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# Avian malaria in introduced, native and endemic New Zealand bird species in a mixed ecosystem

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**Abstract:** Avian malaria, caused by *Plasmodium* spp., has been reported as a cause of morbidity and mortality in New Zealand bird populations. The prevalence of *Plasmodium* lineages in the Waimarino Forest was evaluated in NZ robins (*Petroica longipes*), other passerines, blue ducks (*Hymenolaimus malacorhynchos*), and brown kiwi (*Apteryx mantelli*), using nested PCR. The presence of *P.* sp. lineage LINN1, *P.* (Huffia) e*longatum* lineage GRW06 and *P.* (Novyella) sp. lineage SYAT05 was demonstrated; *Plasmodium* (Haemamoeba) *relictum* lineage GRW4 was not found. The highest prevalence of infection was found in introduced European species (80.5%), followed by native (19%) and endemic species (3.5%), with a significant difference between these groups. All detected *Plasmodium* lineages have previously been identified in New Zealand and introduced species have been suggested as an important reservoir of infection. The results of this study will aid New Zealand conservation managers with disease risk management during bird translocations from the Waimarino forest.

**Key words:** *elongatum*; GRW4; GRW06; LINN1; *Plasmodium*; *relictum*; robin; SYAT05; translocation; Waimarino

## Introduction

New Zealand's ecosystems are considered to be among the most extinction-prone in the world (Myers et al. 2000; Brooks et al. 2002). Due to its high proportion of bird-pollinated plants, New Zealand's terrestrial ecosystems are considered especially sensitive to losses in native bird biodiversity (Sekercioglu et al. 2004). Many New Zealand bird populations are critically endangered (Hitchmough et al. 2007) and efficient wildlife management strategies have been employed (Craig et al. 2000) to safeguard their long-term viability and sustain them as essential functional components of the terrestrial ecosystems. Ongoing predation and habitat changes since European settlement in New Zealand have contributed to population declines in many bird species (O'Donnell 1996; Bogich et al. 2012) even in those still not considered at risk. For example, New Zealand robins (*Petroica longipes*) (henceforth NZ robins) are currently considered to be 'not threatened' (Robertson et al. 2013), despite the IUCN reporting a decreasing population trend for this species (IUCN 2014). Moreover, the distribution pattern of NZ robins has changed from being once widespread throughout the mainland to a patchy distribution (Heather & Robertson 2005). Translocation in the form of assisted colonisation of a new area with suitable habitat is a successful management tool to secure NZ robin numbers (Taylor et al. 2005). According to the IUCN guidelines, monitoring and management of disease should be standard practice when populations are translocated to both maximise the health of translocated birds and minimise the risk of introducing new pathogens to a destination area (IUCN/SSC 2013). Therefore, identification of previously unrecorded pathogens and spatial and temporal tracking of existing pathogens is beneficial for New Zealand conservation managers and provides a rational basis for disease risk management during translocations (Parker et al. 2006).

Avian malaria, caused by various *Plasmodium* species, is an emerging disease in New Zealand (Schoener et al. 2014). A high mortality outbreak occurred after translocation of South Island saddlebacks (*Philesturnus carunculatus*) due to concurrent *Plasmodium* spp. and avipox virus infection (Alley et al. 2010). Furthermore, infection with *Plasmodium* spp. was the confirmed cause of death in five out of eight yellowheads (*Mohoua ochrocephala*) that died after being moved from an area with low *Plasmodium* spp. prevalence to a location with very high prevalence of both the parasite and the appropriate vector (Alley et al. 2008). Other wild bird species in which avian malaria-related mortalities have been documented are brown kiwi (*Apteryx mantelli*) (Banda et al. 2013), great spotted kiwi (*Apteryx haastii*) (Howe et al. 2012), stitchbird (*Notiomystis cincta*) (Howe et al. 2012), and New Zealand dotterel (*Charadrius obscurus*) (Reed 1997). Currently, 17 lineages of *Plasmodium* spp. have been reported in 35 New Zealand wild bird species, including introduced, native, and endemic species (Schoener et al. 2014). The most commonly detected lineages are *P.* (Huffia) e*longatum* lineage GRW06 and *P.* (Novyella) sp. lineage SYAT05, followed by *P.* (Haemamoeba) *relictum* (lineages GRW4 and SGS1) and *P.* sp. lineage LINN1, while *P.* (Novyella) lineage AFTRU08 and *P.* (Haemamoeba) *relictum* lineage LINOLI01 are rare (Schoener et al. 2014). All of these lineages are considered to be non-endemic to New Zealand and were likely introduced into this country with the importation of their avian hosts (Ewen et al. 2012).

