First Report of a Novel *Hepatozoon* sp. in Giant Pandas (*Ailuropoda melanoleuca*)

Jennifer H. Yu,1 Kate L. Durrant,2,9 Songrui Liu,3 Ellen P. Carlin,1,4 Chengdong Wang,3 Juan Rodriguez,3 Ann Bratthauer,6 Tim Walsh,7 Marc T. Valitutto,1 Leah Fine,8 Suzan Murray,1 and Robert C. Fleischer9

1Global Health Program, Smithsonian Conservation Biology Institute, National Zoological Park, Washington, DC 20008
2School of Life Sciences, University of Nottingham, Nottingham, UK
3Chengdu Research Base of Giant Panda Breeding, Chengdu, Sichuan, People’s Republic of China
4EcoHealth Alliance, New York, NY
5Center for Species Survival, Smithsonian Conservation Biology Institute, National Zoological Park, Washington, DC 20008
6Department of Pathology, Smithsonian National Zoological Park, Washington, DC
7College of Veterinary Medicine, Kansas State University, Manhattan, KS
8Washington University in St. Louis, St. Louis, MO
9Center for Conservation Genomics, Smithsonian Conservation Biology Institute, National Zoological Park, Washington, DC 20008

Abstract: The first report of giant pandas (*Ailuropoda melanoleuca*) infected with a novel *Hepatozoon* species is presented. An intraleukocytic parasite was detected via routine blood smear from a zoo-housed giant panda at the National Zoological Park. Ribosomal DNA sequences indicated a previously undescribed *Hepatozoon* species. Phylogenetic and distance analyses of the sequences placed it within its own branch, clustered with Old World species with carnivore (primarily ursid and mustelid) hosts. Retrospective and opportunistic testing of other individuals produced additional positive detections (17/23, 73.9%), demonstrating 100% prevalence (14/14) across five institutions. All animals were asymptomatic at time of sampling, and health implications for giant pandas remain unknown.

Keywords: *Hepatozoon*, *Ailuropoda melanoleuca*, Giant panda, Apicomplexa, Hemoparasite, Conservation management, Polymerase chain reaction

**INTRODUCTION**

*Hepatozoon* spp. are apicomplexan hemoparasites capable of infecting a wide range of vertebrate taxa globally, including canids, ursids, felids, and numerous others (André et al. 2010; East et al. 2008; Kubo et al. 2006, 2008, 2010; Pawar et al. 2011, 2012). In North America, *Hepatozoon* infections have been documented in domestic canids, wild coyotes (*Canis latrans*), and other small carnivores (Kocan et al. 2000; Mercer et al. 1988). Clinical disease is largely uncommon but can affect domestic dogs, causing musculoskeletal lesions and potentially death (Baneth et al. 2003; Barton et al. 1985). *Hepatozoon* spp. have heteroxenous life cycles, requiring definitive invertebrate hosts and intermediate vertebrate hosts (Smith 1996). Unlike the bite-transmission route characteristic of other vector-borne pathogens, *Hepatozoon* spp. are typically...
transmitted via host ingestion of the arthropod during grooming or consumption of infected prey or prey with infected ticks (Allen et al. 2011; Baneth et al. 2003; Smith 1996).

This report describes a novel Hepatozoon parasite found in captive zoo-housed giant pandas (Ailuropoda melanoleuca). Giant pandas are ursids native to central China and an iconic “flagship” species for conservation. Currently categorized as vulnerable by the IUCN, the species faces continued threats from habitat loss and fragmentation, starvation, and infectious diseases (Feng et al. 2015; Swaisgood et al. 2016; Zhang et al. 2008).

In March 2005, an intraleukocytic hemoparasite was detected on a routine blood smear of a male giant panda housed at the Smithsonian National Zoological Park (NZP) in Washington, DC (listed as GP4 in Table 1). The blood smear demonstrated neutrophil-associated parasites with a morphology similar to that of known Hepatozoon species. The specimen could not be identified to species by light microscopy.

