Original article

Cytochrome *b* lineages of *Haemoproteus tinniculi* in the endangered saker falcon (Falco cherrug) in Saudi Arabia

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ABSTRACT

Despite the ecological importance and social popularity of falcons, many elements of their biology remain unknown, including the variety of parasites that infect them in the wild and in captivity. Some of these are from the common alveolate genus *Haemoproteus*, which parasitise birds worldwide. We investigated the genetic diversity of *Haemoproteus tinnunculi* infecting the captive saker falcons (*Falco cherrug*) to describe their phylogenetic relationships. The overall prevalence of *H. tinnunculi* collected between December 2018 and February 2020 was 4.53% (58/1280). A fragment of the mitochondrial cytochrome *b* gene of *H. tinnunculi* was amplified from fifty-eight blood samples. Our findings indicate that two lineages of *H. tinnunculi* infect falcons in Saudi Arabia, one of which is reported here for the first time. These findings will inform the future investigation of phylogenetic relationships and genetic diversity of *Haemoproteus* parasites in falcons.

1. Introduction

Avian haemosporidian parasites are widely distributed across the globe. Three main genera, each consisting of numerous species, are recognised in birds: *Haemoproteus*, *Plasmodium* and *Leucocytozoon* (Atkinson and Van Riper III, 1991). They are transmitted by blood-sucking insects, including biting midges, louse flies, black flies and mosquitoes (Ritchie et al., 1994; Valkiunas, 2004). Haemoparasites are found in many raptors, and their incidence varies by host species and geography (Joseph, 1999). Because raptors are at the top of many food chains, their health can reflect the health of entire ecosystems (Cooper, 2002). Habitat loss and fragmentation have resulted in increased exposure of some raptor populations to parasitism, potentially altering host-parasite balances and increasing parasite pathogenicity.

While blood parasites are generally considered non-pathogenic, some evidence demonstrates that they can be harmful (Remple, 2004). Haemoparasites can cause increased thrombotic thrombocytopenic purpura (TPP) levels, leucocytosis, anaemia and even death (Garvin et al., 2003; Redig, 2003). *Haemoproteus* spp. are the most common blood parasites in raptors (Garvin et al., 2003). Prolonged recuperation time and higher mortality rates are observed in captive raptors with haemoproteid infections (Shihab et al., 2013). During severe infection, clinical signs may include poor flight performance, reduced flight distance, inappetence, weight loss, acute anaemia, dyspnea and lethargy (Butterworth and Harcourt-Brown, 1996; Remple, 2004). Rahim, Bakhiet, and Hussein (2013) found that increased eosinophil count and splenic size were closely associated with haemoproteid infection in captive saker falcons.

From a conservation perspective, the diagnosis and treatment of avian haemosporidians are important for controlling their cumulative effects in raptors (Ciloglu et al., 2016). Nested cytochrome *b* polymerase chain reaction (PCR) analysis is widely used to detect avian haemosporidian parasites in the peripheral blood of infected birds (Bukauskaitė et al., 2015; Hellgren et al., 2004). This molecular diagnostic tool provides greater sensitivity and accuracy than traditional microscopic examination methods. Moreover, the technique has made possible the discovery of a wide diversity of parasites within the traditional morphospecies (Bensch et al., 2009). Despite the popularity of birds of prey, knowledge of their haemosporidian diversity is limited (Valkiunas, 2004), possibly

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attributable to the difficulty of capturing adult raptors in the wild (Pérez-Rodríguez et al., 2013). The avian haemosporidian *Haemoproteus tinnunculi* has been previously reported in captive falcons in Saudi Arabia using only traditional microscopic examination of blood smears (Naldo and Samour, 2004; Rahim et al., 2013). Only one previously published study included molecular analyses of *Haemoproteus tinnunculi* in captive falcons in Saudi Arabia (Alfaleh et al., 2020). However, the reported prevalence of *H. tinnunculi* was quite low (3%) compared to an earlier study based only on microscopic examination (Rahim et al., 2013), possibly due to the study's relatively small sample size. From a total of 100 falcons examined, only three birds were found infected with *H. tinnunculi*. Thus, a larger-scale study of this haemoparasite is needed to improve our understanding of phylogenetic relationships and genetic diversity of avian haemosporidian parasites from captive falcons in Saudi Arabia and more generally, the Middle East.

