Diversity, distribution and biogeographical origins of *Plasmodium* parasites from the New Zealand bellbird (*Anthornis melanura*)

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SUMMARY

Understanding the origin of invasive parasites and ecological transmission barriers on the distribution of mosquito-borne pathogens is enriched by molecular phylogenetic approaches now that large databases are becoming available. Here we assess the biogeographical relationships among haemosporidian blood parasites and an avian host, the New Zealand bellbird (Meliphagidae, *Anthornis melanura*). Four *Plasmodium* haplotypes were identified among 93 infected bellbirds (693 screened) using nested PCR of a mitochondrial DNA cytochrome *b* gene fragment. The most common lineage, LIN1 (11%), is confined to northern New Zealand and falls within a known clade of *Plasmodium* (subgenus *Novyella*) sp. infecting Australian meliphagids. LIN1 differs within that clade by 4·9% sequence divergence suggestive of an endemic lineage to New Zealand. The most widespread lineage, LIN2 (2%), is an exact match with a global cosmopolitan (*P. elongatum* GRW06). Two rare lineages, LIN3 and LIN4 are less abundant, geographically restricted within New Zealand and have <1% sequence divergence with *P.* (*Novyella*) sp. (AFTRU08) and *P. relictum* (LINOLI01) documented from Africa. For the first time, we provide invaluable information on possible rates of entry of invading parasites in New Zealand and their distribution from temperate to cold environments.

Key words: *Plasmodium*, avian malaria, biogeography, New Zealand, host-parasite distribution.

INTRODUCTION

Recent changes in the global distribution of haemosporidians, such as avian malaria parasites *Plasmodium* sp., their arthropod vectors and their avian hosts have been associated with human settlement patterns, global trade and climate change (Garamszegi, 2011). Some parasites have become global cosmopolitans that can utilize multiple vectors and hosts, and these are often more successful or widespread (Valkiunas, 2008*a*). On the other hand, 'ecological transmission barriers', for example, cold temperatures in high latitude and/or altitude climates, can cause differentiation and isolation of parasite lineages between different host groups (Hellgren *et al*. 2008). Parasite lineages with wide host and geographical distributions are considered to be generalist parasites, whereas lineages restricted to particular regions or endemic hosts are considered to be specialist lineages (Ishtiaq *et al*. 2010). In Vanuatu and New Caledonia, for example, close associations between potentially specialist parasite lineages and endemic mosquito species have been described (Ishtiaq *et al*. 2008). If the geographical distribution of the parasite is restricted compared to that of the host, this 'spatial mismatch' might be explained by climatic and ecological limiting factors associated with the invertebrate vector (Valkiunas, 2005). Islands generally have lower parasite diversity owing to isolation and small landmasses that proffer a biogeographical history distinct from that of continents, though species richness will increase with the area of the island (MacArthur and Wilson, 1967; see also Ishtiaq *et al*. 2010). Consequently, islands such as New Zealand provide a good opportunity to discern malaria endemism and the co-evolutionary relationships between blood parasites and their hosts.

The New Zealand bellbird is an abundant and highly vagile passerine endemic to and ubiquitous throughout New Zealand (Robertson *et al*. 2007). These characteristics make the bellbird an ideal candidate for studying parasite distribution, ecological barriers to vectors and host-parasite specificity. In New Zealand, Tompkins and Gleeson (2006) provided evidence for a latitudinal (north to south) decrease in abundance of one lineage of *P. relictum* infecting introduced European bird species. That clinal trend in malaria infections correlated positively with latitudinal trends in its putative mosquito vector, the exotic *Culex quinquefasciatus* (Tompkins and Gleeson, 2006). Recently, a large public avian malaria database (MalAvi; http://mbio-serv4.mbioekol.lu.se/avianmalaria/index.html; see Bensch *et al*. 2009) has been constructed that uses codes for genotypes of

