



Morphological and molecular characterization of *Cystoisospora* sp. from Asian small-clawed otters *Aonyx cinereus*

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ABSTRACT

In this study, we provide the first description of *Cystoisospora* infection in Asian small-clawed otters (*Aonyx cinereus*). In July 2017, three juvenile otters recently imported from the Republic of Indonesia showed severe diarrhea and were diagnosed with coccidian infection; two of them eventually died. Fecal examination revealed the presence of numerous oocysts. Sporulated oocysts showed typical *Cystoisospora* features, measuring 24.6 ± 1.6 (22.0 – 27.0) \times 21.8 ± 1.4 (19.0 – 25.0) μm , with an oocyst length/width ratio of 1.1 ± 0.1 (1.0 – 1.3). Each sporocyst contained four sporozoites in a head-to-tail arrangement. The Stieda body was absent, and the sporocyst residuum was present. These morphological characteristics differentiated this species from the other valid *Cystoisospora* species described from mustelids. Molecular analysis was conducted at two loci: the nuclear 18S ribosomal RNA and mitochondrial cytochrome c oxidase subunit I genes. The 18S sequence showed high similarity with canine *Cystoisospora ohioensis* (1-bp difference, 1422/1423 [99.9%]). At the cytochrome c oxidase subunit I gene locus, the sequence from otters was identical to that of feline *Cystoisospora rivolta* (847/847 [100%]). Phylogenetic analyses using concatenated data demonstrated that *Cystoisospora* sp. from otters and *C. rivolta* grouped together in the same *Cystoisospora* clade. Based on these data, we concluded that *Cystoisospora* sp. detected from otters appeared to be highly similar to *C. rivolta*.

1. Introduction

Coccidia of the genus *Cystoisospora* are common intestinal protozoan parasites found in mammals. Traditionally, this genus was assigned to the genus *Isoospora*, which classified into the family Eimeriidae. Morphological and molecular characterization has differentiated the genus *Isoospora* into two groups of parasites, i.e., the genus *Isoospora* of the family Eimeriidae and the genus *Cystoisospora* of the family Sarcocystidae (Barta et al., 2005). *Isoospora* species are found in birds and reptiles, and their sporocysts possess a Stieda body, whereas *Cystoisospora* oocysts are found in carnivores, primates, and swine and possess sporocysts without a Stieda body (Frenkel, 1977; Barta et al., 2005).

Otters are semiaquatic carnivorous mammals of the subfamily Lutrinae, which is part of the family Mustelidae. The subfamily Lutrinae is represented by 9 genera and 12 extant species. The Asian small-clawed otter (*Aonyx cinereus*, syn. *Lutra cinerea*) is the smallest otter species native to South and Southeast Asia. Statistics by the Japanese Ministry of Health, Labor and Welfare show a total of 13 captive bred

individuals of Asian small-clawed otter were legally imported to Japan from 2015 to 2017 (<http://www.mhlw.go.jp/>, access 2018.7.2).

Reports of *Cystoisospora* infection in otters are rather scarce, and only a single species, *Cystoisospora lutrae*, has been reported in the European otter (*Lutra lutra*) in Spain (Torres et al., 2000). In the present study, we characterized *Cystoisospora* species in three juvenile Asian small-clawed otters using morphological and molecular techniques.

2. Materials and methods

2.1. Animals and specimens

Three juvenile Asian small-clawed otters (2 males and 1 female) were captive breeding littermates born on May 1st, 2017, and had been imported from the Republic of Indonesia to Japan on June 25, 2017. At the Indonesia's breeding farm, they were given milk and cat food in a cat free environment. On July 2, 2017, these three otters were diagnosed with coccidian infection based on the presence of numerous unsporulated oocysts (Fig. 1a) using a standard fecal flotation technique at

