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Molecular diversity of *cox1* and LSU rDNA sequences of *Sarcocystis bertrami* (syn. *S. fayeri*) (Apicomplexa: Eucoccidiorida: Sarcocystidae) in horses

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ABSTRACT

Food poisoning caused by consuming raw horsemeat contaminated with *Sarcocystis* is a significant public health concern. Two morphotypes of sarcocysts in horsemeat, characterized by upright and folded villar protrusions, are typically identified as *Sarcocystis fayeri* and *S. bertrami*, respectively. However, recent molecular studies focusing on the ribosomal RNA gene (rDNA) and mitochondrial cytochrome *c* oxidase subunit I gene (*cox1*) have indicated a conspecific relationship between these two morphotypes using a limited number of specimens. To explore further genetic diversity in equid sarcocysts, *cox1* and large-subunit (LSU) rDNA sequences were analyzed in sarcocysts extracted from horsemeat inspected from 150 horses (76 and 41 horses imported from Canada and France, respectively, and 33 horses reared in Japan). Sarcocysts were detected in the muscles of 71, 2, and 3 horses from Canada, France, and Japan, respectively. Fifty-eight sarcocysts underwent *cox1* and the LSU rDNA sequencing. Newly obtained *cox1* sequences ($n = 53$) and sequences labeled as equid *S. bertrami*, *S. fayeri* and *S. asinus* retrieved from GenBank (n = 53) exhibited conspecific relationships. Inter-individual variation in *cox1* sequences was observed among various sarcocysts, even within a single host animal, although no intra-individual variation was observed. However, nuclear-embedded mitochondrial DNA (NUMT: *cox1* pseudogene) sequences were obtained using inappropriate techniques using certain primers. The LSU rDNA of sarcocysts (211 cloned sequences from 54 sarcocysts) exhibited inter-individual and robust intra-individual variations, indicating significant intragenomic rRNA array mosaicism in *S. bertrami*. These findings confirmed the conspecificity of classically defined species without geographical subpopulations.

1. Introduction

The genus *Sarcocystis* Lankester, 1882 (Apicomplexa: Eucoccidiorida: Sarcocystidae) comprises cyst-forming coccidia with a heteroxenous life cycle involving prey and predator animals (intermediate and definitive hosts, respectively) [1–5]. *Sarcocystis* infection in equids manifests as intra-myofiber cysts (sarcocysts) or as schizonts and merozoites in the spinal cord, leading to protozoal myeloencephalitis [5–8]. The causative agent of the neuronal infection is *Sarcocystis neurona* Dubey et al., 1991. The identity of sarcocysts in horse muscles remains debated with four species proposed: *Sarcocystis bertrami* Doflein, 1901; *Sarcocystis equicanis* Rommel et Geisel, 1975; *Sarcocystis fayeri* Dubey et al., 1977; and *Sarcocystis asinus* Gadaev, 1978 [3,5,9,10]. The sporocysts of *S. bertrami* measure 15.2 (15.0–16.3) μm by 10.0 (8.8–11.3) μm, with a prepatent period of 8 days in dogs, whereas the sporocysts of *S. fayeri* measure 12.0 (11.0–13.0) μm by 7.9 (7.0–8.5) μm, with a prepatent period of 13 days in dogs [5,11]. Matuschka [12] reported that the sporocysts from dogs fed horse (*S. bertrami*) and donkey (*S. asinus*) meats measured 12.2–13.8 μm by 9.2–9.9 μm, with a prepatent period of 9–10 days, and were cross-infective to these two equids.

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Therefore, these two species are considered conspecific [4,5,9,10]. Similarly, *S. equicanis* is regarded as conspecific with *S. bertrami* [3,5,9,10].

In addition to various sporocyst dimensions and prepatent periods, the villar protrusions of *S. bertrami* are folded over the cyst wall, with microtubules that do not extend into the ground substance or reach the plasmalemma of metrocytes/bradyzoites. In contrast, *S. fayeri* has slender, upright villar protrusions with microtubules extending from the villar tips to the plasmalemma of metrocytes/bradyzoites [5,10,13]. However, the fine structure of the sarcocyst wall may vary with age [3,14]. The extension of microtubules from the villar tips through the ground substance to the plasmalemma of metrocyts/bradyzoites depends on the plane of the cut section [10,15,16].

Food poisoning associated with the consumption of raw horsemeat containing sarcocysts has been a significant public health concern in Japan for the past two decades [17–20]. Sarcocysts in horsemeat that caused food poisoning in patients with symptoms such as temporal diarrhea, vomiting, nausea, and ventral pain exhibited two morphotypes with a palisading or thinner wall [17], corresponding to *S. fayeri* and *S. bertrami*, respectively. Following the initial records of the disease in human patients who consumed raw horsemeat, the causative agent was identified as *S. fayeri* based on the sarcocysts' morphology in the causative horsemeat and the size and prepatent period of sporocysts observed after experimental infection in dogs [17–19].

Over the past two decades, molecular genetic studies using small-subunit (SSU) and large-subunit (LSU) ribosomal RNA genes (rDNA), internal transcribed spacer regions (ITS1 and ITS2) of rDNA, mitochondrial cytochrome *c* oxidase subunit 1 gene (*cox1*), and other genes have emerged as effective tools for differentiating *Sarcocystis* spp. [16,21–25]. These approaches have recently been applied to address conflicts regarding equid *Sarcocystis* spp. [15,16,26], highlighting the need for additional isolates and markers to support the finding that *S. bertrami* is a valid species for muscle sarcocysts in horses and donkeys, with *S. fayeri* and *S. asinus* (strictly speaking, a *nomen nudum* at present [10]) recognized as its junior synonyms. The aim of the present study was further clarification of potential variations in the *cox1* and LSU rDNA sequences of *Sarcocystis* isolates from horses reared in Japan and imported from Canada and France.

2. Materials and methods

2.1. Sample collection

Striated muscles surrounding the esophagus and forebody of 150 randomly chosen horses were collected from a slaughterhouse located in Sosaki, Kikuchi, Kumamoto Prefecture, Japan, between June 2020 and May 2022. The sampled horses originally came from Canada ($n = 76$), France ($n = 41$), and Japan ($n = 33$). The majority of them were castrated males and females, aged 2–5 years (average age: 3.1 years), except for eight Japanese-reared horses aged 6–23 years. Imported horses typically arrived as foals and were reared in Japan for 80–890 days (mean ± standard deviation: 313 ± 80 days), except for nine horses that were slaughtered shortly after import quarantine.

Longitudinal muscle slices were assessed individually under a magnifying glass, covering 24–655 cm^2 per animal. Density was calculated as the number of sarcocysts/observed area (cm^2). Representative sarcocysts within myofibers were dissected, sectioned, and separately fixed in 70 % ethanol and 10 % neutral-buffered formalin solution for preservation.

2.2. DNA extraction, polymerase chain reaction (PCR), and nucleotide sequencing

Fifty-nine pieces of muscle containing a sarcocyst, fixed and preserved in 70 % ethanol, were used for DNA extraction. Of these, 57 samples were from seven horses imported from Canada, and one each from France and Japan. Twenty-three samples were isolated from the muscles around the esophagus and 36 from forebody muscles. Muscle pieces containing sarcocysts were individually washed thrice with pure distilled water in 2 mL plastic tubes. DNA was extracted using an Illustra™ tissue and cells genomicPrep Mini Spin Kit (GE Healthcare UK, Buckinghamshire, UK) according to the manufacturer's instructions.

Partial *cox1* sequences were amplified using conventional PCR with a forward primer SF1 (5′-ATG GCG TAC AAC AAT CAT AAA GAA-3′) and reverse primer SR4 (5′-CCA CAC CTG TAG TAC CDC C-3′), or forward primer SF1 and reverse primer SR5 (5′- TAG GTA TCA TGT AAC GCA ATA TCC AT -3′) [21] in 20 μL reaction solutions on a thermal cycler (Model T100; Bio-Rad Laboratories, Inc., Hercules, California, USA). The DNA polymerase used was Blend Taq -Plus- (TOYOBO, Kita-ku, Osaka, Japan), and 1 μL of DNA extraction solution was added to the reaction solution. The following cycling protocol was applied: 3 min at 94 °C; followed by 35 cycles at 94 °C for 30 s, 52 °C for 60 s, and 72 °C for 90 s; with a final extension at 72 °C for 7 min. The PCR products were purified using a FastGene Gel/PCR Extraction Kit (NIPPON Genetics Co., Bunkyo-ku, Tokyo, Japan) and sequenced directly with primers for amplification and sequencing (Sarco_cox1_304F: 5′- CTT CCA GTC GGT TCG GTA CT-3′, Sarco_cox1_375F: 5′- GTA TGC CCC ACT CAG TAC GA-3′, Sarco_cox1_509R: 5′-CCA ACA TAA ACG GCC GTA CC-3′, and Sarco_cox1_592R: 5′-TCG GGA TGG TCA GGA TCA AC-3′) through a commercial service (Fasmac Co., Ltd., Atugi, Kanagawa, Japan). The four sequencing primers were designed using the online software Primer3-web ver. 4.0.0 [27], based on the *cox1* sequence of various equid *Sarcocystis* isolates retrieved from the DDBJ/EMBL/GenBank databases.

Partial LSU rDNA sequences were amplified using conventional PCR with a forward primer KL1 (5′-TAC CCG CTG AAC TTA AGC-3′) and a reverse primer KL3 (5′-CCA CCA AGA TCT GCA CTA G-3′) [28]. The annealing temperature was set to 62 °C for 90 s. Purified PCR products were cloned into the pTA2 plasmid vector (TArget Clone™; TOYOBO, Dojima Hama, Osaka, Japan) according to the manufacturer's instructions. Following propagation, plasmid DNA was extracted using a FastGene Plasmid Mini Kit (NIPPON Genetics Co.), and inserts from at least three independent clones were sequenced using universal M13 forward and reverse primers through the aforementioned commercial service. Additional sequencing was performed using forward primers KL4 (5′-AGC AGG ACG GTG GTC ATG-3′) and 28S-839F/20 (5′-GTT GCG TGC GTG TCA CTA TT-3′), as well as reverse primers Dig.LSU-R1/D2 (5′-TGG TCC GTG TTT CAA GAC-3′) and 28S-1270R/22 (5′-CAG CTA TCC TGA GGG AAA CTTC-3′), as previously described [28,29].