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parasites allowing researchers to deposit, query and download information on haemosporidians from around the world. Data depositions made to this international database (Ruth Brown, *unpublished data*; Bensch *et al*. 2009) show that at least 4 exotic e.g., house sparrow (*Passer domesticus*), European thrushes (*Turdus* spp.), and 6 endemic e.g., South Island robin (*Petroica australis*), saddleback (*Philesturnus carunculatus*), hihi (*Notiomystis cincta*), bird species have been infected by a variety of malarial parasites, including such well known exotic cosmopolitan parasites as SGS1 (*P. elongatum*) and GRW4 (*P. relictum*) in New Zealand. Not much is known about virulence of these parasites in New Zealand, however, but recent outbreaks of avian malaria causing mortality of endemic species have been recorded in New Zealand zoos e.g., mohua (*Mohoua ochrocephala*, Alley *et al*. 2008). The potential vectors for avian malaria in New Zealand are 12 species of endemic and/or native mosquito (Derraik, 2004). One of these species is continuously distributed throughout the country, *Culex pervigilans*; less is known about the other species (Derraik, 2004). At least 27 exotic species of mosquito have been identified from 171 interceptions recorded since 1929 by the Ministry of Agriculture and Fisheries (MAF) at ports of entry into New Zealand (Derraik, 2004). Only 4 species (*Culex quinquefasciatus, Ochlerotatus notoscriptus, Ochlerotatus australis, Ochlerotatus camptorhynchus*) have established successfully, however, and their distribution throughout New Zealand still appears to be patchy (Derraik, 2004; Derraik *et al*. 2008).

The main objectives of this study are to assess (1) the diversity of *Plasmodium* and *Haemoproteus* parasites infecting a highly mobile passerine endemic to New Zealand, (2) the distribution of these parasites relative to that of their host and (3) the worldwide distribution and phylogenetic relationships of bellbird parasite lineages. This study can be useful for comparison with future studies on changes in host-specificity and parasite biogeography associated with environmental change.

MATERIALS AND METHODS

Blood sampling

To assess the diversity and distribution of avian malaria parasites, we collected 693 blood samples from individual bellbirds at 9 sites over a large latitudinal range in New Zealand between 2007 and 2010 (Fig. 1). These sites extend from the northern locations of Poor Knights Islands (Aorangi) and Hauraki Gulf (Hauturu, Tiritiri Matangi and Tawharanui), through to the southern North Island of New Zealand (Tongariro Forest and Kapiti Island), the South Island (Kaikoura and Dunedin), and finally, to the Sub-Antarctic Islands (Adams Island) (Fig. 1). Under permission of the Department of Conservation (DoC) and Massey University Animal Ethics Committee (MUAEC), bellbirds were captured via mist nets, individuals were marked with DOC Banding Office stainless-steel and colour leg bands and blood samples were extracted by venipuncture of brachial vein and samples were stored in either lysis buffer or 95% ethanol. Blood smears were air-dried on microscope slides, preserved with 100% methanol and then stained using Giemsa stain.

PCR detection of the parasite

DNA was extracted from blood samples using a DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA). Samples were screened for *Plasmodium* and *Haemoproteus* infections using a nested polymerase chain reaction (PCR) method that amplifies a 478 base pair (bp) fragment of mitochondrial DNA (mtDNA) cytochrome *b* gene (Hellgren *et al*. 2004). This nested PCR comprises 2 rounds of PCR reactions performed in 15 μl volumes using positive and negative controls. The forward and reverse primers used in the first round of reactions were HaemNFI (5′- CATATATTAAGAGAAITATGGAG-3′) and HaemNR3 (5′-ATAGAAAGATAAGAAATACC-ATTC-3′), respectively. Each 15 μl reaction included 2 μl of genomic DNA, 0·75 mM of each dNTP, 0·6 μM of each primer, 1·5 mM $MgCl_2$ and 0·3 units of *Taq* DNA polymerase (Invitrogen). The thermal profile consisted of a 3-min, 94 °C activation step, followed by 20 cycles of 94 °C for 30 sec, 50 °C for 30 sec and 72 °C for 45 sec, ending with an elongation step of 72 °C for 10 min. In the second round of PCR reactions, primers HaemF (5′-TGGTGCTTTCG-ATATGCATG-3′) and HaemR2 were used (5′-GCATTATCTGGATGTGATAATGGT-3′). The protocol for the second round of PCR was the same as the first, except that 2 μl of PCR product from the first reaction was used as template instead of genomic DNA and the number of cycles in the thermal profile was increased to 35 cycles. Three μl of PCR products were run on 1·5% agarose then stained with ethidium bromide and viewed under UV. PCR products containing bands around 500-bp in size were considered malaria positive and purified using SureClean (Bioline Inc.), then sequenced using the forward primer HaemF on an ABI 3730 DNA Analyzer (Applied Biosystems, Inc.). Sequences edited and aligned in MEGA 5 (Tamura *et al*. 2011).

