

RESEARCH ARTICLE

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Prevalence and Genetic Diversity of Avian Blood Parasites (Apicomplexa: Haemosporidia) in Songbirds from the West of Iran

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Abstract

Haemosporidian parasites, which encompass *Haemoproteus*, *Plasmodium*, and *Leucocytozoon*, are intracellular parasites commonly found in the blood and other tissues of birds worldwide. The prevalence of these parasites can vary depending on the bird species and the habitats they inhabit. Notably, the highest diversity of Haemosporidian parasites has been observed within the Passeriformes order of birds. In our study, we collected 145 blood samples from wild songbirds in the western region of Iran during the period from April to November 2021-2022. Blood smears were prepared using the push-slide method and subsequently stained with Giemsa. The stained smears were then examined under a light microscope, and all samples were screened using a nested PCR protocol that targeted a 479-base-pair segment of the mitochondrial cytochrome b (cytb) gene of *Haemoproteus*, *Plasmodium*, and *Leucocytozoon* species. Among the seven songbird families we sampled, we detected blood parasites in 31 individuals, representing an infection rate of 21.4% (95% CI: 0.1501 to 0.2896). These infected individuals belonged to five different families, and a total of 21 lineages were identified, including thirteen for *Leucocytozoon* spp, seven for *Haemoproteus* spp, and one for *Plasmodium* spp. Notably, the highest infection rates were observed in the Paridae family (9.7%, 95% CI: 0.0537 to 0.1567) and the Motacillidae family (4.8%, 95% CI: 0.0196 to 0.0970), while the lowest infection rates were recorded in the Corvidae family (1.4%, 95% CI: 0.0016 to 0.0490) and the Acrocephalidae family (1.4%, 95% CI: 0.0016 to 0.0490). Additionally, our study documented two new host species for *Leucocytozoon* spp. (*Motacilla flava* and *Acrocephalus melanopogon*), marking the first such observations globally. We also established five new host records (*Motacilla flava*, *Pica pica*, *Acrocephalus melanopogon*, *Poecile lugubris*, and *Acrocephalus agricola*) for avian haemosporidian parasites in the western region of Iran. These findings underscore the remarkable lineage diversity within the *Leucocytozoon* genus compared to other haemosporidian parasites in songbirds in western Iran, setting our study apart from previous research in Iran.

Keywords: *Haemoproteus*, haemosporidian parasites, *Plasmodium*, *Leucocytozoon* spp, wild birds.

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INTRODUCTION

Blood parasites, specifically haemosporidians, are highly prevalent infections in birds and exhibit higher prevalence rates in birds compared to other vertebrates (Valkiūnas et al., 2005). The widespread distribution and significant prevalence of various haemosporidian species underscore the importance of

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their identification and classification (Valkiūnas et al., 2014). These parasites are primarily transmitted by blood-feeding insects, such as *Simulium* and *Culex*, with Passeriformes being particularly susceptible compared to other bird orders (Valkiūnas, 2004, Valkiūnas et al., 2005). The life cycle of avian blood parasites is complex, involving both sexual (gametogenesis and fertilization) and asexual (sporogony) reproduction in the invertebrate host, and asexual reproduction (merogony) in the vertebrate host (Scheuerlein and Ricklefs, 2004, Wood et al., 2007, Jenkins and Owens, 2011). Haemosporidian parasites with low pathogenicity can have ecological and evolutionary effects on wild bird populations, impacting population structure, conservation efforts, physical condition, and reproductive success (Van Riper III et al., 1986, Marzal et al., 2005). Avian Haemosporidian parasites can potentially lead to the extinction of many birds (Van Riper III et al., 1986). The identification of these parasites has historically been performed through microscopic examination, and this method has led to the identification of at least 206 morphological species in 23 bird orders (Valkiūnas, 2004, Valkiūnas et al., 2005). Avian malaria-like diseases are caused by *Leucocytozoon*, *Plasmodium*, and *Haemoproteus* in birds (Medeiros et al., 2013). *Haemoproteus* spp. is considered one of the most significant genera of parasitic organisms and can infect over 50% of bird hosts worldwide (Valkiūnas, 2004). *Leucocytozoon* spp. is also prevalent among wild birds and has more than 100 species (Peirce, 1981, Valkiūnas, 2004). *Plasmodium* spp., with its more than 200 species in 14 subgenera, includes five subtypes of avian malaria (Dimitrov et al., 2010, Medeiros et al., 2013). *Haemoproteus* spp. infect a wide range of birds with 147 known species found in 72 bird families (Bennett et al., 1993). Numerous studies have reported infections with avian haemosporidian parasites in Iranian birds, including Passeriformes (Nourani et al., 2017a, Nourani et al., 2017b, Nourani et al., 2018a, Nourani et al., 2018b, Ghaemitalab et al., 2021), non-Passerines (Nourani et al., 2018b, Nourani et al., 2020), and domestic birds (Bahrami et al., 2012, Nematollahi et al., 2012). Many species have also been reported as hosts for *leucocytozoon* spp. (Dezfoulian et al., 2013) and *plasmodium* spp. (Nazifi et al., 2008, Gorji et al., 2012) in Iran. However, among bird species, songbirds have been found to have the highest prevalence of haemosporidian infections. For example, in Bulgaria, songbirds exhibited an infection rate of 58.04% (Dimitrov et al., 2010), while in Austria, the rate was 43.7% (Himmel et al., 2021). Similar studies conducted in Iran reported a prevalence of haemosporidian infections of 35.75% in northern regions (Nourani et al., 2018b) and 51.1% in southern parts of Iran (Ghaemitalab et al., 2021). Moreover, several studies have shown the high lineage diversity in *Haemoproteus* spp. (Dimitrov et al., 2010, Nourani et al., 2018b, Ghaemitalab et al., 2021). Nevertheless, despite the available research, no prior investigations have addressed the prevalence and molecular detection of avian haemosporidian parasites in songbirds within the western regions of Iran. Consequently, this study is designed to assess the prevalence and genetic diversity of avian haemosporidian parasites in songbirds inhabiting western Iran, employing a combination of light microscopy and molecular techniques.