A set of overlapping DNA segments, bidirectionally sequenced with the different primers mentioned above, were assembled into a contig referencing to representative *S. bertrami* sequences of *cox1* (MH025632) and LSU rDNA (MH025629) using the CLUSTAL W multiple alignment program [30].

2.3. PCR amplification of a nuclear-embedded mitochondrial DNA (NUMT) segment

Our preliminary alignment of equine *Sarcocystis* spp. *cox1* sequences retrieved from the DDBJ/EMBL/GenBank databases, labeled as *S. fayeri*, *S. bertrami*, and *S. asinus cox1* sequences, revealed two loose assemblages: A) a major assemblage of *S. fayeri* and *S. bertrami* sequences, and B) a minor assemblage of Chinese *S. asinus* sequences (OM970235–OM970240) and Brazilian *S. bertrami* sequences (OP887040–OP887043). These assemblages exhibited <85 % sequence identity. Therefore, we hypothesized that sequences belonging to the minor assemblage B may represent a pseudogene, specifically nuclear-embedded mitochondrial DNA (NUMT) segments. We aimed to amplify the NUMT segments using nested PCR with in-house primers (Table 1). Four second-round PCRs (S1–S4 primer combinations in Table 1) were performed using 0.85 μL of products from the first-round PCR (F1 or F2 primer combinations in Table 1) with five sarcocysts collected from four horses in Canada (CA20-2_1; CA20-17_1;

Table 1
Primers used for nested PCR for the amplification of NUMT[a] (*cox1* pseudogene) segments.

		Primer name	Primer sequence	Reference
First round PCR:				
F1)	Forward	SF1	5′-ATGGCGTACAACAATCATAAAGAA-3′	[21]
	Reverse	SR9	5′-ATATCCATACCCCATTGCCCAT-3′	[21]
F2)	Forward	SF1	(see above)	
	Reverse	SarcoNUMTcox1_1041R	5′-CAGGCTGAATAGAATTACGAACGA-3′	Present study
Second round PCR:				
S1)	Forward	SF1	(see above)	
	Reverse	SarcoNUMTcox1_889R	5′-TATCCACCTCCAATCCGACG–3′	Present study
S2)	Forward	SarcoNUMTcox1_120F	5′-AAGAATATTCGGTGTTGATTGCCT-3	Present study
	Reverse	SarcoNUMTcox1_1041R	(see above)	
S3)	Forward	SarcoNUMTcox1_292F	5′-TCGTACTACTTGTTGCCCGT-3′	Present study
	Reverse	SarcoNUMTcox1_1041R	(see above)	
S4)	Forward	SarcoNUMTcox1_311F	5′-TTGGATCGGTATTACTGCTTCATG-3′	Present study
	Reverse	SarcoNUMTcox1_1041R	(see above)	

[a] Nuclear-embedded mitochondrial DNA.

CA20–44_5-f14; CA20-54_1; and CA20-54_9). The nested PCR conditions for NUMT segment amplification were similar to those used for mitochondrial *cox1* amplification. Additionally, gene cloning and sequencing of first-round PCR products were performed.

2.4. Alignment of DNA sequences and cox1 haplotype network analysis

Newly obtained gene sequences in this study and related sequences retrieved from the DDBJ/EMBL/GenBank databases (Supplementary Table S1) were aligned using the CLUSTAL W multiple alignment program [30] equipped in MEGA7 [31]. The relationships among various *cox1* haplotypes were visualized using automated haplotype network layout and visualization software 'HapStar' (http://fo.am/hapstar) [32].

2.5. Phylogenetic analyses of rDNA sequences

In this study, the newly obtained cloned LSU rDNA sequences and related sequences retrieved from the DDBJ/EMBL/GenBank databases (MH025629, MH025630, OM971683, OM971684, and OM971687) were aligned using the CLUSTAL W multiple alignment program [30] equipped in MEGA7 [31] with subsequent manual adjustment. Poorly aligned regions and sites with gaps in any sequence were excluded, resulting in 1427 characters for subsequent analysis. Maximum likelihood (ML) analysis was conducted using the PhyML program [33,34] from the 'phylogeny.fr' website (http://www.phylogeny.fr/). The probability of inferred branching evaluated using the approximate likelihood-ratio test (aLRT), which serves as an alternative to the non-parametric bootstrap estimation of branch support [35]. The LSU rDNA sequences of bovine *Sarcocystis* spp. (*S. bovini*, *S. bovifelis*, and *S. sinensis*) were used as outgroups to construct the ML phylogenetic tree. The accession numbers of the analyzed bovine *Sarcocystis* spp. sequences are provided in the phylogenetic tree.

Similarly, cloned SSU rDNA sequences of *S. fayeri*, *S. bertrami*, and *S. asinus*, retrieved from the DDBJ/EMBL/GenBank databases, were processed to construct an ML phylogenetic tree using the 735 trimmed characters. The SSU rDNA sequences of bovine *S. bovifelis* were used as outgroups for the construction of the ML phylogenetic tree, with the accession numbers provided in the figure.

2.6. Prediction of the secondary structure of the partial LSU rRNA molecule of S. bertrami

Based on the secondary structure of 25S rRNA of the budding yeast *Saccharomyces cerevisiae* [36], a basic secondary structure framework of the LSU rRNA molecule of *S. bertrami* was predicted. Highly conserved regions were identified by aligning the LSU rDNA sequences of *S. bertrami* (DDBJ/EMBL/GenBank accession no. MH025629) and *S. cerevisiae* (NR_132207). To predict the secondary structure of the non-conserved regions of the LSU rRNA molecule in *S. bertrami*, the Mfold web server (http://www.unafold.org/mfold/applications/rna-folding-form.php) was used, where RNA folding structures were proposed based on an energy minimization approach [37].

3. Results

3.1. Sarcocyst prevalence and density in horsemeats

Sarcocysts were observed in the muscles of 93 (71/76), 5 (2/41), and 9 % (3/33) of horses from Canada, France, and Japan, respectively. The density of sarcocysts was higher in Canadian horses (0.002–0.857 sarcocysts/cm^2; geometric means: 0.056–0.061 in three consecutive years from 2020), compared with those in horses from France and Japan (0.005–0.007 sarcocysts/cm^2). Initially 48 collected sarcocysts were lined with palisaded walls featuring upright villar protrusions, consistent with *S. fayeri*. Additionally, 11 more sarcocysts (five lined by thick walls and six with thinner walls exhibiting folded or dispersed villar protrusions) were collected from a Canadian horse (Animal ID: CA20–44).

3.2. Molecular characterization of mitochondrial cox1

Fifty-four *cox1* sequences (42 of 1017 bp and 12 of 1052 bp) were successfully amplified using primer pairs of SF1 and SR4, or SF1 and SR5, respectively. Bi-directional sequencing was conducted using two PCR primers and four sequencing primers to avoid ambiguous nucleotide inclusions in the final contiguous sequences of individual sarcocysts. All sequences, except one (1052 bp) with 100 % identity with the mitochondrial *cox1* sequence of *Sarcocystis speeri* Dubey et Lindsay, 1999 (DDBJ/EMBL/GenBank accession no. KT207461), showed high similarity to the *cox1* sequences of muscular sarcocysts in equids, labeled as *S. bertrami* and *S. fayeri*.

Successful direct sequencing of *cox1* sequences indicated no nucleotide variation in metrocytes/bradyzoites from a single sarcocyst, indicating that the 53 newly obtained *cox1* sequences exhibited no intra-individual nucleotide variation. However, these *cox1* sequences exhibited significant inter-individual variations between each other. On average, 8.8 nucleotide substitutions (± 3.9 [SD]; range 0–23 over 1017 bp) were observed between various sequences (Fig. 1). Only five out of 53 *cox1* sequences were shared by different sarcocysts from three horses. The identities among 53 sarcocysts ranged 97.7–100 (99.1 ± 0.4) %.

Fifty-three newly obtained and 43 retrieved *cox1* sequences of equid muscular sarcocysts at the GenBank, labeled as *S. bertrami* or *S. fayeri* (Supplementary Table S1; Nos. 1–43 for retrieved sequences) were

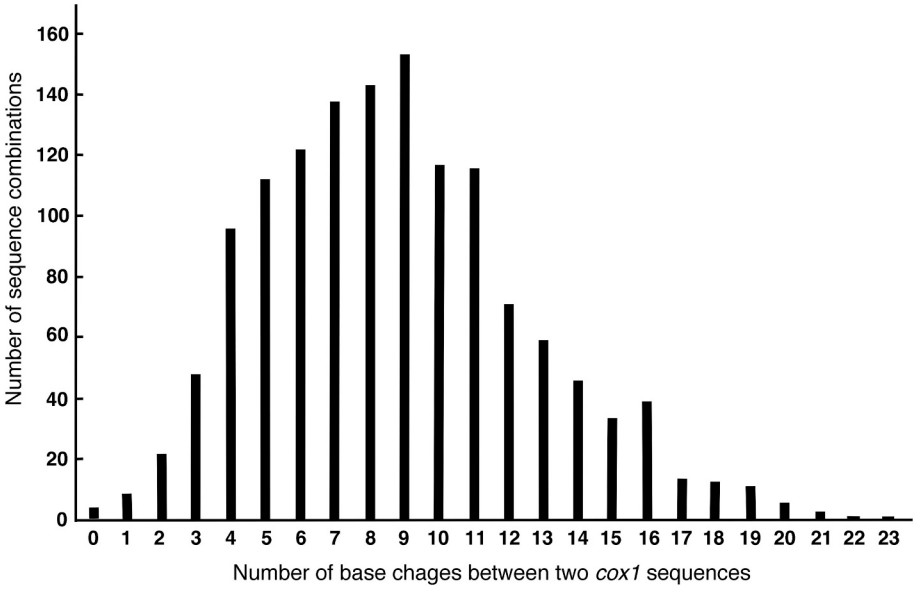

Fig. 1. Inter-individual variations among *cox1* sequences (n = 53) of *Sarcocystis bertrami* assessed in this study. The number of nucleotide substitutions in different combinations between two sequences (n = 1378) is counted.

