Diversity of avian haemosporidians in arid zones of northern Venezuela

NAYARA O. BELO[1]*, ADRIANA RODRÍGUEZ-FERRARO[2,3], ERIKA M. BRAGA[1] *and* ROBERT E. RICKLEFS[2]

[1] *Departamento de Parasitologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil*
[2] *Department of Biology, University of Missouri-St Louis, St Louis, Missouri, USA*
[3] *Departamento de Estudios Ambientales, Universidad Simón Bolívar, Sartenejas, Venezuela*

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SUMMARY

Arid zones of northern Venezuela are represented by isolated areas, important from an ornithological and ecological perspective due to the occurrence of restricted-range species of birds. We analysed the prevalence and molecular diversity of haemosporidian parasites of wild birds in this region by screening 527 individuals (11 families and 20 species) for parasite mitochondrial DNA. The overall prevalence of parasites was 41%, representing 17 mitochondrial lineages: 7 of *Plasmodium* and 10 of *Haemoproteus*. Two parasite lineages occurred in both the eastern and western regions infecting a single host species, *Mimus gilvus*. These lineages are also present throughout northern and central Venezuela in a variety of arid and mesic habitats. Some lineages found in this study in northern Venezuela have also been observed in different localities in the Americas, including the West Indies. In spite of the widespread distributions of some of the parasite lineages found in northern Venezuela, several, including some that are relatively common (e.g. Ven05 and Ven06), have not been reported from elsewhere. Additional studies are needed to characterize the host and geographical distribution of avian malaria parasite lineages, which will provide a better understanding of the influence of landscape, vector abundance and diversity, and host identity on haemosporidian parasite diversity and prevalence.

Key words: *Plasmodium* spp., *Haemoproteus* spp., wild birds, arid zone, Venezuela, South America.

INTRODUCTION

Blood parasites (*Plasmodium* spp. and *Haemoproteus* spp.) are found throughout the world (Young *et al*. 1993; Murata, 2002; Valkiūnas *et al*. 2004; Reullier *et al*. 2006). Although the haematozoa of Neotropical birds have been surveyed in many areas (Woodworth-Lynas *et al*. 1989; Young *et al*. 1993; Valkiūnas, 1997; Matta *et al*. 2004; Ribeiro *et al*. 2005; Durrant *et al*. 2006; Belo *et al*. 2009), some regions and environments in South America remain poorly studied. For example, little is known about the prevalence of blood parasites of wild birds in Venezuela, particularly from the arid Caribbean coast. Habitat quality can affect vector density and the composition of the bird communities (Reiter and Lapointe, 2009; Ramírez-Albores, 2010), and one might expect parasite-host relationships to differ between arid and humid environments. Current land practices in Venezuelan arid zones are causing severe environmental change that threatens the long-term survival of the habitat-specialist birds restricted to these areas (Rodriguez-Ferraro and Blake, 2008). The abundance of some common bird species in dry desert scrub and dense thorny thickets along the Caribbean coasts of Venezuela and Colombia differs considerably among areas as a consequence of anthropogenic factors, such as habitat modification and poaching. Habitat alteration resulting from climate change also might influence the distribution and abundance of wildlife species and thus may be a major driver of change in the ecology of pathogen transmission.

Information on the influence of precipitation on haemoparasite diversity, distribution, and prevalence in tropical environments could help to clarify the effect of habitat and landscape on parasite-host interactions in wild populations, including how parasite species distribution and abundance might respond to climate change in the future.

In this study, we report on the presence and distribution of the haematozoa of birds in the arid zones of northern Venezuela. This region is especially well suited for the study of avian malaria in an arid environment: the area belongs to the 'peri-Caribbean arid belt', which is one of 6 Neotropical arid zones and considered an Endemic Bird Area (EBA; Stattersfield *et al*. 1998) because of the occurrence of restricted-range and habitat-specialist bird species.

