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CHAPTER 7

AVIAN HEMATOZOA IN SOUTH AMERICA: A COMPARISON OF TEMPERATE AND TROPICAL ZONES

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Abstract.—We used screening techniques based on polymerase chain reaction (PCR) to explore the avian hematozoan parasites (*Plasmodium* spp. and *Haemoproteus* spp.) of two previously uninvestigated regions of continental South America. Comparisons of tropical-zone Guyana and temperate-zone Uruguay revealed that overall prevalence of *Plasmodium* and *Haemoproteus* species detected in a diverse sampling of potential hosts was significantly higher in Guyana. The difference in prevalence between the two geographic zones appears to be attributable to ecological differences rather than taxonomic sampling artifacts. Diversity of hematozoan haplotypes was also higher in Guyana. We found no relationship between hematozoan haplotype and host family sampled within or between regions. We found very few *Plasmodium* and no *Haemoproteus* haplotypes shared between the two regions, and evidence of geographic structuring of hematozoan haplotypes between the two regions. We suggest that a lack of hematozoan haplotype transmission between the two regions may be attributable to the migratory patterns of each region's avian hosts. *Received 11 April 2005, accepted 21 November 2005.*

Resumen.—Usamos técnicas de investigación basadas en reacción en cadena de polímeros (RCP) para explorar hemoparásitos avícolas (*Plasmodium* spp. y *Haemoproteus* spp.) en dos regiones no investigadas de Sudamérica. Las comparaciones de la zona tropical de Guyana y de la zona templada de Uruguay revelaron que la frecuencia general de especies de *Plasmodium* y *Haemoproteus* encontrados en una muestra diversa de hospederos potenciales fue significativamente más alta en Guyana. La diferencia en frecuencia entre las dos zonas geográficas aparentemente se debe a diferencias ecológicas que debido al muestreo taxonómico. La diversidad de hematozoos haplotípicos fue también más alta en Guyana. No encontramos una relación entre hematozoos haplotípicos y familias de hospederos muestreados dentro o entre las regiones. Encontramos solo algunos cuantos haplotipos de *Plasmodium* en común entre las dos regiones, pero no se encontraron haplotipos de *Haemoproteus*, ni evidencia de una estructuración geográfica de haplotipos de hematozoos entre las dos regiones. Por lo que sugerimos que la ausencia de transmisión de haplotipos de hematozoos entre las dos regiones puede ser atribuida a los patrones de emigración, para cada región, de las aves hospederas.

Hematozoan parasites (*Plasmodium* spp. and *Haemoproteus* spp.) are commonly found in blood smears from birds on every continent except Antarctica (Bennett et al. 1993). Hematozoan prevalence may differ between geographic locations, and climate may play an important role in this difference by influencing the density of vectors or potential hosts or the ease of transmission. Comparison of hematozoan parasites of temperate and tropical zones may reveal differences related to climatic factors. For example, Ricklefs (1992), surveying results from analyses based on blood smears, found a 2.6× greater infection rate in temperate than in tropical zones. Temperate and tropical

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regions may differ in the types of disease vectors that occur, or in the distribution and patterns of movement of potential hosts during migration (Ricklefs et al. 2005).

Application of genetic techniques based on polymerase chain reaction (PCR) for screening avian hematozoan infections has resulted in a number of recent papers on regional surveys concentrating on a few areas of the globe: Africa (Waldenström et al. 2002), North America (Fallon et al. 2003, Ricklefs et al. 2005), and the Australo-Papuan region (Beadell et al. 2004). Thus far, the South American continent has not been surveyed using these PCR-based screening techniques.

The new screening techniques are also uncovering a wide array of phylogenetically varied lineages of avian hematozoa (Perkins and Schall 2002). It has been proposed that the many newly discovered hematozoan haplotypes may correspond to new species (Perkins 2000, Bensch et al. 2004). Haplotypes of hematozoa are often shared between geographic regions, carried by migratory birds as they travel between wintering and breeding grounds (Waldenström et al. 2002, Ricklefs et al. 2005). Migratory movement is generally between a tropical-zone wintering ground and a temperate-zone breeding ground. Therefore, birds that are found in two disjunct geographic and ecological regions may be expected to share hematozoa with common haplotypes if conditions for transmission are met in each region.

Avian hematozoa were previously classified partially on the basis of the host taxon, and some species were believed to be host specific, particularly in the genus *Haemoproteus* (Atkinson and Van Riper 1991). If this holds, host species that occur in each of two separate geographic and ecological regions may be expected to share phylogenetically similar hematozoan parasites. Alternatively, hematozoan haplotypes found in many different host species may indicate host switching, or a greater range of host sharing than previously expected.

Our aims were: (1) to estimate avian hematozoan prevalence within and between two climatic zones, temperate and tropical, on the South American continent, using PCR-based screening methods; (2) to determine the phylogeny of the hematozoan haplotypes found in the two regions; (3) to explore host specificity of hematozoan haplotypes within and between each region; and (4) to examine the degree of sharing of hematozoan haplotypes between the two zones to determine the potential for transmission between regions.

Methods

Sample collection and processing.—Tissue samples were obtained from birds collected during U.S. National Museum of Natural History (USNM, Smithsonian Institution) collecting expeditions to Guyana (1994–2000) and Uruguay (2002–2003). Voucher specimens for tissues are in the USNM collection (numbers available on request). Samples in each region were subsets of the total number of species collected and were chosen to reflect a variety of potential hosts for avian hematozoa, mostly passerines but also representatives of other groups, such as doves, parrots, and waterfowl. Taxonomy follows that used on the Handbook of the Birds of the World Internet Bird Collection website (see Acknowledgments). Samples came from many locations and habitat types in both regions (Fig. 1). Geographic coordinates of each locality are given in Table 1. Samples from tropical-zone Guyana were collected during the dry season (n = 184) or toward the end of the wet season (n = 11). Samples from temperate-zone Uruguay were collected during August–September 2002 and June–July 2003.

