Journal of Wildlife Diseases, 43(3), 2007, pp. 382–398

PREVALENCE AND DIVERSITY OF AVIAN HEMATOZOAN PARASITES IN ASIA: A REGIONAL SURVEY

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ABSTRACT: Tissue samples from 699 birds from three regions of Asia (Myanmar, India, and South Korea) were screened for evidence of infection by avian parasites in the genera *Plasmodium* and *Haemoproteus*. Samples were collected from November 1994 to October 2004. We identified 241 infected birds (34.0%). Base-on-sequence data for the cytochrome *b* gene from 221 positive samples, 34 distinct lineages of *Plasmodium*, and 41 of *Haemoproteus* were detected. Parasite diversity was highest in Myanmar followed by India and South Korea. Parasite prevalence differed among regions but not among host families. There were four lineages of *Plasmodium* and one of *Haemoproteus* shared between Myanmar and India and only one lineage of *Plasmodium* shared between Myanmar and South Korea. No lineages were shared between India and South Korea, although an equal number of distinct lineages were recovered from each region. Migratory birds in South Korea and India originate from two different migratory flyways; therefore cross-transmission of parasite lineages may be less likely. India and Myanmar shared more host species and habitat types compared to South Korea. Comparison between low-elevation habitat in India and Myanmar showed a difference in prevalence of haematozoans.

Key words: Avian malaria, hematozoan parasites, India, Myanmar, South Korea.

INTRODUCTION

The avian hematozoan parasites *Plasmodium* and *Haemoproteus* are two well-studied and globally distributed genera of blood parasites (Atkinson and Van Riper 1991). Recent molecular studies using DNA sequencing to identify and characterize parasite lineages (Bensch et al., 2000; Ricklefs and Fallon, 2002; Fallon et al., 2003; Beadell et al., 2004) have revealed more detailed information than was provided by morphology alone (Perkins and Schall, 2002). Studies have been conducted on the morphologic classification of these two parasite genera (Atkinson and Van Riper, 1991), host specificity (Bensch et al., 2000; Ricklefs and Fallon, 2002; Waldenström et al., 2002; Beadell et al., 2004), and geographical distribution (Fallon et al., 2005; Durrant et al., 2006). Molecular techniques also have been used to differentiate between native and introduced parasite lineages in native and introduced populations of common myna (*Acridotheres tristis;* Ishtiaq et al., 2006) and to detect cross-species transmissions between migratory and resident birds (Waldenström et al., 2002).

Each year billions of migratory birds migrate between tropical wintering areas to temperate summer breeding grounds (McClure, 1974b). Although Asia is a wintering and staging ground for many migratory birds, and a major source of exotic species that have been introduced into many other regions, it has remained the least-explored region as far as the genetic diversity of avian blood parasites is concerned. The global movements of diverse avian species that may be infected with blood parasites should be a cause of concern

given the potential of migrants and introduced species to spread novel diseases worldwide (McClure, 1974a; Rappole et al., 2000). This may be especially relevant to isolated regions where native species may have a reduced immune defense against such parasites (Van Riper et al., 1986).

Hawaii is an excellent example of biological invasion where the introductions of avian malaria (*Plasmodium relictum*) and avian pox into highly susceptible native populations led to the endangerment and possible extinction of many native birds (Van Riper et al., 1986). About 125 bird species were introduced to the Hawaiian Islands in the late nineteenth and early twentieth centuries (Caum, 1933; Berger, 1981; Pratt, 1994); the majority of these originated from India and Southeast Asia. As part of a global survey to determine the origin of *Plasmodium relictum* in Hawaii, India has been included as a region of interest as at least 12 avian species were introduced from Hawaii from this source (Long, 1981). In this study, we examined the genetic diversity of hematozoan parasites of India, Myanmar, and South Korea. Although McClure et al. (1978) conducted a large-scale microscopy-based survey of the hematozoans in birds from eastern and southern Asia, molecular-based surveys have not been previously conducted in Asia. Our goals were to 1) determine the prevalence of infection within and across regions, and in families of host species shared between regions, 2) determine the phylogenetic relationships of the hematozoan parasites found in these regions, 3) estimate the degree of lineage sharing between regions, and 4) determine parasite diversity among regions.

MATERIALS AND METHODS

Sampling

We collected blood samples from 183 birds in India from seven sites (three sites near Pinjore, in Haryana, Meerut in Uttar Pradesh, and three sites near Guwahati in Assam) between May and June 2003 (before the monsoon during the dry season) that varied from dry deciduous forest to scrubland and marshland (Fig. 1A). Each bird captured was banded for individual identification with rings provided by the Bombay Natural History Society (BNHS), sexed, aged, measured, weighed, and examined for flight or body molt. Approximately 50–100 μl of whole blood was drawn by humeral venipuncture into a microvette with EDTA anticoagulant and used for the preparation of blood smears and DNA analysis. In South Korea, 181 tissue samples were collected in October 2004 from two airbases (Kunsan and Osan) (Fig. 1B). We examined 335 bird tissue samples (all blood impregnated) collected from four locations in Myanmar between November 1994 and March 2001 (in winter–dry season). Myanmar's climate and vegetation vary greatly from the temperate north and high-altitude zones to equatorial regions in the far south. The four sampling locations (Hkakabo Razi National Park, Taungoo area, Alaungdaw Kathapa National Park, and Chatthin Wildlife Sanctuary) varied from altitudes of 200 to 3,500 m and habitats ranging from degraded forest to alpine forest (Fig. 1C).

Screening of blood smears by microscopy

Thin blood smear slides were obtained from each bird sampled in India. These were fixed with methanol, stained for 1 hr with 6% phosphate buffered Giemsa (pH 7.0) and scanned at 400× for 10 min to diagnose malarial infections. Parasite species were identified using morphologic characteristics (Garnham, 1966). A minimum of 50,000 erythrocytes were examined on each slide (Atkinson et al., 1995). The level of parasitemia was based upon results of these examinations. Blood smears were not available for birds sampled in Myanmar and South Korea.

