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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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21

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 mycoplasma¹ 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 km² 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

246 **5. References**

247

248 [1] H. Neimark, K.E. Johansson, Y. Rikihisa, J.G. Tully, Proposal to transfer some members

249 of the genera *Haemobartonella* and *Eperythrozoon* to the genus *Mycoplasma* with
250 descriptions of '*Candidatus Mycoplasma haemofelis*', '*Candidatus Mycoplasma haemomuris*',
251 '*Candidatus Mycoplasma haemosuis*' and '*Candidatus Mycoplasma wenyonii*', *Int. J Syst.*
252 *Evol. Microbiol.* 51 (2001) 891-9.

253 [2] B. Willi, F.S. Boretti, S. Tasker, M.L. Meli, N. Wengi, C.E. Reusch, H. Lutz, R.
254 Hofmann-Lehmann, From *Haemobartonella* to hemoplasma: Molecular methods provide new
255 insights, *Vet. Microbiol.* 125 (2007) 197-209.

256 [3] B. Willi, F.S. Boretti, V. Cattori, S. Tasker, M.L. Meli, C. Reusch, H. Lutz, R. Hofmann-
257 Lehmann, Identification, Molecular Characterization, and Experimental Transmission of a
258 New Hemoplasma Isolate from a Cat with Hemolytic Anemia in Switzerland, *J Clin.*
259 *Microbiol.* 43 (2005) 2581-2585.

260 [4] B. Willi, K. Museux, M. Novacco, E.M. Schraner, P. Wild, K. Groebel, U. Ziegler, G.A.
261 Wolf-Jäckel, Y. Kessler, C. Geret, S. Tasker, H. Lutz, R. Hofmann-Lehmann, First
262 morphological characterization of '*Candidatus Mycoplasma turicensis*' using electron
263 microscopy, *Vet. Microbiol.* 149 (2011) 367-373.

264 [5] S. Tasker, Haemotropic mycoplasmas: what's their real significance in cats?, *J Feline*
265 *Med. Surg.* 12 (2010) 369-81.

266 [6] L. M. Berent, J. B. Messick, S. K. Cooper, Detection of *Haemobartonella felis* in cats with
267 experimentally induced acute and chronic infections, using a polymerase chain reaction assay,
268 *Am. J. Vet. Res* 59 (1998) 1215-20.

269 [7] J. W. George, B. A. Rideout, S. M. Griffey, N. C. Pedersen, Effect of preexisting FeLV
270 infection or FeLV and feline immunodeficiency virus coinfection on pathogenicity of the
271 small variant of *Haemobartonella felis* in cats, *Am. J Vet. Res.* 63 (2002) 1172-8.

272 [8] D. B. Macieira, C. de Menezes Rde, C. B. Damico, N. R. Almosny, H. L. McLane, J. K.
273 Daggy, J. B. Messick, Prevalence and risk factors for hemoplasmas in domestic cats naturally

274 infected with feline immunodeficiency virus and/or feline leukemia virus in Rio de Janeiro--
275 Brazil, J Feline. Med. Surg. 10 (2008) 120-9.

276 [9] B. Willi, C. Filoni, J. L. Catão-Dias, V. Cattori, M. L. Meli, A. Vargas, F. Martínez, M. E.
277 Roelke, M.-P. Ryser-Degiorgis, C. M. Leutenegger, H. Lutz, R. Hofmann-Lehmann,
278 Worldwide Occurrence of Feline Hemoplasma Infections in Wild Felid Species, J Clin.
279 Microbiol. 45 (2007) 1159-1166.

280 [10] M. Haefner, T. J. Burke, B. E. Kitchell, L. A. Lamont, D. J. Schaeffer, M. Behr, J. B.
281 Messick, Identification of *Haemobartonella felis* (*Mycoplasma haemofelis*) in captive
282 nondomestic cats, J Zoo Wildl. Med. : official publication of the American Association of
283 Zoo Veterinarians 34 (2003) 139-43.

284 [11] M. R. André, C. H. Adania, S. M. Allegretti, R. Z. Machado, Hemoplasmas in Wild
285 Canids and Felids in Brazil, J Zoo. Wildl. Med. 42 (2011) 342-347.

