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Authors: Dubey, J. P., de Araujo, Larissa S., Gupta, Aditya, Kwok, Oliver C. H., and Rosenthal, B. M.

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TRICHINELLA AND AT LEAST THREE SPECIES OF *SARCOCYSTIS* PARASITIZE THE MUSCLES OF BOBCATS (*LYNX RUFUS*) FROM MISSISSIPPI

J. P. Dubey, Larissa S. de Araujo, Aditya Gupta, Oliver C. H. Kwok, and B. M. Rosenthal

U.S. Department of Agriculture, Agricultural Research Service, Animal Parasitic Diseases Laboratory, Beltsville Agricultural Research Center, Building 1001, Beltsville, Maryland 20705-2350.

Correspondence should be sent to J. P. Dubey at: *jitender.dubey@usda.gov*

KEY WORDS

Bobcat (*Lynx rufus*)
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Muscles
Trichinella

ABSTRACT

Muscles of 25 bobcats (*Lynx rufus*) from remote areas of Mississippi in 2017 were tested for parasites. Testing for *Sarcocystis* infections included microscopic examination of fresh unstained muscle squashes, pepsin digestion of hearts and tongues, and histological sections of paraffin-embedded tissues. *Sarcocystis* spp. infections were detected in the muscles of 21 (84%) by a combination of methods. Sarcocysts were detected in the unstained tongue squashes of 2 bobcats. *Sarcocystis* sp. bradyzoites were detected in the pepsin digests of 3 of 19 hearts, and 12 of 19 tongues. In paraffin-embedded histological sections, sarcocysts were detected in 7 of 25 hearts, 17 of 25 tongues, and 5 of 23 limb muscles. Based on the character of the cyst wall, at least 3 morphologic types of sarcocysts were detected: those with small spikes on the cyst wall, corresponding to *Sarcocystis felis*, those with long villar protrusions, corresponding to *Sarcocystis neurona*, and those lacking visible cyst wall protrusions, representing an unidentified type of sarcocyst. Myositis associated with sarcocysts was seen in the tongues of 3, and in the limb muscles of 1 bobcat. Multilocus genotyping of the DNA extracted from paraffin-embedded sections from 2 bobcats, employing *18S*, *28S*, *COI*, *ITS-1*, and *5.8S* and *rpoB* genes, diagnosed *Sarcocystis caninum, S. felis, Sarcocystis lutrae*, and *S. neurona*. An encapsulated species of *Trichinella* was identified in the tongue of 1; it represents the first documented occurrences in bobcats from Mississippi. Taken together, these observations suggest intensive exposure of these wild carnivores to *Trichinella* tissue cysts, implies predation or scavenging on these tissues promotes parasite transmission, and raises caution concerning zoonotic risk when such meat is rendered for human consumption.

Protozoa in the genus *Sarcocystis* parasitize virtually all warm-blooded animals, and a few species also occur in cold-blooded animals (Dubey et al., 2016). Of more than 200 named species of *Sarcocystis*, science has established a full life cycle for only a few. Some species of *Sarcocystis* are zoonotic, and some cause economic losses to livestock producers (Dubey et al., 2016). *Sarcocystis* species 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 herbivore host after it ingests water or vegetation contaminated with sporocysts. A carnivore consuming tissues contaminated with sarcocysts releases bradyzoites, 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 blood vessels and finally become encysted as sarcocysts, 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. Those species of *Sarcocystis* benefiting from complete life-cycle descriptions do not exploit the same hosts for completing their asexual and sexual cycles (producing sarcocysts and oocysts, respectively); however, sarcocysts frequently occur in bobcats (Anderson et al., 1992; Dubey et al., 1992; Verma et al., 2015). Anderson et al. (1992) reported sarcocysts in 30 (50%) of 60 Florida bobcats (*Felis rufus floridanus*). Verma et al. (2015) detected sarcocysts in 26 (74.2%) of 35 bobcats (*Lynx rufus*) from Mississippi. In the 2014 survey of bobcats trapped in Mississippi, *Sarcocystis felis*–like sarcocysts were detected in all infected bobcats (Verma et al., 2015). Genetic data also ascribed infections in 2 bobcats to *Sarcocystis neurona*. *Sarcocystis neurona* employs an unusually wide range of intermediate hosts and causes mortality in several animal species, particularly in horses and marine mammals (Dubey et al., 2016). Bobcats are also definitive hosts for unknown species of *Sarcocystis*; sporocysts were detected in the feces of 12 of 56 bobcats from Mississippi (Dubey et al., 2023).

To determine whether such a high prevalence of *Sarcocystis* is typical, we here examined bobcats from Mississippi trapped in 2017. Here, we report the results of this *Sarcocystis* sarcocyst survey.

MATERIALS AND METHODS

Samples from bobcats

During surveys for *Toxoplasma gondii* infections in bobcats, samples of 25 bobcats were obtained from Mississippi in 2017, as reported previously (Dubey et al., 2023). Bobcats were trapped legally in February of 2017. The sampling was from remote counties (Claiborne, Warren, and Jefferson) of Mississippi and included bobcats of both sexes. Samples of the tongue, whole heart, and limb muscle were collected, put in Ziplock bags, and transported to the Animal Parasitic Diseases Laboratory (APDL), U.S. Department of Agriculture (USDA), Beltsville, Maryland for testing. Up to 4 days elapsed between collection and transport of samples to APDL. We previously reported the parasites identified in the feces of these bobcats (Verma et al., 2017; Dubey et al., 2023). This survey, intended to also examine the prevalence of *T. gondii,* was suspended in 2018 when USDA redirected its food safety research efforts; the remaining frozen samples were incinerated and discarded then, but paraffin-embedded muscle samples were maintained, enabling the present report on *Sarcocystis* in these tissues via histology.

