

Coccidia species in endemic and native New Zealand passerines

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Abstract New Zealand native passerines are hosts to a large variety of gastrointestinal parasites, including coccidia. Coccidian parasites are generally host-specific, obligate intracellular protozoan parasites. In passerine birds, members of the genus *Isospora* are most common. Under natural conditions, these parasites seldom pose a threat, but stressors such as quarantine for translocation, overcrowding, or habitat changes may cause an infection outbreak that can severely affect wild populations. Although coccidia are important pathogens and have caused mortalities in kiwi (*Apteryx spp.*) and hihi (*Notiomystis cincta*), their prevalence, epidemiology, life cycles, and taxonomic relationships are still widely unknown in native New Zealand songbirds. Over a period of 3 years (2007–2009), we examined 330 fecal samples of six native passerine species: tui (*Prothemadera novaeseelandiae*), North Island saddleback (*Philesturnus carunculatus rufusater*), North Island robin (*Petroica longipes*), silvereve (*Zosterops lateralis*), and fantail (*Rhipidura fuliginosa*). The overall prevalence by flotation of coccidian infection in the New Zealand bird species examined was 21–38 %, 21 % in North Island robin, 38 % in tui, and 25 % in saddleback. Similar to prior studies in other countries, preliminary sequencing results suggest that coccidia in passerines in New Zealand are members of the family Eimeriidae, unlike the phenotypically similar genus *Cystisospora* of mammals. Using molecular methods, we

identified at least five new genetically distinct *Isospora* species in the examined birds (three in tui and one each in saddlebacks and North Island robins).

Introduction

Although low levels of coccidia are common and harmless in most bird species (Ritchie et al. 1994), severe infections are known to cause serious disease in several captive endemic birds in New Zealand. For example, coccidia have caused mortality in captive kiwi (Thompson 1978) as well as crèche-reared juvenile kiwi (*Apteryx spp.*) that were about to be released (Alley 2002). Systemic coccidial disease has been found to be a grave problem in the captive hihi (*Notiomystis cincta*) population at the Mt. Bruce National Wildlife Centre (Masterton, Wairarapa, North Island) (Twentyman 2001). During a 2-year study, Twentyman found that 100 % of the nestlings of three to five breeding pairs were affected and five young hihi died (Twentyman 2001). Endangered endemic New Zealand passerine species survive today in populations created from translocation of surviving individuals to offshore and mainland islands that are free of introduced mammalian predators (Parker 2008). However, in most cases, these environments have been used for forestry and farming by both Maori and Europeans over the last 1,000 years and contain young, regenerating forest. These disturbed habitats might not be ideal for the translocated birds and may be a source of stress that predisposes them to disease. For example, in 2002, there was a disease outbreak in South Island saddleback (*Philesturnus c. carunculatus*) that wiped out more than 50 % of the population on Motuara Island. Coccidiosis together with avian malaria and avipox infection were implicated in these mortalities. This outbreak was associated with a high population density after the carrying capacity of the island was reached following translocation (Hale 2009). Reasons for these

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serious infections are likely to be twofold: Firstly, any level of captivity poses a form of stress on the birds, and secondly, the birds are often confined to small areas and kept in larger groups than in the wild, which increases the chances of ingesting large numbers of coccidial oocysts. In addition, the stress of translocation and release in a less than optimal habitat, as well as exposure to opportunistic pathogens, such as *Aspergillus*, which prefer highly disturbed habitats (Perrott 2001), may predispose immunologically challenged birds to infection with gastrointestinal parasites, such as coccidia. Taken together, all these factors may, in certain circumstances, be more than enough to endanger the reintroduction success of susceptible passerine species.

Generally, little is known about avian coccidia in wild birds worldwide. Recent studies described a 40 % prevalence of coccidia among clutches of song thrushes (*Turdus philomelos*), blackbirds (*Turdus merula*), and starlings (*Sturnus vulgaris*) in New Zealand (Cassey and John 2008). In contrast, studies in Europe show much higher prevalence, for example, 89 % in greenfinches (*Carduelis chloris*) in Estonia (Horak et al. 2004) and 90 % in blackbirds in Germany (Misof 2004).

Although coccidiosis may in some circumstances be an important cause of mortality in endemic New Zealand passerines, the prevalence, epidemiology, life cycles, and even taxonomic relationships of the organisms involved are still largely unknown. In this study, we examine the feces and perform post-mortem examinations of a small number of New Zealand passerines to establish baseline data on coccidian populations in New Zealand birds.

Material and methods

Mokoia Island (latitude 38°05'S longitude 176°16'E), located in Lake Rotorua in the North Island of New Zealand, was the sampling site for tui (*Prosthemadera novaeseelandiae*), saddlebacks (*Philesturnus carunculatus rufusater*), and NI robins (*Petroica longipes*). Seven trips were made for capturing and sampling of birds on Mokoia Island, in January 2007, May 2007, October 2007, March 2008, November 2008, December 2008, and February 2009. Birds were caught in mist nets; North Island saddlebacks and robins were attracted to the net with the help of recorded songs of their own species. Other species were caught by placing the mist nets at sites frequented by the birds to increase the chances of capture.

After capture, each bird had the following measurements and information taken: weight, tarsus length, wing length, body condition, presence of a brood patch in females, and wattle size in saddlebacks. For assessment of body condition, the amount of muscle and fat tissue covering the sternum and the keel was estimated using standard methods (Melville 2007).

