IJP: Parasites and Wildlife 11 (2020) 88–92

Contents lists available at ScienceDirect

IJP: Parasites and Wildlife

journal homepage: www.elsevier.com/locate/ijppaw

Haemoparasites in endemic and non-endemic passerine birds from central Mexico highlands

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ARTICLE INFO

Keywords:
Haemoparasites
Passeriformes
Endemic
Bird
Mexico
Haemoproteus
Leucocytozoon
Microfilariae

ABSTRACT

Haemosporidian parasites of birds are found worldwide and include the genera *Haemoproteus*, *Plasmodium* and *Leucocytozoon*. Infection with haemosporidian parasites can affect host physical condition and reproductive success. The aim of this study was to identify the blood parasites and parasitaemia in endemic and non-endemic passerine birds from central Mexico highlands. This study included 157 passerines representing 29 species from 17 families. Overall, 30.6% (48/157) of the birds were infected with blood parasites. Of those, *Haemoproteus* spp. were found in 14.0% (n = 22), *Leucocytozoon* spp. 12.1% (n = 19) and microfilariae 0.6% (n = 1). Blood parasites were found in 71.4% (5/7) of endemic bird species and 45.4% (10/22) of non-endemic species. Medium to high parasitaemia (number of parasites/number erythrocytes) was observed in birds with infections of *Haemoproteus* spp. and *Leucocytozoon* spp. Co-infections 3.8% (n = 6) were observed in two species of endemic birds. This study contributes to the knowledge of haemoparasites in endemic and non-endemic passerine birds from central Mexico highlands. Additional investigation on the molecular identification of haemosporidian parasites, pathogenicity and health status of these birds is necessary.

1. Introduction

Parasites are organisms which live for a considerable portion of their lives in (endoparasites) or on (ectoparasites) another different kind of organism, the host. Sometimes, the parasite can cause in the host some harm. Protozoan and metazoan parasites can induce alterations in host behaviour or colouration (Poulin, 2007). Parasites of the Order: Haemosporida are wide-spread and infect many species of vertebrates that are exposed to bites of bloodsucking dipteran insects (Order: Diptera) (Santiago-Alarcon et al., 2012). *Plasmodium*, *Haemoproteus* and *Leucocytozoon* (Phylum: Apicomplexa; and Order: Haemosporida) are the three most common Haemoparasitic protozoan genera found in birds (Valkiūnas, 2005). Additionally, Filarioid nematodes are haemoparasites; the larval microfilariae have been reported in many organs and tissues of numerous terrestrial vertebrates, including birds (Bartlett, 2008).

Mexico has a megadiverse avifauna that includes endemic bird species and species ranging farther north or south in the Americas (Peterson and Navarro-Sigüenza, 2016). Worldwide, there are approximately 10,500 bird species, of which 1,123 to 1,150 are in Mexico; from these, 194 to 212 species of birds are considered endemic (Navarro-Sigüenza et al., 2014). The first information of haemoparasites on Mexican birds was reported in 1940 by Beltrán, he analyzed birds from bird traders (called *pajareros* [derived from *pájaro*, the Spanish word for bird]). Beltrán (1940) found 24.7% of the birds sampled were positive to *Plasmodium* (11.7%), *Haemoproteus* (9.4%) and *Leucocytozoon* (1.1%) with additional co-infection by *Plasmodium* with *Haemoproteus* (2.3%). Further analysis revealed three species of *Plasmodium*: *P. relictum*, *P. cathemerium* and *P. nucleophilum* in Mexican birds (Hewitt, 1940). Beltrán, 1942a also found parasites of *Haemoproteus*, *Leucocytozoon*, *Plasmodium* and *Trypanosoma* from 142 blood smears of birds captured in northern Mexico, and microfilariae in birds from northern and south-eastern Mexico (Beltrán, 1942b). More recent investigations have identified a new lineage of haemosporidian

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https://doi.org/10.1016/j.ijppaw.2019.12.007
Received 18 June 2019; Received in revised form 21 December 2019; Accepted 27 December 2019

parasites unique to Columbiformes from Grey-headed dove (*Leptotila plumbeiceps*) (Valkiūnas et al., 2010) and a new *Haemoproteus* species (*H. paramultipigmentatus*) from the Socorro Common Ground Dove (*Columbina passerina socorroensis*) (Valkiūnas et al., 2013). Prevalence and diversity of haemosporidians from the genera *Parahaemoproteus* and *Leucocytozoon* within an individual are similar for blood, muscle and liver samples; however, *Plasmodium* exhibits differential detectability when screened from different tissue types and was more prevalent in the blood than in muscle and liver tissues (Fecchio et al., 2019). The aim of this study was to determine parasite genera, the proportion of blood parasites and parasitaemia in endemic and non-endemic passerine birds from central Mexico highlands.