Phylogenetic analysis of the haemosporidian parasite

The GTR + I + G model of sequence evolution best fitted our 4 cytochrome *b* mitochondrial DNA haplotype dataset ($N = 93$ individuals) as determined using maximum likelihood analyses and the Akaike Information Criterion (AIC) in JMODELTEST 0.1.1

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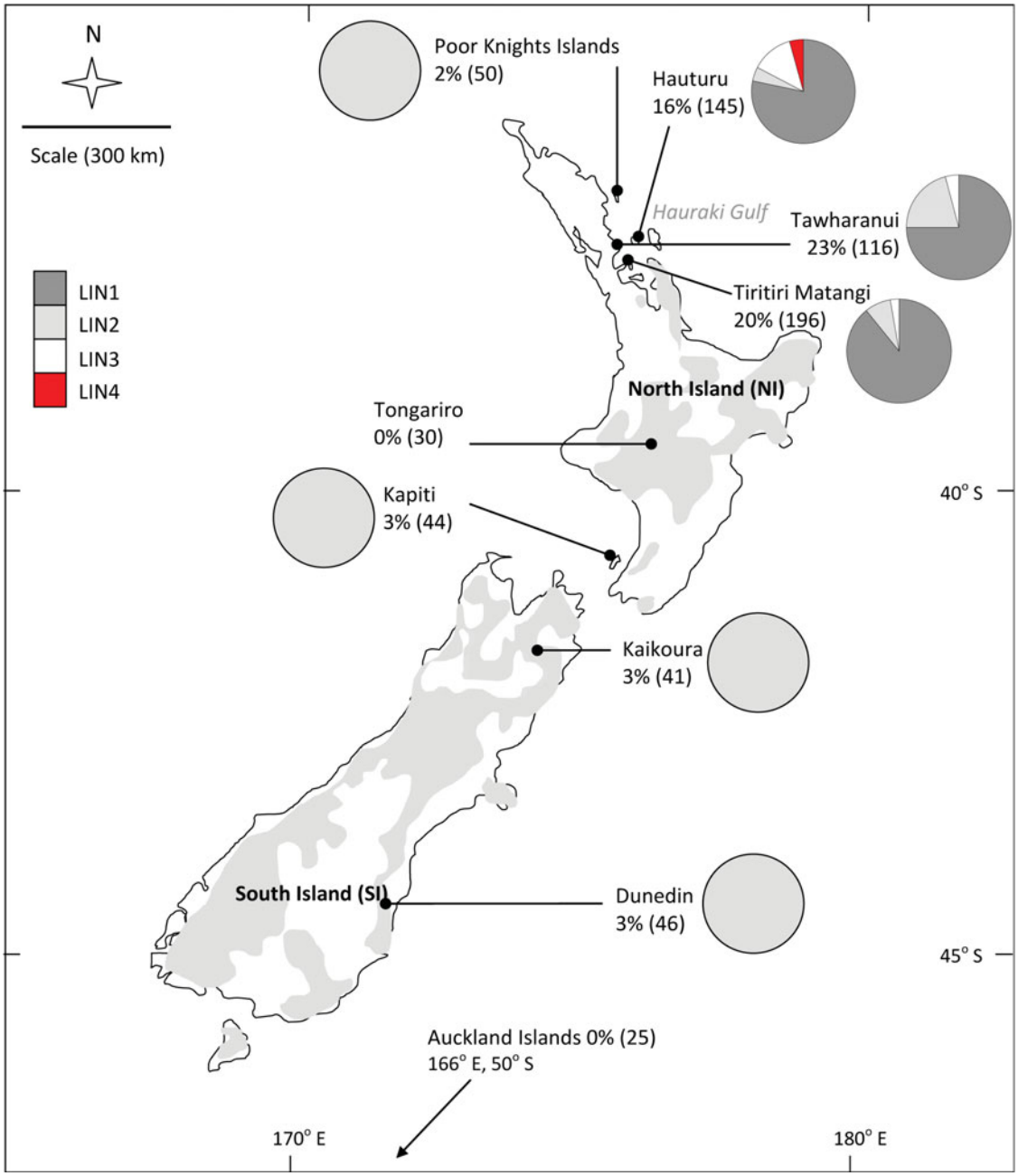

Fig. 1. Location map showing our nine host population sampling locations in New Zealand. The numbers in percentages and parentheses represent *Plasmodium* infection prevalence (number of individuals infected/individuals screened) using PCR. The pie charts show relative proportion of positive infections represented by each *Plasmodium* lineage at each location. The approximate New Zealand bellbird (*Anthornis melanura*) distribution range is shown in light grey shading.