MATERIAL AND METHODS

BLOOD SAMPLES

This study involved the collection of samples from 13 distinct locations in the western region of Iran, including Sanandaj, Sarvabad, and Marivan (Table 1; Figure 1). The sampling period spanned from April to November 2021-2022, during which songbirds were captured using mist nets. Blood samples were collected from each bird by drawing blood from the brachial vein using an insulin syringe. Subsequently, after the preparation of blood smears, the remaining blood was preserved in Queen's buffer for DNA extraction. (Seutin et al., 1991). The blood samples that were collected were utilized to create smears through a push-slide method. Once the smears were prepared, they were air-dried, then fixed with absolute methanol for a duration of five minutes, and allowed to dry once more. Following these steps, the smears were stained with Giemsa stain at a pH of 7.1 for a period of 20-25 minutes. Subsequently, the blood smears were examined under a light microscope, following the method described by Valkiūnas. (2005) (Valkiūnas et al., 2005).

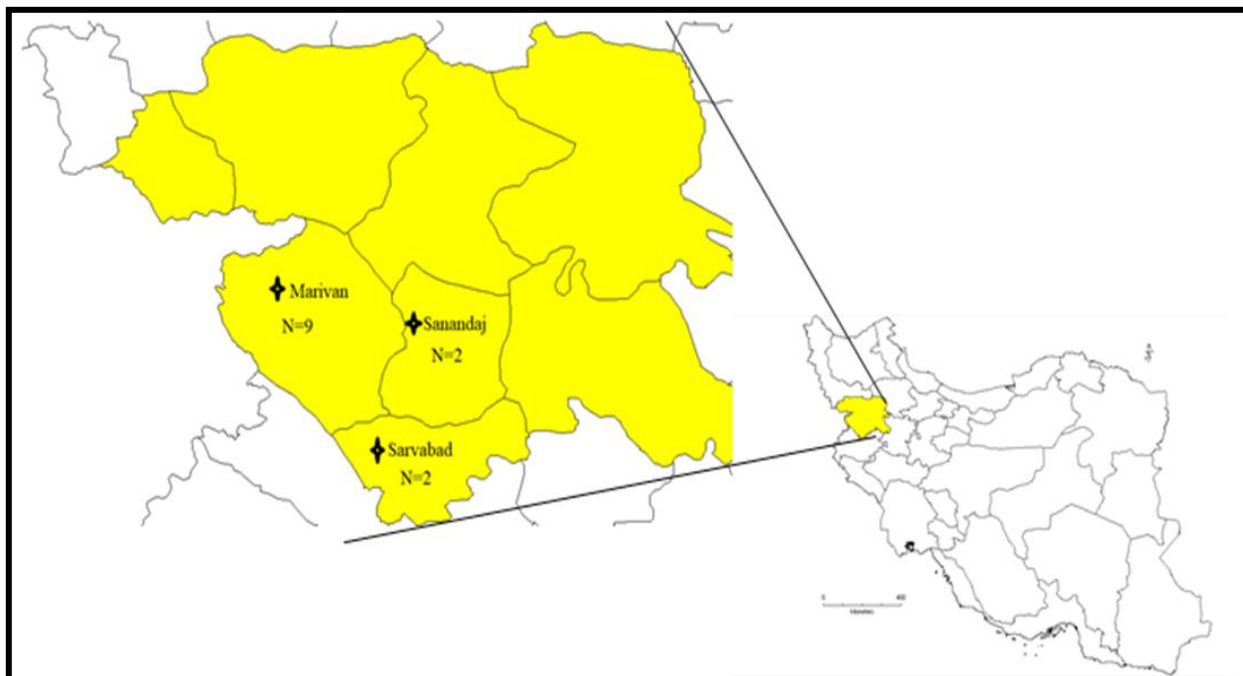


FIGURE 1. Locations of the west of Iran where samples were collected. N: number of sampling sites in each locality. The map was prepared using DIVA-GIS 7.5.0.

STATISTICAL ANALYSIS

In order to evaluate the correlation between mixed infections of *Haemoproteus* and *Leucocytozoon* parasites, we performed a Pearson Correlation Test using SPSS version 22.0 (SPSS for Windows Inc., Chicago, Illinois, 2013) (Statistics, 2013). Additionally, to estimate the 95% Confidence Intervals for prevalence, we employed the Quantitative Parasitology Statistical Tests 3.0 software (<http://www.zoologia.hu/qp/qp.html>) (Reiczigel et al., 2019).

