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Molecular evidence for host specificity of parasitic nematode microfilariae in some African rainforest birds

RAVINDER N. M. SEHGAL,*‡ HUGH I. JONES† and THOMAS B. SMITH‡
*Department of Biology, San Francisco State University, 1600 Holloway, San Francisco, CA, 94132, USA, †Department of Microbiology, University of Western Australia, Nedlands, 6907, Western Australia, ‡Center for Tropical Research, Institute of the Environment, Organismic Biology, Ecology and Evolution, University of California, Los Angeles, CA, 90065, USA

Abstract

Here we describe, determine the prevalence, and examine the host-specificity of some parasitic nematode microfilariae in selected bird species from West and Central Africa. We used microscopy to determine the prevalence of microfilariae in 969 host individuals representing 121 rainforest bird species from Cameroon, Côte d'Ivoire and Equatorial Guinea. Thirteen (11%) of these potential host species harboured microfilariae, and 35 individuals (3.6%) were infected. From the 35 infected individuals, we identified eight distinct morphological microfilarial forms. Sixteen of the 35 infected individuals were of one host species, the Fire-crested Alethe (*Alethe diademata*), at a prevalence rate of 62%. To examine host and geographical specificity, we sequenced a portion of the *LSU rDNA* gene from representative microfilariae drawn from different hosts and collecting locations. Identical sequences of the nematode *LSU rDNA* gene were found in *A. diademata* collected from locations in Côte d'Ivoire and Equatorial Guinea, locations separated by the Dahomey Gap and associated with different hypothesized refugial areas. In contrast, several other bird species collected at the same sites harboured different microfilaria lineages. We sequenced the mitochondrial ATP synthase genes of the host species *A. diademata*, and found a 5.4% sequence divergence between the birds sampled in Côte d'Ivoire, and those from Cameroon. Thus, despite this split between the two populations, they harbour microfilariae with identical lineages. These data provide evidence that the microfilariae found in *A. diademata* may be highly host specific. This apparent specificity may have important implications for the evolutionary and ecological interactions between parasitic nematodes and their avian hosts.

Keywords: birds, disease prevalence, host specificity, host–parasite interactions, microfilariae, parasitic nematodes

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Introduction

Nematodes parasitize both plants and animals. In humans, many are pathogenic, and cause debilitating diseases such as river blindness and elephantiasis. A diverse set of nematode families and genera parasitize birds (Anderson 2000). The life cycles of parasitic nematodes range from simple to complex, and can involve two or three hosts (Olsen 1974). Blood-sucking insects of the Simuliidae, Ceratopogonidae, Tabanidae and Culicidae, are typical intermediate hosts of parasitic nematodes and generally release the nematode's larval stages into the vertebrate host's bloodstream (Greiner *et al*. 1975). These larval forms, termed microfilariae, undergo development to 3rd stage larvae in the arthropod vector before being transmitted to the avian host, where they eventually mature into adults that can inhabit the cardiovascular, pulmonary, or lymphatic systems (Anderson 2000).

In a comprehensive survey of avian haematozoa of North America, the prevalence of microfilariae was reported to be 3.1% (Greiner *et al*. 1975). More recent studies reported a prevalence rate of 5.7% in tyraniid flycatchers in Colombia (Matta *et al*. 2004) and 30% in the Willow

Correspondence: Ravinder Sehgal, Fax: 415-338-2295; E-mail: sehgal@sfsu.edu

Ptarmigan (*Lagopus lagopus*) in Norway (Holmstad *et al*. 2003). In Africa, studies have taken place in a number of sub-Saharan countries with prevalence rates of microfilariae ranging from 0.4% to 6.4% (Bennett *et al*. 1974, 1978; Bennett & Herman 1976; Wink & Bennett 1976; Williams *et al*. 1977; Peirce 1984; Kirkpatrick & Smith 1988). These values are low in comparison to the prevalence of other avian blood parasites. In particular the haemosporids, *Haemoproteus* and *Leucocytozoon* exhibit prevalence rates of over 17% in North American birds (Greiner *et al*. 1975).

Infections with nematodes may have fitness consequences (Morand & Poulin 2000), and at least one case is known of a fatal filarial infection, in red-billed blue magpies (*Urocissa erythrorhynchus*) (Simpson *et al*. 1996). In birds of paradise in Papua New Guinea, studies also showed a significant correlation between blood parasite intensity (including microfilariae) and showiness in males (Pruett-Jones *et al*. 1990). In addition, a study has found a positive correlation between nematode infection and the body weight of bird species suggesting that larger bird species are more likely to be infected (Gregory *et al*. 1991).

Identifying nematode microfilariae to species is problematic due to their high degree of morphological and morphometric similarities (McKeand 1998). For these reasons, many studies utilizing only blood smears have failed to identify the species of infection. The life cycles of filarial parasites in birds are generally not well studied (Smyth 1994; Anderson 2000). In passerine birds, the species of insect vectors that transmit the microfilariae are largely unknown. Moreover, in tropical ecosystems where species diversity is highest, and many insect vectors coexist with many species of birds and parasites, the task of describing life cycles is daunting. In these systems, molecular genetic approaches are required to elucidate the relationships between parasitic nematodes and their avian hosts.

Several studies have reported on the host specificity of parasitic nematodes. For example, two shrew species were shown to be infected with a single species of heligmosomoid nematode (Brant & Ortí 2003). These nematodes are monoxenous; they have a life cycle that does not include an intermediate host. However, parasitic nematodes of the genus *Haemonchus* exhibit low host specificity, and a single species can be found in goats, sheep and cattle (Achi *et al*. 2003). On the other hand, similar nematodes of deer, while having broad geographical distributions, were not found in cattle and other sympatric ruminants (Blouin *et al*. 1995). Recent experimental and phylogenetic studies on parasitic monoxenous nematodes of *Drosophila* have shown high levels of host switching (Perlman & Jaenike 2003; Perlman *et al*. 2003). One filarial species of Splendidofilariinae, *Cardiofilaria pavlovskyi*, has been reported in birds as diverse as Falconiformes, Charadriiformes and Passeriformes (Anderson 2000). Based on these studies, we would predict that in heteroxenous nematodes, host switching might be common, since insect vectors may feed on several bird species, and thus could theoretically spread parasites readily among different avian hosts (i.e. Apperson *et al*. 2004).

The objectives of this study were (i) to assess the prevalence and describe the morphological characteristics of avian microfilariae at 12 sampling localities in three West African countries: Cameroon, Côte d'Ivoire, and Equatorial Guinea, (ii) to conduct a preliminary survey of sequence diversity in the nematodes to assess the relationships between parasite and host lineages in a geographical context, and (iii) to examine in detail the phylogeography of one avian host species, *Alethe diademata*, that exhibited a relatively high prevalence of microfilariae.

Materials and methods

Blood and extraction

All blood samples used in this study were collected over an 11-year period (between 1990 and 2001) as part of an ongoing study of avian evolution in Central and West Africa (Smith *et al*. 1997, 2001, 2005). The avian taxonomy used conforms to Sibley & Monroe (1990). Captured birds were weighed, measured, banded with an aluminium numbered band for ongoing demographic and selection studies, bled, and released following methods described by Smith *et al*. (1997). Blood samples (50–100 μL) were collected from the brachial vein and stored in lysis buffer (10 mM Tris-HCl pH 8.0, 100 mM ethylene-diaminetetraacetic acid, 2% sodium dodecyl sulphate) for subsequent molecular analysis (Sehgal *et al*. 2001). Blood smears were made on site and air dried (Kirkpatrick & Smith 1988). To obtain total DNA, the blood was either extracted following a DNeasy kit protocol (QIAGEN), or with phenol–chloroform followed by ethanol precipitation (Kocher *et al*. 1989).