This study aimed to examine the genetic diversity of *Haemoproteus tinnunculi* obtained from captive saker falcons (*Falco cherrug*) in Saudi Arabia via cytochrome *b* gene sequence analysis.

2. Materials and methods

2.1. Blood sample collection

Blood samples were obtained while the birds were under isoflurane anaesthesia. Blood (0.5 ml) was collected from the brachial vein of each bird using 1 ml disposable syringes and 23 gauge × 5/8 in. needles. After collection, the samples were transferred to pediatric blood collection tubes containing EDTA anticoagulant. Thin blood smears were prepared immediately after blood collection, directly from the tip of syringes, onto glass slides. The smears were air-dried and stained with modified Wright-Giemsa stain and examined microscopically for haemoparasites as described previously (Samour and Silvanose (2000). Diagnosis of *Haemoproteus tinnunculi* was confirmed by identifying the halter-shaped intraerythrocytic gametocytes partially encircling the nucleus, appearing pigmented and occupying more than 50% of the cytoplasm (Beynon et al., 1996). Birds were designated negative if no parasites were observed and positive (infected) if one or more parasites were identified. Blood samples from birds positive for infection were immediately stored at −20 °C for DNA extraction. "All procedures for the samples collection were carried out in strict accordance with the recommendations by the Research Ethics Sub-Committee (REC) of the College of Sciences at the King Saud University (KSU) in Riyadh, Kingdom of Saudi Arabia (KSA) (Ethics Reference No: KSU-SE-19-77)".

2.2. DNA extraction for PCR

Total DNA was extracted from samples infected with *Haemoproteus tinnunculi* using DNAzol® (Invitrogen, Warrington, UK) according to the manufacturer's instructions. Briefly, 25 μl of blood samples were transferred to 1 ml Eppendorf tubes, then 500 μl of DNAzol was added, and the tubes were centrifuged at 10,000g for 3 min at 4 °C. After transferring the resulting supernatant to a new Eppendorf tube, 500 μl of absolute ethanol was added to each tube. The tubes were mixed by vortexing for 20 s, then centrifuged for 4 min at 10,000g and 3 °C. The resulting DNA pellet was washed with 100 μl of 75% ethanol, and samples were centrifuged again for two minutes. The DNA pellets were air-dried for 20 min before resuspension in 500 μl of distilled water. DNA concentrations of each sample were measured by spectrophotometry at 260 nm. The DNA extraction was visualised under UV light in a 1% agarose gel stained with ethidium bromide.

2.3. PCR amplification of the 16S rDNA

PCR amplification of the cytochrome *b* gene employed the following primers: HAEMF (5′ATGGTGCTTTCGATATATGCATG-3′) and HAEMR2 (5′GCATTATCTGGATGTGATAATGGT-3′), as described by Bensch et al. (2000). Each PCR reaction was run with 8.5 μl Green Master Mix (2X; Thermo Fisher Scientific), 0.5 μl (10 pg/μl) of each forward and reverse primer solution (Macrogen, Seoul, Korea), 8.5 μl of distilled water, and 1 μl of sample DNA (total volume 19 μl). Each amplification run included a negative control of purified water. The following cycle conditions were used for the PCR amplification: 94 °C for 3 min, followed by 35 cycles of 94 °C for 30 s, 56 °C for 30 s then 72 °C for 45 s, with a final extension step at 72 °C for 10 min. PCR products were visualised under UV light in 1% agarose gel stained with ethidium bromide. Both DNA strands of all positive PCR products were sequenced by Macrogen (Seoul, Korea).