Pre-translocation health screening of 20 NZ robins from the Waimarino Forest was undertaken by the Greater Wellington Regional Council in 2011. Health screens comprised physical checks for external lesions of avipoxvirus infection, cloacal swabs for bacterial culture of *Salmonella* and *Yersinia*, and blood sample collection for *Plasmodium* spp. PCR. The only detected pathogen of concern for translocation was *P. relictum*

lineage GRW4, which was detected in one NZ robin, while two NZ robins were infected with *P. elongatum* lineage GRW06 (Nikki McArthur, Greater Wellington Regional Council, pers. comm.). *Plasmodium elongatum* lineage GRW06 is widespread in New Zealand and has been found in a large variety of bird species (Alley et al. 2010; Baillie & Brunton 2011; Castro et al. 2011; Marzal et al. 2011; Ewen et al. 2012; Howe et al. 2012; Banda et al. 2013). Although sporadic deaths due to this lineage have been reported in wild birds in New Zealand (Howe et al. 2012; Banda et al. 2013), its pathogenicity in wild birds worldwide is generally considered to be low (Valkiunas 2005). *Plasmodium relictum* lineage GRW4 is considered to be much more pathogenic. This lineage played an important role in the extinction of many endemic bird species in Hawaii. The establishment of the *P. relictum* lineage GRW4 on the Hawaiian Islands, following the introduction of its mosquito-vector *Culex quinquefasciatus* and exotic avian hosts, caused a rapid spread of this lineage amongst the endemic avifauna with catastrophic results (van RiperIII et al. 1986). In Hawaiian bird species that survived, such as the apapane (*Himatione sanguinea*), high mortality is still a problem (Atkinson & Samuel 2010). In New Zealand, this lineage has been identified in wild birds in the northern part of the North Island, including one red-fronted parakeet (*Cyanoramphus novaezelandiae*) from Little Barrier Island (Ortiz-Catedral et al. 2011) and 17 house sparrows (*Passer domesticus*) from Drury, Titiritiri Matangi and Little Barrier Island (Marzal et al. 2011; Ewen et al. 2012). The geographical spread of *P. relictum* lineage GRW4 and its impact on New Zealand's endemic and native bird species are currently not well understood. Therefore, the translocation of NZ robins from the Waimarino Forest was halted until the risk of spreading *Plasmodium* lineages to new areas could be further evaluated.

The aim of this study was to evaluate the presence and prevalence of *Plasmodium* lineages in NZ robins and other bird species in the Waimarino Forest area, as part of the assessment of the area's suitability as a mainland donor site for North Island robin translocations. In addition, routine conservation management activity allowed for opportunistic surveying for *Plasmodium* spp. in brown kiwi and blue duck (*Hymenolaimus malacorhynchos*) living in the area.

## Materials and methods

### Study site

Our study area, the Waimarino Forest, is located in the central North Island of New Zealand (39°26′17.66″ S, 175°8′24.76″ E). It is a privately owned area of 6937 ha, of which 3884 ha are planted in exotic pine (*Pinus radiata*) for logging. The remaining land is made up of indigenous forest, streams, riparian margins, landslip scars and farmland. It is bordered on the south side by the road from Raetihi to Pipiriki, on the north side by the Manganui o te Ao River, a conservation site for the nationally endangered blue duck, and on the west side by the Whanganui River (Fig. 1). Its west side also borders the Whanganui National Park (39°34′59.88″ S, 175°4′59.88″ E), one of the largest remaining tracts of lowland forest with streams and rivers in the North Island. As a consequence, *Plasmodium* lineages found in the Waimarino Forest will also have an impact on birds in the Whanganui National Park (Fig. 1). The mixed landscape within the Waimarino Forest results in adjoining areas with various densities of endemic, native and introduced European bird species within the study site. Among the many endemic species, established breeding populations of NZ robins and brown kiwi are present, which have both been used as source populations for translocations. In 1999, 40 NZ robins were moved to Paengaroa Mainland Island and in 2001, 28 NZ robins were moved to Bushy Park Reserve (Armstrong 2010). Although precise NZ robin counts are lacking, the number of NZ robins in the area is estimated at ca. 1000 individuals, based on a 3-day survey by staff from the Greater Wellington Regional Council in 2010. The population is well established and expected to replenish its numbers easily after removal of up to 60 birds for translocation.