EXTRACTION OF DNA, PCR, AND SEQUENCING

Total DNA was extracted using the standard salt extraction method described by (Bruford et al., 1992), and stored at -20°C until further use. Parasite identification to lineage level was done using the nested PCR protocols developed by (Bensch et al., 2000) and (Hellgren et al., 2004).

To check the success of the PCRs, 3 μl of the final PCR product was run on a 2 % agarose gel with positive (microscopy) and negative (water) controls. All PCR products were purified and sent for sequencing to Microsynth AG (Balgach, Switzerland).

RESULTS

PARASITE PREVALENCE

In our investigation, it was discovered that among 145 host individuals, 31 (21.4%, 95% CI: 0.1501 to 0.2896) were infected with avian haemosporidian parasites. Of the infected individuals, 14 birds were found to have contracted two types of avian haemosporidian parasites, specifically *Haemoproteus* and *Leucocytozoon*. The overall prevalence of avian haemosporidian parasites was determined to be (31.7%, 95% CI: 0.2425 to 0.3997), with *Haemoproteus* accounting for (18.6%, 95% CI: 0.1264 to 0.2593), *Leucocytozoon* for (11.7%, 95% CI: 0.0698 to 0.1811), and *Plasmodium* for (1.4%, 95% CI: 0.0016 to 0.0490) of the infections (Fig 2). The Paridae (9.7%, 95% CI: 0.0537 to 0.1567) and Motacillidae (4.8%, 95% CI: 0.0196 to 0.0970) families exhibited the highest prevalence, while the Corvidae (1.4%, 95% CI: 0.0016 to 0.0490) and Acrocephalidae (1.4%, 95% CI: 0.0016 to 0.0490) families had the lowest prevalence (Table 2).

