Original paper

Blood-parasites (Haemosporida) of wild birds captured at different land uses within a tropical seasonal dry forest matrix

Parásitos sanguíneos (Haemosporida) de aves silvestres capturadas en diferentes tipos de uso de suelo en una matriz de bosque seco tropical de temporal

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Responsible editor: Ricardo Rodríguez-Estrella

ABSTRACT. Avian haemosporidians form a diverse group of vector-borne parasites that can cause detrimental effects on their hosts and threaten the conservation of susceptible species. We explored the prevalence and parasitemia of haemosporidians infecting wild birds from Sierra de Huautla Biosphere Reserve in Morelos, southern Mexico. Birds were caught using mist nets in three habitat conditions (conserved, disturbed, and agricultural) and during two seasons (rainy and

dry). Thin blood smears were prepared from blood samples for microscopic analysis. We captured 142 birds belonging to 17 species. We identified *Haemoproteus* spp., *Plasmodium* spp., and microfilaria. Prevalence was similar among land-use types (conserved (26.3%), disturbed (36.4%) and agricultural (29.9%)), and between seasons (rainy (29.7%) and dry (29.3%)), but varied per parasite genus and group (*Haemoproteus* spp. (28.2%), *Plasmodium* spp. (2.1%), coinfections (5.6%), and microfilaria (4.9%)). Parasitemia was low in most birds (< 0.1% erythrocytes infected), and only one, with an unhealthy appearance, presented high parasitemia (> 0.5%). We reported for the first time 12 species of birds infected with haemosporidian parasites and 16 new host-parasite associations in total. This is the first study of blood parasites in this region and provides fundamental information for future research.

Key words: Avian malaria; Parasite ecology; Landscape parasitology; Avian conservation

RESUMEN. Los hemosporidios aviares forman un grupo diverso de parásitos transmitidos por vectores que pueden perjudicar a sus hospederos y amenazar la conservación de especies susceptibles. Evaluamos la prevalencia y parasitemia de hemosporidios en aves silvestres de la Reserva de la Biósfera Sierra de Huautla, Morelos, al sur de México. Las aves se capturaron usando redes de niebla en tres tipos de hábitat (conservado, perturbado y agrícola) y durante dos temporadas (lluvias y secas). Se realizaron frotis de capa fina para su análisis microscópico. Capturamos 142 aves pertenecientes a 17 especies. Los parásitos presentes fueron *Haemoproteus* spp., *Plasmodium* spp., y microfilarias. La prevalencia fue similar entre tipos de uso de suelo (conservado (26.3 %), perturbado (36.4 %) y agrícola (29.9 %)) y temporadas (lluvias (29.7 %) y secas (29.3 %)), pero varió por género y grupo de parásito (*Haemoproteus* spp. (28.2%), *Plasmodium* spp. (2.1 %), coinfecciones (5.6 %), y microfilarias (4.9 %)). La mayoría de las aves presentó una parasitemia baja (< 0.1% de eritrocitos infectados) y solo una, con apariencia malsana, presentó alta parasitemia (> 0.5 %). Reportamos por primera vez la infección de hemoparásitos en 12 especies de aves y 16 nuevas asociaciones parásito-hospedero. Este es el primer estudio de hemoparásitos en esta región y proporciona información fundamental para investigaciones futuras.

Palabras clave: Malaria aviar; Ecología de parásitos; Parasitología de paisaje; Conservación aviar

INTRODUCTION

Blood parasites of the order Haemosporida have a heteroxenous life cycle requiring a vertebrate host for asexual reproduction (merogony) and a final arthropod vector host (Diptera) to complete sexual reproduction and sporogony (Valkiūnas, 2005; Santiago-Alarcon *et al.*, 2012). Bird species are frequently infected by parasites of three genera *Plasmodium* spp., *Haemoproteus* spp., and *Leucocytozoon* spp. (Santiago-Alarcon & Carbo-Ramírez, 2015). During the last decade, a large genetic diversity was discovered in avian haemosporidians (Bensch *et al.*, 2009; Egerhill *et al.*, 2016), and new morphospecies are continuously described (e.g., Valkiūnas *et al.*, 2010; 2013; Hernández-Lara *et al.*, 2018). The health effects of avian haemosporidian parasites can range from mild (no sign of disease) to severe, such as diminishing reproductive fitness (Knowles *et al.*, 2010) and lower survival rates (Marzal *et al.*, 2008; Martínez-de la Puente *et al.*, 2010), but the consequences for

bird populations in the long term are mainly unknown (Ham-Dueñas *et al*., 2017). *Plasmodium* spp., the causative agent of avian malaria, can produce severe mortality in populations of immunologically naïve species that have not been exposed previously (Foster *et al*., 2007; Atkinson, 2008; LaPointe *et al*., 2012). For example, *Plasmodium relictum* and its vector (*Culex quinquefasciatus*) were introduced to the Hawaiian Islands, and along with other human impacts (i.e., habitat destruction and introduced predators), have contributed to the extinction of a large proportion of endemic bird species (van Riper *et al*., 1986; Foster *et al*., 2007), an effect that can be exacerbated by climate change (Liao *et al*., 2015). Thus, avian haemosporidian parasites are of conservation concern for endemic bird species (Martínez *et al*., 2015). Consequently, avian reintroduction programs should implement monitoring protocols for these parasites before animals are released back into the wild, like the reintroduction of the Socorro dove (*Zenaida graysoni*) on the Socorro Island located in the Mexican Pacific Ocean (Carlson *et al*., 2011; 2013).

The distribution of avian haemosporidian parasites depends on the distribution and abundance of their hosts, which in turn is affected by different natural factors like the landscape, climatic conditions, and by anthropic activities such as the modification, destruction, and fragmentation of natural habitats (Chasar *et al*., 2009; Reinoso-Pérez *et al*., 2016; Hernández-Lara *et al*., 2017). It has been observed that haemosporidian prevalence is higher in conserved habitats like mature forests compared to agricultural areas (Bonneaud *et al*., 2009), but such results change depending on the bird species and haemosporidian genus analyzed (Chasar *et al*., 2009). Haemosporidian prevalence can be lower in urban environments for invasive bird species, particularly in heavily built areas that may be unfavorable for haemosporidian vectors (e.g., Santiago-Alarcon *et al*., 2018). Nonetheless, some studies report a higher prevalence and parasitemia in urban landscapes, especially in urban green spaces (e.g., urban forest reserves and parks), which could be related to the isolation of bird populations and the constant favorable conditions for vector breeding (Hernández-Lara *et al*., 2017). Ultimately, the outcome will depend on the parasite-vector-host system under study, given that current research has found no general patterns for the epizootiology of avian haemosporidians as a function of human impacts (Santiago-Alarcon & Delgado-V, 2017).

The abundance of vectors is influenced by the availability of breeding sites and optimum temperatures. Thus, it is expected that the prevalence and parasitemia of avian haemosporidians increase during the rainy season, which would be particularly relevant in habitats with a contrasting annual seasonality (Santiago-Alarcon *et al*., 2011; Tinajero *et al*., 2019). For example, Mexico's large extensions of dry and arid habitats (e.g., tropical seasonal dry forests) have been dramatically modified for grazing and harvesting (Challenger & Caballero, 1998), which consequently alters the ecological dynamics of host-vector-parasite interactions (Dobson & Foufopoulos, 2001; Ham-Dueñas *et al*., 2017; Tinajero *et al*., 2019). Until now, few studies on blood parasites in wild Mexican birds have been carried out, and the geographical coverage is still limited, particularly for arid regions (Santiago-Alarcon & Carbo-Ramírez, 2015).

We studied the prevalence and parasitemia of avian haemosporidians and analyzed its relation to land use type and seasonality in the state of Morelos, Mexico. About 60% of the original vegetation in the state of Morelos has been lost (Trejo & Dirzo, 2000), but in the southeast of the

state, a preserved area of tropical deciduous forest was declared a natural protected area in 2000, named Sierra de Huautla Biosphere Reserve (CONANP, 2005). This reserve represents the most important remnant of tropical deciduous forest in the south-central region of Mexico and holds an important bird diversity that includes 177 species of which 23 are endemic to Mexico and 24 are under a conservation category (Ramírez-Albores & Ramírez-Cedillo, 2002) and, for this reason, it is a priority area for the conservation of Mexican birds (Benítez *et al*. 1999). We predicted that haemosporidians would be more prevalent in areas with higher human impacts due to the availability of permanent water bodies for vector breeding given cattle and farming activities (e.g., Tinajero *et al*., 2019). Moreover, we expected a higher prevalence during the rainy season when conditions are optimal for vector development (particularly in well-preserved habitats) and when bird immune systems are compromised due to reproductive activities. Among infected birds, parasitemia is expected to be higher during the dry season and in more degraded areas due to harsher environmental conditions (e.g., lower food abundance) (Reinoso-Pérez *et al*., 2016; Ham-Dueñas *et al*., 2017).

