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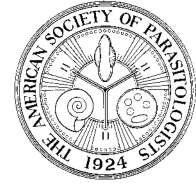

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SARCOCYSTIS INFECTIONS IN RIVER OTTER (*LONTRA CANADENSIS*) IN MICHIGAN

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KEY WORDS

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Sarcocystis caninum
Sarcocystis svanai
Molecular characterization
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ABSTRACT

Sarcocystis infections are common in the muscles of herbivores but were, until recently, considered relatively rare in carnivores. Little is known of sarcocysts in the muscles of river otters in the United States. In a previous epidemiologic study of *Toxoplasma gondii* infections in North American river otters (*Lontra canadensis*) from Michigan in the 2018 and 2019 harvest season, *Sarcocystis* DNA was found in 34 (27.4%) of 124 otter muscles. Tongues from these 34 PCR-positive samples were further examined here for *Sarcocystis* species. An additional batch of frozen 62 samples collected at the end of the season was also tested for *Sarcocystis* herein. Morphologically, sarcocysts were studied in 23 otters (13 of 34 PCR-positive samples from the first batch and 10 of 62 samples of batch 2) in compression smears and paraffin-embedded histologic sections stained with hematoxylin and eosin. Morphologically, at least 2 different kinds of sarcocysts were identified, 1 with a smooth sarcocyst wall and the second with villar protrusions. By transmission electron microscopy, sarcocysts from 1 otter were similar to *Sarcocystis caninum*. Morphologically, sarcocysts from the river otter were different from the European otter (*Lutra lutra*). Sequencing amplification products from *18S* rRNA, *28S* rRNA, and *cox1* genes, suggested *S. caninum*–like, *Sarcocystis svanai*–like, and *Sarcocystis* sp. We detected a third, potentially undescribed species, in 3 otters. Genetic markers for conclusive differentiation of *Sarcocystis* spp. from mustelids should be developed. The samples in the present study had degraded; better preserved samples are needed for further morphologic studies. This is the first report of *S. caninum*–like, *S. svanai*–like, and *Sarcocystis* sp. in the river otter in the United States.

Protozoa in the genus *Sarcocystis* are ubiquitous parasites of ectotherm and endotherm hosts (Dubey et al., 2016). These parasites have an obligatory 2-host life cycle, alternating between an herbivore and a carnivore. The sexual cycle is restricted to the intestines of carnivores; the asexual cycle occurs in extraintestinal tissues of the intermediate host after it ingests water or vegetation contaminated with sporocysts. A carnivore consuming tissues containing viable sarcocysts releases bradyzoites during digestion, which then transform into gamonts in the intestine of the carnivore. Fertilization produces oocysts in the lamina propria, where they sporulate in situ and often rupture, releasing sporocysts in the feces. Once an intermediate host ingests these sporocysts, parasites multiply in endothelium of blood vessels and finally become encysted in tissues, often in muscles. *Sarcocystis* species are generally host specific, especially for the intermediate host (herbivore). In some carnivore or omnivore hosts, sarcocysts occur in extraintestinal muscles; life cycles of these uncommon sarcocysts remain incomplete.

Otters of the subfamily Lutrinae are aquatic animals and feed mainly on fish and invertebrates. They consume enormous amounts of food daily compared with their body weight. They are also exposed to contaminants, including parasites in runoff from land, and thus infections in otters are an indication of environmental contamination (Miller et al., 2002; Shapiro et al., 2012). More is known of protozoan infections of sea otters (*Enhydra lutris*) than of the North American river otters (*Lontra canadensis*) in the United States (Dubey et al., 2016). Two protozoans, *Toxoplasma gondii* (felids as definitive hosts) and *Sarcocystis neurona* (the Virginia opossum, *Didelphis virginiana*, as a definitive host) are common causes of mortality in sea otters in the United States (Dubey et al., 2016; Dubey, 2022).

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Table I. Details of river otter (*Lontra canadensis*) from Michigan.

Batch no.	Otter no.	Michigan ID	Tissue*	Animal Parasitic Diseases Laboratory ID	Compression smear, sarcocyst kind†	No. of sarcocysts studied in histologic sections	PCR results
1	1	2018-296	M	JP-8-11	NP, U	3 (2NP, 1P) (Fig. 2D)	‡
	2	2018-303	M	JP-8-12	P, NP (Fig. 1C)	3 (2NP, 1P) (Figs. 3–5)	‡
	3	2018-311	M, T	JP-8-1	Not recorded	None seen	§
	4	2018-312	M, T	JP-8-2	Not recorded	4 cysts (P), inflammation	§
	5	2018-313	M, T	JP-8-6	Not recorded	None seen	§
	6	2018-314	T	JP-8-7	Not recorded	4 (3P, 1NP) (Fig. 2A, C)	‡
	7	2018-315	M, T	JP-8-8, 13, 16	P, NP (Fig. 1D)	8 (NP) (Fig. 2C, E, F)	‡, ||
	8	2018-316	M, T	JP-8-9	P, NP, U	None seen	§
	9	2018-317	M, T	JP-8-10	Not recorded	None seen	§
	10	2018-321	T	JP-8-17	Not recorded	1	‡
	11	2018-338	M	JP-8-14	Not recorded	2 (P)	‡
	12	2018-449	T	JP-8-18	Not recorded	None seen	‡
	13	2018-463	M, T	JP-8-15, 21	P, NP	4 (1P, 1NP, 2U) (Fig. 2B)	‡
2	14	2018-680	M	JP-8-22	P	1	#
	15	2018-683	M, T	JP-8-3	Not recorded	None seen	#
	16	2018-684	M, T	JP-8-4	Not recorded	None seen	#
	17	2018-685	M, T	JP-8-5	Not recorded	None seen	#
	18	2018-691	M	JP-8-23	NP	1 (P)	#
	19	2018-702	M	JP-8-24	NP	1 (P)	||
	20	2018-707	M	JP-8-19	NP	3 (2P, 1U)	||
	21	2018-709	M	JP-8-20	Not recorded	1 (P), inflammation	#
	22	2018-710	M	JP-8-26	NP (Fig. 1B)	2 (P)	#
	23	2018-712	M	JP-8-27	P, NP (Fig. 1A)	3	||

* M = masseter muscle; T = tongue.
† NP = sarcocysts with no visible protrusions; U = undecided; P = sarcocysts with villar protrusions on cyst wall.
‡ PCR-positive *18S*rRNA gene (Cotey et al., 2022).
§ PCR-negative *18S*rRNA gene (Cotey et al., 2022).
|| PCR positive using several genes in this study.
Not done.

In a recent survey of river otters from the state of Michigan, *Sarcocystis* spp. DNA was detected in the tongues of 34 (27.4%) of 124 animals (Cotey et al., 2022). No details were provided concerning the detection of *Sarcocystis* DNA and morphology of the sarcocysts. Here, we report on the molecular details and morphologically characterize sarcocysts. Also, we add information on another batch of 62 river otters from the same harvest year from Michigan.