DNA was extracted from the sample using DNeasy kits (Qiagen) and amplified via polymerase chain reaction (PCR) using a combination of 18S ribosomal DNA primer sets (Hep18S2-H, Hep18S4-L, Hep18S4-H, BT1-L, BT1-H, BTH1-L, and BTH1-H) in a 25-µL PCR. Sequencing was conducted on an ABI 3730, and sequences were aligned and edited using Sequencher 4.1. A total length of 1092 bp was obtained for GP4. His mate, GP5 (MeiXiang, 113607), was also positive, and we obtained 1111 bp of sequence for her which was identical to that of GP4. The latter sequence was utilized in subsequent analyses. Blasts to GenBank sequences from morphologically identified specimens and phylogenetic analyses confirmed identification of the parasite lineage to genus (Hepatozoon). The program RAxML 8.2.11 (Stamatakis 2014) implemented in Geneious (Kearse et al. 2012) was used to estimate phylogenetic relationships within genera using a maximum likelihood (ML) criterion and a general time-reversible (GTR+I+gamma) model of nucleotide substitution, with 1000 bootstrap replications. jModelTest (Darriba et al. 2012; Guindon and Gascuel 2003) was used to identify the best evolutionary model for the sequence data. Under BIC and DT (decision theory) criteria, the GTR+I+G model was preferred, while the AICc criterion favored the slightly different TPM1uf+I+G model. The estimated I and G values in all models were nearly identical (0.49 and 0.89, respectively). Therefore, we selected the GTR+I+G model from the BIC for the ML analysis.

In the resulting phylogeny, the giant panda-associated Hepatozoon lineage occupied its own distinct, long branch, nested in a clade with Old World species H. felis, H. ursi, and H. martis (Fig. 1). The consensus phylogeny suggested a genetically unique parasite, most closely related to a sister clade containing H. ursi, a parasite of Asiatic bears (Kubo et al. 2008; Pawar et al. 2011), and H. martis, a mustelid parasite (Hodža et al. 2018), but bootstrap support of 52% was marginal for this node, and it essentially collapses to a trichotomy. The giant panda Hepatozoon 18S sequence was 3.2 to 3.6% divergent (uncorrected) from those of H. ursi and H. martis, but ≥ 4.5% divergent from all other carnivore Hepatozoon sequences. Thus, we found support for a single clade containing ursid (including panda) and mustelid Hepatozoon-derived sequences. We also found two paraphyletic H. felis clades, with one falling out as a poorly supported sister clade to H. americanum. However, analyses with additional H. felis sequences sometimes removed the paraphyly and the two H. felis clades became poorly supported sister clades. Regardless, the trees and data support that the giant panda Hepatozoon is a distinct lineage and species and that it is most closely related to Hepatozoon with ursid and mustelid hosts.

Conventional PCR was then performed on 23 archived or opportunistically collected blood and tissue samples acquired from 14 giant pandas between 1982 and 2006. Primer sets BT1 (432 bp amplicon) and BTH1 (751 bp amplicon) provided the greatest amplification and sequencing consistency and were tested on all available samples. Additional sequencing was performed as described, and for most individuals, up to 1113 bp were obtained. Whole blood samples were evaluated where possible, but when unavailable, other tissue types, plasma, or stained blood smears were substituted. For six pandas, multiple samples were used. The samples had been collected from seven adult males, six adult females, and one male neonate from five institutions (three in the USA, one in the United Kingdom, and one in China). All individuals were born in China (either wild-caught or captive-bred) with the exception of the neonate, which was born at the NZP but did not survive beyond a few days. None of the animals were reported to have clinical signs of disease consistent with hepatozoonosis at the time of sampling.