We tested the null hypothesis that haemosporidian parasite lineages do not differ between the arid

* Corresponding author: Universidade Federal de Minas Gerais, Av. Antônio Carlos, 6627. Instituto de Ciências Biológicas, Departamento de Parasitologia. 31270-910. Belo Horizonte, MG. Brazil. Tel: +5531 34992876. Fax: +5531 34992790. E-mail: nayarabelo@yahoo.com.br

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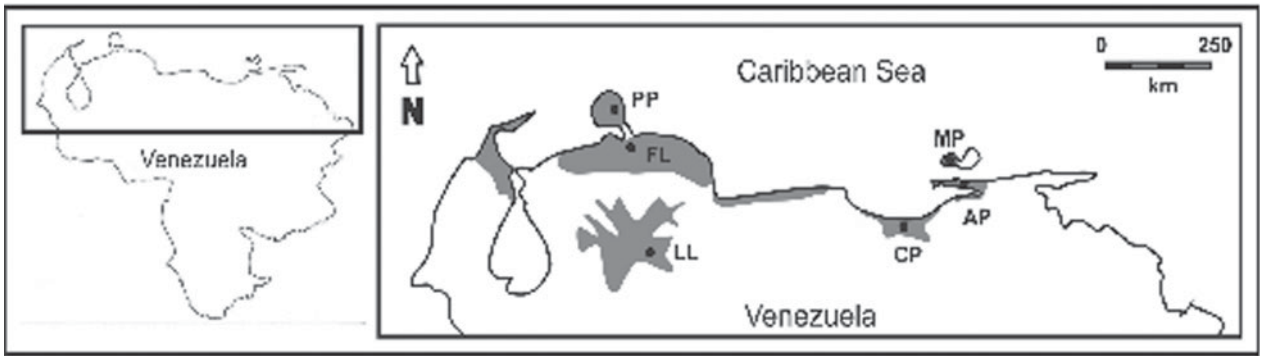

Fig. 1. Location of arid zones (shaded) in northern Venezuela, and areas studied. Study areas of Western region include the following: PP, Paraguaná Peninsula; FL, Falcón Lowlands; LL, Lara Lowlands and MP, Macanao Peninsula. Areas studied of Eastern region: CP, Clarines-Piritu; AP, Araya Peninsula.

regions of Venezuela. We assume that competent vectors occur in every arid region and that generalist host species with wide distributions, such as *Mimus gilvus*, are susceptible to most of the parasite lineages present in the arid lineages of Northern Venezuela. We also tested the hypothesis that some parasite lineages are unique to the arid regions of Venezuela owing to the presence of arid habitat endemic birds. Thus, the avian malaria parasites of this region might potentially reflect the unique avifauna of this arid climate zone.

MATERIALS AND METHODS

Study areas

Sampling was conducted in the arid zones of northern Venezuela, that are characterized by thorn scrubs dominated by species belonging to the Cactaceae, Mimoseae (Fabaceae), and Capparaceae (Sarmiento, 1972). Mean annual temperature is 28 °C, and annual rainfall ranges between 300 and 700 mm, with a long and severe dry season punctuated by 2 brief rainy peaks in July–August and December (Sarmiento, 1976).

Samples were obtained from 6 arid areas in northern Venezuela (Rodríguez-Ferraro and Blake, 2008) (Fig. 1). (1) Paraguaná Peninsula (PP) is located in northwestern Venezuela. It was an island during the Pliocene and extends over 2500 km^2. (2 and 3) Falcon (FL) and Lara (LL) lowlands represent the most extensive arid zone in Venezuela, and are located in the Western region, with an approximate area of 16 000 km^2. (4) The Clarines-Piritu region (CP) extends over about 4500 km^2 in northeastern Venezuela and covers the Unare Depression, between the central and eastern portions of the Coastal Mountain Range. (5) Araya Peninsula (AP) occupies 900 km^2 in northeastern Venezuela and comprises the lowlands to the north of the eastern Coastal Mountain Range. (6) Macanao Peninsula (MP), about 300 km^2, comprises the westernmost portion of Margarita Island. We divided these areas between a western group (PP, FL, LL) and an eastern group (MP, CP, AP).