Cytological and histological examination

Muscle squashes of tongues from 2 bobcats (nos. 4 and 17) were photographed. Additionally, cross section of the heart, 1 cross section of the tongue at midpoint, and 2 × 2 cm of leg muscle of bobcats were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 5 µm, stained with hematoxylin and eosin (HE), and examined microscopically for parasites (Table I). Sarcocysts detected in histological sections were enumerated and photographed using an Olympus AX-70 microscope with DP-73 digital camera (Olympus Optical Ltd., Tokyo, Japan).

Detection of *Sarcocystis* bradyzoites in pepsin digest

Myocardium and tongue (around 30 g each) from 19 of 25 bobcats were homogenized individually and digested in acidic pepsin solution separately, centrifuged, and suspended in saline (0.9% NaCl) as described previously (Verma et al., 2015) to release bradyzoites from sarcocysts (Table I). Drops (50 µl) of digest were screened under the light microscope at ×400 magnification for the presence of *Sarcocystis*-like bradyzoites. The digest stored at −80 C was incinerated after the closure of *Toxoplasma* research.

DNA characterization from paraffin-embedded infected muscle

Attempts were made to extract DNA from selected samples. The extraction of genomic DNA was performed on 5 unstained paraffin sections (10 µm thick) from 2 bobcats (nos. 11 and 14) using the Qiagen (Hilden, Germany) DNeasy® Blood and Tissue Kit following the manufacturer's instructions. The DNA samples were analyzed by PCRs targeting 5 molecular markers: *18S* ribosomal RNA (*18S* rRNA), *28S* ribosomal RNA (*28S* rRNA), cytochrome c oxidase 1 (*COI*), Internal Transcribed Spacer 1 (*ITS-1*), *5.8S* ribosomal RNA (*5.8S* rRNA), and the β-subunit of RNA polymerase (*rpoB*) gene using *Sarcocystis*-specific primers for these genetic markers (Table II). These primers were designed during the present study using NCBI Primer-BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). The 25 µl of PCR mix consisted of a 2-µl DNA template, 12.5 µl of Platinum Hot Start PCR Master mix (Invitrogen, Waltham, Massachusetts), 1 µl of 10 pmol/µl of each primer (Integrated DNA Technology, Coralville, Iowa) and 8.5 µl of molecular grade water. After initial denaturation at 94 C for 3 min, 35 cycles were performed consisting of denaturation at 94 C for 30 sec, annealing at 60 C for 30 sec, and elongation at 68 C for 20 min; terminal elongation incubated products at 68 C for 5 min. The PCR products were analyzed on a 2% agarose gel running at 100 V for 45 min on an electrophoretic unit. and the size of the amplicons was estimated by comparison with the 100–base-pair (bp) DNA Ladder (Promega Corporation, Madison, Wisconsin) followed by the DNA cleaning of the PCR amplicons using the ExoSAP method (Bell, 2008). Sanger sequencing was performed at the DNA sequencing Unit of Psomagen, Rockville, Maryland, on an ABI 3500x1 Genetic Analyzer (Applied Biosystems™, Waltham, Massachusetts). The obtained sequences were visualized, assembled, and edited using Geneious Prime 2023.0.4. The edited sequences were deposited in the GenBank (Table II).

Table I. *Sarcocystis* spp. (*Sarcocystis felis*–like [Sfl], *Sarcocystis neurona*–like [Snl], and undetermined species [UN]) infections (based on histological sections) in bobcats from Mississippi.*

	Bradyzoites in pepsin digest		Sarcocysts in histological sections		
Bobcat no.	Heart	Tongue	Heart	Tongue	Limb muscle
1	−	+	2 (Sfl)	2 (Sfl)	No sample
2	−	−	−	1 (Sfl)	No sample
3	−	−	−	1 (Sfl)	−
4	−	−	−	1 (Sfl)†	−
5	−	+	−	2 (Snl)	−
6	−	−	1 (Sfl)	2 (Sfl)	−
7	−	−	−	2 (Sfl)	2 (Sfl)
8	−	−	−	−	−
9	−	+	−	−	−
10	−	+	−	3 (Sfl)‡	1 (Sfl)
11	−	+	−	19 (Sfl, UN)‡	7 (Sfl, UN)
12	+	+	1 (UN)	−	1 (Sfl)
13	+	+	2 (UN)	1 (UN)	−
14	+	+	1 (Sfl)	6 (Sfl, UN)‡	1 (Sfl)
15	ND	ND	−	−	−
16	ND	ND	−	−	−
17	ND	ND	−	12 (Sfl, UN)†	−
18	ND	ND	−	1 (UN)	−
19	ND	ND	−	1 (UN)	−
20	ND	ND	6 (Sfl, UN)	2 (Sfl, UN)	−
21	−	+	−	1 (UN)	−
22	−	−	−	−	−
23	−	+	1 (UN)	−	−
24	−	+	−	7 (Sfl, UN)	−‡
25	−	+	−	−	−

* Abbreviations: ND = no data; + = present, − = not seen.
† Sarcocysts in squashes of tongue.
‡ Myositis.

Phylogenetic analysis

To reconstruct the phylogenetic position of the sequences, we conducted Blast searches against the NCBI nr database (https://

Table II. Accession numbers for various gene markers of *Sarcocystis* spp. infections in bobcats from Mississippi.