The PCR results from testing 14 captive giant pandas are summarized in Table 1. All individuals (14/14, 100%) sampled demonstrated positive tests for Hepatozoon sp., identical in overlapping sequence to that of GP4 and GP5. Positive detections were made in 17 samples out of 23
Table 1. Summary of *Hepatozoon* PCR results from retrospective and opportunistic testing of captive giant pandas.

| ID  | Sex | Status            | Origin                          | Country      | Location of sampling          | Country       | Sample type                  | Collection date | Positive detection |
|-----|-----|-------------------|--------------------------------|--------------|-------------------------------|---------------|-----------------------------|-----------------|--------------------|
| GP 1| M   | Wild-caught captive | Qionglai Mountains, Baoxing     | China        | National Zoological Park      | USA           | Stained blood smear          | 1983            |                    |
|     |     |                   |                                 |              |                               |               | Stained blood smear          | 1984            |                    |
|     |     |                   |                                 |              |                               |               | Plasma                       | 1982            | x                  |
| GP 2| F   | Wild-caught captive | Qionglai Mountains, Baoxing     | China        | National Zoological Park      | USA           | Stained blood smear          | 1985            |                    |
|     |     |                   |                                 |              |                               |               | Stained blood smear          | 1989            |                    |
|     |     |                   |                                 |              |                               |               | Kidney                       | 1992            | x                  |
| GP 3| M   | Captive           | National Zoological Park        | USA          | National Zoological Park      | USA           | Kidney cell culture          | 1983            | x                  |
| GP 4| M   | Captive           | CCRCGP*                        | China        | National Zoological Park      | USA           | Whole blood                  | 2006            |                    |
|     |     |                   |                                 |              |                               |               | Stained blood smear          |                 | x                  |
| GP 5| F   | Captive           | CCRCGP                         | China        | National Zoological Park      | USA           | Whole blood                  | 2005            |                    |
| GP 6| M   | Captive           | Chongqing Zoological Garden     | China        | Memphis Zoo                   | USA           | Serum                        | 2006            | x                  |
| GP 7| F   | Captive           | Beijing Zoological Garden      | China        | Memphis Zoo                   | USA           | Whole blood                  | 2006            | x                  |
| GP 8| F   | Captive           | Chengdu Research Base          | China        | Zoo Atlanta                   | USA           | Whole blood                  | 2006            | x                  |
| GP 9| M   | Captive           | Chengdu Research Base          | China        | Zoo Atlanta                   | USA           | Whole blood                  | 2006            | x                  |
| GP 10| M  | Wild-caught captive | Qionglai Mountains, Sanjiang    | China        | CCRCGP                        | China         | Plasma                       | 1986            | x                  |
|     |     |                   |                                 |              |                               |               | WBC                          | 1986            | x                  |
| GP 11| F  | Wild-caught captive | Min Mountains, Nanping          | China        | CCRCGP                        | China         | Plasma                       | 1986            | x                  |
|     |     |                   |                                 |              |                               |               | WBC                          | 1986            | x                  |
| GP 12| M  | Wild-caught captive | Qionglai Mountains, Baoxing     | China        | CCRCGP                        | China         | Plasma                       | 1986            | x                  |
| GP 13| F  | Wild-caught captive | Qionglai Mountains, Baoxing     | China        | CCRCGP                        | China         | Plasma                       | 1986            | x                  |
|     |     |                   |                                 |              |                               |               | WBC                          | 1986            | x                  |
| GP 14| M  | Wild-caught captive | Qionglai Mountains, Baoxing     | China        | Zoological Society of London  | UK            | Plasma                       | 1983            | x                  |

*Kidney sample acquired postmortem; individual died due to heart failure.

*Denotes neonate; did not survive beyond a few days. Sample obtained postmortem.

*Denotes the individual from which novel *Hepatozoon* species was first detected on light microscopy.

*This individual had been temporarily transferred to National Zoological Park, USA, in 1981 on breeding loan.

China Conservation and Research Center for the Giant Panda (CCRCGP), Wolong National Nature Reserve.