trimmed to 761 bp and compared for sequence identity: on average, 99.0 % identity (range 97.4–100 %) was observed. Nucleotide sequence identities of muscular sarcocysts collected from Chinese donkeys (labeled as *S. asinus*; OM970235–OM970240) and Brazilian horses (labeled as *S. bertrami*: OP887040–OP887043) exhibited lower identities with the former group of 96 *cox1* sequences: 79.4–81.9 (80.6 ± 0.4)% identities. These values were comparable to 75.0–76.5 (75.9 ± 0.3) % or 73.2–74.8 (74.1 ± 0.3) % identities between *cox1* sequences of equid *Sarcocystis* species (this study) and *S. sinensis* in water buffaloes or *S. medusiformis* in sheep, respectively. Haplotype network analysis was performed on 53 newly obtained *cox1* sequences of horse sarcocysts, 53 retrieved sequences labeled as *S. bertrami*, *S. fayeri*, and *S. aninus* from equids worldwide, and *S. sinensis* from water buffaloes/cats in Egypt (Fig. 2A). The majority of *cox1* haplotypes formed a single major cluster with two side extensions, containing many sarcocyst *cox1* sequences from Chinese donkeys (KY399748, KY399749, KY399757, KY399759, MF152613–MF152615, OM970235–OM970240) along with several sarcocyst sequences from Chinese or Brazilian horses. When translated into amino acid (aa) sequences (254 aa; Fig. 2B), the diversity of *cox1* sequences converged—with a deviated *cox1* sequence group of donkey muscle sarcocysts (KY399748, KY399749, KY399757, and MF152613–MF152615) joining the primary cluster of equid muscle sarcocysts. In contrast, another deviated *cox1* sequence group of donkey muscle sarcocysts (KY399759, and OM970235–OM970240) and Brazilian horse muscle sarcocysts (OP887040–OP887043) remained as a separate cluster.

3.3. PCR amplification of NUMT (cox1 pseudogene) segments

Direct sequencing of NUMT first-round PCR products and their cloned sequences (3–11 in range and 6.3 on average) / first-round amplicon) exhibited complete identities with their aforementioned *cox1* sequences. All four second-round PCR products following different first-round PCRs of one sarcocyst (CA20-54_1) were successfully sequenced, and their 993 bp contiguous sequence (LC856918) had 80.97 % identity (804 / 993) to the mitochondrial *cox1* sequence of the same sarcocyst (LC856906), but 99.40 % identity (987 / 993) to a *cox1* sequence of *S. asinus* from a donkey muscle sarcocyst (OM970235), followed by other donkey muscle sarcocysts (KY399759 and OM970236–OM970240) and Brazilian horse muscle sarcocysts (OP887040–OP887043) with 98.61 % (923 / 936)–99.27 % (951 / 958) (Table 2). All of these sequences, including the newly obtained NUMT sequence, were successfully translated into 312–346 aa, with identities ranging from 98.79 %–99.40 %. The products of the second-round PCR for other sarcocysts (CA20-2_1; CA20-17_1; CA20–44_5-f14; and CA20-54_9), selected arbitrarily, were identical to their mitochondrial *cox1* sequences.

3.4. Molecular characterization of LSU rDNA

Partial LSU rDNA sequences ranging 1570–1607 bp in length were successfully amplified using conventional PCR with KL1 and KL3 primers for 59 sarcocysts. However, direct sequencing of the amplicons consistently failed, indicating intra-individual variation within the sarcocyst gene. Gene cloning of the partial LSU rDNA amplicons produced 211 sequences for 54 sarcocysts: 3.9 ± 1.5 (1–8) [average ± SD (range)] clones per sarcocyst. Finally, 140 genotypes of the partial LSU rDNA (1584 ± 6.7 [mean ± SD] bp in length) were selected after eliminating identical sequences from 38 genotypes observed in clones originating from 34 sarcocysts (Supplementary Table S2). Only one genotype was shared among different sarcocysts in three horses from Canada (n = 2) and France (n = 1), and seven genotypes were detected in an amplicon from a single sarcocyst. The nucleotide sequence similarity of the 140 genotypes ranged 77.5–99.9 %. Of the 1589 sites in the *S. bertrami* LSU rDNA sequence (accession no. MH025629), nucleotides at 756 sites (47.6 % of 1589 sites) were conserved across all 140 newly obtained genotypes in this study, while 647 sites (40.7 %) exhibited nucleotide differences in one to three genotypes compared to the others. Although nucleotide substitutions or insertions/deletions (indels) of different genotypes and a representative *S. bertrami* LSU rDNA sequence were distributed along the length, 69 sites of nucleotide alterations shared by ≥10 genotypes (Supplementary Fig. S1) primarily occurred around the hairpin loops, as depicted in the LSU rDNA secondary structure (Supplementary Fig. S2). Regardless of the high variations in LSU rDNA nucleotide sequences, phylogenetic analysis of the newly obtained sequences in this study and retrieved sequences of *S. fayeri*, *S. bertrami* and *S. asinus* LSU rDNA from the DNA database (MH025629, MH025630, and OM971683–OM971687) demonstrated their monophyletic relationships, as illustrated in Fig. 3. Similarly, SSU rDNA sequences retrieved from the DNA database exhibited monophyletic relationships regardless of their labeling (*S. fayeri*, *S. bertrami* and *S. asinus*), as illustrated in Supplementary Fig. S3.

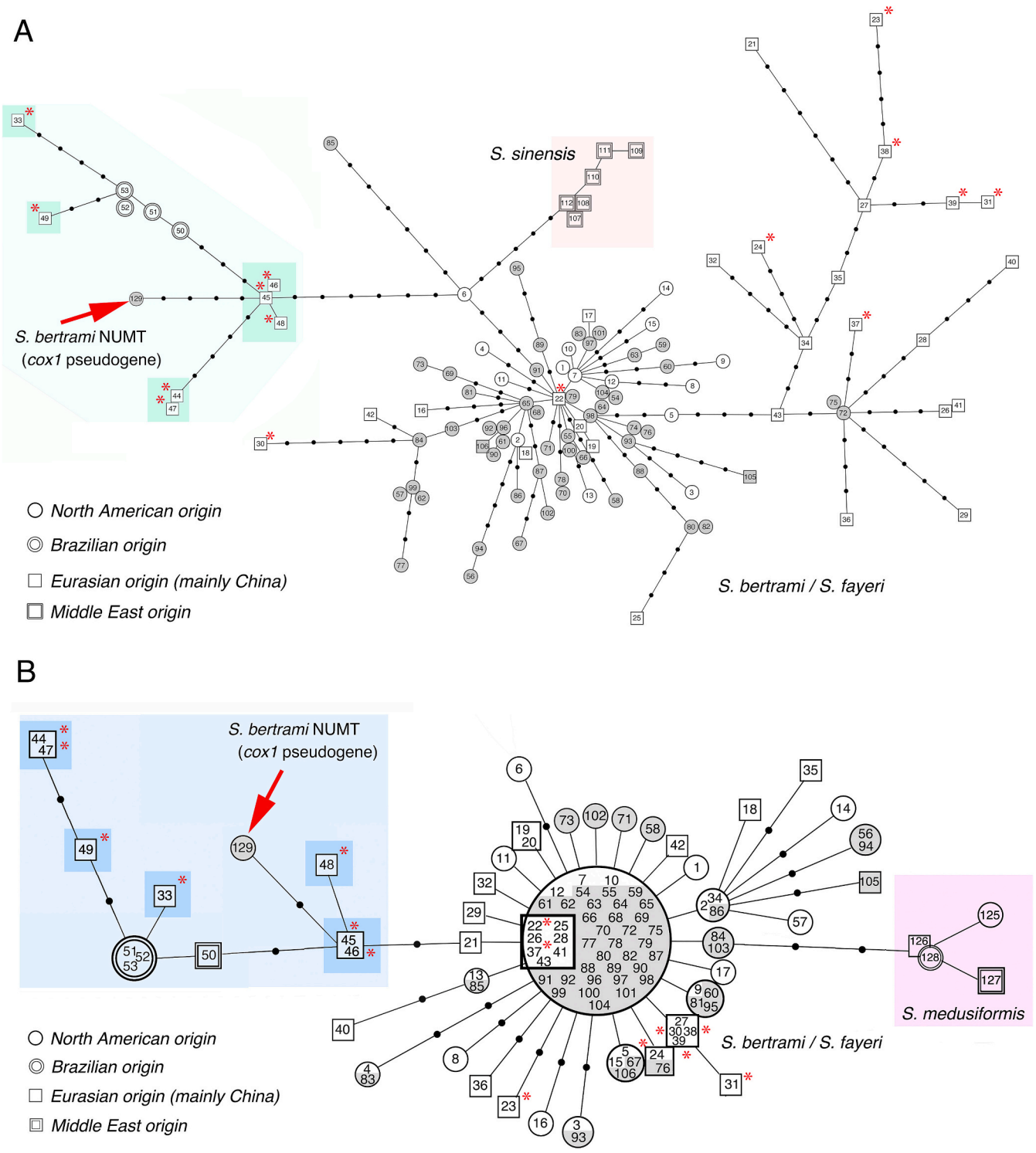

Fig. 2. Haplotype network analysis of 53 newly obtained *cox1* sequences and one NUMT sequence of *S. bertrami* sarcocysts, along with 53 retrieved sequences labeled as *S. bertrami*, *S. fayeri*, and *S. asinus* from equids worldwide in GenBank. Each plot in this figure represents different *cox1* and NUMT sequences detailed in Supplementary Table S1. (A) Nucleotide sequences (761 bp); and (B) amino acid sequences (254 aa). Sequences from sarcocysts of donkeys are indicated by asterisks (*), and sequences newly obtained in this study are shown with a shaded background. *Sarcocystis bertrami* NUMT (*cox1* pseudogene) nucleotide and amino acid sequences newly obtained in this study (DDBJ/EMBL/GenBank accession no. LC856918) are indicated by arrows. Plots for sequences labeled as *S. asinus* in GenBank are shown with a green background in (A), and a blue background in (B). All sequences dispersed in light green and light blue areas are considered to be NUMT sequences of *S. bertrami* as mentioned in the text. Sequences of closely-related but distinct species (*Sarcocystis sinensis* for nucleotide sequences and *Sarcocystis medusiformis* for amino acid sequences) are included for reference purposes only. Plots for *Sarcocystis truncata* sequences are omitted from the figure due to their distant location from the sequences shown here. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2
Comparison of partial NUMT (*cox1* pseudogene) nucleotide and amino acid sequences of *Sarcocystis bertrami*.