MATERIALS AND METHODS

River otter samples

Samples came from otter heads that were collected at the time of registration of the fur and then frozen and submitted to the Michigan Department of Natural Resources (Lansing, Michigan) for the present investigation. The heads were thawed for sampling, and the collected tissues subsequently refrozen. Two batches of muscle samples from tongues or masseter muscles or both of frozen river otter heads were received (Table I). Batch 1 consisted of 124 samples; these were used by Cotey et al. (2022). A second batch of 62 samples collected in the 2018–2019 harvest season was also tested; these samples were not included in Cotey et al. (2022).

Testing of samples at the College of Veterinary Medicine, Oklahoma State University, Stillwater, Oklahoma

For the initial molecular testing performed on 124 samples from batch 1, the following method was used. For detection of *Sarcocystis* DNA, a PCR was performed to amplify a portion of the *18S* rRNA gene, using the primers SarcoF (5′-CGCAAATTACCCAATCCTGA) and SarcoR (5′-ATTTCTCATAAGGTGCAGGAG) that amplify a 700-base-pair (bp) DNA fragment, as previously described by Yang et al. (2002). Briefly, a reaction mixture containing 5 μl (1×) buffer, 1.5 μl of magnesium, 1 μl of deoxynucleoside triphosphate, 0.5 μl each of 10 pmol forward and reverse primers, 0.25 μl of AmpliTaq Gold™ DNA Polymerase, and 2 to 5 μl of DNA containing approximately 10–20 ng of DNA template with water adjusted to a total reaction volume of 25 μl (Applied Biosystems™, Waltham, Massachusetts). PCR was performed in a thermocycler (Applied Biosystems™) with an initial denaturation at 95 C for 5 min, followed by 35 cycles of denaturation at 94 C for 30 sec, annealing at 59 C for 30 sec, extension at 72 C for 60 sec, and a final extension at 72 C for 10 min. These details for PCR performed on 124 samples were not given by Cotey et al. (2022).

Many of the samples from batch 1 were dehydrated; therefore, for the present investigation, we concentrated on testing 34 of the 124 samples that were positive for *Sarcocystis* DNA (Cotey et al., 2022).

Testing samples at the Animal Parasitic Diseases Laboratory (APDL), Beltsville, Maryland

Cytologic and histologic examination: At APDL, samples of muscles were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 5 μm, stained with hematoxylin and eosin (HE), and examined microscopically for parasites. Portions of muscle were stored at −20 C for DNA extraction and further microscopic testing. Around 5 g of muscle was thawed, and compression preparations

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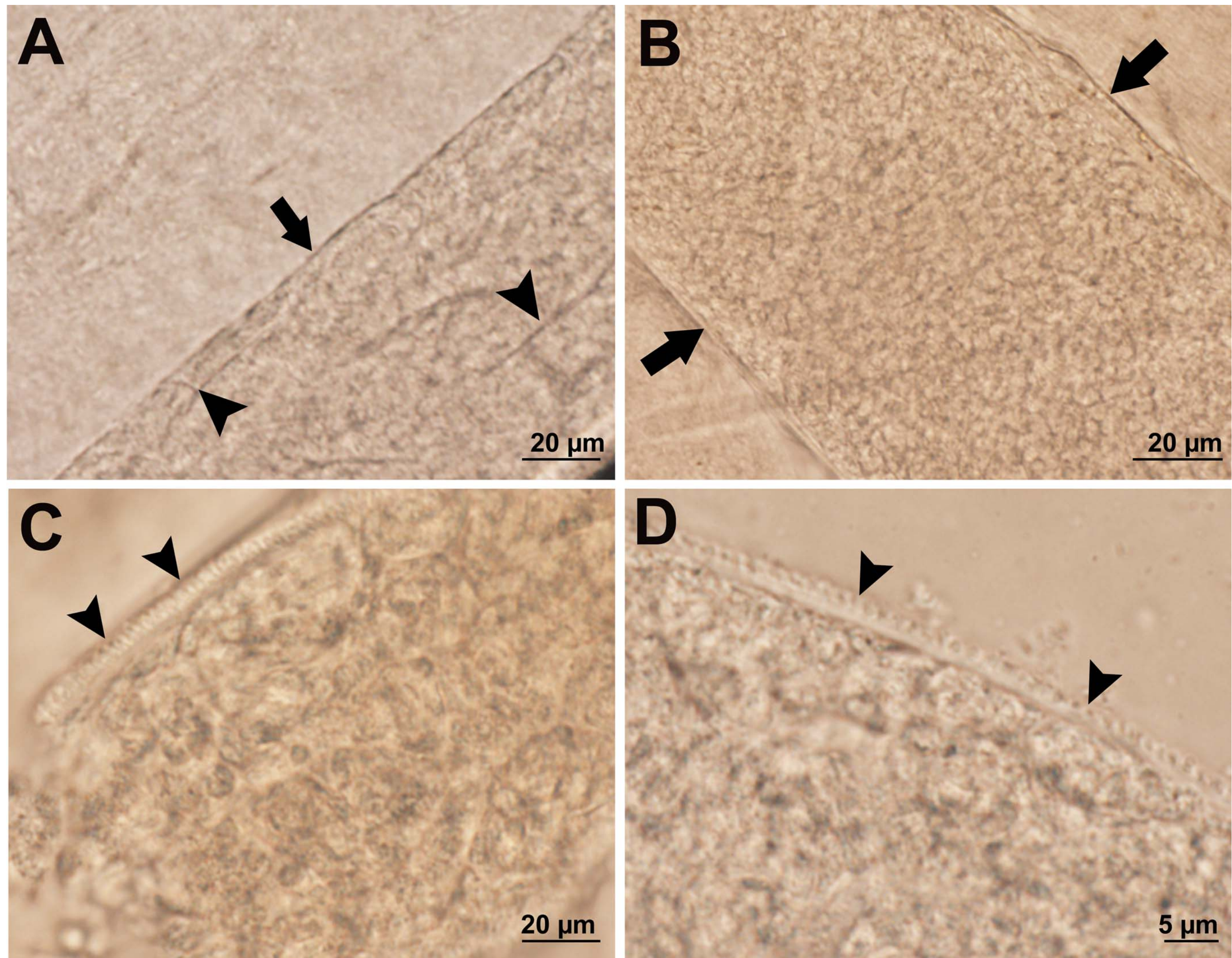

Figure 1. Sarcocysts in compression smears of muscles of river otters (*Lontra canadensis*) from Michigan. Unstained. (**A**) Sarcocyst with a thin smooth wall (arrow) without any visible protrusions. Note septa of the same thickness (arrowheads) as the cyst wall. Otter no. 23. (**B**) Thin-walled sarcocyst (arrows). Otter no. 22. (**C**) Part of a sarcocyst freed from the muscle. Arrowheads point to elongated, slender villar protrusions. Otter no. 2. (**D**) Sarcocyst with conical villar protrusions (arrowheads). Otter no. 7. Color version available online.

were examined microscopically. For this, muscle snips were pressed between coverslips and glass slides (around 30 preparations per 1 g of tissue) and examined microscopically. From 2 otters (nos. 2 and 7; Table I), muscle samples were ground in aqueous 0.85% NaCl solution (saline), and homogenized preparations were examined microscopically (Fig. 1C, D). Portions of muscles containing sarcocysts were also saved in 70% ethanol before DNA extraction. Parasites detected were photographed using an Olympus AX-70 microscope with DP-73 digital camera, and measurements were made digitally using Olympus Imaging Software cellSens Standard 1.18 (Olympus Optical Ltd., Tokyo, Japan).