286 [12] International Union for Conservation of Nature (IUCN), The IUCN Red List of
287 Threatened Species. <http://www.iucnredlist.org> ed2015.

288 [13] M. S. Farhadinia, H. Farahmand, A. Gavashelishvili, M. Kaboli, M. Karami, B. Khalili,
289 S. Montazamy, Molecular and craniological analysis of leopard, *Panthera pardus* (Carnivora:
290 Felidae) in Iran: support for a monophyletic clade in Western Asia, Biol. J Linn. Soc. 114
291 (2015) 721-736.

292 [14] A. Ghoddousi, A. Kh. Hamidi, T. Ghadirian, D. Ashayeri, I. Khorozyan, The status of
293 the Endangered Persian leopard *Panthera pardus saxicolor* in Bamu National Park, Iran, Oryx.
294 44 (2010) 551-557.

295 [15] B. H. Kiabi, B. F. Dareshouri, R. A. Ghaemi, M. Jahanshahi, Population status of the
296 Persian Leopard (*Panthera pardus saxicolor* Pocock, 1927) in Iran, Zool. Middle. East. 26
297 (2002) 41-47.

298 [16] F. Ghazisaeedi, N. Atyabi, T. Zahrai Salehi, F. Gentilini, I. Ashrafi Tamai, H. Akbarein,

299 S. Tasker, A molecular study of hemotropic mycoplasmas (hemoplasmas) in cats in Iran, Vet.
300 Clin. Pathol. 43 (2014) 381-386.

301 [17] A. J. Birkenheuer, M. G. Levy, E. B. Breitschwerdt, Development and Evaluation of a
302 Seminested PCR for Detection and Differentiation of *Babesia gibsoni* (Asian Genotype) and
303 *B. canis* DNA in Canine Blood Samples, J Clin. Microbiol. 41 (2003) 4172-4177.

304 [18] A. Criado-Fornelio, A. Martinez-Marcos, A. Buling-Saraña, J. C. Barba-Carretero,
305 Presence of *Mycoplasma haemofelis*, *Mycoplasma haemominutum* and piroplasmids in cats
306 from southern Europe: a molecular study, Vet. Microbiol. 93 (2003) 307-317.

307 [19] W. A. Jensen, M. R. Lappin, S. Kamkar, W. J. Reagan, Use of a polymerase chain
308 reaction assay to detect and differentiate two strains of *Haemobartonella felis* in naturally
309 infected cats, Am. J Vet. Res. 62 (2001) 604-8.

310 [20] A. P. Santos, J. B. Messick, A. W. Biondo, S. T. Oliveira, V. Pedralli, C. S. Lasta, L. A.
311 Lacerda, V. S. Esteves, R. Hofmann-Lehmann, B. Willi, F. H. D. González, Design,
312 optimization, and application of a conventional PCR assay with an internal control for
313 detection of ‘*Candidatus Mycoplasma turicensis*’ 16S rDNA in domestic cats from Brazil,
314 Vet. Clin. Pathol. 38 (2009) 443-452.

315 [21] C. Pitulle, D. M. Citron, B. Bochner, R. Barbers, M. D. Appleman, Novel bacterium
316 isolated from a lung transplant patient with cystic fibrosis, J Clin. Microbiol. 37 (1999) 3851-
317 5.

318 [22] M. H. M. A. Larsson, P. L. do Espírito Santo, R. M. S. Mirandola, J. D. L. Fedullo, F. H.
319 Ito, P. H. Itikawa, R. B. Pessoa, Hematologic Parameters of Captive Lions (*Panthera leo*) and
320 Siberian Tigers (*Panthera tigris altaica*), Acta Sci. Vet. 43 (2015) 1311.

321 [23] J. Felsenstein, Confidence Limits on Phylogenies: An Approach Using the Bootstrap,
322 Evol. 39 (1985) 783-791.

323 [24] M. Kimura, A simple method for estimating evolutionary rates of base substitutions

324 through comparative studies of nucleotide sequences, J Mol. Evol. 16 (1980) 111-20.

325 [25] N. Saitou, M. Nei, The neighbor-joining method: a new method for reconstructing
326 phylogenetic trees, Mol. Biol. Evol. 4 (1987) 406-425.