Fecal samples from saddlebacks and NI robins were obtained from the holding bags while tui were placed inside a cardboard box (measuring 31×25×22cm) containing a perch and left for 10 min for defecation. Fecal samples from all species were collected in 1.5-ml micro-centrifuge tubes.

The nectar-feeders, saddleback and tui, were given freshly prepared sugar water after handling and prior to release. The fecal samples were either examined within 24 h or stored at 4 °C until examination. Coccidia were removed from feces by flotation using a standard flotation technique using saturated zinc sulfate solution at a specific density of 1.32 and preparations examined at ×100 magnification. A small amount of feces was used for oocyte sporulation. After examination, the coccidia were washed into a 1.5-ml micro-centrifuge tube using water and frozen for later genetic identification. Groups of 10–20 oocysts were collected for DNA extraction. In cases where a sample contained fewer than ten oocysts, the sample was not used for polymerase chain reaction (PCR).

For sporulation, a small amount of feces was placed into a 2 ml Eppendorf tube with a drop of 2 % H₂SO₄ to prevent bacterial overgrowth and left at room temperature for a week. Twice a day, the tube was opened to allow oxygen into the tube. After a week, the sample was checked for successful sporulation, and the oocysts were measured and photographed. The measurements were made using calibrated ocular micrometer scales at ×400 magnification. With few exceptions, at least 20 of each type of oocyst were measured from each bird species.

For pathology, the birds were weighed, the body condition assessed and examined using a standard necropsy technique (Alley, unpublished). Samples of pectoral muscle and all internal organs were collected for histology, and both pectoral muscle and liver were freshly frozen for later PCR analysis. Intestinal contents were collected for parasitological examination. Sections of all visceral organs were fixed in 10 % buffered formalin and routinely processed and embedded in paraffin wax. They were cut at 3 μm and stained with hematoxylin and eosin.

DNA was extracted using the Qiagen QIAamp DNA Stool Mini Kit (Qiagen, Düsseldorf, Germany) according to the manufacturer's instructions with the following minor modifications. The frozen samples were thawed, and the oocysts were concentrated by centrifugation at 150×g for 5 min. The supernatant was carefully removed leaving less than 0.5 ml in the micro-centrifuge tube. In order to break open the walls of the oocysts, the samples were then ground for 2 min with micropestles, before the Qiagen stool kit protocol was applied. As the Qiagen stool kit was developed for mammalian fecal samples which have different PCR inhibitors in the stool from those seen in birds, in which uric acid is the main inhibitor, the InhibitEX tablet included in the kit was not used. All extracted DNA samples were stored at -20 °C until used for PCR analysis.

Previously published primer sets CoC1/CoC2, BSEF/BSER, P3/P5, and CocciA/CocciB were chosen based on their successful use in other studies of coccidia isolated from passerine birds and chickens (Schnitzler et al. 1998; Carreno & Barta 1999; Schrenzel et al. 2005).

All reactions included a previously tested positive control consisting of *Neospora* DNA derived from cell culture by Dr. L. Howe. In all reactions, a negative control (sterile autoclaved Millipore water) was also used.

To confirm successful product amplification, 10 µl of each PCR product was run on a 1.5 % agarose gel (Invitrogen, Carlsbad, California) containing ethidium bromide, with the exception of the products run with the P3/P5 primer set which were run on a 0.5 % agarose gel (Invitrogen). A 100 bp ladder was used to measure amplicon size (Promega Corporation, Madison, Wisconsin). The gels were run at 100 V for 45 min and visualized under UV light and photographed.

All coccidia-positive PCR amplicons were purified using PureLink PCR purification kit (Invitrogen) and subjected to automatic dye-terminator cycle sequencing by BigDye™ Terminator Version 3.1 Ready Reaction Cycle Sequencing kit and the ABI3730 Genetic Analyser (Applied Biosystems Inc., Foster City, California, USA) to confirm genomic sequence.

Analysis of coccidia sequences of approximately 100–300 base pairs depending on primer sets, obtained from the saddlebacks; tui and robins were compared with those of other published sequences available from Genbank. All sequences were trimmed to the same length (450 bases) using Geneious™ (Biomatters, Auckland, New Zealand) and aligned using Clustal W (Higgins et al. 1994). A phylogenetic tree was generated in MrBayes version 3.1 (Ronquist and Huelsenbeck 2003) using Bayesian phylogenetics. A general time-reversible model including invariable sites (GTR+I) was used. The Bayesian phylogeny was

obtained using one cold and three hot Monte Carlo Markov chains, which were sampled every 1,000 generations over 5 million generations; 5,000 trees were generated. Of these trees, 25 % were discarded as burn-in material. The remaining 3,750 trees were used to construct a majority consensus tree. The sequence divergence between and within the different lineages was calculated using a Jukes-Cantor model of substitution implemented in the program PAUP* 4.0 Beta version 10 (Swofford 2002).

Results

We examined 330 feces from five species of New Zealand passerines (Table 1). North Island saddleback and robins had similar rates of coccidian infection 21 % and 25 %, respectively, while tui had a higher rate of 38 %. All samples from silvereye (*Zosterops lateralis*) ($n=1$) and fantail (*Rhipidura fuliginosa*) ($n=2$) were positive for coccidia (Table 1).

The oocysts of silvereyes and NI robins showed a consistent spherical morphology (Table 2), and those of fantail were ellipsoidal (Table 2; Fig. 4). In contrast, oocysts from tui (Figs. 1 and 4) and from saddlebacks from Mokoia (Fig. 3) were sub-spherical and ellipsoidal (Table 2). In several individual birds, oocysts with different shapes and sizes were found, indicating current infection with different species of coccidia (Table 2). Oocysts from all species were sporulated successfully, except from North Island robin. Therefore, all measurements and observations on robin oocysts were performed only on unsporulated oocysts.