Molecular analysis

Host and parasite DNA was extracted from tissue and blood samples using Qiagen DNeasy kits (Qiagen, Valencia, California USA) following manufacturer guidelines. To ensure the quality of DNA extractions, we amplified a small fragment (347 bp) of *cyt b* DNA of host using primers cytb-1 and cytb2 following the methods described in Kocher et al. (1989). These PCRs were successful in all cases. Two primers sets were used for parasite screening of samples; these included primer set F2/R2 (91bp,cytochrome b gene [*cyt b*]) and primer set 850F/1024R (167 bp; Beadell et al., 2004; COIII gene). For positive samples, we attempted to amplify a larger fragment

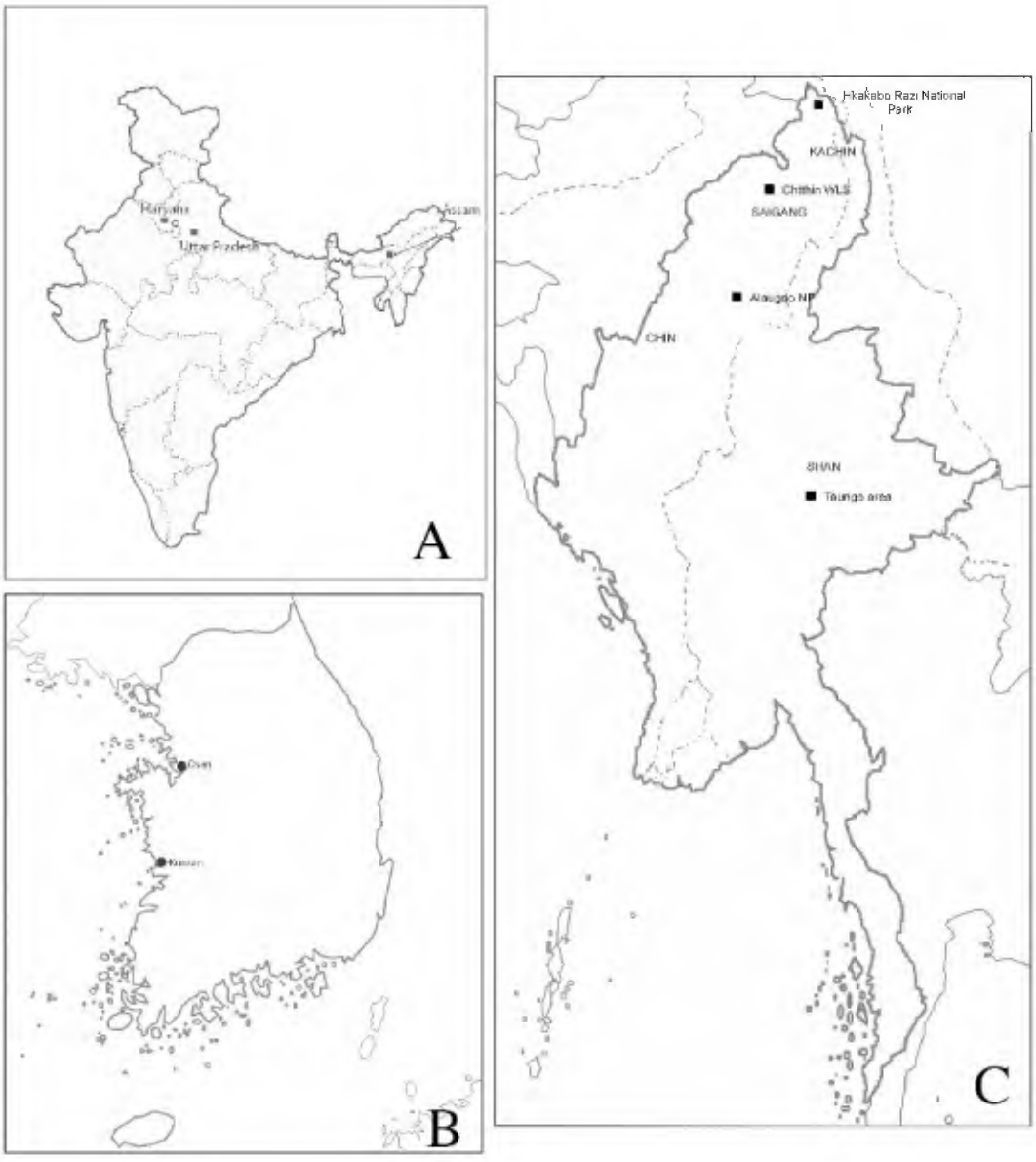

FIGURE 1. Sampling sites in A) India, B) South Korea, and C) Myanmar.

(533 bp) of the *cyt b* sequence using primers 3760F/4292RW2 (Beadell et al., 2004). Because it was not always possible to amplify the larger 533 bp fragments, we attempted to amplify smaller fragments of either 256 bp using primers 3760F with R1 (5′-CTT TTT AAG GTT GGG TCA CTT-3′) or 295 bp using primers F3 (5′-CCA GGA CTT GTT TCA TGG AT-3′) with 4292RW2. When these primer sets failed to amplify DNA, additional reactions using F3 with R3 (5′-GCA TAT CTA TCT ACT GTA ATT GC-3′; 313 bp) and FIF1 (5′-GGG TCA AAT GAG TTT CTGG-3′) with 4292RW2 (351 bp) were used. Details of the PCR protocols are reported in Beadell et al. (2004) and Ishtiaq et al. (2006). Negative and positive controls were included in all reactions. Parasite DNA (*P. relictum*) from an infected Hawaii Amakihi (*Hemignathus virens*) was used as a positive control. Samples from India were screened and amplified in WII's conservation genetics lab, and Myanmar

TABLE I. Prevalence of *Plasmodium* and *Haemoproteus* based on polymerase chain reaction (PCR) detection for sampling sites in Myanmar and India.

Sampling site[a]	Date	Altitude (m)	Habitat	Total samples	*Plasmodium*	*Haemoproteus*
Myanmar						
Hkakabo Razi NP	February–March 2001	>3,500	Alpine	178	29	1
Taungoo Area	November 1994	200–500	Degraded	13	0	1
Alaungdaw Kathapa NP	December 1994, 1997	200–500	Lowland deciduous	23	4	1
Chatthin WLS	November–December 1994	500–1,000	Lowland evergreen	121	35	8
India						
Vulture (H) center	May–June 2003	<200	Dry deciduous	31	7	14
Pinjore (H)	May–June 2003	<200	City	16	3	4
Nagaon (A)	May–June 2003	<200	City	27	5	0
Meerut (UP)	May–June 2003	<200	Scrubland	64	18	11
Guwahati (A)	May–June 2003	<200	City	13	0	1
Deobali Jalah (A)	May–June 2003	<200	Marshland	17	4	0
Ambala (H)	May–June 2003	<200	City	15	8	3

[a] H = Haryana, A = Assam, UP = Uttar Pradesh.

and South Korea samples were analyzed at the genetics program at the Smithsonian Institution.

All samples with positive amplification for larger fragments were purified using Qiaquick kits (Qiagen) and sequenced bidirectionally on an ABI 3100 Sequencer (Applied Biosystems, Inc., Foster City, California, USA). Sequences were edited and aligned using the program SEQUENCHER version 4.1 and are available through Genbank (accession numbers EF380111–EF380209).