2.4. Sequence analysis and phylogenetic trees

All consensus sequences of the cytochrome *b* gene of the forward and reverse DNA strands were obtained and aligned by
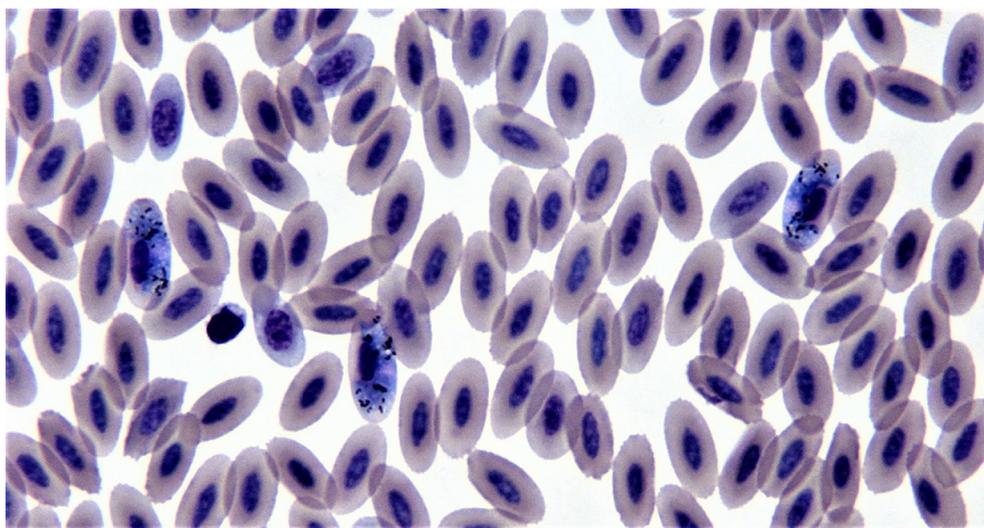
Fig. 1. *Haemoproteus tinnunculi* gametocytes in blood smear from saker falcon (modified wright-giemsa stain).

MEGA X software (Version 10.2.6; Kumar et al., 2018) to compare the evolutionary relationships among the sequences of *Haemoproteus tinnunculi* and build the phylogenetic tree. For cytochrome *b* gene comparisons, multiple reference sequences were downloaded from the National Center for Biotechnology Information (NCBI) GenBank database. The phylogenetic trees of the datasets available from GenBank and those found in this investigation were built by Maximum likelihood (ML) using genetic distance and the Tamura-Nei models, and nucleotide sequence analysis was utilised to examine the relationships between taxa (Jeanmougin et al., 1998; Kumar et al., 2016; Tamura and Nei, 1993). Variance estimation: bootstrap method. Number of bootstrap replications 10,000. The related taxa grouped in the bootstrap test are presented adjacent to the branches using Felsenstein's bootstrap approachEstimates of Evolutionary Divergence between Sequences were calculated as genetic distance matrix (%). Genetic distance heatmap were ploted using CLUSTVIS web tool (Metsalu and Vilo, 2015).

3. Results

A total of 1280 blood samples were collected from captive saker falcons (*Falco cherrug*) in Riyadh, Saudi Arabia, between December 2018 and February 2020. Microscopic examinations showed that

Table 1
Sample details of captive falcons. List of host species, location. All samples were obtained from adult birds, and GeneBank Accession No.

