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 Cai1,2,3, Zeyang Suding1,2,3, Lele Wang1,2,3, Zhaofeng Hou1,2,3, Dandan Liu1,2,3, Siyang Huang1,2,3, Jinjun Xu1,2,3 and Jianping Tao1,2,3*

**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) μm x 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) μm x 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

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**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...
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 1886, 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) × 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) × 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 Residuum | Shape | Size (μm) | Shape Index | Stieda Body | Body | Sporocyst Residuum |          |
| *E. bukidnonensis*  | Cattle         | Pyriform | 47.4 x 33 (43–51 x 30–35) | 1.44 | 2-layered (35) | Present | Absent | Absent | Elongate | 19.6 x 9.8 (18–21 x 9–11) | Present | Absent | Courtney et al., 1976 [35] |
| *E. wyomingensis*   | Cattle         | Pyriform | 39.9 x 28.3 (36–44 x 26–30) | 1.41 | 2-layered (25) | Present | Absent | Absent | Elongate | 18.7 x 8.6 (17–20 x 8–10) | Present | Absent | Courtney et al., 1976 [35] |
| *E. alabamensis*    | Cattle         | Pyriform | 19.5 x 14.3 (16–25 x 12–17) | 1.36 | 1-layered (05) | Absent | N/A   | Absent | Elongate | 8.2 x 3.8 | 2.16 | Tiny | Tiny | Christensen, 1941 [36] |
| *E. subspherica*    | Cattle         | Subspherical | 11.5 x 10.6 (9–13 x 8–12) | 1.08 | N/A | Absent | N/A   | Absent | Elongate | 8.1 x 3.6 | 2.25 | Tiny | Absent | Christensen, 1941 [36] |
| *E. macusaniicensis*| Alpaca         | Ovoid to pyriform | 93.6 x 67.4 (81–107 x 61–80) | 1.39 | 3-layered (8–12) | Prominent | (9–14) | Absent | Elongate to ovoid | 36.3 x 18.3 (33–40 x 16–20) | 1.98 | Faintly perceptible | Faintly perceptible | Guerrero et al., 1971 [37] |
| *E. intricata*     | Sheep          | Ellipsoidal to ovoid | 50 x 38 (39–59 x 27–47) | 1.32 | 2-layered (24–3.8) | Present | Present | Absent | Elongate to ovoid | 17–22 x 9–14 | N/A | Absent | Present | Hao, 2017 [38] |
| *E. mayeri*        | Reindeer       | Spheroidal to ellipsoidal | 17.2 x 14.1 (9–21 x 9–16) | 1.22 | 2-layered (1) | Present | (<1) | Absent | Ovoid | 9.3 x 5.1 (8–11 x 4–6) | 1.82 | Nipple-like | Inconspicuous | Gudmundsdottir and Skirnisson, 2005 [39] |
| *E. rangiferis*    | Reindeer       | Ovoid | 34.9 x 27.6 (31–38 x 25–30) | 1.26 | 2-layered (18–20) | Present | (4–6) | Absent | Spindle shaped | 18.6 x 9.2 (17–20 x 8–10) | 2.02 | Nipple-like | Present | Gudmundsdottir and Skirnisson, 2005 [39] |
| *E. breindyria*    | Reindeer       | Ellipsoidal | 30.0 x 21.1 (24–35 x 18–23) | 1.42 | 2-layered (08–1.2) | Absent | Present | Absent | Spindle-shaped | 15.3 x 6.5 (13–18 x 6–8) | 2.35 | Present | Present | Gudmundsdottir and Skirnisson, 2006 [40] |
| *E. alces*        | Elk            | Ovoid | 38.0 x 26.1 (33–43 x 24–29) | 1.46 | 2-layered (22) | Distinct (~7) | Present | Absent | Ovoid to elongate | 18.6 x 8.9 (17.0–21 x 8–10) | 2.09 | Present | Absent | Pyziel and Demiaszkiewicz, 2013 [24] |
| *E. cervi*        | Sika deer      | Pyriform | 35.2 x 25.1 (26–39 x 20–29) | 1.40 | 2-layered (06–1.0) | Prominent | (4.2) | Absent | Long oval | 18.2 x 9.7 | N/A | Present | Present | Lu, 1995 [41] |
| *E. robusta*      | Sika deer      | Ovoid | 35.3 x 26.1 (27–41 x 21–30) | 1.35 | 2-layered (06–1.1) | Prominent | (5.8) | Absent | Long oval | 18.2 x 9.6 | N/A | Present | Present | Lu, 1995 [41] |
| Species       | Hosts                  | Oocyst | Sporocysts |
|---------------|------------------------|--------|------------|
|               |                        | Shape  | Shape      | Wall   | Micropyle | Polar   | Oocyst  | Residuum | Shape  | Size (μm) | Shape      | Stieda | Body | Residuum |
| E. sordida    | 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] |
| E. austriaca  | 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] |
| E. sp-1       | 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] |
| E. sp-2       | 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 × 100 | N/A | Absent | Present | Lu, 1995 [41] |
| E. scabra     | Pig                    | Ovoid to subspherical | 25–45 × 18–28 | N/A | 2-layered (1.3–3) | Present | Present | Absent | Ovoid | 14–18 × 7–9 | N/A | Prominent | Present | Levine, 1985 [42] |
| E. davidianus | Père David's deer      | Pyriform | 41.2 × 29.5 (39–43 × 26–31) | 1.40 | 2-layered (1.5–2.9) | Prominent (2.8–4.0) | Absent | Spindle shaped | 18.2 × 10.5 (16–20 × 10–12) | 1.73 | Present | Present | Present study |

References:
- Lu, 1995 [41]
- Levine, 1985 [42]
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)

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**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 = sporozoites; 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* (Lavran, 1900) (AY488166) was used as an outgroup (Fig. 4).

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

A 328 bp 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 786 bp 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?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 Haaf ten, 1966), *E. austriaca* (Supperer and Kutzer, 1961), *E. wapiti* (Hones, 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. gallivalieri* (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].

Fig. 4 Evolutionary relationships of *E. davidianusi* inferred by distance analysis of 18S rRNA sequences (1380 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 diarrhoea in Dafeng Milu National Nature Reserve, Jiangsu
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 (K₂Cr₂O₇) [52], mixed well and poured into Erlenmeyer flasks (250 mL) to a depth of less than 1 cm.

Fig. 6 Evolutionary relationships of *E. davidianusi* inferred by distance analysis of COI sequences (786 bp). Percentage support from 1000 pseudoreplicates from Neighbor-joining (NJ) analysis is indicated at the left of the supported node.
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), 10 pM of each primer and 1 μL (10–20 ng) of genomic DNA. The 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 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.

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 ~450 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/).

### Table 2 Sequences of primers

| Name of primer | Sequence (5’ to 3’) |
|----------------|-------------------|
| For 18S rRNA   |                   |
| E18SF          | GAAACTGCGAATGGCTCATT |
| E18SR          | CTTCGGCCTACTAGGCTCTC |
| For ITS1       |                   |
| EIF            | AAGTTGCGTTAAATAGAGCCC |
| EIR            | CAAGACATCCATTGCTGAAA |
| For COI        |                   |
| ECOIF          | GTGGTTGTCAGGTGTTGTTGGAC |
| 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.
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

OFR: oocyst residuum; PG: polar granule; SSBS: 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.

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Authors' contributions

WMC, JPT conceived and designed the study. SYH collected the samples. WMC, ZYSD and LLW performed the laboratory analyses. ZFH, DDL and JJK 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 the manuscript.

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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

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

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