(Guindon and Gascuel, 2003; Posada, 2008). This GTR + I + G model assumes base frequencies A = 0·2994, C = 0·1352, G = 0·1310, T = 0·4345) with proportion of invariable sites (I) <0·001 and a substitution rate matrix A-C = 2·6951, A-G = 1·2005, A-T = 5·8779, C-G = 1·2931, C-T = 20·3541 and G-T = 1·0. Using the GTR + I + G model, we estimated the phylogeny of haplotypes using 5000 bootstrap replicates of the maximum likelihood (ML) analysis in MEGA. The phylogenetic relationships among parasite lineages were estimated using cytochrome *b* sequences ⩾400-bp. We used all avian malaria sequences in the MalAvi database and some additional southern hemisphere Meliphagidae parasite sequences from GenBank (Beadell *et al*. 2004; Bensch *et al*. 2009) to aid in the identification and biogeographical relationships of haemosporidian lineages found in bellbirds. A mammalian *Plasmodium* outgroup sequence was used to root the tree (see Wood *et al*. 2007). Pair-wise LogDet sequence divergence estimates (Lockhart *et al*. 1994) were calculated in MEGA.

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Table 1. Sequence divergence estimates of the bellbird *Plasmodium* parasites detected in this study and the closest sequence matches documented in the MalAvi database and GenBank

(LIN1 through LIN4 is the naming convention adopted for this publication until sequences are resolved. Resolved lineages are listed according to previously published host species codes and GenBank Accession numbers for sequences with which we found a ⩾95% match.)

Bellbird *Plasmodium* haplotypes	Closest parasite haplotype matches		
Lineage code, GenBank Acc. no.	Lineage code, GenBank no.	Genus (subgenus) species	Sequence divergence (%)
LIN1, JN415756	unresolved	*Plasmodium* (*Novyella*) sp.T	4·9
LIN2, JN415757	GRW6, DQ368381	*P. (Huffia) elongatum**	0
LIN3, JN415758	AFTRU08, EU810633	*P. (Novyella)* sp.**	1·2
LIN4, JN415759	LINOLI01, DQ839046	*P. (Haemamoeba) relictum*†	0·7

T This sequence could not be resolved to a known malaria lineage, but belongs to the subgenus *Novyella* and a clade of *Plasmodium* found only in Australopacific honeyeaters (Beadell *et al*. 2004).
* Mitochondrial cytochrome *b* lineages found in bellbirds that were identical to a well-known previously defined cosmopolitan species (Perez-Tris *et al*. 2007; Valkiunas *et al*. 2008*a*).
** This sequence matches most closely to AFTRU08 found in a thrush (*Turdus pelios*) host in Cameroon, western Africa (Beadell *et al*. 2009).
† This sequence matches most closely to LINOLI01 (a *P. relictum* lineage very similar to the cosmopolitan SGS01) collected from a Seychelles sunbird (*Cinnyris dussumieri*) host off the coast of eastern Africa (Beadell *et al*. 2006).