Isolate ID	Case NO.	Host species	Location	Age	GeneBank Accession No.
1	R1	Saker falcon	Riyadh	Adult	MZ571763
2	R2	Saker falcon	Riyadh	Adult	MZ571764
3	R3	Saker falcon	Riyadh	Adult	MZ571765
4	R4	Saker falcon	Riyadh	Adult	MZ571766
5	R5	Saker falcon	Riyadh	Adult	MZ571767
6	R6	Saker falcon	Riyadh	Adult	MZ571768
7	R7	Saker falcon	Riyadh	Adult	MZ571769
8	R8	Saker falcon	Riyadh	Adult	MZ571770
9	R9	Saker falcon	Riyadh	Adult	MZ571771
10	R10	Saker falcon	Riyadh	Adult	MZ571772
11	R11	Saker falcon	Riyadh	Adult	MZ571773
12	R12	Saker falcon	Riyadh	Adult	MZ571774
13	R13	Saker falcon	Riyadh	Adult	MZ571775
14	R14	Saker falcon	Riyadh	Adult	MZ571776
15	R15	Saker falcon	Riyadh	Adult	MZ571777
16	R16	Saker falcon	Riyadh	Adult	MZ571778
17	R17	Saker falcon	Riyadh	Adult	MZ571779
18	R18	Saker falcon	Riyadh	Adult	MZ571780
19	R19	Saker falcon	Riyadh	Adult	MZ571781
20	R20	Saker falcon	Riyadh	Adult	MZ571782
21	R21	Saker falcon	Riyadh	Adult	MZ571783
22	R22	Saker falcon	Riyadh	Adult	MZ571784
23	R23	Saker falcon	Riyadh	Adult	MZ571785
24	R24	Saker falcon	Riyadh	Adult	MZ571786
25	R25	Saker falcon	Riyadh	Adult	MZ571787
26	R26	Saker falcon	Riyadh	Adult	MZ571788
27	R27	Saker falcon	Riyadh	Adult	MZ571789
28	R28	Saker falcon	Riyadh	Adult	MZ571790
29	R29	Saker falcon	Riyadh	Adult	MZ571791
30	R30	Saker falcon	Riyadh	Adult	MZ571792
31	R31	Saker falcon	Riyadh	Adult	MZ571793
32	R32	Saker falcon	Riyadh	Adult	MZ571794
33	R33	Saker falcon	Riyadh	Adult	MZ571795
34	R34	Saker falcon	Riyadh	Adult	MZ571796
35	R35	Saker falcon	Riyadh	Adult	MZ571797
36	R36	Saker falcon	Riyadh	Adult	MZ571798
37	R37	Saker falcon	Riyadh	Adult	MZ571799
38	R38	Saker falcon	Riyadh	Adult	MZ571800
39	R39	Saker falcon	Riyadh	Adult	MZ571801
40	R40	Saker falcon	Riyadh	Adult	MZ571802
41	R41	Saker falcon	Riyadh	Adult	MZ571803
42	R42	Saker falcon	Riyadh	Adult	MZ571804
43	R43	Saker falcon	Riyadh	Adult	MZ571805
44	R44	Saker falcon	Riyadh	Adult	MZ571806
45	R45	Saker falcon	Riyadh	Adult	MZ571807
46	R46	Saker falcon	Riyadh	Adult	MZ571808
47	R47	Saker falcon	Riyadh	Adult	MZ571809
48	R48	Saker falcon	Riyadh	Adult	MZ571810
49	R49	Saker falcon	Riyadh	Adult	MZ571811
50	R50	Saker falcon	Riyadh	Adult	MZ571812
51	R51	Saker falcon	Riyadh	Adult	MZ571813
52	R52	Saker falcon	Riyadh	Adult	MZ571814
53	R53	Saker falcon	Riyadh	Adult	MZ571815
54	R54	Saker falcon	Riyadh	Adult	MZ571816
55	R55	Saker falcon	Riyadh	Adult	MZ571817
56	R56	Saker falcon	Riyadh	Adult	MZ571818
57	R57	Saker falcon	Riyadh	Adult	MZ571819
58	R58	Saker falcon	Riyadh	Adult	MZ571820

58 (4.53%) samples were infected by *Haemoproteus tinnunculi* (Fig. 1). All samples that were positive by microscopy were also positive by PCR. Details of the falcon samples infected by *H. tinnunculi* is summarised in Table 1.