2. Materials and methods

2.1. Ethical approval

Capture methods and sampling of animals were performed under direct supervision of a specialised ornithologist and veterinarian in accordance with the laws and guidelines of Mexico on Animal Welfare and the specific regulations of the Mexican government. The permits (SGPA/DGVS/07613/14, SGPA/DGVS/00955/16) cover all bird handling in this study and were approved by the SEMARNAT (Secretaría de Medio Ambiente y Recursos Naturales) of Mexico.

2.2. Study area

The study was conducted monthly during three-day samplings periods, from 21 June 2014 to 12 November 2017. The study sites (Fig. 1) were Parque Ecológico Ejidal de Cacalomacán (PEEC) in Nevado de Toluca Natural Protected Area (NTNPA) and Parque Ecoturístico Corral de Piedra (PECP) in Valle de Bravo Natural Protected Area (VBNPA). These locations are contained within the Natural Protected Area (PNA) of Central Mexico and the Transverse Neovolcanic Axis (CMTNA). The NTNPA polygon (19°12′37″ N, 99°44′42″ W; 19°12′31″ N, 99°43′51″ W; 19°11′31″ N, 99°44′22″ W; 19°11′47″ N, 99°45′09″ W) is in the State of Mexico at 2,800 to 3,247 m above sea level (m asl). Average annual temperature ranges from 18 °C (minimum 9 °C, maximum 22 °C) at low elevation and 12 °C (minimum −3 °C, maximum 13 °C) at high elevation. The type of vegetation corresponds to a forest with White Cedar (*Cupressus lindleyi*), Tasmanian Blue Gum (*Eucalyptus globulus*), Mexican White Pine (*Pinus ayacahuite*), Mexican Weeping Pine (*P. patula*), and Montezuma Pine (*P. montezumae*) (Sánchez-Jasso et al., 2013). The VBNPA (19°13′25″ N, 99°57′57″ W; 19°13′26″ N, 99°13′76″ W; 19°12′31″ N, 99°57′27″ W; 19°12′39″ N, 99°56′53″ W) is in the State of Mexico at 2,830 to 2,960 m asl. Average annual temperature is 13.4 °C (minimum 0.5 °C, maximum of 29.7 °C). The type of vegetation corresponds to a forest with Fir (*Abies* spp.), Pine (*Pinus* spp.) and grassland at the foothill (Beltrán and Díaz de la Vega, 2010).

2.3. Sample collection and processing

At each study site, eight mist nets were set (8 × 2.5 m) to capture the birds. The nets were placed at relevant points of bird activity as previously reported (Sánchez-Jasso et al., 2013). Birds were captured from 06:00 to 15:00 h. After capture, we identified, measured, banded, took blood samples via brachial venipuncture and then released the birds. The blood was immediately smeared on two slides per bird, air dried, fixed with absolute methanol, and stained with May-Grünwald Giemsa (Piaton et al., 2015). Slides were labelled with the bird species, bird band code, and sampling day. We examined entire smears microscopically (40× and 100×) for the presence of blood parasites using a microscope Primo Star, Carl Zeiss, USA. We followed Valkiūnas (2005) procedures for parasite identification. We took photomicrographs of

