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1           **Detection and molecular characterization of feline hemoplasmas in wild felid**  
2 **species in Iran in the Middle East.**

3  
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22           **Short running title;** Feline hemoplasmas in wild felid species in Iran  
23

24

25 **Abstract**

26 Three feline hemoplasma species exist in felids: *Mycoplasma haemofelis*, ‘*Candidatus*  
27 *Mycoplasma haemominutum*’, and ‘*Candidatus Mycoplasma turicensis*’.

28 The aims of the study were to determine the presence of, and molecularly characterize,  
29 any hemoplasmas in wild felids, including the endangered Persian leopard in Iran, the Middle  
30 East.

31 Blood samples were collected from 19 wild felids, including three Persian leopards.  
32 Using species-specific hemoplasma PCRs and ELISA serological testing for feline leukaemia  
33 virus and feline immunodeficiency virus (FIV), two Persian leopards were found to be  
34 infected with ‘*Ca. M. haemominutum*’ and were seropositive for FIV. Partial 16S rRNA gene  
35 sequences were generated for these ‘*Ca. M. haemominutum*’ species and subsequent  
36 phylogenetic analysis revealed 97.70% to 99.45% sequence identity with those found in  
37 domestic cats from Iran and other countries.

38 This study confirms the presence of ‘*Ca. M. haemominutum*’ and concurrent FIV  
39 antibody in wild felids in Iran. This represents the first report of hemoplasma in wild felids in  
40 the Middle East as well as the first report of infection in Persian leopards.

41 **Key words;** Feline hemoplasma, *Panthera pardus saxicolor*, Persian leopard.

42 **1. Introduction**

43 Hemoplasmas are hemotropic mycoplasma<sup>1</sup> bacteria that infect a wide range of  
44 mammals [1, 2]. At least three feline hemoplasma species have been described in domestic  
45 cats including *Mycoplasma haemofelis*, ‘*Candidatus Mycoplasma haemominutum*’, and  
46 ‘*Candidatus Mycoplasma turicensis*’ [1-4]. The most pathogenic species is *M. haemofelis*,  
47 which can cause hemolytic anemia [5, 6] in immunocompetent cats. Coinfection of  
48 hemoplasmas with other pathogens such as feline leukemia virus (FeLV) and feline

49 immunodeficiency virus (FIV) may worsen the severity of the hemoplasma-induced anemia  
50 and result in anemia following infection with less pathogenic hemoplasmas such as ‘*Ca. M.*  
51 *haemominutum*’, and ‘*Ca. M. turicensis*’ [7, 8].

52 Hemoplasma infection with *M. haemofelis*, ‘*Ca. M. haemominutum*’ and/or ‘*Ca. M.*  
53 *turicensis*’ has been reported in around nine wild felid species worldwide [9-11], with wildlife  
54 isolates showing near identity to those found in domestic feline species [9]. There are,  
55 however, only limited studies of hemoplasma infections in wild felids, and no studies have yet  
56 been performed in countries in the Middle East, such as Iran, and the natural transmission  
57 route for hemoplasmas is not known [9].

58 The Persian leopard is an endangered wild felid, native to Iran and some neighboring  
59 countries. Following the extinction of the lion *Panthera leo persica* and tiger *Panthera tigris*  
60 *virgate* in Iran, it is the only large wild felid now existing in Iran [12-15], and no studies have  
61 yet evaluated this species as a host for feline hemoplasma infection. We have recently  
62 reported the presence and molecular characterization of feline hemoplasma infections in  
63 domestic cats in Iran [16], and the aim of this study was to document the presence and  
64 molecularly characterize of feline hemoplasma species in wild felids in Iran in the Middle  
65 East.