Sarcocystis species identified	Locus	Total bases amplified	Primer name*	Sequence (5′-3′)	Usage	GenBank accession numbers
Sarcocystis caninum	18S	705	11_F	CTTTTGGTCGGCGATGATC	PCR amplification	PP077100
			278_F	CAATTGGAGGGCAAGTCTGG	Sequencing	
			167_R	CCAAGTGCACACCCGTTAAG	Sequencing	
			735_R	CTTCGAGCCCCTAACTTTCG	PCR amplification	
	28S	565	KL1	TACCCGCTGAACTTAAGC	PCR amplification and sequencing	PP077103
			137_R	GGGGACCTACCTTCTGCAC	PCR amplification and sequencing	
	rpoB	390	135_F	AACAGTAGGTGACAAATTATGTGGT	PCR amplification and sequencing	PP078755
			70_R	AGAATACCTTGCAACTCCACAA	PCR amplification and sequencing	
Sarcocystis neurona	18S	507	562_F	TTCCCTCGTGGAAGGGTAGT	PCR amplification and sequencing	PP077106
			433_R	ACACCAATCAGGAGGATGCC	PCR amplification and sequencing	
	28S	552	85_F	TAGCCTCGAGAGGCGTTACC	PCR amplification and sequencing	PP077107
			489_R	TCAGCCAGCATCACAGAACT	PCR amplification and sequencing	
Sarcocystis felis	ITS-1 and 5.8S	200	94_F	TCAACAACTGAATCCCCCGA	PCR amplification and sequencing	PP079422
			118_R	ACATTGTTCATCTTCGCGCC	PCR amplification and sequencing	
Sarcocystis lutrae	COI	494	80_F	GATGCCCGCATTATTTGCAG	PCR amplification	PP078754
			200_R	ACCTAATACAGCCAGGCCAA	Sequencing	
			464_R	ACCTCTGGATGGCCAAAGAAC	PCR amplification	

* All primers were designed during the study except KL1 (Gjerde, 2015).

blast.ncbi.nlm.nih.gov/) on 15 December 2023. The BLAST searches of all the genetic markers revealed 99.82–100% identity with *Sarcocystis caninum*, *S. felis*, *Sarcocystis lutrae*, and *S. neurona*.

The web server Guidance 2 (Sela et al., 2015; (last accessed on 19 December 2023) was used to align and remove ambiguously aligned positions in all the genetic markers. Specifically, the sequences were aligned with the MAFFT algorithm under the options Max-Iterate: 1.000 and Pairwise Alignment Method: –localpair. Positions with a score below 0.93 were removed, as were positions with more than 25% of missing data. Phylogenetic relationships were reconstructed under the maximum likelihood (ML) criteria. 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 1000 -nt 5 (i.e., ModelFinder + tree reconstruction + 1,000 non-parametric bootstrap replicates + tree topology test). The model selected based on the BIC criterion was CAT+GTR+GAMMA 4 model.

Reexamination of type material of *Sarcocystis felis*, Dubey, Hamir, Kirkpatrick, Todd, and Rupprecht, 1992

Until recently, only 1 species of *Sarcocystis*, *S. felis*, was known in the muscles of bobcats. Verma et al. (2015) reported an additional species, *S. neurona*, in 2 of 35 bobcats from Mississippi. *Sarcocystis felis* was originally described from 4 of 6 bobcats from Arkansas (Dubey et al., 1992). To investigate if more than 1 *Sarcocystis* species was present in the original sections of bobcats, histological sections from the original description were retrieved from the personal collection of 1 of us (J.P.D.) and the Type slide in the U.S. National Museum (USNM no. 82095) and reexamined microscopically. Additionally, attempts were made to extract DNA from unstained paraffin-embedded sections; these sections had been stored in a box at room temperature for 31 yr.

RESULTS

Sarcocysts in muscle squashes

In unstained muscle squashes, 3 morphologically distinct sarcocysts were found (Figs. 1, 2). 2 types of sarcocysts were detected in the tongue of bobcat no. 4; 1 type appeared to have a relatively thin wall without any visible projections (Figs. 1A, B; 2A–C), and the second had long villar projections, similar to those of *S. neurona* (Fig. 2D). A slender sarcocyst was found in the tongue squash of bobcat no. 17. The longer part of this broken sarcocyst was 5 mm long (Fig. 1C) and the smaller part around 1 mm long (Fig. 1D); thickness of both parts was around 50 µm. A higher magnification of the sarcocyst wall revealed incisor tooth-like projections on the sarcocyst wall; the wall was around 2 µm thick (Fig. 2E). Our retrospective examination was limited to photographs, because the original samples were disposed of in 2018.

Sarcocystis bradyzoites in pepsin digest

Sarcocystis-like bradyzoites were found in pepsin digests of 3 of 19 hearts, and 12 of 19 tongues.

Sarcocysts in histological slides

In histological sections, sarcocysts were detected in 7 of 25 hearts, 17 of 25 tongues, and 5 of 23 limb muscles. More than 3 types of sarcocyst walls are depicted in Figure 3. It was difficult

