

RESEARCH

Open Access



A new *Eimeria* coccidian species (Apicomplexa: Eimeriidae) from Père David's deer (*Elaphurus davidianus* Milne-Edwards, 1866) in Dafeng Milu National Nature Reserve in Jiangsu Province, eastern China

Weimin Cai^{1,2,3}, Zeyang Suding^{1,2,3}, Lele Wang^{1,2,3}, Zhaofeng Hou^{1,2,3}, Dandan Liu^{1,2,3}, Siyang Huang^{1,2,3}, Jinjun Xu^{1,2,3} and Jianping Tao^{1,2,3*}

Abstract

Background: *Eimeria* coccidiosis is a significant intestinal parasitic disease, which can lead to weight loss, disease and even death of many animals. At present, there is no information about the prevalence of *Eimeria* among the world's endangered species of Père David's deer (*Elaphurus davidianus*). Therefore, the purpose of this study is to identify an unknown *Eimeria* genus in the Père David's deer in Dafeng Milu National Nature Reserve, China.

Results: A new *Eimeria* species is described from Père David's deer. Sporulated oocysts ($n = 54$) are pyriform, with a rough, yellowish brown, 2-layered oocyst wall (2.5 μm thick). A numerous small granules are dispersed randomly on the wall. Oocysts measured 41.2 (39.2–42.8) $\mu\text{m} \times 29.5$ (27.9–30.5) μm , oocyst length/width (L/W) ratio, 1.4. Oocyst residuum, a polar granule and a polar cap are absent. The micropyle (3.5 μm wide) is present. Sporocysts are spindle shaped, 18.2 (16.5–20.0) $\mu\text{m} \times 10.5$ (9.8–11.9) μm , sporocyst L/W ratio, 1.7 (1.5–1.9). A thin convex Stieda body is present and the sporocyst residuum is composed of numerous small granules less than 2.0 μm in diameter dispersed randomly. Each sporocyst contained 2 comma-shaped sporozoites in head-to-tail arrangement. A nucleus is located immediately anterior to the posterior, strong refractive and subspherical refractile body (~8 μm). Molecular analysis was conducted at the 18S, ITS-1 and COI loci.

Conclusion: Based on the morphological and molecular data, this isolate is a new species of coccidian parasite, which is named *Eimeria davidianusi* after its host, the Père David's deer (*Elaphurus davidianus*).

Keywords: Père David's deer, Morphology, *Eimeria davidianusi*, Phylogenetics

Introduction

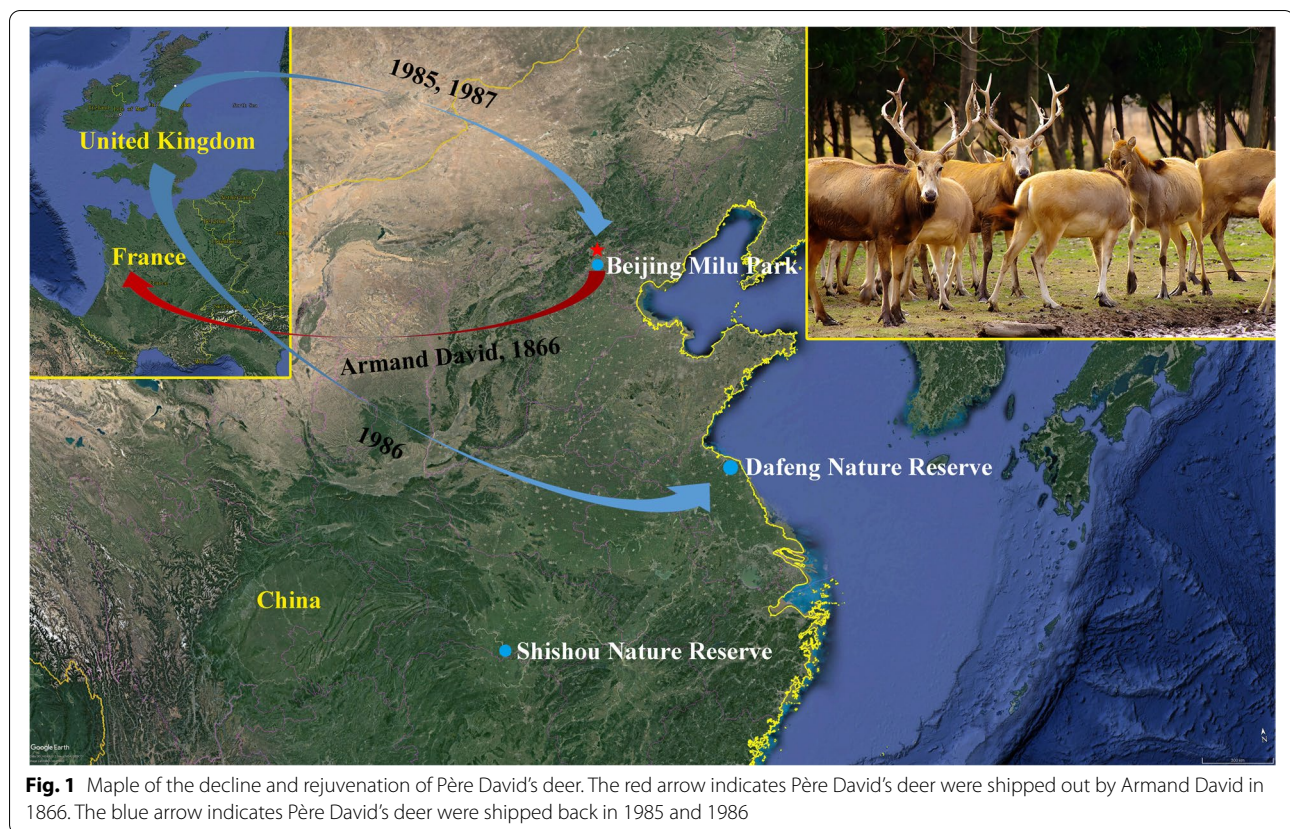
Milu (*Elaphurus davidianus*), also known as Père David's deer, is a species endemic to China, where it is named “four unlike” because it has a head like a horse, a horn like a deer, a hoof like an ox, and a tail like a donkey [1]. Père David's deer, originated from the early Pleistocene about 2 million years ago according to the fossils of antlers and bones excavated now [2, 3], are endangered animals in

*Correspondence: yzjptao@126.com

³ Jiangsu Key Laboratory of Zoonosis, Yangzhou University, Yangzhou 225009, China

Full list of author information is available at the end of the article





the world [4], and belongs to the genus *Elaphurus* in the family Cervidae [3]. Judging from the historical records of human beings, the Père David's deer was first seen in the relevant chapters of Mencius of the Zhou Dynasty [5]. Ever since Armand David, a French missionary, first transported Père David's deer to Europe in 1866, until the early twentieth century, the Père David's deer population was declared extinct in China [6]. The first reintroduction of 38 Père David's deer into China have consisted of 2 donations from the Woburn Abbey herd of England. A herd of 20 (5 males, 15 females) in 1985 followed by a herd of 18 female deer in 1987, and both of these herds went to the Beijing Milu Park [3]. In 1986, an additional 39 deer, chosen from 5 zoological gardens in the UK, were given to the Dafeng Milu National Nature Reserve [7], near the Yellow Sea (Fig.1). Today, more than 8000 Père David's deer are raised in parts of China [8], especially on our nature reserves. According to the report, bacteria [2, 9–11], viruses [12, 13] and parasites such as helminth [14, 15] and protozoa [16–20] are a potential threat to the survival of the Père David's deer. In order to better protect Père David's deer, we collected some feces of Père David's deer for examination, and found an *Eimeria* that had never been described.