327 [26] K. Tamura, G. Stecher, D. Peterson, A. Filipski, S. Kumar, MEGA6: Molecular
328 Evolutionary Genetics Analysis Version 6.0, Mol. Biol. Evol. 30 (2013) 2725-2729.

329 [27] B. Willi, F. S. Boretti, C. Baumgartner, S. Tasker, B. Wenger, V. Cattori, M. L. Meli, C.
330 E. Reusch, H. Lutz, R. Hofmann-Lehmann, Prevalence, Risk Factor Analysis, and Follow-Up
331 of Infections Caused by Three Feline Hemoplasma Species in Cats in Switzerland, J Clin.
332 Microbiol. 44 (2006) 961-969.

333 [28] S. Tasker, C. R. Helps, M. J. Day, T. J. Gruffydd-Jones, D. A. Harbour, Use of real-time
334 PCR to detect and quantify *Mycoplasma haemofelis* and "*Candidatus* Mycoplasma
335 haemominutum" DNA, J Clin. Microbiol. 41 (2003) 439-41.

336 [29] Y. Rikihisa, M. Kawahara, B. Wen, G. Kociba, P. Fuerst, F. Kawamori, C. Suto, S.
337 Shibata, M. Futohashi, Western immunoblot analysis of *Haemobartonella muris* and
338 comparison of 16S rRNA gene sequences of *H. muris*, *H. felis*, and *Eperythrozoon suis*, J
339 Clin. Microbiol. 35 (1997) 823-829.

340 [30] C. Filoni, J. L. Catão-Dias, V. Cattori, B. Willi, M. L. Meli, S. H. R. Corrêa, M. C.
341 Marques, C. H. Adania, J. C. R. Silva, M. F. V. Marvulo, J. S. F. Neto, E. L. Durigon, V. M.
342 de Carvalho, S. D. A. Coutinho, H. Lutz, R. Hofmann-Lehmann, Surveillance using
343 serological and molecular methods for the detection of infectious agents in captive Brazilian
344 neotropic and exotic felids, J Vet. Diagn. Invest. 24 (2012) 166-173.

345 [31] A. Krenzel, M. L. Meli, V. Cattori, B. Wachter, B. Willi, S. Thalwitzer, J. Melzheimer,
346 H. Hofer, H. Lutz, R. Hofmann-Lehmann, First evidence of hemoplasma infection in free-
347 ranging Namibian cheetahs (*Acinonyx jubatus*), Vet. Microbiol. 162 (2013) 972-6.

348 [32] S. Tasker, S. H. Binns, M. J. Day, T. J. Gruffydd-Jones, D. A. Harbour, C. R. Helps, W.

349 A. Jensen, C. S. Olver, M. R. Lappin, Use of a PCR assay to assess the prevalence and risk
350 factors for *Mycoplasma haemofelis* and ‘*Candidatus Mycoplasma haemominutum*’ in cats in
351 the United Kingdom, *Vet. Rec.* 152 (2003) 193-198.

352 [33] C. B. Grindem, W. T. Corbett, M. T. Tomkins, Risk factors for *Haemobartonella felis*
353 infection in cats, *J Am. Vet. Med. Assoc.* 196 (1990) 96-9.

354 [34] F. Gentilini, M. Novacco, M. E. Turba, B. Willi, M. L. Bacci, R. Hofmann-Lehmann,
355 Use of combined conventional and real-time PCR to determine the epidemiology of feline
356 haemoplasma infections in northern Italy, *J Feline Med. Surg.* 11 (2009) 277-285.

357 [35] B. Willi, S. Tasker, F. S. Boretti, M. G. Doherr, V. Cattori, M. L. Meli, R. G. Lobetti, R.
358 Malik, C. E. Reusch, H. Lutz, R. Hofmann-Lehmann, Phylogenetic Analysis of “*Candidatus*
359 *Mycoplasma turicensis*” Isolates from Pet Cats in the United Kingdom, Australia, and South
360 Africa, with Analysis of Risk Factors for Infection, *J Clin. Microbiol.* 44 (2006) 4430-4435.

361 [36] L. C. Aquino, C. A. Hicks, M. C. Scalon, M. G. Lima, S. Lemos Mdos, G. R. Paludo, C.
362 R. Helps, S. Tasker, Prevalence and phylogenetic analysis of haemoplasmas from cats
363 infected with multiple species, *J Microbiol. Methods.* 107 (2014) 189-96.