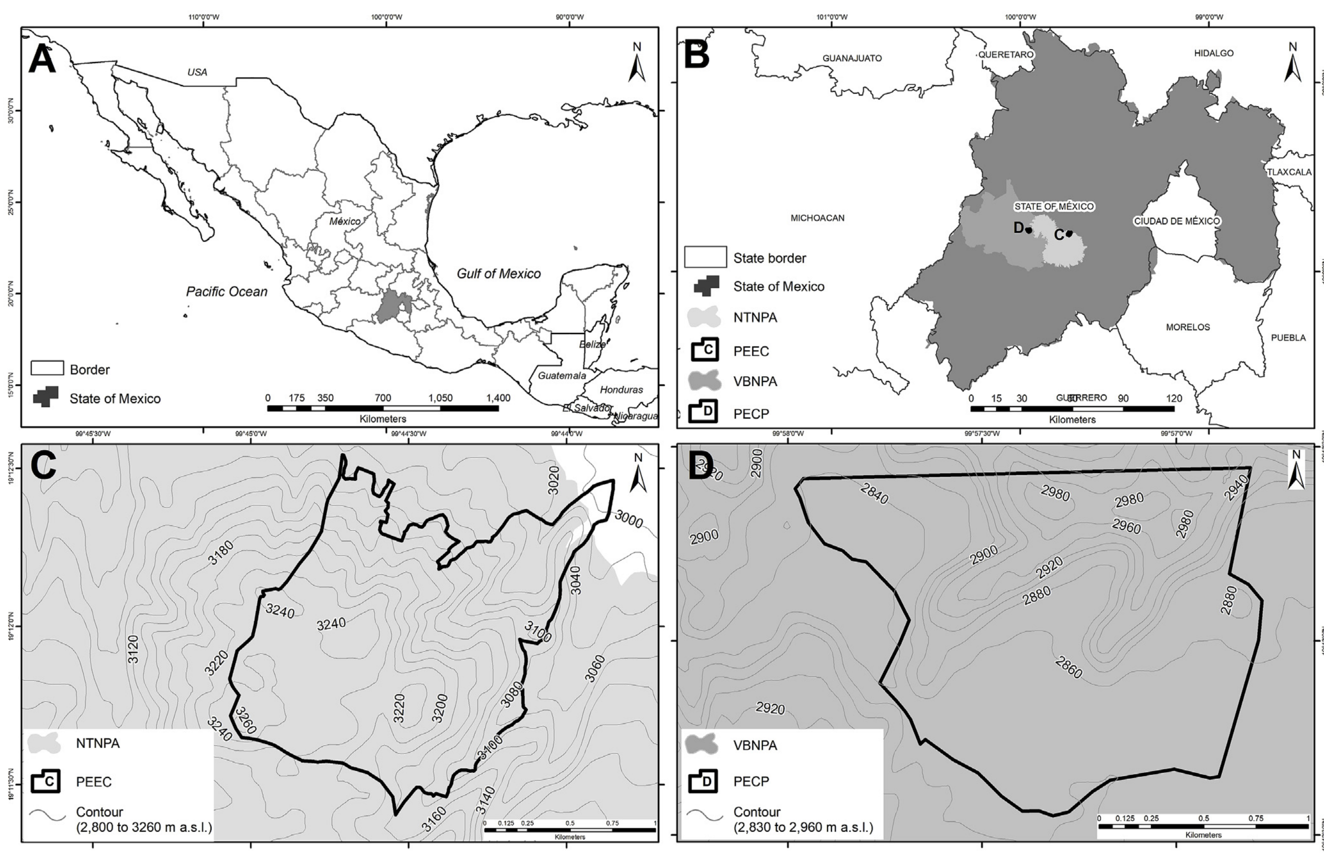

Fig. 1. Location of study sites. (A) Location of the State of Mexico, (B) Location of Nevado de Toluca Natural Protected Area (NTNPA), and Valle de Bravo Natural Protected Area (VBNPA), (C) Parque Ecológico Ejidal de Cacalomacán (PEEC) and (D) Parque Ecoturístico Corral de Piedra (PECP). Datum WGS_1984_UTM Zone 14.

