SARCOCYSTIS STRIXI N. SP. FROM A BARRIED OWL (STRIX VARIA) DEFINITIVE HOST AND INTERFERON GAMMA GENE KNOCKOUT MICE AS EXPERIMENTAL INTERMEDIATE HOST

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ABSTRACT: Here we report a new species of Sarcocystis with a barred owl (Strix varia) as the natural definitive host and interferon gamma gene knockout (KO) mice as an experimental intermediate host. A barred owl submitted to the Carolina Raptor Center, Huntersville, North Carolina, was euthanized because of paralysis. Fully sporulated 12.5 × 9.9 μm sporocysts were found in intestinal scrapings from the owl. Sporocysts from the barred owl were orally fed to laboratory-reared outbred Swiss Webster (SW) (Mus musculus) and 8 KO mice. All mice remained asymptomatic. Microscopic sarcocysts were found in all 5 KO mice euthanized on day 32, 59, 120, 154, and 206 post-inoculation (PI), not in KO mice euthanized on day 4, 8, and 14 PI. Sarcocysts were not found in any SW mice euthanized on day 72, 120, 206, and 210 PI. Sarcocysts were microscopic, up to 70 μm wide. By light microscopy, the sarcocyst wall < 2 μm thick had undulating, flat to conical, protrusions of varying dimensions. Numerous sarcocysts were seen in the histological sections of tongue and skeletal muscles from the abdomen, limbs, and eye but not in the heart. By transmission electron microscopy, the sarcocyst wall was “type 1.” The ground substance layer (gs) was homogenous, up to 2 μm thick, with very fine granules, and a few vesicles concentrated toward the villar projections. No microtubules were seen in the gs. Longitudinally cut bradyzoites at 206 days PI were 7.8 × 2.2 μm. Based on molecular characterization using 18S rRNA, 28S rRNA, and cox1 genes and morphology of sarcocysts, the parasite in the present study was biologically and structurally different from species so far described, and we therefore propose a new species name, Sarcocystis strixi n. sp.

Sarcocystis species have a 2-host life cycle with asexual stages in extra-intestinal tissues (often herbivore) and sexual stages in the intestine (often carnivore). The definitive host becomes infected by ingesting infected tissues of the intermediate hosts containing mature sarcocysts. Bradyzoites released from sarcocysts penetrate into the lamina propria of the small intestine and undergo fertilization to form oocysts within a day. Oocysts sporulate in situ, and sporulated oocysts or sporocysts released from oocysts are excreted in feces. The intermediate host becomes infected by ingesting food and water contaminated with sporocysts. Sporocysts released from sporocysts initiate asexual multiplication, first as schizonts, and then as sarcocysts. Mature sarcocysts contain hundreds of bradyzoites. Bradyzoites are infectious only for the definitive hosts, and oocysts are infectious only for the intermediate hosts. Some Sarcocystis species, such as Sarcocystis muris and Sarcocystis spp. of lizards, can have a diphongenous life cycle where 1 host can act as both intermediate and definitive hosts (reviewed in Dubey et al., 2016).

Among the rodent-infecting Sarcocystis species using raptors as definitive hosts, barn owls (Tyto alba) are definitive hosts for Sarcocystis dispersa (Cerná et al., 1978; Cerná, 1983) with the house mouse (Mus musculus) as intermediate host, while tawny owls (Strix aluco) are definitive hosts for Sarcocystis scotti of the house mouse (Munday, 1977; Levine and Tadros, 1980; Tadros and Laarmann, 1980) and Sarcocystis sebeki of the field mouse (Apodemus sylvaticus) (Tadros and Laarmann, 1976, 1982). Additionally, masked owls (Tyto novaehollandiae) and barn owls (Tyto alba) are reported definitive hosts for another Sarcocystis species present in the house mouse that was not described (Munday, 1977). A species of Sarcocystis has been reported from the northern saw-whet owl (Aegolius acadicus) and experimentally transmitted to deer mice (Peromyscus maniculatus) (Espinosa et al., 1988). Sarcocystis rauschorum was described from snowy owls (Nyctea scandiaca) and experimentally cycled between varying lemmings (Dicrostonyx richardsoni) and snowy owls (Cawthorn et al., 1984; Cawthorn and Brooks, 1985). Sarcocystis species are generally considered host specific for the intermediate hosts (Dubey et al., 2016). For example, S. sebeki of the house mouse (Mus musculus) was not transmissible to field mice (Apodemus sylvaticus) or voles (Clethrionomys glareolus, Microtus arvalis) (Tadros and Laarmann, 1982).

