MOLECULAR IDENTIFICATION OF MALARIAL PARASITES IN AUSTRALIAN BIRDS

Shirley H. Fan[1], Shane Raidal[2], David Phalen[3] and Jan Slapeta[1]

[1] Parasitology, McMaster Bld. (B14), Faculty of Veterinary Science, University of Sydney, Camperdown, NSW 2006

[2] School of Agricultural & Veterinary Sciences, Charles Sturt University, Locked Bag 588, Wagga Wagga, NSW 2678

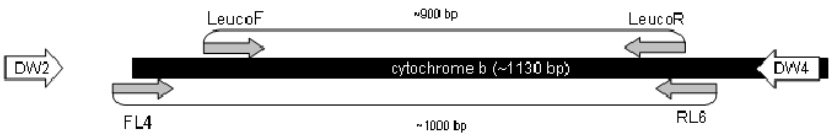
[3] Wildlife Health and Conservation Centre and Avian, Reptile and Exotic Animal Hospital, University of Sydney, 415 Werombi Road, PMB 3, Camden, NSW 2570

The aim of this study was to investigate the prospects of using molecular tools to identify malarial parasites in Australia birds. We applied molecular tools to fresh blood and tissue samples, frozen stored samples and samples from frozen bird collections. The study is the first step in efforts to map the diversity of malarial parasites affecting Australian birds. These data will lead to elucidation of the origin/introduction, hosts specificity, pathology and dispersal of these blood parasites in Australia and worldwide.

Materials and Methods

Blood smears were stained with Diff-Quick and were examined using 100x oil objective. DNA was extracted using the Qiagen DNeasy kit (Qiagen, USA) and the supplier's protocol. Amplification of the target gene segment was achieved with a nested PCR design (Figure 1). Outer reactions were carried out with the primers DW2 and DW4 (Perkins and Schall 2002). We use 2x Master Mix (Fermentas) which includes a complete dNTP, polymerase, and buffer mix for optimal PCR conditions. For all of the samples, an inner reaction was performed using 1 µl of the outer product according to previously published conditions (Martinsen et al. 2006, Sato et al. 2007). PCR products were submitted for sequencing (Supamac, University of Sydney).

Figure 1: Map of the mitochondrial gene cytochrome b of the malarial parasites (*Leucocytozoon*/*Haemoproteus*/*Plasmodium*) ndicating the position of individual primers used for amplification. The first primer set (DW2/DW4) is used in initial PCR reaction and its amplication is subsequently used as the template for the nested PCR reaction using either FL4/RL6 or LeucoR/F primer pair yielding ~1100bp or ~900bp PCR product. Individual primer sequences are listed below the map.



DW2	TAA TGC CTA GAC GTA TTC CTG ATT ATC CAG
DW4	TGT TTG CTT GGG AGC TGT AAT CAT AAT GTG
DW1	TCA ACA ATG ACT TTA TTT GG
DW6	GGG AGC TGT AAT CAT AAT GTG
LeucoR	AGC ATA GAA TGT GCA AAT AAA CC
LeucoF	TCT TAC TGG TGT ATT ATT AGC AAC

Results and Discussion

A total of 43 avian blood or tissue samples were analysed in this study (Table 1). Thirteen of these had been collected over the duration of this study (September – November 2007). The remaining 30 had been

obtained from The Australian Museum, Sydney repository (19 birds) and stored tissue samples (11 Nankeen kestrel brains) previously published by Raidal and Jaensch (2000).

Eight of 43 (19%) birds DNA samples were positive for haemoprotozoan parasite infection. *Leucocytozoon* was seen in a blood smear of an Olive-backed oriole, but for unknown reasons, DNA from this parasite was not amplified. Haematoprotozoa DNA was detected in 4 of the 11 Nankeen kestrel brains, 4 (36%). While positive results from the museum material were significant, prevalence data could not be determined because many of the samples were in advanced states of decomposition and even cellular DNA could not be amplified from some of these samples.

This study has provided the foundation for further molecular characterisation of the malarial parasites. The group welcomes blood samples for morphological and molecular characterisation. If you encounter a malarial parasite please contact us for further details how to submit samples. As a quick guide we recommend:

- storing a blood sample (20-100 µl) at -20 °C
- spotting 1-2 blood drops on a filter paper
- prepare 3 blood smears and stain one with Diff-Quik

Contact address:

Jan Slapeta, Ph.D., MVDr.
Parasitology, McMaster Building, B14
Faculty of Veterinary Science, University of Sydney
Sydney, New South Wales 2006
Tel: ++61-2-935-12025
Fax: ++61-2-935-17348
e-mail: jslapeta@usyd.edu.au