Phylogenetic analysis

Phylogenetic relationships were evaluated using samples for which we had at least 256 bp of cyt *b* sequence. Based on the phylogeny in Perkins and Schall (2002), the phylogenetic trees were rooted using either lizard *Plasmodium* (Genbank accession numbers AY099061, AY099060) or *Haemoproteus* (Genbank accession numbers AY099062, AY099057) sequences as outgroups. We used MODELTEST version 3.6 (Posada and Crandall, 1998) to determine the most appropriate evolutionary model for our data. The hierarchical likelihood ratio test using Akaike Information Criterion (AIC) selected the general time-reversible model (GTR+I+G) for both *Plasmodium* and *Haemoproteus* phylogenies separately. For *Plasmodium*, the proportion of invariable sites equaled 0.6407, and the gamma shape parameter equaled 1.0639; for *Haemoproteus* the invariable sites equaled 0.4514, and the gamma shape parameter equaled 0.4899. These settings were implemented in a maximum likelihood analysis to estimate the topology of the avian malaria parasites. We used 100 replicates and the "fast" heuristic in PAUP* (Swofford, 1999) to estimate bootstrap support.

Statistical analysis

To assess differences in the prevalence of *Plasmodium* spp. and *Haemoproteus* spp. between different regions, we used contingency table analyses in SAS to compare frequency differences using χ^2 or Fisher exact tests. To assess whether prevalence of parasites differed across host families and regions, we performed analysis of variance (ANOVA) on arcsine square-root transformed prevalence rates at the host species level (Scheuerlein and Ricklefs, 2004). We estimated generalized linear models (SAS institute) with prevalence as the dependent variable and region and family as independent variables. Avian families were included in analyses only if they occurred in two or more regions and included ≥5 individuals per regions (Table 1). Individual contribution of variables was summarized as *F*-values and associated probabilities (type III sums of squares). Taxonomy follows the *Handbook of the Birds of the World* (http://

www.hbw.com/ibc/phtml/familia.phtml). EstimateS version 7.0 (2004) was used to calculate Shannon diversity (mean±s.d.) within regions for both parasite lineages and host species.

RESULTS

Prevalence of parasites

We screened a total of 699 birds from Myanmar, India, and South Korea. Of the 335 samples tested in Myanmar, the prevalence of infection was 122 (37.3%). Of these, 113 individuals were positive for *Haemoproteus* spp. (45 [40%]) and *Plasmodium* spp. (68 [60%]). Based on sequence data, nine individuals showed double peaks indicating that these individuals were infected with two parasite lineages. Sequencing results identified 28 unique lineages for *Plasmodium* and 21 for *Haemoproteus;* these were detected in 42 of 133 represented bird species. There was a significant difference in *Plasmodium* spp. (P=0.012, Fisher's exact test) but not in *Haemoproteus* spp. (P=0.079, Fisher's exact test) prevalence among populations at different study sites in Myanmar (Table 1). Significant differences in prevalence of *Haemoproteus* spp. (P=0.232, Fisher's exact Test) or *Plasmodium* spp. (P=0.065, Fisher's exact Test) between the pooled low (500–3,500 m) and high (200–500 m) elevation sites were not detected.

In India, of the 183 samples representing 43 species, 84 individuals of 33 species tested positive for *Plasmodium* spp. and *Haemoproteus* spp. Of the positive samples, 51 (28%) were *Plasmodium* spp. and 33 (18%) were *Haemoproteus* spp. infections. There were 11 individuals showing multiple infections; we were not able to determine sequences for 23 positive samples. Sequencing results indicated that positive samples represented 11 *Plasmodium* and 19 *Haemoproteus* lineages. There was no significant difference in *Plasmodium* spp. (χ^2=12.2, P=0.056, df=6) prevalence among populations in India, but significant difference for the prevalence of *Haemoproteus* spp. between sites was detected (χ^2=26.65, P=0.0002, df=6).

Of the 181 samples of 46 bird species in South Korea, 76 individuals (42%) representing 34 species tested positive for *Plasmodium* spp. and *Haemoproteus* spp. Of the positive samples, 56 (31%) were *Plasmodium* spp. and 20 (11%) were *Haemoproteus* spp. infections. The positive samples included 10 *Plasmodium* and five *Haemoproteus* lineages.

There was no difference (χ^2=0.15, P=2.0, df=1) in the parasite prevalence between host blood (India) and host tissue (Myanmar and South Korea) samples. There were significant differences for *Plasmodium* spp. (χ^2=9.27, P=0.009, df=2) and *Haemoproteus* spp. (χ^2= 29.56, P=0.001, df=2) prevalence among the three regions, with India and South Korea having the higher prevalence for both lineages than Myanmar. Differences in prevalence between Myanmar and India at sites located between 200 and 500 m elevation were not detected for *Plasmodium* spp. (P=0.48, Fisher's exact test) but were observed for *Haemoproteus* spp. (P=0.005, Fisher's exact test). For lower-sited elevations, prevalence differences between South Korea and Myanmar and India (pooled together) also were not detected for *Plasmodium* spp. (P=0.06, Fisher's exact test), but differences were detected for *Haemoproteus* spp. (P=0.04, Fisher's exact test).

Of 52 avian families recorded, 17 were common to all three regions, but there was no overlap at the species level between regions (Table 2). Prevalence of *Plasmodium* spp. (F=1.16, df=16, P=0.310) and *Haemoproteus* spp. (F=1.08, df=16, P=0.383) did not differ among avian families. Among regions, differences in prevalence for the 17 families shared between regions were detected for both *Plasmodium* spp. (F=12.94, df=2, P= 0.001) and *Haemoproteus* spp. (F=12.89, df=2, P=0.001). Regional grouping explained 35% and 27% of the total variance for *Plasmodium* spp. and *Haemoproteus*

TABLE 2. Prevalence of *Plasmodium* and *Haemoproteus* based on PCR detection for selected host families from three Asian regions (Myanmar, India, and South Korea).