364 [37] S. Tasker, J. A. Braddock, R. Baral, C. R. Helps, M. J. Day, T. J. Gruffydd-Jones, R.
365 Malik, Diagnosis of feline haemoplasma infection in Australian cats using a real-time PCR
366 assay, *J Feline Med. Surg.* 6 (2004) 345-354.

367 [38] M. Tanahara, S. Miyamoto, T. Nishio, Y. Yoshii, M. Sakuma, Y. Sakata, K. Nishigaki,
368 H. Tsujimoto, A. Setoguchi, Y. Endo, An epidemiological survey of feline hemoplasma
369 infection in Japan, *J Vet. Med. Sci.* 72 (2010) 1575-81.

370 [39] A. B. Stein, V. Hayssen, *Panthera pardus* (Carnivora: Felidae), *Mammal. Spec.* 45 (2013)
371 30-48.

372 [40] G. A. Balme, R. Slotow, L. T. B. Hunter, Impact of conservation interventions on the
373 dynamics and persistence of a persecuted leopard (*Panthera pardus*) population, *Biol.*

374 Conserv. 142 (2009) 2681-2690.

375 [41] V. Steyn, P. J. Funston, A case of cannibalism in leopards, S Afr. J Wildl. Res. 36 (2006)

376 189–190.

377 [42] R. Hofmann-Lehmann, D. Fehr, M. Grob, M. Elgizoli, C. Packer, J. S. Martenson, S. J.

378 O'Brien, H. Lutz, Prevalence of antibodies to feline parvovirus, calicivirus, herpesvirus,

379 coronavirus, and immunodeficiency virus and of feline leukemia virus antigen and the

380 interrelationship of these viral infections in free-ranging lions in east Africa, Clin. Diagn. Lab.

381 Immunol. 3 (1996) 554-62.

382 [43] C. M. Leutenegger, R. Hofmann-Lehmann, C. Riols, M. Liberek, G. Worel, P. Lups, D.

383 Fehr, M. Hartmann, P. Weilenmann, H. Lutz, Viral infections in free-living populations of the

384 European wildcat, J Wildl. Dis. 35 (1999) 678-86.

385 [44] R. A. Olmsted, R. Langley, M. E. Roelke, R. M. Goeken, D. Adger-Johnson, J. P. Goff,

386 J. P. Albert, C. Packer, M. K. Laurenson, T. M. Caro, Worldwide prevalence of lentivirus

387 infection in wild feline species: epidemiologic and phylogenetic aspects, J Virol. 66 (1992)

388 6008-6018.

389 [45] M. C. Barr, P. P. Calle, M. E. Roelke, F. W. Scott, Feline Immunodeficiency Virus

390 Infection in Nondomestic Felids, J Zoo Wildl. Med. 20 (1989) 265-272.

391 [46] M. J. Daniels, M. C. Golder, O. Jarrett, D. W. MacDonald, Feline Viruses in Wildcats

392 from Scotland, J Wildl. Dis. 35 (1999) 121-124.

393 [47] H. Lutz, E. Isenbügel, R. Lehmann, R. H. Sabapara, C. Wolfensberger, Retrovirus

394 infections in non-domestic felids: serological studies and attempts to isolate a lentivirus, Vet.