MATERIALS AND METHODS

The study was conducted in Sierra de Huautla Biosphere Reserve, a protected natural area located in the south of Morelos state, bordering the states of Guerrero and Puebla (Osorio-Beristain *et al*., 2018). The Reserve (18° 20′ 10″, 18° 34′ 20″ N, 98° 51′ 20″, 98° 08′ 15″ W; 59,030 ha) is a mosaic of primary and secondary tropical seasonal dry forest surrounded by agricultural land and small towns (Fig. 1). In the dry season, from November to May, most trees shed their leaves, and animals are exposed to hydric stress. Precipitation occurs mainly during the rainy season, from June to October, with an annual average of 900 mm (CONANP, 2005).

At the study site, near the town El Limón de Cuauchichinola (1,220 m a.s.l.), large tracts of forest were cleared in the early 1990s, used for maize cultivation for approximately six years, and subsequently abandoned (Martínez-Garza *et al*., 2011). Since then, the resulting secondary forest has been used for wood extraction and extensive livestock ranching. Nearly 600 cattle from neighboring towns are brought in for grazing (~ 7 cattle/ha) in the rainy season. During the dry season, the cattle are kept on farms, but goats, pigs, and horses forage in the forest daily throughout the year (de la O-Toris *et al*., 2012).

We established three sampling areas with different vegetation cover based on a satellite image analysis performed by the Geographical Information Systems Interdisciplinary Laboratory of the Autonomous University of Morelos State that determined the percentage of coverage in the Reserve. (1) A conserved site (18°32′ 47.9′′ N, 98° 56′ 43.9′′ W) characterized by a high tree cover of tropical dry forest, where the most common canopy trees are *Conzattia multiflora*, *Lysiloma acapulcense*, *Lysiloma divaricatum*, *Bursera* spp. and *Ceiba* spp., and vegetation of the tropical sub-deciduous forest is present. (2) A disturbed site (18° 32′ 40.4′′ N, 98° 55′ 35.8′′ W) used for cattle grazing; the most abundant tree species in this site are *Acacia cochliacantha*, *Mimosa benthamii*, and *Ipomoea pauciflora*. Finally, (3) an agricultural site (18° 33′ 10.9′′ N, 98° 56′ 35.7′′ W) that encompasses crops maintained by local people, mainly corn; this area has herbaceous

vegetation, particularly from the Poaceae and Asteraceae families that cover the soil at ground level (CONANP, 2005) (Fig. 1).

The sampling was conducted during March (dry season) and July (rainy season) of 2013. We used six mist nets of 12 × 2.5 m at ground level placed at least 50 m apart inside each sampling area. The nets were in accessible sites where they could be entirely displayed without vegetation interference and opened from 06:00 to 11:00 h approximately. We prepared three thin blood smears per individual, air-dried and fixed in absolute methanol (Valkiūnas, 2005), obtaining the blood by either claw clipping or puncture of the metatarsal vein after disinfecting the area. Birds were released when bleeding was stopped with the aid of a styptic powder (Hemostop®) and were identified with plastic rings to detect recaptures. We did a total of seven sampling days per site in each season (21 sampling days per season for a total of 42 days of fieldwork or 1260 net hours). Birds were identified using field guides (Howell & Webb, 1995; Van Perlo, 2006), and we followed the taxonomical nomenclature suggested by the American Ornithological Society in its Checklist of North and Middle American Birds (Chesser *et al.*, 2018).

The blood smears were stained with Giemsa diluted in a saline solution at a 7.2 pH for 30 minutes and screened for 30 minutes each by light microscopy at 1000× magnification (Santiago-Alarcon & Carbo-Ramírez, 2015). Parasites were identified to the genus level following Valkiūnas (2005). The intensity of infection or parasitemia was estimated as the number of infected red blood cells per 2000 erythrocytes (Godfrey *et al.*, 1987); we subsequently classified them depending on the proportion of infected erythrocytes as low (< 0.1%), medium (0.1 - 0.5%), and high (> 0.5%) (Hauptmanová *et al.*, 2006).
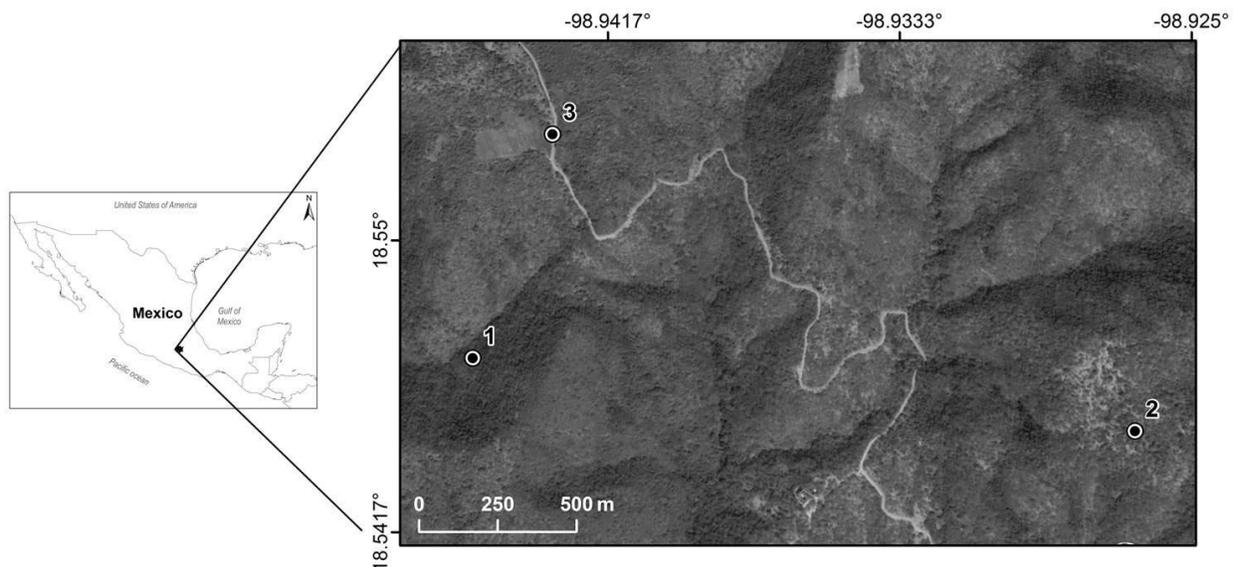

Figure 1. Sampling areas in Sierra de Huautla, Morelos, Mexico. 1) Preserved; 2) Disturbed; 3) Agricultural.

To describe the diversity and structure of the bird community by sampling site, we calculated the Shannon-Wiener (H) and the Pielou evenness (E_H) indexes, made a rank abundance curve and estimated the Jaccard distance. Bird diversity was compared using the Hutcheson t-test for the three combinations of paired sampling areas (preserved/disturbed, preserved/agricultural, and disturbed/agricultural). We built generalized linear models (GLMs) to analyze the response of prevalence (number of infected individuals from the total sample) (binomial distribution) and parasitemia (negative binomial distribution) to the predictor variables habitat type and season. Differences in prevalence by bird families were analyzed with Fisher's exact test. All analyses were done with the R software (v.3.0.2), and diversity analyses were done using the 'vegan' package (v.2.5-7) (R Development Core Team, 2012).