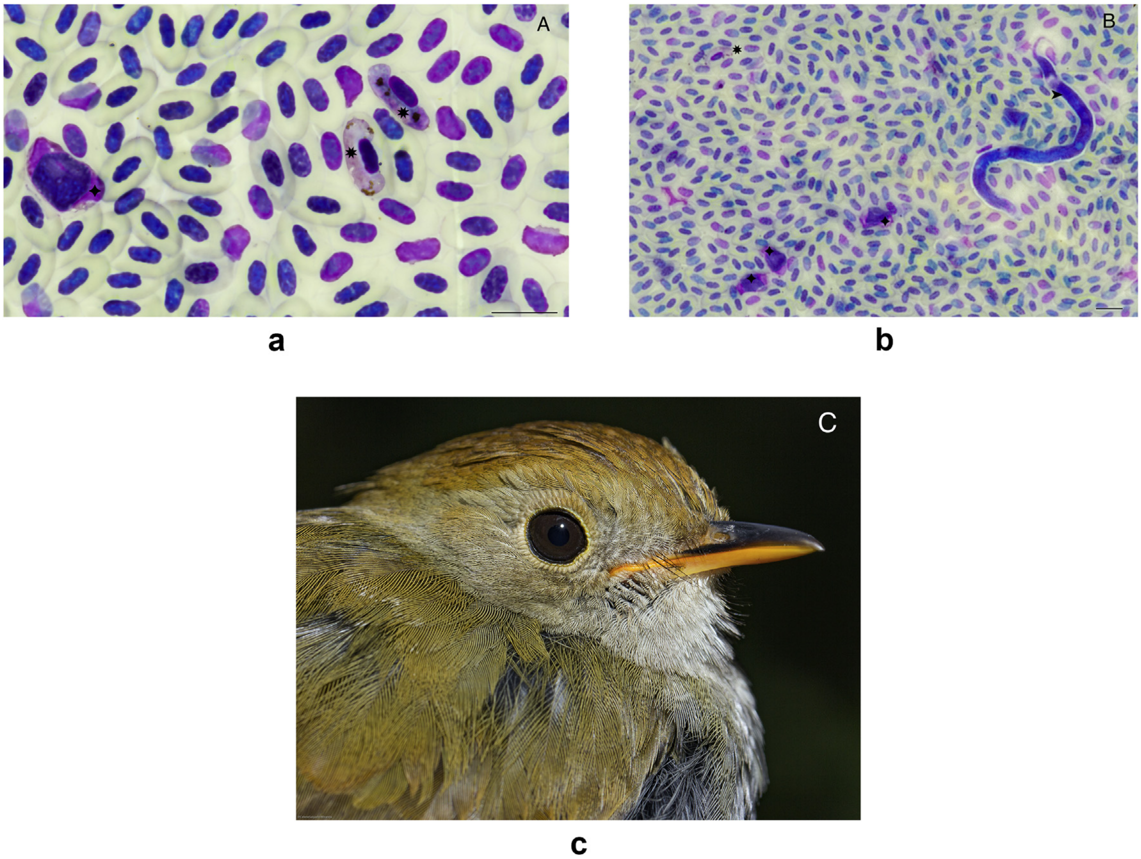

Fig. 2. Triple infection of *Haemoproteus* spp./*Leucocytozoon* spp./microfilaria in a *Catharus occidentalis*. (A). *Leucocytozoon* spp. (✦) and *Haemoproteus* spp. (✱) parasites. (B). Microfilaria (➤), *Leucocytozoon* spp. (✦) and *Haemoproteus* spp. (✱) parasites. (C). *Catharus occidentalis* infected. Photomicrographs. *Scale-bar*: 10 μm.

parasites using a Nikon ECLIPSE 80i binocular microscope coupled to a Nikon DS-Fi2 digital camera. We estimated intensity of infection as a percentage by counting of the number of parasites per 10,000 red blood cells (Godfrey et al., 1987). We classified blood parasite density (number of parasites/number erythrocytes) for infections with *Haemoproteus* (low $<$ 0.1% erythrocytes infected; medium = 0.1–0.5%; high $>$ 0.5%) and *Leucocytozoon* (low $<$ 0.02% erythrocytes infected; medium = 0.02–0.1%, high $>$ 0.1%) following Hauptmanová et al. (2006). We counted the total number microfilaria in a whole blood and categorised infection intensity as low (1-10 microfilariae per slide), medium (11-20 microfilariae per slide), and high ($>$ 20 microfilariae per slide) following Haas et al. (2011). Blood parasites prevalence (percentage of infected birds) and study site, were compared using the Chi-square test. Statistical significance was set at $P < 0.05$ (Zar, 1999).

3. Results

Of the 157 passerine birds from 29 species and 17 families examined, 48 birds (30.6%) were infected with blood parasites including *Haemoproteus* spp. 14.0% (n = 22), *Leucocytozoon* spp. 12.1% (n = 19), microfilariae 0.6% (n = 1) and co-infections 3.8% (n = 6). In this study, seven species of endemic Mexican birds were included, of which 71.4% (5/7 species) were infected. These birds belong to three families: Turdidae (Russet Nightingale-thrush, *Catharus occidentalis*), Passerellidae (Green-Striped Brush Finch, *Arremon virenticeps*; Rufous-capped Brush Finch, *Atlapetes pileatus*; Striped Sparrow, *Oriturus superciliosus*) and Parulidae (Red Warbler, *Cardellina rubra*). We found parasitaemia with *Haemoproteus* spp. at medium (9 birds) and high (13 birds) intensities, *Leucocytozoon* spp. at medium (7 birds) and high (12 birds) intensities, but parasitaemia was low for microfilaria in all birds sampled. In this study, ten species of non-endemic bird species were studied, of which 45.4% (10/22 species) were infected. The prevalence of infected birds by location (study sites) was greater for PEEC (37.6%, 32/85 birds) than for PECP (22.2%, 16/72 birds). Significant differences ($P = 0.036$), were observed in infected birds and location.