For the purpose of this study, the Waimarino Forest, bordering areas of the Whanganui National Park, and farmland surrounding Pipiriki were treated as one epidemiological unit.

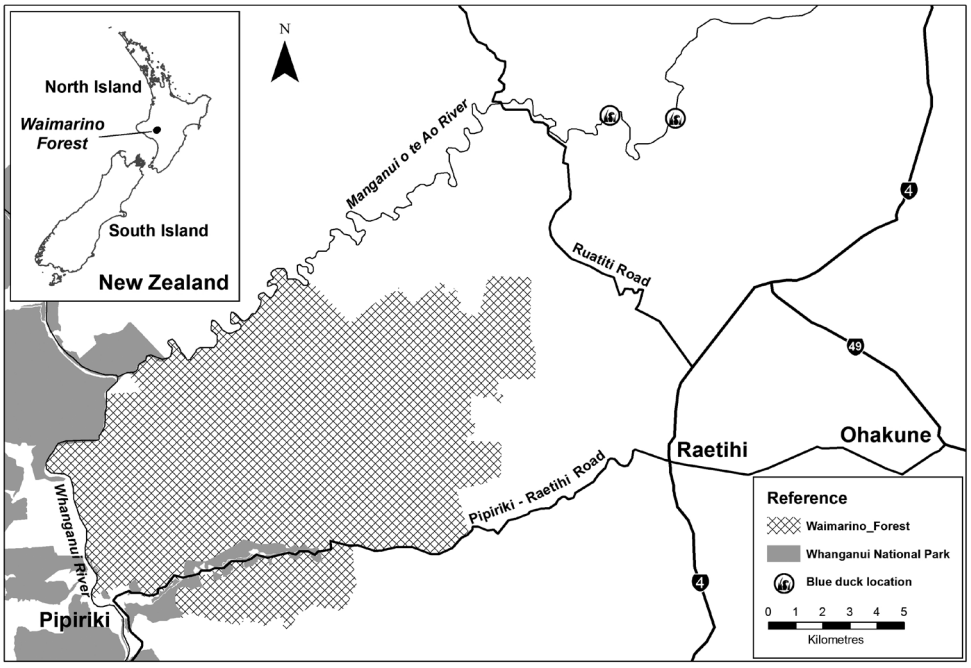


**Figure 1.** Location of the Waimarino Forest within New Zealand and its relation to surrounding geographical features.

Therefore, we assumed that birds within this area were exposed to similar lineages of *Plasmodium* spp.

**Blood sample collection and individual bird processing**

One hundred NZ robins were caught using clap-traps. Standard mist netting techniques were used to catch 88 passerines of various species (excluding robins) and 14 blue ducks. During spring sessions in the first week of October 2012, NZ robins and other passerines were caught in the western half of the forest. During summer sessions in the last week of February 2013, NZ robins were caught in the eastern and northern parts of the forest, while other passerines were caught close to the south-west border of the forest around the village of Pipiriki and neighbouring farmland (Fig. 1). Logging activities inside the Waimarino Forest determined which areas were available to the fieldwork team. The area near Pipiriki was chosen for mist netting during the second fieldwork week because of the higher density of exotic passerine birds in this area.

All passerines were processed at their capture site and identified with uniquely numbered metal leg bands. We collected blood samples using heparinised capillary tubes after puncturing the brachial vein with a sterile 27 gauge needle, ranging in volume from 0.05 to 0.25 ml and equalling less than 1% of each bird's body mass. Body mass (to nearest 0.5 g), tarsometatarsal length (to nearest 0.1 mm) and wing chord (to nearest 0.5 mm) were measured, and a physical health check was performed, before the birds were released. When possible, birds were classified as juvenile or adult, and as male or female according to their plumage characteristics. Individuals recaptured in mist nets were released immediately.