## 66 **2. Materials & Methods**

### 67 **2.1. Sample Collection and Processing**

68 Nineteen EDTA-anticoagulated blood samples (FL Medical K3 EDTA K3E, Lot.  
69 F111332 2.5 mL tube, Torreglia, Italy) were obtained from the following cats; twelve African  
70 lions, four leopards (three Persian leopards and one African leopard), one Eurasian lynx, one  
71 Bengal tiger and one Caracal, using a blowpipe filled with a combination of drugs and dosage  
72 to each species; ketamine (3mg/kg) and medetomidine (0.03 mg/kg) for Bengal tiger, Persian  
73 and African leopards, butorphanol (0.2 mg/kg), medetomidine (0.035mg/kg) and midazolam

74 (0.15 mg/kg) for Eurasian lynx and Caracal caracal and tiletamine/zolazepam (1.5 mg/kg) and  
75 medetomidine (0.015 mg/kg) for African lion. These are given intramuscularly to anesthetize  
76 the animals followed by femoral vein sampling. Approval was granted for the study from the  
77 Iran Veterinary Organization since samples were taken as part of a national and international  
78 cooperative project for conservation of Persian leopards, supported by the Iranian Department  
79 of the Environment, the International Union for Conservation of Nature, The Wildlife  
80 Conservation Society and Panthera. The sampled animals were kept either in Tehran zoo or in  
81 the Tandoureh National Park. Tandoureh National Park has been protected since 1968 and is  
82 located in north eastern Iran and is around 355 km<sup>2</sup> in size. Signalment data for these wild  
83 felids, as well as their origin and current residence are shown in Table 1.

84 Hematological parameters including white blood cell, red blood cell (RBC), Hematocrit  
85 (HCT), hemoglobin concentration (Hb), mean corpuscular volume, mean corpuscular  
86 hemoglobin, mean corpuscular hemoglobin concentration and platelets were measured using  
87 an automatic hemocytometer (Hema-screen 18, Hospitex diagnostic, Florence, Italy). Blood  
88 smears were prepared for differential white blood cells count and examination for  
89 hemoparasites. Plasma was submitted for serological retrovirus testing for FeLV and FIV  
90 using a commercially available rapid diagnostic ELISA kit (Quicking FIV Ab + FeLV Ag  
91 Combined Test, W81099, China), according to the manufacturer's instructions, and were  
92 confirmed by repeat ELISA testing using a different serological retrovirus test (ELISA kit for  
93 serodiagnosis of FeLV and FIV Ab, Biopronix, Agrolabo, Italy).

## 94 **2.2. DNA Extraction**

95 DNA was extracted from 100 µl whole blood from each sample using a commercial kit  
96 (QIAamp cadof pathogen Mini kit, Qiagen, Hilden, Germany), following the manufacturer's  
97 instructions, and stored at -20°C until further use.

98 Distilled water and known positive blood samples for each of the three feline

99 hemoplasma species, obtained from the School of Veterinary Sciences, University of Bristol,  
100 Bristol, UK and Bologna University, Bologna, Italy, were used as negative and positive  
101 controls respectively during each run of DNA extractions.

### 102 **2.3. Diagnostic Polymerase Chain Reaction (PCR) assays**

103 A control conventional PCR to amplify a fragment of feline glyceraldehyde-3-  
104 phosphate dehydrogenase (GAPDH) gene was performed to detect possible PCR inhibitors in  
105 DNA samples [17]. Screening hemoplasma PCR analysis was performed using a previously  
106 described generic universal hemoplasma conventional PCR assay using 5'-  
107 ATACGGCCCATATTCCTACG-3' and 5'-TGCTCCACCACTTGTTCA-3' as forward and  
108 reverse primers, respectively [18].

109 All samples were then subjected to species-specific conventional PCRs for each of the  
110 three feline hemoplasma species using previously described conventional PCR assays [19,  
111 20]. Positive controls of *M. haemofelis*, '*Ca. M. haemominutum*' and '*Ca. M. turicensis*' were  
112 used for both the generic haemoplasma and species specific PCRs.