Electron microscopic examination: Muscle snips (n = 8) containing sarcocysts were fixed with McDowell Trump's fixative (10% formalin 37%, 1% glutaraldehyde in 1.16% sodium phosphate monobasic, and 0.27% NaOH with deionized water), placed in plastic sealed bags, and transported by air to Complutense University of Madrid, Spain, for transmission electron microscopy (TEM) examination. Samples were postfixed, sectioned, and finally contrasted with 4% uranyl acetate and 3% lead citrate, as reported by Dubey et al. (2024b). Ultrathin sections were examined at the Spanish National Centre for Electron Microscopy (Madrid, Spain) using a JEOL JEM 1400 Plus device (JEOL, Tokyo, Japan) at 80 kW.

DNA isolation and amplification: Genomic DNA was extracted from individual sarcocysts excised from 4 positive river otters (nos. 7, 19, 20, and 23; Table I). The extraction was carried out using the DNeasy® Blood and Tissue Kit (Qiagen, Hilden, Germany), following the manufacturer's protocol. DNA was then stored at −20 C. The purity and concentration of the extracted DNA specimens were measured using a Nanodrop spectrophotometer (ThermoFisher Scientific, Waltham, Massachusetts).

PCR amplifications were performed using previously published primers specific to *Sarcocystis* (Table II). Each PCR mixture (14.5 μl total volume) contained 2 μl of DNA template, 6.25 μl of

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Table II. Accession numbers for various gene markers of *Sarcocystis* spp. identified in the tissue cysts of river otter (*Lontra canadensis*) from Michigan.

Gene	River otter ID	*Sarcocystis* species identified	Fragment size (base pair [bp])	Query cover (%)	Highest sequence similarity	GenBank accession no.
18S rRNA*	7	*Sarcocystis svanai*	1,202 bp	74	888/890 (99.8%)	PV231431
	20		2,466 bp	65	1,591/1,594 (99.8%)	PV239786
	23		1,190 bp	72	856/858 (99.8%)	PV239888
	19		1,661 bp	80	1,327/1,328 (99.9%)	PV231833
	7	*Sarcocystis* sp.	592 bp	99	587/587 (100%)	PV231834
	20		593 bp	99	588/588 (100%)	PV231895
	23		576 bp	100	576/576 (100%)	PV231930
28S rRNA	7	*Sarcocystis caninum*	1,509 bp	100	1,506/1,509 (99.8%)	PV232301
	19		1,262 bp	100	1,259/1,262 (99.8%)	PV232315
	20		1,490 bp	100	1,485/1,490 (99.7%)	PV232332
	23		1,400 bp	100	1,397/1,400 (99.8%)	PV239714
cox1	7	*S. svanai*	988 bp	100	982/988 (99.7%)	PV247062
	20		1,007 bp	100	1,001/1,007 (99.7%)	PV247063
	23		1,000 bp	100	994/1,000 (99.7%)	PV247064

* For *18S* rRNA gene, we amplified various fragments of the gene using a different set of primers and sequenced them. The pairing was S3F × BSarc, SU1F × 5.8SR2, and SarCF × SarCR.

Platinum Hot Start PCR Master Mix (Invitrogen, Waltham, Massachusetts), 1 μl of 10 pmol/μl of each primer (Integrated DNA Technologies, Coralville, Iowa), and 4.25 μl of molecular-grade water. The thermal cycling conditions included an initial denaturation step at 94 C for 3 min, followed by 35 cycles of denaturation at 94 C for 30 sec, annealing at 60 C for 30 sec, and extension at 68 C for 2 min, with a final elongation at 68 C for 5 min.

PCR products were visualized on a 2% agarose gel, and fragment size was determined by comparison to the 100-bp Plus DNA Ladder (Promega Corporation, Madison, Wisconsin). PCR products were purified using the ExoSAP method (Bell, 2008), followed by bidirectional Sanger sequencing performed at Psomagen, Inc. (Rockville, Maryland) on an ABI 3500xl Genetic Analyzer (Applied Biosystems™). Sequence data were processed, assembled, and edited using Geneious 11.1.5 (Biomatters Limited), and the final consensus sequences were submitted to GenBank (Table II).

Phylogenetic analysis

Sequences were compared with the NCBI database using Basic Local Alignment Search Tool (BLAST; https://blast.ncbi.nlm.nih.gov/Blast.cgi; Altschul et al., 1990). All sequences were trimmed at each end before phylogenetic reconstruction. Multilocus genotyping using *18S* rRNA, *28S* rRNA, and *cox1* genes was performed, and the results are provided in Table II.

The web server GUIDANCE2 (Sela et al., 2015) was used to align and remove ambiguously aligned positions. Specifically, the sequences were aligned with the MAFFT algorithm under the following options: maxiterate 1,000 and pairwise alignment method –localpair. Positions with a score below 0.93 were removed. Phylogenetic relationships were reconstructed under the maximum-likelihood (ML) criterion. ML analyses were performed with the program IQ-TREE version 1.6.12 (Nguyen et al., 2015). The analyses were run with the options –m MFP –b 1,000. The model selected based on the BIC criterion was T92+G+I, K2+G, and T92 for *18S* rRNA, *28S* rRNA, and *cox1*, respectively.

RESULTS

Cytologic and morphologic findings

Sarcocysts were found in compression smears of 23 otters (13 from the first batch, otter nos. 1–13, and in 10 of 62 samples from batch 2, otter nos. 14–23; Table I). Tongue and masseter were difficult to squash because of partial dehydration. Therefore, details of the sarcocyst wall could be photographed from only 11 of 23 positive samples. In unstained preparations, sarcocysts had smooth sarcocyst walls without any protrusions (Fig. 1A, B). In 2 otters (nos. 2 and 7), sarcocysts could be freed from the host tissue; these sarcocysts had visible serrations to conical villar protrusions (Fig. 1C, D).

Muscles from these 23 samples positive by the compression method were studied histologically in paraffin-embedded sections, and sarcocysts were found in sections of 15 samples. From 2 otters (nos. 7 and 13), several additional muscle samples were studied histologically. Sarcocysts were sparse. In total, 41 sarcocysts were examined at ×1,000 magnification of HE-stained sections. All sarcocysts were mature.

At least 2 morphologic types of sarcocysts were recognized, based on the protrusions of the sarcocyst wall (Fig. 2). Some sarcocysts had conical to slender protrusions (Fig. 1A–D; Table I), but interpretation could be clouded by the angle of the sarcocyst cut.

Before TEM examination, samples underwent several rounds of freezing and thawing, somewhat compromising the morphologic integrity. Nevertheless, ultrastructural morphology could be appreciated in 1 specimen (otter no. 2; Figs. 3–5). In sarcocysts examined by TEM, bradyzoites had degenerated, but amylopectin granules were visible, and the structure of the sarcocyst wall was fairly preserved (Figs. 3–5). The sarcocyst wall consisted of a parasitophorous vacuolar membrane that was wavy and lined with a remarkably thick (94 nm) electron-dense layer (Fig. 5). The sarcocyst wall had pleomorphic villar protrusions, with an average size of 1.78 × 0.59 μm (n = 15), with a smooth 0.77-μm-thick ground substance layer. By TEM, sarcocysts closely resemble the type 9c grouping by Dubey et al. (2016).