Blue duck blood samples of up to 1 ml per bird were collected opportunistically during general health screens with the New Zealand Department of Conservation (DOC) in 2013 at two locations of the Manganui o te Ao River (Fig. 1) during mid-February (39°18′42.900″ S, 175°16′48.301″ E) and mid-May (39°18′49.814″ S, 175°15′12.306″ E). Blood samples were collected using a sterile 27-gauge needle and 1-ml syringe and transferred to BD Microtainer® tubes with Lithium Heparin additive.

Packed cell volume (PCV) was determined for each blood sample on the day of blood collection, using a portable ZIPocrit haematocrit centrifuge (LW Scientific, Georgia, Australia) at 10 000 rpm for 5 min, after which the samples were stored in BD Microtainer® tubes with Lithium Heparin additive at –20ºC until further processing.

To calculate a quantitative body condition index (BCI) for individual blackbirds (*Turdus merula*), silvereyes (*Zosterops lateralis*) and NZ robins, the following formula was used:

$$\text{BCI} = \frac{\text{body weight (g)}}{\text{tarsometatarsal length (mm)}}$$

As part of an ongoing brown kiwi translocation project between the Waimarino Forest and Maungatautari Ecological Reserve (38°01′00″ S, 175°34′00″ E), 20 blood samples for pre-translocation health screens were collected from brown kiwi in the Waimarino Forest during 2012, 2013 and 2014. Results for *Plasmodium* spp. PCR were made available to us by Maungatautari Ecological Island Trust (MEIT).

**Molecular biology**

DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen, Valencia CA, USA), following the manufacturer's instructions for nucleated whole blood. Extracted DNA samples were stored at –20ºC until used for molecular analysis. The presence of the cytochrome b gene of *Plasmodium* spp. was identified using a nested PCR using the primer sets HaemNF1/HaemNR3 and HaemF/HaemR2 as described by Hellgren et al. (2004). A known *Plasmodium* positive blood sample, confirmed through sequencing as *P.* sp. lineage LINN1, was used as a positive control, while nuclease-free water was included as a negative control. To confirm successful amplification, the final PCR products were run on a 1.5% agarose gel (Invitrogen, Carlsbad, CA, USA) containing ethidium bromide for 1 hour at 100 V.

Positive amplicons were purified using a PureLink PCR purification kit (Invitrogen, Auckland, New Zealand) and subjected to automatic dye-terminator cycle sequencing with the BigDyeTM Terminator Version 3.1 Ready Reaction Cycle Sequencing kit and the ABI3730 Genetic Analyzer (Applied Biosystems Inc., Foster City, CA, USA) to confirm the genomic sequences, using both the forward and reverse primers. Chromatograms were aligned using Geneious™ (Biomatters, Auckland, New Zealand) and examined for conspicuous overlapping peaks suggestive of *Plasmodium* spp. co-infection. The *Plasmodium* isolate sequences obtained were compared to other published sequences available from GenBank (Benson et al. 2014) using NCBI Blast and from the MalAvi database (Bensch et al. 2009).

**Statistics**

The apparent prevalence for *Plasmodium* spp. and the 95% confidence interval was determined for various groups and species of birds using the Epitools software, following the Wilson binomial approximation (Brown et al. 2001). Chi-square tests (Preacher 2001) were used to analyse whether prevalence of *Plasmodium* spp. differed significantly between various groups and species of birds. If the frequency in one or more bird categories was below five, a Fisher's exact test was used (Preacher & Briggs 2001). Because data for BCI and PCV in the various bird species were not normally distributed, a non-parametric analysis (Mann-Whitney U tests, SPSS, version 22, IBM Statistics) was used to determine whether there was a significant difference in these parameters between *Plasmodium* spp. negative and positive birds.