Novel *Hepatozoon* sp. in Giant Pandas
tested (73.9%). Of the negative results, four resulted from stained blood smears and two from whole blood samples. These were presumed to be false negatives, attributed to poor quality of archival samples and varying extraction efficiency and PCR sensitivity due to DNA inhibitors (Scopel et al. 2004; Shavey and Morado 2012). The sequence for giant panda 113607 was deposited in GenBank (accession number pending MK645858).

Although the level of parasitemia was not quantified, infrequent findings on routine microscopy and occasional negative results by PCR suggest that the parasite may be present at low levels or intermittently in circulation (Otranto et al. 2011; Scopel et al. 2004). Challenges in detecting the parasite without molecular methods may explain why the parasite was not previously found. It is possible that the parasite is abundant in other tissues, as these may be sites of merogony and cyst formation in mammalian Hepatozoon life cycles (Smith 1996), but tissues were not thoroughly investigated here; testing of other individuals and tissue types may produce additional positive detections.

To our knowledge, this is the first report of a Hepatozoon infection described in giant pandas. The pathogenicity of this novel species and the health and conservation implications for its vertebrate host are unknown. Like many wildlife species found with asymptomatic Hepatozoon infections (Clark et al. 1973; McCully et al. 1975; Pawar et al. 2012), the individuals tested here displayed no apparent clinical illness attributable to hepatozoonosis. The high prevalence and penetration into the population demonstrated here may indicate low pathogenicity (Best et al. 2014).

Given the giant panda’s vulnerable conservation status, however, the presence of any identified pathogen warrants consideration of clinical and conservation management implications. Infectious disease outbreaks can have considerable and disproportionate effects on small populations (De Castro and Bolker 2005; Smith et al. 2006). While Hepatozoon infection may be an incidental finding in otherwise healthy captive giant pandas, it may have significant consequences for juvenile, geriatric, or otherwise immunocompromised individuals (Kocan et al. 2000).
Concomitant disease or coinfections could precipitate increased parasite load and potentially heightened pathogenicity (McCully et al. 1975; Simpson et al. 2013). Giant pandas are prone to a suite of health issues, including gastrointestinal disorders, infectious diseases, infertility, and others which are incompletely understood (Feng et al. 2015; Qiu and Mainka 1993; Williams et al. 2016; Zhang et al. 2008). The extent to which a background Hepatozoon infection may contribute to illness in these cases is unknown.

Considering a common geographic origin for 13/14 individuals, it is likely that the Hepatozoon infections were not locally acquired at receiving institutions but rather were already present in the animals upon arrival. The high prevalence demonstrated here could indicate: an enzootic infection of giant pandas, where they may be the parasite’s natural host; a high rate of exposure; or an elevated susceptibility to a novel infection due to either host or agent factors. The genus has a wide distribution globally, including species identified from China (Wei et al. 2016; Xu et al. 2015). Phylogenetic relationships also support a plausible Asiatic origin, as the species clusters with Old World Hepatozoon species. Its proximity to H. ursi is expected from an evolutionary standpoint and consistent with an Asiatic origin. Hepatozoon ursi is a parasite of other Asiatic ursids like Japanese black bears (Ursus thibetanus japonicas) and Indian sloth bears (Melursus ursinus) (Kubo et al. 2008; Pawar et al. 2011), and to date, North American ursids have not been reported with Hepatozoon infections.

The route of transmission remains to be described. Cases of tick-infested free-ranging giant pandas have been reported (Qiu and Mainka 1993), although possible vector species were not sought or identified in these cases. The positive detection in a neonate suggests a potential vertical transmission route (Allen et al. 2011; Murata et al. 1993). Because host specificity of this novel Hepatozoon species is unknown, the impact of an introduced hemoparasite on local mammalian populations is unclear. The potential risk to both native wildlife and giant pandas may warrant stringent Hepatozoon surveillance in the captive population.