The sequenced PCR products from all samples were edited and aligned, and the resulting alignment of 406 bp was analysed. The ML tree (Fig. 2), genetic distance matrix and heatmap plot (Table 2 and Fig. 3 respectively) clustered them into one distinct group with high confidence. The genetic relationships among these sequences indicated two distinct lineages of *H. tinnunculi*. In 54 samples, the lineages were 100% matches to previously deposited lineages (NCBI accessions MN780909 and MN780908), and all sequences obtained were uploaded into GenBank (MT571763–M571816). We identified a new *H. tinnunculi* lineage in four falcons (MZ571817–-MZ571820). One sequence is reported here for the first time.

4. Discussion

In this study, cytochrome *b* gene was used to compare the genetic variation of Haemoproteus tinnunculi that was collected from captive saker falcons in Saudi Arabia. We found a new lineage of H. tinnunculi that is capable of infecting falcons in Saudi Arabia. This data is crucial for providing a better understanding of the genetic variation of falcon parasites their phylogenetic relationships.

The cytochrome *b* gene is one of the most common markers used for molecular detection and genetic characterisation of avian haemosporidians (Staffan Bensch et al., 2009; Sehgal, 2015; Valkiūnas et al., 2020). Parallel analyses of mtDNA cytochrome *b* provide the best evidence for species lineages, demonstrating consistent gene-trees and apparent absence of recombination for several closely related lineages (Bensch et al., 2004; Hellgren et al., 2007; Pérez-Tris