RESULTS

We captured a total of 142 birds, 41 during the dry season belonging to 12 genera and 17 species, and 101 during the rainy season from 20 genera and 28 species (33 different species in total). All bird species were resident except for *Passerina cyanea* captured in the dry season (n = 7). Seven bird species were captured in the preserved area compared to 20 in the disturbed area and 23 in the agricultural area. The corresponding Shannon-Weiner and Pielou indexes were, for the preserved area H = 1.74, E_H = 0.5, disturbed area H = 2.62, E_H = 0.75, and agricultural area H = 2.79, E_H = 0.8. Species dominance was high in all areas, and the disturbed and agricultural areas had a more similar species composition (Fig. 2). Bird diversity of the preserved area was significantly different when compared to the disturbed and agricultural areas (both p < 0.001), but there was no difference between the disturbed and agricultural areas (p = 0.335).
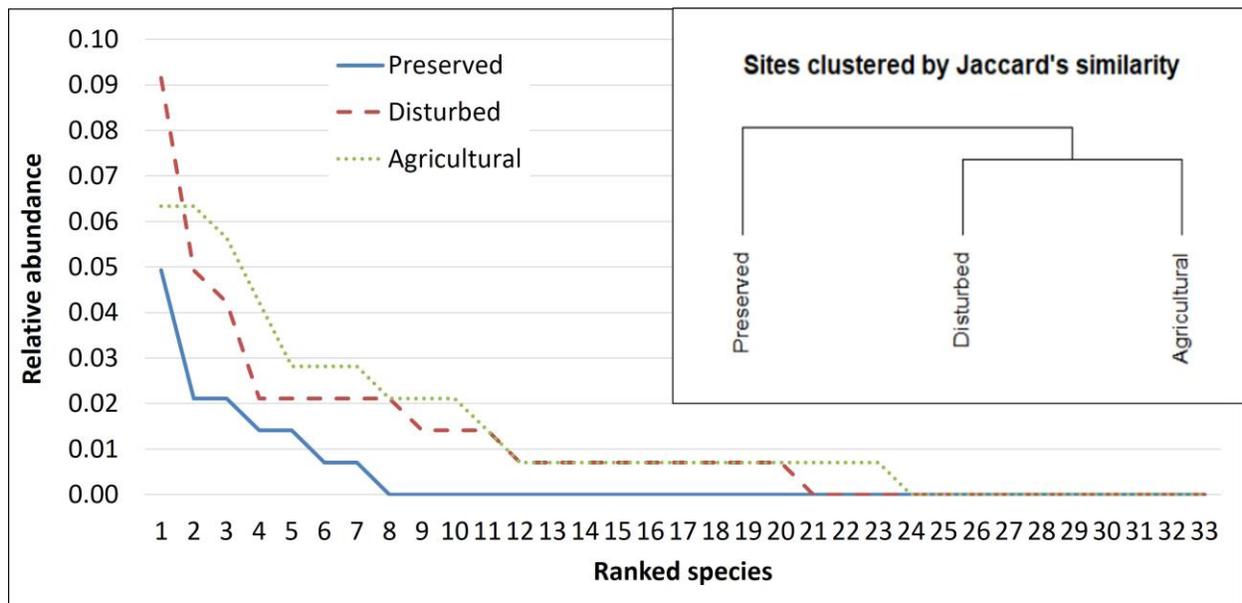

Figure 2. Rank-abundance curve of the bird species by sampling area with a dendrogram of the Jaccard distance in the embedded plot.

Table 1. Blood-parasites from wild birds in Sierra de Huautla, Morelos, Mexico, presented by sampling season.

	Species	Dry Season					Rainy season				
		H	P	M	mx	n	H	P	M	mx	n
	Amazilia beryllina					0					1
*	*Amazilia violiceps*					0	1*				4
*	*Campylorhynchus jocosus*					0	1*				1
*	*Columbina inca*	1	1*	1	1	6	7				16
	Columbina passerine					0	2	1		1	4
	Contopus sordidulus					1					1
	Haemorhous mexicanus					0					1
	Heliomaster constantii					1					0
*	*Icterus pustulatus*	1*		1*	1	1	3				3
	Icterus wagleri					0					1
	Lampornis clemenciae					1					0
	Melanerpes chrysogenys					0					2
	Melanerpes hypopolius					0					11
*	*Molothrus aeneus*					0	1*				6
	Myiarchus tuberculifer					3	1				4
	Myiarchus tyrannulus	1				3					4
	Myiopagis viridicata					0					1
*	*Passerina caerulea*					0	1*				3
	Passerina cyanea (m)	3				7					0
*	*Passerina leclancherii*					1	1*				2
	Passerina versicolor					1					3
	Peucaea botterii					1					0
*	*Peucaea humeralis*	2*				4	2		2*	2	6
*	*Peucaea ruficauda*	1*				2	1				1
*	*Pheucticus chrysopeplus*					0	1*				1
	Pitangus sulphuratus					0					1
	Pyrocephalus rubinus	1				4					3
	Spinus psaltria					0	5		2	2	9
*	*Thryophilus pleurostictus*	1*				1					2
	Toxostoma curvirostre					0					1
*	*Turdus rufopalliatus*					3	2*	1*	1*	1	8
	Xenotriccus mexicanus					1					0
	Xiphorhynchus flavigaster					0					1
	Total	**11**	**1**	**2**	**2**	**41**	**29**	**2**	**5**	**6**	**101**

*, new parasite-host association; (m), migratory species; H, *Haemoproteus* spp.; P, *Plasmodium* spp.; M, microfilariae; mx, mixed infections; n, total number of caught birds.

Table 2. Blood-parasites from wild birds in Sierra de Huautla, Morelos, Mexico by habitat condition.

Species	Preserved					Disturbed					Agricultural				
	H	P	M	mx	n	H	P	M	mx	n	H	P	M	mx	n
Amazilia beryllina					1					0					0
Amazilia violiceps	1				3					0					1
Campylorhynchus jocosus					0					0	1				1
Columbina inca					0	5				13	3	1	1	1	9
Columbina passerina					0	1	1		1	3	1				1
Contopus sordidulus					0					1					1
Haemorhous mexicanus					0					1					0
Heliomaster constantii					0					1					0
Icterus pustulatus					0	3				3	1		1	1	1
Icterus wagleri					0					0					1
Lampornis clemenciae					0					1					0
Melanerpes chrysogenys					2					0					0
Melanerpes hypopolius					0					7					4
Molothrus aeneus					2	1				2					2
Myiarchus tuberculifer					0	1				3					4
Myiarchus tyrannulus					0					1	1				6
Myiopagis viridicata					0					1					0
Passerina caerulea					0	1				2					1
Passerina cyanea (m)					0	3				6					1
Passerina leclancherii					0	1				3					0
Passerina versicolor					0					1					3
Peucaea botterii					0					0					1
Peucaea humeralis					0					2	4		2	2	8
Peucaea ruficauda					0					0	2				3
Pheucticus chrysopeplus					0					0	1				1
Pitangus sulphuratus					0					1					0
Pyrocephalus rubinus					0	1				3					4
Spinus psaltria					0					0	5		2	2	9
Thryophilus pleurostictus	1				3					0					0
Toxostoma curvirostre					0					0					1
Turdus rufopalliatus	2	1	1	1	7					1					3
Xenotriccus mexicanus					0					0					1
Xiphorhynchus flavigaster					1					0					0
Total	**4**	**1**	**1**	**1**	**19**	**17**	**1**		**1**	**56**	**19**	**1**	**6**	**6**	**67**

(m), migratory species; H, *Haemoproteus* spp.; P, *Plasmodium* spp.; M, microfilariae; mx, mixed infections; n, total number of caught birds.

We found blood parasites from the genus *Haemoproteus* spp. (Fig. 3) and *Plasmodium* spp., as well as microfilariae (Order Rhabditidia) (Fig. 4). Parasite prevalence during the dry season was 29.3% and 29.7% during the rainy season. Prevalence was 26.3% in the conserved forest, 36.4% in

the disturbed forest, and 29.9% in the agricultural area. Prevalence per parasite genera was 28.2% for *Haemoproteus* spp., 2.1% for *Plasmodium* spp., 4.9% for microfilariae, and 5.6% of coinfections (*Haemoproteus* spp., *Plasmodium* spp. and microfilariae n = 2, *Haemoproteus* spp. and microfilariae n = 5, and *Haemoproteus* spp. and *Plasmodium* spp. n = 1). The bird species with higher prevalence were *Columbina inca* (9/22), *Spinus psaltria* (5/9), *Icterus pustulatus* (4/4), and *Peucaea humeralis* (4/10) (Tables 1 and 2). There were no differences in prevalence among bird families (Fisher's exact test, p = 0.09) (Table 3). We did not find statistical differences in prevalence between seasons or among sampling areas (Table 4).
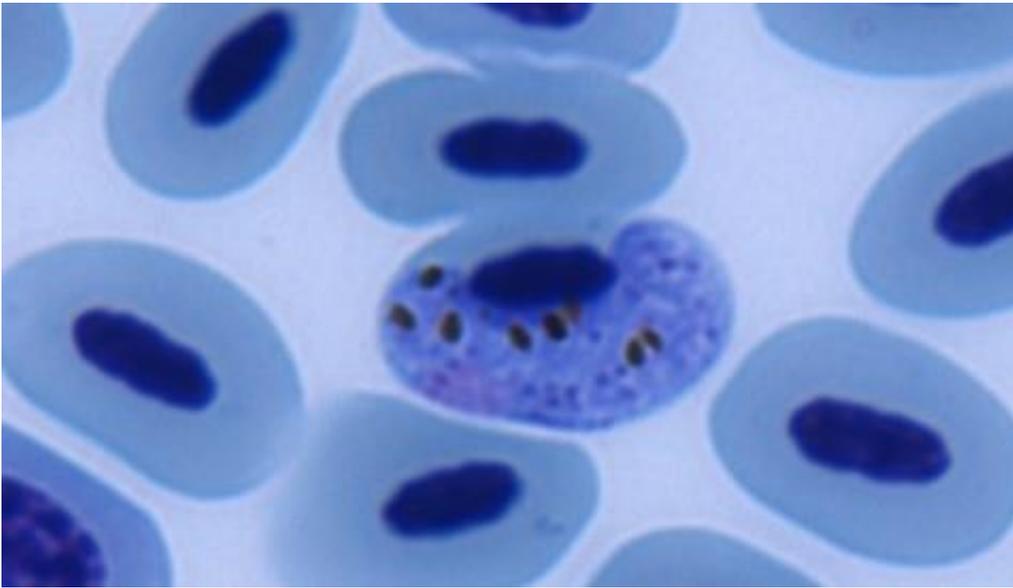

Figure 3. *Haemoproteus* spp. infecting birds in Sierra de Huautla, Morelos, Mexico.

