *Foudia*, which are in the order of 1–3 million years before the present (Warren *et al*. 2003, 2005, 2006). Thus, on the time-scale of mutation in the cytochrome *b* gene, at least some parasite lineages have been exchanged between continental Africa and neighbouring islands after island hosts became isolated.

Parasite exchange may have been facilitated by rare migrants or by human-mediated introductions of species such as common waxbills (*Estrilda estrild*), house sparrows (*Passer domesticus*), common mynas (*Acridotheres tristis*) and red-whiskered bulbuls (*Pycnonotus cafer*), which occur throughout the archipelagos. An interesting exception to this model, however, might be *Haemoproteus* lineage LIN 18H, which was isolated exclusively from *Zosterops* spp. in both Tanzania and the Indian Ocean. Given the apparent host specificity of this lineage and the absence of any introduced species of *Zosterops* in the Indian Ocean, this parasite may have instead arrived in the islands via an infected vector or, alternatively, may have been present in the first *Zosterops* colonist. If the latter is true, then the lack of divergence in LIN 18H will have to be reconciled with the relatively long time of divergence estimated for *Zosterops* spp. in the Indian Ocean (Warren *et al*. 2006). To further clarify the origins of Indian Ocean parasites, future work should be directed towards characterizing the molecular relationships between mainland African parasites and parasites deemed to be endemic to the Indian Ocean region based on morphology (Savage *et al*. 2009).

CONCLUSIONS

Our initial molecular survey of vector-mediated parasites in the western Indian Ocean provided the following insights into the distribution and diversity of parasite lineages: (a) parasite communities exhibited heterogeneity in prevalence and composition across the islands, (b) parasite communities were diverse, consistent with the findings of microscopy-based surveys, (c) the phylogenetic relationships among parasite lineages appear to reflect multiple independent colonizations of the western Indian Ocean region, and (d) in many cases, these colonizations appear to have occurred relatively recently based on the sharing of parasite lineages between mainland Africa and Indian Ocean Islands. Given the relatively small, geographically restricted and sometimes threatened populations of avian hosts present in the Indian Ocean region, assessing the ecological impact of haematozoan parasites on the endemic island avifauna should be a priority.

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APPENDIX 1

List of haematozoan lineages with GenBank Accession numbers. The taxonomy follows Sibley and Monroe (1996)

Lineage	Species	Family	Region	bp	GenBank number
Haemoproteus clade					
1	*Ispidina picta*	Alcedinidae	Botswana	533	JN661907
2A	*Creatophora cinerea*	Sturnidae	South Africa	533	JN661908
2B	*Creatophora cinerea*	Sturnidae	South Africa	295	JN661909
3A	*Hypsipetes madagascariensis*	Pycnonotidae	Grande Comore	533	JN661910
3B	*Pycnonotus nigricans*	Pycnonotidae	Botswana	533	JN661911
3C	*Pycnonotus nigricans*	Pycnonotidae	Botswana	533	JN661912
4A	*Passer melanurus*	Passeridae	Botswana	533	JN661913
4B	*Passer diffusus*	Passeridae	Botswana	533	JN661914
4C	*Passer diffusus*	Passeridae	Botswana	533	JN661915
4D	*Passer diffusus*	Passeridae	Botswana	533	JN661916
4E	*Passer diffusus*	Passeridae	Botswana	533	JN661917
4F	*Passer diffusus*	Passeridae	Botswana	533	JN661918
4G	*Passer diffusus*	Passeridae	Botswana	474	JN661919
4H	*Hirundo abyssinica*	Hirundinidae	Botswana	533	JN661920

Appendix 1 (*Cont.*)