## Results

Blood samples were collected from 15 species in three different avian orders, of which 5 species are classified as introduced, 2 as native, and 8 as endemic to New Zealand (Table 1). All birds showed bright and alert behaviour and were in good physical condition. Overall, 45/222 (20.3%) birds tested positive for the presence of *Plasmodium* DNA using nested PCR. The *Plasmodium* spp. prevalence for introduced bird species was 80.5% (33/41; 95% CI 0.66–0.90), for native species 19% (7/37; 95% CI 0.10–0.34) and for endemic New Zealand bird species 3.5% (5/144; 95% CI 0.02–0.08). The difference in prevalence of avian malaria infection between these three groups was significant ($\chi^2 = 60.04$, d.f. = 2, $P < 0.001$).

DNA sequencing was successful for 30 out of 44 *Plasmodium*-positive passerine blood samples. For the remaining 15 samples sequencing failed due to the PCR product being too weak to sequence, or due to the presence of overlapping peaks throughout the sequence. In all 30 samples,

**Table 1.** The prevalence of avian malaria in birds in the Waimarino Forest area and the *Plasmodium* lineages identified.

| Order | Family | Species* | Positive/ *n* tested | Prevalence | 95% CI | Plasmodium Lineages† |
|---|---|---|---|---|---|---|
| Passeriformes | Turdidae | Song Thrush *Turdus philomelos* (*I*) | 4/4 | 1.00 | 0.51-1.00 | LINN1(1), Elongatum(1) |
| | Prunellidae | Dunnock *Prunella modularis* (*I*) | 1/1 | 1.00 | 0.21-1.00 | |
| | Turdidae | Blackbird *Turdus merula* (*I*) | 28/34 | 0.82 | 0.67-0.92 | LINN1(12), Elongatum(5), SYAT05(4) |
| | Fringillidae | Chaffinch *Fringilla coeleps* (*I*) | 0/1 | 0.00 | 0.00-0.79 | |
| | Fringillidae | Goldfinch *Carduelis carduelis* (*I*) | 0/1 | 0.00 | 0.00-0.79 | |
| | Zosteropidae | Silvereye *Zosterops lateralis* (*N*) | 7/33 | 0.21 | 0.11-0.38 | LINN1(2) |
| | Rhipiduridae | Fantail *Rhipidura fuliginosa* (*N*) | 0/4 | 0.00 | 0.00-0.49 | |
| | Petroicidae | New Zealand Robin *Petroica longipes* (*E*) | 4/100 | 0.04 | 0.02-0.10 | LINN1(1), Elongatum (3) |
| | Petroicidae | Tomtit *Petroica macrocephala toitoi* (*E*) | 0/3 | 0.00 | 0.00-0.56 | |
| | Mohouidae | Whitehead *Mohoua albicilla* (*E*) | 0/3 | 0.00 | 0.00-0.56 | |
| | Acanthizidae | Grey Warbler *Gerygone igata* (*E*) | 0/2 | 0.00 | 0.00-0.66 | |
| | Meliphagidae | Bellbird *Anthornis melanura* (*E*) | 0/1 | 0.00 | 0.00-0.79 | |
| | Meliphagidae | Tui *Prosthemadera novaeseelandiae* (*E*) | 0/1 | 0.00 | 0.00-0.79 | |
| | | Total for all Passeriformes combined | 44/188 | 0.23 | 0.18-0.30 | LINN1(16), Elongatum(9), SYAT05(4) |
| Anseriformes | Anatidae | Blue Duck *Hymenolaimus malacorhynchos* (*E*) | 0/14 | 0.00 | 0.00-0.22 | |
| Apterygiformes | Apterygidae | Brown kiwi *Apteryx mantelli* (*E*) | 1/20 | 0.05 | 0.01-0.24 | LINN1(1) |

* whether an avian species is introduced (*I*), native (*N*), or endemic (*E*) to New Zealand is shown in parenthesis behind the scientific name
† the frequency with which *Plasmodium* lineages have been demonstrated for an avian species is shown in parentheses

**Table 2.** Packed cell volume (PCV) and body condition index (BCI) with standard errors (SE) for species with *Plasmodium* negative (neg) and positive (pos) birds.