### 113 **2.4. 16S rRNA Gene Sequencing**

114 The 16S rRNA gene of positive samples on generic screening hemoplasma PCR was  
115 amplified using primers 8F: 5'-AGAGTTTGATCCTGGCTCAG-3' and 1492R: 5'-  
116 GGTTACCTTGTTACGACTT-3', as previously described, with resulting PCR products then  
117 subjected to sequencing using the Sanger technique (ABI, 96-capillary XL) [21]. After  
118 evaluating the quality of sequence reading in Finch TV software (Geospiza), 5' and 3' ends of  
119 the forward and reverse sequence reading were trimmed. The forward and reverse sequences  
120 of each sample were then overlapped and aligned with available 16S rRNA sequences of '*Ca.*  
121 *M. haemominutum*' in Genbank. Finally, partial 16S rRNA sequences of 1086bp (lacking  
122 about 200 bp from each 5' and 3' end of complete 16S rRNA sequence of '*Ca. M.*  
123 *haemominutum*') were obtained.

## 124           **2.5. Statistical Analysis**

125           Data analysis, including descriptive statistics, was performed using SPSS software (16.0  
126 IBM, New York, USA). African lion hematology reference intervals were calculated using  
127 the mean  $\pm$  SD data available in previously published work [22], using the formula  
128  $\text{mean} \pm 1.96\text{SD}$ . Sequence data analysis and phylogenetic tree construction were performed with  
129 MEGA6 software using the partial 16S rRNA sequences derived in this study as well as other  
130 wild and domestic cat hemoplasma sequences downloaded from Genbank (Accession  
131 numbers shown in Figure 1). Bootstrap testing (1000 replicates) and out-grouping were used  
132 to validate the phylogenetic tree [23]. The evolutionary distances were computed using the  
133 Kimura 2-parameter method [24] and the Neighbor-Joining method [25] used for tree  
134 construction [26].

135           **Nucleotide Sequence Accession Numbers.** The partial 16S rRNA gene sequences  
136 derived from this study were submitted to Genbank with accession numbers KU852586 and  
137 KU852587.

## 138           **3. Results**

139           Of the 19 samples analyzed, all were PCR-positive for GAPDH, and two (10.5%) were  
140 PCR-positive by generic universal hemoplasma conventional PCR. Only the same two  
141 samples were positive on species-specific PCR; both for '*Ca. M. haemominutum*' only. All  
142 positive controls had expected amplified band in the generic universal and the species-specific  
143 conventional haemoplasma PCRs and distilled water as the negative control had none. Both of  
144 the positive samples were from old (14 and 15 years) male Persian leopards (Case numbers 13  
145 and 14 in Table 1), from two different geographical areas of Iran. No hemoplasma organisms  
146 were observed on blood smear examination.

147           The two hemoplasma ('*Ca. M. haemominutum*') infected Persian leopards were both  
148 also FIV seropositive, and one African lion was also FeLV and FIV positive but not

149 hemoplasma infected. No other samples were retrovirus positive.

150 To the authors' knowledge, no hematological reference ranges exist for Persian  
151 leopards, nor for any closely related species (e.g. African leopard, Arabian leopard), limiting  
152 interpretation of the hematology profiles of the Persian leopards in the current study.  
153 However, as shown in Table 2, the hematology profiles of the two '*Ca. M. haemominutum*'  
154 and FIV-seropositive Persian leopards (Case numbers 13 & 14) showed HCT, Hb, and RBC  
155 counts at the lower end of the reference range used for domestic cats, and HCT and RBC  
156 counts below the reference range calculated for African lions based on Larsson et. al 2015  
157 [22], and were lower than those recorded in the non-infected Persian leopard (Case number  
158 15). Thus it is possible that '*Ca. M. haemominutum*' and FIV infection were associated with a  
159 reduction in erythrocyte indices in the infected cats, but further data from larger numbers of  
160 cats would be required to confirm this.