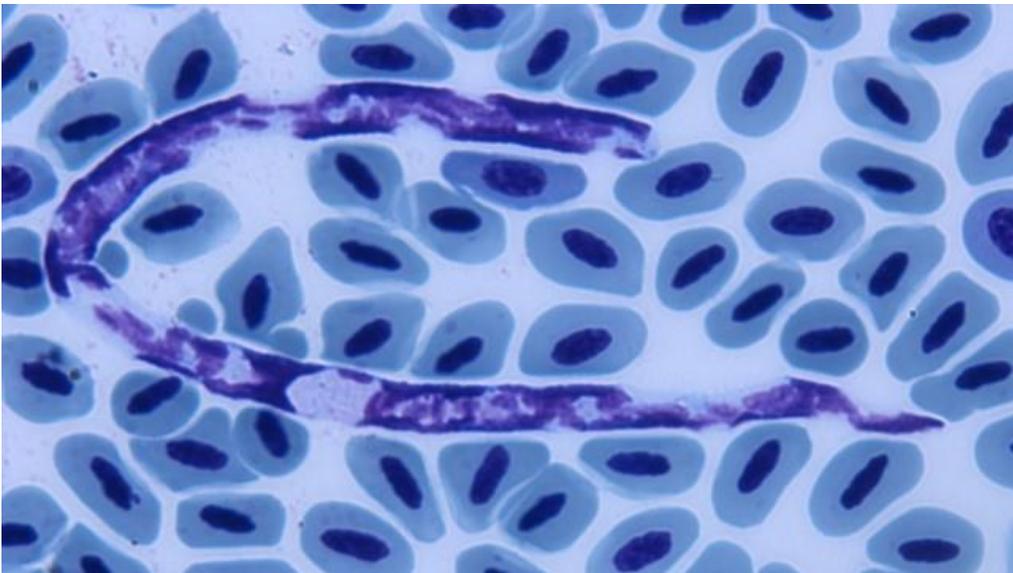**Figure 4.** Microfilariae nematode infecting birds in Sierra de Huautla, Morelos, Mexico.

Parasitemia was low (< 0.1%) in 26 birds, medium (0.1–0.5%) in 13 birds, and high (> 0.5%) in one bird (Table 5). The highest parasitemia was observed in an adult female of *I. pustulatus* infected with *Haemoproteus* spp., its physical appearance was unhealthy as it presented sparse feathers on its head and tail and damaged wing cover feathers (Fig. 5). There were no statistical differences in parasitemia among habitat types or seasons (Table 6).

After reviewing reports by other authors and the databases EID2 (Wardeh *et al.*, 2015) and MalAvi (Egerhill *et al.*, 2016), we found that this is the first time that avian haemosporidian parasites are recorded in 12 of the species that we captured (Table 1). Likewise, this is the first time that *Melanerpes chrysogenys*, *Melanerpes hypopolius*, and *Xenotriccus mexicanus* are examined for blood parasites resulting negative.

Table 3. Prevalence of blood-parasites by families of birds sampled in Sierra de Huautla, Morelos, Mexico.

Order	Family	Infected		Total
		n	%	
Apodiformes	Trochilidae	1	(14.3)	7
Columbiformes	Columbidae	5	(19.2)	26
Passeriformes	Picidae	0	(0)	13
	Cardinalidae	6	(33.3)	18
	Emberizidae	6	(42.9)	14
	Furnaridae	0	(0)	1
	Fringilidae	5	(50.0)	10
	Icteridae	5	(45.5)	11
	Troglodytidae	2	(50.0)	4
	Turdidae	3	(27.3)	11
	Tyrannidae	6	(22.2)	27
	Total	39	(27.0)	142

Table 4. Parameters of the GLM of avian haemosporidian prevalence by site and season.

Factor	Estimate	SE	t	Pr (> |t|)
Intercept	-0.76	0.3	-2.48	0.014*
Preserved	-0.52	0.64	-0.81	0.41
Disturbed	0.07	0.4	0.17	0.86
Season: Dry	-0.37	0.43	-0.86	0.39

Residual deviance: 169.16 on 138 degrees of freedom (quasibinomial family correction).

Table 5. Parasitemia of haemosporidian parasites from Sierra de Huautla, Morelos, Mexico.

Species	*Haemoproteus* spp.	*Plasmodium* spp.
Amazilia violiceps	4/2000	M
Campylorhynchus jocosus	1/2000	L

Species	*Haemoproteus* spp.		*Plasmodium* spp.	
Columbina inca	1/2000	L	< 1/2000	L
	< 1/2000	L		
	1/2000	L		
	2/2000	M		
	1/2000	L		
	4/2000	M		
	< 1/2000	L		
	6/2000	M		
Columbina passerina	< 1/2000	L		
	< 1/2000	L		
			< 1/2000	L
Icterus pustulatus	1/2000	L		
	9/2000	M		
	< 1/2000	L		
	* 63/2000	H		
Myiarchus tuberculifer	< 1/2000	L		
Myiarchus tyrannulus	< 1/2000	L		
Molothrus aeneus	< 1/2000	L		
Passerina caerulea	9/2000	M		
Passerina cyanea	< 1/2000	L		
	< 1/2000	L		
	< 1/2000	L		
Passerina leclancherii	< 1/2000	L		
Peucaea humeralis	2/2000	M		
	2/2000	M		
	4/2000	M		
	8/2000	M		
Peucaea ruficauda	< 1/2000	L		
	10/2000	M		
Pheucticus chrysopeplus	10/2000	M		
Pyrocephalus rubinus	< 1/2000	L		
Spinus psaltria	< 1/2000	L		
	3/2000	M		
	< 1/2000	L		
	< 1/2000	L		
	< 1/2000	L		
Thryophilus pleurostictus	< 1/2000	L		
Turdus rufopalliatus	< 1/2000	L	< 1/2000	L
	< 1/2000	L		

Each row represents an individual. H, high intensity (> 0.5%); M, medium intensity (0.1-0.5%); L, low intensity (< 0.1%); *, this individual had the highest parasitaemia and an unhealthy appearance (see Figure 5).

Table 6. Parameters of the GLM of avian haemosporidian parasitemia by site and season.

Coefficients:	Estimate	SE	z	Pr (> |z|)
Intercept	0.22	0.39	0.57	0.56
Preserved	-1.11	0.8	-1.37	0.17
Disturbed	0.75	0.56	1.33	0.18
Season: Dry	-0.83	0.71	-1.16	0.24
Preserved Site: Season	1.02	2.23	0.45	0.64
Disturbed Site: Season	-1.87	1.16	-1.61	0.11

Residual deviance: 86 on 136 df (negative binomial distribution).

Figure 5. Streak-backed Oriole (*Icterus pustulatus*) captured in Sierra de Huautla, Morelos, Mexico. This individual had low body condition, damaged feathers and high parasitemia.