Six birds with co-infections were observed, accounting for 3.8% (6/157) of all infected birds. Co-infections occurred only in endemic birds belonging to two families (Turdidae and Passerellidae). The co-infections observed were *Haemoproteus* spp./*Leucocytozoon* spp. in three Russet Nightingale-thrushes (*C. occidentalis*) and one Rufous-capped Brush Finch (*A. pileatus*) and triple-infections of *Haemoproteus* spp./*Leucocytozoon* spp./microfilaria in two individuals of *C. occidentalis* (Fig. 2). This is evidence for high levels of co-infections in Passerellidae and Turdidae. Infected birds, intensity of infection, and co-infections of haemoparasites in endemic and non-endemic passerines from central Mexico highlands are shown in Table 1.

4. Discussion

A systematic search in the main scientific databases (Web of Knowledge, PubMed, Searchable Ornithological Research Archive, Scopus, SciELO and Redalyc), showed that this is the second recorded study of haemoparasites of endemic passerine birds from central Mexico highlands. Resident bird species in a shrub-dominated landscape of the Mexican highland plateau in 2012-2013, had prevalence of *Haemoproteus* and *Plasmodium* of 44.3% in House Finches (*Haemorhous mexicanus*) and 47.5% in Canyon towhees (*Melozone fusca*) (Reinoso-Pérez et al., 2016); we found a lower proportion of *Haemoproteus* spp. (14.0%) and *Plasmodium* was not found in blood samples. In this study we found previously unreported hosts for *Haemoproteus* spp. (*A. virenticeps*; *C. occidentalis*; Slate-throated Redstart, *Myioborus miniatus*; *O. superciliosus*; *P. melanocephalus*; *S. townsendi*), *Leucocytozoon* spp. (*C.

Table 1
Infected birds, intensity of infection, and co-infections of haemoparasites in endemic and non-endemic passerines from central Mexico highlands.

Host	Examined birds (157)	Infected birds (48/157)	Parasite									Co-infections	
			Haemoproteus spp. (22/157)			*Leucocytozoon* spp. (19/157)			Microfilariae (1/157)			*Haemoproteus* spp./ *Leucocytozoon* spp. (4/157)	*Haemoproteus* spp./ *Leucocytozoon* spp./ microfilariae (2/157)
Intensity of infection			L	M	H	L	M	H	L	M	H		
Furnariidae													
Lepidocolaptes leucogaster[a]	1	0											
Tyrannidae													
Empidonax hammondii	1	0											
Empidonax affinis	2	0											
Vireonidae													
Vireo huttoni	1	1					1						
Paridae													
Poecile sclateri	6	3					1	2					
Aegithalidae													
Psaltriparus minimus	1	0											
Certhiidae													
Certhia americana	6	0											
Troglodytidae													
Troglodytes aedon	2	2						1	1				
Regulidae													
Regulus satrapa	3	0											
Turdidae													
Myadestes townsendi	1	0											
Catharus occidentalis[a]	34	18		1	5		1	6				3	2
Turdus migratorius	7	0											
Mimidae													
Melanotis caerulescens[a]	1	0											
Ptilogonatidae													
Ptiliogonys cinereus	2	1			1								
Peucedramidae													
Peucedramus taeniatus	3	1						1					
Fringillidae													
Spinus notatus	2	0											
Spinus psaltria	1	0											
Passerellidae													
Arremon virenticeps[a]	7	2		2									
Atlapetes pileatus[a]	20	5		2			2					1	
Pipilo maculatus	4	4		1	2			1					
Oriturus superciliosus[a]	6	2		1			1						
Melospiza lincolnii	1	0											
Junco phaeonotus	6	4			4								
Parulidae													
Setophaga townsendi	1	1			1								
Basileuterus belli	13	0											
Cardellina rubra[a]	14	2					1	1					
Myioborus miniatus	7	1		1									
Cardinalidae													
Pheucticus melanocephalus	2	1		1									
Thraupidae													
Diglossa baritula	2	0											
Total	157	48	22			19			1			4	2
Proportion (%)		30.6	14.0			12.1			0.6			2.5	1.3