More than 1800 species of *Eimeria* have been identified all over the world [21] since Leeuwenhoek found *Eimeria stiedai* in rabbit bile in 1674. Although some studies have been carried out on deer coccidia [22–28], there are few reports on Père David's deer coccidia so far. At the same time, due to the incomplete description and lack of measurement of many *Eimeria* spp. in the deer family in the past, it is difficult to verify the existing species. As a result of these difficulties, molecular tools [29–34] are essential to accurately delimit species and infer phylogenetic relationships among *Eimeria* species. In the present study, we aimed to: morphologically describe and genetically characterize a novel observed species of *Eimeria* as *Eimeria davidianusi* n. sp. isolate in Père David's deer (*Elaphurus davidianus*).

Results

Description

SO ($n=54$) are pyriform, with a yellowish brown, rough and projecting punctate, 2-layered oocyst wall (2.5 thick). Oocysts measured $41.2 (39.2-42.8) \times 29.5 (26.5-30.6)$, oocyst length/width (L/W) ratio, 1.4. OR, PG and PC are absent. The M (3.5 wide) is present. SC are spindle shaped, $18.2 (16.5-20.0) \times 10.5 (9.8-11.9)$,

Table 1 Comparative morphology of *E. davidianusi* from Père David's deer with other *Eimeria* species recorded from ruminants and the pig

Species	Hosts	Oocyst				Sporocysts						References		
		Shape	Size (µm)	Shape Index	Wall (thick/µm)	Micropyle (wide/µm)	Polar Granule	Oocyst Residium	Shape	Size (µm)	Shape Index		Stieda Body	Sporocyst Residium
<i>E. bukidnonensis</i>	Cattle	Pyriform	47.4 × 33 (43–51 × 30–35)	1.44	2-layered (3.5)	Present	Absent	Absent	Elongate	19.6 × 9.8 (18–21 × 9–11)	2.00	Present	Absent	Courtney et al., 1976 [35]
<i>E. wyomin-gensis</i>	Cattle	Pyriform	39.9 × 28.3 (36–44 × 26–30)	1.41	2-layered (2.5)	Present	Absent	Absent	Elongate	18.7 × 8.6 (17–20 × 8–10)	2.17	Present	Absent	Courtney et al., 1976 [35]
<i>E. alabamensis</i>	Cattle	Pyriform	19.5 × 14.3 (16–25 × 12–17)	1.36	1-layered (0.5)	Absent	N/A	Absent	Elongate	8.2 × 3.8	2.16	Tiny	Tiny	Christensen, 1941 [36]
<i>E. sub-spherica</i>	Cattle	Subspheri-cal	11.5 × 10.6 (9–13 × 8–12)	1.08	N/A	Absent	N/A	Absent	Elongate	8.1 × 3.6	2.25	Tiny	Absent	Christensen, 1941 [36]
<i>E. macu-sanien-siensis</i>	Alpaca	Ovoid to pyriform	93.6 × 67.4 (81–107 × 61–80)	1.39	3-layered (8–12)	Prominent (9–14)	Absent	Absent	Elongate to ovoid	36.3 × 18.3 (33–40 × 16–20)	1.98	Faintly per-ceptible	Faintly per-ceptible	Guerrero et al., 1971 [37]
<i>E. intricata</i>	Sheep	Ellipsoidal to ovoid	50 × 38 (39–59 × 27–47)	1.32	2-layered (2.4–3.8)	Present	Present	Absent	Elongate to ovoid	17–22 × 9–14	N/A	Absent	Present	Hao, 2017 [38]
<i>E. mayeri</i>	Reindeer	Spheroidal to ellipsoidal	17.2 × 14.1 (9–21 × 9–16)	1.22	2-layered (1)	Present (<1)	Absent	Absent	Ovoid	9.3 × 5.1 (8–11 × 4–6)	1.82	Nipple-like	Incon-spicuous	Gudmunds-dottir and Skirnisson, 2005 [39]
<i>E. rangiferis</i>	Reindeer	Ovoid	34.9 × 27.6 (31–38 × 25–30)	1.26	2-layered (1.8–2.0)	Present (4–6)	Absent	Absent	Spindle shaped	18.6 × 9.2 (17–20 × 8.0–10)	2.02	Nipple-like	Present	Gudmunds-dottir and Skirnisson, 2005 [39]
<i>E. hreindyria</i>	Reindeer	Ellipsoidal	30.0 × 21.1 (24–35 × 18–23)	1.42	2-layered (0.8–1.2)	Absent	Present	Absent	Spindle-shaped	15.3 × 6.5 (13–18 × 6–8)	2.35	Present	Present	Gudmunds-dottir and Skirnisson, 2006 [40]
<i>E. alces</i>	Elk	Ovoid	38.0 × 26.1 (33–43 × 24–29)	1.46	2-layered (2.2)	Distinct (~7)	Present	Absent	Ovoid to elongate	18.6 × 8.9 (17.0–21 × 8–10)	2.09	Present	Absent	Pyziel and Demiaszk-iewicz, 2013 [24]
<i>E. cervi</i>	Sika deer	Pyriform	35.2 × 25.1 (26–39 × 20–29)	1.40	2-layered (0.6–1.0)	Prominent (4.2)	Absent	Absent	Long oval	18.2 × 9.7	N/A	Present	Present	Lu, 1995 [41]
<i>E. robusta</i>	Sika deer	Ovoid	35.3 × 26.1 (27–41 × 21–30)	1.35	2-layered (0.6–1.1)	Prominent (5.8)	Absent	Absent	Long oval	18.2 × 9.6	N/A	Present	Present	Lu, 1995 [41]

Table 1 (continued)

Species	Hosts	Oocyst					Sporocysts					References		
		Shape	Size (µm)	Shape Index	Wall (thick/µm)	Micropyle (wide/µm)	Polar Granule	Oocyst Residuum	Shape	Size (µm)	Shape Index		Stieda Body	Sporocyst Residuum
<i>E. sordida</i>	Sika deer	Ellipsoidal to ovoid	34.3 × 25.6 (30–38 × 21–28)	1.34	2-layered (0.4–1.1)	Prominent (5.8)	Absent	Absent	Long oval	20.1 × 8.9	N/A	Present	Present	Lu, 1995 [41]
<i>E. austriaca</i>	Sika deer	Ellipsoidal to ovoid	23.3 × 20.0 (19–26 × 15–23)	1.16	layered (0.4–1.1)	Absent	Absent	Absent	Long oval	10.7 × 6.4	N/A	Absent	Absent	Lu, 1995 [41]
<i>E. sp-1</i>	Sika deer	Subspherical	28.0 × 26.3 (24–31 × 21–30)	1.07	2-layered (0.3–1.1)	Absent	Absent/only one	Absent	ovoid	15.0 × 8.6	N/A	Present	Present	Lu, 1995 [41]
<i>E. sp-2</i>	Sika deer	Ellipsoidal	33.4 × 25.7 (27–38 × 20–28)	1.30	2-layered (0.5–1.0)	Absent	Absent	Absent	Long oval	19.1 × 10.0	N/A	Absent	Present	Lu, 1995 [41]
<i>E. scabra</i>	Pig	Ovoid to subspherical	25–45 × 18–28	N/A	2-layered (1.5–3)	Present	Present	Absent	Ovoid	14–18 × 7–9	N/A	Prominent	Present	Levine, 1985 [42]
<i>E. davidianusi</i>	Père David's deer	Pyrriform	41.2 × 29.5 (39–43 × 26–31)	1.40	2-layered (1.5–2.9)	Prominent (2.8–4.0)	Absent	Absent	Spindle shaped	18.2 × 10.5 (16–20 × 10–12)	1.73	Present	Present	Present study

SC L/W ratio, 1.7 (1.5–1.9). A tiny flattened SB is present, SSB is absent and the SR is composed of numerous small granules less than 2.0 in diameter dispersed centrally (Table 1). Each SC contained 2 comma-shaped SZ in head-to-tail arrangement (Fig. 2). One end of the SZ has a large spheroidal posterior refractile body and a nucleus that does not appear to be very clear, which is located in the center of the SZ. A more concise picture of the oocyst pattern is shown in Fig. 3.