Lineage	Species	Family	Region	bp	GenBank number
5	*Passer griseus*	Passeridae	Kenya	533	JN661921
6A	*Ploceus intermedius*	Ploceidae	Botswana	533	JN661922
6B	*Ploceus intermedius*	Ploceidae	Botswana	351	JN661923
6C	*Ploceus intermedius*	Ploceidae	Botswana	351	JN661924
6D	*Ploceus velatus*	Ploceidae	Botswana	533	JN661925
7A	*Foudia madagascariensis*	Ploceidae	Moheli	533	JN661926
7B	*Foudia madagascariensis*	Ploceidae	Moheli	295	JN661927
7C	*Foudia madagascariensis*	Ploceidae	Mayotte	533	JN661928
7D	*Foudia madagascariensis*	Ploceidae	Mayotte	533	JN661929
7E	*Foudia eminentissima*	Ploceidae	Mayotte	533	JN661930
8A	*Ploceus sakalava*	Ploceidae	Madagascar	533	JN661931
8B	*Ploceus sakalava*	Ploceidae	Madagascar	533	JN661932
9	*Foudia madagascariensis*	Ploceidae	Madagascar	533	JN661933
10	*Foudia eminentissima*	Ploceidae	Mayotte	533	JN661934
11	*Euplectes orix*	Ploceidae	South Africa	533	JN661935
12A	*Uraeginthus angolensis*	Estrildidae	Botswana	533	JN661936
12B	*Uraeginthus angolensis*	Estrildidae	Botswana	533	JN661937
13A	*Estrilda melanotis*	Estrildidae	Tanzania	533	JN661938
13B	*Estrilda melanotis*	Estrildidae	Tanzania	533	JN661939
14	*Passer melanurus*	Passeridae	Botswana	533	JN661940
15A	*Foudia madagascariensis*	Ploceidae	Madagascar	533	JN661941
15B	*Foudia madagascariensis*	Ploceidae	Madagascar	533	JN661942
16	*Zosterops senegalensis*	Zosteropidae	Tanzania	351	JN661943
17	*Zosterops poliogastra*	Zosteropidae	Tanzania	351	JN661944
18A	*Zosterops m. analoga*	Zosteropidae	Madagascar	533	JN661945
18B	*Zosterops m. analoga*	Zosteropidae	Madagascar	533	JN661946
18C	*Zosterops m. analoga*	Zosteropidae	Madagascar	533	JN661947
18D	*Zosterops m. analoga*	Zosteropidae	Madagascar	533	JN661948
18E	*Zosterops m. analoga*	Zosteropidae	Madagascar	533	JN661949
18F	*Zosterops m. analoga*	Zosteropidae	Madagascar	533	JN661950
18G	*Zosterops mad. kirki*	Zosteropidae	Grand Comore	533	JN661951
18H	*Zosterops senegalensis*	Zosteropidae	Tanzania	351	JN661952
18I	*Zosterops senegalensis*	Zosteropidae	Tanzania	351	JN661953
18J	*Zosterops senegalensis*	Zosteropidae	Tanzania	351	JN661954
18K	*Zosterops virens*	Zosteropidae	Tanzania	351	JN661955
18L	*Zosterops virens*	Zosteropidae	Tanzania	351	JN661956
18M	*Zosterops poliogastra*	Zosteropidae	Tanzania	351	JN661957
18N	*Zosterops poliogastra*	Zosteropidae	Tanzania	351	JN661958
19	*Sylvia communis*	Sylviidae	Botswana	351	JN661959
20	*Lanius collurio*	Laniidae	Botswana	533	JN661960
21	*Ploceus velatus*	Ploceidae	Botswana	533	JN661961
Plasmodium Clade					
1A	*Ploceus xanthops*	Ploceidae	Botswana	351	JN661962
1B	*Ploceus xanthops*	Ploceidae	Botswana	351	JN661963
1C	*Ploceus velatus*	Ploceidae	South Africa	533	JN661964
1D	*Ploceus velatus*	Ploceidae	South Africa	295	JN661965
1E	*Euplectes orix*	Ploceidae	South Africa	295	JN661966
1F	*Serinus atrogularis*	Fringillidae	South Africa	533	DQ839040
1G	*Serinus atrogularis*	Fringilidae	South Africa	295	JN661967
1H	*Cisticola fulvicapillus*	Cisticolidae	South Africa	295	JN661968
2	*Parisoma subcaeruleum*	Timallidae	Botswana	256	JN661969
3	*Zosterops mad. kirki*	Zosteropidae	Grand Comore	533	DQ839037
3A	*Zosterops mad. kirki*	Zosteropidae	Grand Comore	533	JN661970
3B	*Zosterops mad. kirki*	Zosteropidae	Grand Comore	295	JN661971
3C	*Zosterops mouroniensis*	Zosteropidae	Grand Comore	295	JN661972
3D	*Zosterops mouroniensis*	Zosteropidae	Grand Comore	295	JN661973
3E	*Zosterops bor. mauritianus*	Zosteropidae	Reunion	533	JN661974
3F	*Zosterops m. mayottensis*	Zosteropidae	Mayotte	533	JN661975
3G	*Zosterops m. analoga*	Zosteropidae	Madagascar	295	JN661976
	Zosterops bor. mauritianus	Zosteropidae	Mauritius	295	DQ839035
3H	*Zosterops bor. mauritianus*	Zosteropidae	Mauritius	295	JN661977
	Nectarinia notata	Nectarinidae	Grand Comore	533	DQ839016
3I	*Nectarinia humbloti*	Nectarinidae	Grand Comore	295	JN661978
3J	*Humblotia flavirostris*	Muscicapidae	Grand Comore	295	JN661979
	Foudia eminentissima	Ploceidae	Grand Comore	533	DQ839021
3K	*Foudia eminentissima*	Ploceidae	Grand Comore	295	JN661980