RESULTS

Phylogenetic identification and prevalence of avian malaria lineages

Phylogenetic analysis on cytochrome *b* sequences identified 4 distinct lineages (referred to as lineage LIN1, LIN2, LIN3 and LIN4 for the remainder of the text) of a single genus, *Plasmodium* (Table 1; Fig. S1, online version only). There is strong bootstrap support (91%) that LIN1 falls within a known clade of unresolved *Plasmodium* (subgenus *Novyella*) sp. sampled from Australian and Papua New Guinean Meliphagidae species (Beadell *et al*. 2004) (Fig. 2). LogDet sequence divergence between LIN1 and its 2 closest phylogenetic matches, MELANA01 and MELNOT01, is 4·9% ($22_{\text{BASE-PAIR CHANGES}}/454_{\text{COMMON SITES}}$) and 5·1% ($23_{\text{BASE-PAIR CHANGES}}/454_{\text{COMMON SITES}}$), respectively (Table 1; see Fig. S2, online version only for LogDet pair-wise sequence divergence estimates). LIN1 parasites comprise 80% ($N=74/93$) of positive malaria infections in bellbirds and our country-wide prevalence estimate is 11% ($N=74/693$) (Fig. 1). We identified LIN2 to be the prolific cosmopolitan *P.* (*Huffia*) *elongatum* GRW06 (Perez-Tris *et al*. 2007; see Valkiunas *et al*. 2008*a*). The LIN2 and GRW06 cytochrome *b* sequences are an exact match ($0_{\text{BASE-PAIR CHANGES}}/454_{\text{COMMON SITES}}$) (Table 1). LIN2 is the most geographically widespread infection throughout New Zealand and occurs in 14% ($N=13/93$) of positive infections, but country-wide prevalence is low (2%, $N=13/693$) (Fig. 1). LIN3 is phylogenetically similar to infections previously found in African and Seychelles passerines, AFTRU08 *Plasmodium* (*Novyella*) sp. (Beadell *et al*. 2009) (Fig 1). There is only a 0·7% LogDet sequence difference between LIN3 and AFTRU08 (Table 1). Finally, we found that LIN4 only has a 0·7% ($3_{\text{BASE-PAIR CHANGES}}/478_{\text{COMMON SITES}}$) sequence difference from the *P. relictum* LINOLI01 (Beadell *et al*. 2006) (see Table 1). This lineage is an exact match with both *P. relictum* FOUSEY01 and CINCQ01 sampled in Seychelles passerines (Beadell *et al*. 2006) (Fig. 2). We detected LIN3 and LIN4 in 0·7% ($N=5/693$) and 0·1% ($N=1/693$) of individuals screened throughout New Zealand, respectively (Fig. 1).

DISCUSSION

Diversity and distribution of bellbird Plasmodium *lineages within New Zealand*

We detected 4 lineages of *Plasmodium* and no *Haemoproteus* parasites from a widely distributed endemic passerine host throughout the island archipelago of New Zealand. Our parasite diversity estimates are low compared to studies on continental locations; for example, typically in Europe and North America a host population can have up to 12–15 lineages of malaria within a small geographical area (see Fallon *et al*. 2004; Kimura *et al*. 2006; Bensch *et al*. 2007; Wood *et al*. 2007; Cosgrove *et al*. 2008; Norte *et al*. 2009). The low malaria diversity and prevalence (⩽20%) in the New Zealand bellbird is congruent, however, with findings from other endemic Australopacific island host bird populations (Jarvi *et al*. 2003; Beadell *et al*. 2004).

The distribution of *Plasmodium* parasite, LIN1, was geographically restricted compared to its bellbird host in 2 key ways. First, the most numerous avian malaria parasite, LIN1, was restricted geographically