Family	Region	Host species	Sample size	*Plasmodium* positive[e]	*Haemoproteus* positive[e]
Columbidae[c]	Myanmar	Spotted Dove *Streptopelia chinensis*	2	0	1
		Red Turtle Dove *Streptopelia tranquebarica*	1	1	0
	India	Red Turtle Dove	1	0	1
		Blue Rock Pigeon *Columba livia*	5	0	1
	S. Korea	Rufous Turtle Dove *Streptopelia orientalis*	4	1	0
	Total		13	2 (15)	3 (23)
Corvidae[c]	Myanmar	Collared Treepie *Dendrocitta frontalis*	2	0	0
	India	Rufous Treepie *Dendrocitta vagabunda*	5	0	3
	S. Korea	Black-billed Magpie *Pica pica*	3	1	1
		Jay *Garrulus glandarius*	5	1	0
	Total		15	2 (13)[f]	4 (26)
Meropidae[b]	Myanmar	Little Green Bee-eater *Merops orientalis*	5	2	0
	India	Little Green Bee-eater	1	0	0
	Total		6	2 (33)	0
Motacillidae[a]	Myanmar	Black-backed Wagtail *Motacilla lugens*	1	0	0
	S. Korea	Olive-backed Pipit *Anthus hodgsoni*	3	2	0
		Pipit *Anthus* sp.	2	0	0
		Red-throated Pipit *Anthus cervinus*	3	1	0
		White Wagtail *Motacilla alba*	1	1	0
	Total		9	4 (44)[f]	0
Muscicapidae[c]	Myanmar	Grey-headed Flycatcher *Culicicapa ceylonensis*	1	0	0
		Niltava	1	0	1
		Large Niltava *Niltava grandis*	5	0	0
		Little Forktail *Enicurus scouleri*	1	0	0
		Oriental Magpie-Robin *Cosychus saularis*	7	6	0
		White-rumped Shama *Copsychus malabaricus*	7	2	0
		Orange-flanked Bush Robin *Tarsiger cyanurus*	2	1	0
		Plumbeous Water Redstart *Rhyacornis fuliginosus*	1	0	0
		Daurian Redstart *Phoenicurus auroreus*	1	0	0
		Red-breasted Flycatcher *Ficedula parva*	6	1	4
		Rufous-bellied Niltava *Niltava sundara*	7	1	0
		Rufous-gorgeted Flycatcher *Ficedula strophiata*	5	1	0
		Slaty-backed Forktail *Enicurus schistaceus*	1	0	0
		Small Nilatava *Niltava macgregoriae*	1	0	0
		White-gorgeted Flycatcher *Ficedula monileger*	8	0	0
		White-tailed Robin *Cinclidium leucurum*	1	1	0
		Blue Flycatcher *Cyornis* sp.	1	0	0
	India	Oriental Magpie Robin	5	0	0
		Indian Robin *Saxicoloides fulicata*	2	0	2
		Brown Rock Chat *Cercomela fusca*	3	1	0
	S. Korea	Daurian Redstart *Phoenicurus auroreus*	4	1	0
		Blue-and-white Flycatcher *Cyanoptila cyanomelana*	2	0	1
		Common Stonechat *Saxicola torquata*	1	1	0
		Mugimaki Flycatcher *Ficedula mugimaki*	2	0	1
	Total		75	16 (21)[f]	9 (12)[f]

TABLE 2. Continued.

Family	Region	Host species	Sample size	*Plasmodium* positive[c]	*Haemoproteus* positive[e]
Nectariniidae[b]	Myanmar	Black-throated Sunbird *Aethopyga saturata*	3	0	0
		Crimson Sunbird *Aethopyga siparaja*	2	0	0
		Green-tailed Sunbird *Aethopyga nipalensis*	1	0	0
		Little Spiderhunter *Arachnothera longirostra*	1	0	0
		Streaked Spiderhunter *Arachnothera magna*	2	0	0
	India	Purple Sunbird *Nectarinia sperata*	4	0	2
	Total		14	0	2 (15)[f]
Paridae[a]	Myanmar	Great Tit *Parus major*	1	0	0
		Green-backed Tit *Parus monticolus*	3	0	0
		Sultan Tit *Melanochlora sultanea*	1	0	0
	S. Korea	Coal Tit *Parus ater*	2	1	0
		Great Tit	16	12	1
		Long-tailed Tit *Aegithalos caudatus*	4	0	0
		Marsh Tit *Parus palustris*	2	1	0
		Varied Tit *Parus varius*	7	4	0
	Total		36	18 (50)[f]	1 (3)
Passeridae[d]	India	House Sparrow *Passer domesticus*	21	4	2
		Tree Sparrow *Passer montanus*	3	0	0
	S. Korea	Tree Sparrow	2	0	0
	Total		26	4 (15)	2 (8)
Phasianidae[a]	Myanmar	Himalayan Monal *Lophophorus impejanus*	1	0	0
		Sclater Monal *Lophophorus sclateri*	1	0	0
		Kalij Pheasant *Lophura leucomelanos*	1	0	0
	S. Korea	Japanese Quail *Coturnix japonica*	1	1	0
		Ring-necked Pheasant *Phasianus colchicus*	4	1	0
	Total		8	2 (25)	0
Picidae[a]	Myanmar	Eurasian Wryneck *Jynx torquilla*	2	1	0
		Greater Yellownape Woodpecker *Picus flavinucha*	1	0	0
		Grey-headed Woodpecker *Picus flavinucha*	12	0	0
		Yellow-crowned Woodpecker *Dendrocopos mahrattensis*	2	1	0
		White-browed Piculet *Sasia ochracea*	3	0	0
		Rufous Piculet *Sasia abnormis*	2	0	0
		Common Goldenback Woodpecker *Dinopium javanense*	1	0	0
	S. Korea	Grey-headed Woodpecker	2	1	0
		Japanese Pygmy Woodpecker *Picoides kizuki*	2	0	0
	Total		27	4 (15)	0
Psittacidae[b]	Myanmar	Rose-ringed Parakeet *Psittacula krameri*	2	0	0
	India	Rose-ringed Parakeet *Psittacula krameri*	1	0	1
		Alexandrine Parakeet *Psittacula eupatria*	2	1	0
	Total		5	1 (20)	1 (20)

TABLE 2. Continued.