Appendix 1 (*Cont.*)

Lineage	Species	Family	Region	bp	GenBank number
3L	*Foudia madagascariensis*	Ploceidae	Praslin	533	JN661981
3M	*Foudia madagascariensis*	Ploceidae	Madagascar	295	JN661982
	Foudia madagascariensis	Ploceidae	Grand Comore	295	DQ839020
	Foudia flavicans	Ploceidae	Rodrigues	533	DQ839022
4	*Foudia madagascariensis*	Ploceidae	Madagascar	533	JN661983
5	*Zosterops senegalensis*	Zosteropidae	Tanzania	351	DQ659567
6A	*Nectarinia coquereli*	Nectarinidae	Mayotte	533	JN661984
6B	*Nectarinia coquereli*	Nectarinidae	Mayotte	533	JN661985
	Nectarinia coquereli	Nectarinidae	Mayotte	533	DQ659560
	Nectarinia souimanga	Nectarinidae	Madagascar	533	DQ839045
7	*Foudia sechellarum*	Ploceidae	Fregate	533	DQ659561
	Nectarinia dussumieri	Nectarinidae	Fregate	533	DQ839046
8A	*Foudia madagascariensis*	Ploceidae	Mayotte	533	JN661986
8B	*Foudia madagascariensis*	Ploceidae	Mauritius	295	JN661987
8C	*Foudia madagascariensis*	Ploceidae	Mauritius	295	JN661988
8D	*Foudia madagascariensis*	Ploceidae	Madagascar	295	JN661989
8E	*Foudia madagascariensis*	Ploceidae	Madagascar	295	JN661990
9	*Passer melanurus*	Passeridae	South Africa	533	DQ659566
10	*Passer luteus lutens*	Passeridae	Senegal	533	JN661991
11	*Ploceus velatus*	Ploceidae	South Africa	533	DQ659558
12	*Tockus flavirostris*	Bucerotidae	Botswana	533	JN661992
13	*Turdoides jardineii*	Timallidae	Botswana	256	JN661993
14	*Estrilda astrild*	Estrildidae	Tanzania	256	DQ839068
15	*Pytilia melba*	Estrildidae	Botswana	533	JN661994
16	*Ploceus intermedius*	Ploceidae	Botswana	533	JN661995
17A	*Hypsipetes madagascariensis*	Pycnonotidae	Madagascar	533	JN661996
17B	*Hypsipetes madagascariensis*	Pycnonotidae	Madagascar	533	JN661997
18A	*Hypsipetes madagascariensis*	Pycnonotidae	Madagascar	533	JN661998
18B	*Hypsipetes madagascariensis*	Pycnonotidae	Madagascar	533	JN661999
18C	*Hypsipetes madagascariensis*	Pycnonotidae	Moheli	533	JN662000
19	*Zosterops bor. mauritianus*	Zosteropidae	Mauritius	533	JN662001
20	*Hypsipetes madagascariensis*	Pycnonotidae	Mayotte	533	JN662002