| Species* | PCV% (SE)† | | BCI‡ (SE)† | |
|---|---|---|---|---|
| | neg birds | pos birds | Neg birds | Pos birds |
| Blackbird *Turdus merula* (*I*) | 44.3 (2.3) | 43.9 (0.9) | 2.72 (0.06) | 2.65 (0.06) |
| Silvereye *Zosterops lateralis* (*N*) | 46.0 (0.7) | 44.8 (1.8) | 0.63 (0.02) | 0.73 (0.05) |
| New Zealand Robin *Petroica longipes* (*E*) | 46.4 (1.6) | 51.7 (2.3) | 0.83 (0.01) | 0.82 (0.01) |

* whether an avian species is introduced (*I*), native (*N*), or endemic (*E*) to New Zealand is shown in parenthesis behind the scientific name
† standard error (SE) is shown in parenthesis behind the PCV and BCI values
‡ BCI is defined as bodyweight in grams divided by tarsus length in millimetres

nucleotide sequences of amplified DNA showed > 99% similarity with known sequences from GenBank as determined by NCBI BLAST. *Plasmodium* sp. lineage LINN1 (GenBank GQ471953) was found in blackbirds (12/21), silvereyes (2/2), song thrushes (*Turdus philomelos)* (1/2), NZ robins (1/4), and brown kiwi (1/20). *Plasmodium* e*longatum* lineage GRW06 (GenBank DQ368381) was found in blackbirds (5/21), song thrushes (1/2), and NZ robins (3/4), while *P.* sp. lineage SYAT05 (GenBank DQ847271) was found only in blackbirds (4/21) (Table 1). *Plasmodium relictum* lineage GRW4 (GenBank AY099041) was not found in any of the sequenced PCR products and thus has a maximum true prevalence range within the research area of 0–0.04 in NZ robins, and of 0–0.02 in all sampled avian species combined at the 95% confidence level. For the three detected lineages, the overall true prevalence at the 95% confidence level was 0.05–0.13 for *P.* sp. lineage LINN1, 0.02–0.05 for *P.* e*longatum* lineage GRW06 and 0.01–0.05 for *P.* sp. lineage SYAT05. The difference in prevalence between these lineages was significant ($\chi^2 = 9.04$, d.f. = 2, $P = 0.01$).

Clinical data were further evaluated for bird species for which *Plasmodium* spp. positive as well as negative samples were found, comprising blackbirds, silvereyes and NZ robins. No significant differences in values for PCV and BCI were found between *Plasmodium* spp. negative and positive birds (Table 2), although in silvereyes a trend towards a better BCI in *Plasmodium* spp. positive birds was seen ($n = 33$, U = 120.000, $P = 0.072$).

For the three bird species for which results were further analysed, accurate sex determination by visual inspection of plumage could only be performed for blackbirds, while accurate classification as juvenile or adult was possible for blackbirds and silvereyes. No significant difference in avian malaria prevalence was detected between male and female blackbirds (Fisher's exact, $P = 0.51$), or between juvenile and adult blackbirds (Fisher's exact, $P = 0.30$) or silvereyes (Fisher's exact, $P = 1.00$).

## Discussion

The aim of this study was to evaluate the presence and prevalence of avian malaria lineages in avian species within the Waimarino Forest area to aid assessment of the suitability of this location as a source site for NZ robin translocations. We identified three of the 17 *Plasmodium* lineages currently known in New Zealand, *P.* sp. lineage LINN1, *P. elongatum* lineage

GRW06, and *P.* sp. lineage SYAT05. The establishment of avian *Plasmodium* lineages in an ecosystem and their consequent prevalence of infection in various bird species depend on multiple factors. Susceptibility and tolerance to infection of the avian hosts, presence and competence (transmission efficiency) of arthropod vectors, virulence of the *Plasmodium* spp., spatial and temporal distribution of host and vector, as well as climate, each play an important role (Benning et al. 2002; Bensch & Åkesson 2003; Samuel et al. 2011; Westerdahl 2012). The severity of pathologic effects due to avian malaria infections differs between *Plasmodium* species (Lachish et al. 2011), but also between avian species (Palinauskas et al. 2008). Morbidity and mortality with malarial infections can be severe, especially in immunologically naïve and susceptible hosts, or when co-infections with other infectious agents, such as avipoxvirus or even intestinal parasites, are present (Graham et al. 2005; Alley et al. 2008, 2010; Atkinson & Samuel 2010). Decreased activity and food consumption, pale mucous membranes, respiratory signs, vomiting and behavioural changes can be seen, signs that are caused by destruction of erythrocytes, inflammatory reactions predominantly in spleen, liver and lungs, and infiltration of parasites into the brain (Yorinks & Atkinson 2000; Valkiunas 2005; Dunn et al. 2011).