Host: Père David's deer (*Elaphurus davidianus*, Milne-Edwards, 1866).

Locality: Dafeng Milu National Nature Reserve (32°56'–33°36' N ~ 120°42'–120°51' E), eastern China.

Prevalence: 1 / 1(100%).

Other hosts: Unknown.

Prepatent period: Unknown.

Patent period: Unknown.

Site of infection: Unknown.

Sporulation time: 120–144 h.

Material deposited: the 18S, ITS-1 and COI sequences were submitted to GenBank, and the accession number were MT822711, MT822712, and MT822713.

Etymology: This species is named *Eimeria davidianusi* after its host.

Phylogenetic analysis of *E. davidianusi* at the 18S locus

At the 18S rRNA locus, a 1380bp PCR product of *E. davidianusi* isolate was successfully amplified and sequenced. Phylogenetic analysis of *E. davidianusi* isolate at this locus using Distance, ML and NJ analyses produced similar results (Fig. 3). There are no 18S rRNA sequences from *Eimeria* derived from Père David's deer available in GenBank, therefore phylogenetic analysis could only be conducted using available *Eimeria* 18S rRNA sequences. *Eimeria davidianusi* grouped in a separate clade. It shared 97.76 and 97.69% genetic similarity with *E. alabamensis* (Christensen, 1941) (AB769556) and *E. bukidnonensis* (Tubangui, 1931) (AB769597)

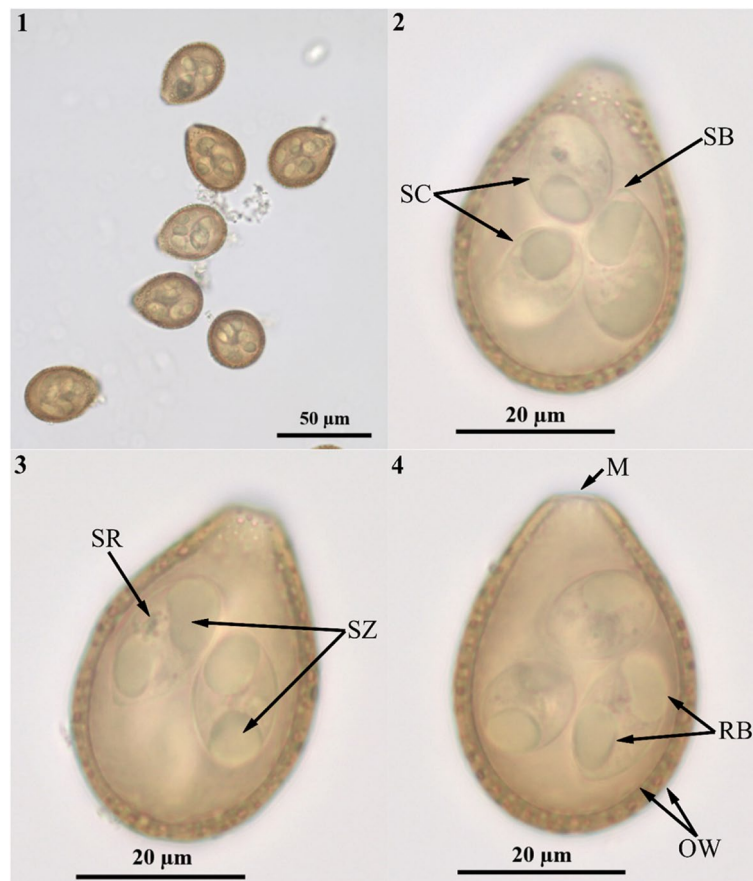
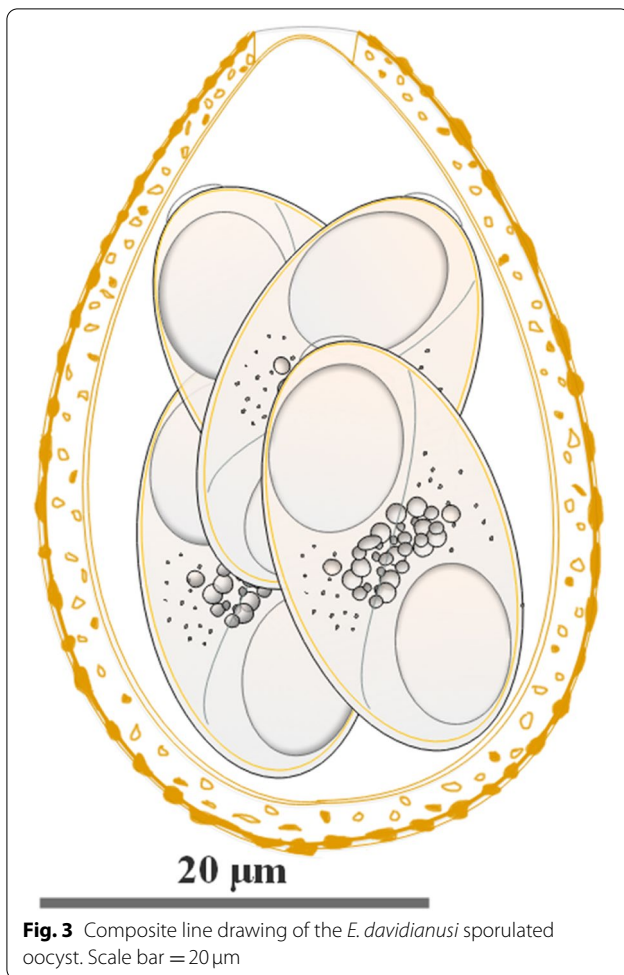


Fig. 2 Photomicrographs of the *E. davidianusi* isolate oocysts. 1, 2 and 3 are the visual field of the *E. davidianusi* isolate oocysts under different magnification lenses, respectively. 4, 5 and 6 are the same oocyst's field of vision under a 100 oil immersion objective. (1 = 10 × objective; 2 = 40 × objective; 3 = 100 × objective; SC = sporocyst; SB = Stieda body; SR = sporocyst residuum; SZ = sporozoties; M = micropyle; RB = refractile body; OW = oocyst wall)



from cattle in Japan (Fig. 3). It exhibited 97.47% genetic similarity to *E. faurei* (Moussu and Marotel, 1901) (AF345998), which was identified from a sheep from Turkey. *Toxoplasma gondii* (Lave ran, 1900) (AY488166) was used as an outgroup (Fig. 4).