Habitats sampled

Samples were collected from three habitat types, forest, ecotone and montane, and over both wet and dry seasons, in Cameroon, Equatorial Guinea and Côte d'Ivoire. Table 1 lists the dates and locations of the collection sites of this study that are also depicted in Fig. 2. The vegetational characteristics of the forest sites include both secondary and mature forest and may be generally classified as lowland rainforest (Letouzey 1968; Louette 1981; Smith *et al*. 1997). The vegetational characteristics of the African ecotone are described by Letouzey (1968) and Louette (1981) as 'shrub–savannah with *Terminala glaucecens*' and 'forest–savannah mosaic', respectively. Additional descriptions of the Central African ecotone may be found elsewhere (Longman & Jenik 1992; Smith *et al*. 2005). Vegetational

Table 1 Dates and locations of collections

Site	Date	Location	Habitat type	Prevalence†
Ivory Coast				
1. CSRS-Abidjan	January 2000	5°19.859′N, 4°07.743′W	Rainforest	2/95
2. Lamto	January 2000	6°12.957′N, 5°01.626′W	Ecotone	0/92
3. Marahoue	January 2000	7°01.680′N, 5°56.852′W	Ecotone	0/64
4. Tai Forest	June 2001	5°49.981′N, 7°20.565′W	Rainforest	8/111
Equatorial Guinea				
5. Mount Alen	May 1998	1°39.4′N, 10°18.9′E	Montane	0/28
6. Elende	May 1998	2°12.98′N, 9°47.57′E	Rainforest	1/43
7. Mokula	May 1998	1°02.86′N, 11°09.78′E	Rainforest	7/35
8. Ncoho	May 1998	1°14.2′N, 9°57.11′E	Rainforest	8/50
Cameroon				
9. Nkwouak*	August 1990	3°52′N, 13°18′E	Rainforest	
10. Zoebefame*	September 1990	2°39′N, 13°23′E	Rainforest	
11. Ndibi	March–May 1989	3°46.583′N, 12°12.616′E	Rainforest	1/275
12. Bouamir*	July 1999	3°03.191′N, 12°12.812′E	Rainforest	
13. Sakbayeme	May 2000	4°02.290′N, 10°34.453′E	Rainforest	6/101
14. Kousse*	July 1997	4°27′N, 11°33′E	Ecotone	
15. Ndikinimeki*	July 1997	4°46′N, 10°50′E	Ecotone	
16. Betare Oya*	May 1995	5°33.795′N, 14°05.475′E	Ecotone	
17. Tibati	June 1995	6°30.260′N, 12°35.280′E	Ecotone	2/75
				Total 35/969

*No blood smears were collected from these sites. †Number of microfilariae positive/number of slides examined.

characteristics of the Mount Alen site that reaches approximately 1000 m in elevation consists of a mixure of lowland forest and of montane vegetation (Letouzey 1968).

Blood smear analysis

Following fixation in methanol, smears were stained with 3% Giemsa for 20 min, and examined using an Olympus BH compound microscope at 100×, 200×, 400×, and 1000× for 20–50 min. Presence and intensity of parasites was recorded. Parasitemias were estimated using methods similar to those of Godfrey *et al*. (1987). Magnifications of 200× and 400× were used to detect microfilariae. Because parasitemias were most often very low, many more than 20 fields were typically examined, and intensity was still usually less than 1/4000 erythrocytes. Only parasitemias greater than 0.025% were quantified.

Representative smears have been deposited in the International Reference Centre for Avian Haematozoa, Queensland Museum, PO Box 3300, South Brisbane, Queensland 4101 Australia.

Amplification of nematode DNA

We used polymerase chain reaction (PCR) to amplify a 782 bp product from the nuclear large subunit rDNA gene (LSU) of nematodes. The PCR primers were designed and described by Nadler & Hudspeth (1998). The primer named Nematode 1 (GCGGAGGAAAAGAAACTAA) corresponds to numbering by *Caenorhabditis elegans* rDNA, 3745–3764, and the primer termed Nematode 2 (ATCCGTGTTTCAAGACGGG) corresponds to bases 4359–4377. Twenty-five-microlitre reaction mixtures contained 10–100 ng of genomic DNA, 0.5 unit of Ampli*Taq* DNA Polymerase (Applied Biosystems), 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 3.0 mM $MgCl_2$, 0.4 μM of each primer and 0.4 mM of each dNTP (QIAGEN). The cycling profile consisted of denaturation at 94 °C for 3 min, followed by 40 cycles of 94 °C denaturation for 30 s, 55 °C annealing for 30 s, and 72 °C extension for 45 s. The samples then went through a final extension at 72 °C for 7 min.

Amplification of avian DNA

We used PCR to amplify an 842 bp region of the mitochondrial genome of the members of the *Alethe* genus. This includes the entire coding sequence of the ATP-synthase 8 (ATPase8; 168 bp) and ATP-synthase 6 (ATPase6; 684 bp) genes. These reactions employed the primer pair CO2GQL (GGACAATGCTCAGAAATCTGCGG) and CO3HMH (CATGGGCTGGGGTCRACTATGTG) (Hunt *et al*. 2001) in a 25 μL reaction mixture which contained 10–100 ng of genomic DNA, 0.5 unit of Ampli*Taq* DNA polymerase (Applied Biosystems), 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 3.0 mM $MgCl_2$, 0.4 μM of each primer and 0.4 mM of each dNTP (QIAGEN). The cycling profile

consisted of denaturation at 94 °C for 3 min, followed by 35 cycles of 94 °C denaturation for 30 s, 54 °C annealing for 30 s, and 72 °C extension for 45 s. The samples were then extended at 72 °C for 10 min.

Sexing of birds

The sexing of *Alethe diademata* was performed using a protocol based on the method described by Fridolfsson & Ellegren (1999). The primer termed MSZ1R (ATCCATCAAGTCTCTAAAGAG) was designed to bind to the *CHD1* gene on the Z chromosome of the streak-necked flycatcher (*Mionectes striaticollis*). This primer was used in conjunction with primer 2550F (GTTACTGATTCGTCTACGAGA) (Fridolfsson & Ellegren 1999). Twenty-five-microlitre reaction mixtures contained 10–100 ng of genomic DNA, 0.5 unit of Ampli*Taq* DNA polymerase (Applied Biosystems), 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.4 mM $MgCl_2$, 0.5 μM of each primer, 1.36 mM of each dNTP (QIAGEN) and 0.8 mg/mL bovine serum albumin (BSA). The cycling profile consisted of denaturation at 94 °C for 3 min, followed by 35 cycles of 94 °C denaturation for 30 s, 54 °C annealing for 30 s, and 72 °C extension for 45 s. The samples were then extended at 72 °C for 10 min. PCR products were visualized on 2% agarose gels stained with ethidium bromide. Two PCR products of sizes of approximately 450 bp and 600 bp are obtained from females, and a single product of 450 bp from males.