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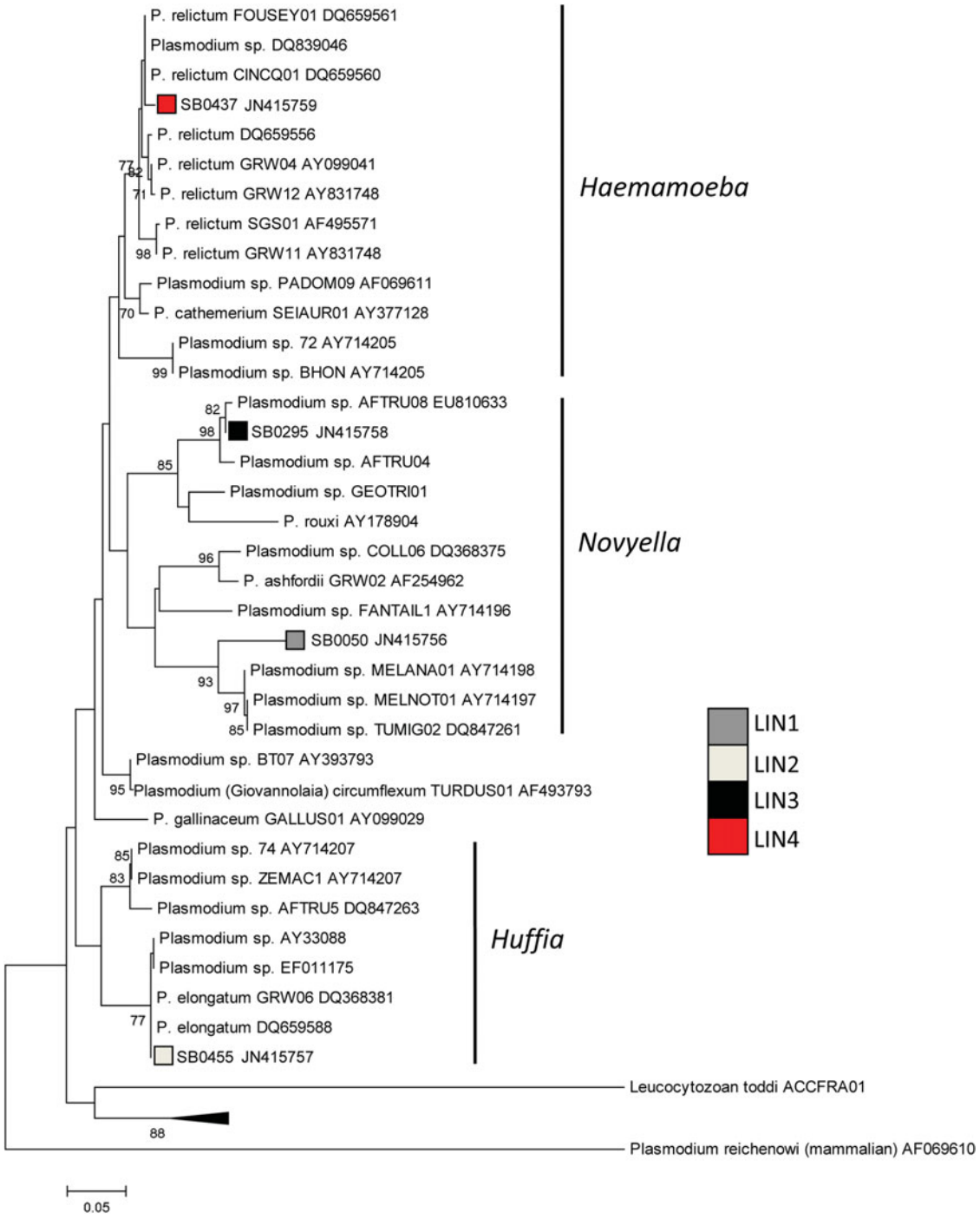

Fig. 2. The maximum likelihood (ML) tree shows the estimated phylogenetic relationships among parasite lineages using cytochrome *b* sequences (length 478-bp). *Leucocytozoon* and a mammalian *Plasmodium* were used as outgroups to root the tree. The numbers at the branches represent ML bootstrap support ⩾70% (5000 replicates). Previously documented sequences have their Latin, MalAvi database and GenBank names at the end of each branch.

to the Hauraki Gulf region and, second, LIN2 (*P. elongatum* GRW06) was the only lineage that reflected the bellbird distribution and was ubiquitous throughout most of New Zealand. As with LIN1, parasite lineages LIN3 and LIN4 were only detected in northern New Zealand. The lack of any *Plasmodium* detected at Tongariro may be due to low parasitaemia and/or high altitude (∼1120 m above sea level), where all other sites were at sea level (Knowles *et al*. 2011). In the Sub-Antarctic Auckland Islands a complete absence of mosquito vectors precludes transmission at that location. The availability of competent vectors or suitable environmental and climatic variables in addition to the geographical distribution of the host likely play an important role in the biogeographical distribution of avian malaria parasites (Hellgren *et al*. 2007; Ishtiaq *et al*. 2010).