To our knowledge barred owls (Strix varia) are not known to be definitive hosts for Sarcocystis species. Here we identified barred owls as natural definitive hosts for a species of Sarcocystis infectious for the interferon gamma gene knockout (KO) mice but not for outbred Swiss Webster (SW) mice.

MATERIALS AND METHODS

Naturally infected owl

A barred owl (no. 19241) was admitted to the Carolina Raptor Center, Huntersville, North Carolina, for treatment on 2

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were fixed in 10% buffered neutral formalin. Fixed tissue samples from the spleen, tongue, eye, brain, kidney, liver, intestine, and muscles of the mice were examined and imaged at 80 kV with a Hitachi HT-7700 transmission electron microscope (Hitachi High Technologies America, Dallas, Texas).

**Cell culture**

African green monkey kidney (CV-1) cells (ATCC CCL-70, Manassas, Virginia) were grown in 25 cm² cell culture flasks in RPMI 1640 cell culture medium (Mediatech, Manassas, Virginia) containing 100 IU penicillin/ml, 100 μg/ml streptomycin/ml, and 10% (v/v) fetal bovine serum (FBS). Cells were maintained in the same medium except the concentration of FBS was 2%. Living cell cultures were examined in 25 cm² flasks using an inverted microscope equipped with phase-contrast optics (Zeiss Inverted, Thornwood, New York) for 60 days post-inoculation (PI) of parasites. Cell culture medium was replenished once or twice a week PI based on these observations.

**Sporocysts**: Sporocysts in 0.5 ml of 37 C HBSS were suspended in 0.5 ml of 37 C excystation solution (1.5% (w/v) sodium taurocholic acid and 0.5% (w/v) trypsin (Sigma Chemical Co., St. Louis, Missouri) in HBSS in a 15 ml sterile test tube. Sterile 2 mm glass beads were added to the tube and vortexed for 15 sec, and then the mixture was incubated at 37 C in a water bath for 2 hr. The excystation solution was washed off by repeated centrifugation in HBSS and the pellet used to inoculate in 4 (25 cm²) cell culture flasks containing CV-1 cells (2 flasks at 6 hr and 2 flasks at 12 hr). The inoculation media was washed off cultures 2 hr PI, and fresh maintenance medium was added.

**Schizonts**: *Sarcocystis* species with mice as intermediate hosts often complete schizogony exclusively in liver around the second week after infection (Dubey et al., 2016). Therefore, special attention was focused on livers of mice 8 days PI (Table 1). For this, most (90%) of the liver was fixed in formalin, and the entire liver was sliced 2 mm thick and all pieces embedded in paraffin for histological sectioning. The remainder of the liver was homogenized in saline with pestle and mortar, and the homogenate was inoculated subcutaneously into 2 KO mice and seeded on to CV-1 cells. The KO mice were observed for 32 and 72 days PI, necropsied, and studied histologically (Table 1). The cell cultures were observed for 60 days PI for schizonts.

