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Sarcocystis sp. shed by the common boa snake (*Boa constrictor*) in Brazil

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ABSTRACT

The genus *Sarcocystis* contains around 200 species and 25 of these infect snakes. Two *Sarcocystis* spp. shed by snakes have called special attention of the scientific community. *S. nesbitti*, which is shed by scrub pythons (*Simalia amethistina*), causes myopathy in humans that consume water or food contaminated with the parasite. Sporocysts of *S. singaporensis*, excreted by reticulated pythons (*Malayopython reticulatus*), is letal for rats and was successfully tested in the biological control of these rodents. A high biodiversity of snakes is found in Brazil, however, scarce information is available about *Sarcocystis* spp. in Brazilian snakes. Herein, we investigated *Sarcocystis* sp. in feces of the common boa (*Boa constrictor*) from Salvador, as it is widely distributed in Brazil and it is also bred in other countries. Feces of 65 boas were examined, and *Sarcocystis* sp. was found in 1/65 (1.53%) snakes. All snakes were alive, and for this reason, intestinal scrapping, which is the most sensitive method to detect the parasite, was not performed. Morphometric evaluation of sporocysts showed significant differences in their sizes. PCR and multilocus sequencing of four genetic markers (*cox1, 18S*, ITS1, and *28S*) revealed that sporocysts corresponded to a new *Sarcocystis* species. Sequences of *cox1* and *18S* had identities of 100% and higher than 98%, respectively, with sequences obtained from the rodent *Lagostomus maximus* in Argentina. ITS1 and *28S* sequences did not match with any known *Sarcocystis* sp. No ITS1 and *28S* sequences were available for the *Sarcocystis* sp. found in the Argentinian *L. maximus*. Bioassay using the boa sporocysts was conducted in three mouse lineages and in *Rattus norvegicus*, but no parasitic stages were detected in these rodents. We concluded that the common boa is probably the definitive host of a new species of *Sarcocystis* sp. that has *L. maximus* or related rodents as intermediate hosts.

1. Introduction

Sarcocystis spp. are cyst-forming coccidian parasites that infect mostly mammals, birds and reptiles (Fayer, 2004; Munday et al., 1979). Encysted bradyzoites of *Sarcocystis* spp. are infective solely to their definitive hosts (Fayer, 2004). More than 200 *Sarcocystis* spp. have been described and 22 of these species use snakes as definitive hosts (Dubey et al., 2016; Verma et al., 2017). Classification of most *Sarcocystis* spp. derived from snakes were based on morphological aspects; as molecular confirmation was not attempted for the majority of sporocysts or oocysts detected in these animals, validation of the species names require further analysis (More et al., 2014).

Identification and differentiation of *Sarcocystis* spp. by morphology of sarcocysts, including the size of cysts (macroscopic or microscopic), as well as the conformation of the sarcocyst walls, although quite informative, is not enough for a robust classification at the species level (Dubey et al., 2016; Fischer and Odening, 1998; Fayer, 2004). Descriptions of *Sarcocystis* spp. based on sporocyst morphology is impossible, as sporocysts from different species show very minor differences in size (Slapeta et al., 2003); in these cases, molecular analysis is imperative for genus or species identification (Yang et al., 2001).

The gene *18S* of the rDNA is a largely used tool in studies involving *Sarcocystis* spp. (Carreno et al., 1998; Fischer and Odening, 1998; Tenter, 1995; Tenter and Johnson, 1997), and for decades, it was the mostly frequently employed genetic marker for *Sarcocystis* spp. in snakes (Slapeta et al., 2003; Hu et al., 2012; Abe et al., 2015; Wassermann et al., 2017). Due to the broad use of *18S* for *Sarcocystis* spp., a large number of nucleotide sequences of this marker is available in genetic databases,