et al., 2007). We revealed two distinct lineages of *H. tinnunculi* in saker falcons based on cytochrome *b* analysis. One of these lineages had not been previously registered in GenBank. The cytochrome *b* gene was shown to be useful in building well-supported phylogenies for *H. tinnunculi* species.

Phylogenetic analysis revealed two distinct sequences that were clearly split into multiple branches depending on the cytochrome *b* gene (Fig. 1). Sequence alignment indicated that 54 *H. tinnunculi* samples were 100% identical to a previously identified *H. tinnunculi* sequence (GenBank MN780909) found in a saker falcon in Saudi Arabia (Alfaleh et al., 2020). One new detected lineage was found in four falcons, which had not been described previously. This sequence shared 99.75% identity with previously sequenced *H. tinnunculi* (GenBank MN780908) (Alfaleh et al., 2020).
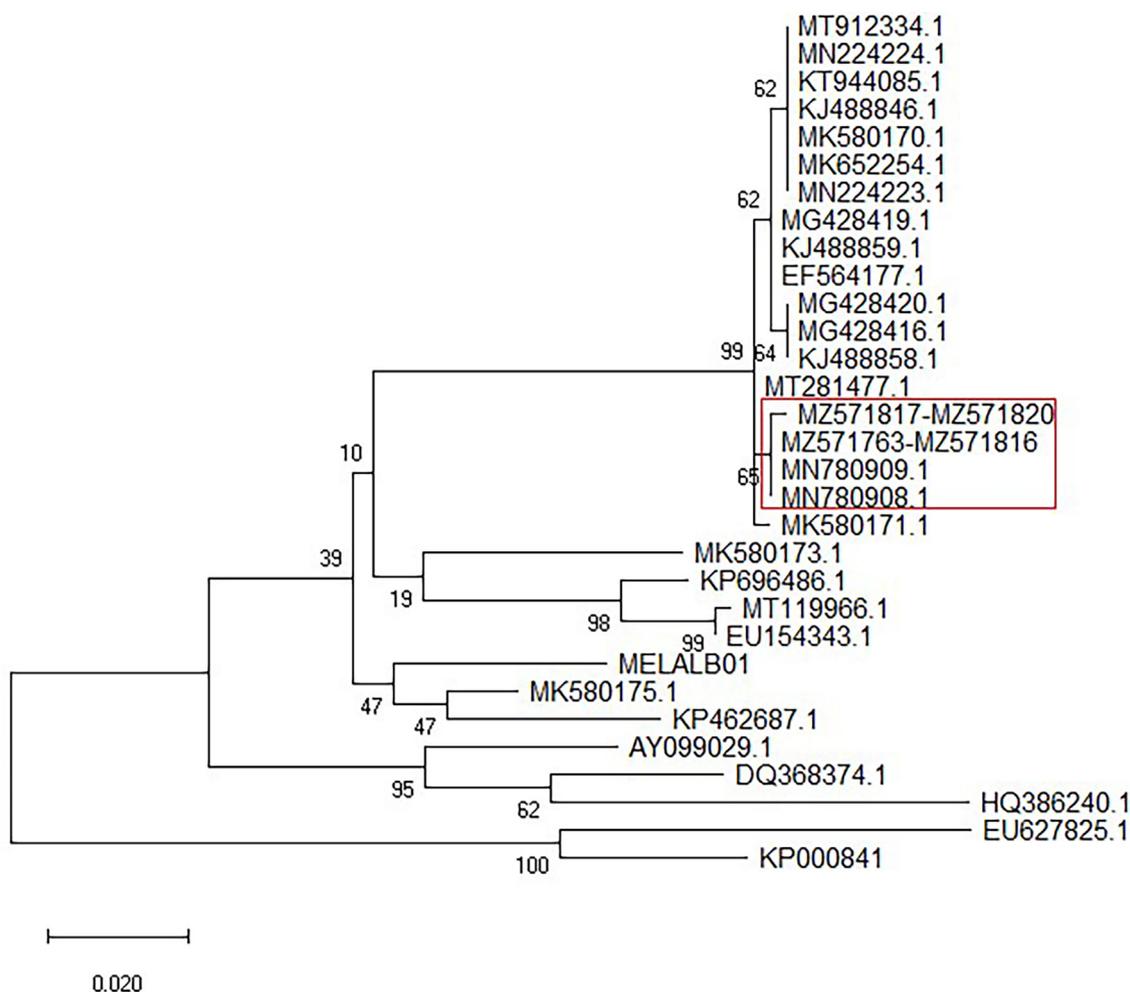
Fig. 2. Maximum likelihood phylogeny of *Haemoproteus tinnunculi*. Phylogenetic tree was done using 406 bp of cytochrome *b* gene. The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model (Tamura and Nei, 1993). The tree with the highest log likelihood (−1745.73) is shown. Tree is showed as traditional tree in straight view. Values at nodes represent bootstrap probabilities (bootstrap 10000). Evolutionary analyses were conducted in MEGA X (Kumar et al., 2018). The sequences used in constructing the tree were obtained from GenBank. Red rectangle: subgroup composed of accessions MN780909, MN780908, MT571763-M571816 and MZ571817-MZ571820.