Some birds collected in Uruguay had thin blood smears prepared from a small volume of peripheral blood for hematozoan detection (n = 141). These samples were fixed in 95% ethanol and stained with Giemsa as described in Garnham (1966). Blood smears were scanned under a microscope for 10 min at 400× magnification. In this time limit, ~20,000 erythrocytes could be examined, the estimate for passerines being 455 erythrocytes per field at this magnification (Gering and Atkinson 2004). Intense infections may have as many as one parasitemia per 2,000 erythrocytes (Godfrey et al. 1987), whereas extremely mild infections may have <1 parasitemia per 50,000 erythrocytes (Jarvi et al. 2002); our method falls between the two, striving for maximum efficiency. We noted the presence or absence of parasitemia for each individual, but not the intensity of infection. Each hematozoan detected was digitally photographed using a Nikon Coolpix 4500 camera and identified from these photos by M. Peirce to genus and species where possible using hematozoan morphological characteristics and host taxonomy. A subset of the samples was examined both by blood smear analysis and PCR-based screening techniques (n = 85).

We extracted host and parasite DNA from the tissue samples using the manufacturer's protocols supplied with DNeasy kits (Qiagen, Valencia, California). We tested the quality of each DNA extraction by amplifying a small fragment of avian cytochrome-*b* (cyt *b*) DNA using primers cytb-2RC and cytb-wow (268 base pairs [bp]) (Dumbacher et al. 2003). This amplification was successful in all cases.

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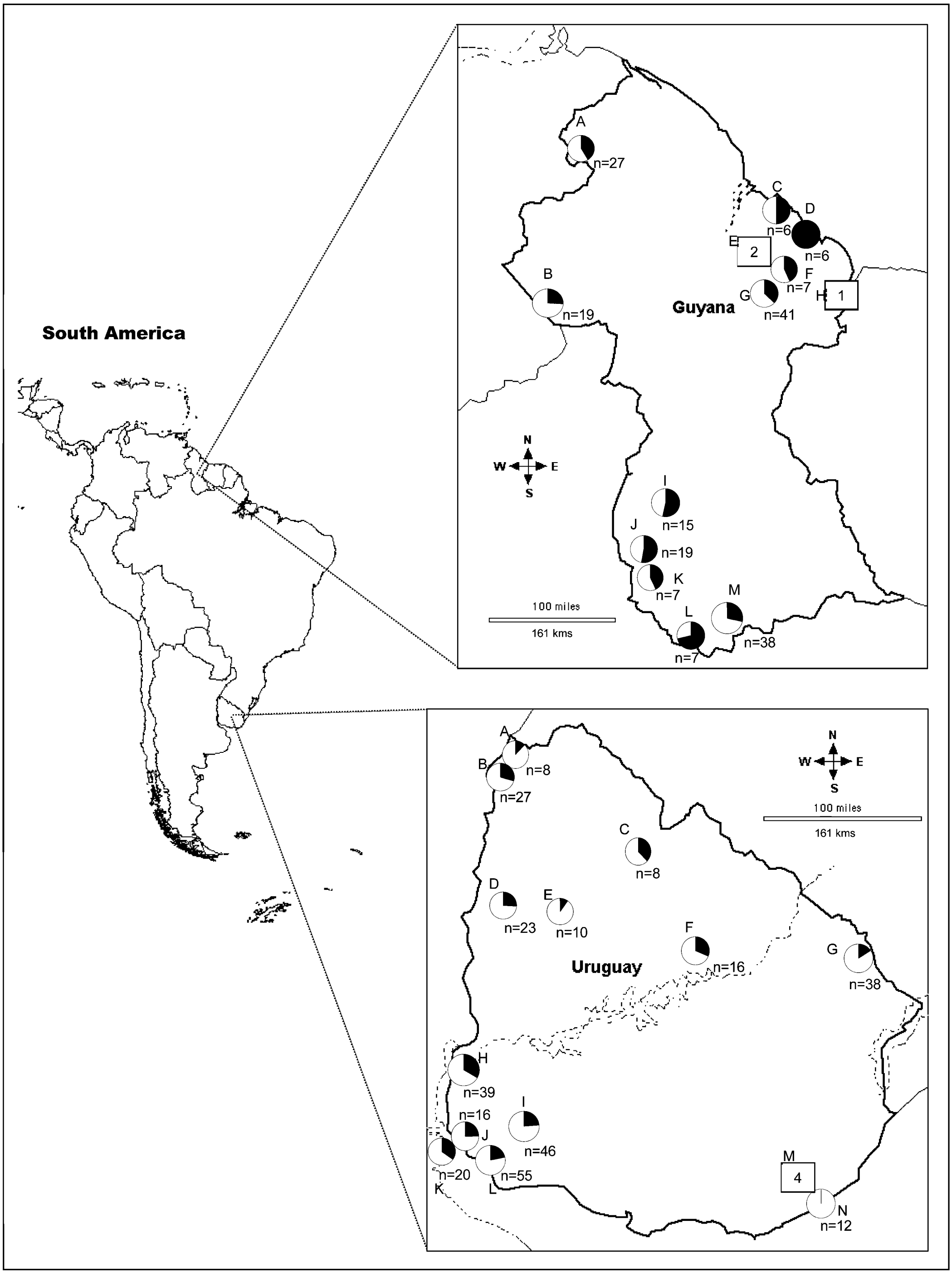

FIG. 1. Map showing prevalence at each sampling location for (A) tropical-zone Guyana and (B) temperate-zone Uruguay. Numbers in gray boxes are sample sizes too low for prevalence charts at certain sites. Sites are designated by letters that refer to Table 1, where site names and site coordinates are given.

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TABLE 1. Sampling sites from Figure 1. Localities and latitude and longitude coordinates for bird species sampled in Guyana and Uruguay.

Country	Site	Nearest named locality	Latitude	Longitude
Guyana	A	Baramita	07°22′N	–60°29′W
	B	Waruma River	05°30′N	–60°47′W
	C	Hope	06°45′N	–57°56′W
	D	Onverwagt	06°27′N	–57°38′W
	E	Linden Highway	06°19′N	–58°12′W
	F	Wiruni River	05°45′N	–57°56′W
	G	Berbice River	05°40′N	–57°53′W
	H	South of Corriverton	05°46′N	–57°11′W
	I	Wiwitau Mountain	02°52′N	–59°16′W
	J	Karaudanawa	02°22′N	–59°27′W
	K	Parabara Savannah	02°12′N	–59°22′W
	L	Acarai Mountains	01°23′N	–58°56′W
	M	Upper Essequibo River	01°39′N	–58°73′W
Uruguay	A	Bella Union	–30°19′S	–57°37′W
	B	Colonia Palma	–30°30′S	–57°45′W
	C	Tacuarembo	–31°17′S	–56°01′W
	D	Quebracho	–31°49′S	–57°39′W
	E	Colonia Guaviyu	–31°48′S	–57°01′W
	F	Rio Negro	–32°17′S	–55°27′W
	G	Rio Branco	–32°17′S	–53°47′W
	H	Isla de Lobos and Viscaino	–33°22′S	–58°20′W
	I	Cardona	–33°47′S	–57°20′W
	J	Carmelo	–33°59′S	–58°18′W
	K	Isla Juncal	–33°59′S	–58°22′W
	L	Conchillas	–34°11′S	–58°00′W
	M	Rocha	–34°29′S	–54°20′W
	N	La Paloma	–34°39′S	–54°07′W