GenBank accession no.	GenBank Labeling	Length (bp)	ID no. in Fig. 2	Nucleotide position where any changes are observed[a]															
				93	123	363	365	385	450	487	507	543	564	597	637	641	645	652	762
LC856918	*S. bertrami*	993	129	T	A	T	T	A	T	G	T	C	T	G	C	C	G	G	G
OM970235	*S. asinus*	1038	46	·	·	C	·	·	·	·	·	·	C	T	·	·	·	·	·
OM970236	*S. asinus*	1038	47	·	·	C	·	·	·	G	G	·	·	T	T	T	·	·	·
OM970237	*S. asinus*	1038	48	·	·	C	·	G	·	·	·	·	C	T	·	·	·	·	·
OM970238	*S. asinus*	1038	49	·	·	A	C	·	C	G	G	·	·	T	·	·	·	·	A
OM970239	*S. asinus*	1038	44	·	·	C	·	·	·	G	G	·	·	T	T	T	·	·	·
OM970240	*S. asinus*	1038	45	·	·	C	·	·	·	·	·	·	C	T	·	·	·	·	·
OP887040	*S. bertrami*	958	50	·	G	C	·	·	·	G	·	·	·	T	·	·	·	·	A
OP887041	*S. bertrami*	958	51	·	G	A	·	·	·	G	·	·	·	T	·	·	·	·	A
OP887042	*S. bertrami*	949	52	·	G	A	·	·	C	G	·	·	·	T	·	·	·	·	A
OP887043	*S. bertrami*	949	53	·	G	A	·	·	C	G	·	·	·	T	·	·	·	·	A
KY399759	*Sarcocystis* sp.	936	33	A	G	A	C	·	C	G	·	T	·	T	·	·	A	A	A

GenBank accession no.	GenBank Labeling	Length (bp)	ID no. in Fig. 2	*(continued)*					Length (aa)	Amino acid position where any changes are observed[b]							
				798	888	927	975	985		50	105	129	163	169	214	218	329
LC856918	*S. bertrami*	993	129	A	C	C	T	A	331	T	F	N	G	C	A	V	T
OM970235	*S. asinus*	1038	46	G	·	T	C	·	346	·	L	·	C	·	·	·	·
OM970236	*S. asinus*	1038	47	G	·	T	C	G	346	·	L	·	·	W	V	·	A
OM970237	*S. asinus*	1038	48	G	T	T	C	·	346	·	L	D	C	·	·	·	·
OM970238	*S. asinus*	1038	49	G	·	T	C	·	346	·	I	·	·	W	·	·	·
OM970239	*S. asinus*	1038	44	G	·	T	C	G	346	·	L	·	·	W	V	·	A
OM970240	*S. asinus*	1038	45	G	T	T	C	·	346	·	L	·	C	·	·	·	·
OP887040	*S. bertrami*	958	50	G	·	T	C	·	320	A	L	·	·	·	·	·	·
OP887041	*S. bertrami*	958	51	G	·	T	C	·	320	A	I	·	·	·	·	·	·
OP887042	*S. bertrami*	949	52	G	·	T	C	·	317	A	I	·	·	·	·	·	·
OP887043	*S. bertrami*	949	53	G	·	T	C	·	317	A	I	·	·	·	·	·	·
KY399759	*Sarcocystis* sp.	936	33	G	·	T			312	A	I	·	·	·	·	I	

[a] Nucleotide position is expressed relative to the 5′-end of NUMT of *S. bertrami* collected from a horse (DDBJ/EMBL/GenBank accession no. LC856918). Dots denote a base identical to that of the *S. bertrami* NUMT sequence in the top row.
[b] Amino acid position is expressed relative to the amino acid sequence of NUMT of *S. bertrami* collected from a horse (DDBJ/EMBL/GenBank accession no. LC856918). Dots denote an amino acid identical to that of the *S. bertrami* NUMT sequence in the top row.

3.5. Incidental detection of *S. cf. speeri* DNA in a horsemeat sample

A *cox1* nucleotide sequence (LC856919; 1052 bp) with 100 % identity to the mitochondrial *cox1* sequence of *S. speeri* sporocysts in an opossum (*Didelphis alviventris* Lund, 1840) in Argentina (KT207461) was obtained from the esophageal muscle of a 5-year-old castrated horse imported from Canada (2020–44), which spent 113 days in Japan before slaughter. Additionally, this newly obtained sequence exhibited 100 % identity with the *cox1* sequences of *S.* cf. *falcatula* schizonts in the liver of *Eclectus roratus* (Müller, 1776) or in the lungs of *Polytelis alexandrae* Gould, 1863 in Argentina (MZ962976 and MZ962977, respectively), or *S. falcatula* schizonts in the lungs of *Trichoglossus moluccanus* (Gmelin, 1788) in Philadelphia, USA (MH665257) as illustrated in Table 3A. Two LSU rDNA genotypes (LC857066 and LC857067; 1527-bp) were sequenced from the same muscle sample, exhibiting 99.80 % identity (1524/1527) with each other. These newly obtained sequences exhibited 99.86–99.93 % identity (1521/1522, or 1520/1522) with the LSU rDNA sequence of *S. speeri* sporocysts in an opossum in Argentina (KT207460) and cerebral schizonts of *S. neurona* in a horse with protozoan myeloencephalitis in USA (AF092927) with 99.80–99.87 % identity (1525/1527, or 1524/1527) as illustrated in Table 3B.

4. Discussion

Phenotypical traits, such as different dimensions and prepatent periods of sporocysts, microscopic and ultrastructural differences in villar protrusions of sarcocysts, and different geographical distributions with prominence (Old World vs. New World), have divided *Sarcocystis* species in equid muscles into *S. bertrami* and *S. fayeri* after synonymizing *S. equicanis* with *S. bertrami* [5]. Owing to its inadequate original description by Gadaev [38], *Sarcocystis asinus*, which is still used for sarcocysts in the muscles of donkeys, has been indicated as a *nomen*

Table 3A
Partial *cox1* nucleotide sequences closely associated with *S.* cf. *speeri* detected molecularly in a horse imported from Canada.

Parasite species	GenBank accession no.	Length (bp)	Host species	Country	Nucleotide position where any changes are observed[a]	
					197	347
S. cf. *speeri*	**LC856919**	1052	*Equus caballus*	Japan	T	A
S. speeri	KT207461	1057	*Didelphis albiventris*	Argentina	·	·
S. falcatula	MH665257	1002	*Trichoglossus moluccanus*	USA	·	·
S. cf. *falcatula*	MZ962974	975	*Eclectus roratus*	Argentina	·	·
S. cf. *falcatula*	MZ962976	1055	*Eclectus roratus*	Argentina	·	·
S. cf. *falcatula*	MZ962977	1055	*Polytelis alexandrae*	Argentina	·	·
S. neurona	MN175967	547	*Felis silvestris catus*	Brazil	A	C

[a] Nucleotide position is expressed relative to the *cox1* nucleotide sequence of *S.* cf. *speeri* collected from a horse (DDBJ/EMBL/GenBank accession no. LC856919). Dots denote a nucleotide identical to the *S.* cf. *speeri cox1* sequence in the top row.

Table 3B
Partial LSU rDNA nucleotide sequences closely associated with *S.* cf. *speeri* detected molecularly in a horse imported from Canada.

Parasite species	GenBank accession no.	Length (bp)	Host species	Country	Nucleotide position where any changes are observed[b]			
					473	482	896	1224
S. cf. *speeri*	**LC857066**	1052	*Equus caballus*	Japan	A	C	T	C
S. cf. *speeri*	**LC857067**	1052	*Equus caballus*	Japan	G	·	C	T
S. speeri	KT207461	1057	*Didelphis albiventris*	Argentina	·	·	·	T
S. neurona	MZ962974	975	*Eclectus roratus*	Argentina	·	G	·	T

[b] Nucleotide position is expressed relative to the LSU rDNA nucleotide sequence of *S.* cf. *speeri* collected from a horse (DDBJ/EMBL/GenBank accession no. LC857066). Dots denote a nucleotide identical to the *S.* cf. *speeri cox1* sequence in the top row.

nudum [10]. Recent genetic studies using mitochondrial *cox1* and nuclear rDNA as molecular markers have demonstrated the conspecificity of *S. bertrami* and *S. fayeri* [15,16]. An ultrastructural study by Dubey et al. [10] observed solely *S. bertrami*-type villar protrusions lining sarcocysts in the muscle of Egyptian donkeys, whereas Zeng et al. [15] noted both *S. bertrami*- and *S. fayeri*-type of villar protrusions in the muscle of Chinese donkeys, corresponding to type 11c and type 11a as described by Dubey et al. [5], respectively. These upright (*S. fayeri*, type 11a) and folded (*S. bertrami,* type 11c) villar protrusions create a thicker or thinner sarcocyst wall under light microscopy. Additionally, while the extension of microtubules from the villar tips to the ground substance and the abundance of ovoid osmiophilic bodies along the length of the microtubules within the villar protrusion are indicated as distinguishing features between these two species [5,10], Ma et al. [16] observed these ultrastructural characteristics in sarcocysts from donkeys and horses in China. This suggests that morphological traits thought to distinguish the two species for half a century following Dubey et al. [11] in 1977 are indeed variations within a single species, rather than discontinuous features morphologically separating the two species [15,39]. Similarly, Erber and Geisel [40] recovered two types of sarcocysts in the muscle of horses slaughtered in München, Germany: sarcocysts with folded hair-like villar protrusions (5–11 μm long by <0.5 μm wide) of *S. bertrami* (syn. *S. equicanis*) and sarcocysts with upright finger-like villar protrusions (2.5–4.5 mm long by 0.8–1.0 mm wide) of *S. fayeri*. In this study, we also collected two types of sarcocysts from horses originating in Canada.