Table 2

Genetic distance matrix (%). Estimates of Evolutionary Divergence between Sequences. The number of base substitutions per site from between sequences are shown. Analyses were conducted using the Tamura-Nei model (Tamura and Nei, 1993). This analysis involved 31 nucleotide sequences. Evolutionary analyses were conducted in MEGA X (Kumar et al., 2018).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
1 MZ571763-MZ571816																															
2 MZ571817-MZ571820	0.2																														
3 MN780909.1	0.0	0.2																													
4 MN780908.1	0.0	0.2	0.0																												
5 MT281477.1	0.2	0.5	0.2	0.2																											
6 MK580171.1	0.5	0.8	0.5	0.5	0.2																										
7 MG428419.1	0.5	0.8	0.5	0.5	0.2	0.5																									
8 KJ488859.1	0.5	0.8	0.5	0.5	0.2	0.5	0.0																								
9 EF564177.1	0.5	0.8	0.5	0.5	0.2	0.5	0.0	0.0																							
10 MK652254.1	0.8	1.0	0.8	0.8	0.5	0.8	0.2	0.2	0.2																						
11 MK580170.1	0.8	1.0	0.8	0.8	0.5	0.8	0.2	0.2	0.2	0.0																					
12 MG428420.1	0.8	1.0	0.8	0.8	0.5	0.8	0.2	0.2	0.2	0.5	0.5																				
13 MG428416.1	0.8	1.0	0.8	0.8	0.5	0.8	0.2	0.2	0.2	0.5	0.5	0.0																			
14 KJ488858.1	0.8	1.0	0.8	0.8	0.5	0.8	0.2	0.2	0.2	0.5	0.5	0.0	0.0																		
15 KJ488846.1	0.8	1.0	0.8	0.8	0.5	0.8	0.2	0.2	0.2	0.0	0.0	0.5	0.5	0.5																	
16 KT944085.1	0.8	1.0	0.8	0.8	0.5	0.8	0.2	0.2	0.2	0.0	0.0	0.5	0.5	0.5	0.0																
17 MT912334.1	0.8	1.0	0.8	0.8	0.5	0.8	0.2	0.2	0.2	0.0	0.0	0.5	0.5	0.5	0.0	0.0															
18 MN224224.1	0.8	1.0	0.8	0.8	0.5	0.8	0.2	0.2	0.2	0.0	0.0	0.5	0.5	0.5	0.0	0.0	0.0														
19 MN224223.1	0.8	1.0	0.8	0.8	0.5	0.8	0.2	0.2	0.2	0.0	0.0	0.5	0.5	0.5	0.0	0.0	0.0	0.0													
20 EU627825.1	19.2	19.6	19.2	19.2	19.2	18.8	18.8	18.8	18.8	19.2	19.2	18.5	18.5	18.5	19.2	19.2	19.2	19.2	19.2												
21 MK580173.1	9.6	9.9	9.6	9.6	9.2	9.6	9.6	9.6	9.6	9.9	9.9	9.9	9.9	9.9	9.9	9.9	9.9	9.9	9.9	20.6											
22 MK580175.1	8.6	9.0	8.6	8.6	8.3	8.6	8.6	8.6	8.6	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	18.7	6.0										
23 DQ368374.1	13.7	14.0	13.7	13.7	13.3	13.3	13.7	13.7	13.7	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	21.3	11.1	9.0									
24 AY099029.1	12.9	13.2	12.9	12.9	12.6	12.6	12.9	12.9	12.9	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	21.4	11.4	7.9	6.8								
25 MT119966.1	10.3	10.6	10.3	10.3	9.9	10.3	10.3	10.3	10.3	10.6	10.6	10.6	10.6	10.6	10.6	10.6	10.6	10.6	10.6	19.7	7.6	7.1	12.5	11.0							
26 KP696486.1	9.5	9.8	9.5	9.5	9.2	9.5	9.5	9.5	9.5	9.8	9.8	9.9	9.9	9.9	9.8	9.8	9.8	9.8	9.8	18.9	7.0	6.2	12.1	11.0	2.5						
27 HQ386240.1	15.0	15.4	15.0	15.0	14.7	15.0	15.0	15.0	15.0	15.4	15.4	14.7	14.7	14.7	15.4	15.4	15.4	15.4	15.4	21.9	11.9	9.8	8.1	10.2	12.5	11.0					
28 MELALB01	9.4	9.8	9.4	9.4	9.1	9.4	9.4	9.4	9.4	9.8	9.8	9.8	9.8	9.8	9.8	9.8	9.8	9.8	9.8	20.0	7.1	4.9	11.6	10.6	8.3	7.9	11.3				
29 EU154343.1	10.0	10.3	10.0	10.0	9.7	10.0	10.0	10.0	10.0	10.3	10.3	10.3	10.3	10.3	10.3	10.3	10.3	10.3	10.3	19.4	7.3	6.8	12.2	10.7	0.2	2.3	12.2	8.0			
30 KP462687.1	10.4	10.7	10.4	10.4	10.0	10.4	10.4	10.4	10.4	10.7	10.7	10.7	10.7	10.7	10.7	10.7	10.7	10.7	10.7	20.8	7.1	4.1	11.9	10.9	8.2	7.3	11.5	5.7	7.9		
31 KP000841	18.0	18.3	18.0	18.0	18.0	18.3	18.3	18.3	18.3	17.9	17.9	18.0	18.0	18.0	17.9	17.9	17.9	17.9	17.9	8.8	19.6	16.1	18.7	16.8	17.9	17.1	18.3	18.2	17.6	17.9	

Fig. 3. Genetic distance (%) heatmap. Data from Table 2 were ploted as a heatmap using CLUSTVIS web tool (Metsalu and Vilo, 2015). A) Genetic distance of 31 accession. Red square: subgroup of 19 accessions re-plottered in B. B) Genetic distance of 19 accession. Red square: subgroup composed of accessions MN780909, MN780908, MT571763-M571816 and MZ571817-MZ571820.