Family	Region	Host species	Sample size	*Plasmodium* positive[c]	*Haemoproteus* positive[e]
Pycnonotidae[b]	Myanmar	Black-crested Bulbul *Pycnonotus melanicterus*	3	0	1
		Red-vented Bulbul *Pycnonotus cafer*	6	0	0
		Grey-eyed Bulbul *Iole propinqua*	1	0	0
		Alophoixus (*Criniger* auct.) spp.	4	0	0
	India	Red-vented Bulbul	10	3	2
	Total		24	3 (12)[f]	3 (12)
Paradoxornithidae[a]	Myanmar	Black-throated Parrotbill *Paradoxornis nipalensis*	2	0	0
		Lesser Rufous-throated Parrotbill *Paradoxornis atrosuperciliaris*	1	0	0
	S. Korea	Vinous-throated Parrotbill *Paradoxornis webbianus*	9	1	0
	Total		12	1 (8)	0
Sturnidae[b]	Myanmar	Asian Pied Starling *Sturnus contra*	1	0	0
		Black-collared Starling *Sturnus nigricollis*	1	0	0
	India	Asian Pied Starling	4	0	1
		Common Myna *Acridotheres tristis*	20	8	2
		Bank Myna *Acridotheres gingianianius*	4	2	1
		Jungle Myna *Acridotheres fuscus*	1	0	0
	Total		31	10 (32)	4 (13)
Sylviidae[c]	Myanmar	Common Tailorbird *Orthotomus sutorius*	5	1	0
		Mountain Tailorbird *Orthotomus cuculatus*	2	0	0
		Thick-billed Warbler *Acrocephalus aedon*	1	1	0
		Rufous-faced Warbler *Abroscopus albogularis*	1	0	0
		Blyth's Leaf Warbler *Phylloscopus reguloides*	2	0	0
		Yellow-browed Warbler *Phylloscopus inornatus*	3	0	1
		Mountain Leaf Warbler *Phylloscopus trivirgatus*	2	0	0
		Leaf Warbler *Phylloscopus* spp.	6	0	0
		Grey-cheeked Warbler *Seicercus poliogenys*	6	1	0
		Jungle Babbler *Turdoides striatus*	2	0	2
		Black-chinned Babbler *Stachyris pyrrhops*	2	2	0
		Striated Grassbird *Megalurus pryeri*	2	2	0
		Bush Warbler *Cetti* sp.	1	0	0
	India	Common Tailorbird	2	1	0
		Yellow-eyed Babbler *Chrysomma sinense*	4	2	1
	S. Korea	Yellow-browed Warbler	5	0	0
		Arctic Warbler *Phylloscopus borealis*	1	0	1
		Oriental Great Reed Warbler *Acrocephalus orientalis*	1	1	0
		Leaf Warbler	9	2	0
	Total		57	13 (23)[f]	5 (9)

TABLE 2. Continued.

Family	Region	Host species	Sample size	*Plasmodium* positive[c]	*Haemoproteus* positive[c]
Timaliidae[b]	Myanmar	Coral-billed Scimitar Babbler *Pomatorhinus ferrufinosus*	2	1	0
		Eyebrowed Wren Babbler *Napothera epilepidotai*	1	0	0
		Long-billed Wren Babbler *Rimator malaoptilus*	2	1	0
		Golden Babbler *Stachris chrysaea*	3	1	0
		Grey-cheeked Fulvetta *Alcippe morrisonia*	11	1	0
		Rufous-throated Fulvetta *Alcippe rufogularis*	6	0	0
		Yellow-throated Fulvetta *Alcippe cinerea*	3	0	0
		Rufous-winged Fulvetta *Alcippe castaneceps*	5	0	0
		Grey-throated Babbler *Stachyris nigriceps*	7	1	0
		Spot-necked Babbler *Stachyris striolata*	2	1	0
		Rufous-capped Babbler *Stachyris ruficeps*	4	0	0
		Greater Necklaced Laughingthrush *Garrulax pectoralis*	3	2	0
		Lesser Necklaced Laughingthrush *Garrulax monileger*	2	0	0
		Scaly Laughingthrush *Garrulax subunicolor*	1	0	0
		Striated Laughingthrush *Garrulax striatus*	1	1	0
		White-crested Laughingthrush *Garrulax leucolophus*	2	0	0
		Black-faced Laughingthrush *Garrulax affinis*	1	0	0
		Chestnut-backed Laughingthrush *Garrulax nuchalis*	2	0	0
		Chestnut-crowned Laughingthrush *Garrulax erythrocephalus*	4	0	0
		Grey-sided Laughingthrush *Garrulax caerulatus*	1	0	0
		Long-tailed Sibia *Heterophasia picaoides*	5	1	0
		Puff-throated Babbler *Pellorneum ruficeps*	9	1	0
		Pygmy Wren Babbler *Pnoepyga pusilla*	1	0	0
		Red-faced Liocichla *Liocichla phoenicea*	1	0	0
		Red-tailed Minla *Minla ignotincta*	1	0	0
		Silver-eared Mesia *Leiothrix argentauris*	3	0	0
		Streaked Wren Babbler *Napothera breavicaudata*	1	0	0
		Wedge-billed Wren Babbler *Sphenocichla humei*	1	0	0
		White-bellied Yuhina *Yuhina zantholeuca*	2	0	0
		Black-chinned Yuhina *Yuhina nigrimenta*	1	0	0
		White-naped Yuhina *Yuhina bakeri*	1	0	0
		White-browed Scimitar Babbler *Pomatorhinus schisticeps*	1	0	0
		White-throated Babbler *Turdoides gularis*	2	0	0
		Yellow-bellied Warbler *Abroscopus superciliaris*	1	0	0
		Yellow-eyed Babbler *Chrysomma sinense*	7	3	0
	Total		35	14 (40)	0

TABLE 2. Continued.

Family	Region	Host species	Sample size	*Plasmodium* positive[e]	*Haemoproteus* positive[e]
Turdidae[a]	Myanmar	Long-tailed Thrush *Zoothera dixoni*	2	0	0
		Lesser Shortwing *Brachypteryx leucophrys*	2	0	0
		Blue Rock Thrush *Monticola solitarius*	1	0	0
		Blue Whistling Thrush *Myophonus caeruleus*	1	0	0
	S. Korea	Grey Thrush *Turdus cardis*	1	0	1
	Total		7	0	1 (14)

[a] Family shared between Myanmar and South Korea.

[b] Family shared between Myanmar and India.

[c] Family shared among Myanmar, India, and South Korea.

[d] Family shared between India and South Korea.

[e] *n* (% positive).

[f] $P \leq 0.001$, Fisher's exact test (among regions).

spp., respectively. Prevalence of parasites observed in families represented in a single region was analyzed separately from families shared among regions (Table 3). As found for shared families, the prevalence of *Plasmodium* spp. and *Haemoproteus* spp. did not differ significantly among these families. We tested the prevalence of parasites for families shared between two or more regions separately and found significant differences in only a few cases (Table 2).

Microscopy and PCR detection

Of 183 blood smears collected in India, 61 individuals were positive for either *Plasmodium* spp. or *Haemoproteus* spp. Of these, 22 (12%) *Plasmodium* and 39 (21.3%) *Haemoproteus* positive samples were detected by examination of blood smears. Results from blood smear examination and PCR where in agreement for only 98 (54%) of the 183 samples.