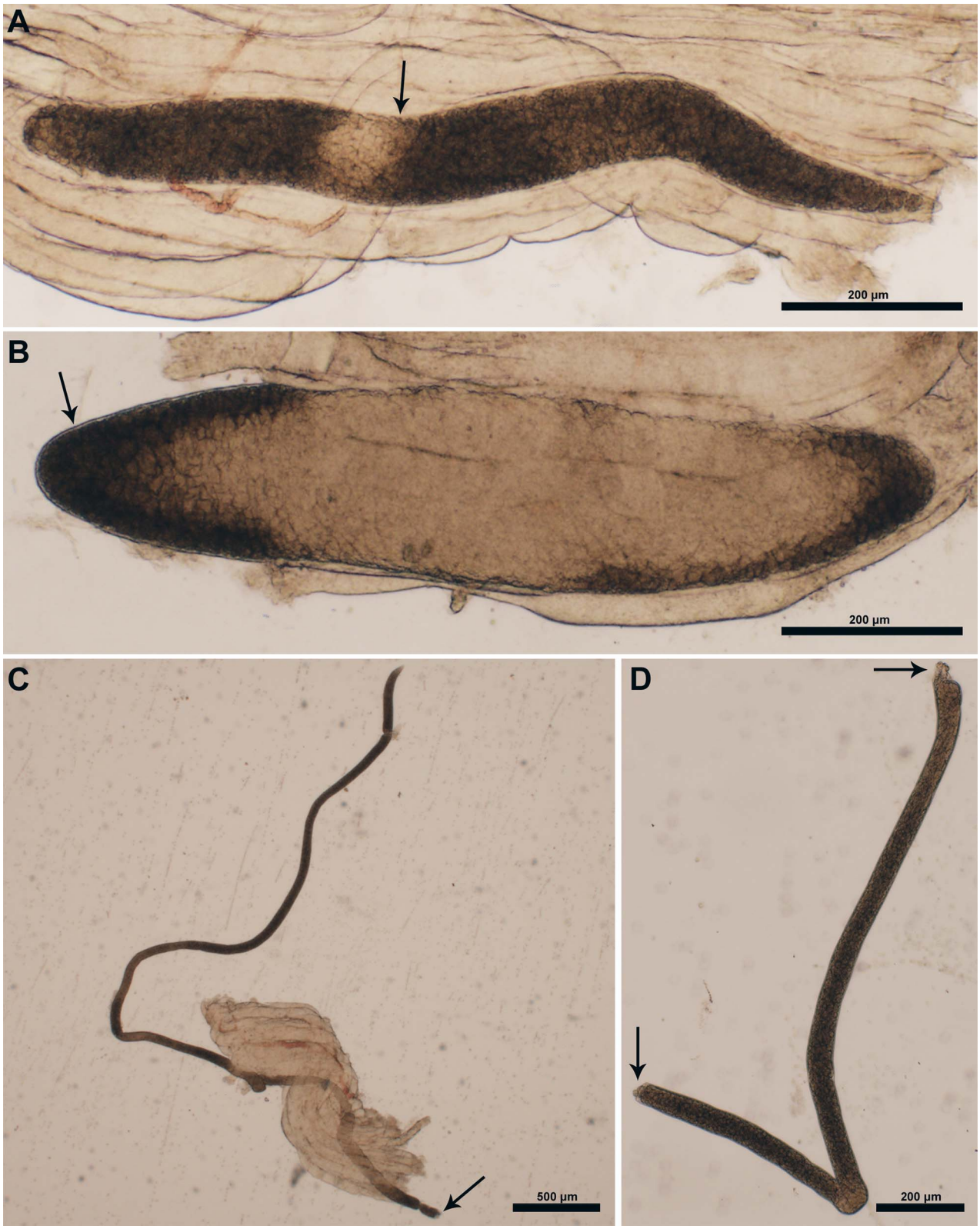

Figure 1. *Sarcocystis* spp. sarcocysts in squash of tongue muscle of bobcats from Mississippi. Unstained. (**A**), (**B**) 2 sarcocysts from bobcat no. 4; villar protrusions were not visible on the sarcocyst wall (arrows). (**C**), (**D**) Slender sarcocyst around 6.5 mm long and around 50 µm wide; the sarcocyst was broken (arrows). Bobcat no. 17.