161 The hematology profiles of the 12 African lions were also compared to the reference  
162 range calculated for African lions based on Larsson et. al 2015 [22], and 11 of the 12 lions  
163 had HCT and Hb values within or above the reference range. The FeLV and FIV seropositive  
164 but hemoplasma PCR negative lion had a hypochromic normocytic anemia (HCT 20.7%).  
165 The four remaining cases (Case numbers 16, 17, 18 and 19) could not have their  
166 hematological profiles determined due to sample hemolysis.

167 The partial (1086 bp) '*Ca. M. haemominutum*' 16S rRNA gene sequences derived for  
168 the two hemoplasma infected Persian leopards in the current study (KU852586 and  
169 KU852587) showed high sequence identity (97.7-99.45%) with, and were closely related to  
170 the '*Ca. M. haemominutum*' sequences in Genbank derived from worldwide wild felids and  
171 domestic cats [9, 27-29], including Iranian domestic cats. Data are shown in Figure 1. The  
172 '*Ca. M. haemominutum*' Persian leopard sequence KU852586 was slightly more closely  
173 related to the Iranian domestic cat sequence KU852585 than the other Persian leopard



174 sequence KU852587, with 99.26% sequence identity. The sequence identity between the two  
175 Persian leopards '*Ca. M. haemominutum*' (KU852586 and KU852587) was 98.43%. The  
176 highest sequence identities of 99.63% and 98.62% were between '*Ca. M. haemominutum*'  
177 sequences KU852586 and KU852587 derived from Persian leopards and DQ825452 from a  
178 lion in Tanzania.

#### 179 **4. Discussion**

180 This study documents the presence of hemoplasmas in wild felids for the first time in  
181 Iran in the Middle East. It is also the first documentation of hemoplasma infection in the  
182 endangered Persian leopard species [12]. The prevalence of hemoplasma infection in wild  
183 felids has varied in different studies but is not frequently high. In a surveillance study in  
184 Brazil on neotropic and exotic felids, 9.2% of 109 felids were hemoplasma positive (all '*Ca.*  
185 *M. haemominutum*') [30], whilst in free-ranging Cheetahs in Namibia, only one of 63  
186 Cheetahs was positive [31]. However higher prevalence was reported in another study  
187 evaluating a large sample size (275) from worldwide geographical areas where prevalence of  
188 18%, 32% and 20% were found for *M. haemofelis*, '*Ca. M. haemominutum*', and '*Ca. M.*  
189 *turicensis*', respectively [9]. In the current study, hemoplasma infection was confirmed in just  
190 two of 19 samples (10.5%), although the sample size was small since access to wild felid  
191 samples in the Middle East is very limited due to the difficulties in access to hosts and  
192 collection of blood. Both '*Ca. M. haemominutum*' infected wild felids in the current study  
193 were old male Persian leopards, an indigenous species in Iran. This is in agreement with other  
194 studies in domestic cats showing that being male and, older, are risk factors for '*Ca. M.*  
195 *haemominutum*' infection [27, 32-37]. Fighting behavior is also regarded as a risk factor for  
196 hemoplasma infection [27, 36, 38], although the fighting behavior of the cats sampled was not  
197 completely known and leopards (*Panthera pardus*) are generally not regarded as an  
198 aggressive species [39]. However, in the literature there are cases of intraspecific killing

199 among leopards over a kill, territory or cannibalism, so aggression is possible [40, 41]. There  
200 are also two reports of intraspecific killing from Persian leopards in Tandoureh in 2007 and  
201 2016 over food and territory (Memarian. I, personal communication).