Phylogenetic analysis of *E. davidianusi* at the ITS-1 locus

A 328bp sequence of ITS-1 from *E. davidianusi* was used for phylogenetic analysis. There are no ITS-1 sequences from *Eimeria* derived from Père David's deer available in GenBank, therefore phylogenetic analysis could only be conducted using available *Eimeria* ITS-1 rRNA sequences. *Toxoplasma gondii* (AJ628254) was used as an outgroup. Phylogenetic analysis grouped the *E. davidianusi* isolate in a separate clade and shared 97.50 and 96.38% genetic similarity with *E. bukidnonensis* (AB769599) and *E. subspherica* (Christensen, 1941) (AB769642) from cattle in Japan (Fig. 5).

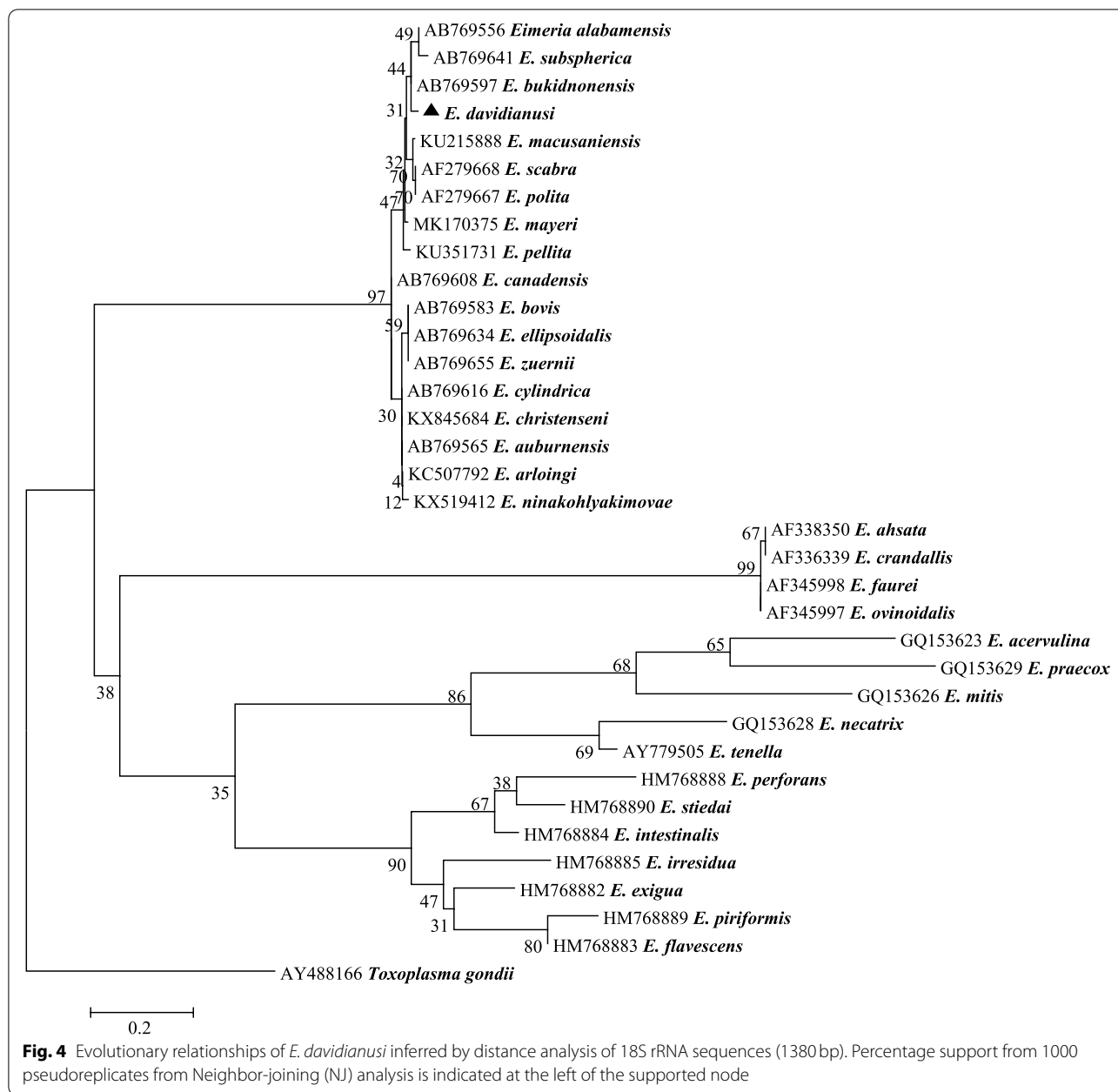
Phylogenetic analysis of *E. davidianusi* at the COI locus

Phylogenetic analysis of the 786bp COI sequence placed *E. davidianusi* in a clade with *E. bukidnonensis* (KU351700) and *E. alabamensis* (KU351690, KT184376) (50.0% similarity). There are no COI sequences from *Eimeria* derived from Père David's deer available in GenBank, therefore phylogenetic analysis could only be conducted using available *Eimeria* COI sequences. It exhibited 90.29% genetic similarity to *E. bukidnonensis* (KU351700), which was identified from cattle from Turkey. *Toxoplasma gondii* (KM657810) was used as an outgroup (Fig. 6).

Discussion

Traditionally, identification of *Eimeria* species has been based largely on sporulated oocyst morphology and some biological characteristics such as pathological changes, incubation period, sporulation time [22–25]. In practice, a small portion of oocysts may differ from the expected typical morphology, and all these biological characteristics can present a variable level of overlap, making it difficult in some cases to identify *Eimeria* species accurately. In view of the limitations of microscopic identification, molecular techniques have been developed as a method of detection and specific identification of species of the genus *Eimeria* [43–47]. For this reason, we used three genes (18S rRNA, ITS-1 and COI) as molecular markers to ensure the accuracy of identification and phylogeny of the new *Eimeria* species in this paper.

The family Cervidae within the Artiodactyla includes 19 genera with 51 species of deer (<http://www.departments.bucknell.edu/biology/resources/msw3/browse.asp?s=y&id=14200205>). Based on the mitochondrial and nuclear phylogenies of Cervidae, *Elaphurus* is most closely related to the genus *Cervus* [48]. Currently, 37 *Eimeria* species have been named from the family Cervidae, of which 11 species, including *E. elaphi* (Jansen and van Haaften, 1966), *E. austriaca* (Supperer and Kutzer, 1961), *E. wapiti* (Honess, 1955), *E. zuernii* (Rivolta, 1878), *E. sordida* (Supperer and Kutzer, 1961), *E. cervi* (Galli-Valerio, 1927), *E. robusta* (Supperer and Kutzer, 1961), *E. asymmetrica* (Supperer and Kutzer, 1961), *E. gallivalerioi* (Rastegaieff, 1930), *E. hegneri* (Rastegaieff, 1930) and *E. schoenbuchi* (Boch, 1963) were described from genus *Cervus* (<https://www.k-state.edu/parasitology/worldcoccidia/CERVIDAE>). In China, 6 *Eimeria* species, including *E. austriaca*, *E. cervi*, *E. robusta*, *E. sordida* and two indeterminate species had been reported from sika deer [41], but their morphology is different from this new species (Table 1). The new species, *Eimeria davidianusi*, represents the first coccidian species described from the Père David's deer.