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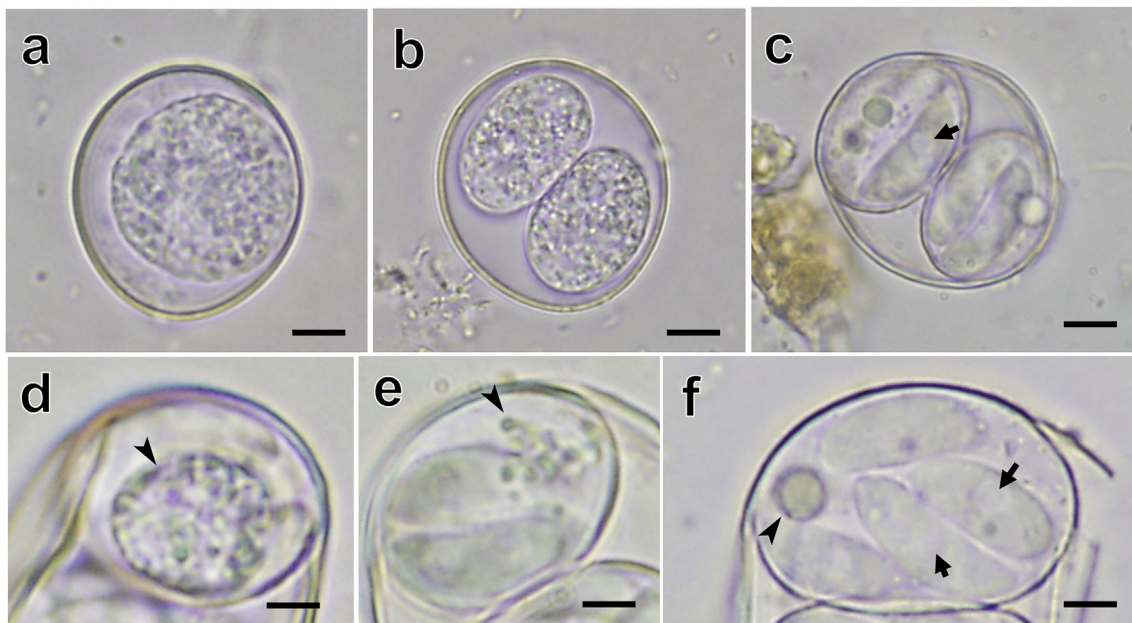


Fig. 1. Photographs of *Cystoisospora* oocysts detected from Asian small-clawed otters. (a and b) Immature oocysts containing one (a) or two sporoblasts (b). (c) Mature oocysts containing two sporocysts, each with four club-shaped sporozoites. Sporozoites contained rounded retractile vacuoles (arrow). (d–f) Sporozoites containing sporocyst residuum, which was composed of numerous small granules (d and e) or a rounded granule (f).

Den-en-chofu Animal Hospital, Japan. Two of the three animals showed anorexia, green diarrhea, and vomiting and were administered a single dose of toltrazuril (15 mg/kg, p.o.). Although these two animals showed reduced oocyst excretion, they showed persistent diarrhea and died after 2 and 7 days, respectively.

Fecal specimens were collected from these animals, placed in separate vials with 2.0% (w/v) aqueous potassium dichromate ($K_2Cr_2O_7$) solution. Oocysts sporulation was induced by incubation at room temperature for 10 days and at 4 °C for prolonged storage. For histological examination, the animal that died 7 days after diagnosis was necropsied in Nippon Veterinary and Life Science University, and the intestines, liver, lung, kidney, brain, and spleen were fixed in 10% neutral-buffered formalin. Formalin-fixed tissue specimens were embedded in paraffin, cut into 5- μ m-thick sections, and stained with hematoxylin and eosin (H&E).

2.2. Microscopic analysis

Oocysts were observed with a BX41 microscope (Olympus, Japan), and photomicrographs were captured using a DP71 photomicroscope (Olympus). Line drawings were made using FireAlpaca Ver. 2.0 (<http://firealpaca.com/>). Measurements were made with a calibrated ocular micrometer under an oil immersion objective and are reported as means \pm standard deviations with the range in parentheses.

2.3. Molecular analysis

Oocysts were purified by flotation methods from each specimens separately, and a single oocyst was isolated using a manually pulled glass micropipette under an Olympus SZX stereomicroscope (Olympus). Genomic DNA was extracted using a Power Soil DNA isolation kit (MoBio Laboratories, USA) as previously described (Tokiwa et al., 2017). Double distilled water (no fecal specimen) was included as a negative control. The resulting DNA specimens were used as templates for polymerase chain reaction (PCR) amplification.