202         Neither of the two '*Ca. M. haemominutum*' infected Persian leopards identified in the  
203 current study lived in zoos. As reported in an extensive study on feline hemoplasma infection  
204 in wild felid species worldwide[9], free-ranging felids had higher hemoplasma infection  
205 prevalence [9, 42, 43] than captive felids. This may be because free-ranging felids have more  
206 fighting and hunting habits and/or more exposure to vectors, than captive or zoo-based felids.  
207 In the same study described, a significant correlation between FeLV PCR positivity and  
208 hemoplasma infection was found in European wild cats[9]. There are several reports of  
209 retrovirus infections in free-ranging and captive wild felids [44-47], and multiple other  
210 concurrent infections such as feline calicivirus, feline herpesvirus, feline parvovirus, and  
211 feline coronavirus [42, 43]. In the current study both '*Ca. M. haemominutum*' Persian  
212 leopards were FIV seropositive and this is, to the authors' knowledge, the first report for such  
213 a co-infection in a wild felid species.

214         It is not known if the '*Ca. M. haemominutum*' infection in the Persian leopards caused  
215 anemia. This was difficult to assess since no reference ranges exist for hematological  
216 parameters in this species, and it is known that greater anemia can occur in cats with  
217 concurrent '*Ca. M. haemominutum*' and retrovirus infection compared to '*Ca. M.*  
218 *haemominutum*' alone [7]. The very small sample size did not permit a statistical comparison  
219 between '*Ca. M. haemominutum*' FIV-seropositive and FIV-seronegative Persian leopards.  
220 Nevertheless, it was of note that the HCT, Hb and RBC counts of the two '*Ca. M.*  
221 *haemominutum*' FIV-seropositive leopards were lower than the Persian leopard free from  
222 hemoplasmas and retroviral infection, suggesting that coinfection of '*Ca. M. haemominutum*'  
223 and FIV could have been associated with reduced RBC parameters.

224 The partial 16S rRNA gene phylogenetic analysis found that the ‘*Ca. M.*  
225 *haemominutum*’ isolates derived from this study were closely related to those from different  
226 geographical origins and from both domestic and wild felids. In a previous phylogenetic study  
227 of domestic feline hemoplasmas, using both 16S rRNA gene and RNaseP genes, almost 100%  
228 identity was reported between Europe, Asia, Africa and United States species [48]. In a  
229 Japanese study, the identities of the detected hemoplasma sequences was very high, such that  
230 it was not possible to assume the origin of *M. haemofelis* and ‘*Ca. M. turicensis*’ from  
231 endangered Iriomote cats. In agreement with our findings, previous studies describing feline  
232 hemoplasma phylogenetic analysis based on the RNaseP gene revealed similar close  
233 relationships between the hemoplasma species of both domestic and wild felids [9, 31, 48].

234 A limitation of this study is the small sample size, but despite this, it is interesting to  
235 note that two of the three Persian leopards tested were ‘*Ca. M. haemominutum*’ positive,  
236 suggesting that hemoplasma infection may be prevalent in this species, especially as the two  
237 positive Persian leopards were from geographically distinct areas.

238 In conclusion, we have documented that hemoplasma infections occur in wild felids and we  
239 have reported, for the first time, hemoplasma infection in wild felids in the Middle East and  
240 hemoplasma infection in Persian leopards. Interestingly the two ‘*Ca. M. haemominutum*’  
241 infected Persian leopards were seropositive for FIV. The prevalence of infectious diseases in  
242 wild felids is difficult to assess and monitor but should be considered by those working to  
243 save endangered animal species such as the Persian leopard.