Sequencing

Amplification products were confirmed visually on agarose gels stained with ethidium bromide, and were cleaned using the QIAquick PCR Purification Kit (QIAGEN). Sequencing reactions were conducted using the amplification primers via dye terminator cycle sequencing (ABI PRISM, Perkin-Elmer) following the manufacturer's protocols. For the sequencing of avian mitochondrial DNA, an additional primer, A8PWL (CCTGAACCTGACCATGAAC) (Hunt *et al*. 2001) was also used. Sequences were then obtained using an Applied Biosystems 377 automated DNA sequencer. Sequences were aligned and proofread using SEQUENCHER 3.1 (Gene Codes Corporation 1998). No insertions or deletions were present and therefore sequence alignments were unambiguous. Sequencing products had a high signal-to-noise ratio and no double peaks at single nucleotide locations. Sequences are deposited in GenBank with the following accession numbers for microfilariae AY770047–AY770064, for *A. diademata* AY772490–AY772511 and AY772516–AY772531, and for *A. poliocephala* AY772512–AY772515. All unique sequences were compared to sequences in GenBank using a BLAST search. The sequences of three nematodes were used for comparison: *Heterocheilus tunicatus* U94759, *Cruzia americana* U94757, and *Contracaecum septentrionale* AF226588.

Phylogenetic analysis

We based our phylogenetic analysis of nematode microfilariae on sequences (519 nucleotides) of the *LSU rDNA* from 18 individuals. For the genus *Alethe* we used the complete coding sequence (842 nucleotides) of the overlapping mitochondrial ATPase 8 and ATPase 6 genes from 41 individuals. Phylogenetic analyses using maximum-parsimony techniques were conducted using PAUP*4.0b10 (Swofford 2002). Searches used the bootstrap search option with 500 stepwise addition replicates using the TBR branch-swapping algorithm. Simple consensus trees were constructed to summarize the results.

Results

Epizootiology of microfilariae in some African rainforest birds

Thirty-five of the 969 individuals studied harboured microfilariae, a prevalence rate of 3.6%. Table 2 lists the prevalence rates according to avian families. Of 121 avian species examined, microfilariae were detected in only 13 species. The prevalence rates of each of these species are tabulated in Appendix I. We found relatively high prevalence rates in the Fire-crested Alethe, *Alethe diademata* (16/26 = 61.5%). No microfilariae were found in the congeneric Brown-crested Alethe, *Alethe poliocephala* (0/8).

Samples were analysed from material collected from seven contiguous rainforest sites, and three ecotone sites (Table 1). At rainforest sites, the proportion of detected microfilaria infections (33/710 = 4.6%) was significantly greater ($\chi^2 = 6.962$, d.f. = 1, $P \leq 0.01$) than at ecotone sites (2/231 = 0.87%), suggesting the prevalence of microfilarial infections is higher in contiguous rainforest habitats than ecotone forest fragments. No infections were found in the 28 birds sampled from the one montane site (Mount Alen, Equatorial Guinea). Two sites in Equatorial Guinea had the highest prevalence rates. These were Mokula (7/35 = 20% infected) and Ncoho (8/50 = 16% infected). We did not detect any correlation between infection and sampling date or year.

Eight distinct forms of microfilariae were identified based on morphological characteristics in 34 of the infected birds. Their descriptions are presented in the Appendix.

Association of microfilarial infection with body mass

With field data that compiles the weights of all caught birds, we sought to determine how nematode infection might affect individuals of the host species *A. diademata*. All individuals collected were judged by appearance to be adults in the field; juvenile birds exhibit immature plumage patterns (Borrow & Demey 2001). However, because

Table 2 Prevalence of avian microfilariae classified by family

Family	No. of species examined	No. of individuals examined	No. of individuals infected	%
Accipitridae	1	1	0	0
Alcedinidae	7	37	0	0
Centropodidae	1	1	0	0
Cisticolidae	8	37	0	0
Columbidae	4	36	0	0
Corvidae	9	40	0	0
Cuculidae	3	3	0	0
Eurylaimidae	1	1	0	0
Hirundinidae	1	2	0	0
Indicatoridae	3	3	0	0
Lybiidae	5	19	0	0
Meropidae	1	5	0	0
Muscicapidae	13	74	18	24.3
Nectarininiidae	6	154	1	0.7
Passeridae	20	147	3	2.0
Phasianidae	1	1	0	0
Picathartidae	1	1	0	0
Picidae	3	11	0	0
Pycnonotidae	17	333	12	3.6
Rallidae	2	2	0	0
Sylviidae	14	61	1	1.6
Total	121	969	35	3.6

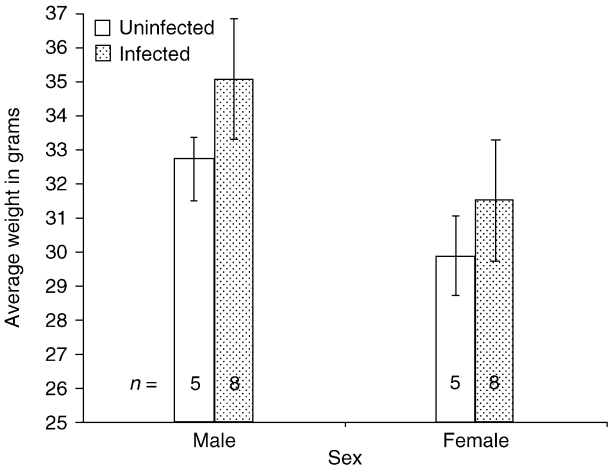

Fig. 1 Schematic representation of the correlation of microfilarial presence and bird weight. Of the 26 individuals of the host species *Alethe diademata* studied by microscopy, 13 were male and 13 were female, as determined by PCR. The prevalence of infection was identical between males and females (8/13 = 62% in each case). Regression analysis revealed that infected birds had a higher body mass than uninfected birds, and males were heavier than females.

A. diademata is sexually monomorphic for plumage, we used PCR to determine the sex of 26 individuals, and compared the mass of infected with uninfected individuals (Fig. 1). The average weight for the five uninfected males was 32.2 g, and for the eight infected males, 35.1 g. The average weight of the five uninfected females was 29.9 g, and for the eight infected females, 31.5 g. An equal number of males and females were infected with microfilariae. Regression analysis revealed that the difference between infected and uninfected individuals was significant, with infected individuals weighing more than uninfected (regression coefficient = 2.54, $t = 2.81$, $P = 0.010$). In addition, males weighed more than females (regression coefficient = 3.19, $t = 3.62$, $P = 0.001$).

Host–parasite interactions

We used primers described by Nadler & Hudspeth (1998) to amplify a fragment of the large subunit (LSU) rDNA from avian blood samples that were infected with microfilariae, as shown by microscopy. In addition, we used PCR to screen 12 blood samples of *A. diademata* where no blood smears were available, and found an additional two infected individuals that were collected from Nkwouak, Cameroon (site 9 in Table 1 and Fig. 2). We were able to obtain DNA sequences of 519 bp from 18 of the infected individuals representing seven host species. Figure 2 depicts a phylogenetic tree of the *LSU rDNA* alleles. Eight of the individuals had identical sequences, and were found entirely in the avian host *A. diademata*. These sequences correspond to the form A described in the Appendix. Interestingly, these samples were collected from sites in Cameroon, Equatorial Guinea, and Côte d'Ivoire, with the collection site in Côte d'Ivoire separated by the Dahomey Gap, and a distance of over 1500 km, from