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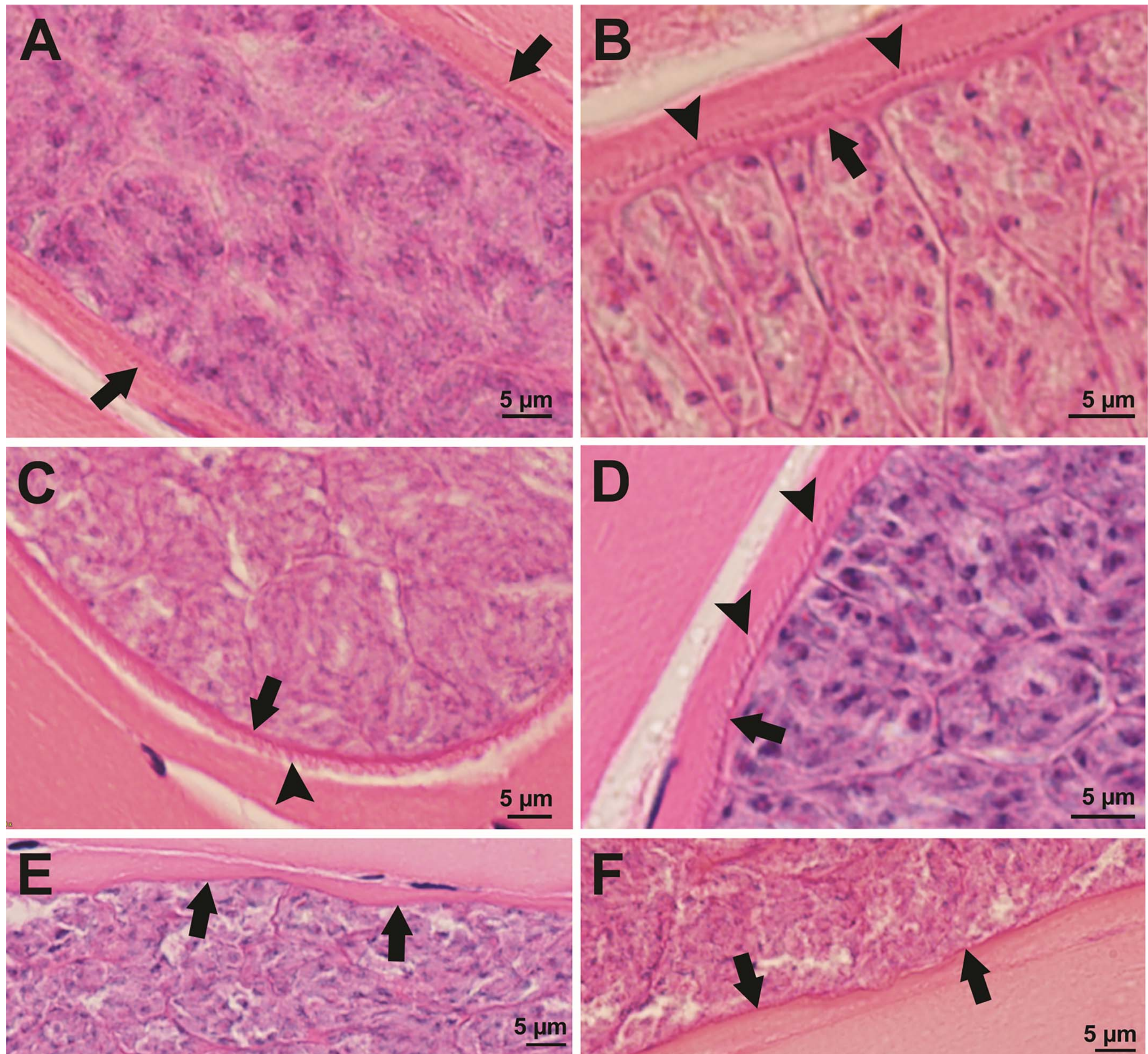

Figure 2. Sarcocysts in histologic sections of the tongues of river otters (*Lontra canadensis*) from Michigan. Hematoxylin and eosin stain. Sarcocysts have degraded, but cyst wall protrusions are recognizable. (**A**) Indistinct protrusions (arrow) on the wall. Otter no. 6. (**B**) Eosinophilic sarcocyst wall (arrow) with villar protrusions (arrowhead). Note prominent septa divide bradyzoites into groups. Otter no. 13. (**C**) Eosinophilic sarcocyst wall (arrow) with villar protrusions (arrowhead). Otter no. 5. (**D**) Slender villar protrusions (arrowheads) on the sarcocyst wall (arrow). Otter no. 1. (**E, F**) Degenerated sarcocysts with smooth cyst walls (arrowheads). Otter no. 7. Color version available online.

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Molecular and phylogenetic analysis

The molecular analyses based on the conserved regions of the *18S* rRNA, *28S* rRNA, and *cox1* genes confirm the close genetic relationship of the *Sarcocystis* isolates from river otters to sequences of *Sarcocystis caninum* and *Sarcocystis svanai* derived from domestic dogs with identities of >99%. The *18S* rRNA gene also showed absolute identity to *Sarcocystis* sp. previously described from fisher (*Martes pennanti*) in Pennsylvania (Larkin et al., 2011). The *cox1* gene, a more variable marker, provided high-resolution insights into interspecies differences and affirmed the identity of these *Sarcocystis* species. The sequence types obtained during the study appeared closely related (but not identical) to each other: 98.8% (for *18S* rRNA); 98.4% (for *28S* rRNA); and 98.8% (for *cox1*).

Based on the *18S* rRNA alignment using undescribed species from *M. pennanti* (GenBank accession nos. HQ709138 and HQ709139) and some sequences of *S. caninum* (GenBank accession nos. MH469238 KM362427), *Sarcocystis arctica* (GenBank accession

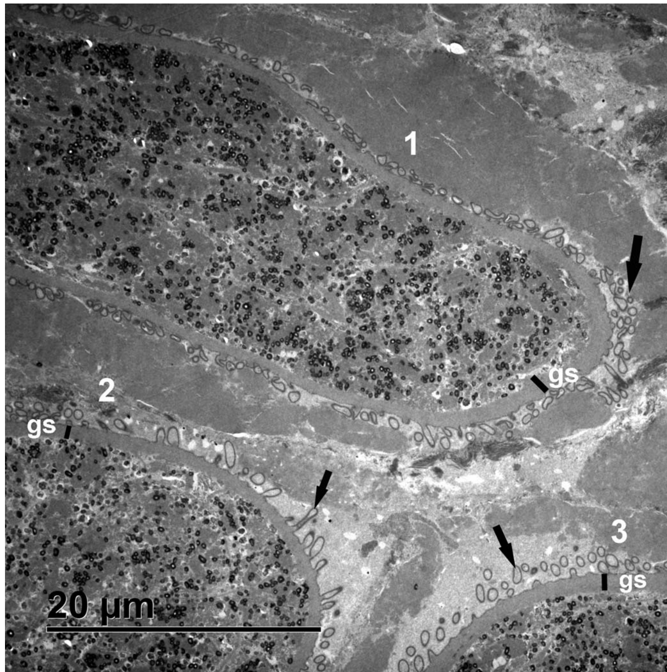

Figure 3. Transmission electron micrograph of *Sarcocystis caninum*–like sarcocysts in the tongue of river otter no. 2. Note 3 sarcocysts (1, 2, and 3). Sarcocyst marked 1 is slender, and the sarcocyst wall is visible on both sides. Note homogenous ground substance layer (gs) and pleomorphic villar protrusions (arrows).