**DNA extractions and PCR amplification**

Individual sarcocysts isolated mechanically under light microscope were directly transferred into ATL buffer (Qiagen, Valencia, California). Genomic DNA from sarcocysts was extracted using DNeasy Blood and Tissue Kit (Qiagen) according to the manufacturer’s instructions. DNA quantification and quality were determined by Thermo Scientific NanoDrop Lite Spectrophotometer (Thermo Scientific, Waltham, Massachu-

| Table 1. Experimental transmission of *Sarcocystis strixi* sporocysts from a barred owl into laboratory-reared mice. |
|-------------------------------------------------------------|
| **Mice Type** | **Sporocysts dose (× 100)** | **Days post-inoculation** | **Sporocysts found** |
| Swiss Webster | 420 | 210 | No |
| Gamma interferon gene knockout mice | 420 | 206 | No |
| 475* | 42 | 120 | No |
| 966* | 42 | 72 | No |
| 744 | 420 | 154 | Yes |
| 745 | 420 | 32 | Yes |
| 967* | 42 | 120 | Yes |
| 969* | 42 | 59 | Yes |
| 968* | 42 | 14 | No |
| 474* | 42 | 8 | No |
| 475* | 42 | 4 | No |
| 499 Sub-passage* | 32 | No |
| 500 Sub-passage* | 72 | No |

* Sub-passage = liver tissues of mice (ID 474) were homogenized in normal saline and inoculated subcutaneously into 2 KO mice.
PCR amplification and sequencing were done at 2 nuclear ribosomal DNA units, 18S rRNA and 28S rRNA, and the mitochondrial cytochrome c oxidase subunit 1 (cox1) locus. The complete regions of 18S rRNA and 28S rRNA were amplified using overlapping fragments and primer pairs: ERIB1/S2r, SSF/S4r, SSF/Primer Bsarc, and KL1/LS2r, LS1F/KL3, respectively, as described previously (Gjerde and Josefsen, 2015). In addition, the partial sequence of cox1 locus was amplified using primer pair SF1/SR5 (Gjerde and Josefsen, 2015). The PCR amplifications were performed in 50 µl total reaction volume containing 10 pmol of each primer and 1 × Taq PCR Master Mix Kit (Qiagen). The PCR amplicons of 18S rRNA, 28S rRNA, and cox1 were excised from the gel and purified using QIAquick Gel Extraction Kit (Qiagen) according to the manufacturer’s recommendation. The purified PCR products were sent to Macrogen Corporation (Rockville, Maryland) for direct sequencing using the amplification primers, and sequences were analyzed accurately because sarcocysts were twisted in myocytes. The sarcocyst wall had undulating flat to conical protrusions of varying dimensions under a light microscope. The wall varied in width (<1 to 2 µm thick), depending on the area (Fig. 1C). In sections stained with HE, numerous sarcocysts were seen in sections of tongue and skeletal muscles from the abdomen, limbs, and eye (Fig. 1D–E). No sarcocysts were seen in the heart. In HE-stained sections the sarcocyst wall was eosiophilic and often without projections (Fig. 1F). Sarcocysts were partitioned by septa into compartments that contained metrocytes and bradyzoites at 32 days PI and bradyzoites at 206 days PI. Focal myositis was seen associated with degenerating sarcocysts (Fig. 1D, E). The inflammatory response consisted of mixed leukocytes. In the KO mouse at 32 days PI, free bradyzoites were seen apparently without the sarcocyst wall (Fig. 1G). Live bradyzoites were 7–8 µm long. In histological sections, bradyzoites were approximately 4 µm long. Bradyzoites were not found in the pepsin digest of mice negative for sarcocysts by histological examination.