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facilitating the conduction of phylogentic studies (Slapeta et al., 2002; Hu et al., 2014). The *28S* rDNA or large subunit of rRNA is less employed than the *18S* rDNA (Hu et al., 2016), however its use may complement or reinforce results obtained by sequencing the *18S* rDNA (Mugridge et al., 2000; Porter and Golding, 2012). The internal transcribed spacer 1 (ITS1) is a region in the rDNA that evolves much faster than the *18S* and *28S* genes (Olias et al., 2011; Gjerde and Schulze, 2014; Prakas et al., 2014). Despite the low number of studies on *Sarcocystis* spp. involving the ITS1, this marker has been successfully used to differentiate closely related species in the genus *Sarcocystis* (Gjerde, 2013; Gjerde and Schulze, 2014). A potential problem when ITS1 is used alone as a genetic marker, is the possibility of intraspecific variation in this *locus* (Gjerde and Josefsen, 2015). The mitochondrial gene coding for cytochrome *c* oxidase subunit 1 (*cox1*) has been largely used in recent years to discriminate closely related species (Gjerde, 2013; Rubiola et al., 2018; Gondim et al., 2021), as it has high interspecific variation and low intraspecific variability (Hebert and Gregory, 2005). According to Hebert et al. (2003), mitochondrial genes evolve faster than those in nucleus and *cox1* was considered particularly useful to resolve problems related to closely related *Sarcocystis* species in numerous animal hosts (Gjerde, 2013). Despite the relevance of *cox1* (Gjerde, 2013), there are still few reports with this marker for *Sarcocystis* spp., and just one paper on its application for *Sarcocystis* sp. in snakes (Verma et al., 2016).

Among *Sarcocystis* spp. that infect snakes, *S. singaporensis* and *S. nesbitti* have attracted special attention of the scientific community due to their impact in public health. The former uses the reticulated pythons (*Malayopython reticulatus*) as definitive hosts, and rodents of the genus *Rattus* and *Bandicota* as intermediate hosts; sporocysts of *S. singaporensis* have an interesting action as a natural rodenticide (Jakel et al., 1996, 2019); when high doses of sporocysts are ingested by rats, they usually die (Zaman and Colley, 1975; Brehm and Frank, 1980; Jakel et al., 1996). *S. nesbitti* was reported as the causative agent of muscular sarcocystosis in humans in the southeast of Asia (Abubakar et al., 2013; Italiano et al., 2014; Lau et al., 2014) and was firstly described as forming sarcocysts in a non-human primate (*Macaca mulata*) (Mandour, 1969). Snakes were suggested as definitive hosts of *S. nesbitti* based on phylogenetic studies conducted by Tian et al. (2012). Lau et al. (2013) studied this hypothesis and found additional molecular evidence pointing snakes as definitive hosts of *S. nesbitti*, but their results were inconclusive. In a study performed by Wassermann et al. (2017), *S. nesbitti* was identified in scrub pythons (*Simalia amethistina*) using morphological and molecular tools, and confirmed the role of snakes as definitive hosts of the parasite.

In Brazil, wild reptiles are frequently rescued from the urban and periurban environments. The common boa (*Boa constrictor*) is one of the most commonly rescued species, due to its large distribution in the country and its habit to search for food and shelter close nearby humans' habitation (Costa, 2015). Despite the increasing relevance of snakes as natural hosts for *Sarcocystis* spp., scarce information is available about these parasites in Brazilian snakes. To the authors' knowledge, there is no data about *Sarcocystis* spp. in common boas. The aim of the present study was to identify and to characterize *Sarcocystis* spp. in *B. constrictor* and to evaluate the infectivity of the detected parasites by bioassay in rodents.

2. Material and methods

2.1. Animals and survey for sporocysts

Fecal samples from 65 *B. constrictor* were collected during 2018 and 2020. All snakes were rescued in the municipality of Salvador and were maintained in the Clinics of Wild and Exotic Animals at Federal University of Bahia (UFBA) or in the Zoobotanic Garden from Salvador, Bahia. The animals were kept in these two locations until improvement of their health status and then released into the wild. The maintenance of the snakes in captivity lasted from weeks to several months. The maximum time of maintenance in captivity occurred for one snake, which was kept for 9 months at UFBA. Single fecal samples were collected from 64 snakes, except for one snake, which was kept longer in captivity and had six fecal samples obtained for the experiment. Snakes were fed laboratory or commercially raised rodents, including mice, hamsters and rats, which were not SPF animals.

Fecal samples were examined by conventional flotation in saturated sucrose solution (density: 1.15 g/mL) as previously described (Gondim et al., 2019). In brief, each fecal sample was fractionated in aliquots of 5 g, and each aliquot was mixed with 12 ml of distilled water, filtered in gauze, and the volume completed with distilled water up to 14 ml of a 15-ml plastic tube. The tube was centrifuged at 1200 *g* for 10 min at 24 °C, brake 3 (0–9 scale), the supernatant was aspirated using a vacuum pump, and the sediment was suspended with sucrose solution. The solution was centrifuged as above and a volume of 50 µl was collected from the very top of each tube and examined for *Sarcocystis* sp. by light microscopy. Samples were re-examined using a fluorescent microscope to detect autofluorescent sporocysts or oocysts. A microscope magnification of 200 × was used in the screening for parasites (Dubey et al., 2016).