The oocyst morphology of *E. davidianusi* is very similar to that of *E. bukidnonensis*, a species of bovine coccidia. The oocysts of *E. bukidnonensis* were pyriform and measured 47.4 (43–51) × 33.0 (30–35); sporocysts were 19.6 (18–21) × 9.8 (9–11). M is present (about 3–5 in diameter) and PG, OR, and SR are absent. Oocyst wall of 2 layers, 3.5 thick, and dark brown [35]. In phylogenetic relationships of two species, the pairwise genetic distance of *E. davidianusi* and *E. bukidnonensis* at the 18S rRNA, ITS-1 and COI locus is 97.69, 97.50 and 90.29%, respectively, and phylogenetic analysis revealed that *E.*

davidianusi was closely to *E. bukidnonensis*. Therefore, we speculated that *E. davidianusi* and *E. bukidnonensis* may evolve from a common ancestor that parasitized some ancient ancestor of deer and cattle, thus co-speciating with their respective hosts, while still maintaining plesiomorphic features. Surprisingly, the existing research results suggest that Père David’s deer and cattle may have a common ancestor, such as ancient deer or ancient cattle [49].

Similar to oocysts of *E. davidianusi*, *E. wyomingensis* (Huizinga and Winger, 1942) from cattle, *E. intricata*

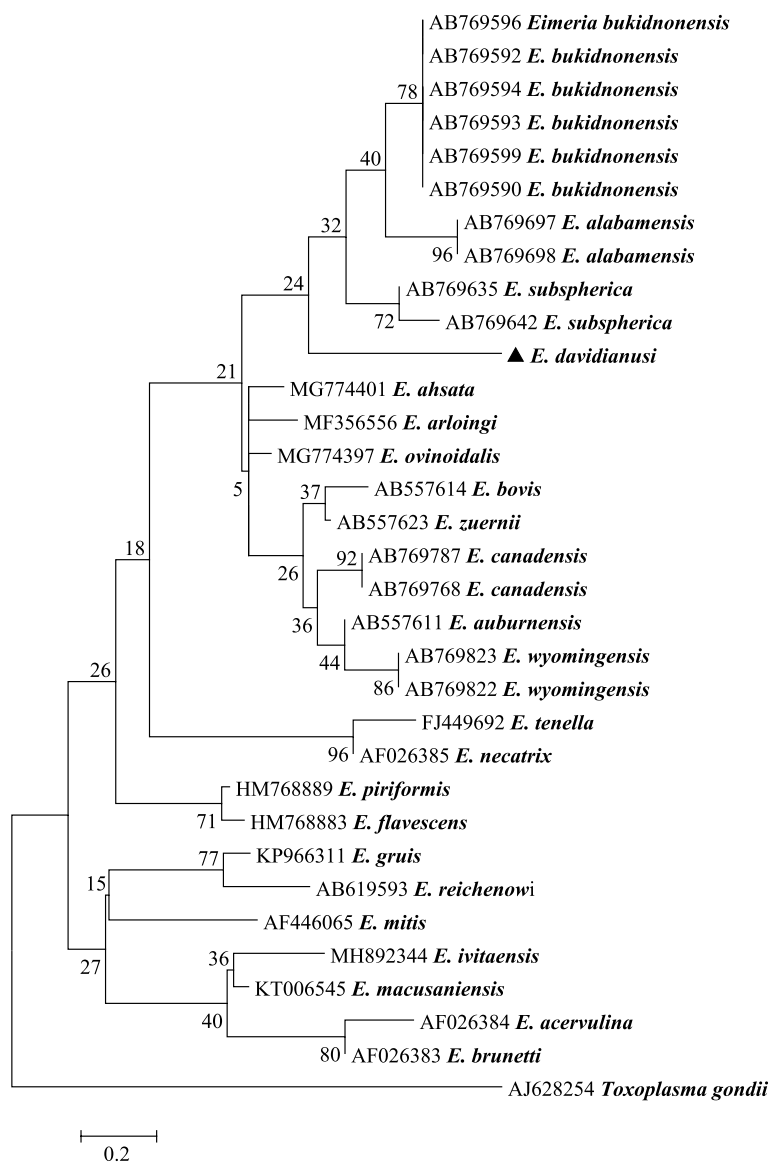


Fig. 5 Evolutionary relationships of *E. davidianusi* inferred by distance analysis of ITS-1 sequences (328 bp). Percentage support from 1000 pseudoreplicates from Neighbor-joining (NJ) analysis is indicated at the left of the supported node

(Spiegl, 1925) from sheep, *E. macusaniensis* (Hernandez, Bazalar and Alva, 1971) from alpacas and *E. scabra* (Henry, 1931) from pigs have a rough wall and a micropyle (Table 1), but these species differ greatly from *E. davidianusi* in molecular characteristics. On the contrary, *E. alabamensis* from cattle and *E. subspherica* from cattle are similar to *E. davidianusi* in molecular characteristics but far apart in morphology (Table 1). These results are consistent with Ogedengbe’s findings [50]. Owing to no sequences of 18S, ITS-1 and COI of *Eimeria* from the family Cervidae in GenBank, the phylogenetic relationships between Cervidae coccidia and this new species couldn’t be analysed.