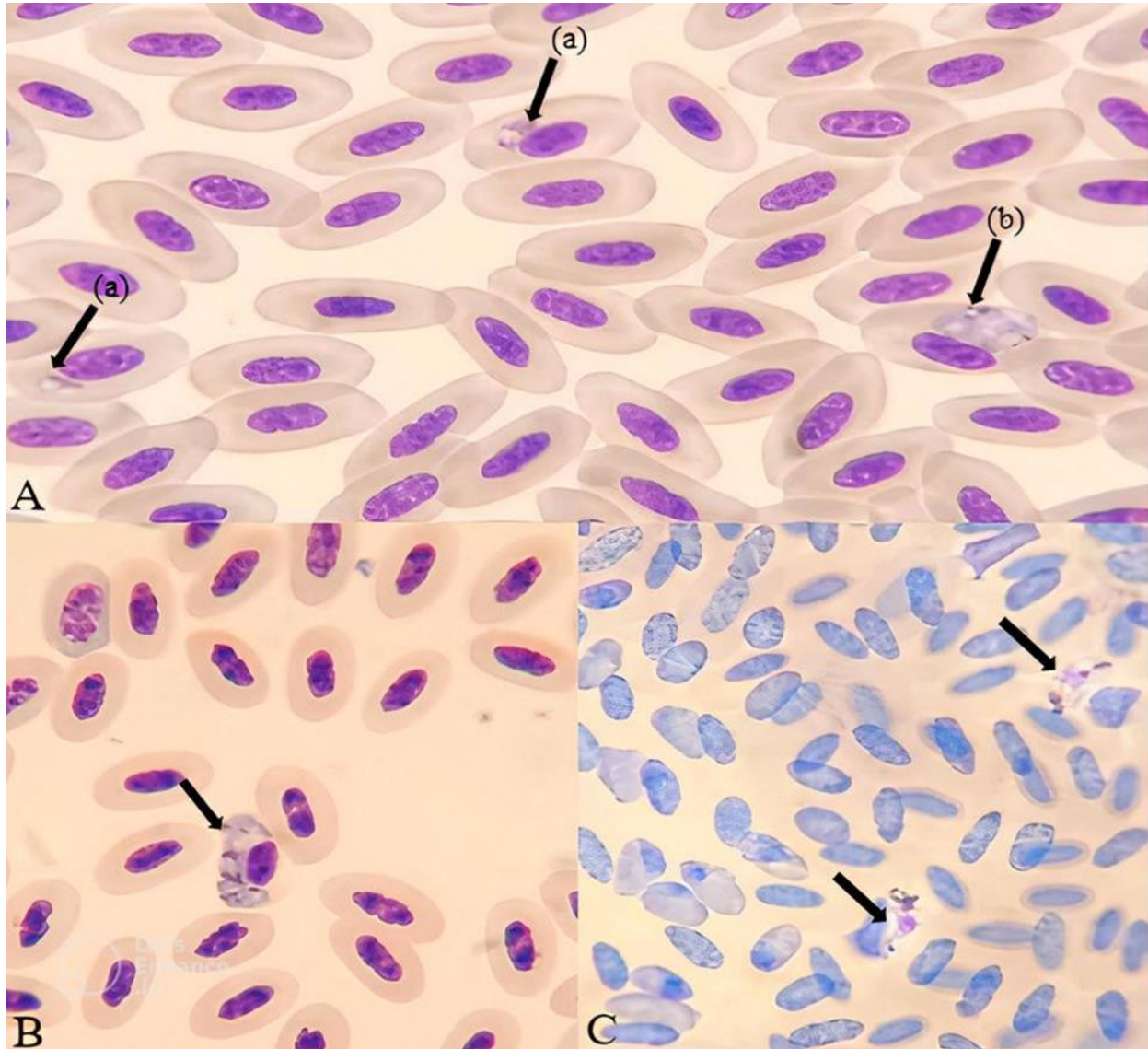


FIGURE 2. A) Early (left a) and late (b) trophozoites of *Plasmodium relictum* (SYCONO2) in *Acrocephalus agricola*, B) *Haemoproteus motacilla* (YWT1) in *Motacilla flava*, and C) *Leucocytozoon* sp. (BT5) in *Motacilla flava*. (Stained with the Giemsa stain, Magnification 1000×).

LINEAGE DIVERSITY

Thirteen lineages of *Leucocytozoon* spp., seven lineages of *Haemoproteus* spp. and one lineage of *Plasmodium* sp. were recorded. The families Paridae and Passeridae exhibited the highest lineage diversity, with ten and five different lineages found, respectively. *Cyanistes caeruleus*, *Parus major*, and *Passer domesticus* showed the highest lineage diversity. Furthermore, based on the MalAvi database, two new hosts for *Leucocytozoon* spp. (*Motacilla flava* and *Acrocephalus melanopogon*) were recorded, representing the first documentation of their infection worldwide. Additionally, four hosts (*Pica pica*, *Acrocephalus agricola*, *Motacilla flava*, and *Acrocephalus melanopogon*) were found for three genus parasites in Iran. Four parasite species, namely *Haemoproteus majoris* (PARUS1), *Haemoproteus motacilla* (YWT1), *Haemoproteus vireonis* (VIGIL01), and *Plasmodium relictum* (SYCONO2), were also discovered (Table 2).

TABLE 1. Geographic coordinates of sampling in this study.