Further research is necessary to assess whether this Hepatozoon species constitutes a potential pathogen of giant pandas and to characterize the extent of threat to the species. Epidemiologic studies may help determine whether there is a correlation between the degree of parasitemia and any occurrence of clinical signs. Prospective studies could investigate prevalence in captive individuals held globally in other facilities. The parasite’s infectivity, pathogenicity, range, prevalence, and vector species in free-ranging populations are unknown, but should remain priorities for future investigation. Caution may also be indicated in the international movement of captive individuals. In domestic canids, hepatozoosmosis is typically managed using antiprotozoal and palliative treatments (Allen et al. 2011). Efforts could be directed at identifying safe and effective antiprotozoal therapies and parasite management for giant pandas for implementation during routine quarantine procedures prior to animal transport. Thus, additional studies can elucidate pathogen, host, and vector relationships and identify the need for targeted conservation management actions for this vulnerable species.

**Acknowledgements**

The authors would like to thank our global partner zoological institutions—Smithsonian’s National Zoological Park, the Memphis Zoo, Zoo Atlanta, the Zoological Society of London Zoo, and the Chengdu Research Base of Giant Panda Breeding—for the collection of biological material and providing access to their samples. A multitude of staff and interns—including Lauren Helgen, Gracia Syed, Melanie O’Day, Kristin Stewardson, Tabitha Viner, and Beya Hull—also provided valuable assistance with this project, for which the authors are very grateful. This project was made possible with support from David M. Rubenstein and family, Judy and John W. McCarter, Jr., and the Smithsonian Institution. Generous support was also provided by the Morris Animal Foundation and Dennis and Connie Keller through our training partnership.

**Compliance with Ethical Standards**

**Conflict of Interest** The authors declare no conflict of interest.

**Appendix**

List of all GenBank sequences used in comparative analysis in Figure 1.