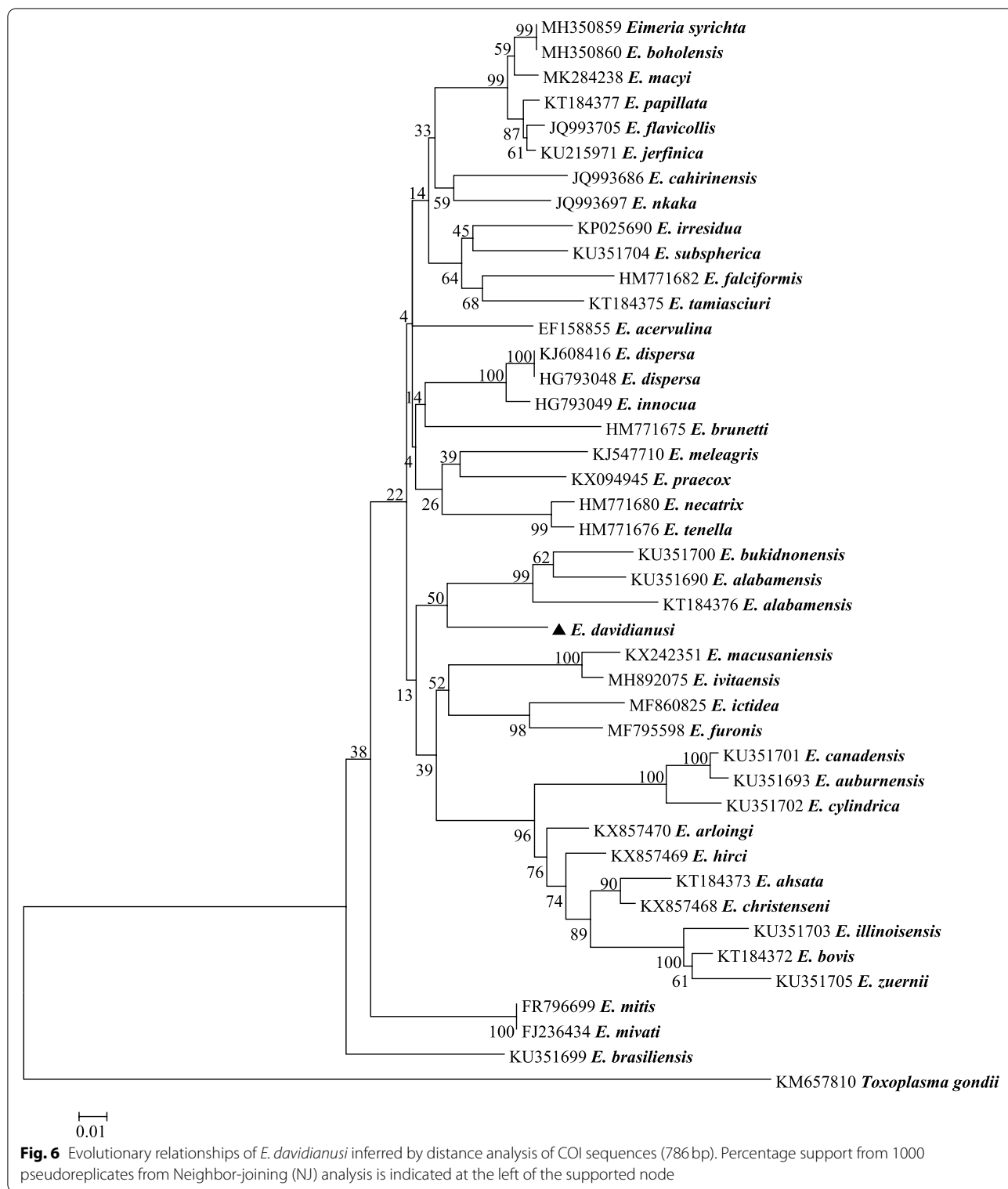
Conclusion

In summary, this is the first report of the morphological and molecular characterization of an *Eimeria* sp. in Père David’s deer worldwide. A new *Eimeria* coccidian species (Apicomplexa: Eimeriidae) from Père David’s deer in Dafeng National Nature Reserve in eastern China has been identified which is named *E. davidianusi*.

Materials and methods

Sample collection

In May 2018, a juvenile Père David’s deer occurred diarrhea in Dafeng Milu National Nature Reserve, Jianguo



Province. We collected fecal samples and stored them in an insulated field box until processed, which was no later than 5 hours after collection. Shortly after returning to the laboratory and microscopy revealed unsporulated coccidian oocysts.

Fecal flotation was conducted using a saturated sodium chloride and 50% sucrose (w/v) solution [51]. A portion of feces was placed in 2.5% (w/v) potassium dichromate solution ($K_2Cr_2O_7$) [52], mixed well and poured into Erlenmeyer flasks (250 mL) to a depth of less than 1 cm

and kept at room temperature in the dark to facilitate sporulation.

Morphological analysis

Fifty-four sporulated *Eimeria* spp. oocysts of a consistent, novel morphology (obtained from a Père David's deer) were observed using a Carl Zeiss AxioCam ICc 5 (Jena, Germany) digital microimaging camera and photographed with a 100× oil immersion objective. It was observed that there was only one species of coccidial oocysts in the fecal samples. Images were analyzed using ZEN 2012 (blue edition) software, to obtain measurements of oocyst length and width, oocyst wall thickness and sporocyst length and width. Due to the compacted nature of this species of *Eimeria*, measurements were only taken from one sporocyst per oocyst and the sporocyst was subjectively identified as being positioned laterally. Where no sporocysts could be manipulated into lateral position within the oocyst, sporocyst length measurement was not taken. The oocysts were compared with some published coccidia of Artiodactyla to observe the morphological similarities and differences [24, 35, 37–42].

DNA isolation

Six hundred oocysts were ground in liquid nitrogen for five times. Grinded oocyst fragments were transferred to a 1.5 mL centrifugal tube and the remaining operations were performed according to MiniBEST Universal Genomic DNA Extraction Kit Ver.5.0 (TaKaRa Biomed, Beijing, China) instructions for DNA extraction (<https://www.takarabiomed.com.cn/ProductShow.html>).

PCR amplification

A standard PCR with the primers E18SF and E18SR (Table 2) [47] was used for amplification of the 18S ribosomal RNA (abbreviated 18S rRNA) gene. The expected PCR product was ~1500 bp. The PCR reaction (50 µL) were performed in 1 µL (10–20 ng) of genomic DNA, 10 pM of each primer and 2.5 U Premix Taq polymerase (TaKaRa, Tokyo, Japan) in a thermocycler (Bio-Rad, CA, USA) under the following conditions: 94 °C for 4 min (initial denaturation), followed by 30 cycles of 94 °C for 60 s (denaturation), 59 °C for 45 s (annealing), 72 °C for 60 s (extension), and then a final extension of 72 °C for 10 min.

The PCR for the first internal transcribed spacer (ITS-1) locus was carried out with the primers EIF and EIR (Table 2) [53]. The expected PCR product was ~450 bp. The PCR reaction contained 2.5 U Premix

Table 2 Sequences of primers

Name of primer	Sequence (5' to 3')
For 18S rRNA	
E18SF	GAAACTGCGAATGGCTCATT
E18SR	CTTGCGCCTACTAGGCATT
For ITS1	
EIF	AAGTTGCGTAAATAGAGCCC
EIR	CAAGACATCCATTGCTGAAA
For COI	
ECOIF	GTTTGGTTGAGGTTGGTTGGAC
ECOIR	ATCCAATAACCGCACCAAGAGATA

Taq polymerase (TaKaRa, Tokyo, Japan), 10 pM of each primer and 1 µL (10–20 ng) of genomic DNA. The PCR was conducted using the following cycling conditions: 1 cycle of 94 °C for 4 min, followed by 30 cycles of 94 °C for 60 s, 56 °C for 30 s and 72 °C for 60 s and a final extension of 72 °C for 10 min.

A partial cytochrome c oxidase subunit I (COI) gene sequence was amplified using a standard PCR with the following primers ECOIF and ECOIR (Table 2) [54]. The expected PCR product was ~900 bp. The PCR reaction (50 µL) were performed in 1 µL (10–20 ng) of genomic DNA, 10 pM of each primer and 2.5 U Premix Taq polymerase (TaKaRa, Tokyo, Japan) in a thermocycler (Bio-Rad, CA, USA) under the following conditions: 94 °C for 5 min (initial denaturation), followed by 30 cycles of 94 °C for 60 s (denaturation), 50 °C for 30 s (annealing), 72 °C for 60 s (extension), and then a final extension of 72 °C for 10 min.

Sequence analysis

Samples without DNA (no-DNA controls) were included in each amplification run, and in no case were amplicons detected in the no-DNA controls. Each amplicon (10 µL) was examined by agarose (1%) gel electrophoresis, stained with ethidium bromide and photographed using a gel imaging system (Bio-Rad, CA, USA). All PCR products yielded a single band and were purified by MiniBEST DNA Fragment Purification Kit Ver.4.0 (TaKaRa, Tokyo, Japan). Purified PCR products were sent to GenScript (Nanjing, China) for sequencing from both directions by using a primer walking strategy.

The results of the sequencing reactions were analyzed and edited using DNASTAR software, compared to existing *Eimeria* sp. 18S, ITS-1 and COI sequences on GenBank using BLAST searches and aligned with reference genotypes from GenBank using Clustal W in MegAlign and MAFFT (<https://www.ebi.ac.uk/Tools/msa/mafft/>).

Phylogenetic analysis

Phylogenetic trees were constructed for *Eimeria* sp. at the 18S, ITS-1 and COI loci with additional isolates from GenBank. Parsimony analyses were conducted using MEGA (Molecular Evolutionary Genetics Analysis software, version 5, Arizona State University, Tempe, Arizona, USA). Neighbor-joining (NJ) and maximum likelihood (ML) analyses were conducted using Tamura-Nei based on the most appropriate model selection using ModelTest in MEGA 5 [55]. Bootstrap analyses were conducted using 1000 replicates to assess the reliability of inferred tree topologies.