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245

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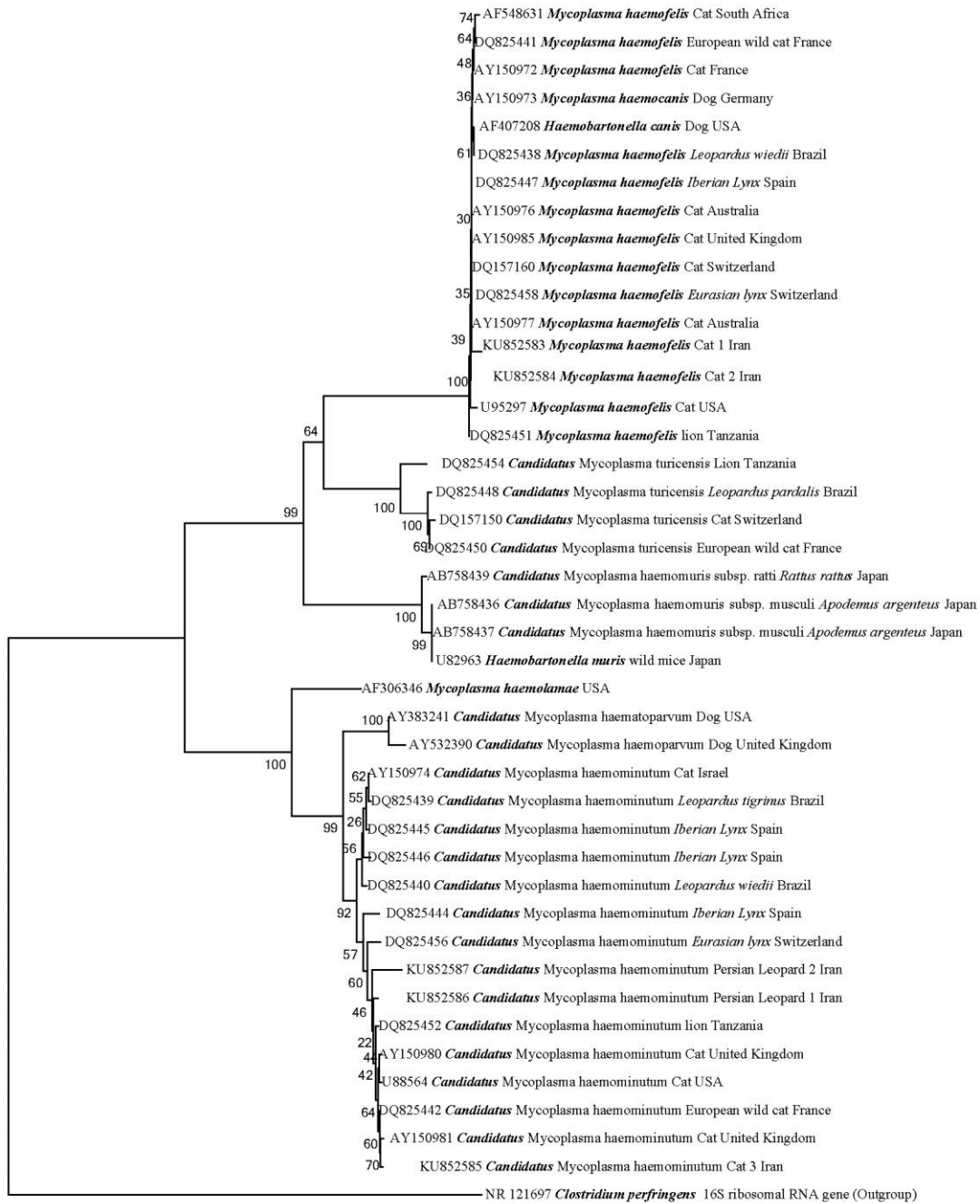
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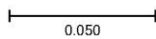
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402 Figure. 1. Phylogenetic analysis of partial 16S rRNA gene sequences from “*Candidatus*  
403 *Mycoplasma haemominutum*” isolates from Persian leopards. Sequences from this study are  
404 shown all in bold. Bootstrap values are given at the nodes of the tree. The following  
405 sequences are shown: *Mycoplasma haemofelis* (Cat, South Africa AF548631; Cat, Iran,  
406 KU852584; Eurasian Lynx, Switzerland, DQ825458; European Wildcat, France, DQ825441;  
407 *Leopardus Weidii*, Brazil, DQ825438; Cat, Switzerland, DQ157160; Cat, United Kingdom,  
408 AY150985; Cat, Australia, AY150977; Cat, Australia, AY150976; Cat, France, AY150972;  
409 Iberian Lynx, Spain, DQ825447; Cat, Iran, KU852583; Cat, United States, U95297, Lion,  
410 Tanzania, DQ825451), *Mycoplasma haemocanis* (Dog, United States, AF407208; Dog  
411 Germany AY150973), “*Candidatus Mycoplasma haemomuris*” (*Apodemus argenteus*, Japan,  
412 AB758437; wild mouse, Japan, U82963; *Apodemus argenteus*, Japan, AB758436; *Rattus*  
413 *rattus*, Japan, AB758439) “*Candidatus Mycoplasma turicensis*” (Lion, Tanzania, DQ825454;  
414 *Leopardus Pardalis*, Brazil, DQ825448; Cat Switzerland, DQ157150; European Wildcat,  
415 France, DQ825450; *Mycoplasma haemolamae* AF306346, “*Candidatus Mycoplasma*  
416 *haematoparvum*” (Dog, United States, AY383241; Dog, United Kingdom, AY532390)  
417 “*Candidatus Mycoplasma haemominutum*” (*Leopardus tigrinus*, Brazil, DQ825439; Iberian  
418 Lynx, Spain, DQ825445; Cat, Israel, AY150974; *Leopardus Weidii*, Brazil, DQ825440;  
419 Iberian Lynx, Spain, DQ825446; Iberian Lynx, Spain, DQ825444; Euroasian Lynx, Switzerland,  
420 DQ825456; Persian Leopard, Iran, KU852587; Persian Leopard, Iran, KU852586; Lion,  
421 Tanzania, DQ825452; Cat, United Kingdom, AY150980; Cat United States, U88564;  
422 European Wildcat, France, DQ852442; Cat, United Kingdom, AY150981; Cat, Iran,  
423 KU852585), *Clostridium perfringens* NR 121697.  
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**Table 1.** Signalment data, origin and residence of sampled wild felids