- HQ224959.1 Haemogregarina balli clone SAS_1 18S ribosomal RNA gene, partial sequence
- HQ224958.1 Dactylosoma ranarum clone 1B1_6 18S ribo-
Hepatozoon canis, partial sequence
HQ224957.1 Dactylosoma ranarum clone 1A2_2 18S ribosomal RNA gene, partial sequence
EF622096.1 Hepatozoon canis isolate Pelotas 1 18S ribosomal RNA gene, partial sequence
AY461376.2 Hepatozoon canis isolate Curupira 1 18S ribosomal RNA gene, partial sequence
DQ439541.1 Hepatozoon canis isolate Spain 4 18S ribosomal RNA gene, partial sequence
LC331054.1 Hepatozoon canis gene for 18S ribosomal RNA, partial sequence, clone: MoM24
KX712128.1 Hepatozoon canis isolate 3470 18S ribosomal RNA gene, partial sequence
KX712124.1 Hepatozoon canis isolate 1 18S ribosomal RNA gene, partial sequence
KX712127.1 Hepatozoon canis isolate 2734 18S ribosomal RNA gene, partial sequence
KX712125.1 Hepatozoon canis isolate 2480 18S ribosomal RNA gene, partial sequence
KX712129.1 Hepatozoon canis isolate 3474 18S ribosomal RNA gene, partial sequence
AY150067.2 Hepatozoon canis isolate Spain-1 18S ribosomal RNA gene, partial sequence
AY461375.2 Hepatozoon canis isolate Curupira 3 18S ribosomal RNA gene, partial sequence
KX712123.1 Hepatozoon canis isolate 939 18S ribosomal RNA gene, partial sequence
LC169075.2 Hepatozoon canis gene for 18S ribosomal RNA, partial sequence, isolate: MT208
KX712126.1 Hepatozoon canis isolate 2733 18S ribosomal RNA gene, partial sequence
DQ439540.1 Hepatozoon canis isolate Venezuela 2 18S ribosomal RNA gene, partial sequence
LC331053.1 Hepatozoon canis gene for 18S ribosomal RNA, partial sequence, clone: LuM2
AY461378.2 Hepatozoon canis isolate Spain 2 18S ribosomal RNA gene, partial sequence
AY731062.1 Hepatozoon canis isolate Spain 3 18S ribosomal RNA gene, partial sequence
KC138531.2 Hepatozoon canis clone 9617 18S ribosomal RNA gene, partial sequence
KC138532.2 Hepatozoon canis clone 9618 18S ribosomal RNA gene, partial sequence
DQ111754.1 Hepatozoon canis isolate Dog-26 18S ribosomal RNA gene, partial sequence
LC331052.1 Hepatozoon canis gene for 18S ribosomal RNA, partial sequence, clone: ZD7
KU893120.1 Hepatozoon canis isolate fox 3-2 18S ribosomal RNA gene, partial sequence
KU893124.1 Hepatozoon canis isolate fox 9 18S ribosomal RNA gene, partial sequence
KU893127.1 Hepatozoon canis isolate dog 4 18S ribosomal RNA gene, partial sequence
KU893121.1 Hepatozoon canis isolate fox 4-2 18S ribosomal RNA gene, partial sequence
KU893126.1 Hepatozoon canis isolate dog 3 18S ribosomal RNA gene, partial sequence
KU893123.1 Hepatozoon canis isolate fox 6 18S ribosomal RNA gene, partial sequence
KU893119.1 Hepatozoon canis isolate fox 2-2 18S ribosomal RNA gene, partial sequence
KU893118.1 Hepatozoon canis isolate fox 1-2 18S ribosomal RNA gene, partial sequence
KU893125.1 Hepatozoon canis isolate fox 33 18S ribosomal RNA gene, partial sequence
KU893122.1 Hepatozoon canis isolate fox 5-2 18S ribosomal RNA gene, partial sequence
HQ829429.1 Hepatozoo ursi isolate LaCONES/Indian sloth bear 01 18S ribosomal RNA gene, partial sequence
EU041718.1 Hepatozoo ursi isolate Gifu 2 18S ribosomal RNA gene, partial sequence
HQ829434.1 Hepatozoo ursi isolate LaCONES/Indian sloth bear 06 18S ribosomal RNA gene, partial sequence
HQ829430.1 Hepatozoo ursi isolate LaCONES/Indian sloth bear 02 18S ribosomal RNA gene, partial sequence
EU041717.1 Hepatozoo ursi isolate Gifu 1 18S ribosomal RNA gene, partial sequence
AF176836.1 Hepatozoo americanum 18S ribosomal RNA gene, partial sequence
AY461377.2 Hepatozoo sp. - Curupira 2 18S ribosomal RNA gene, partial sequence
KC127679.1 Hepatozoo sp. F3 18S ribosomal RNA gene, partial sequence
LC169077.2 Hepatozoo sp. 235 gene for 18S ribosomal RNA gene, partial sequence
LC169077.2 Hepatozoo sp. 235 gene for 18S ribosomal RNA gene, partial sequence
LC169076.2 Hepatozoo sp. MT456 gene for 18S ribosomal RNA gene, partial sequence
EF222257.1 Hepatozoo sp. European pine marten 1 18S ribosomal RNA gene, partial sequence
KU198330.1 Hepatozoo sp. badger isolate 04/00284 18S ribosomal RNA gene, partial sequence
MG136688.1 Hepatozoo martis isolate 446/17 18S ribosomal RNA gene, partial sequence
MG136687.1 Hepatozoo martis isolate 197/16 18S ribosomal RNA gene, partial sequence
MK645858 Hepatozoo (panda) 113607 MeiXiang
References

Allen KE, Johnson EM, Little SE (2011) Hepatozoon spp infections in the United States. Veterinary Clinics of North America: Small Animal Practice 41:1221–1238