DNA sequencing and phylogenetic analysis

The PCR amplicons of 18S rRNA, 28S rRNA, and cox1 were sequenced from the gel and purified using QIAquick Gel Extraction Kit (Qiagen) according to the manufacturer’s recommendation. The purified PCR products were sent to Macrogen Corporation (Rockville, Maryland) for direct sequencing using the amplification primers, and sequences were analyzed accurately because sarcocysts were twisted in myocytes. The sarcocyst wall had undulating flat to conical protrusions of varying dimensions under a light microscope. The wall varied in width (<1 to 2 µm thick), depending on the area (Fig. 1C). In sections stained with HE, numerous sarcocysts were seen in sections of tongue and skeletal muscles from the abdomen, limbs, and eye (Fig. 1D–E). No sarcocysts were seen in the heart. In HE-stained sections the sarcocyst wall was eosiophilic and often without projections (Fig. 1F). Sarcocysts were partitioned by septa into compartments that contained metrocytes and bradyzoites at 32 days PI and bradyzoites at 206 days PI. Focal myositis was seen associated with degenerating sarcocysts (Fig. 1D, E). The inflammatory response consisted of mixed leukocytes. In the KO mouse at 32 days PI, free bradyzoites were seen apparently without the sarcocyst wall (Fig. 1G). Live bradyzoites were 7–8 µm long. In histological sections, bradyzoites were approximately 4 µm long. Bradyzoites were not found in the pepsin digest of mice negative for sarcocysts by histological examination.

By TEM, the sarcocyst wall consisted of a highly undulating parasitophorous vacuolar membrane (pvm); the undulations were at irregular distances, up to 2.5 µm apart from each other (Figs. 2, 3). The pvm was lined by an uneven electron dense layer (edl) that was up to 50 nm thick. The edl appeared denser at the tips of the fold (Fig. 3). The pvm was invaginated into the interior of the sarcocyst, and invaginations lacked edl at irregular distances, giving the appearance of pores in the wall (Fig. 3). The ground substance layer (gs) was homogenous with very fine granules and few vesicles vs. concentrated towards the villar projections. No microtubules were seen in villar projections. The gs was up to 2 µm thick and continued into the interior of the sarcocyst as septa (Figs. 2, 3). Metrocytes were 5 µm in size with only a few organelles (Fig. 3). Longitudinally cut bradyzoites at day 206 PI were 7.8 × 2.2 (7.1–8.4 × 1.5–2.9; n = 25) µm in size. Bradyzoites contained a conoid, and numerous irregularly arranged microvillae occupying the anterior of the bradyzoite. No more than 2 rhoptries were seen in any bradyzoite section (Fig. 3). There were several dense granules, amylopectin granules, and a sub-terminally located nucleus. The amylopectin granules were few and often located at the non-conoidal end. Both metrocytes and bradyzoites divided by endodyogeny.
FIGURE 1. Life cycle stages of Sarcocystis strixi n. sp. (A) Sporocysts from the intestine of a naturally infected barred owl (Strix varia). Note thin sporocyst wall (sw), 4 elongated sporozoites (sz) and granules of the residual body (rb). (B) Microscopic mature sarcocyst isolated from the muscles of experimentally infected KO mice, 206 days PI, unstained. (C) Sarcocyst wall (cw) with villar protrusions (vp). Also note septa (se). (D) Numerous sarcocysts in abdominal muscle. Arrow points to an inflammatory focus. (E) Severe inflammatory response around sarcocysts in leg muscle. (F) Cross section of a mature sarcocyst with a thin sarcocyst wall, without any visible protrusions. (G) Bradyzoites (br) and metrocytes (me) apparently without a cyst wall. (C–F) Sarcocysts in experimentally infected KO mice, C–F = 206 days PI. G = 32 days PI. B, C-unstained, D–G = histological sections of muscle, stained with hematoxylin and eosin. Color version available online.
FIGURE 2. TEM of a Sarcocystis strixi n. sp. sarcocyst in cross section, 32 days PI. The sarcocyst has undulating surface. Note variability in thickness (arrowheads) of the cyst wall (cw). The ground substance layer (gs) is smooth and continued in the interior of the sarcocyst as septa (se). Most organisms are metrocytes (me) and 1 is dividing by endodyogeny (double arrows). Also note 2 bradyzoites (br) and the host cell (hc).
FIGURE 3. TEM of sarcocysts of Sarcocystis strixi n. sp. (A) Sarcocyst with relatively flat vp. (B) Sarcocyst with angular vp. (C) Note projections (pr) on vp. (D) Metrocyte, probably transforming to bradyzoite. (E) Details of vp. Note pvm lined by edl of uneven thickness, almost missing in areas of invaginations of the pvm in the gs (arrowheads). Also note the juxtaposition of a bradyzoite plasma lemma with outer (om) and inner membrane (im) and amylopectin granules (am). (F) Bradyzoite with a conoid (co), numerous micronemes (mn), 2 rhoptries (rh1, rh2), and posteriorly located nucleus (nu) and amylopectin granules (am). A = 32 days PI, B–E = 206 days PI. Note host cell (hc), parasitophorous vacuolar membrane (pvm) lined by electron dense layer (edl), villar protrusion (vp), ground substance layer (gs), vesicles (vs), metrocytes (me), and bradyzoites. Also note variability in the appearance of vp in 5 images depicted here.
**In vitro cultivation**