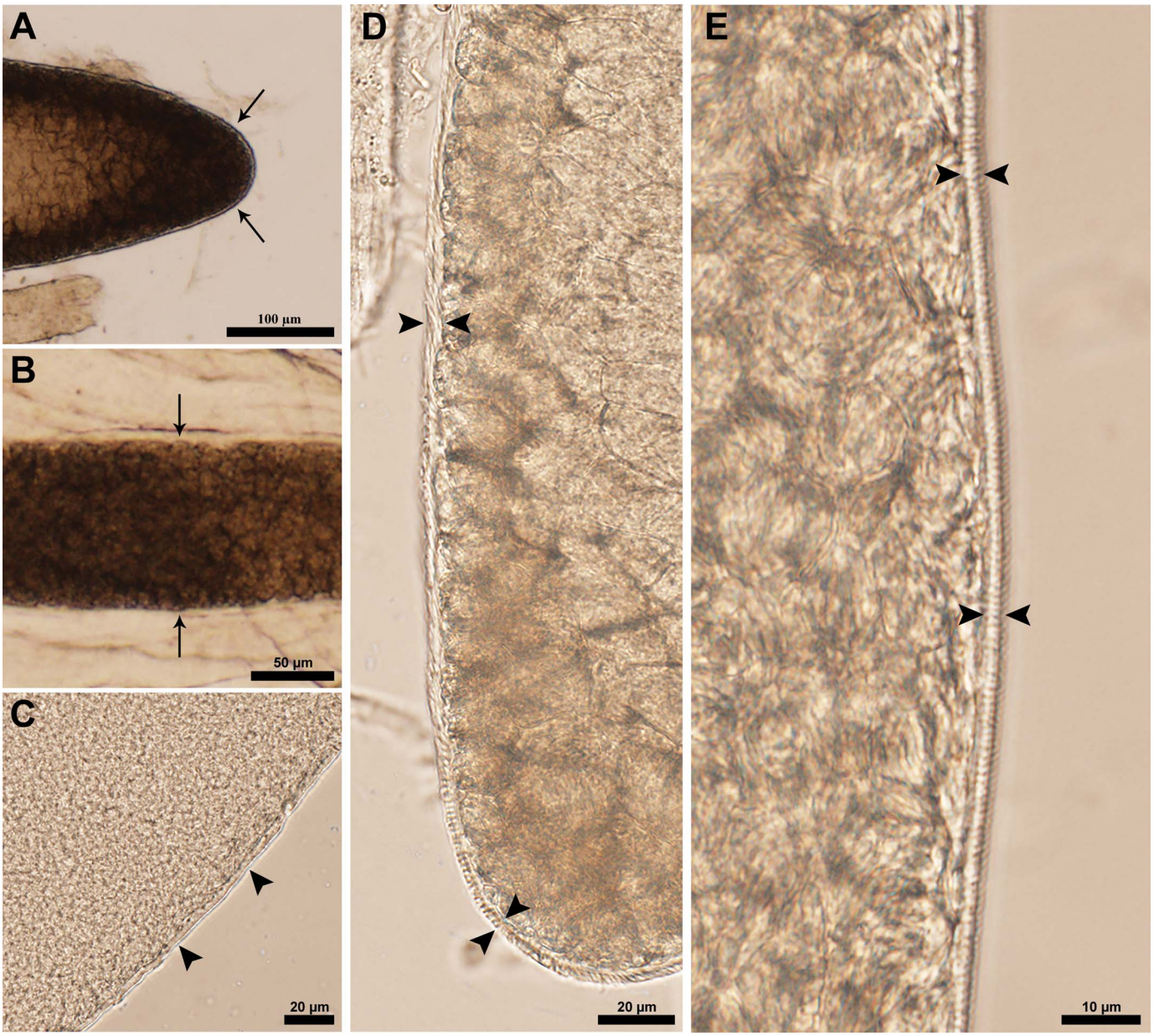

Figure 2. Higher magnification of the sarcocyst walls of *Sarcocystis* spp. in squash of tongue muscle of bobcats from Mississippi. Unstained. (**A**), (**B**) Sarcocysts in the tongue of bobcat no. 4. Note smooth sarcocyst wall without visible protrusions (arrows). (**C**) Thin sarcocyst wall (arrowheads) of *Sarcocystis* sp. in bobcat no. 4. (**D**) A *Sarcocystis neurona*–like sarcocyst with relatively thicker cyst wall and with elongated villar protrusions (arrowheads). (**E**) Higher magnification of the sarcocyst wall of the sarcocyst in Figure 1C. Note incisor teeth–like closely packed villar protrusions (arrowheads).

to discern the projections on the sarcocyst wall even in hematoxylin and eosin (HE) –stained sections examined at ×1,000 magnification. Some sarcocysts had elongated villi described for *S. neurona* (Fig. 3A), some had incisor teeth-like protrusions (Fig. 3B), and some had hobnail-type projections described for *S. felis*. 2 types of sarcocysts appeared to lack recognizable projections; some had thicker cyst walls (Fig. 3D) than others (Fig. 3E).

The density of sarcocysts in histological sections was low (Table I). Except for the 4 bobcats, typical sections contained 1–3 sarcocysts (Table I). Most sarcocysts were seen in 2 bobcats (nos. 11 and 17). Sarcocysts in histological sections were up to 1,045 µm long and up to 109 µm wide (n = 89). Foci of inflammation were detected in the tongues of 2 and the limb muscles of 2 bobcats (Fig. 4; Table I).

Molecular characterization of sarcocysts

Multilocus genotyping was performed on the paraffin-embedded sections from 2 bobcats (nos. 11 and 14) employing primers targeting *18S* rRNA, *28S* rRNA, *COI* rRNA, *ITS-1* and *5.8S* rRNA, and *rpoB* genes (Table II). The resultant sequences from the 2 paraffin sections produced identical reads for each gene.