No.	Species	Scientific name	Gender	Age (years)	Origin	Residence at time of sampling
1	African lion	<i>Panthera leo</i>	Female	6	Born in zoos in Iran	Zoo of Pardisan, Tehran, Iran
2	African lion	<i>Panthera leo</i>	Male	3	Born in zoos in Iran	Zoo of Pardisan, Tehran, Iran
3	African lion	<i>Panthera leo</i>	Female	7	Born in zoos in Iran	Zoo of Pardisan, Tehran, Iran
4	African lion	<i>Panthera leo</i>	Female	1	Born in zoos in Iran	Zoo of Pardisan, Tehran, Iran
5	African lion	<i>Panthera leo</i>	Female	1	Born in zoos in Iran	Zoo of Pardisan, Tehran, Iran
6	African lion	<i>Panthera leo</i>	Female	7	Born in zoos in Iran	Zoo of Pardisan, Tehran, Iran
7	African lion	<i>Panthera leo</i>	Female	2	Born in zoos in Iran	Zoo of Pardisan, Tehran, Iran
8	African lion	<i>Panthera leo</i>	Male	3	Born in zoos in Iran	Zoo of Pardisan, Tehran, Iran
9	African lion	<i>Panthera leo</i>	Male	1	Born in zoos in Iran	Zoo of Pardisan, Tehran, Iran
10	African lion	<i>Panthera leo</i>	Male	2	Born in zoos in Iran	Zoo of Pardisan, Tehran, Iran
11	African lion	<i>Panthera leo</i>	Female	5	Born in zoos in Iran	Zoo of Pardisan, Tehran, Iran
12	African lion	<i>Panthera leo</i>	Female	3	Born in zoos in Iran	Zoo of Pardisan, Tehran, Iran
13	Persian leopard	<i>Panthera pardus</i> ssp. <i>saxicolor</i>	Male	15	Born in wild in Khorasan, Iran	In wild in Iran (transferred to National Park of Tandooreh, Iran at time of sampling)
14	Persian leopard	<i>Panthera pardus</i> ssp. <i>saxicolor</i>	Male	14	Born in wild in Mazandaran, Iran before being transferred to National Park of Tandooreh, Iran	National Park of Tandoureh, Iran
15	Persian leopard	<i>Panthera pardus</i> ssp. <i>saxicolor</i>	Female	4	Born in wild in Golestan, Iran before being transferred to National Park of Tandooreh, Iran	National Park of Tandoureh, Iran
16	African leopard	<i>Panthera pardus</i> ssp. <i>pardus</i>	Male	22	Born in wild in Kenya before being transferred to National Park of Tandooreh, Iran	National Park of Tandoureh, Iran
17	Eurasian lynx	<i>Lynx lynx</i>	Male	8	Born in wild in Iran before being transferred to National Park of Tandooreh, Iran	National Park of Tandoureh, Iran
18	Caracal	<i>Caracal caracal</i>	Male	6	Born in wild in Iran before being transferred to National Park of Tandooreh, Iran	National Park of Tandoureh, Iran
19	Bengal tiger	<i>Panthera tigris</i> ssp. <i>Tigris</i>	Male	1.5	Born in zoo in Denmark	Zoo of Pardisan, Tehran, Iran