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Table 2. Worldwide biogeographical distribution of *Plasmodium* lineages most closely matching each parasite sequence found in New Zealand bellbirds (*Anthornis melanura*) in this study (information only for sequences with <2% LogDet sequence divergence from LIN1-4)

Bellbird parasite lineage	Host country	Number of avian host species	Publication
LIN1 *P.* (*Novyella*) sp.	New Zealand	1	This study
LIN2 *P. elongatum* GRW06	Australia	1	Beadell *et al*. (2004)
	Bulgaria	4	Valkiunas *et al*. (2008*a*); Marzal *et al*. *unpubl. data*; Zethindjiev, *unpubl. data*
	Cameroon	2	Beadell *et al*. (2009)
	Gabon	1	Hellgren *et al*. (2007)
	Galapagos I.	1	Santiago-Alarcon *et al*. (2008, 2010)
	Myanmar	2	Ishtiaq *et al*. (2007)
	New Zealand	9	Ruth Brown, unpubl. data; this study
	Papua New Guinea	1	Beadell *et al*. (2004)
	United States	8	Beadell *et al*. (2006); Ishak *et al*. (2008); Outlaw and Ricklefs (2009); Kimura *et al*. (2006)
	Spain	1	Fernandez *et al*. (2010)
	Sweden	2	Bensch *et al*. (2007); Hellgren *et al*. (2007)
LIN3 *P.* (*Novyella*) sp. AFTRU08	Cameroon	1	Beadell *et al*. (2009)
	New Zealand	1	This study
LIN4 *P. relictum* LINOLI01	New Zealand	1	This study
	Bioko	1	Beadell *et al*. (2006)
	Botswana	1	Beadell *et al*. (2006)
	South Africa	1	Beadell *et al*. (2006)
	Zimbabwe	1	Beadell *et al*. (2006)
CINQO01	Mayotte	1	Beadell *et al*. (2006)
	Madagascar	1	Beadell *et al*. (2006)
FOUSEY	Fregate	1	Beadell *et al*. (2006)

Biogeographical origins of bellbird Plasmodium *parasites*

Our detailed phylogenetic analysis using the complete MalAvi database (Bensch *et al*. 2009) and GenBank provides no mitochondrial DNA sequence match close enough to resolve the LIN1 lineage to the species level, let alone a particular lineage for LIN1. However, we found that the LIN1 lineage belongs to a clade of parasites known to infect only Australopacific meliphagids: the yellow-spotted honeyeater (*Meliphaga notata*) that inhabits Australia; Lewin's honeyeater (*Meliphaga lewinii*) that inhabits northeastern Australia and Papua New Guinea (Beadell *et al*. 2004). In a recent study, Ricklefs and Outlaw (2010) demonstrated that the rate of molecular evolution of haemosporidian parasites is ~1·2% sequence divergence per million years. Bellbirds are thought to have evolved ~2·9 million years ago (see Beadell *et al*. 2004; Driskell and Christidis, 2004). Thus our results here of a 4·9% sequence divergence between Australian and New Zealand Meliphagid *Plasmodium* spp. date the origin of the LIN1 parasite to the historical Meliphagidae radiation from Australia to New Zealand. A convincing argument would be that the ancestral bellbird carried its parasites from Australia and that the bellbird and LIN1 have been locked in an ancient evolutionary arms race (Dawkins and Krebs, 1979). However, Ricklefs and Outlaw (2010) concluded that a new disease might emerge in a host, through host switching, soon after its origin and at any time thereafter, thus it is still plausible that the modern-day bellbird *Plasmodium* LIN1 lineage may have originated in New Zealand earlier than 3 million years ago, independently of the evolutionary history of the bellbird.

LIN2 was an exact match with the *P. elongatum* GRW06 lineage originally described in the great reed warbler (*Acrocephalus arundinaceus*; Perez-Tris *et al*. 2007; see also Valkiunas *et al*. 2008*a*) and is a global cosmopolitan species found in many species of avian hosts throughout Europe, Africa, Australia and North America (Table 2). Despite its relatively low prevalence in bellbirds, the geographical distribution of LIN2 is ubiquitous throughout New Zealand (this study) and this same parasite has been sampled from several endemic and exotic avian species throughout New Zealand (Ruth Brown, *unpublished data*). Furthermore, it is unlikely that the more rare lineages LIN2-4 maintain their population solely within the bellbird (Bensch *et al*. 2007). The most probable reservoir for LIN2-4 parasite lineages are the European and Asian passerines, and indeed *Passer domesticus* is the most common host listed in the MalAvi database for LIN2 infections. Taken together with the lack of nucleotide base pair substitutions, we suggest that GRW06 (LIN2) has arrived in New Zealand relatively recently.