The partial fragment of the 18S rRNA gene (18S) and the mitochondrial gene encoding cytochrome c oxidase subunit 1 (*cox1*) were amplified using the primer sets EF (5'-GAACTGCGAATGGCTC ATT-3')/ER (5'-CTTGCGCCTACTAGGCATTC-3') (Kvicerová et al.,

2008) and Sdae-Cox1_260F (5'-GATCTTTATGTTYTTRATGCC-3')/Sdae-Cox1_1147R (5'-CATTACCCATAACYACACC-3') (Ogedengbe et al., 2016), respectively. The PCR mixture contained 2.5 μ L of 10 \times Ex Taq buffer (Takara Bio Inc., Japan), 0.2 mM dNTPs (Takara Bio, Inc.), 0.2 μ M each primer, 1 U Ex Taq polymerase (Takara Bio, Inc.), and 1 μ L DNA extract in a total volume of 20 μ L. PCR conditions consisted of initial denaturation at 94 °C for 5 min; 35 cycles at 94 °C for 30 s, 54 °C (18S) or 50 °C (*cox1*) for 1 min, and 72 °C for 1 min; and a final extension step at 72 °C for 7 min (18S) or 1 min (*cox1*). The amplified DNA was applied to 2% agarose gels, electrophoresed, and visualized under an LED transilluminator. PCR products were sent to a sequence service (Macrogen Corp., Kyoto, Japan) and analyzed with an ABI3730xl DNA Analyzer (Thermo Fisher Scientific, USA) using the same PCR primers.

2.4. DNA sequence analyses

Sequence analyses were conducted on nucleotide sequences of the 18S and *cox1* genes using MEGA version 7 software (Kumar et al., 2016). Sequence similarity was determined separately using the BLASTN program with the National Center for Biotechnology Information database (<http://www.ncbi.nlm.nih.gov/Blast.cgi>).

Phylogenetic trees of concatenated 18S and *cox1* sequence data were generated using MEGA. Each sequence was derived from the same isolate/strain available in the GenBank/DDBJ/EMBL databases, as described by Ogedengbe et al. (2016). In total, 14 concatenated sequences, including two new sequences derived from *Cystoisospora* sp. detected from the otters in the present study, were aligned using MAFFT version 7 (Katoh and Standley, 2013) with the Q-I setting and ClustalW (Larkin et al., 2007), followed by manual editing. All positions with less than 95% site coverage were eliminated, and a total of 875 positions were included in the dataset. The phylogenetic tree was reconstructed by neighbor joining (NJ) and maximum likelihood (ML) methods using the Tamura-Nei model (Tamura and Nei, 1993) and General Time Reversible model with gamma distribution plus invariant sites, respectively. Bootstrap values for NJ and ML were obtained from 2000 to 1000 replicates, respectively.

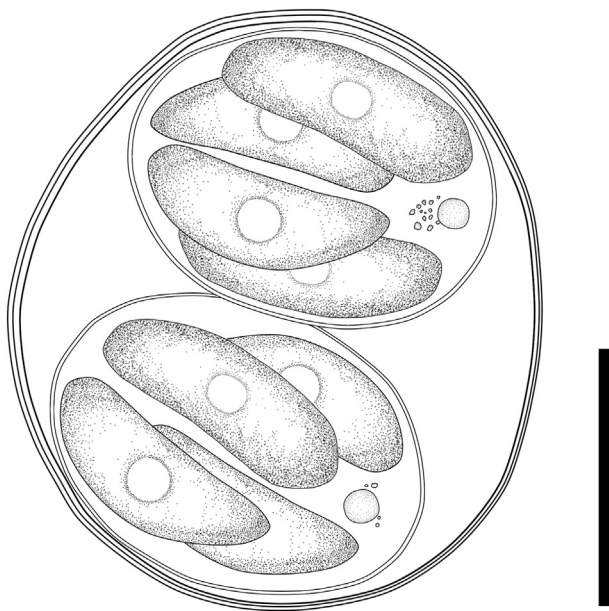


Fig. 2. Line drawing of sporulated oocysts of *Cystoisospora rivolta*-like oocysts from small-clawed otters. Bar = 10 µm.