We screened each potential host's sample multiple times using different primer sets designed to amplify *Plasmodium* and *Haemoproteus*. Short fragments were initially amplified using each of three primer sets: F2/R2 (132 bp, mitochondrial DNA [mtDNA] cyt *b*); 850F/1024R (167 bp, mtDNA COIII) (see Beadell et al. 2004); and 213F/372R (160 bp, mtDNA cyt *b*) (Beadell and Fleischer 2005). Polymerase chain reactions typically followed conditions developed for "ancient" DNA, to increase the probability of successful amplification of possibly degraded samples or samples with low levels of parasitemia (Fleischer et al. 2000). Samples that produced ultraviolet-visible bands after electrophoresis of the PCR product, indicating infection with one or more hematozoa, were re-amplified with primers designed to target longer fragments of mtDNA cyt *b*: 3760F/4292R (574 bp); or a combination of either F1 or F3 with 4292R (475 bp or 336 bp, respectively) (Beadell et al. 2004); or FIFI/FIRI (423 bp) or a combination of FIFI with another reverse primer (Ishtiaq et al. 2006). Use of multiple primers that amplify a variety of hematozoan mitochondrial haplotypes likely reduced bias toward parasites from a particular region within South America. In addition, the sensitivity of the tests we conducted suggests that we were able to identify infections that might have low or variable levels of parasitemia and not be visible on smears.

Polymerase chain reaction products that produced the largest fragment for an infected sample were purified using Qiaquick kits (Qiagen) and bidirectionally sequenced on an ABI 3100 Sequencer (Applied Biosystems, Foster City, California). Sequences were assembled, aligned, and edited using the SEQUENCHER, version 4.1 (Gene Codes, Ann Arbor, Michigan). The sequences returned were of high quality and there were no gaps in the resulting alignments.

To identify the genus of hematozoan present (*Plasmodium* or *Haemoproteus*), we used both the phylogenetic alignment of sequences and the results of a restriction enzyme test on positive amplifications using the 213F/372R primer pair (Beadell and Fleischer 2005). This resulted in identification of the genera of hematozoa in most infected samples, including mixed infections.

Phylogenetic analysis.—Samples with sequences >186 bp long were used to estimate parasite phylogenetic relationships. One or more base differences between sequences defined a unique haplotype, and we combined unique haplotypes from both regions into a single data set. We reconstructed a phylogenetic tree

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using a heuristic search method, a distance criterion, and a Kimura two-parameter evolutionary model in PAUP* (Swofford 1999). We rooted the tree with mammalian *Plasmodium* cyt-*b* sequences (GenBank accession nos. AY069614, AF069624, AF055587, AY099051, AY283019, and AF069610), following the phylogeny developed by Perkins and Schall (2002).

Statistical analysis.—Basic statistics were performed using SPSS, version 10.0.5 (SPSS, Chicago, Illinois). A Shannon diversity index and its standard deviation were calculated for the bird host species sampled in each region and the hematozoan haplotypes detected in each region, using the program ESTIMATES, version 7.00 (see Acknowledgments). The Shannon diversity index gave a cumulative estimate of the diversity of each sample, given the sample size and the number of individuals representing each species. We designed a nested analysis of variance (ANOVA) using SAS, version 8.2 (SAS Institute, Cary, North Carolina), to determine whether there was variation in prevalence among host family groupings. This was done to account for the potential of differential prevalence of infection among various species within each family to drive apparent family-level infection-prevalence differences. The nested ANOVA was composed of the proportion of infected individuals (arcsine-transformed square root of the number of infected individuals, divided by total number of that species sampled) nested within host species, and species nested within families. The analysis was split by the genus of the infecting hematozoa, and only non-mixed infections that were positively identified as either *Plasmodium* or *Haemoproteus* were included. Only host families that were well sampled (>10 individuals) in each region were included in the analysis.

Results

Prevalence within regions.—In Guyana, we sampled 195 birds belonging to 53 species, 35 genera, and 10 families; 82 (42.1%) of these birds were infected with a hematozoan (Table 2). Of non-mixed infections that could be positively identified to one genus or the other (n = 54), 64.8% were *Plasmodium* and 35.2% were *Haemoproteus*, and there were significantly more infections by *Plasmodium* than by *Haemoproteus* ($\chi^2 = 4.74$, $P = 0.029$). Prevalence did not vary significantly across sampling sites within Guyana ($\chi^2 = 17.73$, $P = 0.060$; see Fig. 1A).

In Uruguay, we sampled 322 birds, belonging to 111 species, 89 genera, and 41 families; 78 (24.2%) of these were infected with a hematozoan (Table 2). Of non-mixed infections that could be positively identified to one genus or the other (n = 59), 81.3% were *Plasmodium* and 18.6% were *Haemoproteus*, and there were significantly more infections by *Plasmodium* than by *Haemoproteus* ($\chi^2 = 23.20$, $P < 0.001$). The prevalence did not vary significantly across sampling sites within Uruguay ($\chi^2 = 11.90$, $P = 0.454$; see Fig. 1B). We also found no difference in prevalence across sampling months in Uruguay ($\chi^2 = 4.69$, $P = 0.196$).

Smear data: Uruguay.—Of 141 blood-smear samples from Uruguay, 4 (3%) were infected with hematozoa, 2 with *Plasmodium*, and 2 with *Haemoproteus*. This represents a much lower overall prevalence for the region than indicated by PCR-based screening techniques. Of 85 Uruguayan samples that were tested using both techniques, only 2 (2%) were detected as infected using blood-smear analysis, versus 22 (26%) using PCR-based screening. Only one of the smear-based positives was also positive on PCR analysis, and both techniques identified the parasite as belonging to the genus *Plasmodium*. The other identified a *Haemoproteus* on the smear that was not amplified with PCR screening techniques.