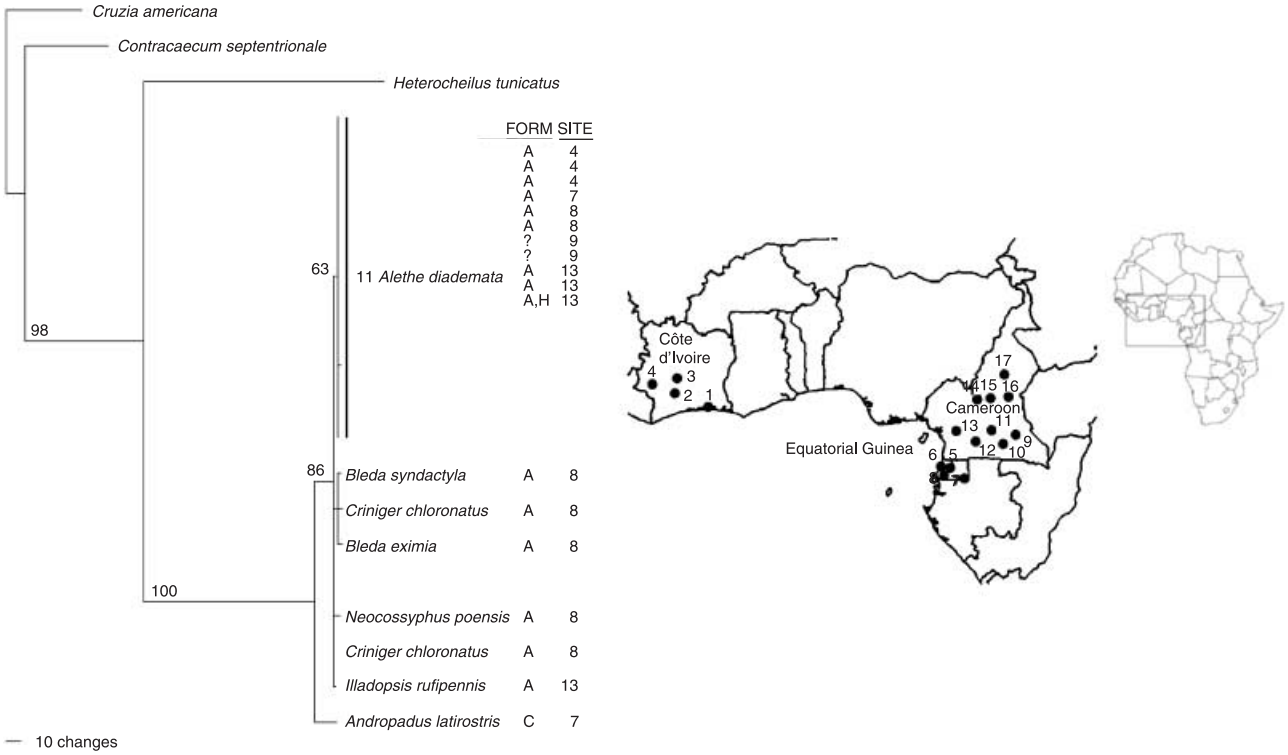

Fig. 2 Map of the locations of the collection sites and consensus maximum parsimony bootstrap tree based on sequences (519 bp) of microfilarial *LSU rDNA* gene sequences from 18 individuals. Numbers correspond to sites described in Table 1. The tree was rooted by the nematodes *Heterocheilus tunicatus*, *Contracaecum septentrionale*, and *Cruzia americana*. Bootstrap percentages of clades (500 iterations) are shown above internal nodes. Of the 18 sequences, 15 represent nematode microfilariae with the morphological description of form A (see Appendix). The lineage found in the individual *Andropadus latirostris* from Mokula, site 7, is from a form C nematode. Two sequences (taken from two individuals of *Alethe diademata* collected at site 9, Nkwouak) had no corresponding blood smear, so the morphological descriptions are unknown. In the host species *A. diademata*, identical microfilaria lineages were found from locations in Côte d'Ivoire, Cameroon, and Equatorial Guinea. In contrast, microfilariae found in a host in the same family, *Neocossyphus poensis*, differ from those found in *A. diademata* by four fixed base changes, even though the two birds were caught in the same nets at Ncoho, Equatorial Guinea on the same day.

the sites in Equatorial Guinea. In addition, none of the other hosts sampled harboured microfilariae that shared this sequence, even if they were collected at the same location on the same day as some of the individuals of *A. diademata*. For example, the microfilaria *LSU rDNA* sequence found in the host *Neocossyphus poensis* (which is also in the family Muscicapidae and is also described as a form A microfilaria) differs from the sequences found in *A. diademata* by four fixed base changes, even though the two individuals were caught in the same nets at Ncoho, Equatorial Guinea. Similarly, the sequence found in the host *Andropadus latirostris* that corresponds to form C microfilariae, is significantly different than the other sequences (Fig. 2), even though the individual was caught at Mokula, Equatorial Guinea, where one infected *A. diademata* was also sampled. These data suggest that the parasitic nematodes found in the avian host *A. diademata* may be highly host-specific, although form A microfilaria can be found in several different hosts.

Phylogeography of Alethe diademata

Based on the relatively high prevalence and host specificity of microfilariae in *A. diademata*, to better understand the evolutionary relationship between the parasite and its host, we set out to describe the phylogeography of this host species. We sequenced the mitochondrial ATP-synthase 6 and ATP-synthase 8 genes from blood samples of 38 individuals of *A. diademata*. A maximum-parsimony tree (Fig. 3) shows a clear split between the birds sampled from Côte d'Ivoire and those from Cameroon and Equatorial Guinea. Sequence divergence between the two domains was 5.4%, substantially more than within lineage divergences of 1.7% and 0.5% for Lower and Upper Guinea, respectively. The Dahomey Gap separates these two populations, and these data support the subspecific designation *A. diademata diademata* found west of the Dahomey Gap, and *A. diademata castanea* found in Central Africa (Keith *et al*. 1992). Based on our data, we find that

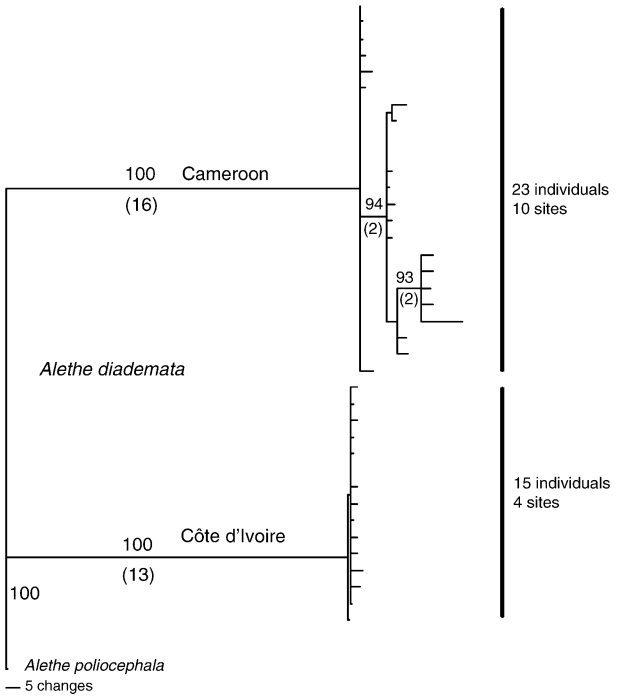

Fig. 3 Consensus maximum-parsimony bootstrap tree obtained from analysis of the mitochondrial ATPase 8 and ATPase 6 genes (842 bp combined) from 38 individuals of *Alethe diademata* and three individuals of *Alethe poliocephala*. The tree was rooted by the *Alethe poliocephala* outgroup. Bootstrap percentages of clades (500 iterations) are shown above internal nodes. Below the bootstrap percentages in parentheses are values for the fixed number of DNA base differences among the clades. Results show that an evolutionary split exists in *A. diademata* between populations in Côte d'Ivoire and those of Cameroon and Equatorial Guinea. Twenty of the individuals were collected from seven sites in Cameroon (sites 9, 10, 12, 13, 14, 15, and 16 in Table 1 and Fig. 2), three from two sites (7 and 8) in Equatorial Guinea (included in the Cameroon clade), and 15 from three sites in Côte d'Ivoire (sites 2, 3, and 4).

despite the evolutionary split between the birds sampled from Côte d'Ivoire and Cameroon, the *LSU rDNA* microfilarial sequences are in several cases identical in these two populations.