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The 2 remaining lineages grouped within 2 *Plasmodium* clades representing species reported from African birds only: LIN3, *Plasmodium* (*Novyella*) sp. AFTRU08 (Beadell *et al*. 2006); and LIN4, *P*. (*Haemamoeba*) *relictum* LINOLI01. A single bellbird screened positive for *Plasmodium relictum*, LIN4, at Hauturu and it is unknown to date whether this exact lineage has been detected in other New Zealand birds. The *P. relictum* lineages documented by Tompkins and Gleeson (2006) cannot be compared directly to our results because the cytochrome *b* markers they used differed from those in our study and those generally used in the MalAvi database. The *P. relictum* lineage that we detected in bellbirds may be a distant variant of the well-known GRW04 *P. relictum* lineage (LIN4 *vs* GRW04: 1·7% sequence divergence; LIN4 *vs* LINOLI01: 0·7% sequence divergence). GRW04 is well known to be responsible for the decimation of Hawaiian avifauna (Perkins and Schall, 2002). That tragedy has not occurred in New Zealand likely because mosquitos are endemic, unlike in Hawaii (Derraik, 2004). Thus, endemic New Zealand birds should have some acquired resistance to exotic strains of malaria as well as endemic strains (Jarvi *et al*. 2002).

Lack of Haemoproteus *infections and underestimation of parasite prevalence*

We found no *Haemoproteus* infections in bellbirds despite the fact that their usual hippoboscid vector (Valkiunas, 2005) was observed on most individuals captured in this study (S.M.B., *unpublished data*). The New Zealand bellbird host lineage originated from Australian meliphagids (Driskell *et al*. 2007), in which *Haemoproteus* infections are common (Beadell *et al*. 2006). Thus, we suggest that *Haemoproteus* infections in bellbirds (1) might have been lost through the process of host population bottleneck, (2) are too low in numbers to detect, or (3) have never been part of the New Zealand meliphagid system. PCR detection of parasites has been experimentally shown to underestimate prevalence by 30%, especially (1) in cases of low parasitaemia characteristic of chronic infection and (2) if infections temporarily evacuate the peripheral blood stream (Jarvi *et al*. 2002; Valkiunas *et al*. 2008*b*). This large margin of error alone may explain the absence of *Haemoproteus* in our study and means that our *Plasmodium* prevalence estimates are underestimated. Microscopy yields varying results depending on both the skill level of the observer and quality of specimens. For most non-specialized researchers microscopy techniques will underestimate prevalence by 70% (Jarvi *et al*. 2002). In this study, we found that in our initial 97 samples, 39% (16/26) of the malaria detected by nested PCR was not detected by microscopy performed by a professional haematologist experienced in malaria detection. A more sensitive malaria detection technique involves serological assay of antibodies, but current infection status and parasite lineage identification is not possible (Jarvi *et al*. 2002). The most promising blood parasite detection approach for future studies is a relatively new quantitative PCR (qPCR) that has been shown to have higher detection sensitivity than nested PCR and it can be used to estimate parasitaemia, or concentration of parasites in a given amount of blood (see Knowles *et al*. 2011).

Conclusions

Students of biogeography have long centred their studies on islands. Because of its large almost continental sized islands, range of climatic and altitudinal variation and long distance from source areas, New Zealand is an ideal island to study replicate natural experiments that allow a focus on a wide variety of issues including ecological transmission barriers, area and distance effects, endemic versus widespread species and extinction rates. Relative to continental bird populations, we found a low diversity of haematozoan parasites in New Zealand bellbirds. Avian malaria parasite diversity and abundance was concentrated in northern New Zealand and the only malaria lineage present throughout the country was an exotic *P. elongatum* GRW06 (LIN2, this study). Our phylogenetic analysis provides strong evidence that the LIN2 parasite (as well as LIN3 and LIN4) has been recently introduced to New Zealand. On the other hand, sequence divergence of the LIN1 parasite within its clade indicates that LIN1 may have existed in New Zealand for several millions of years. The mismatch we reveal between host and parasite spatial distributions suggests that this putative endemic parasite is limited by vector distribution, possibly for climatic reasons. As climatic conditions change both in New Zealand and around the world, the use of the MalAvi database, as well as phylogenetics and qPCR techniques will be important tools in future studies on rates of entry of invading parasites in New Zealand, parasite virulence and ecological transmission barriers across variable climatic environments.

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