Statistical analysis

Measurements of 54 sporulated oocysts were analyzed using Statistical Package for the Social Sciences (SPSS Version 22) and results are presented in micrometres as the mean, with the observed range in parentheses. Since all measurement units in the article are microns, all length units except those in the summary have been omitted in order to follow the standardized format.

Line drawing

The oocyst line drawing was constructed using the software of Edraw Max (<https://www.edrawsoft.cn/>).

Abbreviations

OR: oocyst residuum; PG: polar granule; SSB: sub Stieda body; 18S: 18S ribosomal RNA; ITS-1: internal transcribed spacer 1; COI: cytochrome c oxidase subunit I.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12917-022-03308-2>.

Additional file 1.

Acknowledgments

The author would like to thank the staff of Dafeng Milu National Nature Reserve for their help in collecting fecal samples, as well as their professional treatment of Père David's deer.

Authors' contributions

WMC, JPT conceived and designed the study. SYH collected the samples. WMC, ZYSD and LLW performed the laboratory analyses. ZFH, DDL and JJX analyzed the data. All authors critically appraised and interpreted the results. WMC drafted the first version of the manuscript. All authors read and approved the final version of manuscript.

Funding

This study was supported by the National Natural Science Foundation of China (31972698), and the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD).

Availability of data and materials

The datasets generated and/or analysed during the current study are available in the GenBank repository (MT822711 to MT822713). All data and additional files are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

This study was approved by the Animal Ethics Committee of Yangzhou University and the sample collection was permitted by Ethics Committee of Dafeng Milu National Nature Reserve. All Père David's deer were handled in accordance with good animal practices required by the Animal Ethics Procedures and Guidelines of the People's Republic of China.

Consent for publication

Not applicable.

Competing interests

All authors declare that they have no competing interests.

Author details

¹College of Veterinary Medicine, Yangzhou University, 12 East Wenhui Road, Yangzhou, Jiangsu 225009, People's Republic of China. ²Jiangsu Co-innovation Center for Prevention and Control of Important Animal Infectious Diseases and Zoonoses, Yangzhou University, Yangzhou 225009, China. ³Jiangsu Key Laboratory of Zoonosis, Yangzhou University, Yangzhou 225009, China.

Received: 28 January 2022 Accepted: 20 May 2022

Published online: 03 June 2022

References

- Zhang L. History of the Père David's deer. *China Nat.* 1999;6:3–4. (in Chinese).
- Zhang M, Shi M, Fan M, Xu S, Li Y, Zhang T, et al. Comparative analysis of gut microbiota changes in Père David's deer populations in Beijing Milu Park and Shishou, Hubei Province in China. *Front Microbiol.* 2018;9:1258.
- Bai J, Zhang Y, Zhong Z, Cheng Z, Cao M, Meng Y. The 35th anniversary of the reintroduction of Milu deer to China: history, population status, achievements and challenges. *Biodivers Sci.* 2021;29(2):160–6.
- Jiang Z, Harris RB. *Elaphurus davidianus*. The IUCN Red List of Threatened Species 2016, <https://doi.org/10.2305/IUCN.UK.2016-2.RLTS.T7121A22159785.en>
- Cao K. Chinese Milu. Shanghai: Xueling Press; 1990. (in Chinese).
- Wang L, Ding J, Yang Z, Chen H, Yao R, Dai Q, et al. Père David's deer gut microbiome changes across captive and translocated populations: implications for conservation. *Evol Appl.* 2019;12(3):622–35.
- Dayuan X, Yuanyuan Z, Zhibin C, Zhenyu Z, Ming C, Mengdi F, et al. Père David's deer (*Elaphurus davidianus*) in China: population dynamics and challenges. *J Resour Ecol.* 2022;13(1):41–50.
- Zhang Y, Bai J, Zhu A, Chen R, Xue D, Zhong Z, et al. Reversing extinction in China's Père David's deer. *Science.* 2021;371(6530):685.
- Lu X, Xiao X, Liu Y, Huang S, Li R, Wang Z. Widespread prevalence of plasmid-mediated colistin resistance gene *mcr-1* in *Escherichia coli* from Père David's Deer in China. *mSphere.* 2020;5(6):e01221–20.
- Zhong Z, Shan Y, Zhang L, Cheng Z, Wang L, Zhang P, Y L. Pathology observation of Père David's deer (*Elaphurus davidianus*) sudden death syndrome. *J Shanxi Agricult Univ.* 2015;35(3):290–6.
- Wang M, Zhou D, Guo D, Wen H, Li P, Guo R, Chang Y, Fang R. Investigation of Important Pathogenic Bacterial Parasites in Shishou Milu National Nature Reserve. *Chin J Wildlife.* 2018;39(1):146–50.
- Frölich K, Flach EJ. Long-term viral serology of semi-free-living and captive ungulates. *J Zoo Wildlife Med.* 1998;29(2):165–70.
- Cooley AJ, Taus NS, Li H. Development of a management program for a mixed species wildlife park following an occurrence of malignant catarrhal fever. *J Zoo Wildlife Med.* 2008;39(3):380–5.
- Goossens E, Verduyck J, Boomker J, Verdammen F, Dorny P. A 12-month survey of gastrointestinal helminth infections of cervids kept in two zoos in Belgium. *J Zoo Wildlife Med.* 2005;36(3):470–8.
- Huang SY, Gong JZ, Ren YJ, Pan M, Cai WM, Fan YM, et al. First report on the prevalence of *Fasciola hepatica* in the endangered Père David's deer (*Elaphurus davidianus*) in China. *BMC Vet Res.* 2020;16(1):473.