After sporulation, the saddleback, tui, and silvereye oocysts resembled *Isospora*-type oocysts, containing two sporocysts, and each sporocyst contained four sporozoites (Fig. 2). However, the sporulated oocysts collected from fantail were similar to *Caryospora*-type oocysts, containing

Table 1 Results of the examination of 330 fecal samples of New Zealand passerines for *Coccidia*

Family	Species	Common name (E, N)	Sampling site	Number sampled	Coccidia positive by		Coccidia sequences obtained
					Flotation	PCR	
Callaeidae	<i>Philesturnus carunculatus</i>	Saddleback (E)	Mokoia	233	58/233 (24.9 %)	7/47 (14.9 %)	1/7
Meliphagidae	<i>P. novaeseelandiae</i>	Tui (E)	Mokoia	42	16/42 (38.1 %)	7/15 (46.67 %)	3
			Mainland	7			
			Raoul Island	7			
Eopsaltriidae	<i>P. australis longipes</i>	NI Robin (E)	Mokoia	38	8/38 (21 %)	2/3	1/2
Monarchidae	<i>R. fuliginosa</i>	Fantail (N)	Mainland	1	1/1		
Zosteropidae	<i>Z. lateralis</i>	Silvereye (N)	Mainland	2	2/2		
Total				330	89	16	5

N native, E endemic

Table 2 Descriptive statistics of unsporulated oocysts measured in micrometers

	North Island saddleback (SS)			Tui (E)			North Island robin (S)			Fantail (E)			Silvereye (S)
	Length	Width	L/W ratio	Length	Width	L/W ratio	Length	Width	L/W ratio	Length	Width	L/W ratio	Diameter
N	20	20	20	26	26	26	16	16	16	21	21	21	14
Mean	19.73	18	1.11	28.65	23.69	1.22	17.81	17.66	1.01	19.64	17.32	1.14	27.14
SD	1.83	2.24	0.13	2.67	2.54	0.15	1.33	1.20	0.04	1.06	0.72	0.10	1.14
SE Mean	0.41	0.5	0.03	0.52	0.50	0.03	0.33	0.30	0.01	0.23	0.16	0.02	0.31
Min	15	15	1	20	16	1	15	15	1	17.5	15	1	25
Max	25	20	1.33	30	25	1.56	20	20	1.14	21.25	18.75	1.42	28.75

E ellipsoidal, *S* spherical, *SS* sub-spherical

one sporocyst with up to eight sporozoites. All the sporocysts had Stieda bodies (Figs. 3 and 4), a diagnostic characteristic of oocysts of the family Eimeriidae.

The sporocysts of silvereyes were elliptical in shape; sporocysts in the saddleback and tui showed elliptical as well as sub-spherical sporocysts (Table 3, Figs. 3 and 4).

Twenty-one birds were examined post-mortem, including fourteen tui, five saddleback, and two silvereyes. Three of the saddleback cases presented with a severely dilated gallbladder (up to 4 mm diameter) which was associated with trematode infection. No gross abnormalities due to gastrointestinal parasites were observed in the remaining birds, but on histology, one saddleback and one tui, both from Mokoia Island, had a moderate number of sexual coccidial stages in the duodenum, causing no apparent inflammatory reaction.

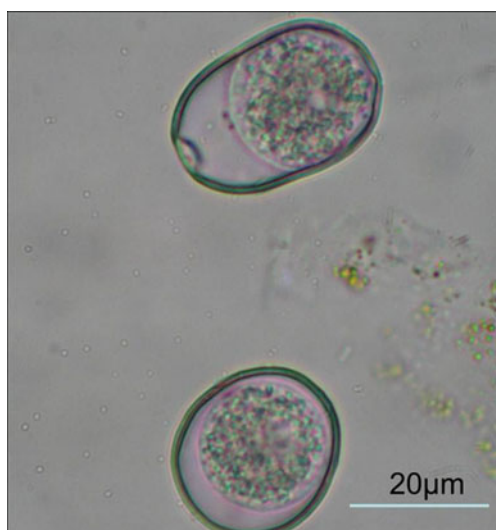


Fig. 1 Unsporulated oocysts from a Tui; note the two different types (ellipsoidal and sub-spherical). The bird was sampled on Mokoia Island ($\times 400$)

Sequencing and phylogenetics

COC1 and COC2 primer sets

DNA was amplified by PCR, using the primers COC1 and COC2 from seven of the fifteen (46.67 %) tui fecal samples that were positive for coccidia on flotation. Of these seven positive samples, three (20 %) could be sequenced with conclusive results and were subjected to a Blast search on the NCBI database. The results revealed that one isolate, “Tui 1”, had 99 % sequence homology to different *Eimeria* spp. in bats (GenBank AF307876) and rodents (GenBank AF307879, AF307878) as well as 98 % sequence homology to *Isospora* and *Atoxoplasma* spp. in passerine birds of North America (GenBank AF080612) and Asia (GenBank AY283829, AY283828). The second isolate, “Tui 4”, had 94 % similarity to *Eimeria* spp. in bats (GenBank AF307876) as well as a 93 % similarity to *Isospora* and *Atoxoplasma* spp. in North America (GenBank AF080612) and Asia (GenBank AY283829, AY283828).