3. Results

3.1. Description of *Cystoisospora* sp. from Asian small-clawed otters

Fecal examination revealed the presence of numerous *Cystoisospora* oocysts. Although the accurate sporulation time could not be identified, some oocysts were mature at 70 h after sampling. The sporulated oocysts were morphologically identical in three fecal specimens. Sporulated oocysts were subspherical to spherical and measured 24.6 ± 1.6 (22.0–27.0) \times 21.8 ± 1.4 (19.0–25.0) µm, with an oocyst length/width ratio of 1.1 ± 0.1 (1.0–1.3), and contained two sporocysts (Fig. 1b and c, 2), each of which harbored four sporozoites (Figs. 1f and 2). The oocyst wall was smooth and measured 0.3 µm thick, with a colorless outer layer and light yellowish-brown inner layer. The oocyst residuum, polar granule, and micropyle were absent. Sporocysts are elongate-ovoid with a smooth wall and measured 16.3 ± 1.6 (14.0–20.0) \times 12.3 ± 1.0 (11.0–15.0) µm, with a sporocyst length/width ratio of 1.3 ± 0.1 (1.1–1.6) µm. Each sporocyst contained four sporozoites in a head-to-tail arrangement. Sporozoites were club-shaped, possessed a rounded refractile body at the center part, and measured 9.6 ± 0.6 (9.0–11.0) \times 4.1 ± 0.3 (4.0–5.0) µm, with a length/width ratio of 2.4 ± 0.2 (2.0–2.8). The Stieda body and substieda body were absent. Sporozoites in the sporocyst residuum were initially compact masses of small granules (Fig. 1d), but gradually

decreased in size and became a single rounded granule (2–5 µm in diameter) with small granules (Fig. 1c, e, 1f, 2).

Postmortem histology analysis of the otter after toltrazuril treatment showed bacterial pneumonia and degeneration and necrosis of the epithelial cells in the mucosa of the intestine. No developmental stages of *Cystoisospora* sp. were observed in these legions (data not shown).

3.2. Sequence analyses of *Cystoisospora* sp. from Asian small-clawed otters

A 1423-bp sequence of the 18S gene obtained from the three otters was identical in each otter and was deposited in the DDBJ database under accession no. LC377842. Sequence comparisons revealed that the present sequence from otters shared the highest identity (99.9%) for *C. ohioensis* (1422/1423, AY618555; 1421/1423, KT1843651, KT184359, KT184366) and *C. suis* (1421/1423, KF854252, KF854251). Similarities of 99.8% for *C. belli* (1420/1423, U94787, JX025649, AB268326, DQ060659, DQ060661), 99.6% for *C. canis* (1417/1423, KT184368), 99.4% for *C. timoni* (1415/1423, AY279205, EU200732) and *C. orlovi* (1414/1423, AY365026), and 99.2% for *C. felis* (1412/1423, KT134364) and *C. canis* (1412/1423, KT184362, KT184363, TK184360) were also noted. Sequence identity with the near complete 18S sequence of *C. rivolta* (AY618554) was 81.1% (1154/1447); however, this low similarity was thought to be due to incorrect registration of the sequence because high similarities (> 99%) were observed with the red yeast *Rhodospiridium* spp. The partial 18S sequence showed 100% identity (401/401) with that of *C. rivolta* (KT184367) and was identical to those of *C. canis* (KT184360, KT184362, KT184368), *C. ohioensis* (KT184351, KT184359, KT184366), *C. suis* (KF854252, KF854251), *C. belli* (U94787, JX025649, AB268326, DQ060659, DQ060661), and *C. timoni* (EU200792, AY279205).

An 847-bp sequence of the *cox1* gene obtained from three otters was identical in each otter and deposited in the DDBJ database under accession no. LC377843. Sequence comparisons using BLAST revealed that the new sequence from otters was completely identical to that from *C. rivolta* (KT184383) and showed 99.8% (845/847) similarity to *C. ohioensis* (KT184384), 98.7% (836/847) similarity to *C. canis* (KT184385), and 98.4% (814/827) similarity to *C. felis* (KT184386). Table 1 shows the multiple alignment of *cox1* sequences from 5 *Cystoisospora* species and the present species. A single nonsynonymous substitution occurred in between the present species and *C. ohioensis* (362: Ile to Leu), *C. canis*, and *C. felis* (371: Ile to Leu), and 2 nonsynonymous substitutions (362: Ile to Leu, 374: Leu to Ile) occurred in *C. suis*.

3.3. Phylogenetic analyses of *Cystoisospora* spp.

Phylogenetic analyses using NJ and ML methods with concatenated sequences produced topologically similar trees that supported the monophyletic clade of the genus *Cystoisospora* (Fig. 3). The present sequence from Asian small-clawed otters was placed within the

Table 1
Alignment of *cox1* sequences of *Cystoisospora* spp.