nos. MF596237, MF596227, KX022100, and KX022101), and *S. svanai* (GenBank accession nos. OR921255, KM362428, and OR921256), 8 single nucleotide polymorphisms (SNPs) were found comparing HQ709138 and HQ709139 (100% identity to

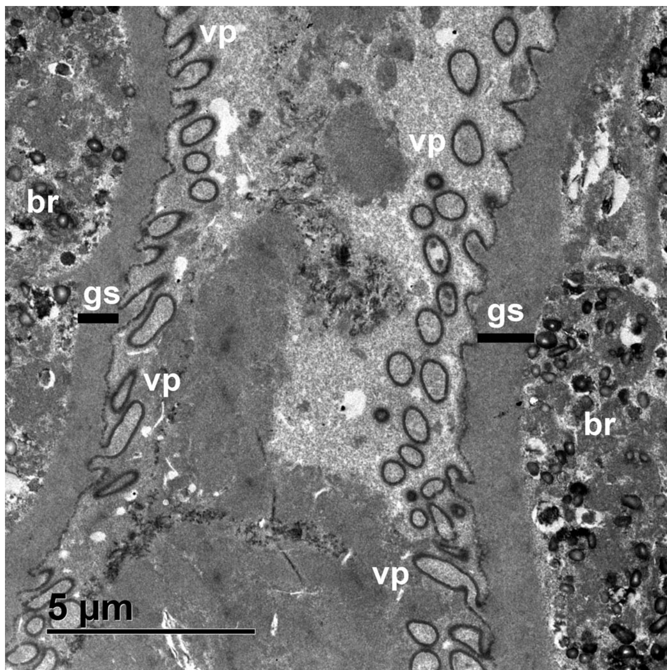

Figure 4. Transmission electron micrograph of 2 adjacent *Sarcocystis caninum*–like sarcocysts in tongue of river otter no. 2. The ground substance layer (gs) is smooth. Note pleomorphic villar protrusions. The bradyzoites are degenerated, but amylopectin is visible as dark spots.

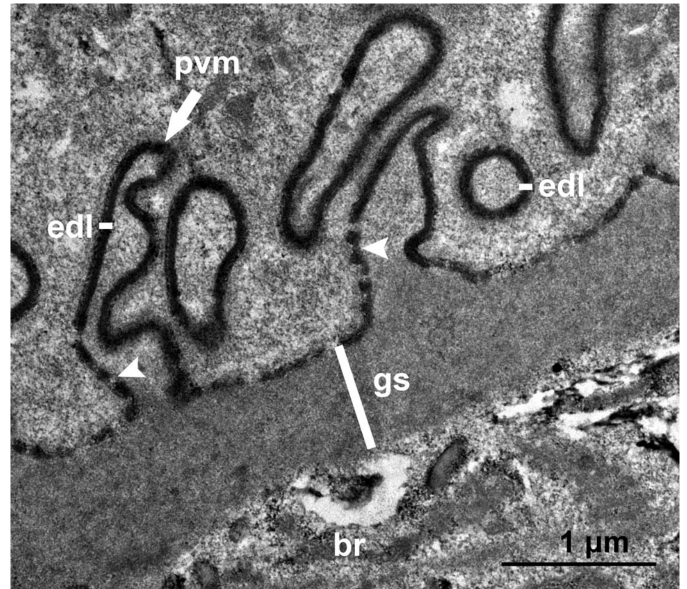

Figure 5. Transmission electron micrograph of a *Sarcocystis caninum*–like sarcocyst in the tongue of river otter no. 2. Higher magnification of the sarcocyst wall. The parasitophorous vacuolar membrane (pvm) is wavy and lined by a thick electron-dense layer (edl). The villar protrusions are pleomorphic, and there are no microtubules. The ground substance layer (gs) is smooth without granules. The bradyzoite (br) is degenerated.

18S rRNA sequences isolated from otter nos. 7, 20, and 23 and *S. svanai*). Only 1 fixed SNP comparing HQ709138, HQ709139 with *S. caninum* or *S. arctica* (Y = C or T) was also found. Such a small difference does not rule out the possibility that it is an intraspecific variation.

The phylogenetic analysis of *18S* rRNA included 18 taxa and 1,678 positions, 13 taxa and 1,085 positions for *cox1*, and 15 taxa and 1,509 positions for *28S* rRNA. Isolates of *Sarcocystis myodes* were used as an out-group (Fig. 6).

The *18S* rRNA gene

The *18S* rRNA sequences isolated from otters (nos. 7, 20, and 23) were identical to previously reported isolates of an undescribed species from *M. pennanti* (GenBank accession nos. HQ709138, HQ709139, and HQ709143). These formed a group, supported in 67 of 100 bootstrap replicates, distinct from sequences attributed previously to *S. caninum* and *S. arctica* (GenBank accession nos. KM362427, KX022102, and MH469238). Strong bootstrap support (97/100) distinguishes the 2 groups discussed previously as a monophyletic group to the exclusion of all others, including the *18S* rRNA sequence amplified from river otter no. 20 that resembles, but appears slightly distinct from, prior exemplars of *S. svanai* (GenBank accession nos. OR921254 and KM362428). Excluded from this analysis are 3 partial *18S* rRNA sequences obtained from parasites infecting river otters nos. 7, 19, and 23. Over their length, these 3 sequences are identical to the sequence obtained from river otter no. 20 whose phylogenetic position appears in Fig. 6A.

The *cox1* gene

Sarcocystis svanai sequences (GenBank accession nos. PQ490734 and PP819578) grouped with *S. svanai* sequences isolated during