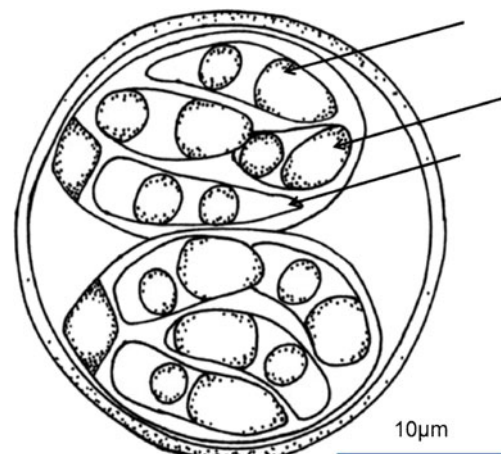


Fig. 2 Sporulated oocyst from a silvereye from Massey campus/Manawatu. Note the sporozoites in the sporocysts (arrows) ($\times 400$)

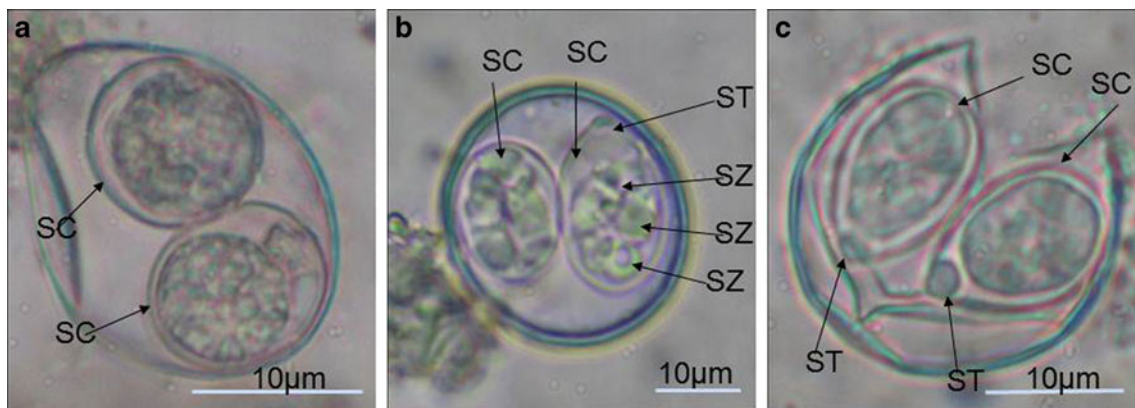


Fig. 3 Sporulated oocysts obtained from **a**. Domestic cat (for comparison). Note the absence of Stieda bodies on the sporocysts as typical of mammalian *Isospora* spp. of the family Sarcocystidae. **b** North Island

saddleback. **c** Silvereye (partly ruptured oocyst wall). $\times 400$ SC sporocyst, SZ sporozoite, ST Stieda body

A multiple alignment was performed using a Bayesian analysis on 205 bp of the 18S small subunit ribosomal gene of the two coccidia isolates “Tui1” and “Tui4” and other *Eimeria* species available from GenBank. *Theileria parva* was used as the out-group. A phylogenetic tree was constructed which resulted in the isolates “Tui 1” and “Tui 4” clustering with *Eimeria* from rodents from North America (GenBank AF307879, AF307878) as well as passerines from North America (GenBank AF080612) and Asia (GenBank AY283829, AY283828) (Fig. 5), as suggested by the BLAST search results. As a result, “Tui 1” was clustering with *Eimeria* from rodents from North America (GenBank AF307879, AF307878) as well as passerines from North America (GenBank AF080612) and Asia (GenBank AY283829, AY283828) (Fig. 5) as suggested by the BLAST search results. Tui 1 had a sequence divergence with sequences from bats and rodents (AF311643, AF307877, AF307878, AF307879, and AF324214) of 0.48 % to 1.4 %, so they were relatively closely related. The sequence from the “Tui 4” isolate had a sequence divergence with sequences from bats and rodents (AF311643, AF307877, AF307878, AF307879, AF324214) of 6.6 % to 7.2 % and a divergence

of 7.2 % to *Isospora robini* (AF080612) and to coccidia of a woodpecker (FN298443) of 8.7 %. The divergence analysis showed a sequence divergence of 7.1 % between the isolates “Tui1” and “Tui4”. This suggests that two distinct species of *Eimeria* are present in Tui.

Unfortunately, the CocciA/B primers amplify a different region of the 18S gene from the Cocci1/2 primer set. Therefore, a direct comparison between “Tui1” and “Tui4” below mentioned Tui coccidia, “Tui Waikato”, cannot be made.

CocciA and CocciB primer sets

The limited amount of sample material did not allow PCR using these primers on many samples, as most material was already used with the COC1/2 primers. However, coccidia DNA was successfully amplified with PCR from 1/1 saddleback and 1/1 (100 %) fecal samples using the primers CocciA and CocciB. The coccidia isolates “NI saddleback 44” and “Tui Waikato” sequences were subjected to a BLAST search on the NCBI database. The results revealed that the coccidia isolated from saddleback and tui were most closely related to coccidia of the *Eimeria* genus in North

Fig. 4 Sporulated oocysts obtained from **a** Tui, **b** fantail. $\times 400$ SC sporocyst, SZ sporozoite, ST Stieda body