Species	GenBank/DDBJ/EMBL accession Nos.	Nucleotide variable site																			
		32	40	112	115	148	175	179	253	271	298	362	371	374	382	472	475	521	575	706	847
Present species	LC377843	C	A	T	A	A	G	C	T	T	T	A	A	T	T	A	A	C	C	A	A
<i>Cystoisospora rivolta</i>	KT184383
<i>Cystoisospora ohioensis</i>	KT184384	T	C*
<i>Cystoisospora suis</i>	KF854265	-	-	-	-	-	C*	.	A*	-	-
<i>Cystoisospora canis</i>	KT184385	.	.	A	C	.	.	T	A	C	C	.	C*	.	A	C	.	.	.	A	G
<i>Cystoisospora felis</i>	KT184386	T	C	.	C	.	A	T	A	C	C	.	C*	.	.	.	G	T	A	G	T
Codon position		1	3	3	3	3	3	1	3	3	3	1	1	1	3	3	3	1	1	3	3

Dot represent matches with nucleotides present in *cox1* sequence of *Cystoisospora* sp. from Asian small-clawed otters (LC377843). Asterisk represent nonsynonymous substitution sites in which amino acid replacement.

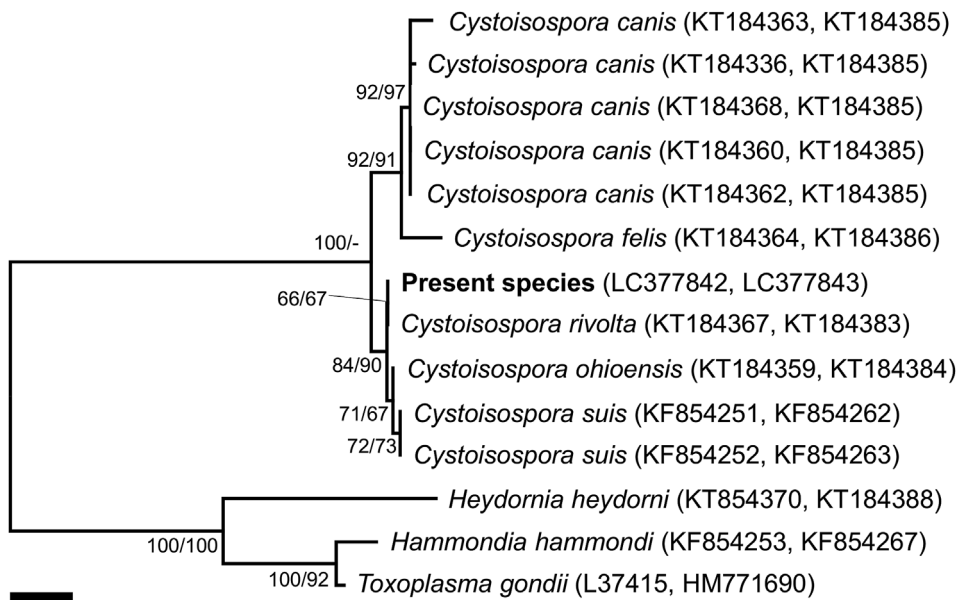


Fig. 3. Mid-point rooting tree of *Cystoisospora* spp. inferred from concatenated alignment of two nucleotide sequences (18S and *cox1*). Nucleotide sequences of *Cystoisospora* spp. GenBank/DBJ/EMBL accession numbers for 18S and *cox1* are shown next to species names. Nodal support values (NJ/ML) greater than 60% are represented on ML branches. Scale bar represents 0.01 nucleotide substitutions per sites.

monophyletic group with *C. rivolta* detected from a domestic cat (*Felis catus*) in Canada.

4. Discussion

Here, we present the first description of a coccidian from the Asian small-clawed otter. All animals showed typical symptoms of cystoisosporiosis, also known as isosporiosis, including severe diarrhea and oocyst excretion. Although a single treatment with toltrazuril reduced the oocyst shedding, two otters eventually died. The cause of death in these two otters was estimated exhaustion due to diarrhea, followed by aspiration pneumonia.