For the *Plasmodium* lineages detected in the Waimarino Forest, information regarding their virulence and pathogenicity in New Zealand's wild bird species is limited. All three lineages detected in this study are common in exotic passerines, in which they are expected to have a relatively low pathogenicity, based on the high prevalence of chronic infections in apparently healthy birds (Tompkins & Gleeson 2006). The lower prevalence in native and endemic bird species, combined with reported mortality cases, strengthens the belief that the impact on these New Zealand species is different (Tompkins & Gleeson 2006; Ewen et al. 2012; Howe et al. 2012). One explanation might be that many endemic and native species, which did not evolve with the introduced *Plasmodium* spp., might have less immunocompetence to infection with these parasites, resulting in a lower percentage of infected birds surviving until the chronic phase of infection.

*Plasmodium* sp. lineage LINN1 (GenBank GQ471953) was detected most frequently in the widest range of bird species. To our knowledge, this is the first study to show the presence of *P.* sp. lineage LINN1 infection in native silvereyes and endemic NZ robins. *Plasmodium* sp. lineage LINN1 is a cosmopolitan generalist parasite and has been isolated from a wide range of avian species and mosquitoes throughout Europe, Asia and America (Bentz et al. 2006; Wood et al. 2007; Cosgrove et al. 2008; Kimura et al. 2010; Hellgren et al. 2011; Szoellosi et al. 2011; Ferraguti et al. 2013). *Plasmodium* sp. lineage LINN1 forms a cluster with the 99% genetically similar lineages *P.* sp. lineages AFTRU5 (GenBank DQ847263) and WA39 (GenBank EU810610), which have also been reported in New Zealand (Howe et al. 2012). Within New Zealand, the lineage LINN1 has been previously reported in blackbirds, great spotted kiwi, song thrush and bellbird (*Anthornis melanura*) (Ewen et al. 2012; Howe et al. 2012).

*Plasmodium elongatum* lineage GRW06 was the second most common lineage in our study and appears to be the most prevalent *Plasmodium* lineage in NZ robins from the Waimarino area. It is a cosmopolitan parasite with a wide host range, and has been detected in over 60 avian species of at least nine orders, including waterfowl (Anseriformes), raptors (Falconiformes), owls (Strigiformes) and particularly passeriformes, which are considered to act as reservoirs (Valkiunas 2005; Valkiunas et al. 2008). Within New Zealand, *P. elongatum* lineage GRW06 is considered the most common malarial parasite with the widest host range (Schoener et al. 2014). It is present throughout New Zealand (Baillie & Brunton 2011) and has been described in the North Island saddleback (*Philesturnus rufusater*), South Island saddleback (*P. carunculatus*), silvereye, brown kiwi, blackbird, house sparrow, songthrush, bellbird, yellowhammer (*Emberiza citronella*), whitehead (*Mohoua albicilla*), and NZ robin (Alley et al. 2010; Baillie & Brunton 2011; Castro et al. 2011; Marzal et al. 2011; Ewen et al. 2012; Howe et al. 2012; Banda et al. 2013). In a captive rearing program of brown kiwi, an outbreak resulted in the death of one kiwi and parasitaemia in 25 out of 32 (78%) of the concurrent captive population (Banda et al. 2013). Infection with this lineage has contributed to a high level of mortality in translocated South Island saddlebacks with concurrent avipox infection (Alley et al. 2010). This indicates that although the pathogenicity of the *P. elongatum* lineage GRW06 in exotic passerines appears to be moderate to low, its impact on endemic species can be severe.