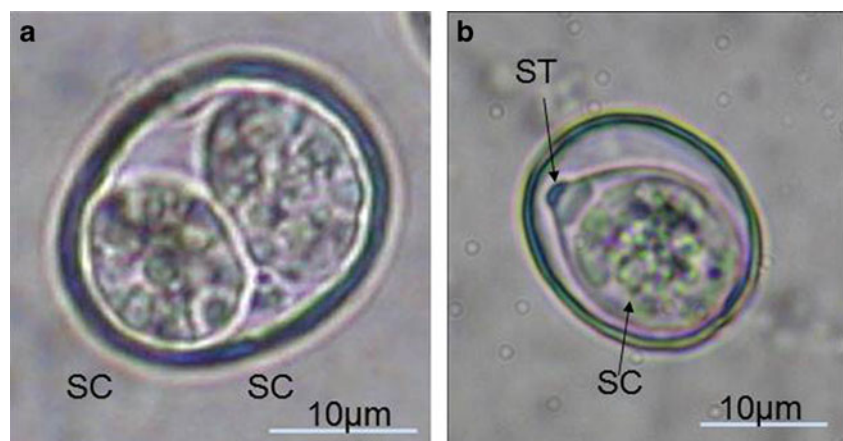


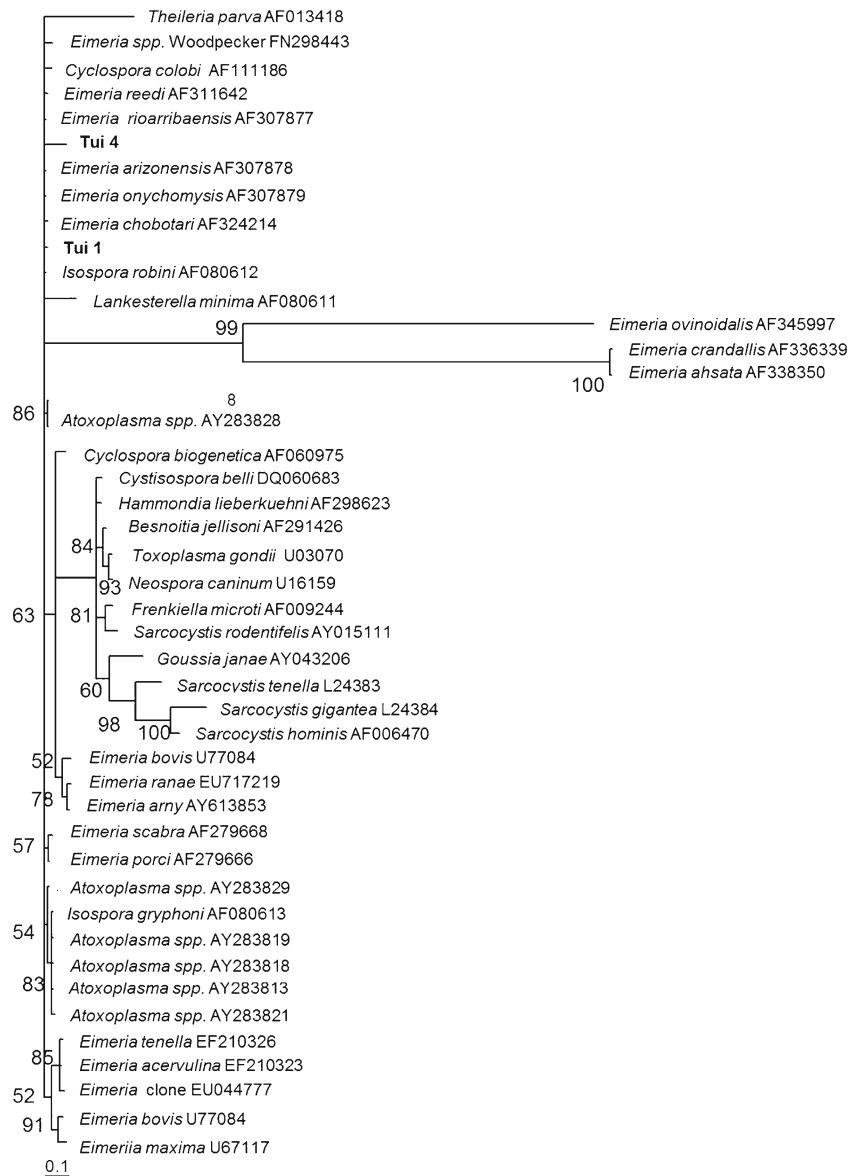
Table 3 Descriptive statistics of the spherical (S) and ellipsoidal (E) and sub-spherical (SS) sporulated oocysts, with measurements in micrometers

	North Island Saddleback (SS)			North Island Saddleback (E)			Silvereye (S)	Tui (with spherical sporocysts)			Tui (with ellipsoidal sporocysts)		
	Length	Width	L/W ratio	Length	Width	L/W ratio	Diameter	Length	Width	L/W ratio	Length	Width	L/W ratio
N	12	12	12	12	12	12	9	11	11	11	11	11	11
Mean	24.79	22.5	1.12	19.17	15.83	1.21	28.19	25.69	22.5	1.14	27.05	23.07	1.18
SD	1.29	3.02	0.20	1.23	1.23	0.09	1.55	1.52	1.85	0.06	1.16	2.12	0.11
SE Mean	0.37	0.87	0.06	0.36	0.36	0.03	0.52	0.46	0.56	0.02	0.35	0.64	0.03
Min	22.5	15	1	17.5	15	1.14	25	22.5	17.5	1.1	25	20	1.05
Max	27.5	25	1.67	20	17.5	1.33	30	27.5	25	1.29	28.75	26.25	1.44

American rodents with 97 % similarity (e.g., GeneBank AF 339489, AF 313642) and North American passerines with 96 % similarity (e.g., GeneBank AF 080612, AY331573).