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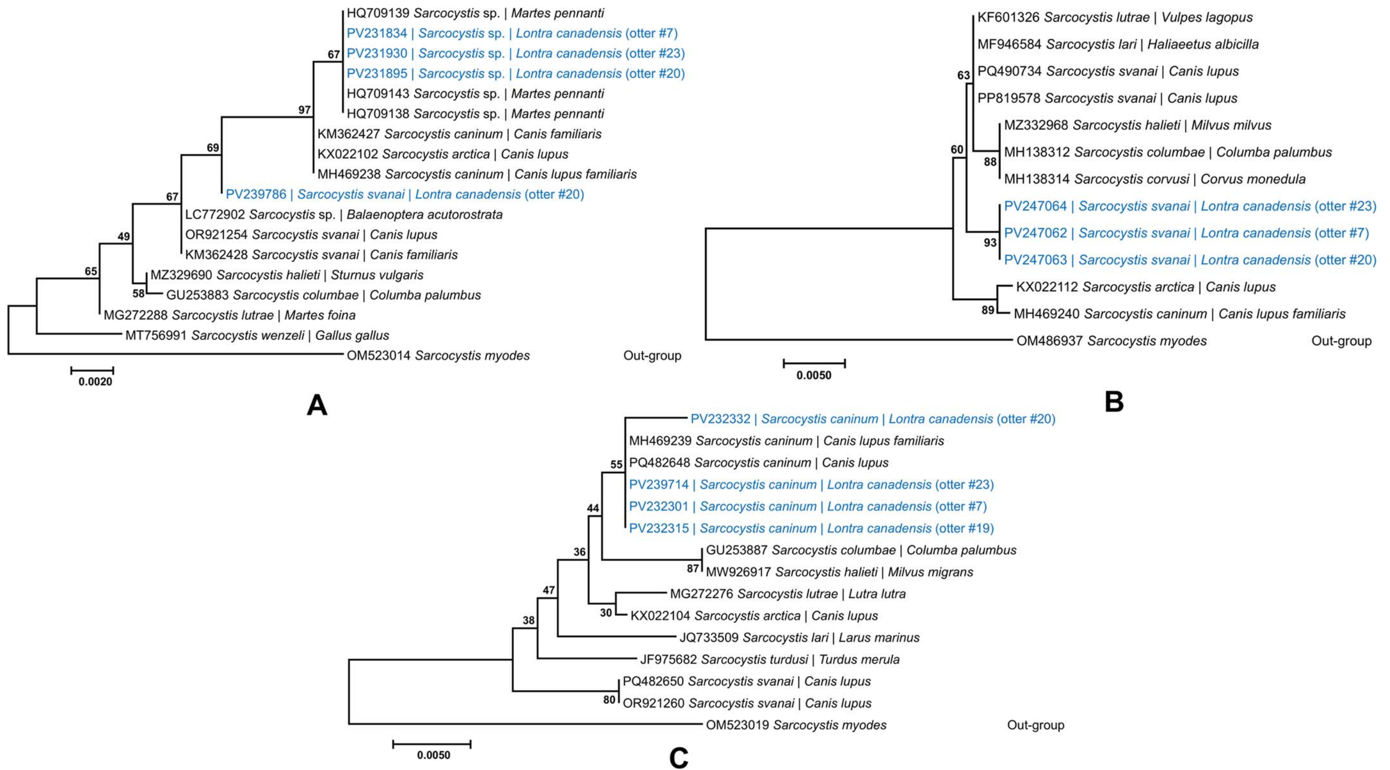

Figure 6. Phylogenetic relationships of the various *Sarcocystis* species identified from river otter (*Lontra canadensis*) in Michigan, inferred using multilocus genotyping: (**A**) *18S* rRNA, (**B**) *cox1*, and (**C**) *28S* rRNA. Branch support values are shown near the respective nodes. Species highlighted represent those identified in this study. Color version available online.

the study from river otters (nos. 7, 20, 23), but this assemblage does not form a strictly monophyletic clade due to the presence of other *Sarcocystis* species within the grouping. The high bootstrap support value (93) for the *S. svanai* cluster suggests strong confidence in the relationships within the *cox1* dataset. *Sarcocystis caninum* and *S. arctica* formed a distinct grouping, separate from other species, consistent with their taxonomic identity. Also, *Sarcocystis halieti*, *Sarcocystis corvusi*, and *Sarcocystis columbae* clustered together, separating them from *S. arctica* and *S. caninum*, further supporting their evolutionary divergence (Fig. 6B).

The *28S* rRNA gene

Sarcocystis caninum sequences obtained from 4 river otters (nos. 7, 19, 20, and 23) during the study formed a closely related cluster with other isolates of *S. caninum* (GenBank accession nos. MH469239 and PQ482648) with strong support, demonstrating genetic consistency within the species for the *28S* rRNA gene. Moreover, the sequences of *S. columbae* and *S. halieti* are grouped distinctly, reflecting species-specific differences. Also, *S. svanai* sequences showed clear clustering, differentiating them from other species such as *Sarcocystis lutrae*, *S. arctica*, *Sarcocystis turdusi*, and *Sarcocystis lari* (Fig. 6C).

Phylogenetic trees reconstructed also indicated a close relationship between *S. caninum* and *S. arctica*, as previously discussed by Gupta et al. (2024) and can be seen in the present study. This finding further suggests that *S. arctica* cannot be genetically differentiated from *S. caninum*. Therefore, sequencing additional loci, such as the *ITS2*, *cox3,* or *cytb* genes, can provide finer resolution for phylogenetic insights and confirm transmission pathways.

DISCUSSION

There is considerable uncertainty concerning the *Sarcocystis* species in otters and the relationship with *Sarcocystis* species in other mustelids. *Sarcocystis* infections were first recognized in 4 European otters (*Lutra lutra*) from Norway (Wahlström et al., 1999; Gjerde and Schulze, 2014; Table III). During an introduction project, 70 otters were imported into Sweden from Norway. One of these otters died in captivity before it could be released to the wild, and sarcocysts were found in the skeletal muscle; however, sarcocysts were not found in the remaining 69 otters (Wahlström et al., 1999). Sarcocysts were up to 2.3 mm long and 0.25 mm wide. With a light microscope, the sarcocyst wall was serrated. Ultrastructurally, the sarcocyst wall had small blebs at regular intervals, corresponding to the type 1 classification of Dubey et al. (2016). Bradyzoites were 8 × 2 μm (Wahlström et al., 1999). Wahlström et al. (1999) did not name the parasite. Gjerde and Josefsen (2014) found sarcocysts in 3 otters in Norway and named the parasite *S. lutrae*, primarily based on molecular characteristics. Sarcocysts were up to 970 μm long. By light microscopy, the sarcocyst wall was thin (<0.5 μm) and smooth without any serrations or projections; TEM examination was not performed. Sarcocyst DNA was characterized using *18S* and *28S* ribosomal and mitochondrial *cox1* genes (Gjerde and Josefsen, 2014). Molecularly, *S. lutrae* resembled *Sarcocystis kalvikus* from wolverines in Canada (Gjerde and Josefsen, 2014). Since then,

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Table III. Molecular details of *Sarcocystis* species found in river otter (*Lontra canadensis*) during the study.