16. Huang SY, Fan YM, Yao N, Yang Y, Pei MY, Ren YJ, et al. Seroprevalence of toxoplasma gondii infection in the endangered Père David's deer (*Elaphurus davidianus*) in China. *Microb Pathog.* 2020;149:104282.
17. Xie F, Zhang Z, Zhao A, Jing B, Qi M, Wang R. Molecular characterization of *Cryptosporidium* and *Enterocytozoon bienewsi* in Père David's deer (*Elaphurus davidianus*) from Shishou, China. *Int J Parasitol Parasites Wildlife.* 2019;10:184–7.
18. Sedlak K, Bartova E. Seroprevalences of antibodies to *Neospora caninum* and *Toxoplasma gondii* in zoo animals. *Vet Parasitol.* 2006;136(3–4):223–31.
19. Zhang Z, Huang J, Karim MR, Zhao J, Dong H, Ai W, et al. Zoonotic *Enterocytozoon bienewsi* genotypes in pere David's deer (*Elaphurus davidianus*) in Henan, China. *Exp Parasitol.* 2015;155:46–8.
20. Moreno-Torres KI, Pomeroy LW, Moritz M, Saville W, Wolfe B, Garabed R. Host species heterogeneity in the epidemiology of *Nesopora caninum*. *PLoS One.* 2017;12(8):e0183900.
21. Duszynski DW. *Eimeria*. In: eLS. Chichester: Wiley; 2011.
22. Foreyt WJ, Lagerquist JE. Experimental infections of *Eimeria wapiti* and *E. zuernii*-like oocysts in Rocky Mountain elk (*Cervus elaphus*) calves. *J Wildl Dis.* 1994;30(3):466–9.
23. Skirnisson K, Cuyler C. A new *Eimeria* species (Protozoa: Eimeriidae) from caribou in Ameralik, West Greenland. *Parasitol Res.* 2016;115(4):1611–5.
24. Pyziel AM, Demiaszkiewicz AW. *Coccidia* (Apicomplexa: Eimeriidae) of elk (*Alces alces*) in Poland. *Parasitol Res.* 2013;112(5):2083–5.
25. Kowal J, Kornas S, Nosal P, Wajdzik M, Basiaga M, Lesiak M. Parasite infections in red deer *Cervus elaphus* from Krakow area, southern Poland. *Ann Parasitol.* 2015;61(1):49–52.
26. Kuligowska I, Demiaszkiewicz AW, Kowalczyk R. A new occurrence of *Eimeria alces* (Apicomplexa: Eimeriidae) in elk (*Alces alces*) in East Poland. *Ann Parasitol.* 2014;60(4):277–9.
27. Filip KJ, Demiaszkiewicz AW. A new occurrence of *Eimeria catubrina* (Apicomplexa: Eimeriidae) in elk (*Alces alces*) from the Kampinos Forest. *Ann Parasitol.* 2016;62(4):345–7.
28. Jokelainen P, Moroni B, Hoberg E, Oksanen A, Laaksonen S. Gastrointestinal parasites in reindeer (*Rangifer tarandus tarandus*) calves from Fennoscandia: An epidemiological study. *Vet Parasitol Regional Stud Rep.* 2019;16:100277.
29. Ogedengbe JD, Hanner RH, Barta JR. DNA barcoding identifies *Eimeria* species and contributes to the phylogenetics of coccidian parasites (Eimeriorina, Apicomplexa, Alveolata). *Int J Parasitol.* 2011;41(8):843–50.
30. Tan L, Li Y, Yang X, Ke Q, Lei W, Mughal MN, et al. Genetic diversity and drug sensitivity studies on *Eimeria tenella* field isolates from Hubei Province of China. *Parasit Vectors.* 2017;10(1):137.
31. Ekawasti F, Kitagawa K, Domae H, Wardhana AH, Shibahara T, Uni S, et al. Molecular identification of *Eimeria hestermani* and *Eimeria prionotemni* from a red-necked wallaby (*Macropodidae*; *Macropus rufogriseus*) in Japan. *Parasitol Res.* 2020;119(4):1271–9.
32. Dia A, Cheeseman IH. Single-cell genome sequencing of protozoan parasites. *Trends Parasitol.* 2021;37(9):803–14.
33. Capewell P, Krumrie S, Katzer F, Alexander CL, Weir W. Molecular epidemiology of *Giardia* infections in the genomic era. *Trends Parasitol.* 2021;37(2):142–53.
34. Bawm S, Win TZB, Win SY, Htun LL, Nakao R, Katakura K. First detection of *Eimeria* species in Myanmar domestic goats with both microscopic and molecular methods. *Parasite.* 2020;27:38.
35. Courtney CH, Ernst JV, Benz GW. Redescription of oocysts of the bovine coccidia *Eimeria bukidnonensis* Tubangui 1931 and *E. wyomingensis* Huizinga and winger 1942. *J Parasitol.* 1976;62(3):372–6.
36. Christensen JF. The oocysts of coccidia from domestic cattle in Alabama (U.S.A.), with descriptions of two new species. *J Parasitol.* 1941;27(3):203–20.
37. Guerrero CA, Hernández J, Bazalar H, Alva J. *Eimeria macusaniensis* n. sp. (Protozoa: Eimeriidae) of the alpaca *Lama pacos*. *J Protozool.* 1971;18(1):162–3.
38. Hao GY. Advances in coccidiosis of goats and sheep. *Chin J Vet Sci.* 2017;37(3):577–84 (in Chinese).
39. Gudmundsdottir B, Skirnisson K. Description of a new *Eimeria* species and redescription of *Eimeria mayeri* (Protozoa: Eimeriidae) from wild reindeer *Rangifer tarandus* in Iceland. *J Parasitol.* 2005;91(2):353–7.
40. Gudmundsdottir B, Skirnisson K. The third newly discovered *Eimeria* species (Protozoa: Eimeriidae) described from wild reindeer, *Rangifer tarandus*, in Iceland. *Parasitol Res.* 2006;99(6):659–62.
41. Lu G. Preliminary study on coccidia species of sika deer in Hanshan County. *Chin J Vet Parasitol.* 1995;3(4):41–3 (in Chinese).
42. Levine N. *Veterinary protozoology*. Iowa: Iowa State University Press; 1985.
43. Sogin ML, Silberman JD. Evolution of the protists and protistan parasites from the perspective of molecular systematics. *Int J Parasitol.* 1998;28(1):11–20.
44. Cantacessi C, Riddell S, Morris GM, Doran T, Woods WG, Otranto D, et al. Genetic characterization of three unique operational taxonomic units of *Eimeria* from chickens in Australia based on nuclear spacer ribosomal DNA. *Vet Parasitol.* 2008;152(3):226–34.
45. Ogedengbe ME, El-Sherry S, Ogedengbe JD, Chapman HD, Barta JR. Phylogenies based on combined mitochondrial and nuclear sequences conflict with morphologically defined genera in the eimeriid coccidia (Apicomplexa). *Int J Parasitol.* 2018;48(1):59–69.
46. Morris GM, Gasser RB. Biotechnological advances in the diagnosis of avian coccidiosis and the analysis of genetic variation in *Eimeria*. *Biotechnol Adv.* 2006;24(6):590–603.
47. Kvicerova J, Pakandl M, Hyspa V. Phylogenetic relationships among *Eimeria* spp. (Apicomplexa, Eimeriidae) infecting rabbits: evolutionary significance of biological and morphological features. *Parasitology.* 2008;135(4):443–52.
48. Gilbert C, Ropiquet A, Hassanin A. Mitochondrial and nuclear phylogenies of Cervidae (Mammalia, Ruminantia): systematics, morphology, and biogeography. *Mol Phylogenet Evol.* 2006;40(1):101–17.
49. Wang Y, Zhang C, Wang N, Li Z, Heller R, Liu R, Zhao Y, Han J, Pan X, Zheng Z, et al. Genetic basis of ruminant headgear and rapid antler regeneration. *Science.* 2019;364(6446):eaav6335.
50. Ogedengbe ME, El-Sherry S, Ogedengbe JD, Chapman HD, Barta JR. Phylogenies based on combined mitochondrial and nuclear sequences conflict with morphologically defined genera in the eimeriid coccidia (Apicomplexa). *Int J Parasitol.* 2018;48(1):59–69.
51. Foreyt WJ. Diagnostic parasitology. *Vet Clin North Am Small Anim Pract.* 1989;19(5):979–1000.
52. Williams RB, Thebo P, Marshall RN, Marshall JA. Coccidian oocysts as type-specimens: long-term storage in aqueous potassium dichromate solution preserves DNA. *Syst Parasitol.* 2010;76(1):69–76.
53. Gu X, Sun X, Liu H, Cui P, Suo X, S F. Phylogenetic analysis of five rabbit *Eimeria* species based on the ITS-1 sequence. *Acta Veterinaria et Zootecnica Sinica* 2012;43(7):1123–1128.
54. Lin RQ, Qiu LL, Liu GH, Wu XY, Weng YB, Xie WQ, et al. Characterization of the complete mitochondrial genomes of five *Eimeria* species from domestic chickens. *Gene.* 2011;480(1–2):28–33.
55. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol.* 2011;28(10):2731–9.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more biomedcentral.com/submissions