Morphological observation of the sporulated oocysts from three individuals showed infection with a single coccidian species. The sporulated oocysts contained two sporocysts without a Stieda body, which each harbored four sporozoites, representing typical *Cystoisospora* features. According to the guideline by Duszynski and Wilber (1997), coccidian species should be compared morphologically in detailed with other species having similar features and belonging to the host family. Tenter et al. (2002) discussed morphological and molecular characters and host-related parameters to be considered for the classification of coccidia. Therefore, the morphological characteristics of *Cystoisospora* oocysts found in otters were compared with those of other *Cystoisospora* spp. previously described in carnivores of the family Mustelidae. Table 2 shows a list of 12 valid species of the genus *Cystoisospora* (syn. *Isoospora*) detected from mustelids. Only a single species of *Cystoisospora* has been described for otter species (subfamily Lutrinae), i.e., *C. lutrae*, from the European otter (*Lutra lutra*) originating from Spain (Torres et al., 2000). However, *C. lutrae* has significantly larger oocysts. Unidentified *Cystoisospora* oocysts were reported from the North American otter (*Lontra canadensis*) (Hoover et al., 1985); however, measurements of oocysts, line drawings, and photomicrographs have not been reported. In *Cystoisospora* species described from non-otter mustelids (subfamily Mustelinae), the oocysts of *C. melis*, *C. altaica*, *C. pavlovskyi*, and *C. laidlawi* are much larger (Hoare, 1927; Kotlán and Pospesch, 1933; Svanbaev, 1956; Svanbaev and Rachmatullina, 1971), and the oocysts of *C. evermanni* and *C. goussevi* are much smaller (Svanbaev, 1956; Musaev and Veison, 1983). *C. hoogstraali* has much larger oocysts and has sporocysts with prominent Stieda bodies (Prasad, 1961). The latter features indicate that *C. hoogstraali* may an *Isoospora* pseudoparasite passing through the intestine

after consumption of birds, reptiles, or feces. The oocysts of *C. martessii* and *C. nivalis* are similar in size, whereas the sporocysts of these organisms differ in size (Nukerbaeva, 1981; Musaev and Veison, 1983). Finally, the oocysts of *C. africana* resemble those of the present species, whereas the sporozoites of *C. africana* are elongated and have refractile vacuoles at their ends (Prasad, 1961). These features support that this may be a novel *Cystoisospora* species in mustelids.

Numerous phylogenetic studies have focused on the analysis of 18S, 28S, ITS, and *cox1* genes, and 18S and *cox1* genes have been commonly used for coccidians belonging to the families Sarcocystidae and Eimeriidae (Carreno et al., 1998; Morrison et al., 2004; Ogedengbe et al., 2011, 2016). The coccidian 18S sequence is phylogenetically useful for distant taxa, but seems to be much less variable than that in other protozoan parasites and to be insufficient for resolving the relationships among closely related coccidian species (Morrison et al., 2004). In contrast, the *cox1* sequence provides sufficient sequence divergence for studying phylogenetic relationships among closely related coccidian species. Ogedengbe et al. (2016) made specific *cox1* primers for Sarcocystidae groups and suggested that *cox1* sequences from sarcocystids (i.e., *Toxoplasma*, *Neospora*, and *Hammondia*) appeared well suited for species delimitation; however, sequences from *Cystoisospora* spp. showed remarkably limited sequence variation. In this study, because no sequence data exist for *Cystoisospora* species infecting mustelids, we compared *Cystoisospora* sequences from other animals. Interestingly, *Cystoisospora* sp. from otters showed the highest *cox1* sequence similarity to *C. rivolta*. Phylogenetic analyses confirmed the genus *Cystoisospora* as being a monophyletic clade comprising two morphologically supported clades: (1) one including feline *C. felis* and canine *C. canis*, which possess large (> 30 µm long) and egg-shaped oocysts (Wenyon, 1923; Lepp and Todd, 1974); and (2) the other including feline *C. rivolta*, canine *C. ohioensis*, swine *C. suis*, and otter *Cystoisospora*, which possess small oocysts (< 30 µm long) (Biester and Murray, 1934; Dubey, 1975, 1979). We cannot molecularly differentiate the present *Cystoisospora* species from *C. rivolta*. Furthermore, the sporulated oocysts of the present species morphologically resembles those of *C. rivolta* from domestic cats, measuring 25.4 (23–29) × 23.4 (20–26) µm in diameter (Dubey, 1979); thus, we have not proposed this as a novel species and have designated this species as *Cystoisospora* cf. *rivolta*.