DISCUSSION

This is the first study of avian haemosporidian parasites on wild birds from the state of Morelos in Mexico, within Sierra de Huautla Biosphere Reserve. We found a higher overall prevalence to that reported in other studies in Mexico conducted in similar arid ecosystems (e.g., Coahuila 11.8%, San Luis Potosi 22.1%, Nuevo Leon 11.8%, and Tamaulipas 15.4%) (Bennett *et al.*, 1991; Ham-Dueñas *et al.*, 2017). Other surveys have recorded higher prevalence at both the assemblage and population levels, including areas of arid habitats (49%) (Blanco *et al.*, 2001) (44.3–47.5%) (Reinoso-

Pérez *et al.*, 2016) (47.3%) (Hernández-Lara *et al.*, 2017) (76.3%) (Cadena-Ortiz *et al.*, 2019) (32.2%) (Tinajero *et al.*, 2019). In our study, *Haemoproteus* spp. was the most common parasite genus, which is consistent with other works in Mexico (49%) (Blanco *et al.*, 2001) and across tropical regions (e.g., McClure *et al.*, 1978; White *et al.*, 1978). Some tropical countries have reported lower *Haemoproteus* spp. prevalence; for instance, 0.8-4% in two localities in Costa Rica (Benedikt *et al.*, 2009) and 2.6-6.7% in Colombia (Rodríguez & Matta, 2001; Londoño *et al.*, 2007), which may be related to habitat type and elevation. These differences suggest that local biotic and abiotic conditions have a differential effect on the same parasite system, making epizootiological generalizations at small spatial scales unfeasible (e.g., Santiago-Alarcon *et al.*, 2019). Moreover, the diversity and structure of bird communities differ among studies, which could imply that birds' susceptibility is different in each case, affecting overall parasite prevalence.

Regarding parasites of the genus *Plasmodium* spp., we found a similar prevalence to previous reports for areas with arid vegetation in Mexico (1%) (Bennett *et al.*, 1991) (4.8%) (Ham-Dueñas *et al.*, 2017) and in general across tropical regions (Santiago-Alarcon & Marzal 2020). *Plasmodium* parasites are transmitted by mosquitoes (Diptera: Culicidae) and arid conditions may restrict their successful development limiting their presence and successful transmission. For example, the immature stages of mosquitoes of the genus *Culex* spp., which are primary vectors of *Plasmodium* spp., develop in a diverse range of shallow still water bodies rich in organic matter (Becker *et al.*, 2010) that we did not observe near our sampling areas. There are two damns for agricultural purposes near our sampling sites, both of which are deep, thus not ideal for *Culex* spp. development. Likewise, at high elevations, the temperature may limit the development of both mosquito vectors and parasites (LaPointe *et al.*, 2012). Therefore, the combination of dry conditions and lower temperatures could restrict the prevalence of *Plasmodium* parasites (Freed *et al.*, 2005; LaPointe *et al.*, 2012; Abella-Medrano *et al.*, 2018).

Trypanosoma spp. and microfilariae are exoerythrocytic blood parasites belonging to orders different of Haemosporida. We did not observe *Trypanosoma* spp., and in some previous studies in México, both parasite groups have been recorded at low prevalence (Bennett *et al.*, 1991; Ham-Dueñas *et al.*, 2017). The prevalence of microfilariae in our study suggests that the conditions of our sampling area are favorable for its transmission. All the microfilaria infections that we observed were in individuals infected with other blood-parasite (i.e., coinfections). The prevalence of coinfections is better described in recent years (Marinov *et al.*, 2017; Carlson *et al.*, 2018), yet some studies report low proportions suggesting that coinfection prevalence will depend on the host-parasite system under study (Murata, 2002; Marzal *et al.*, 2011). This is possibly due to the specificity of parasites to particular hosts (Huang *et al.*, 2018) and the combined probability for a bird to be bitten by more than one infected vector carrying different parasite species (Marzal *et al.*, 2008). Coinfections are expected to cause higher costs for the immune system and generate more detrimental effects on birds' health, but data suggesting the opposite has also been reported (Marzal *et al.*, 2008; Cadena-Ortiz *et al.*, 2019).

When comparing microscopy with nested PCR techniques, there is a high agreement between them (almost 80%) and a significant correlation; however, PCR is more sensitive to low-intensity infections but underestimates mixed infections (Valkiūnas *et al.*, 2006; Okanga *et al.*,

2013). Nevertheless, the advantages of microscopy techniques are the ability to observe parasite stages (e.g., gametocytes, which are required for successful transmission), detecting mixed infections with haemosporidians and other types of parasites (e.g., microfilaria) and estimating parasitemia. Our data derived only from blood smears (due to funding limitations); therefore, it would be ideal to complement our survey using molecular methods (e.g., Pacheco *et al.*, 2018) to improve prevalence estimates and describe haemosporidian lineages at our study sites.

Humidity and mild temperatures promote the reproduction of Diptera vectors and facilitate their survival (Belo *et al.*, 2012; Santiago-Alarcon *et al.*, 2012). For this reason, we expected to find a higher prevalence during the rainy season in comparison to the dry season. However, we did not find differences between seasons, a result like that of Belo *et al.* (2012) in arid regions of Venezuela. However, Ham-Dueñas *et al.* (2017) found a seasonal difference in prevalence and parasitemia in dry habitats of the northern portion of the Mexican plateau, and Hernández-Lara *et al.* (2017) reported prevalence differences between seasons among habitat types within a cloud forest matrix. This variability in prevalence is related to the different climatic and environmental conditions of each location, which influence the development of both parasites and vectors, and affect the encounter rate between parasites and hosts (Lachish *et al.*, 2011; Pulgarín-R *et al.*, 2018; Cadena-Ortiz *et al.*, 2019). Therefore, it is necessary to consider the habitat type, vegetation structure (e.g., leaf litter cover of forest ground and canopy cover) and environmental variables (e.g., temperature, humidity, precipitation, and host abundance) in which blood-parasites are surveyed to evaluate their effects on the epizootiological process (LaPointe *et al.*, 2012; Reinoso-Pérez *et al.*, 2016; Abella-Medrano *et al.*, 2018; Cadena-Ortiz *et al.*, 2019; van Hoesel *et al.*, 2019).

Passerina cyanea was the only migratory species captured at our study sites, and it was positive for parasites of the genus *Haemoproteus* spp. It has been proposed that migratory species might be more susceptible to infections because the physiological demands of migration could compromise their immune system (Jenkins *et al.*, 2012). Also, it has been shown that long-distance migrants in the Americas can connect haemosporidian communities in their breeding and wintering areas, and exchanged parasites lineages reflect the taxonomic similarity between resident and migratory species (Ricklefs *et al.*, 2017; Ishtiaq & Renner, 2020). Thus, because migrants are likely to encounter diverse avian, parasite, and vector assemblages at stopover sites, they could have a relevant role in blood parasite introductions at distant locations (Jenkins *et al.*, 2012; Santiago-Alarcon *et al.*, 2013; Reinoso-Pérez *et al.*, 2016). However, the role of migrant birds on the development of parasite communities needs to be assessed over a broad time, space, and taxonomic scales (Ricklefs *et al.*, 2017); moreover, the study of vector communities in breeding and wintering sites must be integrated, as well as the influence of environmental variables in both the parasite and vector life cycles. All this represents an extensive research effort that is not complete yet and the differences, for example, in the American and Euro-African migration systems cannot be completely explained (Ricklefs *et al.*, 2017).

It is worth mentioning that the *I. pustulatus* individual with the highest parasitemia in this study was infected with *Haemoproteus* spp. and microfilaria nematodes, having an unhealthy appearance (Fig. 5). The relevance of this observation hinges on the infrequent recording of disease signs in wild-caught birds with mist nets (Valkiūnas, 2005; Travis *et al.*, 2006), even when

having a high parasitemia, probably because such individuals have already survived the initial acute phase of infection and commonly carry chronic infections (Hauptmanová *et al.*, 2004; Benedikt *et al.*, 2009; Santiago-Alarcon *et al.*, 2018; Valkiūnas & Atkinson 2020). Furthermore, the prevalence in this species was 100%, although our sample number was small, n = 4, and Belo *et al.* (2012) also recorded a 100% prevalence in a species of the same genus in Venezuela (*Icterus nigrogularis*, n = 19). Thus, current data suggests that *Icterus* spp. could be highly susceptible to haemosporidian infections, but studies targeting species of this genus are needed to confirm such findings. The health impacts that haemosporidian parasites can have on birds may jeopardize wild bird conservation. This is paramount in countries with high diversity and endemism like Mexico, a megadiverse country with a high proportion of endemic wild birds, many of which are included in some category of endangerment (Bertelli *et al.*, 2017).