Site Name	Coordinates		Characteristics
Marivan	35°31'37.30"N, 46°08'37.47"E	1299 m a.s.l.	Zarivar Lake
	35°31'24.09"N, 46°06'55.25"E	1289 m a.s.l.	Zarivar Lake
	35°31'02.50"N, 46°08'23.05"E	1289 m a.s.l.	Zarivar Lake
	35°31'21.82"N, 46°08'50.51"E	1290 m a.s.l.	Zarivar Lake
	35°31'09.25"N, 46°07'04.71"E	1288 m a.s.l.	Zarivar Lake
	35°31'42.25"N, 46°10'36.19"E	1348 m a.s.l.	Inside City
	35°31'42.40"N, 46°10'37.26"E	1350 m a.s.l.	Inside City
	35°31'39.99"N, 46°10'36.52"E	1344 m a.s.l.	Inside City
	35°31'39.03"N, 46°10'39.95"E	1354 m a.s.l.	Inside City
Sanandaj	35°16'05.02"N, 46°59'52.62"E	1453 m a.s.l.	Inside City
	35°16'04.04"N, 46°59'51.00"E	1454 m a.s.l.	Inside City
Sarvabad	35°18'05.43"N, 46°21'33.14"E	1086 m a.s.l.	Sirwan River
	35°18'04.28"N, 46°21'32.56"E	1085 m a.s.l.	Sirwan River

LEUCOCYTOZOON AND HAEMOPROTEUS NEW LINEAGES

In this study, we found three new lineages including one lineage for *Haemoproteus* sp. and two lineages for *Leucocytozoon* spp. We recorded the lineage PICPIC03 in *Pica pica*, and lineages PARUS95, PARUS96 in *Parus major* (Table 2).

MIXED INFECTION

We detected 14 mixed infections of *Haemoproteus* spp. and *Leucocytozoon* spp. We also identified ten lineages for *Leucocytozoon* spp. and five lineages for *Haemoproteus* spp. (Table 3). The highest number of mixed infections was detected in the Paridae and Corvidae families, with eight and four mixed infections found, respectively. Among the hosts, *Parus major* and *Parus caeruleus* showed the highest number of mixed infections (Table 3). Based on the Pearson correlation Test, the probability of mixed infections by *Leucocytozoon* and *Haemoproteus* parasites were correlated (Sig = 0.00, a correlation was weighed significantly at the < 0.01 level).

DISCUSSION

This study showed a range of infection rates among different avian species, with the highest prevalence of *Haemoproteus* parasite infection being (18.6%, 95 % CI: 0.1264 to 0.2593), and the lowest prevalence of *Plasmodium* spp. infection at (1.4%, 95 % CI: 0.0016 to 0.0490). *Cyanistes caeruleus* had the highest prevalence (5.5%, 95% CI: 0.0241 to 0.1059), followed by *Motacilla flava* (4.8%, 95% CI: 0.0196 to 0.0970) and *Parus major* (4.1%, 95% CI: 0.0153 to 0.0879). Additionally, *Motacilla flava* showed the highest prevalence of *Haemoproteus* spp., while *Parus major* and *Cyanistes caeruleus* showed the highest prevalence of *Leucocytozoon* spp. (Table 2). Based on the MalAvi database we identified three novel lineages and 18 previously recorded lineages in songbirds in western Iran (Table 2).

The overall prevalence of infection in our study aligns with the findings in north Iran (Nourani et al., 2018b), which may be due to similar weather conditions between the regions. However, the high prevalence in southern Iran (Ghaemitalab et al., 2021) can be attributed to the relatively high temperature and humidity in that area. Furthermore, our study found a high prevalence of *Haemoproteus* spp., consistent with other studies (Dimitrov et al., 2010, Nourani et al., 2018b, Ghaemitalab et al., 2021, Himmel et al., 2021). However, our study and the findings in north Iran (Nourani et al., 2018b) revealed a low prevalence of *Plasmodium* (1.37% and 1.26%, respectively), contrasting with the higher rates observed in Bulgaria (24.7%), southern Iran (14.5%) and Austria (13.8%) (Dimitrov et al., 2010, Ghaemitalab et al., 2021, Himmel et al., 2021). Our findings show the highest lineage diversity for *Leucocytozoon* sp. (13 lineages, 61.9 %), and the lowest lineage diversity was observed in *Plasmodium* sp. (one lineage, 4.76%) this finding is similar to the result reported by Himmel et al. (2021), who also

TABLE 2. Prevalence and genetic diversity of the avian haemosporidian parasites in songbirds from western Iran. **N** = Number examined, **Inf (n)** = number infection, **Pre (%)** = Prevalence %, **Z** = Zarivar lake, **Si** = Sirwan river, **IC** = Inside City, **M** = Marivan, **Sn** = Sanandaj and **Sa** = Sarvabad .New host records for Iran (+), new host records for the world (*), and new lineages (∞) are specified, **95 % CI** = 95 % confidence limits.