Fig. 3 (*continued*)

Haemoproteus parasites are well-known avian haematozoa that can cause decreased productivity and increased mortality in birds (Elahi et al., 2014). Blood-sucking dipteran insects transmit avian haemoproteids (Friend et al., 1999; Ritchie et al., 1994; Valkiunas, 2004). Microscopic examination of a Giemsa-stained peripheral blood smear is usually used to diagnose *Haemoproteus* infection. Although the parasites resemble *Plasmodium*, the pigment inside the intra-erythrocytic gametocytes is more scattered, and there are no schizonts visible in peripheral blood smears. The pigment granules are formed by the digestion of haemoglobin in the host's RBCs and appear as refractile yellow to brown granules inside the RBCs (Friend et al., 1999). *Haemoproteus tinnunculi* was the most prevalent hematozoan parasite found in captive falcons in Saudi Arabia and the Middle East ((Naldo and Samour, 2004; Samour and Silvanose, 2000). According to Rahim et al. (2013), haemoproteid infection is more common at the end of the winter season, which lasts from January to April and usually coincides with the rainy season; this could be due to increased density and activity of *Culicoides* spp. and other biting insects that could serve as vectors for *Haemoproteus* spp. Molecular detection of *H. tinnunculi* has included extracted DNA amplification by PCR as an extremely sensitive and specific tool for avian haemosporidians. However, there is no common naming of lineages, thus making it difficult to find and compare information (Valkiūnas et al., 2019). There have been minimal molecular investigation of *H. tinnunculi* in falcons. Although a smear-based diagnosis is necessary, it is often inadequate or inaccurate in identifying and diagnosing *Haemoproteus* species (Ortiz-Catedral et al., 2019; Valkiūnas et al., 2020). Previous investigations have shown that PCR has a comparably high sensitivity to microscopy in diagnosing avian *Haemoproteus* (Tavassoli et al., 2018; Valkiūnas et al., 2019).

In this study, we only examined *H. tinnunculi* that infected saker falcons. Rahim et al. (2013) and Alfaleh et al. (2020) reported that

this falcon species experienced a greater risk of infection with *H. tinnunculi*. This species has also been reported in peregrine falcons (*F. peregrinus*) and gyrfalcons (*F. rusticolus*) in Kuwait and *F. sparverius* from Pennsylvania, USA (Apanius and Kirkpatrick, 1988; Tarello, 2007). These results indicate that *F. cherrug* may experience greater exposure than other falcons or that there are host species variations in *H. tinnunculi* infection. Meixell et al. (2016) reported that several variables, including vector exposure, host body size (a larger size possibly attracting more insects), and plumage color may influence host-specific vectors. Further analyses of genetic diversity of haemoproteid parasites and exploration of relevant clinical and subclinical effects and possible pathogenicity in other falcon species are needed.

Valkiūnas et al. (2019) established the first genetic markers for identifying haemoproteids parasitising falconiform birds overwintering in Africa. They described three new *Haemoproteus* species using morphological and molecular markers. Phylogenetic study of haemoproteid lineages obtained from falcons, as well as available morphological data, show that *H. tinnunculi* and *H. brachiatus* have similar cytochrome *b* gene sequences.

This study will be valuable for researchers interested in the evolutionary history of *H. tinnunculi* parasites and to falconry managers, clinicians, and microbiologists. Our results will inform avian ecology and contribute to the study of an important molecular toolbox in examining a disease of increasing importance.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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