L = Low, M = Medium, H = High.
[a] Endemic birds of Mexico.

rubra; *O. superciliosus*; Olive Warbler, *Peucedramus taeniatus*; *P. sclateri*) and microfilariae (House Wren, *Troglodytes aedon*). Using molecular diagnostic on 83 Mexican bird specimens, Fecchio et al. (2019) found infection proportions similar to those of this study of *Parahaemoproteus* (18.0%), *Leucocytozoon* (19.2%), and *Plasmodium* (14.4%). However, Fecchio et al. (2019) also identified *Plasmodium* in seven bird species that were not detected in this study: *C. occidentalis, Peucedramus taeniatus*, *Poecile sclateri*, *Psaltriparus minimus*, *Regulus satrapa*, *Troglodytes aedon* and *Vireo huttoni*. Non-identification of *Plasmodium* could be due to study site location, since high altitude and low temperatures restrict mosquito abundance of and slow parasite development in these vectors (LaPointe et al., 2012). Furthermore, altitude governs the distribution of parasites belonging to different genera, with higher prevalence of *Plasmodium* at lower altitudes; and *Leucocytozoon* and *Haemoproteus* at higher altitudes (van Rooyen et al., 2013). In Himalayan passerine birds, *Plasmodium* remained absent in high elevation environments in resident birds and showed distinctly low prevalence in migrant populations (Ishtiaq and Barve, 2018). Prevalence (63.3%) and parasitaemia (19.7%) of *Haemoproteus* and *Plasmodium* infections were higher in the Chestnut-capped Brush Finch (*Arremon brunneinucha*) at a low altitude in the urban forest (1,427-1,467 m asl) (Hernández-Lara et al., 2017). These results contrast with our study, since the birds were captured from 2,800 to 3,247 m asl (NTNPA), and from 2,830 to 2,960 m asl (VBNPA). Both study sites belong at the Transverse Neovolcanic Axis, nevertheless, birds of PEEC had higher blood parasite prevalence (37.6%). This may be due to a positive effect of forest fragmentation on blood parasite prevalence (Chasar et al., 2009; Hernández-Lara et al., 2017; Pérez-Rodríguez et al., 2018), since PEEC is a forested island surrounded by agricultural areas, within the fragmented forest of the NTNPA (Sánchez-Jasso et al., 2013).

Worldwide, the presence of microfilariae has been reported in 97 bird families (Bartlett, 2008). In the present study, we reported microfilariae in two families Troglodytidae and Turdidae, including the previously unreported Russet Nightingale-thrush (*C. occidentalis*), an endemic Mexican bird.

In our study, birds of Passerellidae and Turdidae families presented a high level of co-infections. Significant associations for haemosporidian species that infect the four most common avian host families (Turdidae, Fringillidae, Passerellidae and Parulidae), has been previously reported (Galen et al., 2019). The percentage of co-infections found in the birds included in this study is lower than the reported in *Zosterops* spp. (Family: Zosteropidae, Order: Passeriformes), but it has been observed that wildlife co-infections are frequent, and can be present in up to 35.9% of infected birds (Clark et al., 2016). Russet Nightingale-thrush (*C. occidentalis*) were the most captured birds and 18 of 34 (52.9%) were infected with blood parasites with co-infections also found in this endemic species. Further research on *C. occidentalis* populations is necessary to determine the causes behind this result.

The results of this study provide baseline information about haemosporidian parasites in endemic and non-endemic passerine birds from central Mexico highlands. New hosts for *Haemoproteus* spp., *Leucocytozoon* spp., and microfilaria were identified; however, we did not find *Plasmodium* in these bird species. We recommend further genomic studies based on partial mitochondrial cytochrome b to confirm these findings.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgements

Daphne Estefany Villalva-Pasillas received the support of a Master's scholarship from CONACYT (Consejo Nacional de Ciencia y Tecnología). We would like to thank to SEMARNAT for scientific collection permits. A special thanks to Cacalomacán Ejidal Community and Amanalco de Becerra Ejidal Community for their support. This work was funded by Universidad Autónoma del Estado de México, project 4328/2017/CI, and Institute for Biodiversity Research, Development and Sustainability (iBIRDS). The reading and revision of the grammar in this manuscript by P. MVZ. Luis Eduardo Ruiz Lozano (Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México) is greatly appreciated.

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