Host Species and Family	Study site	N	Inf (n)	<i>Haemoproteus</i>	<i>Leucocytozoon</i>	<i>Plasmodium</i>
Corvidae <i>Pica pica</i> +	M	4	2	CXPIP28(1) PARUS6(1)	PICPIC01(1) PICPIC03 (1) ∞	0
Pycnonotidae <i>Pycnonotus leucotis</i>	Sn	1	0	0	0	0
Passeridae <i>Passer domesticus</i>	M,Sn	74	5	YWT1(1) PARUS1(1) PAHIS2(1)	CWT8(1)	SYCON02(1)
Motacillidae <i>Motacilla flava</i> *+	M	10	7	YWT1 (7)	BT5 (2) PICPIC01(1)	0
Acrocephalidae <i>Acrocephalus agricola</i> +	M	3	1	0	0	SYCON02(1)
<i>Acrocephalus melanopogon</i> *+	M	1	1	VIGIL01(1)	COCORO9(1)	0
Turdidae <i>Turdus merula</i>	Sn	1	0	0	0	0
Paridae <i>Parus major</i>	Sa	22	6	PARUS1(5) PARUS95(1) ∞	PARUS34(1) PARUS20(1) PARUS74(1) PARUS7(1) PARUS96 (1) ∞	0
<i>Poecile lugubris</i> +	M	1	1	0	PARUS4 (1)	0
<i>Cyanistes caeruleus</i>	M,Sa	28	8	PARUS1(8)	PARUS87(1) PARUS74(1) PARUS12(1) PARUS4 (1)	0
Total		145	31	27	17	2
Prevalence, %			21.4 %	18.6%	11.7 %	1.4 %
95 % CI			(0.1501 to 0.2896)	(0.1264 to 0.2593)	(0.0698 to 0.1811)	(0.0016 to 0.0490)

found a high diversity of lineage in *Leucocytozoon* spp. while our results differ from a study conducted by Ghaemitalab et al. (2021) in which the highest lineage diversity was reported for *Haemoproteus* sp. (63.6 %) but aligns with the result of the same study, Ghaemitalab et al. (2021), which reported that the lowest lineage diversity for *plasmodium* sp. (14.5 %). Contrary to our results, in a study conducted by Nourani et al. (2018) the highest lineage diversity was found in *Haemoproteus* sp. (33.3 %) and the

lowest for *Leucocytozoon* sp. (0.6 %) (Nourani et al., 2018b). Moreover, in this study, we found that *Parus major*, *Cyanistes caeruleus*, and *Passer domesticus* showed the highest lineage diversity among the hosts, with seven, six, and five lineages, respectively. In a study conducted by Nourani et al. (2018b) in north Iran, *Acrocephalus dumetorum*, *Iduna pallida*, and *passer domesticus* showed the highest lineage diversity with three, four, and three lineages, respectively, on the other hand, Ghaemitalab et al. (2021) reported the highest lineage diversity in the genera *Cymnoris xanthocollis*, *Pycnonotus leucotis*, *Sylvia althaea*, *Sylvia atricapilla*, and *Passer domesticus* with ten, nine, eight, seven, and six lineages, respectively. We found low genetic diversity with 21 lineages in western Iran compared to southern Iran with 55 lineages (Ghaemitalab et al., 2021) and north Iran with 43 lineages (Nourani et al., 2018b). The high prevalence for lineage in this study was *Haemoproteus majoris* PARUS1 with 14 infections (Table 2), while in north Iran, Nourani et al. (2018b) reported only three infections with this lineage, and Ghaemitalab et al. (2021) found no infection with this lineage in southern Iran. Our results showed that six lineages were infecting multiple host species. Of which, three lineages belonged to *Leucocytozoon* (PARUS4, PARUS20, and PARUS74), two lineages belonged to *Haemoproteus* (PARUS1, and YWT1), and one lineage belonged to *Plasmodium* (SYCONO2) (Table 2). This result is consistent with the results reported in southern Iran (Ghaemitalab et al., 2021).

Table 3. Mixed infection of haemosporidian parasites (*Leucocytozoon* spp. and *Haemoproteus* spp. lineages) in songbirds in western Iran.