Prevalence between regions.—There were significantly more hematozoan infections in Guyana than in Uruguay ($\chi^2 = 18.06$, $P < 0.001$). There were significantly more infections of *Haemoproteus* in Uruguay than in Guyana ($\chi^2 = 17.83$, $P < 0.001$), but no highly significant difference in the proportion of *Plasmodium* infections between the two regions ($\chi^2 = 3.59$, $P = 0.058$).

There were 7 potential host species shared between the two geographic regions and an additional 13 shared genera, though with different species in each region. Of this pool, 96 individuals from Guyana (41.7%) were infected with either *Haemoproteus* or *Plasmodium*. Guyanan birds that also had representatives at the generic level in Uruguay were infected with *Plasmodium* in 57.5% of cases, and with *Haemoproteus* in 25.0% of cases. Overall prevalence did not differ from that in Uruguay (n = 60), where 26.7% of shared bird genera were infected with a hematozoan parasite ($\chi^2 = 3.61$, $P = 0.057$). *Haemoproteus* in Uruguay was represented at a similar frequency as in Guyana: 25.0% ($\chi^2 = 1.80$, $P = 0.180$). The frequency of *Plasmodium* infections, comprising 50.0% of infections in Uruguay, was significantly less than the frequency found in Guyana ($\chi^2 = 5.21$, $P = 0.02$).

There were four well-sampled families of birds common to both regions (n = 8 to 57

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TABLE 2. Number and percentage of individuals infected with each type of hematozoan in each sampling region. Percentages for types of infections indicate the proportion of total infections that type comprised. "Unknown genera" refers to parasite infections that could not be amplified with primers other than F2/R2 to provide information on generic status. Mixed infections occurred when there was more than one sequence returned for an infected individual or restriction enzyme tests indicated that both *Plasmodium* and *Haemoproteus* were present.

Region	Total sample (n)	Infected individuals	*Plasmodium* only (single infection)	*Haemoproteus* only (single infection)	Unknown genera	Mixed infections[a]
Guyana	195	82 (42.1%)	35 (42.7%)	19 (23.2%)	12 (14.6%)	6 P/P
						2 P/?
						5 P/H
						3 H/?
Total						16 (19.5%)
Uruguay	322	78 (24.2%)	48 (61.5%)	11 (14.1%)	10 (12.8%)	6 P/P
						2 P/?
						1 P/H
						0 H/?
Total						9 (11.5%)

[a] P = *Plasmodium*, H = *Haemoproteus*, ? = either P or H.

individuals per family). The proportion of birds infected with hematozoa did not differ between regions, except for a greater prevalence of infection among Guyanan Cardinalidae (Fig. 2).

Prevalence across families.—Nested ANOVA showed that there was no variation in prevalence among the family grouping of sampled hosts for either *Plasmodium* or *Haemoproteus* infections in either region (Table 3).

Phylogenetics.—We detected 23 distinct haplotypes of *Plasmodium* and 15 of *Haemoproteus* in the sample from Guyana (GenBank Acession nos. DQ241508–DQ241559, inclusive). We subsequently removed four of these (three *Plasmodium* and one suspected *Haemoproteus*) from the phylogenetic analyses, because the sequences recovered from 10 host individuals were only 91 bp long and their distinctiveness could not be affirmed when the sequences were compared with those from Uruguay. The mitochondrial haplotypes used in the phylogenetic analysis were detected in 60 host individuals (Appendix).

We detected 14 distinct haplotypes of *Plasmodium* and 7 of *Haemoproteus* in the sample from Uruguay. The mitochondrial haplotypes were detected in 48 host individuals (Appendix). All sequences were of sufficient length (>186 bp) to be included in the combined data for both regions. There were no significant differences between regions in numbers of *Plasmodium* ($\chi^2 = 2.19$, $P = 0.139$) or *Haemoproteus* ($\chi^2 = 2.91$, $P = 0.88$) haplotypes detected.

The phylogenetic tree produced for both regions combined resulted in a single clade of 31 *Plasmodium* haplotypes, and a single well-supported clade of 21 *Haemoproteus* haplotypes (Fig. 3). Some subclades were unique to one region or the other but were not well supported by bootstrap values. The well-supported clade of *Haemoproteus* haplotypes numbered 49–52 (Fig. 3) in Guyana may reflect an unequal sampling across families rather than a unique grouping, given that they appear to be restricted to the host family Columbidae, and fewer doves and pigeons were sampled in Uruguay ($n = 8$) than in Guyana ($n = 28$). Only three haplotypes (1, 23, and 31) were shared between the two regions, all *Plasmodium*. None of these occurred in the same host species in the two regions and, in some cases, they were not found in the same host family, which suggests that these may be highly generalized lineages of parasites (Table 4). Shannon diversity indices indicate that diversity was higher in the Uruguayan sampling of potential bird hosts but that diversity of hematozoa haplotypes was higher in the Guyanan sample (Table 5).

DISCUSSION

Prevalence of hematozoan infection was significantly higher overall in Guyana. Genera and species common to both regions had higher rates of infection in Guyana than in Uruguay. This is in contrast to findings based