Table 1. Samples collected and analysed over the duration of the study

ID	*Bird species*	Common name	Locality	M	PCR
SHF0001	*Gymnorhina tibicen*	Australian Magpie	Killara, NSW	H	H
SHF0002	*Gymnorhina tibicen*	Australian Magpie	Killara, NSW	-	-
SHF0003	*Haliaeetus leucogaster*	Sea Eagle	Sydney, NSW	-	-
SHF0004	*Chenonetta jubata*	Wood Duck	NSW	-	-
SHF0005	*Oriolus sagittatus*	Olive-backed oriole	Cobbity, NSW	L	-
SHF0006	*Strepera graculina*	Currawong	Turramurra, NSW	L	L
SHF0007	*Aquila audax*	Wedge-tailed eagle	NSW	-	-
SHF0008	*Gymnorhina tibicen*	Australian Magpie	Camden, NSW	L	L
SHF0009	*Gymnorhina tibicen*	Australian Magpie	Belrose, NSW	HP	HP
SHF0010	*Gymnorhina tibicen*	Australian Magpie	Australian Museum*	n.a.	-
SHF0011	*Strepera graculina*	Currawong	Australian Museum*	n.a.	-
SHF0012	*Strepera graculina*	Currawong	Australian Museum*	n.a.	-
SHF0013	*Strepera graculina*	Currawong	Australian Museum*	n.a.	-
SHF0014	*Corvus coronoides*	Black Crow	Australian Museum*	n.a.	-
SHF0015	*Podargus strigoides*	Tawny Frog mouth	Australian Museum*	n.a.	-
SHF0016	*Dacelo novaeguinaea*	Kookaburra	Australian Museum*	n.a.	-
SHF0017	*Centropus phasianinus*	Pheasant Coucal	Australian Museum*	n.a.	-
SHF0018	*Ptilonorhycnhus violaceus*	Saturn Bower bird	Australian Museum*	n.a.	-
SHF0019	*Ocyphaps lophotes*	Crested Pigeon	Australian Museum*	n.a.	-
SHF0020	*Cacatua roseicapilla*	Galah	Australian Museum*	n.a.	-
SHF0021	*Manorina melanocephala* (juv.)	Noisy Minor	Camperdown, NSW	-	-
SHF0022	*Manorina melanocephala* (juv.)	Noisy Minor	Camperdown, NSW	-	-
SHF0023	*Gymnorhina tibicen*	Australian Magpie	Australian Museum*	n.a.	-
SHF0024	*Gymnorhina tibicen* (juv.)	Australian Magpie	St. Ives, NSW	-	-
SHF0025	*Falco cenchroides*	Nankeen Kestral	Murchison Region, WA	n.a.	-
SHF0026	*Falco cenchroides*	Nankeen Kestral	Murchison Region, WA	n.a.	-
SHF0027	*Falco cenchroides*	Nankeen Kestral	Murchison Region, WA	n.a.	-
SHF0028	*Falco cenchroides*	Nankeen Kestral	Murchison Region, WA	n.a.	L
SHF0029	*Falco cenchroides*	Nankeen Kestral	Murchison Region, WA	n.a.	-
SHF0030	*Falco cenchroides*	Nankeen Kestral	Murchison Region, WA	n.a.	-
SHF0031	*Falco cenchroides*	Nankeen Kestral	Murchison Region, WA	n.a.	L
SHF0032	*Falco cenchroides*	Nankeen Kestral	Murchison Region, WA	n.a.	-
SHF0033	*Falco cenchroides*	Nankeen Kestral	Murchison Region, WA	n.a.	L
SHF0034	*Falco cenchroides*	Nankeen Kestral	Murchison Region, WA	n.a.	L
SHF0035	*Falco cenchroides*	Nankeen Kestral	Murchison Region, WA	n.a.	-
SHF0036	*Podargus strigoides*	Tawny Frog mouth	Australian Museum*	n.a.	-
SHF0037	*Podargus strigoides*	Tawny Frog mouth	Australian Museum*	n.a.	-
SHF0038	*Podargus strigoides*	Tawny Frog mouth	Australian Museum*	n.a.	-
SHF0039	*Gymnorhina tibicen*	Australian Magpie	Australian Museum*	n.a.	-
SHF0040	*Gymnorhina tibicen*	Australian Magpie	Australian Museum*	n.a.	H
SHF0041	*Dacelo novaeguinaea*	Kookaburra	Australian Museum*	n.a.	-
SHF0042	*Ninox strenua*	Powerful Owl	Australian Museum*	n.a.	-
SHF0043	*Gymnorhina tibicen*	Australian Magpie	St. Ives, NSW	n.a.	-

* Specimens obtained from The Australian Museum, Sydney collection, original location of bird unknown; n.a. - not available; LHP - indicates the type of parasite detected via morphology (M) or PCR/sequence (L – *Leucocytozoon* spp., H – *Haemoproteus* spp., P – *Plasmodium* spp.); age of bird is adult unless otherwise specified. Sample collection has been approved by the Animal Ethics Committee (University of Sydney).

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