Field sampling

Bird samples were collected during bimonthly trips to each area between September 2004 and August 2005. Birds were captured using mist nets (12 m × 2·8 m × 36 mm mesh). Blood was collected using heparinized microcapillary tubes following venipuncture of the brachial vein with a sterile syringe needle (Gaunt and Oring, 1997) and stored in lysis buffer.

DNA extraction

Approximately 20 μl of blood sample was stored at room temperature (22–25 °C) in cell lysis solution (Quiagen Inc, Valencia, California) before processing in the laboratory at the University of Missouri-St Louis. DNA from blood samples was extracted using a PureGene® commercial kit according to the manufacturer's protocol (Gentra systems, Minneapolis, MN, USA). The DNA pellet was re-suspended in 100 μl of hydration solution and kept at −20 °C until use.

Screening

We used screening primers designed to amplify a 154-nucleotide segment of RNA-coding mitochondrial DNA (Fallon *et al*. 2003). PCR reactions were run in 10 μl volumes that contained the following final concentrations: 0·4 mM of each primer, 200 mM of each dNTP (PROMEGA), 10 mM Tris–HCl, pH 8·5, 50 mM KCl, and 1 U of Taq DNA polymerase (PHONEUTRIA, Minas Gerais, Brazil). Thermal cycling conditions were as follows: initial denaturation of 2 min at 94 °C followed by 35 cycles with 1 min denaturation at 94 °C, 1 min annealing at 62 °C, and extension at 72 °C for 1 min 10 sec. This was followed by a final extension of 3 min at 72 °C.

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PCR products were screened on 1·5% agarose gels, stained with ethidium bromide and visualized with a UV light source.

Cytochrome b amplification and sequencing

From samples in which we detected hematozoan infection (mitochondrial DNA amplification), we amplified a fragment of 591 bp of the cyt b gene (Perkins and Schall, 2002) under the following conditions: with 1 μl of genomic DNA was subjected to an initial denaturation of 4 min at 94 °C, followed by 35 cycles of 94 °C for 20 sec, 49 °C for 10 sec, and 68 °C for 45 sec, and a final extension at 68 °C for 3 min. For most samples, a 0·5-μl aliquot of this product was used as a template for a nested reaction with primers described by Ricklefs *et al*. (2005) under initial denaturation of 94 °C for 1 min and 28 cycles of 94 °C for 20 sec, 52 °C for 10 sec, and 68 °C for 50 sec and then 68 °C for 7 min. PCR products were screened on 1% agarose gels, stained with ethidium bromide, and visualized with a UV light source.

Positive PCR products were purified for cycle sequence reactions using ExoSAP-IT® (USB Corporation, Cleveland, Ohio) following the manufacturer's instructions. Bi-directional sequencing using primers 413F and 926R with dye-terminator fluorescent labelling was performed in an ABI Prism 3100 automated sequencer ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). We sequenced approximately 500 base pairs of the cyt b gene for *Plasmodium* spp. and *Haemoproteus* spp.

Phylogenetic analysis

DNA sequences were aligned using CLUSTALX (Thompson *et al*. 1997) and edited using Seq Man II version 4 (DNASTAR Inc.) and are available through GenBank (Accession numbers JN819517-JN819533). Sequences were compared for identification to their closest matches in GenBank using the NCBI nucleotide Blast search, and to unpublished sequences using a local blast search in the laboratory of R. E. Ricklefs. We used MODELTEST version 3.6 (Posada and Crandall, 1998) to determine the most appropriate evolutionary model for our data. A maximum likelihood phylogenetic tree was produced for the parasite sequences using RAxML (Stamataxis, 2006) with the GTR + gamma model of nucleotide evolution and 100 bootstrap replications.