Discussion

Parasitic nematode microfilariae in African rainforest birds

We detail here the prevalence of microfilariae in some African rainforest birds. Analysis of blood smears by microscopy revealed a prevalence rate of 3.6%, which is within the range of results described in other studies of African birds (Bennett *et al*. 1974, 1978; Bennett & Herman 1976; Wink & Bennett 1976; Williams *et al*. 1977; Peirce 1984; Kirkpatrick & Smith 1988). It must be noted that, as in many avian studies, we collected blood from the brachial vein. This practice may underestimate the prevalence of microfilariae. In a study of the Willow Ptarmigan (*Lagopus lagopus*) in Norway, many more microfilariae were detected when blood was sampled from the pulmonary artery, as compared to blood taken from the brachial vein (Holmstad *et al*. 2003). In addition, few African birds have been examined for the presence of adult filarial nematodes, and nothing is known about circadian rhythms affecting the appearance of microfilariae in the peripheral blood, to coincide with vector feeding habits. Therefore any microfilariae exhibiting nocturnal periodicity may not have been detected, and as a result the true prevalence may be higher than reported here.

We found a relatively high prevalence of microfilariae in the Fire-crested Alethe (62% infected vs. 2% for the remainder of the birds tested). More information on the behavioural ecology of this species will be required before we can infer why this species may have relatively high parasite loads. In addition, we have little knowledge about the insect vectors specific to these bird populations. It is possible that there may also exist a specific vector that has coevolved with the host species *A. diademata* and its parasitic nematode. A recent study of blood-feeding blackflies in Sweden (Diptera: Simuliidae) revealed a high degree of specificity of feeding preferences (Malmqvist *et al*. 2004). Studies on the ecology of vector transmission of microfilariae will be necessary to address these questions.

We found that *A. diademata* with patent filarial infections were heavier than uninfected birds. Little is known about how nematode parasites affect the health and fitness of rainforest birds. Studies have shown that nematode species diversity correlates with body weight, meaning that larger bird species harbour a greater array of parasites (Gregory *et al*. 1991). To our knowledge, this is the first example of a comparison of infected and uninfected individuals within a single rainforest species. Why the body mass might increase with filarial infection is unknown, however, adults of the bird-inhabiting filarial genera *Chandlerella* and *Splendidofilaria* may be found in many tissues and organs of their hosts, principally in the connective tissue around arteries, and in the walls of arteries (Anderson 2000). The possibility that the significantly greater weight in infected birds might be due to pathological processes related to the worms, such as peritoneal effusions, enlarged liver or other viscera, should be considered. It must be noted, however, that many other factors (i.e. age, availability of food, presence or absence of other infections) could contribute to increased body weight, and only extensive experimental investigations can confirm the correlation between filarial infection and increased body mass.

We found a higher prevalence of microfilariae in birds collected from dense rainforest sites than ecotone sites.

There are many factors that may contribute to these differences, including differences in rainfall, soil moisture, forest fragment size, and species composition, both of the avian hosts and the insect vectors. In addition, we did not detect infected birds at the one montane site, Mount Alen. Similarly, no microfilaria infections were found in birds collected at montane sites in Papua New Guinea (Jones 1985). Future studies utilizing remote sensing data will allow a more direct assessment of which ecological factors best correlate with the prevalence of blood-borne pathogens. However, with this work, we have established an important baseline of parasite prevalence for little-studied rainforest birds of western Africa. With the high degree of deforestation and land use changes presently occurring in Africa, these data will be relevant to studies in the ecology of infectious disease, and especially how human-induced changes may affect host–parasite interactions.

Studies on host–parasite–geography relationships

We found 11 nematode *LSU rDNA* sequences among the 18 samples that were sequenced. These lineages were found in seven bird species representing three families, and of the 11 lineages, eight were of the microfilariae with the morphology of form A (see Appendix), two were unknown (no blood smear was available for the two individuals of *A. diademata* collected at site 9, Nkwouak) and one was of form C. We found four closely related sequences from the 11 samples obtained from the host species *A. diademata*. Because identical sequences were found in animals collected from Côte d'Ivoire and Cameroon, this suggests that there is a high degree of host specificity exhibited by the nematodes in this host. Other host species collected in the exact same locations harboured nematodes of different lineages (seven lineages found in six species). These data provide the first molecular-based evidence that a vector transmitted parasitic nematode might be host specific. It seems paradoxical that some monoxenous species of nematode can be found in several hosts (i.e. Perlman *et al*. 2003), and that the filarial nematodes described here, that are spread by an insect vector, may be host specific. Little is known about the relative host specificity of heteroxenous parasitic nematodes, and possible explanations may include specific feeding preferences for the insect vector, or immune tolerance in the avian host. Much further research is necessary on this topic.

Our data show a distinct evolutionary split between populations of *A. diademata* in Côte d'Ivoire (in Upper Guinea), and those of Cameroon and Equatorial Guinea (Lower Guinea). Separating the two populations is the savannah region between Benin and Ghana which forms a break in the zonal rainforest of West Africa: the Dahomey Gap. Other avian species exhibit a similar evolutionary split. For example, based on mitochondrial DNA sequences, it was estimated that the split between the Upper and Lower Guinea lineages of the little greenbul, *Andropadus virens*, occurred approximately 2 million years ago (Ma) (Smith *et al*. 2001). In that study, the sequence divergence was 5.6% between birds from the Upper Guinea and Lower Guinea refugia. The mitochondrial sequence divergence between the Upper and Lower Guinea populations of *A. diademata* in this study is 5.4%, so the split may have occurred at roughly the same time. Molecular systematic work on the genus *Alethe* by Beresford (2002) also supports differentiation between Upper and Lower Guinea populations.

A similar evolutionary split between Upper and Lower Guinea populations was not evident of the microfilariae in their hosts. The sequence data collected from the microfilariae was from the nuclear *LSU rDNA* gene, which has been used in other studies to determine species-level phylogenetic relationships in nematodes (Nadler *et al*. 2000). We hypothesize that the analysis of faster evolving mitochondrial genes taken from the nematodes might also reveal a significant evolutionary split between Upper and Lower Guinea. In order to strengthen our arguments with additional sequence data, we tried to amplify DNA from several other genes; the internal transcribed spacer (ITS) region of the rDNA, and mitochondrial genes (data not shown), but in all cases we were unsuccessful in either obtaining PCR products or reproducible results. In addition, we were unable to obtain LSU PCR products from all the hosts infected with microfilariae. Furthermore, we obtained sequence data only from Form A and Form C microfilariae. In one case, an individual infected with both form A and form H microfilariae yielded a sequence identical to other form A sequences. Thus, there is a possibility that the PCR primers preferentially amplify DNA of form A microfilariae. However, in several cases, it was noted that we obtained PCR amplification from samples that had relatively high parasitemias, as noted by microscopy. We suspect the failure to obtain PCR amplification from all samples was due to the low concentration of nematode DNA relative to avian DNA.