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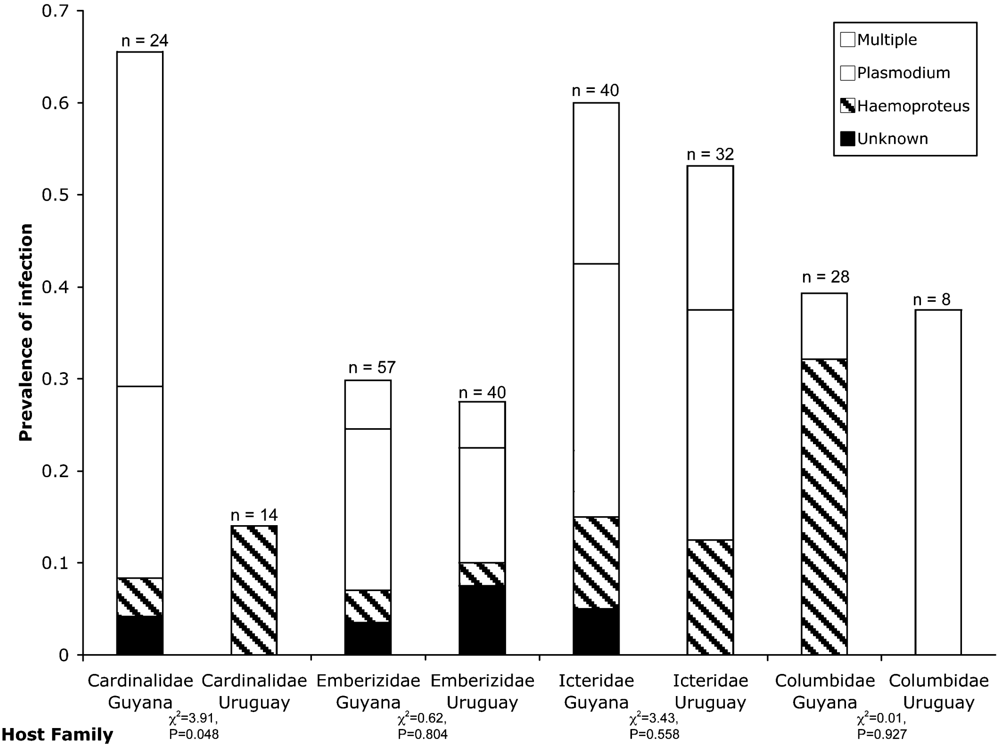

FIG. 2. Prevalence of hematozoan infection detected within selected host family groups that were sampled in each region. Chi-square tests indicate significant differences in prevalence within families between regions. "Unknown" refers to parasites that were not amplified by any other primer than F2/R2, so that assignation of genus was impossible to do with certainty.

TABLE 3. Results of nested ANOVAs designed to estimate the effect of the sampled host family on the prevalence of *Plasmodium* and *Haemoproteus* lineage infections for each region.

Region	Families (*n*)	Species (*n*)	Individuals (*n*)	Infection	Percentage of variation attributable to family	Percentage of variation attributable to other sources	*F*	*P*
Guyana	6	47	174	*Haemoproteus*	0.00	100.00	0.18	0.97
				Plasmodium	10.11	89.89	1.81	0.13
Uruguay	9	62	313	*Haemoproteus*	3.76	96.24	1.10	0.38
				Plasmodium	9.82	90.18	1.70	0.12

on blood-smear analyses, in which birds from temperate zones showed higher rates of infection than birds in tropical zones (Ricklefs 1992). Our pattern is not conclusively carried through to the family level of organization for the host species; although all families well-represented in both regions displayed higher prevalence in Guyana, only the Cardinalidae showed significantly higher prevalence of hematozoan infection in Guyana. This may have resulted from a biased sampling regime in one region or the other, so that potential host species of birds were missed in the region that displayed lower prevalence. However, because the Uruguayan sample of potential host species is actually more diverse than the sample from Guyana, this does not appear to be the case. The higher prevalence in temperate zones found by earlier researchers (Ricklefs 1992) may be an artifact of the blood-smear technique, in which infections are more easily detected when parasitemia levels are high, whereas low-intensity infections

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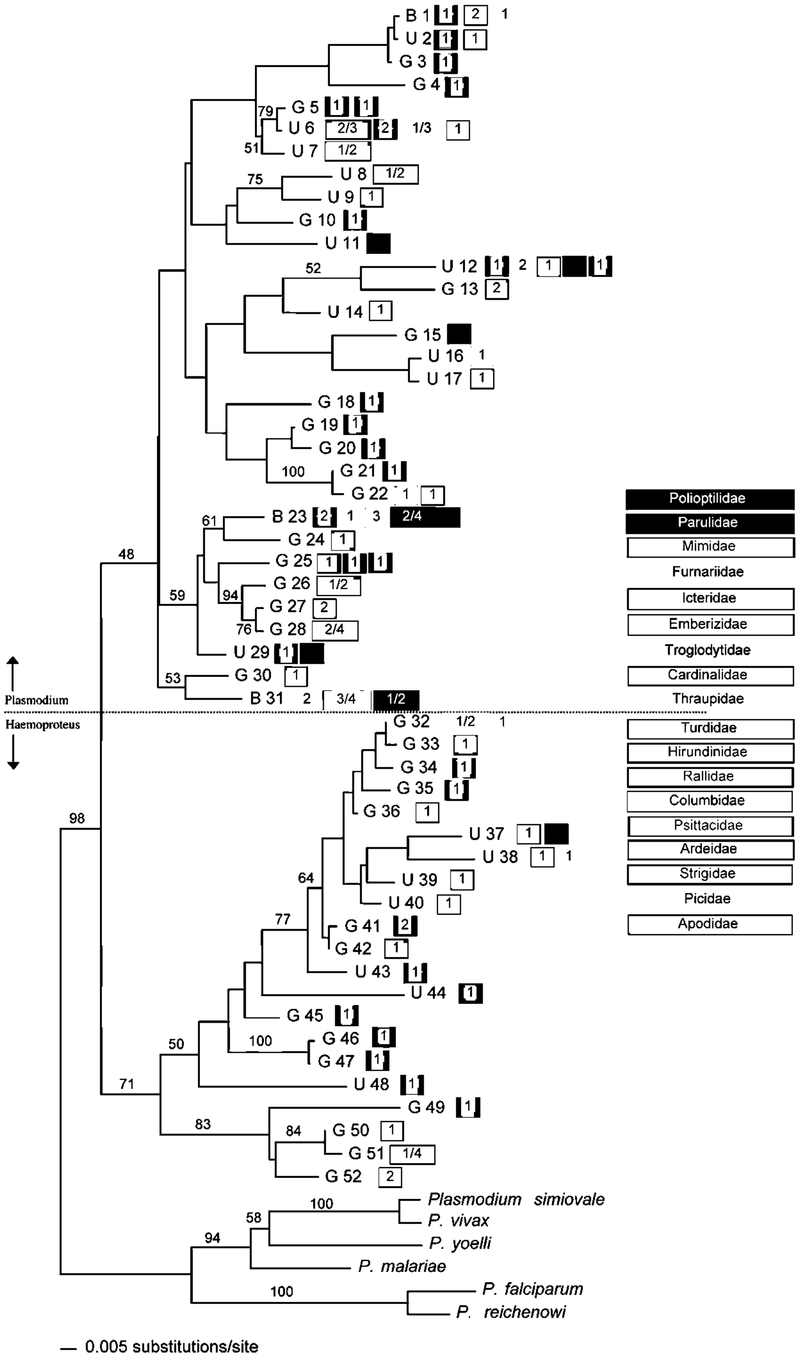

FIG. 3. Neighbor-joining tree created with a heuristic search method and a Kimura two-parameter evolutionary model, rooted with mammalian *Plasmodium* outgroups. Bootstrap (PAUP* "fast" heuristic search) values ≈/> 50% are included on relevant branches. Haplotype numbers are preceded by the region in which they were detected in (G = Guyana, U = Uruguay, B = both regions). Haplotypes are related to host species in which they were found; numbers refer to the number of species in which haplotypes were detected (where there is >1 individual per species, number of individuals follows the backslash).