The definitive host specificity of *Cystoisospora* species is relatively narrow, perhaps at the host genus or family level. Nukerbaeva (1981)

Table 2
Comparative morphology of *Cystoisospora* spp. recorded from mustelids.

Cystoisospora species	Type species	Oocyst	Description	Sporocyst	Description	Sporozoite	References
	Species (Subfamily)	Size		Size			
<i>C. arctopithecii</i> (Rodhain, 1933)	<i>Eira barbara</i> (Mustelinae)	L: 27.7 (22.7–32.7) W: 24.3 (20.5–27.3) L/W: 1.14 (1.05–1.30)	Subspherical to ellipsoidal M: absent OR: absent PG: absent	L: 17.6 (13.1–20.5) W: 12.5 (9.8–15.9)	SR: present SB: absent		Hendricks, 1974
<i>C. martessii</i> (Nukerbaeva, 1981)	<i>Martes zibellina</i> (Mustelinae)	L: 25.2–28.0 W: 16.8–22.4	Ovoid, short oval to spherical M: absent OR: absent	L: 11.2–16.8 W: 8.4–11.2	SR: present	Elongated	Nukerbaeva, 1981
<i>C. melis</i> (Kotlán and Pospesch, 1933)	<i>Meles meles</i> (Mustelinae)	L: 32.8 (25.6–37.8) W: 26.9 (24–29.6) L/W: 1.22 (1.10–1.57)	Oval M: absent OR: absent PG: absent	L: 14–16 W: 12	SR: present SB: absent	Round at one end and the other end tapered L: 14.2 W: 4.0	Kotlán and Pospesch, 1933, Anwer et al., 2000
<i>C. altaica</i> (Svanbaev and Rachmatullina, 1971)	<i>Mustela altaica</i> (Mustelinae)	L: 33.3 (28.0–33.6) W: 27.7 (25.2–28.0) L/W: 1.24 (1.11–1.24)	Oval to spherical M: absent OR: absent PG: absent	L: 14.0–16.8 W: 11.1–16.8	SR: present		Svanbaev and Rachmatullina, 1971
<i>C. eversmanni</i> (Svanbaev, 1956)	<i>Mustela eversmanni</i> (Mustelinae)	L: 18.5 (16–20) W: 14.8 (12–16) L/W: 1.3 (1.1–1.6)	Oval M: absent OR: absent PG: absent	L: 11.5 (10–13.5) W: 9.8 (9–11)	SR: present SB: absent		Yi-Fan et al., 2012
<i>C. pavlowskyi</i> (Svanbaev, 1956)	<i>Mustela eversmanni</i> (Mustelinae)	L: 32.2 (29–36) W: 27.3 (26.5–28.5) L/W: 1.2 (1.1–1.4)	Oval M: absent OR: absent PG: absent	L: 19.5 (18–21) W: 14.4 (12–15)	SR: present SB: absent		Yi-Fan et al., 2012
<i>C. laidlawi</i> (Hoare, 1927)	<i>Mustela putorius furo</i> (Mustelinae)	L: 34 (32.0–36.8) W: 29 (27.2–30.4)	Ovoid M: absent OR: absent PG: absent	L: 20.8 W: 14.4	SR: present (coarse granules) SB: absent	Sausage shaped	Hoare, 1927
<i>C. goussevi</i> (Musaev and Veison, 1983)	<i>Mustela nivalis</i> (Mustelinae)	L: 22.4 (22–25) W: 17.4 (16–19) L/W: 1.35 (1.33–1.37)	Ovoid M: absent OR: present PG: present	L: 12 (10–13) W: 7 (6–8)	SR: present SB: present	Elongated	Musaev and Veison, 1983
<i>C. nivalis</i> (Musaev and Veison, 1983)	<i>Mustela nivalis</i> (Mustelinae)	L: 20.6 (20.0–23.0) W: 18.4 (18.0–21.0) L/W: 1.1 (1.09–1.11)	Oval M: absent OR: absent PG: absent	L: 12.5 (12.0–13.0) W: 8.0 (7.0–9.0)	SR: present SB: absent	Lemon or pear shaped	Musaev and Veison, 1983
<i>C. africana</i> (Prasad, 1961)	<i>Ictonyx libycus</i> (Mustelinae)	LW: 26 (25–27)	Spherical M: absent OR: absent PG: absent	LW: 15.5–15.6	SR: present (prominent) SB: absent	Elongated L: 13.5 W: 3.0	Prasad, 1961
<i>C. hoogstraali</i> (Prasad, 1961)	<i>Ictonyx libycus</i> (Mustelinae)	L: 38 (37–40) W: 33 (32–34)	Ellipsoid M: present (small button-like) OR: absent PG: present	L: 19.5–21 W: 13–15	SR: present (bulky) SB: absent	Club-shaped L: 18–19W: 4–6	Prasad, 1961
<i>C. lutrae</i> (Torres et al., 2000)	<i>Lutra lutra</i> (Lutrinae)	L: 31.2 (27.5–32) W: 29.6 (28–31) L/W: 1.04 (1.0–1.12)	Spherical to subspherical M: absent OR: absent PG: absent	L: 18.2 (17–19) W: 14.4 (14–16)	SR: present	Spindle-shaped L: 12.4 W: 2.5	Torres et al., 2000
Present species	<i>Aonyx cinereus</i> (Lutrinae)	L: 24.6 (22.0–27.0) W: 21.8 (19.0–25.0) L/W: 1.1 (1.0–1.3)	Subspherical to spherical M: absent OR: absent PG: absent	L: 16.3 (14.0–20.0) W: 12.3 (11.0–15.0)	SR: present SB: absent	Club-shaped L: 9.6 W: 4.1	This study