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Table 2. Hematological parameters for Persian leopards

Species	Persian leopard Case no. 13*	Persian leopard Case no. 14*	Persian leopard Case no. 15	Domestic cat Reference range [49]	African lion Reference range [22](mean ± SD)	Derived African lion Reference range**	Unit
Hct <sup>+</sup>	29,4	28,2	34,15	29-45	42,38 ± 4,73	33,11-51,65	%
Hb	10,2	8,8	12,1	8-14	14,11 ± 1,63	10,92-17,30	g/dl
RBC	6,1	6,59	7,92	6-10	8,97 ± 1,43	6,17-11,77	10 <sup>6</sup> /μl
MCV	48	50	48,5	41.0-54	47,70 ± 4,53	38,82-56,58	fl
MCH	12,8	15,7	14,53	13.3-17.5	15,48 ± 1,25	13,03-17,93	pg
MCHC	26,6	31	29,38	31-36	33,3 ± 2,02	29,34-37,26	%
Plt	139	102	98,5	2.3-6.8	-	-	10 <sup>5</sup> /μl
WBC	9,53	4,06	10,75	5.5-19.5	9,73 ± 1,43	6,93-12,53	10 <sup>3</sup> /μl
Seg.	7,1475	2,436	7,821	2.5-12.5	7,748 ± 1,209	5,38-10,12	10 <sup>3</sup> /μl
Band	0,1906	0,0406	0,131	0-0.3	0	0,00-0,00	10 <sup>3</sup> /μl
Lymph				1,5-7	894 ± 456	0,24-	10 <sup>3</sup> /μl
	1,906	1,421	2,281			1787,76	
Mono	0,0953	0,0406	0,067	0-0.85	365 ± 193	0-743,28	10 <sup>3</sup> /μl
Eos	0,1906	0,0812	0,101	0-1.5	372 ± 364	0-1085,44	10 <sup>3</sup> /μl
Baso	0	0	0	Rare	0	0,00-0,00	10 <sup>3</sup> /μl

NB. No hematology reference range is available for Persian leopards.

\* '*Ca. M. haemominutum*' FIV infected Persian leopards

+Hematocrit (HCT), hemoglobin concentration (Hb), Red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) mean corpuscular hemoglobin concentration (MCHC), platelets (Plt), white blood cell count (WBC), segmented neutrophil (seg), band cell (Band), lymphocyte (Lymph), monocyte (Mono), eosinophil (Eos), basophil (Baso).

\*\* Reference range for African lion derived using mean ± 1.96SD from Larrson et al <sup>23</sup>