Sarcocystis species	Intermediate hosts	Molecular characterization and references				
		18S rRNA	*28S* rRNA	*cox1*	*ITS1*	*rpoB*
Sarcocystis caninum	Originally described from muscles of domestic dogs (*Canis familiaris*) from the United States and Canada (Dubey et al., 2015). Later, similar sarcocysts reported from domestic dog in China (Ye et al., 2018) and in Finland (Hagner et al., 2018) and also reported in gray wolf (*Canis lupus*) from the United States (Gupta et al., 2024) and Lithuania (Juozaitytė-Ngugu et al., 2024)	Dubey et al. (2015); Hagner et al. (2018); Ye et al. (2018); Gupta et al. (2024); Juozaitytė-Ngugu et al. (2024)	Ye et al. (2018); Gupta et al. (2024); Juozaitytė-Ngugu et al. (2024)	Ye et al. (2018); Gupta et al. (2024); Juozaitytė-Ngugu et al. (2024)	Ye et al. (2018); Gupta et al. (2024); Juozaitytė-Ngugu et al. (2024)	Dubey et al. (2015); Ye et al. (2018); Gupta et al. (2024); Juozaitytė-Ngugu et al. (2024)
Sarcocystis lutrae	Eurasian otters from Norway (Gjerde and Josefsen, 2014), beech martins (*Mates foina*), European pole cat (*Mustela putorius*), European badger (*Meles meles*) in Europe (Lepore et al., 2017; Kirillova et al., 2018; Máca, 2018; Prakas et al., 2018).	Gjerde and Josefsen, (2014); Lepore et al. (2017); Kirillova et al. (2018); Máca, (2018); Prakas et al. (2018)	Gjerde and Josefsen, (2014); Kirillova et al. (2018); Máca 2018; Prakas et al. (2018)	Gjerde and Josefsen, (2014); Kirillova et al. (2018); Máca (2018); Prakas et al. (2018)	Gjerde and Josefsen, (2014); Kirillova et al. (2018); Máca (2018); Prakas et al. (2018)	Kirillova et al. (2018)
Sarcocystis svanai	Originally described from muscles of the domestic dogs (*Canis familiaris)* from the United States and Canada (Dubey et al., 2015). Later, similar sarcocysts reported from domestic dog in Finland (Hagner et al., 2018), gray wolf (*Canis lupus*) from the United States (Gupta et al., 2024) and Lithuania (Juozaitytė-Ngugu et al. 2024), in Pampas fox (*Lycalopex gymnocercus*) from Argentina (Scioscia et al., 2017).	Dubey et al. (2015); Scioscia et al. (2017); Hagner et al. (2018); Gupta et al. (2024); Juozaitytė-Ngugu et al. (2024)	Gupta et al. (2024); Juozaitytė-Ngugu et al. (2024)	Gupta et al. (2024); Juozaitytė-Ngugu et al. (2024)	Gupta et al. (2024); Juozaitytė-Ngugu et al. (2024)	Gupta et al. (2024); Juozaitytė-Ngugu et al. (2024)
Sarcocystis sp.	From fisher *Martes pennanti* from Pennsylvania, USA (Larkin et al. (2011).	Larkin et al. (2011)	No data	No data	No data	No data

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based on molecular characteristics, *S. lutrae* has been reported from other mustelids in Europe, including the American mink (*Neovison vison*), beech martins (*Mates foina*), European pole cat (*Mustela putorius*), and the European badger (*Meles meles*) (Lepore et al., 2017; Kirillova et al., 2018; Máca, 2018; Prakas et al., 2018). In conclusion, it is uncertain if there is more than 1 *Sarcocystis* species infecting European otters.

Máca and González-Solís (2022) found *Sarcocystis* sporocysts in the intestines of a white-tailed eagle (*Haliaeetus albicilla*) in the Czech Republic; molecularly, these (*18S* rRNA, *28S* rRNA, *ITS1*, and *cox1*) were considered *S. lutrae*, thus confirming the eagle as its definitive host (Table III). Molecularly, *S. lutrae* has been also identified in the small intestines of the hooded crow (*Corvus cornix*) from Lithuania; however, only oocysts not sporocysts were seen in this omnivorous bird (Juozaitytė-Ngugu et al., 2021).

Little is known of *Sarcocystis* infections in the North American river otter in the United States. As stated earlier, using PCR (*18S* rRNA gene), Cotey et al. (2022) found *Sarcocystis* sp. DNA in 34 of 124 otters from Michigan; sarcocyst morphology was not described.

Sarcocystis svanai and *S. caninum* are primarily known to infect canids and other intermediate hosts (Dubey et al., 2015; Hagner et al., 2018; Gupta et al., 2024; Table III). The presence of parasites similar to these species in river otters, which are not their expected typical hosts, broadens the understanding of their host range. This finding might indicate the spread or introduction of *Sarcocystis* species into aquatic ecosystems, potentially through definitive hosts (e.g., dogs or wild canids) defecating near water sources. Nevertheless, our TEM observations indicated the presence of a *Sarcocystis* that resembled *S. caninum* (Dubey et al., 2015); therefore, additional descriptive studies would be of interest. The *Sarcocystis* spp. with characteristics of an *S. caninum*–like sarcocyst is structurally different from *S. lutrae* named from European otters from Norway by Gjerde and Josefsen (2014) and *Sarcocystis* sp. reported by Wahlström et al. (1999). Here, we are postponing naming the species awaiting further studies.

The use of *18S*, *28S*, and *cox1* genes as molecular markers provided robust genetic evidence for species identification. The *cox1* gene proved valuable due to its high resolution in distinguishing closely related species (Gjerde, 2013, 2016; Dubey et al., 2024a; Gupta et al., 2024). Further genotyping studies using microsatellites or more discriminatory markers by multilocus sequencing typing assays could provide additional insight into *Sarcocystis* species that are closely related and are host and tissue specific.

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LITERATURE CITED

Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman. 1990. Basic local alignment search tool. Journal of Molecular Biology 215: 403–410. doi:10.1016/D0022-2836(05)80360-2.

Bell, J. R. 2008. A simple way to treat PCR products prior to sequencing using ExoSAP-IT. BioTechniques 44: 834. doi:10.2144/000112890.

Cotey, S. R., R. Scimeca, L. Chang, A. L. Carpenter, E. E. Will, C. Ott-Conn, A. L. Mayer, and M. V. Reichard. 2022. *Toxoplasma gondii* prevalence, partial genotypes, and spatial variation in North American river otters (*Lontra canadensis*) in the upper peninsula of Michigan, USA. Journal of Wildlife Diseases 58: 869–881. doi:10.7589/JWD-D-22-00021.

Dubey, J. P. 2022. Toxoplasmosis of Animals and Humans, 3rd ed. CRC Press, Boca Raton, Florida, 564 p. doi:10.1201/9781003199373.

Dubey, J. P., R. Calero-Bernal, B. M. Rosenthal, C. A. Speer, and R. Fayer. 2016. Sarcocystosis of Animals and Humans, 2nd ed. CRC Press, Boca Raton, Florida, 501 p. doi:10.1201/b19184.

Dubey, J. P., L. S. de Araujo, A. Gupta, O. C. H. Kwok, and B. M. Rosenthal. 2024a. *Trichinella* and at least three species of *Sarcocystis* parasitize the muscles of bobcats (*Lynx rufus*) from Mississippi. Journal of Parasitology 110: 402–411. doi:10.1645/24-6.

Dubey, J. P., A. Gupta, R. Calero-Bernal, L. S. de Araujo, M. L. García-Gil, J. Battle, A. Ankarah, K. Van Why, J. D. Brown, and B. M. Rosenthal. 2024b. Gray fox (*Urocyon cinereoargenteus*) identified as a new intermediate host for *Sarcocystis neurona*. Veterinary Parasitology: Regional Studies and Reports 56: 101122. doi:10.1016/j.vprsr.2024.101122.

Dubey, J. P., J. E. Sykes, G. D. Shelton, N. Sharp, S. K. Verma, R. Calero-Bernal, J. Viviano, N. Sundar, A. Khan, and M. E. Grigg. 2015. *Sarcocystis caninum* and *Sarcocystis svanai* n. spp. (Apicomplexa: Sarcocystidae) associated with severe myositis and hepatitis in the domestic dog (*Canis familiaris*). Journal of Eukaryotic Microbiology 62: 307–317. doi:10.1111/jeu.12182.