Host species	<i>Leucocytozoon</i> lineage	<i>Haemoproteus</i> lineages	Number
<i>Pica pica</i>	PICPIC01	PARUS6	1
	PICPIC03 _∞	CXPIP28	1
<i>Parus major</i>	PARUS7	PARUS1	1
	PARUS74	PARUS1	1
	PARUS34	PARUS1	1
	PARUS 20	PARUS1	1
<i>Cyanistes caeruleus</i>	PARUS74	PARUS1	1
	PARUS87	PARUS1	1
	PARUS4	PARUS1	1
	PARUS12	PARUS1	1
<i>Motacilla flava</i>	BT5	YWT1	2
	PICPIC01	YWT1	1
<i>Acrocephalus melanopogon</i>	COCOR09	VIGIL01	1
Total	10 lineages	5 lineages	14

The overall mixed infection rate of 14 individuals in our study aligns with the results of a study conducted by Ghaemitalab et al. (2021), which reported 13 cases of mixed infection with *Haemoproteus* spp. and *Leucocytozoon* spp. (Ghaemitalab et al., 2021). However, in the study by Nourani et al. (2018), only one sample was found to be mixed infected with *Haemoproteus* spp. and *Leucocytozoon* spp. (Nourani et al., 2018b). The higher occurrence of mixed infection in the Paridae family in our study is consistent with a study by van Rooyen et al. (2013), which reported a high incidence of mixed infection in great tits (Rooyen et al., 2013). In sum, our results demonstrate a high genetic diversity of the *Leucocytozoon* parasite and high mixed infection for Haemosporidian parasites in western Iran. In future research, it would be valuable to investigate more studies about genetic diversity for Haemosporidian parasites in these areas. This could provide deeper insights into the dynamics and distribution of these parasites, as well as their potential impact on the local bird populations.

CONCLUSION

In conclusion, our results indicated a higher lineage diversity of *leucocytozoon* spp. compared to other studies conducted in north and southern Iran. These differences in lineage diversity could potentially be related to specific weather conditions and environmental factors such as cold climate and altitude in the study area. These factors might create a more suitable transmission environment for the *leucocytozoon* parasite, leading to higher lineage diversity in our study area.

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LITERATURE CITED

- Bahrami, A. M., A. L. Monfared, and M. Razmjoo (2012), Pathological study of parasitism in racing pigeons: An indication of its effects on community health, *African Journal of Biotechnology*, 11(59), 12364-12370.
- Bennett, G., M. Peirce, and R. Ashford (1993), Avian haematozoa: mortality and pathogenicity, *Journal of Natural History*, 27(5), 993-1001.
- Bensch, S., M. Stjernman, D. Hasselquist, Ö. Örjan, B. Hansson, H. Westerdahl, and R. T. Pinheiro (2000), Host specificity in avian blood parasites: a study of *Plasmodium* and *Haemoproteus* mitochondrial DNA amplified from birds, *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 267(1452), 1583-1589.
- Bruford, M. W., O. Hanotte, J. Brookfield, and T. Burke (1992), Single-locus and multilocus DNA fingerprinting, *Molecular genetic analysis of populations: a practical approach.*, 225-269.
- Dezfoulian, O., M. Zibaei, H. Nayebzadeh, M. Haghgoo, A. Emami-Razavi, and K. Kiani (2013), Leucocytozoonosis in domestic birds in southwestern Iran: an ultrastructural study, *Iranian journal of parasitology*, 8(1), 171.
- Dimitrov, D., P. Zehindjiev, and S. Bensch (2010), Genetic diversity of avian blood parasites in SE Europe: Cytochrome b lineages of the genera *Plasmodium* and *Haemoproteus* (Haemosporida) from Bulgaria, *Acta Parasitologica*, 55, 201-209.
- Ghaemitalab, V., O. Mirshamsi, G. Valkiūnas, and M. Aliabadian (2021), Prevalence and genetic diversity of avian haemosporidian parasites in southern Iran, *Pathogens*, 10(6), 645.
- Gorji, S. F., B. Shemshadi, H. Habibi, S. Jalali, M. Davary, and M. Sepehri (2012), Survey on parasites in sparrows of Amol (Mazandaran Province, Iran), *Journal of Life Sciences*, 6(7), 783.
- Hellgren, O., J. Waldenström, and S. Bensch (2004), a new PCR assay for simultaneous studies of *Leucocytozoon*, *Plasmodium*, and *Haemoproteus* from avian blood, *Journal of Parasitology*, 90(4), 797-802.

Himmel, T., J. Harl, J. Matt, and H. Weissenböck (2021), A citizen science-based survey of avian mortality focusing on haemosporidian infections in wild passerine birds, *Malaria Journal*, 20, 1-13.

Jenkins, T., and I. P. Owens (2011), Biogeography of avian blood parasites (*Leucocytozoon spp.*) in two resident hosts across Europe: phylogeographic structuring or the abundance–occupancy relationship?, *Molecular Ecology*, 20(18), 3910-3920.

Marzal, A., F. d. Lope, C. Navarro, and A. P. Møller (2005), Malarial parasites decrease reproductive success: an experimental study in a passerine bird, *Oecologia*, 142, 541-545.