Avian blood parasites exhibit differing levels of host switching and specificity. For example, studies on avian malaria (in these cases classifying *Haemoproteus* as well as *Plasmodium* as malaria) have found relatively low levels of host switching (Bensch *et al*. 2000; Ricklefs & Fallon 2001; Fallon *et al*. 2003). However in a study in Africa, 44% of haemosporidian lineages were found in more than a single host species, indicating that host switching may be relatively common in African birds (Waldenström *et al*. 2002). Our previous findings revealed that trypanosomes exhibit no apparent host or geographical specificity in African rainforest birds (Sehgal *et al*. 2001). Here we identify a parasitic nematode that appears to be highly host specific

and relatively prevalent in one avian host species. Based on our prevalence and sequence data, we would expect host switching of heteroxenous microfilariae among birds to be relatively uncommon, since at identical sampling locations, we found relatively high prevalence rates in one host species, and very low prevalence rates in others. Further studies on parasitic nematodes and their avian hosts will reveal more about their phylogenetic relationships and evolutionary history.

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References

Achi YL, Zinsstag J, Yao K, Yeo N, Dorchies P, Jacquiet P (2003) Host specificity of *Haemonchus* spp. for domestic ruminants in the savanna in northern Ivory Coast. *Veterinary Parasitology*, **116**, 151–158.

Anderson RC (2000) *Nematode Parasites of Vertebrates*. CABI Publishing, New York.

Apperson CS, Hassan HK, Harrison BA *et al*. (2004) Host feeding patterns of established and potential mosquito vectors of West Nile Virus in the Eastern United States. *Vector-Borne and Zoonotic Diseases*, **4**, 71–82.

Bennett GF, Herman CM (1976) Blood parasites of some birds from Kenya, Tanzania and Zaire. *Journal of Wildlife Diseases*, **12**, 59–65.

Bennett GF, Okia NO, Cameron MF (1974) Avian hematozoa of some Ugandan birds. *Journal of Wildlife Diseases*, **10**, 458–465.

Bennett GF, Blancou J, White EM, Williams NA (1978) Blood parasites of some birds from Senegal. *Journal of Wildlife Diseases*, **14**, 67–73.

Bensch S, Stjernman M, Hasselquist D *et al*. (2000) Host specificity in avian blood parasites: a study of *Plasmodium* and *Haemoproteus* mitochondrial DNA amplified from birds. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **267**, 1583–1589.

Beresford P (2002) *Molecular systematics and biogeography of certain Guineo-Congolian passerines*. PhD Thesis, City University of New York.

Blouin MS, Yowell CA, Courtney CH, Dame JB (1995) Host movement and the genetic structure of populations of parasitic nematodes. *Genetics*, **141**, 1007–1014.

Borrow N, Demey R (2001) *A Guide to the Birds of Western Africa*. Princeton University Press, Princeton, New Jersey.

Brant SV, Ortí G (2003) Evidence for gene flow in parasitic nematodes between two host species of shrews. *Molecular Ecology*, **12**, 2853–2859.

Chabaud AG (1979) *Chandlerella inversa* n. sp., Filaire parasite de Nectariniidés au Gabon. *Bulletin Du Muséum National d'Histoire Naturelle*, **1**, 295–297.

Chabaud AG, Bain O (1990) Trois nouvelles Filaires parasites d'Oiseaux de forêts équatoriales humides Africaines. *Bulletin Du Muséum National d'Histoire Naturelle*, **12**, 9–18.

Fallon SM, Bermingham E, Ricklefs RE (2003) Island and taxon effects in parasitism revisited: avian malaria in the Lesser Antilles. *Evolution*, **57**, 606–615.

Fridolfsson A-K, Ellegren H (1999) A simple and universal method for molecular sexing of non-ratite birds. *Journal of Avian Biology*, **30**, 116–121.

Godfrey RD, Jr, Fedynich AM, Pence DB (1987) Quantification of hematozoa in blood smears. *Journal of Wildlife Diseases*, **23**, 558–565.

Gregory RD, Keymer AE, Harvey PH (1991) Life history, ecology and parasite community structure in Soviet birds. *Biological Journal of the Linnean Society*, **43**, 249–262.

Greiner EC, Bennett GF, White EM, Coombs RF (1975) Distribution of the avian hematozoa of North America. *Canadian Journal of Zoology*, **53**, 1762–1787.

Holmstad PR, Anwar A, Iezhova T, Skorping A (2003) Standard sampling techniques underestimate prevalence of avian hematozoa in willow ptarmigan (*Lagopus lagopus*). *Journal of Wildlife Diseases*, **39**, 354–358.

Hunt JS, Bermingham E, Ricklefs RE (2001) Molecular systematics and biogeography of Antillean thrashers, tremblers, and mocking birds (Aves: Mimidae). *Auk*, **118**, 35–55.

Jones HI (1985) Hematozoa from montane forest birds in Papua New Guinea. *Journal of Wildlife Diseases*, **21**, 7–10.

Keith S, Urban EK, Fry CH (1992) *Birds of Africa*, Vol. IV, p. 442. Academic Press, San Diego, California.

Kirkpatrick CE, Smith TB (1988) Blood parasites of birds in Cameroon. *Journal of Parasitology*, **74**, 1009–1013.

Kocher TD, Thomas WK, Meyer A *et al*. (1989) Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proceedings of the National Academy of Sciences of the United States of America*, **86**, 6196–6200.

Letouzey R (1968) *Etude Phytogeographique Du Cameroun*. Lechevalier, Paris.

Longman KA, Jenik J (1992) Forest-savanna boundaries: general considerations. In: *Nature and Dynamics of Forest-Savanna Boundaries* (eds Furley PA, Proctor J, Ratter JA), pp. 3–20. Chapman & Hall, New York.

Louette M (1981) The birds of Cameroon. An annotated checklist. Verhandelingen van de Koniklijke Academie voor Wetenschappen, Letteren Schone Kunsten van Belgie. *Klasse de Wetenschappen*, **43**, 1–295.

Malmqvist B, Strasevicius D, Hellgren O, Adler PH, Bensch S (2004) Vertebrate host specificity of wild-caught blackflies revealed by mitochondrial DNA in blood. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **271**, S152–S155.

Matta NE, Basto N, Gutierrez R, Rodriguez OA, Greiner EC (2004) Prevalence of blood parasites in Tyrannidae (flycatchers) in the eastern plains of Colombia. *Memórias do Instituto Oswaldo Cruz*, **99**, 271–274.

McKeand JB (1998) Molecular diagnosis of parasitic nematodes. *Parasitology*, **117**, S87–S96.

Morand S, Poulin R (2000) Nematode parasite species richness and the evolution of spleen size in birds. *Canadian Journal of Zoology*, **78**, 1356–1360.

Nadler SA, D'Amelio S, Fagerholm HP, Berland B, Paggi L (2000) Phylogenetic relationships among species of *Contracaecum* Railliet & Henry, 1912 and *Phocascaris* Høst, 1932 (Nematoda: Ascaridoidea) based on nuclear rDNA sequence data. *Parasitology*, **121**, 455–463.

Nadler SA, Hudspeth DSS (1998) Ribosomal DNA and phylogeny of the ascaridoidea (Nemata: Secernentea): implications for morphological evolution and classification. *Molecular Phylogenetics and Evolution*, **10**, 221–236.