Gjerde, B. 2013. Phylogenetic relationships among *Sarcocystis* species in cervids, cattle and sheep inferred from the mitochondrial cytochrome c oxidase subunit I gene. International Journal for Parasitology 43: 579–591.

Gjerde, B. 2016. 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*). Parasitology Research 115: 1473–1492. doi:10.1007/s00436-015-4881-5.

Gjerde, B., and T. D. Josefsen. 2014. Molecular characterisation of *Sarcocystis lutrae* n. sp. and *Toxoplasma gondii* from the musculature of two Eurasian otters (*Lutra lutra*) in Norway. Parasitology Research 114: 873–886. doi:10.1007/s00436-014-4251-8.

Gjerde, B., and J. Schulze. 2014. Muscular sarcocystosis in two arctic foxes (*Vulpes lagopus*) due to *Sarcocystis arctica* n. sp.:

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Sarcocyst morphology, molecular characteristics and phylogeny. Parasitology Research 113: 811–821. doi:10.1007/s00436-013-3711-x.

Gupta, A., L. de Araujo, R. Calero-Bernal, C. Humpal, M. Schrage, M. Carstensen, B. M. Rosenthal, and J. P. Dubey. 2024. Molecular and morphological characterization of *Sarcocystis* infections in muscles of gray wolves (*Canis lupus*) from Minnesota suggest they may serve as reservoirs for infection in domesticated dogs. Journal of Parasitology 110: 471–485. doi:10.1645/24-72.

Hagner, K., T. S. Jokinen, A. Lavikainen, and A. Sukura. 2018. Acute fulminant necrotizing myopathy in a dog caused by co-infection with ultrastructural *Sarcocystis caninum* and *Sarcocystis svanai*-like apicomplexan protozoa. Veterinary Parasitology 252: 153–156. doi:10.1016/j.vetpar.2018.02.011.

Juozaitytė-Ngugu, E., E. Maziliauskaite, M. Kirjusina, P. Prakas, R. Vaitkeviciute, J. Stankeviciute, and D. Butkauskas. 2024. Identification of *Sarcocystis* and *Trichinella* species in muscles of gray wolf (*Canis lupus*) from Lithuania. Veterinary Sciences 11: 85. doi:10.3390/vetsci11020085.

Juozaitytė-Ngugu, E., S. Švažas, D. Šneideris, E. Rudaitytė-Lukošienė, D. Butkauskas, and P. Prakas. 2021. The role of birds of the family Corvidae in transmitting *Sarcocystis* protozoan parasites. Animals 11: 3258. doi:10.3390/ani11113258.

Kirillova, V., P. Prakas, R. Calero-Bernal, I. Gavarāne, J. L. Fernández-García, M. Martínez-González, E. Rudaityte-Lukosiene, M. A. H. Martínez-Estéllez, D. Butkauskas, and M. Kirjusina. 2018. Identification and genetic characterization of *Sarcocystis arctica* and *Sarcocystis lutrae* in red foxes (*Vulpes vulpes*) from Baltic States and Spain. Parasites & Vectors 11: 173. doi:10.1186/s13071-018-2694-y.

Larkin, J. L., M. Gabriel, R. W. Gerhold, M. J. Yabsley, J. C. Wester, J. G. Humphreys, R. Beckstead, and J. P. Dubey. 2011. Prevalence to *Toxoplasma gondii* and *Sarcocystis* spp. in a reintroduced fisher (*Martes pennanti*) population in Pennsylvania. Journal of Parasitology 97: 425–429.

Lepore, T., P. M. Bartley, F. Chianini, A. I. Macrae, E. A. Innes, and F. Katzer. 2017. Molecular detection of *Sarcocystis lutrae* in the European badger (*Meles meles*) in Scotland. Parasitology 144: 1426–1432. doi:10.1007/S0031182017000762.

Máca, O. 2018. Molecular identification of *Sarcocystis lutrae* in the European otter (*Lutra lutra*) and the European badger (*Meles meles*) from the Czech Republic. Parasitology Research 117: 943–945. doi:10.1007/s00436-018-5793-y.

Máca, O., and D. González-Solís. 2022. White-tailed eagle (*Haliaeetus albicilla*) as the definitive host of *Sarcocystis lutrae* in the Czech Republic. Frontiers in Veterinary Science 9: 981829. doi:10.3389/fvets.2022.981829.

Miller, M. A., I. A. Gardner, C. Kreuder, D. M. Paradies, K. R. Worcester, D. A. Jessup, E. Dodd, M. D. Harris, J. A. Ames, A. E. Packham, et al. 2002. Coastal freshwater runoff is a risk factor for *Toxoplasma gondii* infection of southern sea otters (*Enhydra lutris nereis*). International Journal for Parasitology 32: 997–1006.

Nguyen, L. T., H. A. Schmidt, A. von Haeseler, and B. Q. Minh. 2015. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Molecular Biology and Evolution 32: 268–274. doi:10.1093/molbev/msu300.

Prakas, P., Ž. Strazdaite-Želienė, E. Rudaityte-Lukošienė, E. Servienė, and D. Butkauskas. 2018. Molecular identification of *Sarcocystis lutrae* (Apicomplexa: Sarcocystidae) in muscles of five species of the family Mustelidae. Parasitology Research 117: 1989–1993. doi:10.1007/s00436-018-5880-0.

Scioscia, N. P., L. Olmos, A. Gorosábel, L. Bernad, J. Pedrana, Y. P. Hecker, I. Gual, M. L. Gos, G. M. Denegri, D. P. Moore, et al. 2017. Pampas fox (*Lycalopex gymnocercus*) new intermediate host of *Sarcocystis svanai* (Apicomplexa: Sarcocystidae). Parasitology International 66: 214–218. doi:10.1016/j.parint.2017.01.021.

Sela, I., H. Ashkenazy, K. Katoh, and T. Pupko. 2015. GUIDANCE2: Accurate detection of unreliable alignment regions accounting for uncertainty of multiple parameters. Nucleic Acids Research 43: W7–W14. doi:10.1093/nar/gkv318.

Shapiro, K., M. Miller, and J. Mazet. 2012. Temporal association between land-based runoff events and California sea otter (*Enhydra lutris nereis*) protozoal mortalities. Journal of Wildlife Diseases 48: 394–404.

Wahlström, K., T. Nikkilä, and A. Uggla. 1999. *Sarcocystis* species in skeletal muscle of otter (*Lutra lutra*). Parasitology 118: 59–62.

Yang, Z. Q., Q. Q. Li, Y. X. Zuo, X. W. Chen, Y. J. Chen, L. Nie, C. G. Wei, J. S. Zen, S. W. Attwood, X. Z. Zhang, et al. 2002. Characterization of *Sarcocystis* species in domestic animals using a PCR-RFLP analysis of variation in the 18S rRNA gene: A cost-effective and simple technique for routine species identification. Experimental Parasitology 102: 212–217. doi:10.1016/s0014-4894(03)00033-x.

Ye, Y., Y. Liang, J. Hu, Z. Huang, and Y. Zhang. 2018. First isolation of *Sarcocystis caninum* sarcocysts from two domestic dogs (*Canis familiaris*) from China. Parasitology Research 117: 3613–3618. doi:10.1007/s00436-018-6060-y.

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