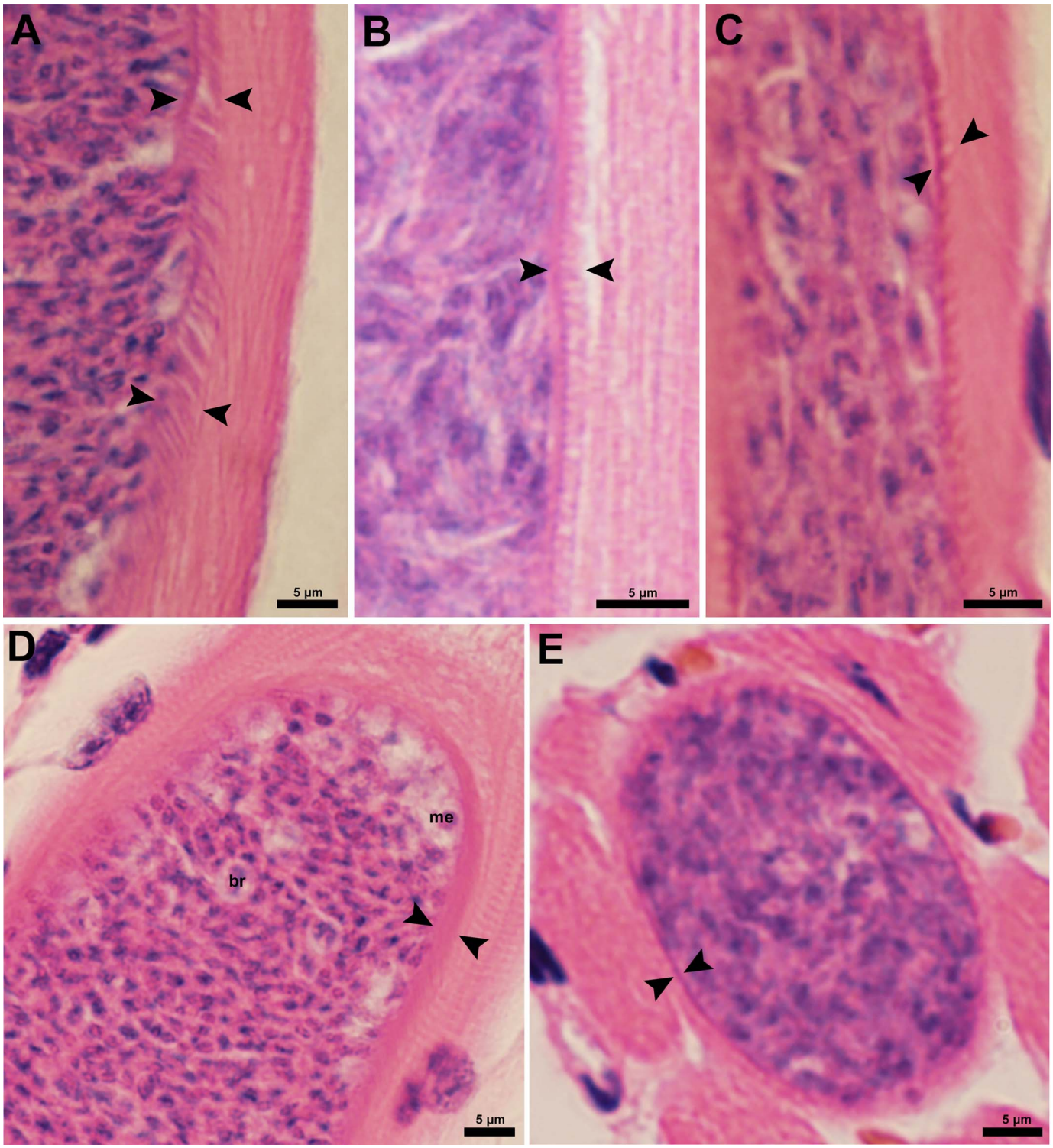

Figure 3. *Sarcocystis* spp. sarcocysts in histological sections of the tongues of bobcats from Mississippi. Arrowheads point to villar protrusions on cyst walls. Hematoxylin and eosin stain. (**A**) Longitudinal section of a *Sarcocystis neurona*–like sarcocyst wall. Note elongated villar protrusions. (**B**) Sarcocyst in the tongue of bobcat no. 1. Note *Sarcocystis felis*–like villar protrusions on the sarcocyst wall. (**C**) An oblique section of a sarcocyst in the tongue of bobcat no. 7. (**D**) A sarcocyst with a relatively thicker cyst wall and with no visible protrusions in the tongue of bobcat no. 14. Note, peripherally located metrocytes (me) and bradyzoites (br) occupy most of the sarcocyst. (**E**) Section of a sarcocyst in myocardium of bobcat no. 20. Note thin sarcocyst wall without villar protrusions.

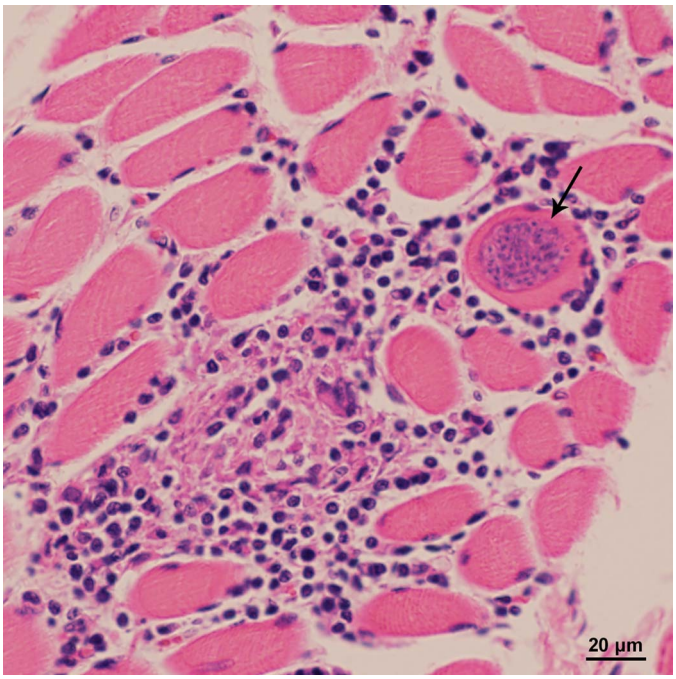

Figure 4. Focal infiltrations of mononuclear cells in histological section of the tongue of bobcat no. 5. Arrow points to cross-section of a sarcocyst. Hematoxylin and eosin stain.

Therefore, we included only 1 sequence for each gene for the molecular analysis. A few chromatograms showed double picks and a background signal, precluding the construction of a consensus sequence; we presume these represent mixed infections and excluded them from further analysis. Since we did not know which sequences could relate to which *Sarcocystis* species, the BLAST of the obtained sequences were done, and, surprisingly, the BLAST searches corresponded almost identically to the already identified species based on morphology and histology (mentioned in the foregoing) such as *S. caninum* (*18S* rRNA, *28S* rRNA, and *rpoB* genes; 705 bp, 565 bp, and 390 bp, respectively); *S. felis* (*ITS-1* gene; 200 bp); *S. lutrae* (*COI* gene; 494 bp); *S. neurona* (*18S* and *28S* genes; 507 bp and 552 bp, respectively). Furthermore, the resultant sequences showed 100% (*18S* rRNA) and 99.29% (*28S* rRNA) identity with *S. caninum* (acc. no. MH469238). In the case of *ITS-1* and *5.8S*, it showed identical homologies with *S. felis* (acc. no. AY190081) whereas in the case of *COI*, it showed 99.80% homology with *S. lutrae* (acc. no. MT036254; 1 single nucleotide polymorphism [SNP] was found, and it was C instead of A in present species). For *S. neurona*, the identities were 100% (*18S* rRNA) and 99.82% (*28S* rRNA). Though it also showed 100% identity with *Sarcocystis speeri* in the case of *28S* rRNA gene, it is noted that based on morphology, histology, and cyst structure, the species is *S. neurona*. Strangely, the *rpoB* gene also showed 100% similarity with *Sarcocystis arctica* and 99.74% with *S. caninum* (1 SNP was found; C instead of T in present species). In this case, also, the species identified is *S. caninum*. Possible reasons could be the limited molecular information available on NCBI from the previously described *Sarcocystis* species which precluded further comparison.