A multiple alignment was performed a Bayesian analysis on 260 bp of the 18S small subunit ribosomal gene of the two New Zealand coccidia isolates and other *Eimeria* species

Fig. 5 Phylogenetic analysis of *Isoospora* spp. isolated from tui. Neighbor-joining phylogeny of the 18S small subunit ribosomal gene. The tree is rooted on a lineage of *T. parva*. Numbers above the branches indicate bootstrap support based on 1,000 replicates. Names of the lineages (when available) and Genbank accession numbers of the sequences are given after the species names of the parasites



available from the GenBank database. A phylogenetic tree was constructed which clustered the sequence of the “NI saddleback 44” isolate with the “Tui Waikato” isolate (Fig. 6). Both the “NI saddleback 44” and “Tui Waikato” isolates clustered on a separate branch from the *Eimeria* species from rodents (e.g., GenBank AF324214, AF339491), chickens (GenBank EU025116), and North American robin (GenBank AF080612) (Fig. 6). Sequence divergence analysis revealed a difference of 1.9 % between the “Tui Waikato” and “NI saddleback 44” isolates. This suggests that, although there is some limited sequence variation, the coccidia from tui and saddleback, both sampled on Mokoia Island, are quite similar. The divergence analysis also showed that the Tui and saddleback isolates had a 6.6 % and 6.7 % divergence, respectively, from *Eimeria* in North American rodents (*Eimeria reedi*, GenBank AF311642) as well as a 6.6 % and 6.7 % divergence, respectively, to *I. robini* (GenBank AF080612) isolated from a North American Robin. In addition, there was

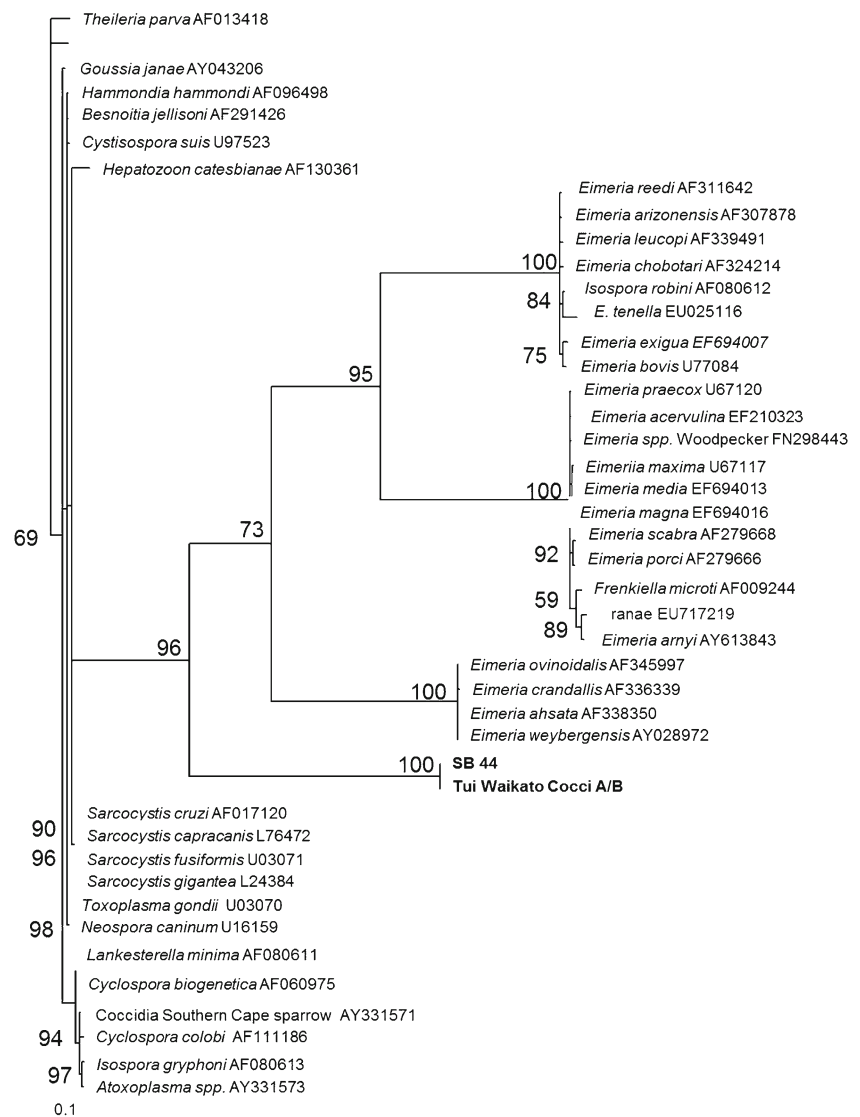
a large sequence divergence of 64 % with an *Atoxoplasma* species isolated from Northern house sparrows (GenBank AY331573).

BSEF and BSER primer set

Coccidia DNA was amplified by PCR, using the BSEF and BSER primers, from two of the three samples from North Island robin, but only one sample could be sequenced with conclusive results. A Blast search on the NCBI database revealed a 95 % sequence homology to *Atoxoplasma* spp. from sparrows (GenBank AY331573, with 96 % sequence coverage) as well as 98 % sequence homology (ranging from 77–82 % sequence coverage) with to a group of *Eimeria* spp. in poultry (GenBank FJ449586, FJ449688).