Phylogenetics

We identified 241 infected birds and obtained sequence for *cyt b* from 221 of these. Within the 197 samples for which we had at least 256 bp of sequence, 34 lineages of *Plasmodium* and 41 of *Haemoproteus* were identified. Within the *Plasmodium* tree (Fig. 2), there were four lineages (LIN 8, LIN 26, LIN 28, LIN 29) shared between India and Myanmar, and one lineage (LIN 15) shared between Myanmar and South Korea. In *Haemoproteus* (Fig. 3), a single lineage (LIN 1) was shared between India and Myanmar. The proportion of parasite lineages sharing identical sequences in more than one host species occurs more frequently ($\chi^2=15.1$, $P=0.035$) in the *Plasmodium* tree than in the *Haemoproteus* tree. This may indicate higher rates of host-switching and reduced host-specificity in *Plasmodium* (see Figs. 2 and 3 for details on host species).

For 20 additional birds, short (91 bp) sequences were obtained from primers F2/R2. These were used to identify parasite genera but were not included in the phylogenetic analysis. These results, however, suggest the presence of the following *Plasmodium* lineages in host species in South Korea: LIN 7—great spotted woodpecker [*Dendrocopos major*], common stonechat [*Saxicola torquata*], red throated pipit [*Anthus cervinus*]; LIN 10—bull-headed shrike [*Lanius bucephalus*], varied tit [*Parus varius*], great tit [*Parus major*], coal tit [*Parus ater*], marsh tit [*Parus*

TABLE 3. Prevalence of hematozoan parasites for avian families recorded within one region.

Region	Host family	Species	Sample size	*Plasmodium* positive (*n*)	*Haemoproteus* positive (*n*)
Myanmar	Alcedidae	Common Kingfisher *Alcedo atthis*	6	1	0
	Strigidae	Asian Barred Owlet *Glaucidium cuculoides*	4	1	0
		Oriental Scops Owl *Otus sunia*	2	0	0
		Spotted Owlet *Athene brama*	1	1	0
	Dicruridae	Ashy Drongo *Dicrurus leucophaeus*	2	2	0
		Bronzed Drongo *Dicrurus aeneus*	1	0	0
		Hair-crested Drongo *Dicrurus hottentottus*	2	0	0
		Lesser Racket-tailed Drongo *Dicrurus remifer*	2	0	0
		Common Green Magpie *Cissa chinensis*	2	0	0
		Gold-billed Magpie *Urocissa flavirostus*	1	0	0
	Aegithinidae	Common Iora *Aegithina tiphia*	1	2	0
	Prionopidae	Common Woodshrike *Tephrodornis pondicerianus*	1	3	1
	Campephagidae	Rosy Minivet *Pericrocotus roseus*	1	0	0
		Scarlet Minivet *Pericrocotus flammeus*	3	0	0
		Small Minivet *Pericrocotus cinnamomeus*	3	1	0
		White-bellied Minivet *Pericrocotus erythropygius*	2	1	0
	Rhipiduridae	White-browed Fantail *Rhipidura aureola*	3	1	0
		White-throated Fantail *Rhipidura albicollis*	3	1	0
	Sittidae	Beautiful Nuthatch *Sitta formosa*	2	0	0
		Chestnut-bellied Nuthatch *Sitta castanea*	5	0	0
India	Cisticolidae	Jungle Prinia *Prinia sylvatica*	1	0	0
		Plain Prinia *Prinia inornata*	4	2	2
		Ashy Prinia *Prinia socialis*	5	1	2
		Grey-breasted Prinia *Prinia hodgsonii*	1	0	0
		Prinia spp.	2	0	2
		Streaked Fantail Warbler *Cisticola juncidis*	2	0	0
	Zosteropidae	Oriental White-eye *Zosterops palpebrosus*	7	2	1
	Estrildidae	Red Munia *Estrilda amandava*	31	11	0
		Spotted Munia *Lonchura punculata*	11	1	0
		White-throated Munia *Lonchura malabarica*	1	0	0
		Black-headed Munia *Lonchura malaca atricapilla*	4	1	0

TABLE 3. Continued.

Region	Host family	Species	Sample size	*Plasmodium* positive (*n*)	*Haemoproteus* positive (*n*)
South Korea	Emberizidae	Black-faced Bunting *Emberiza spodocephala*	9	4	0
		Chestnut Bunting *Emberiza rutila*	7	1	3
		Rustic Bunting *Emberiza rustica*	2	0	0
		Tristram's Bunting *Emberiza tristrami*	2	1	1
		Yellow-throated Bunting *Emberiza elegans*	1	1	0
	Laridae	Black-tailed Gull *Larus crassirostris*	5	0	2
	Laniidae	Bull-headed Shrike *Lanius bucephalus*	10	3	1
	Fringillidae	Common Rosefinch *Carpodacus erythrinus*	1	1	0
		Oriental Greenfinch *Carduelis sinica*	7	1	1
	Scolopacidae	Dunlin *Calidris alpina*	5	0	0
		Red-necked Stint *Calidris ruficollis*	5	0	0
	Anatidae	Mallard *Anas platyrhynchos*	2	0	1
		Spot-billed Duck *Anas poecilorhyncha*	5	0	0

palustris], Daurian redstart [*Phoenicurus auroreus*], leaf warbler [*Phylloscopus* sp.], black-billed magpie [*Pica hudsonia*]; LIN 11—bull-headed shrike [*Lanius bucephalus*], ring-necked pheasant [*Phasianus colchicus*], yellow-browed warbler [*Phylloscopus inornatus*], great tit [*Parus major*]; LIN 12—great tit; LIN 34—vinous-throated parrotbill [*Paradoxornis webbianus*]; LIN 35—great tit. Results suggested the presence of one additional *Haemoproteus* lineage, LIN 41, from a black-billed magpie (*Pica hudsonia*).

Host and parasite diversity

The Shannon diversity indices indicate that host diversity was higher in Myanmar (5.86±0.01) than in India (5.20±0.03) or South Korea (5.20±0.02); parasite diversity was lower in South Korea (3.91±0.01) as compared to Myanmar (4.32±0.02) or India (4.30±0.03). The host and parasite ratio was 82% in India followed by South Korea (75%) and Myanmar (73%).