Olsen OW (1974) *Animal Parasites, Their Life Cycles and Ecology*. Dover Publications, Mineola, New York.

Peirce MA (1984) Hematozoa of Zambian birds. *Journal of Natural History*, **18**, 105–122.

Perlman SJ, Jaenike J (2003) Infection success in novel hosts: an experimental and phylogenetic study of *Drosophila*-parasitic nematodes. *Evolution*, **57**, 544–557.

Perlman SJ, Spicer GS, Shoemaker DD, Jaenike J (2003) Associations between mycophagous *Drosophila* and their *Howardula* nematode parasites: a worldwide phylogenetic shuffle. *Molecular Ecology*, **12**, 237–249.

Pruett-Jones SG, Pruett-Jones MA, Jones HI (1990) Parasites and sexual selection in birds of paradise. *American Zoologist*, **30**, 287–298.

Ricklefs RE, Fallon SM (2001) Diversification and host switching in avian malaria parasites. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **269**, 885–892.

Sehgal RNM, Jones HI, Smith TB (2001) Host specificity and incidence of *Trypanosoma* in some African rainforest birds: a molecular approach. *Molecular Ecology*, **10**, 2319–2327.

Sibley CG, Monroe BL, Jr (1990) *Distribution and Taxonomy of Birds of the World*. Yale University Press, New Haven, Connecticut.

Simpson VR, MacKenzie G, Harris EA (1996) Fatal microfilarial infection in red-billed blue magpies (*Urocissa erythrorhynchus*). *Veterinary Record*, **138**, 522–523.

Smith TB, Calsbeek R, Wayne RK, Holder KH, Pires D, Bardeleben C (2005) Testing alternative mechanisms of evolutionary divergence in an African rainforest passerine bird. *Journal of Evolutionary Biology*, **18**, 257–268.

Smith TB, Schneider CJ, Holder K (2001) Refugial isolation versus ecological gradients. *Genetica*, **112–113**, 383–398.

Smith TB, Wayne RK, Girman DJ, Bruford MW (1997) A role for ecotones in generating rainforest biodiversity. *Science*, **276**, 1855–1857.

Smyth JD (1994) *Introduction to Animal Parasitology*. Cambridge University Press, Cambridge.

Swofford DL (2002) *PAUP* Phylogenetic Analysis Using Parsimony (*and Other Methods)*, Version 4.0b10. Sinauer Associates, Sunderland, Massachusetts.

Waldenström J, Bensch S, Kiboi S, Hasselquist D, Ottosson U (2002) Cross-species infection of blood parasites between resident and migratory songbirds in Africa. *Molecular Ecology*, **11**, 1545–1554.

Williams NA, Bennett GF, Troncy PM (1977) Avian hematozoa of some birds from Tchad. *Journal of Wildlife Diseases*, **13**, 59–61.

Wink M, Bennett GF (1976) Blood parasites of some birds from Ghana. *Journal of Wildlife Diseases*, **12**, 587–590.

Ravinder Sehgal is an adjunct professor in the Department of Biology at San Francisco State University and a research fellow at the Center for Tropical Research at the University of California, Los Angeles. His interests lie in the evolution and ecology of blood-borne diseases in birds, both in Africa and in California. Tom Smith heads the Center for Tropical Research at UCLA and is involved in research studying alternative speciation hypotheses in tropical rainforests. Hugh Jones is an adjunct senior lecturer at The University of Western Australia. His research interests are in the taxonomy and evolution of wildlife parasites; he did the blood smear and morphological analyses in this work.

Appendix

We identified eight morphologically distinct forms of parasitic microfilariae from 34 infected birds. Since few adult filarial worms have been described from these birds, these microfilariae are referred to by letters. Appendix I documents which of the eight morphologically identified forms of microfilariae were found in which host species, Appendix II details the morphometrics of the microfilariae, and Appendix III shows illustrations of these microfilariae

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Morphological descriptions of microfilariae

Form A. Hosts: Muscicapidae: 16 *Alethe diadematа*; Pycnonotidae: 1 *Neocossyphus poensis*, 2 *Bleda eximia*, 1 *B. syndactyla*, 2 *Criniger chloronatus*, 1 *Pycnonotus barbatus*; Sylviidae: 1 *Illadopsis rufipennis*. Small, stout unsheathed microfilariae, length 42–45 μm cephalic end pointed, short cephalic space. 'Kinked' appearance. Maximum width a short distance behind anterior end, narrowing uniformly posteriorly until near the posterior end, with a sudden diminution in width to a short bluntly pointed tail, which is often curved 180° into a hook-like shape. Cell nuclei densely packed, and nerve ring and excretory pore often difficult to see. G1 cell a narrow band across the full width of the microfilaria, up to 2 μm long. The single infected *Neocossyphus poensis* also had many worms which were identical in morphology and proportions but were much longer (59–73 μm), with no overlap in length with the smaller forms.

Form B. Host: Passeridae: 2 *Nigrita canicapilla*. Unsheathed microfilariae 65–92 μm, almost uniform width to level of anus, and tapering thereafter to a blunt, tail; some rounded, and others with almost knobbed rounded posterior end. Very short cephalic space. Nerve ring and excretory pore conspicuous, G1 cell usually up to 3 μm in length, rounded.

Form C. Host: Pycnonotidae: 3 *Andropadus latirostris*, 1 *Bleda syndactyla*. Fairly short worms (59–70 μm), unsheathed, often disposed in hairpin position. Two conspicuous parallel rows of nuclei behind cephalic space. Worms uniform width to the level of G1 cell, thereafter tapering evenly to a very thin attenuated tail, posteriormost portion of which is free of cell nuclei. G1 cell a narrow band which does not usually extend across full width of worm, up to 2 μm long. G4 cell conspicuous, excretory pore inconspicuous. The infected *Bleda syndactyla* also contained a small number of microfilariae which were similar in morphology and proportions, but were considerably longer (100–105 μm).

Form D. Host: Pycnonotidae: 1 *Pynonotus barbatus*. Length 75–88 μm. A distinctive worm, unsheathed, with a large cephalic space and large, clear, key features. G1 cell particularly conspicuous, extending across full width of worm, approximately same length or longer than width and appearing square, 4 μm long. Usually disposed extended or undulating. Tail narrows suddenly beyond anus to a pointed tip which is often recurved into a hook-like shape.

Form E. Host: Pycnotidae: 1 *Nicator chloris*. Very distinctive, unsheathed (only three seen). Length 95–110 μm. Worms almost uniform width throughout with truncated rounded tail (possibly short tightly turned back tail, but this cannot be seen). Excretory pore conspicuous and large, G cells appear as large irregular lacunae, 20–25 μm in length commencing near midpoint of worm; anal cell also appears as large lacuna in 2/3 specimens seen, 3–5 μm long.

Form F. Host: Passeridae: 1 *Motacilla clara*. Relatively large stout worms, without sheaths, 95–120 μm in length. In 16/19 individuals, posterior ends abruptly rounded; in 3/19, short tapering tail; no evidence that the tail was bent back and hidden in other specimens. Large cephalic space, nerve ring often difficult to differentiate, excretory pore large and conspicuous, and very elongated clear G1 cell, 8–11 μm long.

Form G. Host: Nectariniidae: 1 *Nectarinia olivacea*. Long worms, 108–130 μm, without sheath, usually extended, irregularly sinuous, kinked appearance, often with sharp bend about 10–15 μm from anterior end. Width rather uneven, maximum width in anterior quarter, tapering gradually to a bluntly tipped tail. Short cephalic space. G1 cell small, 1–2 μm, often not extending across width of worm. Anal cell usually large and conspicuous.