André MR, Adania CH, Teixeira RHF, Vargas GH, Falcade M, Sousa L, Salles AR, Allegretti SM, Felippe PAN, Machado RZ (2010) Molecular detection of Hepatozoon spp. in Brazilian and exotic wild carnivores. Veterinary Parasitology 173:134–138. https://doi.org/10.1016/j.vetpar.2010.06.014

Baneth G, Mathew JS, Macintire DK, Barta JR, Ewing SA (2003) Canine hepatozoonosis: two disease syndromes caused by separate Hepatozoon spp. Trends in Parasitology 19(1):27–31. https://doi.org/10.1016/s1471-4922(02)00016-8

Barton CL, Russo EA, Craig TM, Green RW (1985) Canine hepatozoonosis: A retrospective study of 15 naturally occurring cases. Journal of the American Animal Hospital Association 21(1):125–134

Best A, White A, Boots M (2014) The coevolutionary implications of host tolerance. Evolution 68(5):1426–1435

Clark KA, Robinson RM, Weishuhn LL, Galvin TJ, Horvath K (1973) Hepatozoon procyonis infections in Texas. Journal of Wildlife Diseases 9:182–193

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest2: more models, new heuristics and parallel computing. Nature Methods 9(8):772

De Castro F, Bolkert B (2005) Mechanisms of disease-induced extinction. Ecology Letters 8:117–126

East ML, Wibbelt G, Lieckfeldt D, Ludwig A, Goller K, Wilhelm K, Scharcs G, Thierer D, Hofer H (2008) A Hepatozoon species genetically distinct from H. canis infecting spotted hyenas in the Serengeti ecosystem, Tanzania. Journal of Wildlife Diseases 44(1):45–52

Feng N, Yu Y, Wang T, Wilker P, Wang J, Li Y, Sun Z, Gao Y, Xia X (2015) Fatal canine distemper virus infection of giant pandas in China. Scientific Reports 6:27518. https://doi.org/10.1038/srep27518

Guindon S, Gascuel O (2003) A simple, fast, and accurate method to estimate large phylogenies by maximum likelihood. Systematic Biology 52(5):696–704

Hodžića A, Alić A, Beck R, Beck A, Huber D, Otranto D, Baneth G, Duschera G (2018) Hepatozoon martis n. sp. (Adeleorina: Hepatozoidae): Morphological and pathological features of a Hepatozoon species infecting martens (family Mustelidae).Ticks and Tick-borne Diseases 9(4):912–920

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28(12):1647–1649

Kocan AA, Cummings CA, Panciera RJ, Mathew JS, Ewing SA, Barker RW (2000) Naturally occurring and experimentally transmitted Hepatozoon americanum in coyotes from Oklahoma. Journal of Wildlife Diseases 36(1):149–153

Kubo M, Jeong A, Kim S, Kim Y, Lee H, Kimura J, Yanai T (2010) The first report of Hepatozoon species infection in leopard cats (Prionailurus bengalensis) in Korea. The Journal of Parasitology 96(2):437–439

Kubo M, Miyoshi N, Yasuda N (2006) Hepatozoonosis in two species of Japanese wild cat. The Journal of Veterinary Medical Science 68(8):833–837

Kubo M, Uni S, Agatsuma T, Nagataki M, Panciera RJ, Tsubota T, Kubo M, Miyoshi N, Yasuda N (2006) Hepatozoonosis in two naturally occurring cases. Journal of the Veterinary Medical Association 21(1):125–134

McCully RM, Basson PA, Bigalke RD, De Vos V, Young E (1975) Observations on naturally acquired hepatozoonosis of carnivores and dogs in the Republic of South Africa. Onderstepoort Journal of Veterinary Research 42(4):117–134

Merrer SH, Jones LP, Rappole JH, Twedt D, Laack LL, Craig TM (1988) Hepatozoon sp. in wild carnivores in Texas. Journal of Wildlife Diseases 24(3):574–576

Murata T, Inoue M, Tateyama S, Taura Y, Nakama S (1993) Vertical transmission of Hepatozoon canis in dogs. Journal of Veterinary Medical Science 55(5):867–868