Regarding the bimodal separation of sporocyst dimensions and the prepatent period between typical *S. bertrami* and *S. fayeri* [11,41], subsequent studies failed to demonstrate such a clear separation. Temporal alterations in oocyst dimensions during the patent period or different sharp indices (i.e., length / width) of excreted oocysts from hosts inoculated with single-oocyst-driven lines of avian coccidia (*Eimeria dispersa* and *E. adnoeides* in turkeys) were reported [42,43]. These parameters may affect the different sporocyst dimensions observed by researchers, even if they are reported for a single species.

The phenotypic features of commonly studied equid *Sarcocystis* spp. (*S. bertrami* and *S. fayeri*) overlap, making species identification challenging and often reliant on the researcher's discretion. SSU rDNA, LSU rDNA, ITS-1, and mitochondrial *cox1* sequences are effective molecular markers for the identification of *Sarcocystis* species [21–25,28,44–48]. To infer accurate taxonomic relationships between different *Sarcocystis* isolates from equids, a substantial collection of nucleotide sequences of the aforementioned molecular markers has been compiled. It is now widely accepted that *cox1* sequencing of equid sarcocysts is a reliable species identification approach owing to its direct sequencing from a single sarcocyst [15,16,26]. In contrast, divergent rDNA genes, even in a single bradyzoite/sarcocyst, require gene cloning to obtain unambiguous nucleotide sequences, as in other *Sarcocystis* spp. [21–23,25].

This study revealed significant variation in the *cox1* and LSU rDNA sequences of equid sarcocysts, in contrast to *S. speeri*, which exhibited highly consistent sequences with the gene sequences reported in previous studies [49]. This indicates that the nucleotide variation range depends on specific *Sarcocystis* species [22–24,50,51]. Murata et al. [26] revealed intraspecific variation of up to 9.8 % in *S. fayeri* SSU rDNA sequences from eight individual bradyzoites from a single sarcocyst, whereas *cox1* sequences from the six fragments of a single sarcocyst were 100 % identical. The present study further explored the variation in *cox1* sequences in individual sarcocysts of a single species isolated from an individual host animal, highlighting the broad intraspecific *cox1* sequence variations of different *Sarcocystis* spp., excluding species such as *S. speeri*, *S. falcata*, and *S. neurona*. The high intraspecific variation of rDNA sequences in equid sarcocysts, derived from individual bradyzoites, aligns with the divergent rDNA sequences of other apicomplexan parasites, such as *Plasmodium* spp., *Babesia bigemina*, *Cryptosporidium parvum*, and *Eimeria meleagrimitis* [52–57]. Additionally, a similar significant rRNA array mosaicism has been observed in the human nuclear genome, although it was previously accepted that the ribosomal genes were tandemly arrayed and underwent concerted homogenization in most eukaryotic organisms [58–61]. Although intentional sequencing of rDNA formed an artificial phylogenetic lineage, unintentional sequencing of rDNA segments demonstrated diversity and significant rRNA array mosaicism unique to each species (Fig. 3).

Gjerde [23] reported that the intraspecific *cox1* sequence identities of *S. bovifelis*, *S. bovini* and *S. sinensis* were 98.4–100 % (n = 45), 99.3–100 % (n = 24), and 99.5–100 % (n = 7), respectively. These values are comparable to the intraspecific *cox1* sequence identity of equid sarcocysts observed in this study: 97.7–100 % (99.1 ± 0.4) (n = 53). Simultaneously, Gjerde [23] recorded an aberrant type of *cox1* sequences with only 91–92 % identity to the predominant *cox1* type of the same species, and approximately 98 % identity to the aberrant types of the other two species. These sequences were classified as non-functional pseudogenes (NUMTs), as previously noted in *Toxoplasma gondii*, *Neospora caninum*, *Sarcocystis gigantea*, *S. rangiferi*, *S. silva*, *S. tarandi* and several other *Sarcocystis* spp. [21,62,63]. This study demonstrated several *cox1* sequence derivatives forming a minor assemblage distinct from the major *cox1* assemblage of equid sarcocysts as NUMT segments. Therefore, excluding these NUMT sequences (KY399759, OM970235–OM970240, OP887040–OP887043, and LC856918), all *cox1* sequences of equid sarcocysts, including those obtained in this study, are true *cox1* haplotypes of a single species that exhibit the morphological features of either *S. bertrami* or *S. fayeri*. Taxonomically, the species forming sarcocysts in equids (horses, mules, and donkeys) is *Sarcocystis bertrami* Doflein, 1901, with *Sarcocystis fayeri* Dubey et al., 1977 as its junior synonym, as indicated recently [15,16,64]. Additionally, it should be highlighted that unique *cox1*-like sequences of donkey muscle sarcocysts [15,65] and horse muscle sarcocysts [66] are the NUMT segments of *S. bertrami*. This explains why the SSU and LSU rDNA sequences of the isolates reported by Zhang et al. [65] (OM971696–OM971701) formed a minor subclade within the major monophyletic assemblage of the equid *S. bertrami* (syn. *S. fayeri*).

From one of 21 sarcocysts collected from a 5-year-old castrated horse imported from Canada (2020–44), the partial *cox-1* (LC856919; 1052 bp) and LSU rDNA (LC857066 and LC857067; 1527 bp) sequences of *S.* cf. *speeri* were molecularly detected. In contrast to the divergent *cox1* and rDNA sequences of *S. bertrami*, these three newly obtained sequences exhibited 100 % or 99.9 % identity with the deposited *S. speeri* sequences of each gene in the GenBank (KT207461, and KT207460, respectively), as shown in Table 3. *Sarcocystis* spp. that use opossums

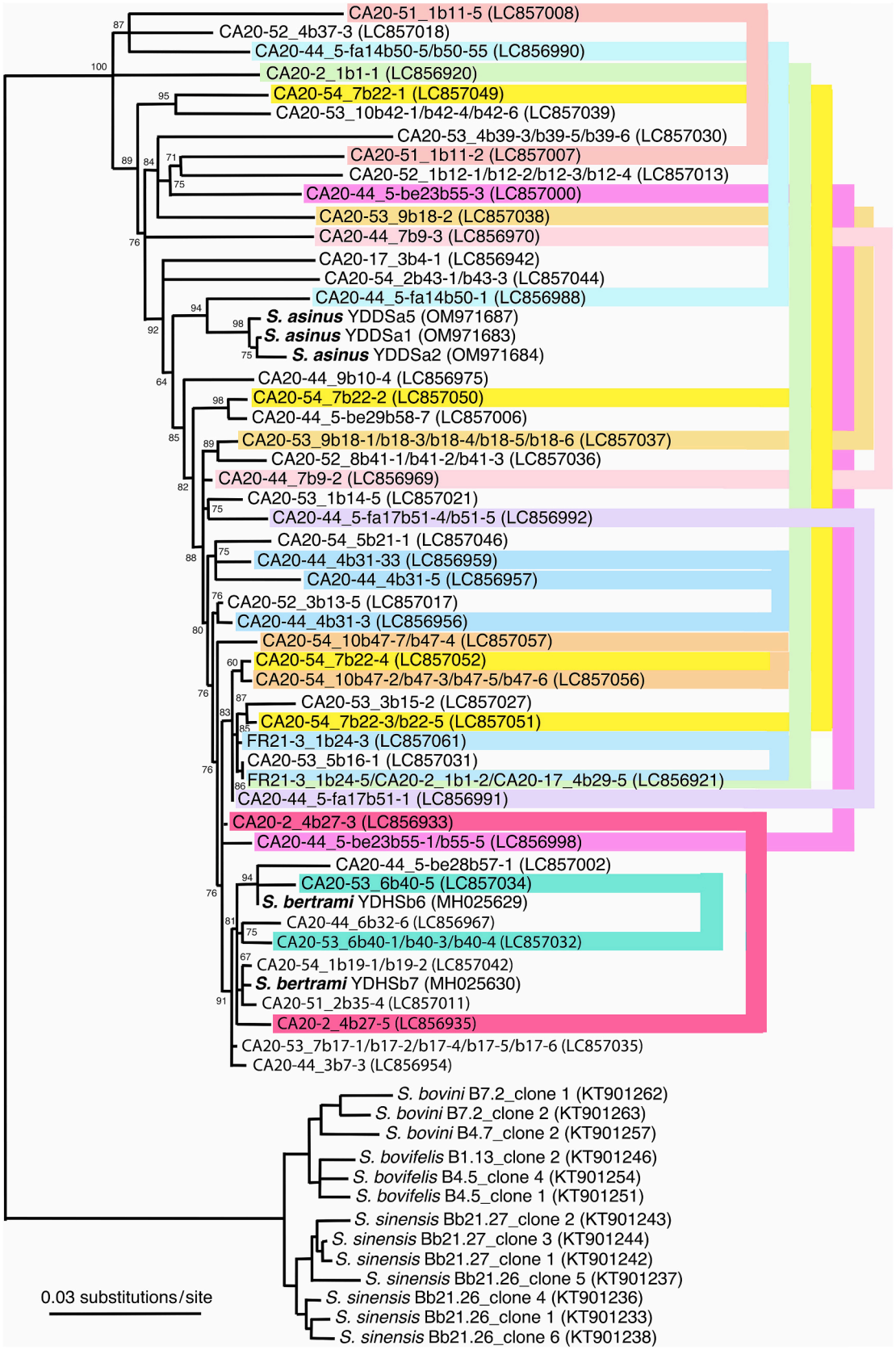

Fig. 3. Maximum Likelihood phylogenetic tree of cloned LSU rDNA sequences (1427 characters) of newly collected sarcocysts in this study (*Sarcocystis bertrami*) and retrieved LSU rDNA sequences of *S. bertrami* and *S. asinus* from the DNA database (MH025629, MH025630, OM971683, OM971684, and OM971687). Each isolate name is followed by its clone name, and then accession number from DDBJ/EMBL/GenBank in parentheses. Cloned LSU rDNA sequences obtained from the same sarcocyst (isolate) are connected by lines of the same color.