DISCUSSION

The overall prevalence (*Haemoproteus* or *Plasmodium*) was higher in India (tropical zone); the highest number of unique *Haemoproteus* lineages also was observed among birds sampled in India. The lower parasite prevalence observed for Myanmar could be attributed to differences in habitat as most of the birds from Myanmar were sampled from high altitudes where less exposure to vectors may have occurred. On the other hand, the comparison between low-elevation habitats of India and Myanmar (tropical zone) and South Korea (temperate zone) showed no significant difference in prevalence for either parasite. Our results are not entirely consistent with previous studies that have used PCR-based techniques. Durrant et al. (2006) found that tropical zones had higher parasite prevalence than temperate zones, whereas Ricklefs (1992) showed that temperate

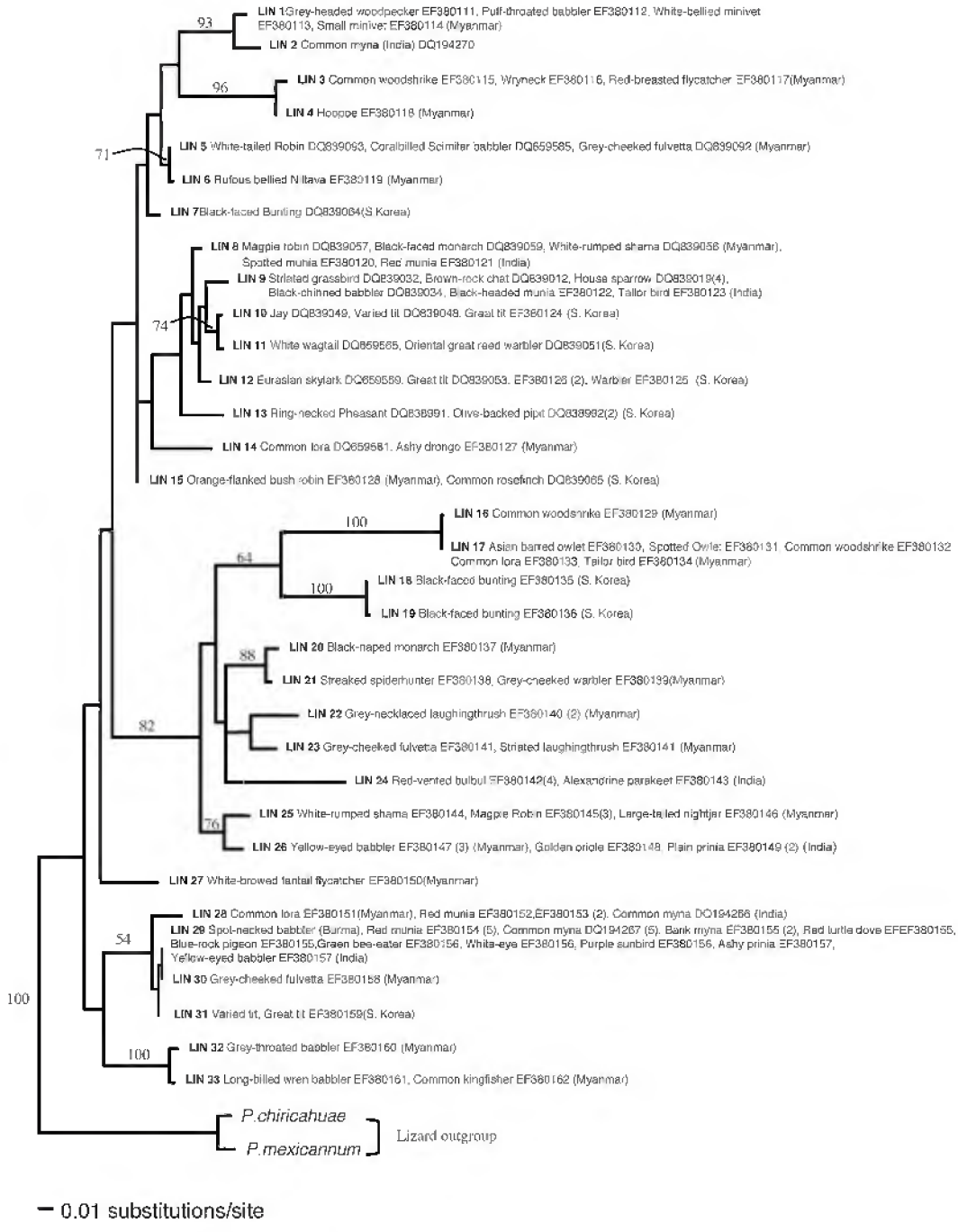

FIGURE 2. Relationships of *Plasmodium* lineages based on maximum likelihood using the model GTR+I+G. Each lineage represents host species and GenBank number.

birds had higher parasite prevalence. These differences may result from seasonal differences in timing of sampling or diagnostic techniques. In this study the correspondence between PCR and microscopy results for samples from India was poor. This may have resulted from a low-intensity peripheral parasitemia and