No developmental stages of *Sarcocystis* were observed in any of the 4 flasks of CV-1 cell cultures inoculated with sporozoites/sporocysts or liver homogenates.

**PCR and DNA analysis**

Molecular analysis with sarcocysts DNA as the template yielded amplicons of the expected size for the 18S rRNA, 28S rRNA, and *cox1* loci. PCR-DNA sequencing of amplicons resulted the unambiguous sequences of 2 nuclear DNA regions; 18S rRNA (1,782 bp), 28S rRNA (1,493 bp), and the mitochondrial DNA locus, *cox1* (1,045 bp). These sequences were submitted to NCBI GenBank with accession numbers MF162315 (18S rRNA), MF162316 (28S rRNA), and MF162317 (*cox1*) and designated as *S. strixi* n. sp. Analysis of 18S rRNA sequence obtained from *S. strixi* n. sp. confirmed its membership among the genus *Sarcocystis* and indicated an especially close relationship to other parasites in this genus that employs birds as their hosts: *S. dispersa* n. sp. (JN256117), *S. corvusi* (FJ232949), *S. lari* (JQF134580), *Sarcocystis* sp. ex *Phalacrocorax carbo* (JQ733511), *Sarcocystis* sp. ex *Columba livia* (GQ246570.1), and *Sarcocystis* sp. ex *Anser albirotrix* (EU052869).

Relationships among 18S rRNA sequences from various species of *Sarcocystis* were reconstructed to understand the evolutionary position of *S. strixi* with respect to its congener. Almost complete identity with several other species at this slow-evolving molecule precluded complete resolution of this tree, but sufficed to identify *S. strixi* as a member of a clade containing several other parasites known or suspected to use raptors as definitive hosts and their avian and mammalian prey as intermediate hosts. Although phylogenetic information in this molecule is too limited to allow definitive conclusion, our results suggest that among sampled parasite taxa to date, the most closely related species to *S. strixi* may be *S. dispersa*, which completes its life cycle in another species of owl (*Asio otus*) (*Fig. 4*).

The partial 28S rRNA sequence of *S. strixi* n. sp. shared 97% identity with sequences of *Sarcocystis* ex *Columba livia* (FJ232949), *Sarcocystis* (*Frenkelia*) *glareoli* (AF044251), *Sarcocystis* (*Frenkelia*) *microti* (AF044252), *Sarcocystis lutrae* (KM657771, KM657772), *Sarcocystis arctica* (KX022104-7, KF601312), *S. lari* (JQ733509), *Sarcocystis tundrai* (KF795682) *Sarcocystis* sp. ex *Accipter nisus* (GU253888), *Sarcocystis calchas* (KU220951), and many other species of *Sarcocystis*.

The partial *cox1* sequence of *S. strixi* n. sp. (1,045 bp) shared 99% identity with *S. lutrae* (KM657808, KF601326), *S. tundrai* (KT588511-KT588518), *S. arctica* (KX022112-KX022115, KF601318-KF601321), and *Sarcocystis sperei* (KT207461) and 98% identity with *Sarcocystis rileyi* (KT184389, KJ396582) and *S. neurona* (KF854272).