The phylogenetic tree was reconstructed for each gene using the closely related *Sarcocystis* spp. found in the top hits of the BLAST search including the present species. *Toxoplasma gondii* (accession numbers KM657810, JX456456, KJ159879, and XR_001974116), *Sarcocystis rangi* (accession number KJ396590) and *Sarcocystis tarandi* (accession number EF056018) were used as outgroups. For each tree, the obtained isolates were highlighted in bold and a well-supported and highly consensus clustering of the target isolates with the obtained sequences was formed (Fig. 5). Because we used the highly conserved regions for our molecular studies, these clustering patterns are in sync with the morphological and histological identification of the parasites.

Trichinella in histological section

An encapsulated *Trichinella* sp. cyst (258 × 195 µm including the capsule) was found in the tongue of bobcat no. 5 (Fig. 6).

DISCUSSION

Sarcocystis spp. were reported previously from Florida bobcats (*Felis rufus floridanus*), considered a subspecies of *Lynx rufus* (Anderson et al., 1992). In the same year, sarcocysts were found in 4 of 6 bobcats from Arkansas and recognized as a new species, *Sarcocystis felis* (Dubey et al., 1992). Only 1 morphologic type of sarcocyst was found in each report; we reexamined those materials here and confirm that assessment. Those sarcocysts were up to 2.1 mm long and had a thin (around 1 µm) wall with small hobnail-type projections on the cyst wall. Verma et al. (2015) found sarcocysts in histological muscle sections of 26 of 35 bobcats from Mississippi; they, too, were considered *S. felis*. Additionally, in 2 of these bobcats, sarcocysts with thicker walls bearing finger-like villi were detected; these resembled sarcocysts of *S. neurona* sarcocyst, a diagnosis confirmed by characterizing *ITS-1* (Verma et al., 2015). The domestic cat (*Felis catus*) is an intermediate host of *S. neurona,* both experimentally and naturally (Dubey et al., 2000; Turay et al., 2002). *Sarcocystis felis*–like sarcocysts have been reported in muscles from several species of wild felids in the Americas (Cañón-Franco et al., 2016; Dubey et al., 2016). Molecularly, based on *ITS-1, S. felis* is distinct from *S. neurona* and related species that use opossums as definitive hosts (Gillis et al., 2003; Verma et al., 2015; Cañón-Franco et al., 2016). Here, we document more than 3 morphological types of sarcocysts in bobcats. Further studies using the transmission electron microscopic examination and molecular characterization using more discriminatory markers will be needed to clarify how many discrete entities occur, and further fieldwork and experimental transmission studies will be needed to clarify their epizootiology.

The data from Mississippi indicates that bobcats are intermediate hosts for several species of *Sarcocystis*. Of these, a full life cycle is known only for *S. neurona*; the Virginia opossum (*Didelphis virginianus*) is its definitive host. Natural predators for bobcats are unknown. However, bobcat carcasses are probably scavenged by several species of carnivores. Molecular data from *S. felis*–like sarcocysts in wild felids and domestic cats suggest that opossums are unlikely to host *S. felis*.

Based on the molecular analysis, we conclude that the parasites derived from the paraffin-embedded sections from 2 bobcats (nos. 11 and 14) are probably *S. caninum*, *S. felis*, *S. lutrae*, and *S. neurona*, but we do not know which of these cysts correspond