*Plasmodium* sp. lineage SYAT05 was found only in blackbirds. This lineage is very closely related to the African lineages W38 (GenBank EU810633, MalAvi AFTRU08) and W37 (GenBank EU810632, MalAvi AFTRU08) (Beadell et al. 2009). Hellgren et al. (2007) have reported the presence of *P.* sp. lineage SYAT05 in multiple passerine species from Africa and Europe, and in 13 species of intercontinental migrants. European blackbirds have a high prevalence of *P.* sp. lineage SYAT05, while lower prevalences were found in other European and American passerine species (Bentz et al. 2006; Dimitrov et al. 2010; Hellgren et al. 2011; Santiago-Alarcon et al. 2011). Within New Zealand, *P.* spp. lineage SYAT05 is a common lineage present in European blackbirds in the North Island; however, data regarding its geological spread in the South Island are lacking (Howe et al. 2012). Additionally, the *P.* sp. lineage SYAT05 has been isolated from kereru (*Hemiphaga novaeseelandiae*), tomtits (*Petroica macrocephala*), and bellbirds (Baillie & Brunton 2011; Ewen et al. 2012; Howe et al. 2012). This lineage is considered to have low pathogenicity in exotic passerines, but it is currently unclear what effect *P.* sp. lineage SYAT05 has on the unique range of New Zealand's native and endemic species.

To evaluate the impact of *Plasmodium* infection on the birds' physical parameters, BCIs and PCVs were compared between *Plasmodium* spp. positive and negative blackbirds, silvereyes, and NZ robins, but no significant differences were found. Changes in PCV and BCI are more likely seen in the acute, high parasitaemia phase of infection, coinciding with an increased destruction of erythrocytes and reduced food intake (Moller & Nielsen 2007). The *Plasmodium* positive birds we screened were likely in the chronic phase of infection, as studies suggest that acutely infected birds move around less, are more likely to be caught by predators and less likely to be captured in mist nets (Yorinks & Atkinson 2000; Moller & Nielsen 2007). It was interesting that in silvereyes we found a non-significant trend towards higher BCIs for *Plasmodium* infected birds compared with non-infected birds. It is plausible that in silvereyes a higher pre-infection weight to size ratio coincided with a more efficient immune response and a higher survival rate during the acute phase of infection (Atkinson et al 1995, Moller & Saino 2004). An initial deterioration in BCI and PCV could have occurred in the acute phase of infection, followed by a return to the pre-infection condition once surviving birds reached a chronic, low parasitaemia

phase. Nevertheless, as long as there are no existing data regarding the empirical validation of a relationship between BCI and survival of avian malaria in silvereyes, speculations regarding this association have to be made with extreme caution (Barnett et al. 2015). To confirm this theory, a higher number of birds would need to be tested (Power = 0.637 in our analysis. G3Power 3.1.7), and a quantitative analysis of parasite load would need to be performed. The fact that all birds with positive PCR results for avian malaria were also in good physical condition suggests that they were either in the chronic or latent phase of infection, or that the infection was non-pathogenic.

*Plasmodium relictum* lineage GRW4, the lineage that raised concerns after it was identified in a NZ robin in 2011, was not found during this study. Because the geographical spread of *P. relictum* lineage GRW4 and its impact on New Zealand's endemic and native bird species are currently not well understood, bird translocations from areas where this lineage is confirmed to be established to areas that are potentially free of the linage GRW4 should be avoided. The absence of *Plasmodium relictum* lineage GRW4 in the 100 NZ robins and in the 144 birds of other species sampled during this study suggests that this lineage is either not established within the Waimarino Forest or that its prevalence in NZ robins in the area is less than 4%.

In summary, the three *Plasmodium* lineages that we detected during this study are widespread throughout New Zealand, with a high prevalence in introduced bird species (Baillie & Brunton 2011; Ewen et al. 2012; Howe et al. 2012), and *P. relictum* lineage GRW4 was not found in any of the tested birds. Based on our data, we contend that there is little chance of introducing *Plasmodium* lineages from the Waimarino Forest to new areas during NZ robin translocations. The high incidence of introduced bird species co-inhabiting with native and endemic species appears to create a melting pot for *Plasmodium* spp., an aspect of the Waimarino Forest that may be reflected in other ecosystems across New Zealand. Therefore, further research is needed to monitor the appearance of new pathogens in this area and to clarify the impact of *Plasmodium* spp. on endemic and native NZ bird species.

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