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TABLE 4. Host family, genus, and species by region for *Plasmodium* haplotypes detected in both regions.

Haplotype	Region	Family	Genus	Species
1	Guyana	Emberizidae	*Dolospingus*	*fringilloides*
		Icteridae	*Icterus*	*nigrogularis*
				chrysocephalus
	Uruguay	Troglodytidae	*Troglodytes*	*aedon*
23	Guyana	Emberizidae	*Volatinia*	*jacarina*
		Icteridae	*Cacicus*	*cela*
				haemorrhous
		Psittacidae	*Ara* (*Diopsittaca*)	*nobilis*
	Uruguay	Emberizidae	*Zonotrichia*	*capensis*
		Furnariidae	*Cranioleuca*	*pyrrhophia*
		Icteridae	*Gnorimopsar*	*chopi*
		Paraulidae	*Basileuterus*	*culicivorous*
				leucoblepharus
				leucoblepharus
				leucoblepharus
31	Guyana	Cardinalidae	*Cyancompsa*	*cyanoides*
			Pitylus	*grossus*
			Saltator	*maximus*
				maximus
		Icteridae	*Cacicus*	*cela*
	Uruguay	Icteridae	*Agelaius*	*ruficapillus*
		Polioptilidae	*Polioptila*	*dumicola*
				dumicola

TABLE 5. Sample sizes and Shannon diversity indices (means ± SD) for bird host samples in both regions and hematozoan haplotypes detected in both regions.

Region	Sample	Individuals (n)	Shannon diversity index
Guyana	Bird host species (n = 54)	195	3.85 ± 0.01
Uruguay	Bird host species (n = 111)	322	4.44 ± 0.01
Guyana	Hematozoan haplotypes (n = 34)	60	3.37 ± 0.01
Uruguay	Hematozoan haplotypes (n = 21)	48	2.70 ± 0.02

may not be detected in blood smears—in which case, PCR-based analyses may be more reliable. It may be that parasitemia levels as well as prevalence differ between the two zones and the difference in prevalence previously observed is actually reversed, with higher rates in tropical zones and lower in temperate zones.

Although seasonality of sampling may have affected our results, there were no temporal differences in prevalence nor evidence of a spring-time spike in rates of hematozoan infection in Uruguay. In Guyana, most samples were collected during the dry season, when density of mosquito vectors for *Plasmodium* is comparatively lower than during the wet season, yet hematozoan prevalence was still higher than in Uruguay.

The Uruguayan specimens that were examined using both PCR-based techniques and blood smears showed an approximately 10-fold difference in incidence of hematozoa, with PCR-based techniques detecting many more infections. Supposed differences in prevalence of infection claimed to be attributable to seasonality may be another artifact of the blood-smear technique, which may be biased toward high levels of parasitemia. Among avian hosts from American Samoa examined using both blood smears and PCR-based techniques, no infections were detected in the smears, whereas 59% of the sample showed infection using PCR-based techniques (Jarvi et al. 2003). Thus, PCR-based screening techniques should be superior for detecting low-intensity infections, regardless of seasonality.

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The overall prevalence of avian hematozoa detected using PCR screening techniques in tropical zones is generally high: 41% in northeastern Australia and Papua New Guinea (Beadell et al. 2004), 30% in Nigeria (Waldenström et al. 2002), 42% in the Lesser Antilles (Fallon et al. 2003), and 59% (*Plasmodium* only) in American Samoa (Jarvi et al. 2003). In host species sampled in temperate zones, the infection rate is often lower: 22% in North America (Fallon et al. 2006) and 24% in Norway and Sweden (Hellgren 2005); however, exceptions exist in temperate North America (higher prevalence) when compared with the Caribbean (lower prevalence, *Haemoproteus* only) (Ricklefs et al. 2005). This apparent difference in prevalence is most likely attributable to a combination of factors. The habits of the host species must be taken into account; many migratory bird species winter in tropical zones and breed in temperate zones, and the aggregation of individuals on wintering grounds may facilitate transmission of hematozoan parasites in tropical zones. Warmer, more humid climates may increase the density of *Culex* or other mosquito vectors of *Plasmodium* and facilitate transmission of this parasite in tropical zones.

The avian hematozoan haplotypes that we detected in tropical and temperate zones of South America were not limited to particular families of hosts, nor does prevalence of infection vary by host family. This is in accordance with recent research indicating that avian hematozoa are less host-specific than previously believed (Bensch et al. 2000, Ricklefs and Fallon 2002, Beadell et al. 2004 [*Plasmodium* only]) and that the identity of a host species may no longer be considered a valid criterion for identifying taxa of hematozoa (especially *Plasmodium*) in birds.

We found a marked lack of shared hematozoan haplotypes between the tropical and temperate zones, indicating that avian hematozoa have undergone different evolutionary processes in each region. There were only three *Plasmodium* haplotypes shared between the two regions, and they were found in several different host species, genera, and even families. This indicates that these shared haplotypes are not restricted to a particular host and appear in the sample more commonly than the haplotypes restricted to one region.

Vector incompatibilities may also be operating between the two regions. Avian hematozoan haplotypes may be carried between the two regions, but the vectors present in each region may not be able to transmit the parasite to a new host. Among *Culex pipiens* mosquitoes, Huff (1934) found individual variation in susceptibility to infection by *Plasmodium cathemerium* and *P. relictum*. Those that were not susceptible were incapable of transmitting infection to new hosts. Similarly, Li et al. (2001) found that while *Anopheles albimanus* and *A. freeborni* mosquitoes were both susceptible to infection by "New World" strains of *P. vivax*, only *A. freeborni* was susceptible to infection by "Old World" *P. vivax* strains, limiting the transmission of that strain. Investigation of the different potential vectors of avian hematozoa present in Guyana and Uruguay could answer questions regarding geographic structuring of haplotypes.