Our results suggest that different species of the bird community could have different susceptibilities to haemosporidian infection and possibly different roles in the endemic transmission process (e.g., Svensson-Coelho *et al.*, 2016; Santiago-Alarcon *et al.*, 2019). Considering the habitat classification of Ramírez-Albores and Ramírez-Cedillo (2002), only two of our species (*Pyrocephalus rubinus* and *Haemorhous mexicanus*) are not typically associated with tropical seasonal dry forest but to open or perturbed areas instead. These authors also reported a low number of species associated exclusively with riparian habitats, and some of our nets in the preserved area were placed in that kind of habitat. According to the classification of Ramírez-Albores and Ramírez-Cedillo (2002), the species that we captured in riparian conditions were common or abundant; only one was irregular (*Xenotriccus mexicanus*), and another one was scarce (*Toxostoma curvirostre*). Therefore, our sampling may have misrepresented the bird diversity in the preserved area; this is also noticeable by the diversity indexes, the rank-abundance curve, the Jaccard distance and the Hutcheson t-tests among sampling areas, which showed that the disturbed and agricultural areas are similar between each other but different from the preserved area where significantly less bird diversity was recorded. Moreover, a high proportion of the species that we found are considered common (42%) and a low proportion as rare (9%) or irregular (9%) compared to the proportions reported by Ramírez-Albores and Ramírez-Cedillo (2002), which were 20%, 27%, and 25%, respectively; indicating that our sampling failed to record the diversity of uncommon species. Furthermore, the lack of prevalence differences between seasons and sampling areas in our study could be influenced by the number of shared species among sampling localities, as we captured 12 (36%) species in both seasons and 13 (39%) in more than one kind of land-use type, most of which were shared between the disturbed and agricultural sites (n = 11, 33%). Another factor to consider is that the local vegetation matrix separating land use types is highly connected via wooded habitat; the slopes are mostly covered with tropical dry forest trees and tropical sub-deciduous forest, which for the dispersal ability of birds does not represent a limitation in their distribution or habitat use. Overall, our study area is one of the best regarding vegetation cover within the reserve, and thus, the observed similarity in prevalence and parasitemia may be due to its connectivity. Finally, it must be considered that we did not evaluate the vectors' community. A great diversity of vectors could be involved in the transmission of haemosporidian parasites, which have different preferred habitats, developing cycles, host preference, feeding times, and choice of vegetation strata within the forest (Santiago-Alarcon *et al.*, 2012). In future studies, the distance among sampling areas should be increased and located

in more extensive dissimilar areas and sampling should be carried out for longer periods to improve the bird community description. Likewise, simultaneous surveillance of potential vectors would contribute to understanding their role in the parasite transmission cycle.

The present work provides vital information about the relationship of wild birds and blood parasites in Sierra de Huautla Biosphere Reserve, being the first survey of Haemosporida parasites in the Mexican state of Morelos, adding sixteen new bird-haemosporidian associations for twelve bird species.

LITERATURE CITED

Abella-Medrano, C. A., Ibáñez-Bernal, S., Carbó-Ramírez, P., Santiago-Alarcon, D. (2018) Blood-meal preferences and avian malaria detection in mosquitoes (Diptera: Culicidae) captured at different land use types within a neotropical montane cloud forest matrix. *Parasitology International*, 67, 313–320.
https://doi.org/10.1016/j.parint.2018.01.006

Atkinson, C. T. (2008) Avian malaria. Pp. 35–53. *In:* C. T. Atkinson, N. J. Thomas, D. B. Hunter (Eds.). *Parasitic diseases of wild birds*. Wiley-Blackwell. US, Iowa.

Belo, N. O., Rodríguez-Ferraro, A., Braga, E. M., Ricklefs, R. E. (2012) Diversity of avian haemosporidians in arid zones of northern Venezuela. *Parasitology*, 139, 1021–1028.
https://doi.org/10.1017/S003118201200039X

Becker, N., Petric, D., Zgomba, M., Boase, C., Dahl, C., Madon, M., Kaiser, A. (2010) *Mosquitoes and Their Control*. Springer-Verlag, Berlin, 557 pp.

Benedikt, V., Barus, V., Capek, M., Havlicek, M., Literak, I. (2009) Blood parasites (*Haemoproteus* and microfilariae) in birds from the Caribbean slope of Costa Rica. *Acta Parasitologica*, 54, 197–204.
https://doi.org/10.2478/s11686-009-0043-1

Benítez, H., Arizmendi, C., Márquez, L. (1999) *Base de datos de las áreas de importancia para la conservación de aves*. México: Consejo Internacional para la Protección de las Aves, Sección México, Comisión Nacional para el Conocimiento y uso de la Biodiversidad, Fondo Mexicano para la Conservación de la Naturaleza y Comisión para la Cooperación Ambiental de América del Norte. Mexico, D.F.

Bennett, G. F., Aguirre, A. A., Cook, R. S. (1991) Blood parasites of some birds from Northeastern Mexico. *Journal of Parasitology*, 77, 38–41.

Bensch, S., Hellgren, O., Perez-Tris, J. (2009) MalAvi: a public database of malaria parasites and related haemosporidians in avian hosts based on mitochondrial cytochrome *b* lineages. *Molecular Ecology Resources*, 9, 1353–1358.
https://doi.org/10.1111/j.1755-0998.2009.02692.x

Bertelli, S., Szumik, C., Goloboff, P. A., Giannini, N. P., Navarro-Siguenza, A. G., Peterson, A. T., Cracraft, J. (2017) Mexican land birds reveal complexity in fine-scale patterns of endemism. *Journal of Biogeography*, 44, 1836–1846.
https://doi.org/10.1111/jbi.12987

Blanco, G., Rodríguez-Estrella, R., Merino, S., Bertellotti, M. (2001) Effects of spatial and host variables on hematozoa in white-crowned sparrows wintering in Baja California. *Journal of Wildlife Diseases*, 37, 786–790.
https://doi.org/10.7589/0090-3558-37.4.786

Bonneaud, C., Sepil, I., Milá, B., Buermann, W., Pollinger, J., Sehgal, R. N. M., Valkiūnas, G., Iezhova, T. A., Saatchi, S., Smith, T. B. (2009) The prevalence of avian *Plasmodium* is higher in undisturbed tropical forests of Cameroon. *Journal of Tropical Ecology*, 25, 439–447.
https://doi.org/10.1017/S0266467409006178

Cadena-Ortiz, H., Mantilla, J. S., de Aguilar, J. R., Flores, D., Bahamonde, D., Matta, N. E., Bonaccorso, E. (2019) Avian haemosporidian infections in rufous-collared sparrows in an Andean dry forest: diversity and factors related to prevalence and parasitaemia. *Parasitology*, 146, 765–773.
https://doi.org/10.1017/S0031182018002081

Carlson, J. S., Martínez-Gómez, J. E., Cornel, A., Loiseau, C., Sehgal, R. N. M. (2011) Implications of *Plasmodium* parasite infected mosquitoes on an insular avifauna: the case of Socorro Island, México. *Journal of Vector Ecology*, 36, 213–220.
https://doi.org/10.1111/j.1948-7134.2011.00159.x

Carlson, J. S., Martínez-Gómez, J. E., Valkiūnas, G., Loiseau, C., Bell, D. A., Sehgal, R. N. (2013) Diversity and phylogenetic relationships of hemosporidian parasites in birds of Socorro Island, Mexico, and their role in the re-introduction of the socorro dove (*Zenaida graysoni*). *Journal of Parasitology*, 99, 270–276.
https://doi.org/10.1645/GE-3206.1

Carlson, J. S., Nelms, B., Barker, C. M., Reisen, W. K., Sehgal, R. N. M., Cornel, A. J. (2018) Avian malaria co-infections confound infectivity and vector competence assays of *Plasmodium homopolare*. *Parasitology Research*, 117, 2385–2394.
https://doi.org/10.1007/s00436-018-5924-5

Challenger, A., Caballero, J. (1998) *Utilización y conservación de los ecosistemas terrestres de méxico: pasado, presente y futuro*. CONABIO, Mexico, 847 pp.

Chasar, A., Loiseau, C., Valkiūnas, G., Iezhova, T., Smith, T. B., Sehgal, R. N. M. (2009) Prevalence and diversity patterns of avian blood parasites in degraded African rainforest habitats. *Molecular Ecology*, 18, 4121–4133.
https://doi.org/10.1111/j.1365-294X.2009.04346.x

Chesser, R. T., Burns, K. J., Cicero, C., Dunn, J. L., Kratter, A. W., Lovette, I. J., Rasmussen, P. C., Remsen, J. V., Stotz, D. F., Winger, B. M., Winker, K. (2018) Check-list of North and Middle American birds. *American Ornithological Society*. Available at: http://checklist.aou.org/taxa (accessed on 15 July 2019).

CONANP (2005) *Programa de conservación y manejo Reserva de la Biosfera Sierra de Huautla, México*. CONANP (Comisión Nacional de Áreas Naturales Protegidas), Mexico.

de la O-Toris, J., Maldonado, B., Martínez-Garza, C. (2012) Efecto de la perturbación en la comunidad de herbáceas nativas y ruderales de una selva estacional mexicana. *Botanical Sciences*, 90, 469–480.