(*Didelphis* spp.) as definitive hosts, including *S. neurona*, *S. falcatula* Stiles, 1893, and *S. speeri*, have non-variable *cox1* and rDNA sequences but exhibit distinct phenotypic traits, such as different natural and experimental intermediate hosts and varying pathogenicities in aberrant hosts [5]. *Sarcocystis neurona* is the primary cause of equine protozoal myeloencephalitis (EPM) in aberrant host horses, where schizonts and merozoites are observed in the central nervous system [6,7]. This species uses armadillos, raccoons, sea otters, skunks, and cats as natural intermediate hosts, with sarcocysts forming in the muscles [5,7,67–70]. Natural intermediate hosts of *S. falcatula* or *S. falcatula*-like species include New World passerines, columbiformes and psittacines [71,72], causing pulmonary and/or neurological diseases in Old World psittacines in captivity [73–77]. The intermediate hosts of *S. speeri* remain unknown, but sarcocyst growth has been reported in immunodeficient mice fed sporocysts from opossums [49,78]. Similarly, *S. neurona* infects immunodeficient mice but not birds, as in case of *S. speeri* [79]. Cutler et al. [80] demonstrated that *S. falcatula* is unable to infect horses, because it does not infect immunodeficient mice [79].

Although horses are considered aberrant hosts for *S. neurona*, which causes EPM and is unable to develop forming sarcocysts in the muscle [6,7], Mullaney et al. [81] described putative *S. neurona* sarcocysts in the tongue and its schizonts in the brain of a naturally infected horse based on specific differentiation using ultrastructure, immunohistochemistry and molecular evidence. Molecular analysis of the *cox1* and LSU rDNA indicated that a sample from the esophageal muscle of a 5-year-old horse from Canada (2020–44) was more likely *S. speeri* rather than *S. neurona.* Because the horse with this infection was slaughtered after 113 days' stay in Japan before slaughter, it had ingested *S. speeri* or its relative species' sporocysts from an environment contaminated with opossum feces on the North American continent.

In this study, we demonstrated notably divergent *cox1* and LSU rDNA sequences of *S. bertrami* (syn. *S. fayeri*) in equid hosts. The *cox1* marker offers greater specificity for identifying species in host animals compared to the rDNA marker. A single isolated sarcocyst revealed a uniform mitochondrial *cox1* sequence through conventional PCR, whereas divergent rDNA sequences were observed in a single sarcocyst or even a single bradyzoite, due to nuclear rRNA array mosaicism in most *Sarcocystis* spp. To ensure accurate species identification, it is vital to carefully differentiate mitochondrial *cox1* sequences from the NUMT segments (*cox1* pseudogenes), which can be easily co-amplified with commonly used specific primers (e.g. SF1/SR9 [23]).

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Author's contribution

JT, JM, HO, and HS conceived and designed the study. JT, JM, and HO conducted sample collection and data analyses of horses. KM, MY, RA, BKS, and HS conducted molecular analyses and data organization. JT and HS wrote the original draft, and all authors contributed to refining the article.

CRediT authorship contribution statement

Junko Toda: Writing – original draft, Validation, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Jiro Miyasaka:** Project administration, Methodology, Investigation, Data curation. **Hideo Osako:** Project administration, Methodology, Investigation, Data curation, Conceptualization. **Koichi Murata:** Visualization, Methodology, Investigation, Data curation. **Muchammad Yunus:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Reski Amalia:** Visualization, Methodology, Investigation, Data curation. **Babi Kyi Soe:** Writing – original draft, Software, Methodology, Investigation, Formal analysis, Data curation. **Hiroshi Sato:** Writing – review & editing, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Ethical standards

Not applicable.

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Declaration of competing interest

The authors declare that there are no conflicts of interest.

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Data availability

The nucleotide sequences (*cox1*, LSU rDNA, and NUMT) reported in this study are available in the DDBJ/EMBL/GenBank databases under the accession numbers LC856865– LC857068.

References

[1] J.P. Dubey, A review of *Sarcocystis* of domestic animals and of other coccidia of cats and dogs, J. Am. Vet. Med. Assoc. 169 (1976) 1061–1078, https://doi.org/10.2460/javma.1976.169.10.1061.

[2] J.K. Frenkel, *Besnoitia wallacei* of cats and rodents: with a reclassification of other cyst-forming isosporoid coccidia, J. Parasitol. 63 (1977) 611–628, https://doi.org/10.2307/3279560.

[3] N.D. Levine, The taxonomy of *Sarcocystis* (Protozoa, Apicomplexa) species, J. Parasitol. 72 (1986) 372–382, https://doi.org/10.2307/3281676.

[4] K. Odening, The present state of species-systematics in *Sarcocystis* Lankester, 1882 (Protista, Sporozoa, Coccidia), Syst. Parasitol. 41 (1998) 209–233, https://doi.org/10.1023/A:1006090232343.

[5] J.P. Dubey, R. Calero-Bernal, B.M. Rosenthal, C.A. Speer, R. Fayer, Sarcocystosis of Animals and Humans, 2nd ed., CRC Press, Florida, USA, 2016.

[6] J.P. Dubey, S.W. Davis, C.A. Speer, D.D. Bowman, A. de Lahunta, D.E. Granstrom, M.J. Topper, A.N. Hamir, J.F. Cummings, M.M. Suter, *Sarcocystis neurona* n. sp. (Protozoa: Apicomplexa), the etiologic agent of equine protozoal myeloencephalitis, J. Parasitol. 77 (1991) 212–218, https://doi.org/10.2307/3283084.

[7] J.P. Dubey, D.K. Howe, M. Furr, W.J. Saville, A.E. Marsh, S.M. Reed, M.E. Grigg, An update on *Sarcocystis neurona* infections in animals and equine protozoal myeloencephalitis (EPM), *Vet.* Parasitol. 209 (2015) 1–42, https://doi.org/10.1016/j.vetpar.2015.01.026.

[8] C.K. Fenger, D.E. Granstrom, A.A. Gajadhar, N.M. Williams, S.A. McCrillis, S. Stamper, J.L. Langemeier, J.P. Dubey, Experimental induction of equine protozoal myeloencephalitis in horses using *Sarcocystis* sp. sporocysts from the opossum (*Didelphis virginiana*), Vet. Parasitol. 68 (1997) 199–213, https://doi.org/10.1016/s0304-4017(96)01112-0.

[9] N.D. Levine, W. Tadros, Named species and hosts of *Sarcocystis* (Protozoa: Apicomplexa: Sarcocystidae), Syst. Parasitol. 2 (1980) 41–59, https://doi.org/10.1007/BF00015094.