**DESCRIPTION**

*Sarcocystis strixi* n. sp.  
(Figs. 1–4)

**Diagnosis:** Sporocysts in the intestine of barred owl, 12.5 × 9.9 (11.2–13.7 × 8.8–10.9, n = 15) μm in size. Sarcocysts in KO mice microscopic, with thin (<2 μm) sarcocyst wall with wavy outline and conical to flat projections. Ultrastructurally, the sarcocyst wall “type 1” with wavy parasitophorous vacuolar membrane lined by electron dense layer. Microtubules absent in villar projections of the pvm and in the smooth ground substance layer. Bradyzoites 7.84 × 2.19 μm in size.

**Taxonomic summary**

Type definitive host: Barred owl (*Strix varia*).
Natural intermediate host: Unknown.
Experimental intermediate host: Interferon gamma KO mouse.
Type locality: Indian Trail, Union County, North Carolina, USA.
Other localities: Unknown.
Etymology: Species named after the genus of the definitive host, barred owl (*Strix varia*).

**Remarks**

*Sarcocystis* species are generally host-specific for the intermediate host, especially those using rodents as intermediate hosts (Dubey et al., 2016). The ultrastructure of the sarcocyst wall is a useful taxonomic criterion for differentiating *Sarcocystis* species within a given host. The sarcocyst wall of the species in the present study is structurally distinct from other species described. Dubey et al. (2016) proposed 42 types of sarcocyst walls with many subdivisions within some of the wall types. By light microscopy, sarcocysts were grouped as thin-walled (<1 μm) or thick-walled (>2 μm). The “type 1” sarcocyst wall is thin and has small blebs on the wall; it was subdivided into 8 subtypes (1a–1h) depending on the villar protrusions (Dubey et al., 2016). To this description a new “type 1l” was added for *Sarcocystis jamaicensis* from red-tailed hawks (Verma et al., 2017). Type 1 sarcocyst wall has knoblike blebs with rounded ends. Here we have added another new type “type 1j” in the present study. In *S. strixi* n. sp., the undulations were at irregular distances, up to 2.5 μm apart from each other. The pvm was invaginated into the interior of the sarcocyst, and invaginations lacked edl at irregular distances, giving the appearance of pores in the wall.

**DISCUSSION**

Based on the structure of sarcocysts, non-infectivity for house mice, and molecular characteristics, *S. strixi* n. sp. described in the present study is different from other *Sarcocystis* species from rodents that use raptors as definitive hosts. The structures of *S. strixi* n. sp. sarcocysts in KO mice in the present study vaguely resemble those of *S. sebeki* from tawny owls (*Strix aluco*) in muscles of long-tailed field mice (*Apodemus sylvaticus*). There is considerable confusion and uncertainty regarding *S. sebeki*; its description is incomplete and spread over 3 reports by Tadros and Laarmann (1976, 1978b, 1979). This information is summarized
here for the benefit of future investigators. Sporocysts measuring 10 × 14 μm found in intestinal scrapings of a tawny owl (no. 1) in the Netherlands were fed to long-tail field mice (*A. sylvaticus*); the source and number of mice and the source of the owls were not reported. Macroscopic (several centimeters long sarcocysts) were found in skeletal muscle of 2 mice 3 mo later. Infected mouse tissues were fed to an adult tawny owl (no. 2) at the Artis Zoo in Amsterdam. Eight days later, the owl excreted sporocysts similar to those found in owl no. 1 (Tadros and Laarman, 1976). They provided a limited description of the ultrastructure of sarcocysts in a second paper (Tadros and Laarman, 1978a). By light microscopy, the sarcocyst wall was undulated but without projections. By TEM, the “outer unit membrane” was 6 nm thick and had 110 nm osmiophilic invaginations; bradyzoites or metacytes were not described (Tadros and Laarman, 1978a). Subsequently, Tadros and Laarman (1979) reported mouse-to-mouse transmission of *S. sebeki* based on the following experiment. Sporocysts (400,000) from owl no. 2 were fed to 3 *A. sylvaticus*; 8 days later 2 mice died, and the third mouse was euthanized. Schizonts (18 μm in diameter) were detected in smears of liver of all 3 mice; homogenate of livers of all 3 mice were inoculated intraperitoneally into 2 laboratory-bred *A. sylvaticus*. Several-centimeter-long macroscopic sarcocysts were found in skeletal muscles of both mice euthanized 4 mo later; only a few sarcocysts developed in these mice compared with heavy infections induced by feeding sporocysts. In the present study