Statistics

Contingency table analyses were used to detect interactions between location, sex (where distinguished), season, and parasite prevalence. Statistics were carried out using Prism 5.0 for Mac OS (GraphPad Software, Inc.). The estimator *Mao Tau* (Colwell *et al*. 2004) was used to compare parasite species richness between two regions using the scores of 200 randomizations produced by EstimateS 8.2 (Colwell, 2008) to estimate parasite lineage richness in each study site.

Table 1. Prevalence of *Plasmodium*/*Haemoproteus* according to regions studied and the sex in two species wild birds

(Prevalence reported as percent infected (%) and number of host individuals examined (*N*).)

Parasite prevalence % (*N*)			
	Region		
Species	Eastern	Western	*P*-value
Cardinalis phoeniceus	72·7 (36)	75 (44)	0·3
Tiaris bicolor	22·5 (80)	19·7 (61)	0·3
	Sex		
	Male	Female	
Cardinalis phoeniceus	69·2 (39)	80·5 (41)	0·9
Tiaris bicolor	16·9 (65)	24·7 (76)	0·2

RESULTS

Samples from 527 wild birds (11 families and 20 species) were screened for haemosporidian parasites. Of these, 41% were infected with *Plasmodium* spp. (13 individuals) or *Haemoproteus* spp. (148 individuals), using PCR as the diagnostic method; 55 individuals that presented mixed infections were not considered in this study (data not shown).

The species with the highest prevalence of infection were *Icterus nigrogularis* ($n=19$, 100%), *Mimus gilvus* ($n=51$, 84·3%), *Melanerpes rubricapillus* ($n=14$, 78·6%), and *Cardinalis phoeniceus* ($n=80$, 73·8%). The overall frequency of haemosporidian infection did not differ between the western (45%; $n=252$) and eastern (37·5%; $n=275$) regions ($P=0·1$). Homogeneity between western and eastern regions was also apparent in the two most abundant species in our sample, *Cardinalis phoeniceus* and *Tiaris bicolor* (Table 1). Haemosporidian prevalence was not heterogeneous with respect to dry versus rainy seasons, either in the total sample ($P=0·07$) or in *C. phoeniceus* and *T. bicolor* ($P>0·5$). Blood parasite prevalence did not differ between males and females of *C. phoeniceus* and *T. bicolor* ($P=0·3$) (Table 1).

General absence of regionalization of parasite lineages

Cytochrome b sequences revealed 7 *Plasmodium* lineages and 10 *Haemoproteus* lineages in the entire sample (Fig. 2). Of the lineages recovered from 5 or

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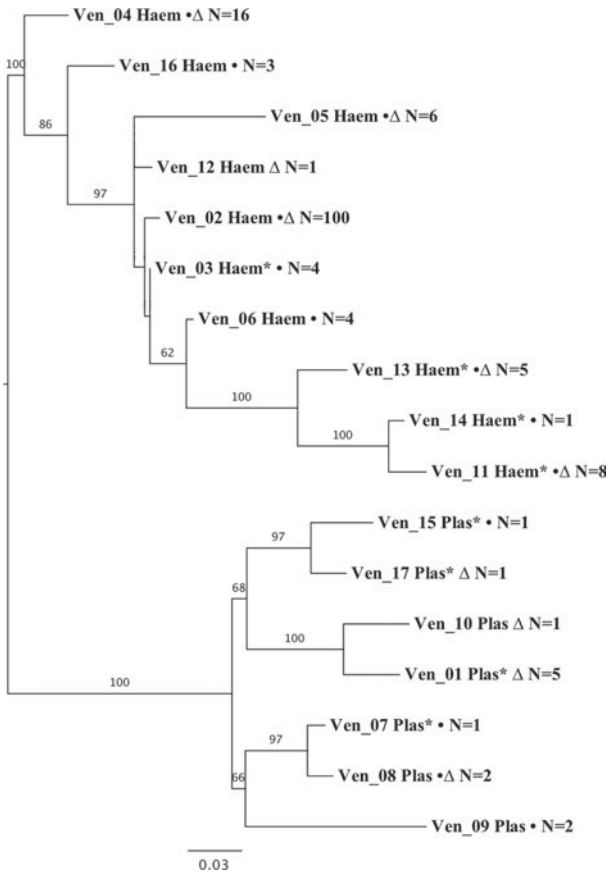