A multiple alignment was performed using a Bayesian analysis on the 104 bp of the ITS1 16S small subunit ribosomal gene of coccidia isolated from the fecal sample

Fig. 6 Phylogenetic analysis of *Isospora* spp. isolated from North Island saddleback and tui. Neighbor-joining phylogeny of the 18S small subunit ribosomal gene. The tree is rooted on a lineage of *T. parva*. Numbers above the branches indicate bootstrap support based on 1,000 replicates. Names of the lineages (when available) and Genbank accession numbers of the sequences are given after the species names of the parasites



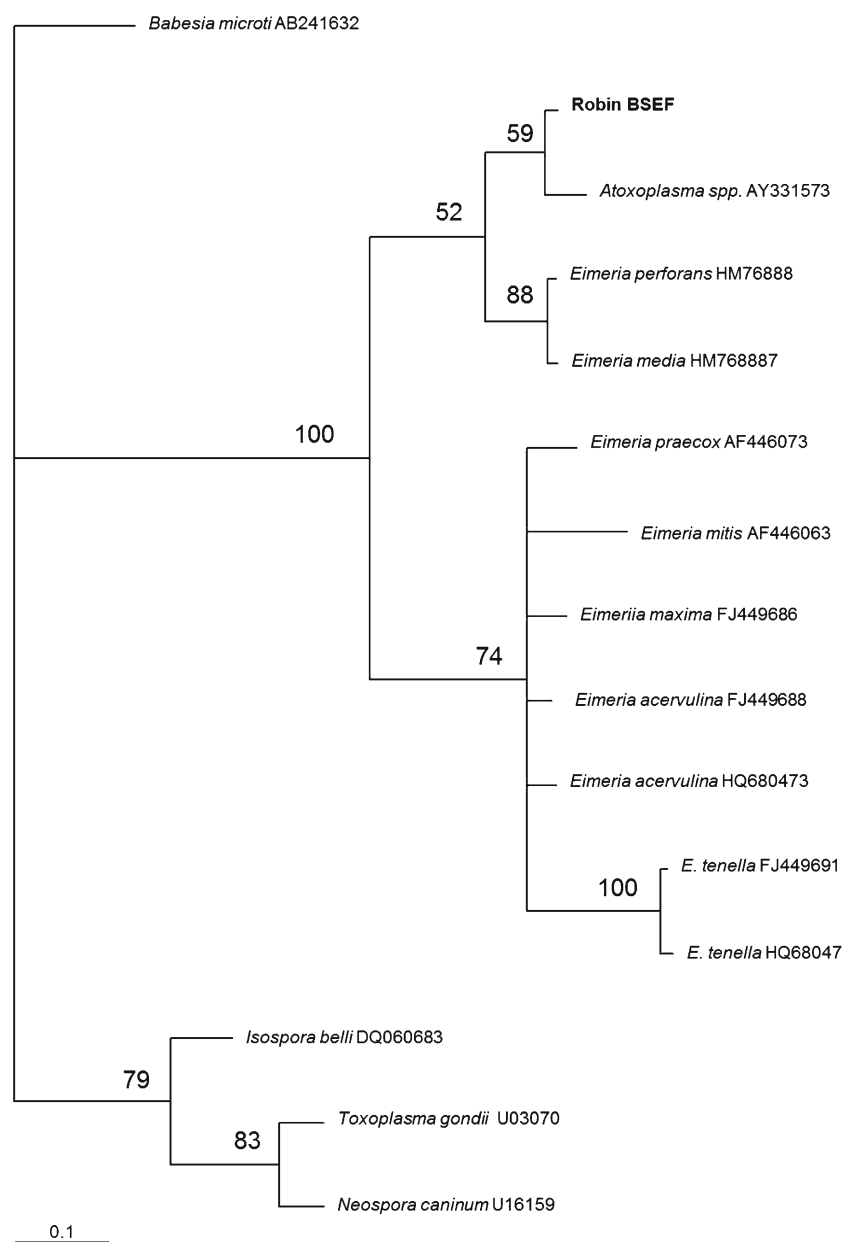
of North Island robin from Mokoia and other *Eimeria* species available from GenBank. A phylogenetic tree constructed resulted in the sequence of *Eimeria* DNA from the North Island robin being placed on a unique location among the *Eimeria* genus (Fig. 6). As a result, the isolated coccidia clusters with “*Atoxoplasma*” from northern house sparrows (AY331573) and has only a 5 % sequence divergence from *Eimeria* spp. of the rabbit (*Eimeria media* HM768887 and *Eimeria perforans* HM768888) but is different from *Eimeria* species isolated from chickens worldwide (Fig. 7).

The findings using multiple alignment are not consistent with the results from the BLAST search because of the lower

sequence coverage in the NCBI database for *Eimeria* species when compared with *Atoxoplasma* species. For example, while *Atoxoplasma* has coverage of 98 % matching 95 % of our robin sequence, the *Eimeria* spp. sequence only covers 77–82 % of the robin sequence.

Sequence divergence results support the findings in the tree, with “*Atoxoplasma*” from northern house sparrows (AY331573) having a divergence of 3 % from the North Island robin sample and between 5 and 15 % divergence to the *Eimeria* species from poultry (for example, *Eimeria tenella* HQ680474 to North Island robin 15.4 %, *Eimeria acervulina* HQ680473 11.2 %, and *E. media* HM768887 5.2 %).