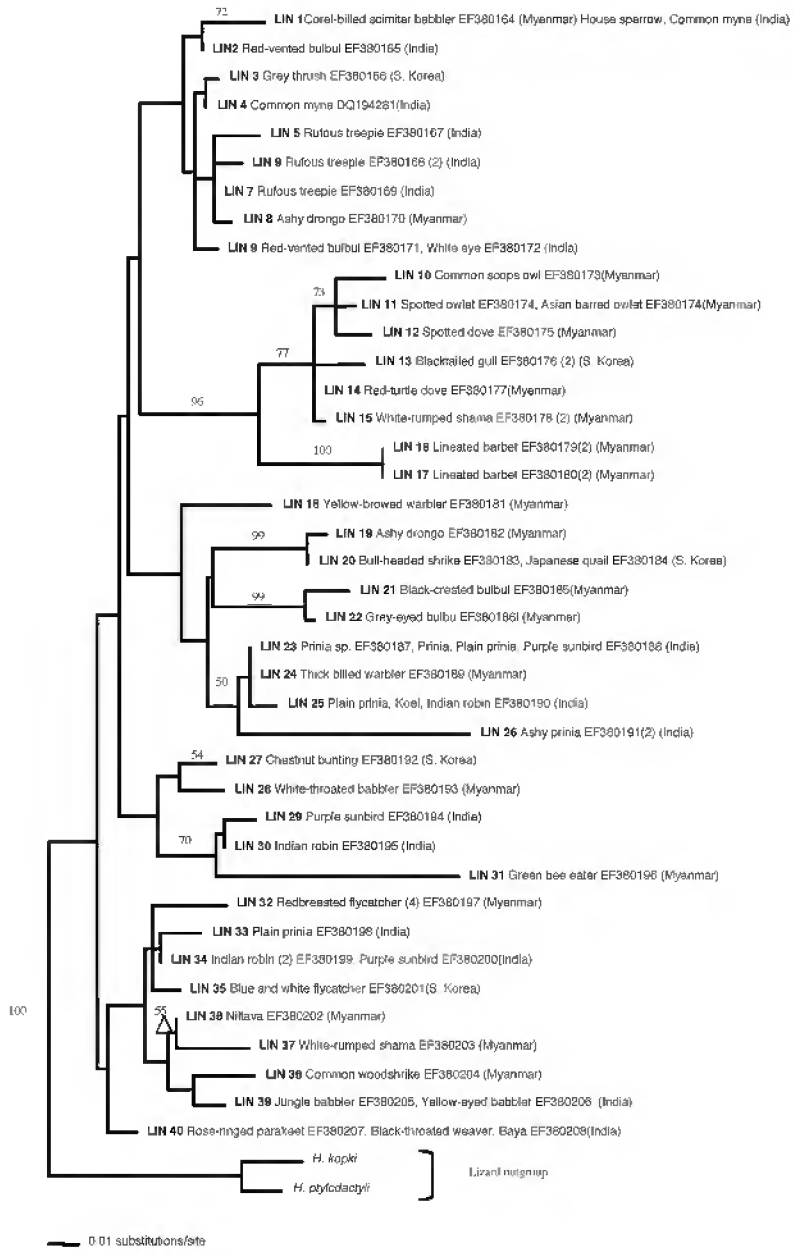

FIGURE 3. Relationships of *Haemoproteus* lineages based on maximum likelihood using the model GTR+I+G. Each lineage represents host species and GenBank number.

variation in diagnostic sensitivity and is consistent with results from the previous studies where blood smears were evaluated by both methods (Jarvi et al., 2002; Richard et al., 2002). We are confident that the prevalence results from this study are not biased by testing a combination of blood and tissue samples; such differences have not been apparent in our previous studies (Ishtiaq et al., 2006; Pagenkopp, 2006) using such samples.

The prevalence of parasites differed at the regional level but not at host family level. This finding is consistent with pre-

vious studies (Hellgren, 2005; Durrant et al., 2006). Temporal and spatial variation in parasite prevalence has been demonstrated (Schall and Marghoob, 1995; Bensch and Akesson, 2003), and it is likely that different parasite lineages are associated with particular vector communities. This factor may explain the differences in the geographic distribution of parasite lineages at different sites. The *Haemoproteus* tree shows regional clades, whereas the *Plasmodium* clades are "scattered" between regions and host species.

Plasmodium and *Haemoproteus* are widely distributed blood parasites that have been reported in many families of birds. Migrants can form a link for the transmission of parasites between tropical and temperate resident birds, especially among taxonomically similar hosts. Some sharing of parasite lineages between resident and migratory songbirds has been observed in Nigeria, indicating parasite transmission between migrants and residents on the wintering grounds (Waldenström et al., 2002). As a major wintering ground for hundreds of avian species, we would expect more shared parasite lineages between India and Myanmar because migrants utilizing these area originate from the same biogeographic region and share similar host families. The *Plasmodium* phylogenetic tree shows four shared lineages between these two regions (Fig. 1A). Birds migrating to South Korea do not appear to use the same flyway as birds migrating to India and Myanmar, and this is reflected by the few shared lineages between these regions; only one *Plasmodium* lineage was shared between Myanmar and South Korea (Fig. 1A). Although we found similar indices of parasite diversity in India and South Korea, the host-parasite ratio was higher in India, indicating more parasites infecting several host species than in South Korea or Myanmar. India had high parasite prevalence and highest number of unique parasite lineages as well.

The phylogenetic tree differentiates *Haemoproteus* and *Plasmodium* lineages; a well-supported topology is not necessary for our analyses, but it clearly defines lineages shared between different regions. There is some evidence of geographic structuring of parasite lineages in the *Plasmodium* tree as indicated in other studies (e.g., Beadell et al., 2006). In our parasite phylogeny (Fig. 2), more lineages of *Plasmodium* are shared between multiple host species than are of *Haemoproteus*, which is indicative of host switching (Bensch et al., 2000; Ricklefs and Fallon, 2002; Waldenström et al., 2002; Beadell et al., 2004) and supports *Haemoproteus* as being relatively more host specific than *Plasmodium* (Atkinson and van Riper, 1991).

Variation in the abundance of dipteran vectors at a particular geographic location is positively correlated with variation in the prevalence of parasites in the avian host community (Bennett and Cameron, 1974) and also may be related to variation in the intensity of parasitic infection. Host habitat choice is known to influence parasite load in host species as it can affect the frequency of contact with flying vectors of hematozoan parasites, which are mainly associated with wet and closed habitats such as the forest canopy (Scott and Edman, 1991). We did find a slight difference in prevalence of *Haemoproteus* in high- compared with low-elevation habitat in Myanmar and India but prevalence of *Plasmodium* remained the same, suggesting more exposure to ceratopogonids and hippoboscids at low elevations. Comparison between low-elevation habitats in India and Myanmar may have been affected by the timing of sampling in two regions. Samples from India were collected between May and June, which is the peak breeding season for lowland resident species, while winter (November–March) was the sampling period in Myanmar and October in South Korea. Parasite load is known to increase in spring–summer owing to the increased abundance of flying vectors at that time (Bennett and Ca-

meron, 1974; Atkinson and Van Riper, 1991) and because higher levels of sexual steroid hormones are circulating in the bird's blood, which in general depresses the immune system and allows parasites to survive (Wedekind and Følstad, 1994; Saino et al., 1995).

The Asian region harbors a diverse community of avian hematozoan lineages that were distributed among 41% of the birds sampled in this study. Prevalence varied between regions but not at the host family level. An in-depth study on the distribution of vector communities and their relationship to hematozoa in these regions would be useful and may provide some insight into the regional distribution of specific hematozoan lineages.

ACKNOWLEDGMENTS

Part of this study was funded by a National Institutes of Health grant (1R01GM063258). We would like to thank the Ministry of Environment and Forests, Government of India; Myanmar Forestry Ministry, Nature and Wildlife Conservation Division (Myanmar Collection and Export Permit 11853-55/96, UYin-Office/TWMC/2303/2002, SI/4697/2004) for permits to catch birds for blood and tissue samples. The Smithsonian expedition to South Korea was supported by a grant from the U.S. Department of Defense–Legacy Resource Management Program (DACA87-03-H-0006). We thank C. McIntosh, K. Durrant, and D. K. Sharma for facilitating laboratory analyses. We would also like to thank R. Jakati, A. Choudhury, R. Bharagava, H. Singha, K. Lahkar, B. P. Lahkar, and S. Barua for help in organizing field camps in Assam, and Cho Sok Hun, 51 Fighter Wing, Osan, Air Base for organizing field work in South Korea.

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Received for publication 17 August 2005.