reported that *C. martessii* from sables (*Martes zibellina*) and *C. laidlawi* from minks (*Mustela vison*) were not transmissible between their respective hosts, even if in the same family Mustelidae. Similarly, cross-infection of *C. ohioensis* from domestic dogs and *C. rivolta* from domestic cats does not occur among these species (Dubey, 1975). *Cystoisospora rivolta* was first named from domestic cats. Subsequently, Wenyon (1923) detected *Cystoisospora* oocysts morphologically similar to those of *C. rivolta* from domestic dogs and considered *C. rivolta* from dogs and cats to be identical. Dubey (1975) separated canine *C. rivolta* on the basis of crossinfection experiments and proposed *C. ohioensis*. Feline *C. rivolta* oocysts are commonly found in domestic cats worldwide; however, structurally similar oocysts have also been reported from carnivores belonging to the family Felidae; i.e., cheetahs (*Acinonyx jubatus*), jungle cats (*F. chaus*), European wildcats (*F. silvestris*), clouded leopards (*Neofelis nebulosi*), leopards (*Panthera pardus*), lions (*Panthera leo*), tigers (*Panthera tigris*), and leopard cats (*Prionailurus bengalensis*) (Yakimoff et al., 1933; Penzhorn et al., 1994; Bjork et al., 2000). Recently, Dubey et al. (2015) reported transmission of *C. felis*-like organisms collected from the bobcat (*Lynx rufus*), a small wildcat found in North America, to domestic cats by feeding of monozyotic cysts in mouse tissues. These studies suggested that feline *Cystoisospora* are family and not genus specific. In contrast, however, Hendricks (1977) studied the host range of the primate species *C. arctopitheci* and demonstrated the susceptibility of six genera of New World nonhuman primates, one marsupial species, and four families of carnivores, including tayras (*Eira barbar*) belonging to the family Mustelidae. The sporulated oocysts from otters morphologically resembled those of *C. arctopitheci*, measuring $27.7 (22.7\text{--}32.7) \times 24.3 (20.5\text{--}27.3) \mu\text{m}$ in diameter (Hendricks, 1974), but differed in that they showed an elliptical shape, in contrast to those of the present species, which were mostly spherical.

In conclusion, this is the first description of morphological and molecular characteristics of *Cystoisospora* sp. detected from Asian short-clawed otters. This coccidian can cause diarrheal disease in juveniles. We conclude that *Cystoisospora* sp. detected from otters appeared to be highly similar to *C. rivolta*, but there is still room for discussion of general hypothesis for definitive host specificity of the genus *Cystoisospora*. Future characterization of *Cystoisospora* species infecting mustelids is necessary using a combination of morphological, molecular, epidemiological, and cross-transmission experiments.

Conflicts of interest

None.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.ijppaw.2018.07.001>.

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