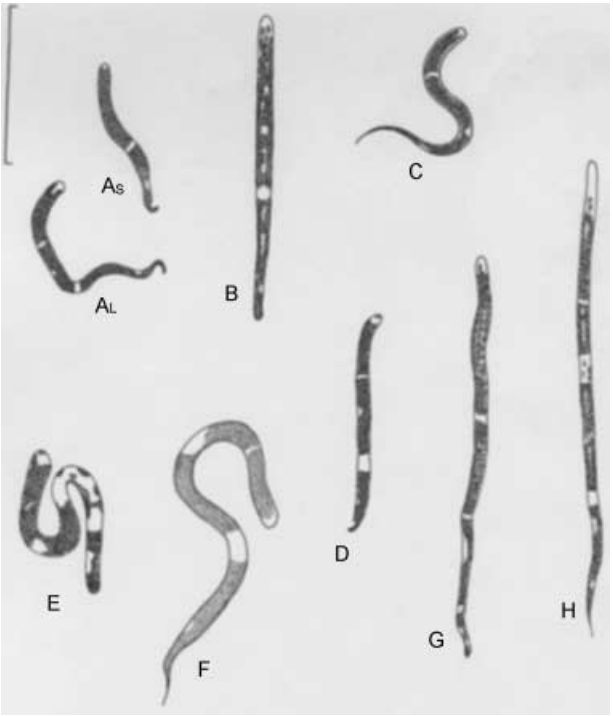
Form H. Host: Muscicapidae: 2 *Alethe diadematа*. Long, thin sinuous worms (149 and 152 μm), unsheathed, with large cephalic space. Uniform width to level of anal cell, with tapering tail terminating in a fine point. Two worms only were seen on one slide (occurring concurrently with the more numerous form A). Nerve ring conspicuous. Large excretory pore and G1 cell 2–5 μm long, full width of worm. In another host, the worms were similar in morphology but smaller (110–124 μm). A single microfilaria seen in a third host was not in a suitable condition to ascertain the internal morphology.

Most filarial parasites of birds occur in the onchocercid subfamilies Splendidofilariinae and Lemdaninae (Anderson 2000). Only two filarial worms have been described from the birds examined in this study; *Cardiofilaria erardi* was described from *Alethe diadematа* in Gabon by Chabaud & Bain (1990). However the microfilaria were considerably larger (length 225–245 μm) than those described from this host in our study (110–152 μm), and we cannot assign our specimens (form H) with certainty to this species. *Chandlerella inversa* was described from the sunbird *Cyanomitra olivacea cephaelis* (= *Nectarinia olivacea*) also from Gabon (Chabaud 1979), but no description was provided for the microfilariae beyond their length in the uterus, which was larger (mean, 132 μm) than the specimens recovered in this host in our study. They were not reported to be sheathed, as is normally the case in *Cardiofilaria* (Anderson 2000), and no sheaths were discerned in our specimens. It is therefore again not possible to assign with certainty the specimens we recovered to this species. Without knowledge of the adult worms, or information on the degree of difference between microfilariae of different species, the specimens described herein cannot be further identified.

We detected eight morphologically distinct forms of microfilariae in the birds of this study. Form A, a short microfilaria, occurred in 23 individual birds of seven species in three families (Sylviidae, Pycnonotidae and Muscicapidae). This may, however, represent more than one species of filarial worm. It was present concurrently with form H in two *A. diadematа*, with form D in one *Pycnonotus barbatus* and with form C in one *Bleda eximia*. In *Illadopsis poensis* a larger form, similar in morphology but significantly longer, was present concurrently with the smaller form. Form C in *Bleda syndactyla* also occurred in two size ranges, with a small number of microfilaria, being up to 60% longer than the smaller morphs. The other four microfilarial forms each occurred in one individual bird of one species. Thus despite the low apparent prevalence of microfilariae in these bird populations, our findings illustrate the variety of filarial worms present, albeit in a few bird families and species. They emphasize the incomplete knowledge of parasites in African birds that therefore hinders our understanding of the role that parasitic infections may play in the overall ecology and evolution of the avifauna.

Appendix I Presence of eight forms of microfilaria in 12 bird species (no. of birds of each species infected follows name)

	Microfilariae							
Host	A	B	C	D	E	F	G	H
Sylviidae:								
Illadopsis rufipennis 1/6	+							
Pycnonotidae:								
Pycnonotus barbatus 2/5	+			+				
Nicator chloris 1/9					+			
Criniger chloronatus 2/4	+							
Bleda syndactyla 2/4	+		+					
Bleda eximia 2/14	+							
Andropadus latirostris 3/103			+					
Passeridae:								
Nigrita canicapilla 2/2		+						
Motacilla clara 1/1						+		
Nectariniidae:								
Nectarinia olivacea 1/124							+	
Muscicapidae:								
*Neocossyphus poensis*1/5	+							
Alethe diademata 16/26	+							+
Turdus pelios 1/1 (not characterized)								



Appendix III Illustration of the eight forms of microfilariae described in Appendices I and II. The scale bar represents 50 μm.

Appendix II Measurements of eight forms of microfilariae, including two large variants, recovered from 34 birds of 12 species

Microfilaria	Host sp.	*N*	length	MW	NR	EP	G1 cell	Anal pore
A	(8 species)	6	44.5 (42–47)	3.5–4.0	20.5 (17.8–23.8)	32.2 (28.9–35.7)	54.7 (46.6–58.1)	3–6
A (large form)	*N. poensis*	7	63.9 (59–73)	3.0–3.5	23.4 (21.2–24.2)	36.1 (31.8–41.2)	58.4 (54.5–60.3)	?
B	*N. canicapilla*	15	78.4 (65–112)	3.0–4.0	26.0 (20.6–31.2)	39.7 (36.7–43.1)	61.6 (56.0–71.0)	8–15
C	*A. latirostris*, *B. syndactyla*	11	61.7 (59–70)	3.0–4.0	25.4 (21.7–32.2)	37.7 (33.3–47.5)	59.1 (51.5–71.7)	7–15
C (large form)	*B. syndactyla*	2	102 (100–105)	4.5–5.0	24.9 (23.8–26.0)	34.6 (34.0–35.2)	58.5 (57.1–60.0)	12–14
D	*P. barbatus*	8	79 (75–83)	3.0–4.0	25.2 (21.3–28.2)	35.4 (31.4–44.0)	57.7 (53.3–60.0)	10–14
E	*N. chloris*	3	99.3 (95–110)	4.0–4.5	22.1 (20.0–25.2)	32.2 (29.5–35.0)	52.6–74.8* (47.0–58.2; 70.0–80.6)	?
F	*M. clara*	8	105.9 (90–120)	4.5–6.0	21.9 (18.9–25.0)	33.2 (30.0–37.5)	57.0–66.7* (50.5–63.3; 63.1–68.4)	8–20
G	*N. olivacea*	10	118.6 (108–130)	3.5–5.0	25.7 (21.3–29.1)	37.5 (33.3–40.1)	63.5 (50.0–68.7)	10–17
H	*A. diademata*	4	134 (110–152)	2.5–3.0	25.7 (22.6–27.6)	39.8 (36.3–42.8)	65.5 (61.8–70.0)	16–18

Lengths, maximum width, and tail in μm; mean with range in brackets. Other measurements are percentages of distance from anterior end. MW, maximum width; NR, nerve ring; EP, excretory pore; G1 cell; anterior border.