Dobson, A., Foufopoulos, J. (2001) Emerging infectious pathogens of wildlife. *Philosophical Transactions of the Royal Society B-Biological Sciences*, 356, 1001–1012.
https://doi.org/10.1098/rstb.2001.0900

Egerhill, M., Canbäck, B., Bensch, S. (2016) MalAvi, version 2.2.9. Lund University, Department of Biology. Available at: http://130.235.244.92/Malavi/ (accessed on 10 November 2019).

Foster, J. T., Woodworth, B. L., Eggert, L. E., Hart, P. J., Palmer, D., Duffy, D. C., Fleischer, R. C. (2007) Genetic structure and evolved malaria resistance in Hawaiian honeycreepers. *Molecular Ecology*, 16, 4738–4746.
https://doi.org/10.1111/j.1365-294X.2007.03550.x

Freed, L. A., Cann, R. L., Goff, M. L., Kuntz, W. A., Bodner, G. R. (2005) Increase in avian malaria at upper elevation in Hawai'i. *Condor*, 107, 753–764.
https://doi.org/10.1650/7820.1

Godfrey, R. D., Fedynich, A. M., Pence, D. B. (1987) Quantification of hematozoa in blood smears. *Journal of Wildlife Diseases*, 23, 558–565.
https://doi.org/10.7589/0090-3558-23.4.558

Ham-Dueñas, J. G., Chapa-Vargas, L., Stracey, C. M., Huber-Sannwald, E. (2017) Haemosporidian prevalence and parasitaemia in the Black-throated sparrow (*Amphispiza bilineata*) in central-Mexican dryland habitats. *Parasitology Research*, 116, 2527–2537.
https://doi.org/10.1007/s00436-017-5562-3

Hauptmanová, K., Baruš, V., Literák. I., Benedikt, V. (2004) Haemoproteids and microfilariae in hawfinches in the Czech Republic. *Helminthologia*, 41, 125–133.

Hauptmanová, K., Benedikt, V., Literák, I. (2006) Blood parasites in passerine birds in Slovakian East Carpathians. *Acta Protozoologica*, 45, 105–109.

Hernández-Lara, C., González-García, F., Santiago-Alarcon, D. (2017) Spatial and seasonal variation of avian malaria infections in five different land use types within a Neotropical montane forest matrix. *Landscape and Urban Planning*, 157, 151–160.
https://doi.org/10.1016/j.landurbplan.2016.05.025

Hernández-Lara, C., Espinosa de los Monteros, A., Ibarra-Cerdeña, C. N., García-Feria, L., Santiago-Alarcon, D. (2018) Combining morphological and molecular data to reconstruct the phylogeny of avian Haemosporida. *International Journal for Parasitology*, 48, 1137–1148.
https://doi.org/10.1016/j.ijpara.2018.10.002

Howell, S. N. G., Webb, S. (1995) *A guide to the birds of Mexico and Northern Central America*. Oxford University Press, Oxford, 1010 pp.

Huang, X., Ellis, V. A., Jönsson, J., Bensch, S. (2018) Generalist haemosporidian parasites are better adapted to a subset of host species in a multiple host community. *Molecular Ecology*, 27, 4336–4346.
https://doi.org/10.1111/mec.14856

Ishtiaq, F., Renner, S. C. (2020) Bird migration and vector-borne parasite transmission. Pp. 513–526. *In*: D. Santiago-Alarcon, A. Marzal (Eds). *Avian malaria and related parasites in the tropics*. Springer, Cham.

Jenkins, T., Thomas, G. H., Hellgren, O., Owens, I. P. F. (2012) Migratory behavior of birds affects their coevolutionary relationship with blood parasites. *Evolution*, 66, 740–751.
https://doi.org/10.1111/j.1558-5646.2011.01470.x

Knowles, S. C., Palinauskas, V., Sheldon, B. C. (2010) Chronic malaria infections increase family inequalities and reduce parental fitness: experimental evidence from a wild bird population. *Journal of Evolutionary Biology*, 23, 557–569.
https://doi.org/10.1111/j.1420-9101.2009.01920.x

Lachish, S., Knowles, S. C., Alves, R., Wood, M. J., Sheldon, B. C. (2011) Infection dynamics of endemic malaria in a wild bird population: parasite species-dependent drivers of spatial and temporal variation in transmission rates. *Journal of Animal Ecology*, 80, 1207–1216.
https://doi.org/10.1111/j.1365-2656.2011.01893.x

LaPointe, D. A., Atkinson, C. T., Samuel, M. D. (2012) Ecology and conservation biology of avian malaria. *Annals of the New York Academy of Sciences*, 1249, 211–226.
https://doi.org/10.1111/j.1749-6632.2011.06431.x

Liao, W., Elison, T. O., Zhang, C., Atkinson, C. T., LaPointe, D. A., Samuel, M. D. (2015) Will a warmer and wetter future cause extinction of native Hawaiian forest birds? *Global Change Biology*, 21, 4342–4352.
https://doi.org/10.1111/gcb.13005

Londoño, A., Pulgarín-R, P. C., Blair, S. (2007) Blood parasites in birds from the lowlands of Northern Colombia. *Caribbean Journal of Science*, 43, 87–93.

Marinov, M. P., Marchetti, C., Dimitrov, D., Ilieva, M., Zehtindjiev, P. (2017) Mixed haemosporidian infections are associated with higher fearfulness in Yellow Wagtail (*Motacilla flava*). *Canadian Journal of Zoology*, 95, 405–410.
https://doi.org/10.1139/cjz-2016-0121

Martínez, J., Vásquez, R. A., Venegas, C., Merino, S. (2015) Molecular characterisation of haemoparasites in forest birds from Robinson Crusoe Island: is the austral thrush a potential threat to endemic birds? *Bird Conservation International*, 25, 139–152.
https://doi.org/10.1017/S0959270914000227

Martínez-de la Puente, J. M., Merino, S., Tomás, G., Moreno, J., Morales, J., Lobato, E., García-Fraile, S., Belda, E. J. (2010) The blood parasite *Haemoproteus* reduces survival in a wild bird: a medication experiment. *Biology Letters*, 6, 663–665.
https://doi.org/10.1098/rsbl.2010.0046

Martínez-Garza, C., Osorio-Beristain, M., Valenzuela-Galván, D., Nicolás-Medina, A. (2011) Intra and inter-annual variation in seed rain in a secondary dry tropical forest excluded from chronic disturbance. *Forest Ecology and Management*, 262, 2207–2218.
https://doi.org/10.1016/j.foreco.2011.08.013

Marzal, A., Bensch, S., Reviriego, M., Balbontin, J., de Lope, F. (2008) Effects of malaria double infection in birds: one plus one is not two. *Journal of Evolutionary Biology*, 21, 979–987.
https://doi.org/10.1111/j.1420-9101.2008.01545.x

Marzal, A., Ricklefs, R. E., Valkiūnas, G., Albayrak, T., Arriero, E., Bonneaud, C., Czirják, G. A., Ewen, J., Hellgren, O., Hořáková, D., Iezhova, T. A., Jensen, H., Križanauskienė, A., Lima, M. R., de Lope, F., Magnussen, E., Martin, L. B., Møller, A. P., Palinauskas, V., Pap, P. L., Pérez-Tris, J., Sehgal, R. N., Soler, M., Szöllősi, E., Westerdahl, H., Zetindjiev, P., Bensch, S. (2011) Diversity, loss, and gain of malaria parasites in a globally invasive bird. *PLoS ONE*, 6 (7), e21905.
https://doi.org/10.1371/journal.pone.0021905

McClure, H. E., Poonswad, P., Greiner, E. C., Larid, M. (1978) *Haematozoan in the birds of Eastern and Southern Asia*. St. Jhon's Memorial University of Newfoundland, Canada.