Fig. 2. Phylogram of *Plasmodium* spp. and *Haemoproteus* spp. lineages in community birds. Phylogenetic relationships of the 17 haemosporidian parasite lineages found in 2 different regions, based on cyt b sequences. Numbers located on the top of the branches indicate ML bootstrap support (100 replications, only values above 50% are shown). The presence of each parasite lineage in the two areas studied is indicated by: western region (●) and eastern region (△). Parasite lineages described in others studies (*). The values of 'N' are the samples size of each lineage.

more host individuals, only 1 was restricted to a single region (*Plasmodium* Ven01 in the east). Thus, there is little evidence of regionalization in the parasite faunas across the arid northern coast of Venezuela.

Ven01 was restricted to the host species *Mimus gilvus*, which had similar representation in our samples from 2 regions (east, $n=28$; west, $n=23$). Four of 5 examples of the *Haemoproteus* lineage Ven13 were restricted to *Mimus gilvus* in the western region (the other example was from *Xiphorhynchus picus* in the east). This appears to be the only potential case of partitioning of host individuals among parasite lineages on a regional basis. Two other lineages recovered from *M. gilvus* (Ven04 and Ven05) occurred in both regions.

Eight of the lineages found in this study had previously been described in other studies (Table 2). Nine lineages are described for the first time in this study: 3 *Plasmodium* and 6 *Haemoproteus*, although several of these lineages are additionally known from unpublished work in the laboratory of R. E. Ricklefs (see Discussion section).

Parasite diversity was higher in the eastern region (82% of the combined diversity) than in the western region (65%), but the differences were not statistically significant ($P>0{\cdot}05$) (Table 3). The rarefaction curve and *Mao Tau* estimator indicated that likely we have not sampled all of the lineages expected to occur in 2 regions in arid zones of Venezuela, suggesting the need for additional sampling (Fig. 3).

The Table 4 lists the parasite lineages and host species and families parasitized.

DISCUSSION

In this study we observed high prevalence (41%) of malaria parasites in arid zones of coastal northern Venezuela, representing 17 mitochondrial lineages: 7 of *Plasmodium* and 10 of *Haemoproteus*. Of those lineages, 8 have been described in other studies, while 9 lineages are described for the first time in this study. We found some lineages in northern Venezuela that also were observed in different localities in the Americas. However, other lineages, including some that are relatively common (e.g. Ven05 and Ven06), have not been reported from elsewhere. The prevalence and diversity of parasite lineages did not differ between the isolated eastern and western regions of arid coastal habitat in Venezuela.

Other studies in Neotropical regions that used molecular analysis have reported high prevalence values consistent with this study (Belo *et al*. 2009; Santiago-Alarcon *et al*. 2010; Belo *et al*. 2011). In contrast, several studies that have screened haematozoan infections by scanning blood smears have typically reported low overall prevalence in the Neotropics (approximately 10%), significantly less than in any other zoogeographical region (Greiner *et al*. 1975; Winchell, 1978; White *et al*. 1978; Bennett *et al*. 1991; Valkiūnas *et al*. 2003; Rodriguez and Matta, 2001). More studies of blood parasites in the Neotropical region, directly comparing association PCR and microscopy for diagnosis, are required to fully characterize the distribution of haemosporidian parasites in this region.

The higher occurrence of *Haemoproteus* infections compared to *Plasmodium* infections in the arid environments considered in this study could be due to the relative abundance of vectors of *Haemoproteus* in this region. Biting midges (Ceratopogonidae) and mosquitoes (Culicidae), the primary vectors known for *Haemoproteus* spp. and *Plasmodium* spp., respectively, are common in the Neotropics (Garnham, 1966; Linley, 1985) but their relative abundance in the arid coastal zones of northern Venezuela is not known.