The restriction of many hematozoan haplotypes to one region might also be attributable to haplotypes not being carried between the two regions by migrants. Many avian hematozoan haplotypes appear to be transported between geographic locations by migratory birds (Ricklefs et al. 2005). Waldenström et al. (2002) detected many hematozoan haplotypes in their African sample that had also been detected in breeding populations of migratory birds in Europe. The Fennoscandian Bluethroat (*Luscinia svecica*) harbors hematozoan parasites that are transmitted both on its tropical southern Asian wintering grounds and its temperate European breeding grounds (Hellgren 2005). The austral migrant system that operates between temperate and tropical zones in South America does not include many species that move between Guyana and Uruguay. Very few species that migrate out of temperate South America winter farther north than Amazonia (Chesser 2005). In addition, relatively few species of birds exhibit this pattern of migration. For example, 122 species of South American passerines are austral migrants, compared with 211 species that are Nearctic–Neotropical migrants (Chesser 2005). Given the lack of movement of hosts between the two regions, geographic differentiation of the hematozoan parasite populations between these regions of temperate and tropical South America is not surprising.

This work could be extended by examining the hematozoan haplotypes of more South American austral migrant birds to investigate whether parasites are transmitted along migration routes

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and between wintering and breeding grounds as they are with other, better-studied migration systems, such as the Nearctic–Neotropical or Palearctic–Oriental systems.

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Appendix. Host family, genus, species, and GenBank accession number for each sequence recovered for all mitochondrial hematozoan haplotypes in Figure 3.

Haplotype	Region	Family	Scientific name	Common name	GenBank accession number
1	Guyana	Emberizidae	*Dolospingus fringilloides*	White-naped Seedeater	DQ241508
	Guyana	Icteridae	*Icterus nigrogularis*	Yellow Oriole	
	Guyana	Icteridae	*Icterus chrysocephalus*	Moriche Oriole	
	Uruguay	Troglodytidae	*Troglodytes aedon*	House Wren	
2	Uruguay	Emberizidae	*Poospiza lateralis*	Red-rumped Warbling Finch	DQ241509
	Uruguay	Icteridae	*Icterus cayanensis*	Epaulet Oriole	
3	Guyana	Cardinalidae	*Cyanocompsa cyanoides*	Blue-black Grosbeak	DQ241510
4	Guyana	Cardinalidae	*Saltator maximus*	Buff-throated Saltator	DQ241511
5	Guyana	Emberizidae	*Emberizoides herbicola*	Wedge-tailed Grassfinch	DQ241512
	Guyana	Icteridae	*Sturnella militaris*	Red-breasted Blackbird	
6	Uruguay	Icteridae	*Gnorimopsar chopi*	Chopi Blackbird	DQ241513
	Uruguay	Icteridae	*Gnorimopsar chopi*	Chopi Blackbird	
	Uruguay	Icteridae	*Pseudoleistes guirahuro*	Yellow-rumped Marshbird	
	Uruguay	Thraupidae	*Tangara preciosa*	Chestnut-backed Tanager	
	Uruguay	Thraupidae	*Stephanophorus diadematus*	Diademed Tanager	
	Uruguay	Troglodytidae	*Troglodytes aedon*	House Wren	
	Uruguay	Troglodytidae	*Troglodytes aedon*	House Wren	
	Uruguay	Troglodytidae	*Troglodytes aedon*	House Wren	
	Uruguay	Turdidae	*Turdus rufiventris*	Rufous-bellied Thrush	
7	Uruguay	Icteridae	*Sturnella superciliaris*	White-browed Blackbird	DQ241514
	Uruguay	Icteridae	*Sturnella superciliaris*	White-browed Blackbird	
8	Uruguay	Emberizidae	*Embernagra platensis*	Great Pampa Finch	DQ241515
	Uruguay	Emberizidae	*Embernagra platensis*	Great Pampa Finch	
9	Uruguay	Icteridae	*Pseudoleistes virescens*	Brown and Yellow Marshbird	DQ241516
10	Guyana	Apodidae	*Streptoprocne zonaris*	White-collared Swift	DQ241517
11	Uruguay	Rallidae	*Aramides ypecaha*	Giant Wood Rail	DQ241518
12	Uruguay	Emberizidae	*Poospiza lateralis*	Red-rumped Warbling Finch	DQ241519
	Uruguay	Furnariidae	*Coryphistera alaudina*	Lark-like Bushrunner	
	Uruguay	Furnariidae	*Limnornis curvirostris*	Curve-billed Reedhaunter	
	Uruguay	Icteridae	*Gnorimopsar chopi*	Chopi Blackbird	
	Uruguay	Mimidae	*Mimus saturninus*	Chalk-browed Mockingbird	
	Uruguay	Thraupidae	*Stephanophorus diadematus*	Diademed Tanager	

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APPENDIX. Continued.