395 Immunol. Immunopath. 35 (1992) 215-224.

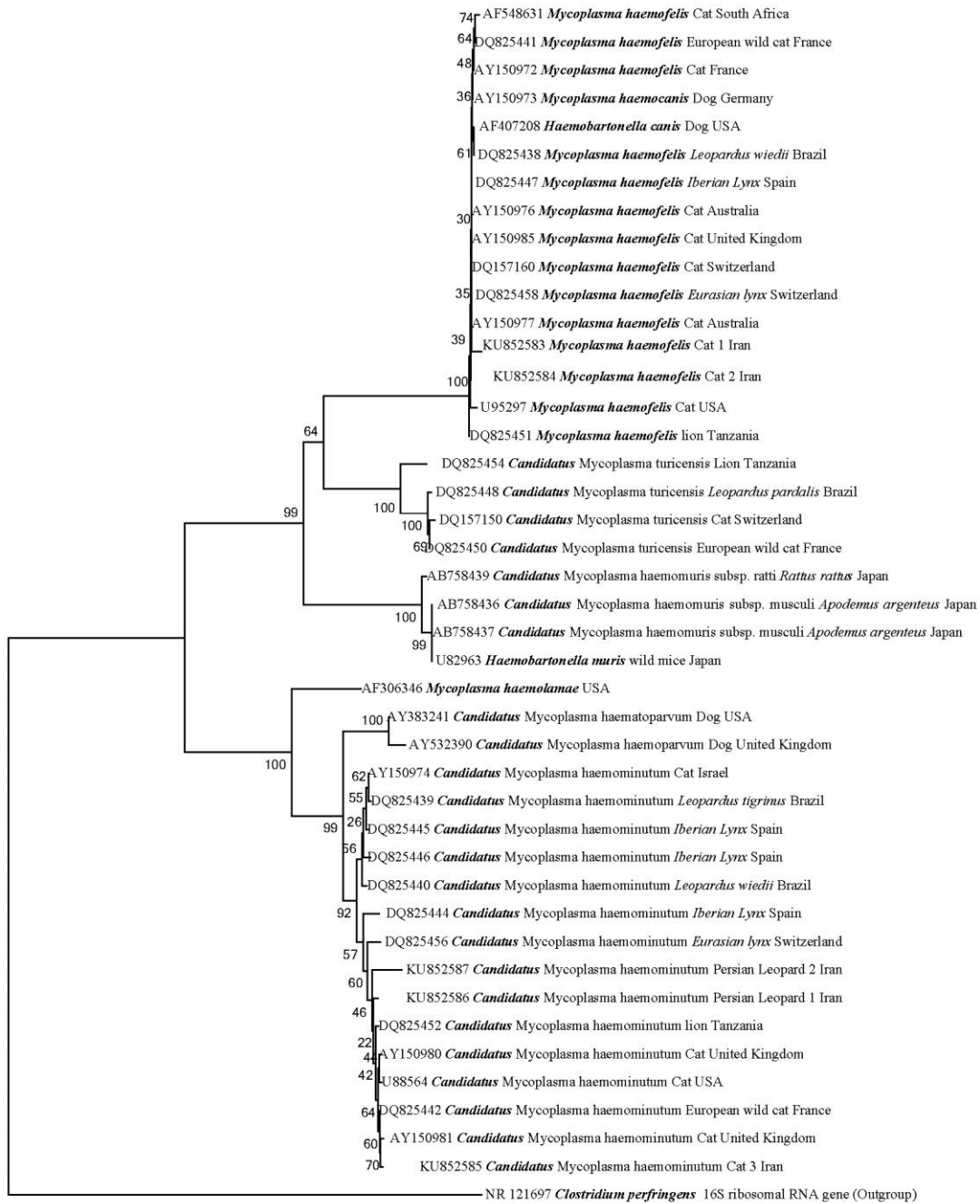
396 [48] S. Tasker, C. R. Helps, M. J. Day, D. A. Harbour, S. E. Shaw, S. Harrus, G. Baneth, R.

397 G. Lobetti, R. Malik, J. P. Beaufils, C. R. Belford, T. J. Gruffydd-Jones, Phylogenetic

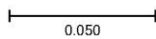
398 analysis of hemoplasma species: an international study, J Clin. Microbiol. 41 (2003) 3877-80.

399 [49] D. J. Weiss, J. K. Wardrop, Schalm's Veterinary Hematology, 6th ed., Wiley-Blackwell
400 2010.
401

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 ⁺	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 ⁶ /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 ⁵ /μl
WBC	9,53	4,06	10,75	5.5-19.5	9,73 ± 1,43	6,93-12,53	10 ³ /μl
Seg.	7,1475	2,436	7,821	2.5-12.5	7,748 ± 1,209	5,38-10,12	10 ³ /μl
Band	0,1906	0,0406	0,131	0-0.3	0	0,00-0,00	10 ³ /μl
Lymph				1,5-7	894 ± 456	0,24-	10 ³ /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 ³ /μl
Eos	0,1906	0,0812	0,101	0-1.5	372 ± 364	0-1085,44	10 ³ /μl
Baso	0	0	0	Rare	0	0,00-0,00	10 ³ /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 ²³