Parasite prevalence did not vary in relation to time of year in this study. Reproduction is highly seasonal in arid northern Venezuela, and the increased stress of reproduction has been shown to depress immune

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Table 2. Relation of parasite lineages to those previously reported in other areas

Parasite lineage	GenBank number Identical Lineages	Source
Ven_01 Plas	AF465559	Ricklefs and Fallon (2002)
Ven_03 Haem	GQ395658	Levin *et al*. (2009)
Ven_07 Plas	DQ838997	Beadell *et al*. (2006)
Ven_11 Haem	AF465568	Ricklefs and Fallon (2002)
Ven_13 Haem	HM222483	Ricklefs and Outlaw (2010)
Ven_14 Haem	GQ395647	Levin *et al*. (2009)
Ven_15 Plas	GQ395654	Levin *et al*. (2009)
Ven_17 Plas	EU627831	Ishak *et al*. (2008)

Table 3. Parasite lineage richness as estimated by *Mao Tau* (S) in Western and Eastern regions in arid zones of Venezuela

Areas	S	S.D.	95% CI		%
Richness total	17	2·42	12·26	21·74	100
Eastern region	14	2·33	9·43	18·57	82
Western region	11	2·14	6·8	15·2	65

defences and lead to increased parasite prevalence, particularly in females (Møller and Saino, 1994; Weatherhead *et al*. 1993; Zuk, 1996; Zuk and McKean, 1996; Hughes and Randolph, 2001). However, blood parasite prevalence in females and males of the two most abundant species in this study did not differ, consistent with the results of a similar study in North America (Ricklefs *et al*. 2005). Based on 33 studies of blood smears from Europe and North America, McCurdy *et al*. (1998) also failed to find a significant difference in parasitism between the sexes. Recently, studies in blue tits (*Cyanistes caeruleus*) demonstrated differences in parasite prevalence related to host sex depending on the species of *Plasmodium*. Male and female blue tits differed in prevalence of *P. circumflexum* (Lachish *et al*. 2011), but not other *Plasmodium* lineages (Szöllosi *et al*. 2011).

Prevalence of haemoparasites has been found to vary over the annual cycle and between years (Bennett and Lopes, 1980; Woodworth-Lynas *et al*. 1989). Seasonal differences in the prevalence of haemoparasites occur more frequently in temperate regions (Kirkpatrick and Suthers, 1988, Weatherhead and Bennett, 1992; Hatchwell *et al*. 2000), where climate seasonality is more pronounced, limiting the transmission of the parasites to the warm months of the year (Atkinson, 1988). However, one study of haematozoan prevalence throughout the year in a seasonally dry forest in Puerto Rico found no significant seasonal variation in the prevalence of infection or predominant parasite lineages (Fallon *et al*. 2004). We also did not find seasonal variation in parasite prevalence in this study. Because the prevalence of different parasite species can vary individually in response to environmental and host factors (e.g. Lachish *et al*. 2011), it is important to consider abiotic factors (climate and habitat change), biotic factors (age/sex, host species, population density), and vectors, as well as parasite species, when characterizing parasite prevalence.

In this study, we found 17 parasite lineages in arid zones, of which 9 (3 *Plasmodium*/6 *Haemoproteus*) had not been described previously. This is the first study on avian malaria in arid environments in South America, and it is important because it reports the high parasite prevalence and parasite diversity present in this area. Temperature and moisture are fundamental determinants of the distribution and abundance of most vector species. Among mosquito species described in this region of Venezuela (Heinemann and Belkin, 1978), *Culex quinquefasciatus* and *Anopheles strode* are likely avian malaria vectors (Valkiūnas, 2005). The high parasite prevalence might be associated with anomalous rainfall, which might have promoted vector breeding and survival. In Botswana, Africa, more than two thirds of the variability observed between years in human malaria incidence during January–May could be explained by variation in rainfall during December–February (Thomson *et al*. 2005). In Venezuela there are reports of anomalous rainfall, such as a torrential rainfall during December 1999, described by Lyon (2003) that resulted in devastating floods and landslides along the northern coast of Venezuela. These events that occurred in an area with a predominantly dry climate, took place during what is regionally the dry season, and were preceded by unusually heavy seasonal rainfall. More studies in arid zones must be undertaken to understand the parasite-vector-host relationships in these areas.