Murata, K. (2002) Prevalence of blood parasites in Japanese wild birds. *Journal of Veterinary Medical Science*, 64, 785–790.
https://doi.org/10.1292/jvms.64.785

Okanga, S., Cumming, G. S., Hockey, P. A. R., Grome, M., Peters, J. L. (2013) A comparison of techniques employed in detection of avian malaria infection, South Africa. *African Zoology*, 48 (2), 309–317.
https://doi:10.3377/004.048.0215

Osorio-Beristain, M., Rodríguez, A., Martínez-Garza, C., Alcalá, R. E. (2018) Relating flight initiation distance in birds to tropical dry forest restoration. *Zoologia*, 35, 1–6.
https://doi.org/10.3897/zoologia.35.e12642

Pacheco, M. A., Cepeda, A. S., Bernotienė, R., Lotta, I. A., Matta, N. E., Valkiūnas, G., Escalante, A. A. (2018) Primers targeting mitochondrial genes of avian haemosporidians: PCR detection and differential DNA amplification of parasites belonging to different genera. *International Journal for Parasitology*, 48, 657–670.
https://doi.org/10.1016/j.ijpara.2018.02.003

Pulgarín-R, P. C., Gómez, J. P., Robinson, S., Ricklefs, R. E., Cadena, C. D. (2018) Host species, and not environment, predicts variation in blood parasite prevalence, distribution, and diversity along a humidity gradient in northern South America. *Ecology and Evolution*, 8, 3800–3814.
https://doi.org/10.1002/ece3.3785

R Development Core Team (2012) R: a language and environment for statistical computing. (v. 3.0.2). R Foundation for Statistical Computing, Vienna.

Ramírez-Albores, J. E., Ramírez-Cedillo, M. G. (2002) Avifauna de la región oriente de la sierra de Huautla, Morelos, México. *Anales del Instituto de Biología, Serie Zoología*, 73, 91–111.

Reinoso-Pérez, M. T., Canales-Delgadillo, J. C., Chapa-Vargas, L., Riego-Ruiz, L. (2016) Haemosporidian parasite prevalence, parasitemia, and diversity in three resident bird species at a shrubland dominated landscape of the Mexican highland plateau. *Parasites & Vectors*, 9, 307.
https://doi.org/10.1186/s13071-016-1569-3

Ricklefs, R. E., Medeiros, M., Ellis, V. A., Svensson-Coelho, M., Blake, J. G., Loiselle, B. A., Soares, L., Fecchio, A., Outlaw, D., Marra, P. P., Latta, S. C., Valkiūnas, G., Hellgren, O.,

Bensch, S. (2017) Avian migration and the distribution of malaria parasites in New World passerine birds. *Journal of Biogeography*, 44, 1113–1123.
https://doi.org/10.1111/jbi.12928

Rodríguez, O. A., Matta, N. E. (2001) Blood parasites in some birds from eastern plains of Colombia. *Memorias Do Instituto Oswaldo Cruz*, 96, 1173–1176.
http://dx.doi.org/10.1590/S0074-02762001000800026

Santiago-Alarcon, D., Bloch, R., Rolshausen, G., Schaefer, H. M., Segelbacher, G. (2011) Prevalence, diversity, and interaction patterns of avian haemosporidians in a four-year study of blackcaps in a migratory divide. *Parasitology*, 138, 824–825.
https://doi.org/10.1017/S0031182011000515

Santiago-Alarcon, D., Palinauskas, V., Schaefer, H. M. (2012) Diptera vectors of avian Haemosporidian parasites: untangling parasite life cycles and their taxonomy. *Biological Reviews of the Cambridge Philosophical Society*, 87, 928–964.
https://doi.org/10.1111/j.1469-185X.2012.00234.x

Santiago-Alarcon, D., Mettler, R., Segelbacher, G., Schaefer, H. M. (2013) Haemosporidian parasitism in the blackcap *Sylvia atricapilla* in relation to spring arrival and body condition. *Journal of Avian Biology*, 44, 521–530.
https://doi.org/10.1111/j.1600-048X.2013.00181.x

Santiago-Alarcon, D., Carbo-Ramírez, P. (2015) Malaria parasites and related genera (order: Haemosporida) in birds from Mexico: methodological recommendations for the field and laboratory. *Ornitologia Neotropical*, 26, 59–77.

Santiago-Alarcon, D., Delgado-V, C. A. (2017) Warning! Urban threats for birds in Latin America. Pp. 125–142. *In*: I. MacGregor-Fors, J. F. Escobar-Ibáñez (Eds.). *Avian ecology in Latin American cityscapes*. Springer, Cham.

Santiago-Alarcon, D., Carbó-Ramírez, P., Macgregor-Fors, I., Chávez-Zichinelli, C. A., Yeh, P. J. (2018) The prevalence of avian haemosporidian parasites in an invasive bird is lower in urban than non-urban environments. *Ibis*, 162, 201–214.
https://doi.org/10.1111/ibi.12699

Santiago-Alarcon, D., Macgregor-Fors, I., Falfán, I., Lüdtke, B., Segelbacher, G., Schaefer, H. M., Renner, S. (2019) Parasites in space and time: a case study of haemosporidian spatiotemporal prevalence in urban birds. *International Journal for Parasitology*, 49, 235–246.
https://doi.org/10.1016/j.ijpara.2018.08.009

Santiago-Alarcon, D., Marzal, A. (2020) *Avian malaria and related parasites in the tropics: ecology, evolution and systematics*. Springer, Cham, 575 pp.

Svensson-Coelho, M., Loiselle, B. A., Blake, J. G., Ricklefs, R. E. (2016) Resource predictability and specialization in avian malaria parasites. *Molecular Ecology*, 25, 4377–4391.
https://doi.org/10.1111/mec.13758

Tinajero, R., Chapa-Vargas, L., Ham-Dueñas, J. G., Santiago-Alarcon, D. (2019) Haemosporidian infection of the American kestrel in the Southern Chihuahuan Desert, Mexico: relationship with land use. *Journal of Ornithology*, 160, 699–710.
https://doi.org/10.1007/s10336-019-01640-3

Travis, E. K., Vargas, F. H., Merkel, J., Gottdenker, N., Miller, R. E., Parker, P. G. (2006) Hematology, serum chemistry, and serology of Galapagos penguins (*Spheniscus mendiculus*) in the Galapagos Islands, Ecuador. *Journal of Wildlife Diseases*, 42, 625–632. https://doi.org/10.7589/0090-3558-42.3.625

Trejo, I., Dirzo, R. (2000) Deforestation of seasonally dry tropical forest: a national and local analysis in Mexico. *Biological Conservation*, 94, 133–142. https://doi.org/10.1016/S0006-3207(99)00188-3

Valkiūnas, G. (2005) *Avian malaria parasites and other haemosporidia*. CRC Press. Florida, 946 pp.

Valkiūnas, G., Bensch, S., Iezhova, T. A., Križanauskienė, A., Hellgren, O., Bolshakov, C. (2006) Nested cytochrome B polymerase chain reaction diagnostics underestimate mixed infections of avian blood haemosporidian parasites: microscopy is still essential. *Journal of Parasitology*, 92, 418–422. https://doi.org/10.1645/GE-3547RN.1

Valkiūnas, G., Iezhova, T. A., Evans, E., Carlson, J. S., Martínez-Gómez, J. E., Sehgal, R. N. (2013) Two new *Haemoproteus* species (Haemosporida: Haemoproteidae) from columbiform birds. *Journal of Parasitology*, 99, 513–521. https://doi.org/10.1645/12-98.1

Valkiūnas, G., Santiago-Alarcon, D., Levin, I. I., Iezhova, T. A., Parker, P. G. (2010) A new *Haemoproteus* species (Haemosporida: Haemoproteidae) from the endemic Galapagos dove *Zenaida galapagoensis*, with remarks on the parasite distribution, vectors, and molecular diagnostics. *Journal of Parasitology*, 96, 783–792. https://doi.org/10.1645/GE-2442.1

Valkiūnas, G., Atkinson, C. T. (2020) Introduction to life cycles, taxonomy, distribution, and basic research techniques. Pp. 45–80. *In*: D. Santiago-Alarcon, A. Marzal (Eds.). *Avian malaria and related parasites in the tropics.* Springer, Cham.

van Hoesel, W., Marzal, A., Magallanes, S., Santiago-Alarcon, D., Ibáñez-Bernal, S., Renner, S. C. (2019) Management of ecosystems alters vector dynamics and haemosporidian infections. *Scientific Reports*, 9, 8779.

van Perlo, B. (2006) *Birds of Mexico and Central America*. Princeton University Press, London, 336 pp.

van Riper III, C., van Riper, S. G., Goff, M. L., Laird, M. (1986) The epizootiology and ecological significance of malaria in Hawaiian land birds. *Ecological Monographs*, 56, 327–344.

Wardeh, M., Risley, C., Mcintyre, M. K., Setzkorn, C., Baylis, M. (2015) Database of host-pathogen and related species interactions, and their global distribution. *Scientific Data*, 2, 150049. https://doi.org/10.1038/sdata.2015.49

White, E. M., Greiner, E. C., Bennett, G. F., Herman, C. M. (1978) Distribution of the hematozoa of Neotropical birds. *Revista de Biología Tropical*, 26, 43–102.