Medeiros, M. C., G. L. Hamer, and R. E. Ricklefs (2013), Host compatibility rather than vector–host-encounter rate determines the host range of avian *Plasmodium* parasites, *Proceedings of the Royal Society B: Biological Sciences*, 280(1760), 20122947.

Nazifi, S., S. Razavi, F. Yavari, M. Rajaifar, E. Bazyar, and Z. Esmailnejad (2008), Evaluation of hematological values in indigenous chickens infected with *Plasmodium gallinaceum* and *Aegyptianella pullorum*, *Comparative Clinical Pathology*, 17, 145-148.

Nematollahi, A., M. Ebrahimi, A. Ahmadi, and M. Himan (2012), Prevalence of *Haemoproteus columbae* and *Trichomonas gallinae* in pigeons (*Columba domestica*) in Isfahan, Iran, *Journal of Parasitic Diseases*, 36, 141-142.

Nourani, L., M. Aliabadian, N. Dinparast-Djadid, and O. Mirshamsi (2017a), New host records for *Haemoproteus spp.*(Apicomplexa: Haemosporidiasina) in passeriformes from north-west of Iran, *Journal of arthropod-borne diseases*, 11(2), 236.

Nourani, L., M. Aliabadian, O. Mirshamsi, and N. D. Djadid (2017b), Detection of the avian blood parasites from northeast of Iran, paper presented at 1st International Congress on Vector-Borne Diseases and Climate Change.

Nourani, L., M. Aliabadian, N. D. Djadid, and O. Mirshamsi (2018a), Occurrence of *Haemoproteus spp.*(Haemosporida: Haemoproteidae) in new host records of passerine birds from the east of Iran, *Iranian Journal of Parasitology*, 13(2), 267.

Nourani, L., M. Aliabadian, O. Mirshamsi, and N. Dinparast Djadid (2018b), Molecular detection and genetic diversity of avian haemosporidian parasites in Iran, *PloS One*, 13(11), e0206638.

Nourani, L., M. Aliabadian, H. Amini, and O. Mirshamsi (2020), Prevalence of haemosporidian parasite infections in raptors of Iran, *Journal of Raptor Research*, 54(4), 455-462.

Peirce, M. (1981), Distribution and host-parasite check-list of the haematozoa of birds in Western Europe, *Journal of Natural History*, 15(3), 419-458.

Reiczigel, J., M. Marozzi, I. Fábíán, and L. Rózsa (2019), Biostatistics for parasitologists—a primer to quantitative parasitology, *Trends in parasitology*, 35(4), 277-281.

Rooyen, J. v., F. Lalubin, O. Glaiot, and P. Christe (2013), Avian haemosporidian persistence and co-infection in great tits at the individual level, *Malaria Journal*, 12(1), 1-8.

- Scheuerlein, A., and R. E. Ricklefs (2004), Prevalence of blood parasites in European passeriform birds, *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 271(1546), 1363-1370.
- Seutin, G., B. N. White, and P. T. Boag (1991), Preservation of avian blood and tissue samples for DNA analyses, *Canadian journal of zoology*, 69(1), 82-90.
- Statistics, I. (2013), IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp, Google Search.
- Valkiunas, G. (2004), *Avian malaria parasites and other haemosporidia*, CRC press.
- Valkiūnas, G., A. M. Anwar, C. T. Atkinson, E. C. Greiner, I. Paperna, and M. A. Peirce (2005), What distinguishes malaria parasites from other pigmented haemosporidians?, *Trends in parasitology*, 21(8), 357-358.
- Valkiūnas, G., V. Palinauskas, M. Ilgūnas, D. Bukauskaitė, D. Dimitrov, R. Bernotienė, P. Zehtindjiev, M. Ilieva, and T. A. Iezhova (2014), Molecular characterization of five widespread avian haemosporidian parasites (Haemosporida), with perspectives on the PCR-based detection of haemosporidians in wildlife, *Parasitology research*, 113, 2251-2263.
- Van Riper III, C., S. G. Van Riper, M. L. Goff, and M. Laird (1986), The epizootiology and ecological significance of malaria in Hawaiian land birds, *Ecological monographs*, 56(4), 327-344.
- Wood, M. J., C. L. Cosgrove, T. A. Wilkin, S. C. Knowles, K. P. Day, and B. C. Sheldon (2007), Within-population variation in prevalence and lineage distribution of avian malaria in blue tits, *Cyanistes caeruleus*, *Molecular Ecology*, 16(15), 3263-3273.