Haplo-type	Region	Family	Scientific name	Common name	GenBank accession number
13	Guyana	Icteridae	*Icterus nigrogularis*	Yellow Oriole	DQ241520
	Guyana	Icteridae	*Cacius cela*	Yellow-rumped Cacique	
14	Uruguay	Columbidae	*Leptotila verreauxi*	White-tipped Dove	DQ241521
15	Guyana	Psittacidae	*Diopsittaca nobilis*	Red-shouldered Macaw	DQ241522
16	Uruguay	Turdidae	*Turdus rufiventris*	Rufous-bellied Thrush	DQ241523
17	Uruguay	Turdidae	*Turdus rufiventris*	Rufous-bellied Thrush	DQ241524
18	Guyana	Ardeidae	*Agamia agami*	Agami Heron	DQ241525
19	Guyana	Emberizidae	*Volatinia jacarina*	Blue-black Grassquit	DQ241526
20	Guyana	Cardinalidae	*Saltator maximus*	Buff-throated Saltator	DQ241527
21	Guyana	Ardeidae	*Butorides striatus*	Green-backed Heron	DQ241528
22	Guyana	Cardinalidae	*Cyanocompsa cyanoides*	Blue-black Grosbeak	DQ241529
	Guyana	Icteridae	*Icterus cayanensis*	Epaulet Oriole	
23	Guyana	Emberizidae	*Volatina jacarina*	Blue-black Grassquit	DQ241530
	Guyana	Icteridae	*Cacicus cela*	Yellow-rumped Cacique	
	Guyana	Icteridae	*Cacicus haemorrhous*	Red-rumped Cacique	
	Guyana	Psittacidae	*Diopsittaca nobilis*	Red-shouldered Macaw	
	Uruguay	Emberizidae	*Zonotrichia capensis*	Rufous-collared Sparrow	
	Uruguay	Furnariidae	*Cranioleuca pyrrhophia*	Stripe-crowned Spinetail	
	Uruguay	Icteridae	*Gnorimopsar chopi*	Chopi Blackbird	
	Uruguay	Paraulidae	*Basileuterus culicivorous*	Golden-crowned Warbler	
	Uruguay	Paraulidae	*Basileuterus leucoblepharus*	White-browed Warbler	
	Uruguay	Paraulidae	*Basileuterus leucoblepharus*	White-browed Warbler	
	Uruguay	Paraulidae	*Basileuterus leucoblepharus*	White-browed Warbler	
24	Guyana	Icteridae	*Cacicus cela*	Yellow-rumped Cacique	DQ241531
25	Guyana	Cardinalidae	*Saltator coerulescens*	Greyish Saltator	DQ241532
	Guyana	Emberizidae	*Sicalis luteola*	Grassland Yellow Finch	
	Guyana	Icteridae	*Sturnella militaris*	Red-breasted Blackbird	
26	Guyana	Icteridae	*Molothrus oryzivorus*	Giant Cowbird	DQ241533
	Guyana	Icteridae	*Molothrus oryzivorus*	Giant Cowbird	
27	Guyana	Icteridae	*Icterus nigrogularis*	Yellow Oriole	DQ241534
	Guyana	Icteridae	*Sturnella militaris*	Red-breasted Blackbird	
28	Guyana	Icteridae	*Sturnella supercilliaris*	White-browed Blackbird	DQ241535
	Guyana	Icteridae	*Icterus nigrogularis*	Yellow Oriole	
	Guyana	Icteridae	*Icterus nigrogularis*	Yellow Oriole	
	Guyana	Icteridae	*Icterus nigrogularis*	Yellow Oriole	
29	Uruguay	Ardeidae	*Ardea alba*	Great Egret	DQ241536
	Uruguay	Strigidae	*Otus choliba*	Tropical Screech Owl	
30	Guyana	Cardinalidae	*Cyanocompsa cyanoides*	Blue-black Grosbeak	DQ241537
31	Guyana	Cardinalidae	*Pitylus grossus*	Slate-colored Grosbeak	DQ241538
	Guyana	Cardinalidae	*Saltator maximus*	Buff-throated Saltator	
	Guyana	Cardinalidae	*Saltator maximus*	Buff-throated Saltator	
	Guyana	Cardinalidae	*Cyanocompsa cyanoides*	Blue-black Grosbeak	
	Guyana	Icteridae	*Cacicus cela*	Yellow-rumped Cacique	
	Uruguay	Icteridae	*Agelaius ruficapillus*	Chestnut-capped Blackbird	
	Uruguay	Polioptilidae	*Polioptila dumicola*	Masked Gnatcatcher	
	Uruguay	Polioptilidae	*Polioptila dumicola*	Masked Gnatcatcher	
32	Guyana	Apodidae	*Chaetura spinicauda*	Band-rumped Swift	DQ241539
	Guyana	Icteridae	*Psarocolius viridis*	Green Oropendola	
	Guyana	Icteridae	*Psarocolius viridis*	Green Oropendola	
33	Guyana	Icteridae	*Psarocolius viridis*	Green Oropendola	DQ241540
34	Guyana	Icteridae	*Psarocolius viridis*	Green Oropendola	DQ241541
35	Guyana	Thraupidae	*Lamprospiza melanoleuca*	Red-billed Pied Tanager	DQ241542
36	Guyana	Columbidae	*Leptotila rufaxilla*	Grey-fronted Dove	DQ241543

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APPENDIX. Continued.

Haplo-type	Region	Family	Scientific name	Common name	GenBank accession number
37	Uruguay	Icteridae	*Molothrus badius*	Bay-winged Cowbird	DQ241544
	Uruguay	Rallidae	*Rallus sanguinolentus*	Plumbeous Rail	
38	Uruguay	Icteridae	*Pseudoleistes virescens*	Brown and Yellow Marshbird	DQ241545
	Uruguay	Picidae	*Colaptes campestris*	Campo Flicker	
39	Uruguay	Icteridae	*Icterus cayanensis*	Epaulet Oriole	DQ241546
40	Uruguay	Turdidae	*Turdus amaurochalinus*	Creamy-bellied Thrush	DQ241547
41	Guyana	Cardinalidae	*Caryothraustes canadensis*	Yellow-green Grosbeak	DQ241548
	Guyana	Cardinalidae	*Saltator coerulescens*	Greyish Saltator	
42	Guyana	Icteridae	*Psarocolius decumanus*	Crested Oropendola	DQ241549
43	Uruguay	Emberizidae	*Paroaria coronata*	Red-crested Cardinal	DQ241550
44	Uruguay	Hirundinidae	*Tachycineta leucorrhoa*	White-rumped Swallow	DQ241551
45	Guyana	Apodidae	*Streptoprocne zonaris*	White-collared Swift	DQ241552
46	Guyana	Psittacidae	*Aratinga pertinax*	Brown-throated Parakeet	DQ241553
47	Guyana	Emberizidae	*Paroaria gularis*	Red-capped Cardinal	DQ241554
48	Uruguay	Cardinalidae	*Saltator aurantiirostris*	Golden-billed Saltator	DQ241555
49	Guyana	Columbidae	*Geotrygon montana*	Ruddy Quail-Dove	DQ241556
50	Guyana	Columbidae	*Columbina talpacoti*	Ruddy Gound-Dove	DQ241557
51	Guyana	Columbidae	*Columbina passerina*	Common Ground-Dove	DQ241558
	Guyana	Columbidae	*Columbina passerina*	Common Ground-Dove	
	Guyana	Columbidae	*Columbina passerina*	Common Ground-Dove	
	Guyana	Columbidae	*Columbina passerina*	Common Ground-Dove	
52	Guyana	Columbidae	*Columbina passerina*	Common Ground-Dove	DQ241559
	Guyana	Columbidae	*Columbina talpacoti*	Ruddy Ground-Dove	

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