Otranto D, Dantas-Torres F, Weigl S, Latrofa MS, Stannack D, Decaparrias D, Capelli G, Baneth G (2011) Diagnosis of Hepatozoon canis in young dogs by cytology and PCR. Parasites and Vectors 4:55. https://doi.org/10.1186/1756-3305-4-55

Pawar RM, Poornachandar A, Arun AS, Manikandan S, Shivaji S (2011) Molecular prevalence and characterization of Hepatozoon ursi infection in Indian sloth bears (Melursus ursinus). Veterinary Parasitology 182:329–332

Pawar RM, Poornachandar A, Srinivas P, Rao KR, Lakshminikan U, Shivaji S (2012) Molecular characterization of Hepatozoon spp. infection in endangered Indian wild felids and canids. Veterinary Parasitology 186:475–479

Qiu X, Mainka SA (1993) Review of mortality of the giant panda (Ailuropoda melanoleuca). Journal of Zoo and Wildlife Medicine 24(4):425–429
Scopel KKG, Fontes CJF, Nunes AC, De Fátima Horta M, Braga EM (2004) Low sensitivity of nested PCR using *Plasmodium* DNA extracted from stained thick blood smears: an epidemiological retrospective study among subjects with low parasitemia in an endemic area of the Brazilian Amazon region. *Malaria Journal* 3:8. https://doi.org/10.1186/1475-2875-3-8

Shavey CA, Morado JF (2012) DNA extraction from archived Giemsa-stained blood smears using polymerase chain reaction to detect host and parasitic DNA. *Journal of Histotechnology* 35(30):105–109

Simposon VR, Hargreaves J, Butler HM, Davison NJ, Everest DJ (2013) Causes of mortality and pathological lesions observed post-mortem in red squirrels (*Sciurus vulgaris*) in Great Britain. *BioMed Central Veterinary Research* 9:229. https://doi.org/10.1186/1746-6148-9-229

Smith KF, Sax DF, Lafferty KD (2006) Evidence for the role of infectious disease in species extinction and endangerment. *Conservation Biology* 20(5):1349–1357

Smith TG (1996) The genus *Hepatozoon* (Apicomplexa: Adeleina). *The Journal of Parasitology* 82(4):565–585

Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30(9):1312–1313

Swaisgood R, Wang D, Wei F (2016) *Ailuropoda melanoleuca* (errata version published in 2017). The IUCN Red List of Threatened Species 2016. Available: http://www.iucnredlist.org/details/712/0 [accessed on 26 July 2018]

Wei F, Song M, Liu H, Wang B, Wang S, Wang Z, Ma H, Li Z, Zeng Z, Qian J, Liu Q (2016) Molecular detection and characterization of zoonotic and veterinary pathogens in ticks from northeastern China. *Frontiers in Microbiology* 7:1913. https://doi.org/10.3389/fmicb.2016.01913

Williams CL, Dill-McFarland KA, Vandewege MW, Sparks DL, Willard ST, Kouba AJ, Suen G, Brown AE (2016) Dietary shifts may trigger dysbiosis and mucous stools in giant pandas (*Ailuropoda melanoleuca*). *Frontiers in Microbiology* 7:661. https://doi.org/10.3389/fmicb.2016.00661

Xu D, Zhang J, Shi Z, Song C, Zheng X, Zhang Y, Hao Y, Dong H, Wei L, El-Mahallawy HS, Kelly P, Xiong W, Wang H, Li J, Zhang X, Gu J, Wang C (2015) Molecular detection of vector-borne agents in dogs from ten provinces of China. *Parasites and Vectors* 8:301. https://doi.org/10.1186/s12377-015-1120-y

Zhang J, Daszak P, Huang H, Yang G, Kilpatrick AM, Zhang S (2008) Parasite threat to panda conservation. *EcoHealth* 5:6–9. https://doi.org/10.1007/s10393-007-0139-8