Fig. 7 Phylogenetic analysis of *Eimeria* spp. isolated from North Island robin. Neighbor-joining phylogeny of the 18S small subunit ribosomal gene. The tree is rooted on a lineage of *Eimeria bovis*. Numbers above the branches indicate bootstrap support based on 1,000 replicates. Names of the lineages (when available) and Genbank accession numbers of the sequences are given after the species names of the parasites



Fantail and silvereye

To date, PCR has been unsuccessful in amplifying coccidia from fecal samples from this group of birds using any of the primers listed above. Further primer sets are currently under evaluation.

Discussion

This study identified five species of coccidia, three in tui and one each in saddleback and NI robin. The overall prevalence of coccidian infection in the New Zealand bird species examined was 21–38 %—21 % in North Island robin, 38 % in tui, and 25 % in saddleback. This result is different to findings elsewhere in the world, where a higher prevalence of coccidia in passerines was found, for example, 89 % in greenfinches (*C. chloris*) in Estonia (Horak et al. 2004) and 90 % in blackbirds in Germany (Misof 2004). Previous studies in blackbirds, song thrushes, and starlings in New Zealand found a prevalence of 40 % in fledglings (Cassey 2008) which is also lower than that of European birds. Large numbers of coccidian oocysts were also found in saddleback that were translocated by Thorne (2007), although the prevalence in these birds was not recorded back then.

In the study presented here, one juvenile tui captured twice in the period of 1 week was found to have a very high fecal count of oocysts. Although not weighed the second time so as not to cause additional handling stress, its body condition score was only 1 as opposed to 3 at the first time it was caught. This suggests that young birds might be affected by the parasites, whereas in adult birds infection is usually asymptomatic. Dorresteijn (1998) reported that acute disease due to coccidia is the most common parasitic disease in juvenile canaries in breeding aviaries in Germany, with a mortality rate of up to 80 %.

The methods used in this research, namely fecal flotation and the Qiagen stool kit (as recommended by Schrenzel et al. 2005), were sensitive enough to obtain parasite DNA even from low parasite counts. This finding is contrary to the opinion of Dolnik et al. (2009) who described a low efficiency for the Qiagen stool kit and regarded it as unsuitable for single cell DNA extraction. In the present study, it was possible to extract DNA even from very few oocysts.

We found morphological differences in the oocysts of the fecal samples from individual birds, particularly in the tui and saddleback, supporting the genetic findings indicating the presence of several different species of coccidia in these birds. In the future, more samples of individual birds containing oocysts should be collected, and single sporulated oocysts with documented morphology should be used for PCR analysis and sequencing. If this is done, it would be

possible to link the morphology of the oocysts with their phylogeny. A similar study has been done before by Dolnik et al. (2009) who isolated single oocysts from the feces of wild blackcaps (*Sylvia atricapilla*), photographed them, isolated DNA and obtained the sequence. These authors also took blood samples and analyzed the extra-intestinal coccidial stages found in the blood to relate their findings to the different oocysts shed in the feces. By doing this, it was possible to determine which species of coccidia had extra intestinal stages in their life cycle.

Formerly, all coccidian parasites with two sporocysts (“diplosporocystic”) in their oocyst have been placed together in the genus *Isoospora* due to their morphological similarity. Our findings confirm those of Carreno and Barta (1999) who have postulated that diplosporocystic coccidian parasites should be placed in different genera, or even families, based on their relationships established using sequence homologies in the genes of the small subunit rRNA. These authors suggested that species with diplosporocystic, tetrasporozoic oocysts possessing Stieda bodies in their sporocysts should be placed in the genus *Isoospora*, family Eimeriidae. These species have been described in the feces of many birds. Diplosporocystic, tetrasporozoic oocysts without Stieda bodies in their sporocysts, as found in mammals, should be placed in the genus *Cystisosporea*, family Sarcocystidae. More recently, Barta and co-workers (2005) examined oocysts from the genus “*Atoxoplasma*” in passerines and suggested that the term *Atoxoplasma* should be a synonym for the genus “*Isoospora*” in birds, due to their genetic similarity. Sporulated coccidia from the New Zealand passerines examined in this study all showed a Stieda body on their sporocysts. So they too are distinguishable from mammal “*Isoospora*” that lack a Stieda body. The morphology and general characteristics of the coccidia found in this study are similar to those of coccidia infecting other passerines in the world. For example, *Isoospora spp.* found in New World passerines (Berto et al. 2011), Turdidae species from Costa Rica (Keeler et al. 2012) and *Caruelis-* species worldwide (Ball et al. 2012), show oocysts and sporocysts sizes very similar found to those in New Zealand (for a review, see Berto et al. 2011).

Additional primer sets will have to be developed to amplify further regions of the 18S gene which would provide more specific phylogenetic identification. In addition, the similarities of the coccidia found in this study to those of rodents and bats in other parts of the world also have to be viewed with caution. There has been little work done on coccidia and their genetic relationships in general and much less so in birds, so the database available for comparing sequences to date is far from complete.

Transmission experiments would provide much needed information on host specificity, pathogenicity, and life cycle of the coccidian species found in this study. This type of

study, however, using vulnerable species like North Island saddleback and robin, would be difficult to obtain permits for.

This study is a first step in understanding coccidian parasitism in New Zealand passerines, and it is evident that there is a range of species yet to be identified. As we know from chickens, a variety of different species of coccidia can infect a single host species, and even a single individual (Augustine 1996; 1999), a phenomenon that seems to be repeated in passerine birds around the world, as shown in the studies of Box (1977), Cringoli and Quesada (1991), Ball et al. (1998), Barta et al. (2005), and Dolnik et al. (2009). It would have been surprising to find a different situation in passerine birds native to New Zealand.

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