[10] J.P. Dubey, E. Van Wilpe, S.K. Verma, M. Hilali, Ultrastructure of *Sarcocystis bertrami* sarcocysts from a naturally infected donkey (*Equus asinus*) from Egypt, Parasitology 143 (2016) 18–23, https://doi.org/10.1017/S0031182015001432.
[11] J.P. Dubey, R.H. Streitel, P.C. Stromberg, M.J. Toussant, *Sarcocystis fayeri* sp. n. from the horse, J. Parasitol. 63 (1977) 443–447, https://doi.org/10.2307/3279997.
[12] F.R. Matuschka, Infectivity of *Sarcocystis* from donkey for horse via sporocysts from dogs, Z. Parasitenkd. 69 (1983) 299–304, https://doi.org/10.1007/BF00927871.
[13] S.P. Tinling, G.H. Cardinet III, L.L. Blythe, M. Cohen, S.L. Vonderfecht, A light and electron microscopic study of sarcocysts in a horse, J. Parasitol. 66 (1980) 458–465, https://doi.org/10.2307/3280748.
[14] B.L. Munday, D.L. Obendorf, Morphology of *Sarcocystis gigantea* in experimentally-infected sheep, Vet. Parasitol. 16 (1984) 193–199, https://doi.org/10.1016/0304-4017(84)90036-0.
[15] W. Zeng, L. Sun, Z. Xiang, N. Li, J. Zhang, Y. He, Q. Li, F. Yang, J. Song, J. Morris, B.M. Rosenthal, L. Sun, H. Liu, Z. Yang, Morphological and molecular characteristics of *Sarcocystis bertrami* from horses and donkeys in China, Vet. Parasitol. 252 (2018) 89–94, https://doi.org/10.1016/j.vetpar.2018.01.024.
[16] C.-L. Ma, Y.-L. Ye, T. Wen, Z.-M. Huang, J. Pan, J.-J. Hu, J.-P. Tao, J.-L. Song, Prevalence and morphological and molecular characteristics of *Sarcocystis bertrami* in horses in China, Parasite 27 (2020) 1, https://doi.org/10.1051/parasite/2019078.
[17] M. Saito, Food poisoning caused by *Sarcocystis fayeri* associated with ingestion of raw horse meat, J. Vet. Epidemiol. 16 (2012) 114–125 (in Japanese with English summary), https://doi.org/10.2743/jve.16.114.
[18] S. Harada, M. Furukawa, E. Tokuoka, K. Matsumoto, S. Yahiro, J. Miyasaka, M. Saito, Y. Kamata, M. Watanabe, D. Irikura, H. Matsumoto, Y. Sugita-Konishi, Control of toxicity of *Sarcocystis fayeri* in horsemeat by freezing treatment and prevention of food poisoning caused by raw consumption of horsemeat, Shoku-eisei-shi [J. Food Hyg. Soc. Jpn.]. 54 (2013) 198–203 (in Japanese with English summary).
[19] Y. Kamata, M. Saito, D. Irikura, Y. Yahata, T. Ohnishi, T. Bessho, T. Inui, M. Watanabe, Y. Sugita-Konishi, A toxin isolated from *Sarcocystis fayeri* in raw horsemeat may be responsible for food poisoning, J. Food Prot. 77 (2014) 814–819, https://doi.org/10.4315/0362-028X.JFP-13-351.
[20] D. Irikura, M. Saito, Y. Sugita-Konishi, T. Ohnishi, K.-I. Sugiyama, M. Watanabe, A. Yamazaki, S. Izumiyama, H. Sato, Y. Kimura, R. Doi, Y. Kamata, Characterization of *Sarcocystis fayeri*'s actin-depolymerizing factor as a toxin that causes diarrhea, Genes Cells 22 (2017) 825–835, https://doi.org/10.1111/gtc.12515.
[21] B. Gjerde, Phylogenetic relationships among *Sarcocystis* species in cervids, cattle and sheep inferred from the mitochondrial cytochrome *c* oxidase subunit I gene, Int. J. Parasitol. 43 (2013) 579–591, https://doi.org/10.1016/j.ijpara.2013.02.004.
[22] B. Gjerde, *Sarcocystis* species in red deer revisited: with a re-description of two known species as *Sarcocystis elongata* n. sp. and *Sarcocystis truncata* n. sp. based on mitochondrial *cox1* sequences, Parasitology 141 (2014) 441–452, https://doi.org/10.1017/S0031182013001819.
[23] B. Gjerde, Molecular characterisation of *Sarcocystis bovifelis*, *Sarcocystis bovini* n. sp., *Sarcocystis hirsuta* and *Sarcocystis cruzi* from cattle (*Bos taurus*) and *Sarcocystis sinensis* from water buffaloes (*Bubalus bubalis*), Parasitol. Res. 115 (2016) 1473–1492, https://doi.org/10.1007/s00436-015-4881-5.
[24] B. Gjerde, T.D. Josefsen, Molecular characterisation of *Sarcocystis lutrae* n. sp. and *Toxoplasma gondii* from the musculature of two Eurasian otters (*Lutra lutra*) in Norway, Parasitol. Res. 114 (2015) 873–886, https://doi.org/10.1007/s00436-014-4251-8.
[25] N. Abe, K. Matsuo, J. Moribe, Y. Takashima, T. Irie, T. Baba, B. Gjerde, Morphological and molecular characteristics of seven *Sarcocystis* species from sika deer (*Cervus nippon centralis*) in Japan, including three new species, Int. J. Parasitol. Parasites Wildl. 10 (2019) 252–262, https://doi.org/10.1016/j.ijppaw.2019.10.002.
[26] R. Murata, J. Suzuki, A. Hyuga, T. Shinkai, K. Sadamasu, Molecular identification and characterization of *Sarcocystis* spp. in horsemeat and beef marketed in Japan, Parasite 25 (2018) 27, https://doi.org/10.1051/parasite/2018026.
[27] A. Untergasser, I. Cutcutache, T. Koressaar, J. Ye, B.C. Faircloth, M. Remm, S. G. Rozen, Primer 3—new capabilities and interfaces, Nucleic Acids Res. 40 (2012) e115, https://doi.org/10.1093/nar/gks596.
[28] N.B. Mugridge, D.A. Morrison, A.M. Johnson, K. Luton, J.P. Dubey, J. Votýpka, A. M. Tenter, Phylogenetic relationships of the genus *Frenkelia*: a review of its history and new knowledge gained from comparison of large subunit ribosomal ribonucleic acid gene sequences, Int. J. Parasitol. 29 (1999) 957–972, https://doi.org/10.1016/s0020-7519(99)00062-4.
[29] H. Sato, S. Ihara, O. Inaba, Y. Une, Identification of *Euryhelmis costaricensis* metacercariae in the skin of Tohoku hynobiid salamanders (*Hynobius lichenatus*), northeastern Honshu, Japan, J. Wildl. Dis. 46 (2010) 832–842, https://doi.org/10.7589/0090-3558-46.3.832.
[30] J.D. Thompson, D.G. Higgins, T.J. Gibson, Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice, Nucleic Acids Res. 22 (1994) 4673–4680, https://doi.org/10.1093/nar/22.22.4673.
[31] S. Kumar, G. Stecher, K. Tamura, MEGA7: molecular evolutionary genetics analysis version 7/0 for bigger datasets, Mol. Biol. Evol. 33 (2016) 1870–1874, https://doi.org/10.1093/molbev/msw054.
[32] A.G. Teacher, D.J. Griffiths, HapStar: automated haplotype network layout and visualization, Mol. Ecol. Resour. 11 (2011) 151–153, https://doi.org/10.1111/j.1755-0998.2010. 02890.x.
[33] S. Guindon, O. Gascuel, A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood, Syst. Biol. 52 (2003) 696–704, https://doi.org/10.1080/10635150390235520.
[34] A. Dereeper, V. Guignon, G. Blanc, S. Audic, S. Buffet, F. Chevenet, J.F. Dufayard, S. Guindon, V. Lefort, M. Lescot, J.-M. Claverie, O. Gascuel, Phylogeny.fr: robust phylogenetic analysis for the non-specialist, Nucleic Acids Res. 36 (2008) W465–W469, https://doi.org/10.1093/nar/gkn180.
[35] M. Anisimova, O. Gascuel, Approximate likelihood-ratio test for branches: a fast, accurate, and powerful alternative, Syst. Biol. 55 (2006) 539–552, https://doi.org/10.1080/10635150600755453.
[36] Q. Xie, Y. Wang, J. Lin, Y. Qin, Y. Wang, W. Bu, Potential key bases of ribosomal RNA to kingdom-specific spectra of antibiotic susceptibility and the possible archaeal origin of eukaryotes, PLoS One 7 (2012) e29468, https://doi.org/10.1371/journal.pone.0029468.
[37] M. Zuker, Mfold web server for nucleic acid folding and hybridization prediction, Nucleic Acids Res. 31 (2003) 3406–3415, https://doi.org/10.1093/nar/gkg595.
[38] A. Gadaev, On sarcocysts of ass (*Equus asinus*), Dokl. Akad. Nauk Uzb. (1978) 47–48. SSR. 1. (in Russian).
[39] G. Passantino, R.P. Lia, S. Latrofa, G. Annoscia, J. Šlapeta, D. Otranto, R. Rossi, N. Zizzo, *Sarcocystis bertrami* in skeletal muscles of donkeys (*Equus africanus asinus*) from southern Italy, Vet. Parasitol. Reg. Stud. Rep. 16 (2019) 100283, https://doi.org/10.1016/j.vprsr.2019.100283.
[40] M. Erber, O. Geisel, Vorkommen und Entwicklung von 2 Sarkosporidienarten des Pferdes, Z. Parasitenkd. 65 (1981) 283–291 (in German with English summary), https://doi.org/10.1007/BF00926722.
[41] M. Rommel, O. Geisel, Untersuchungen über die Verbreitung und den Lebenszyklus einer Sarkosporidienart des Pferdes (*Sarcocystis equicanis* n. spec.), Berl. Münch. Tierarztl. Wochenschr. 88 (1975) 468–471 (in German).
[42] P.L. Long, B.J. Millard, Studies on *Eimeria dispersa* Tyzzer 1929 in turkeys, Parasitology 78 (1979) 41–52, https://doi.org/10.1017/s0031182000048575.
[43] S. El-Sherry, M.E. Ogedengbe, M.A. Hafeez, M. Sayf-Al-Din, N. Gad, J.R. Barta, Re-description of a genetically typed, single oocyst line of the turkey coccidium, *Eimeria adenoeides* Moore and Brown, Parasitol. Res. 113 (2014) (1951) 3993–4004, https://doi.org/10.1007/s00436-014-4066-7.
[44] Z.-Q. Yang, Y.-X. Zuo, Y.-G. Yao, X.-W. Chen, G.-C. Yang, Y.-P. Zhang, Analysis of the 18S rRNA genes of *Sarcocystis* species suggests that the morphologically similar organisms from cattle and water buffalo should be considered the same species, Mol. Biochem. Parasitol. 115 (2001) 283–288, https://doi.org/10.1016/s0166-6851(01)00283-3.
[45] R. Kolenda, M. Ugorski, M. Bednarski, Molecular characterization of *Sarcocystis* species from Polish roe deer based on ssu rRNA and *cox1* sequence analysis, Parasitol. Res. 113 (2014) (2014) 3029–3039, https://doi.org/10.1007/s00436-014-3966-x.
[46] B. Gjerde, M. Hilali, S.A. Mawgood, Molecular characterization of three regions of the nuclear ribosomal DNA unit and the mitochondrial *cox1* gene of *Sarcocystis fusiformis* from water buffaloes (*Bubalus bubalis*) in Egypt, Parasitol. Res. 114 (2015) 3401–3413, https://doi.org/10.1007/s00436-015-4566-0.
[47] J.-J. Hu, S. Huang, T. Wen, G.W. Esch, Y. Liang, H.-L. Li, *Sarcocystis* spp. in domestic sheep in Kunming City, China: prevalence, morphology, and molecular characteristics, Parasite 24 (2017) 30, https://doi.org/10.1051/parasite/2017025.
[48] S. Rubiola, T. Civera, E. Ferroglio, S. Zanet, T. Zaccaria, S. Brossa, R. Cipriani, F. Chiesa, Molecular differentiation of cattle *Sarcocystis* spp. by multiplex PCR targeting 18S and COI genes following identification of *Sarcocystis hominis* in human stool samples, Food Waterborne Parasitol. 18 (2020) e00074, https://doi.org/10.1016/j.fawpar. 2020.e00074.
[49] J.P. Dubey, S.K. Verma, D. Dunams, R. Calero-Bernal, B.M. Rosenthal, Molecular characterization and development of *Sarcocystis speeri* sarcocysts in gamma interferon gene knockout mice, Parasitology 142 (2015) 1555–1562, https://doi.org/10.1017/S0031182015001109.
[50] B.M. Rosenthal, D.B. Dunams, B. Pritt, Restricted genetic diversity in the ubiquitous cattle parasite, *Sarcocystis cruzi*, Infect. Genet. Evol. 8 (2008) 588–592, https://doi.org/10.1016/j.meegid.2008.04.004.
[51] B. Gjerde, M. Hilali, I.E. Abbas, Molecular differentiation of *Sarcocystis buffalonis* and *Sarcocystis levinei* in water buffaloes (*Bubalus bubalis*) from *Sarcocystis hirsuta* and *Sarcocystis cruzi* in cattle (*Bos taurus*), Parasitol. Res. 115 (2016) 2459–2471, https://doi.org/10.1007/s00436-016-4998-1.
[52] T.F. McCutchan, V.F. de la Cruz, A.A. Lal, J.H. Gunderson, H.J. Elwood, M.L. Sogin, Primary sequences of two small subunit ribosomal RNA genes from *Plasmodium falciparum*, Mol. Biochem. Parasitol. 28 (1988) 63–68, https://doi.org/10.1016/0166-6851(88)90181-8.
[53] G.R. Reddy, D. Chakrabarti, C.A. Yowell, J.B. Dame, Sequence microheterogeneity of the three small subunit ribosomal RNA genes of *Babesia bigemina*: expression in erythrocyte culture, Nucleic Acids Res. 19 (1991) 3641–3645, https://doi.org/10.1093/nar/19.13.3641.
[54] V. Corredor, V. Enea, The small ribosomal subunit RNA isoforms in *Plasmodium cynomolgi*, Genetics 136 (1994) 857–865, https://doi.org/10.1093/genetics/136.3.857.
[55] S.H. Qari, I.F. Goldman, N.J. Pieniazek, W.E. Collins, A.A. Lal, Blood and sporozoite stage-specific small subunit ribosomal RNA-encoding genes of the human malaria parasite *Plasmodium vivax*, Gene 150 (1994) 43–49, https://doi.org/10.1016/0378-1119(94)90855-9.
[56] S.M. Le Blancq, N.V. Khramtsov, F. Zamani, S.J. Upton, T.W. Wu, Ribosomal RNA gene organization in *Cryptosporidium parvum*, Mol. Biochem. Parasitol. 90 (1997) 463–478, https://doi.org/10.1016/s0166-6851(97)00181-3.
[57] S. El-Sherry, M.E. Ogedengbe, M.A. Hafeez, J.R. Barta, Divergent nuclear 18S rDNA paralogs in a turkey coccidium, *Eimeria meleagrimitis*, complicate molecular