The common lineages of *Haemoproteus* and *Plasmodium* occurred in both the eastern and western portions of the arid Venezuelan coast. Geographically widespread birds can make the parasite community homogeneous across the arid regions. In this area, habitat degradation occurs at a very local scale and does not promote contact between eastern and western regions, because humid regions between sampling areas act as barriers for arid specialist birds.

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Table 4. Parasite lineages and host species in arid northern Venezuela

Parasite lineages	Parasite lineage names used previously (see Table 2 for GenBank numbers)	Haemosporidian genus	Host family	Host species
Ven01	*Plasmodium* sp. haplotype 64	*Plasmodium* sp	Mimidae	*Mimus gilvus*
Ven02		*Haemoproteus* sp	Cardinalidae	*Cardinalis phoeniceus*
			Icteridae	*Icterus nigrogularis*
			Picidae	*Melanerpes rubricapillus*
			Psittacidae	*Aratinga pertinax*
			Furnariidae	*Xiphorhynchus picus*
			Thamnophilidae	*Sakesphorus canadensis*
			Cardinalidae	*Saltator coerulescens*
			Thraupidae	*Tiaris bicolor*
				Thraupis glaucocolpa
Ven03	*Haemoproteus* sp. LA07GD29	*Haemoproteus* sp	Icteridae	*Icterus nigrogularis*
Ven04		*Haemoproteus* sp	Mimidae	*Mimus gilvus*
			Psittacidae	*Aratinga pertinax*
Ven05		*Haemoproteus* sp	Mimidae	*Mimus gilvus*
Ven06		*Haemoproteus* sp	Thraupidae	*Coryphospingus pileatus*
				Tiaris bicolor
Ven07	*Plasmodium* sp. P11	*Plasmodium* sp	Tyrannidae	*Sublegatus arenarum*
Ven08		*Plasmodium* sp	Cardinalidae	*Cardinalis phoeniceus*
			Thraupidae	*Thraupis glaucocolpa*
Ven09		*Plasmodium* sp	Thraupidae	*Thraupis glaucocolpa*
			Icteridae	*Icterus nigrogularis*
Ven10		*Plasmodium* sp	Cardinalidae	*Cardinalis phoeniceus*
Ven11	*Haemoproteus* sp. haplotype 7	*Haemoproteus* sp	Thraupidae	*Tiaris bicolor*
Ven12		*Haemoproteus* sp	Cardinalidae	*Cardinalis phoeniceus*
Ven13	*Haemoproteus* sp. YU2MEX510	*Haemoproteus* sp	Mimidae	*Mimus gilvus*
			Furnariidae	*Xiphorhynchus picus*
Ven14	*Haemoproteus* sp. GD1GD24	*Haemoproteus* sp	Furnariidae	*Xiphorhynchus picus*
Ven15	*Plasmodium* sp. JA7J725	*Plasmodium* sp	Furnariidae	*Xiphorhynchus picus*
Ven16		*Haemoproteus* sp	Cardinalidae	*Saltator orenocensis*
			Thamnophilidae	*Formicivora intermedia*
			Picidae	*Melanerpes rubricapillus*
Ven17	*Plasmodium* sp. GHOW3489	*Plasmodium* sp	Tyrannidae	*Sublegatus arenarum*