**Figure 4.** Phylogenetic tree based on 18S rRNA sequences was reconstructed under the criterion of maximum likelihood using Tamura-Nei 93 + G + I model of sequence evolution. Variability among sites was assumed to be gamma distributed with a shape parameter = 0.38 and 73% of sites as invariant. PhyML, as implemented in Geneious 7.0, was used to reconstruct relationships from this alignment and using this model on 100 bootstrap replicates of the data (Guindon et al., 2010).
with *S. strixi* n. sp., schizonts were not identified, and sarcocysts were not detected in muscles of KO mice inoculated with liver homogenates of liver from acutely infected mice. Not all species of *Sarcocystis* are transmissible to KO mice. The parasite in the present study resembles *S. neurona* and *S. speeri* with respect to sporocyst infectivity; *S. neurona* forms sarcocysts in KO mice, but *S. neurona* does not (Dubey et al., 2016). The KO mice are considered an aberrant host for *S. neurona* because only schizonts are produced. In this respect, finding of sarcocysts in KO mice orally inoculated with sporocysts from barred owl is noteworthy and might assist in finding the natural intermediate host for *S. strixi* n. sp. In a prey selection experiment (Graham, 2012), barred owls prefer small prey (*Mus musculus*) over larger prey (*Rattus norvegicus* or *Rattus rattus*); however, Hindmarch and Elliott (2015) documented no house mice (*Mus musculus*) in the diet (remains of 688 prey items) of barred owls inhabiting urban environments in the Lower Fraser Valley of southwestern British Columbia, Canada. Whether the house mouse is a natural host of *S. strixi* n. sp. requires investigation.

Analysis of 18S rRNA, 28S rRNA, and cox1 sequences confirmed *S. strixi* n. sp. membership among the genus *Sarcocystis*. None of these 3 commonly used marker sequences were matched 100% with any sequence deposited in the GenBank. The 18S rRNA sequences are mostly available in GenBank and commonly use in the differentiation of many species of *Sarcocystis*. However, the 18S rRNA sequence of *S. strixi* n. sp. is 99% identical with many species of *Sarcocystis*: *S. corvus*, *S. lari*, *Sarcocystis* sp. ex Phalacrocorax carbo, *S. columbae*, *Sarcocystis* sp. ex *Columba livia*, *Sarcocystis* sp. ex *Anser albifrons*, and many other species of *Sarcocystis*. In the phylogenetic analysis based on 18S rRNA sequences, *S. strixi* n. sp. clustered consistently in a separate clade. These high sequences identity indicated a close relationship with these species but a difference from them. The 28S rRNA sequence of *S. strixi* n. sp. did not share the sequence identity more than 97% with any sequence deposited in the GenBank. The cox1 sequences of *S. strixi* n. sp. shared the highest identity (99–98%) with *S. lutreæ*, *S. turdusi*, *S. arctica*, and *S. speeri*, *S. rileyi*, and *S. neurona*. The uses of cox1 gene as a genetic marker for *Sarcocystis* species discrimination has been proposed recently, so presently only a limited number of sequences of cox1 genes from different *Sarcocystis* are now available for comparisons (Gjerde, 2013). These data sufficed to conclude, however, that there were certain molecular and phenotypic characteristics (i.e., development of sarcocysts in KO mice and bearing a new type “type 1j” wall structure that does not change with age) that set them apart from other known species.

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