systematics and identification, Int. J. Parasitol. 43 (2013) 679–685, https://doi.org/10.1016/j.ijpara.2013.03.005.
[58] D. Liao, Concerted evolution: molecular mechanism and biological implications, Am. J. Hum. Genet. 64 (1999) 24–30, https://doi.org/10.1086/302221.
[59] E. Smirnov, N. Chmúrčiaková, F. Liška, P. Bažantová, D. Cmarko, Variability of human rDNA, Cells 10 (2021) 196, https://doi.org/10.3390/cells10020196.
[60] W. Fan, E. Eklund, R.M. Sherman, H. Liu, S. Pitts, B. Ford, N.V. Rajeshkumar, M. Laiho, Widespread genetic heterogeneity of human ribosomal RNA genes, RNA 28 (2022) 478–492, https://doi.org/10.1261/rna.078925.121.
[61] W. Wang, X. Zhang, S. Garcia, A.R. Leitch, A. Kovařík, Intragenomic rDNA variation—the product of concerted evolution, mutation, or something in between? Heredity (Edinb) 131 (2023) 179–188, https://doi.org/10.1038/s41437-023-00634-5.
[62] P.N. Ossorio, L.D. Sibley, J.C. Boothroyd, Mitochondrial-like DNA sequences flanked by direct and inverted repeats in the nuclear genome of *Toxoplasma gondii*, J. Mol. Biol. 222 (1991) 525–536, https://doi.org/10.1016/0022-2836(91)90494-q.
[63] B. Gjerde, Characterisation of full-length mitochondrial copies and partial nuclear copies (numts) of the cytochrome *b* and cytochrome *c* oxidase subunit I genes of *Toxoplasma gondii*, *Neospora caninum*, *Hammondia heydorni* and *Hammondia triffittae* (Apicomplexa: Sarcocystidae), Parasitol. Res. 112 (2013) 1493–1511, https://doi.org/10.1007/s00436-013-3296-4.
[64] C.D.P. Marques, B.W.S. da Silva, Y.V.S. Nogueira, T.L. Bezerra, W. Borges-Silva, R. M. Soares, L.F.P. Gondim, Sporocysts of *Sarcocystis bertrami* (syn. *Sarcocystis fayeri*) shed by dogs: molecular analysis, morphometry and pattern of excretion, Vet. Parasitol. 331 (2024) 110269, https://doi.org/10.1016/j.vetpar.2024.110269.
[65] M. Zhang, K. Wei, Z. Wu, J. Sun, J. Hu, S. Deng, J. Tao, Morphological and molecular characterization of a *Sarcocystis* species infecting donkeys from China, Parasitol. Res. 121 (2022) 2917–2926, https://doi.org/10.1007/s00436-022-07616-2.
[66] C. Marques, B. da Silva, Y. Nogueira, T. Bezerra, A. Tavares, W. Borges-Silva, L. Gondim, Brazilian horses from Bahia state are highly infected with *Sarcocystis bertrami*, Animals (Basel) 12 (2022) 3491, https://doi.org/10.3390/ani12243491.
[67] B.J. Rosonke, S.R. Brown, S.J. Tornquist, S.P. Snyder, M.M. Garner, L.L. Blythe, Encephalomyelitis associated with a *Sarcocystis neurona*-like organism in a sea otter, J. Am. Vet. Med. Assoc. 215 (1999) 1839–1842, https://doi.org/10.2460/javma.1999.215.12.1839.
[68] J.P. Dubey, W.J. Saville, D.S. Lindsay, R.W. Stich, J.F. Stanek, C.A. Speert, B. M. Rosenthal, C.J. Njoku, O.C. Kwok, S.K. Shen, S.M. Reed, Completion of the life cycle of *Sarcocystis neurona*, J. Parasitol. 86 (2000) 1276–1280, https://doi.org/10.1645/0022-3395(2000)086[1276:COTLCO]2.0.CO;2.
[69] J.P. Dubey, W.J. Saville, J.F. Stanek, D.S. Lindsay, B.M. Rosenthal, M.J. Oglesbee, A.C. Rosypal, C.J. Njoku, R.W. Stich, O.C. Kwok, S.K. Shen, A.N. Hamir, S.M. Reed, *Sarcocystis neurona* infections in raccoons (*Procyon lotor*): evidence for natural infection with sarcocysts, transmission of infection to opossums (*Didelphis virginiana*), and experimental induction of neurologic disease in raccoons, Vet. Parasitol. 100 (2001) 117–129, https://doi.org/10.1016/s0304-4017(01)00500-3.
[70] M.A. Miller, B.C. Barr, R. Nordhausen, E.R. James, S.L. Magargal, M. Murray, P. A. Conrad, S. Toy-Choutka, D.A. Jessup, M.E. Grigg, Ultrastructural and molecular confirmation of the development of *Sarcocystis neurona* tissue cysts in the central nervous system of southern sea otters (*Enhydra lutris nereis*), Int. J. Parasitol. 39 (2009) 1363–1372, https://doi.org/10.1016/j.ijpara.2009.04.014.
[71] E.D. Box, J.H. Smith, The intermediate host spectrum in a *Sarcocystis* species of birds, J. Parasitol. 68 (1982) 668–673, https://doi.org/10.2307/3280926.
[72] H.A.B. Llano, H. Zavatieri Polato, L. Borges Keid, T.M. Ferreira de Souza Oliveira, T. Zwarg, A.S. de Oliveira, T.C. Sanches, A.M. Joppert, L.F.P. Gondim, R. Martins Soares, Molecular screening for Sarcocystidae in muscles of wild birds from Brazil suggests a plethora of intermediate hosts for *Sarcocystis falcatula*, Int. J. Parasitol. Parasites Wildl. 17 (2022) 230–238, https://doi.org/10.1016/j.ijppaw.2022.03.002.
[73] E.D. Box, D.W. Duszynski, Experimental transmission of *Sarcocystis* from icterid birds to sparrows and canaries by sporocysts from the opossum, J. Parasitol. 64 (1978) 682–688, https://doi.org/10.2307/3279960.
[74] E.D. Box, J.L. Meier, J.H. Smith, Description of *Sarcocystis falcatula* Stiles, 1893, a parasite of birds and opossums, J. Protozool. 31 (1984) 521–524, https://doi.org/10.1111/j.1550-7408.1984.tb05495.x.
[75] R. Ecco, M.M. Luppi, M.C. Malta, M.R. Araújo, R.M. Guedes, H.L. Shivaprasad, An outbreak of sarcocystosis in psittacines and a pigeon in a zoological collection in Brazil, Avian Dis. 52 (2008) 706–710, https://doi.org/10.1637/8303-040408-Case.1.
[76] G. Konradt, M.V. Bianchi, R.V. Leite-Filho, B.Z. da Silva, R.M. Soares, S.P. Pavarini, D. Driemeier, Necrotizing meningoencephalitis caused by *Sarcocystis falcatula* in bare-faced ibis (*Phimosus infuscatus*), Parasitol. Res. 116 (2017) 809–812, https://doi.org/10.1007/s00436-016-5341-6.
[77] J. Origlia, F. Unzaga, M. Piscopo, G. Moré, Fatal sarcocystosis in psittacine birds from Argentina, Parasitol. Res. 121 (2022) 491–497, https://doi.org/10.1007/s00436-021-07375-6.
[78] J.P. Dubey, D.S. Lindsay, *Sarcocystis speeri* n. sp. (Protozoa: Sarcocystidae) from the opossum (*Didelphis virginiana*), J. Parasitol. 85 (1999) 903–909, https://doi.org/10.2307/3285830.
[79] J.P. Dubey, D.S. Lindsay, Isolation in immunodeficient mice of *Sarcocystis neurona* from opossum (*Didelphis virginiana*) faeces, and its differentiation from *Sarcocystis falcatula*, Int. J. Parasitol. 28 (1998) 1823–1828, https://doi.org/10.1016/s0020-7519(98)00166-0.
[80] T.J. Cutler, R.J. MacKay, P.E. Ginn, E.C. Greiner, R. Porter, C.A. Yowell, J.B. Dame, Are *Sarcocystis neurona* and *Sarcocystis falcatula* synonymous? A horse infection challenge, J. Parasitol. 85 (1999) 301–305, https://doi.org/10.2307/3285638.
[81] T. Mullaney, A.J. Murphy, M. Kiupel, J.A. Bell, M.G. Rossano, L.S. Mansfield, Evidence to support horses as natural intermediate hosts for *Sarcocystis neurona*, Vet. Parasitol. 133 (2005) 27–36, https://doi.org/10.1016/j.vetpar.2005.05.016.
[82] V. Watthanakaiwan, M. Sukmak, K. Hamarit, N. Kaolim, W. Wajjwalku, Y. Muangkram, Molecular characterization of the ribosomal DNA unit of *Sarcocystis singaporensis*, *Sarcocystis zamani* and *Sarcocystis zuoi* from rodents in Thailand, J. Vet. Med. Sci. 79 (2017) 1412–1418, https://doi.org/10.1292/jvms.16-0086.