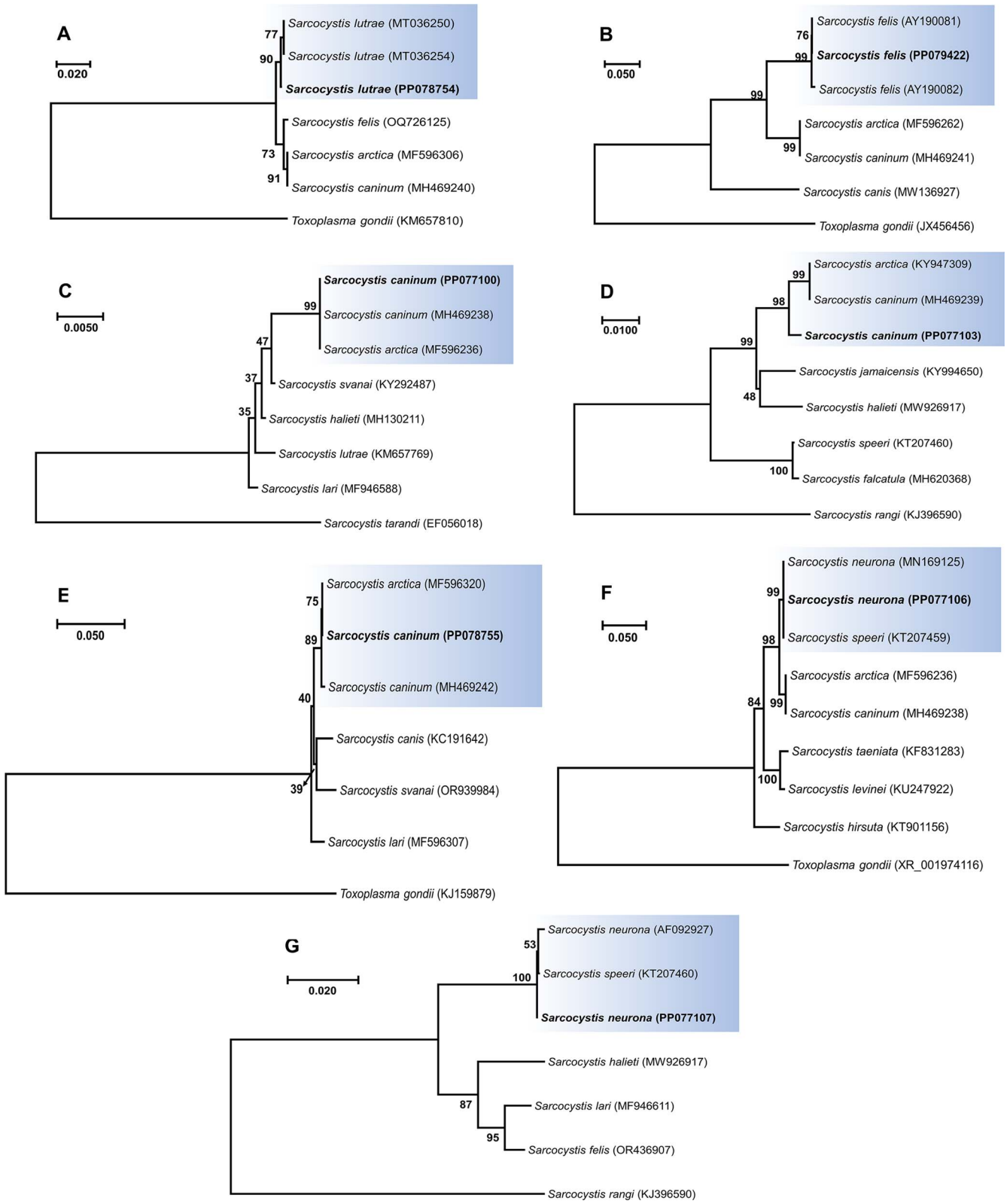

Figure 5. Phylogenetic relationships of the sequences obtained from the muscles of bobcat with other *Sarcocystis* species inferred from various genetic markers (**A**) = *COI* [for *Sarcocystis lutrae*], (**B**) = *ITS-1* and *5.8S* [for *Sarcocystis felis*], (**C**) = *18S* [for *Sarcocystis caninum*], (**D**) = *28S* [for *S. caninum*], (**E**) = *rpoB* [for *S. caninum*], (**F**) = *18S* [for *Sarcocystis neurona*], and (**G**) = *28S* [for *S. neurona*]) under the Bayesian CAT+GTR+GAMMA 4 model. Branch supports are indicated near the corresponding nodes. Species in bold are the ones obtained during the study and the highlighted part shows the cluster of isolates with the present species.

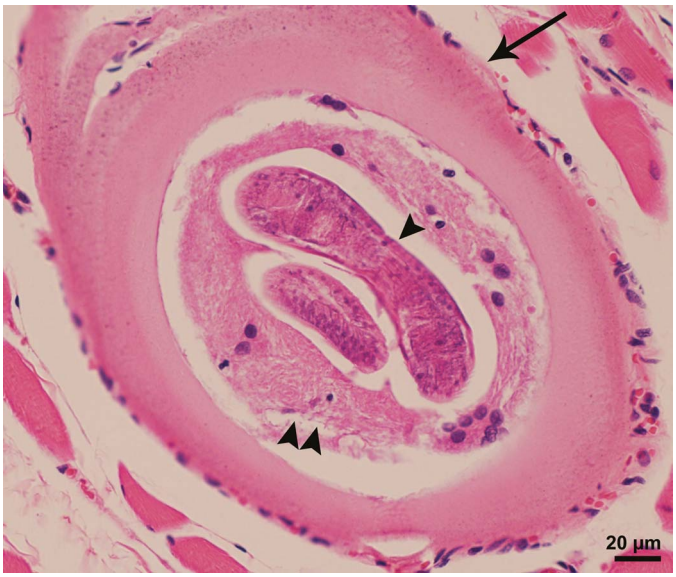

Figure 6. *Trichinella* sp. cyst in tongue section of bobcat no. 5. The larva (arrowhead) is surrounded by nurse myocyte within an empty space (double arrowheads) surrounded by a thick capsule (arrow).

to which parasites. The markers used during the study are mostly employed to study the conserved regions of a particular species, and our results supported the same by forming a cluster of the identified *Sarcocystis* spp. with the *Sarcocystis* spp. under study. Uncertainty remains concerning the identity of the *Sarcocystis* species that have been reported from muscles of domestic and wild carnivores, including wild felids, because (except *S. neurona*) their life cycles remain unknown (reviewed in Dubey et al., 2016). Therefore, further studies are needed to confirm these parasites' identity from the bobcats.

Trichinella spp. infections occur in many species of wildlife, including bobcats (Gajadhar and Forbes, 2010; Reichard et al., 2021). By using pepsin digestion, Reichard et al. (2021) detected *Trichinella* larvae in the tongues of 18 (5.9%) of 301 bobcats from Oklahoma. There are several species (genotypes) of *Trichinella* (Zarlenga et al., 2020). Of the 18 infected bobcats from Oklahoma, 17 had *Trichinella murrelli* and 1 was *Trichinella pseudospiralis* (Reichard et al., 2021). *Trichinella murrelli* is an encapsulated species and common in U.S. wildlife, whereas *T. pseudospiralis* is noncapsulated and comparatively rare. The most common species (genotype) associated with clinical trichinosis in humans is *Trichinella spiralis*, and pigs are the hosts for it, but other species, such as *T. murrelli*, can also cause clinical trichinosis in humans (Dupouy-Camet et al., 2001). Here, we report *Trichinella* sp. in the tongue of a bobcat from Mississippi for the first time. The parasite was most likely *T. murrelli*, but encapsulated species of *Trichinella* do not bear morphological distinctions from one another. Genetic means are required for definitive diagnosis, and this was not possible in this case, because the infection was recognized, from histological sections, long after the disposal of frozen tissues. Finding a parasite of zoonotic potential in these bobcats bears emphasis because, though hunted for fur, their meat was exported for human consumption.

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