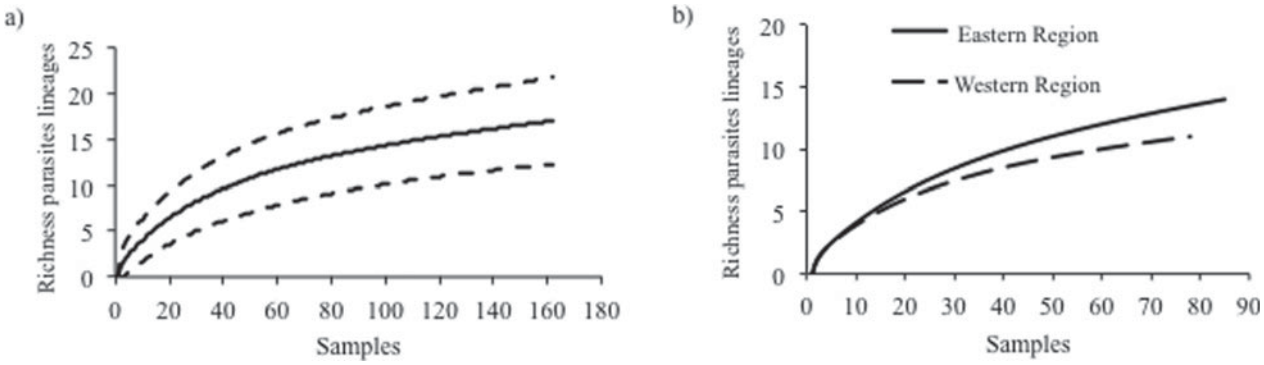

Fig. 3. *Mao Tau* species accumulation curves for the parasite lineages of 2 regions. (a) Total richness of parasite lineages, (--) is the 95% CI and (—) is Total richness. (b) Richness of parasite lineages in the eastern and western regions separately (the 95% confidence intervals for the two regions overlap; see Table 3).

However, only 2 parasite lineages (Ven01 and Ven13) appeared to be partitioned between the eastern and western regions in a single host species, *Mimus gilvus*, which is present throughout northern and central Venezuela and uses a variety of arid and mesic habitats. The partitioning might be a sampling effect as we recovered only 5 examples of each of these lineages. Other, more common parasite lineages were distributed across the region.

Some lineages found in this study in northern Venezuela have been observed in different localities in the Americas. Among *Haemoproteus* lineages previously reported from other species and areas, Ven03 has been recovered from other *Icterus* spp. in

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Jamaica and the Yucatan Peninsula of Mexico. Ven04 differs by a single nucleotide from a lineage found in several endemic Mimidae in the Lesser Antilles and in the catbird *Dumetella carolinensis* in eastern North America. Ven11 had already been described in another study of *Tiaris bicolor* in Venezuela (Ricklefs and Fallon, 2002; their lineage H07). Ven13 is also common in *M. gilvus* in the Mexican state of Yucatan. Ven14 is an abundant parasite of the bananaquit *Coereba flaveola* throughout the West Indies. Ven16 was found as single infections in 4 species in this study, but also in the flycatcher *Hemitriccus margaritaceiventer* in Tocantins State, Brazil (Belo *et al.* 2011, HQ287536).

Four of the 7 *Plasmodium* lineages observed in this study also occur in other regions. Ven01 infecting *Mimus gilvu*s in our study was obtained from *Vireo griseus* in Missouri, USA, but has also been recovered from a variety of species in several locations around the Caribbean Basin. Ven07 was observed in this study infecting *Sublegatus arenarum*, and Beadell *et al.* (2006) recovered this parasite lineage from *Troglodytes aedon* in Uruguay; this lineage is also frequently encountered in a variety of species in the Caribbean Basin. We obtained Ven15 from *Xiphorhynchus picus*, but it has also been recorded in North Carolina infecting a captive Great Horned Owl *Bubo virginianus* as well as in many species in the Caribbean Basin and North America. Ven17 was detected in *Bubo virginianus* in northern California.

In spite of the widespread distributions of some of the parasites found in northern Venezuela, several of the lineages, including some that are relatively common (e.g. Ven05 and Ven06) have not been reported from elsewhere. Clearly, additional studies are needed to characterize the host and geographical distribution of avian malaria parasite lineages, which will provide a foundation for a better understanding of the influence of landscape, vector abundance and diversity, and host identity on haemosporidian parasite diversity and prevalence.

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