For fecal samples that were positive for sporocysts or oocysts, 5 mL of the sucrose solution containing parasites were aspirated from the very top of the 15 mL tube and placed in a 50 mL tube. The 5 mL sucrose solution was mixed with distilled water until completion of the tube (Dubey et al., 2016). The tube was centrifuged at 1600 *g* for 10 min (brake 1) and the sediment was treated with 5 mL of a 2–2.5% sodium hypochlorite solution and homogenized for 10 min. Then, the solution was washed with distilled water in a 50 mL tube and centrifuged as above (1600 *g*, 10 min, brake 1). This washing step was repeated two more times for removal of sodium hypochlorite. After the last washing, the sediment was mixed with 5 ml of antibiotic/antimycotic containing 10.000 U/mL of penicillin, 10.000 µg/mL of streptomycin, and 25 µg/mL of amphotericin B (Gibco-Invitrogen, Carlsbad, CA, USA) in a 15 mL tube and kept at 4 °C (Gondim et al., 2019).

The observed sporocysts were quantified using a hemocytometer and their dimensions were recorded (n = 50) employing a Nikon Eclipse CiL microscope coupled with a Nikon DS-Qi2 camera and the software NIS-Elements (Nikon Instruments Inc.).

2.2. DNA extraction and PCR

DNA was extracted from sporocysts of the last day of sheding (sample 06) using a commercial extraction kit (ZR Fecal DNA MiniPrep™, USA), according to the manufacturer's instructions. Samples containing 100, 1,000, 2,000, 4,000 and 10,000 sporocysts were prepared from a concentrated antibiotic/antimycotic solution and employed for DNA extraction. The obtained DNA was diluted in the buffer contained in the kit and stored 4 °C until analysis.

A conventional PCR was conducted with DNA derived from sporocysts and targeted to four regions of *Sarcocystis* sp. genome. The targets of the PCR and reaction mixtures were as follows: *18S* rRNA (Li et al., 2002), ITS1 (Soares et al., 2011), *cox1* (Gondim et al., 2019) and *28S* (Ellis et al., 1999). Reactions were performed in volumes of 25 µl: 13.8 µl of UltraPure™ water (DEPC Treated Water - Invitrogen®), 2.5 µl of 10x PCR Buffer (50 mM KCl; 10 mM Tris-HCl; pH 9.0), 0.5 µl of dNTP mixture (10 mM of each nucleotide), 1.0 µl of MgCl2 (50 mM), 2.5 µl of each primer (10 pmol/µl), 0.2 µl de Taq DNA Polimerase Platinum, Invitrogen® (5 U/µl), and 2 µl of DNA extracted from sporocysts. The primers used in each reaction were summarized in Table 1. Amplifications were conducted in a Veriti 96-well Thermal Cycler (Applied Biosystems) under the following conditions: (i) initial denaturation at 94 °C for 3 min; (ii) denaturation at 94 °C for 30 s; (iii) annealing at 54/56 °C for 30 s (ITS1, *18S* and *28S* at 56 °C, and *cox1* at 54 °C); (iv) extension at 72 °C for 50 s. A total of 35 cycles were conducted starting from the 30 s denaturation step, followed by a final extension at 72 °C for 5 min.

PCR products were analysed by horizontal electrophoresis in a 2%

Table 1
Primers employed in the *Sarcocystis* sp. PCR for four genetic markers.

Locus	Primer	Sequence	Reference
18S	*18S*9L	GGATAACCTGGTAATTCTATG	Li et al. (2002)
	*18S*1H	GGCAAATGCTTTCGCAGTAG	
ITS1	JS4b	AGTCGTAACAAGGTTTCCGTAGG	Soares et al. (2011)
	CT2b	TTGCGCGAGCCAAGACATC	
cox1	*COX1*-275F22	TGTACCCACGAATTAATGCAGT	Gondim et al. (2019)
	COX1-844R21	GTGTGCCCATACTAGAGAACC	
28S	CR1	CTGAAATTGCTGAAAAGGAA	Ellis et al. (1999)
	CR2	CCAGCTACTAGATGGTTCGA	

agarose gel in TBE buffer (Tris-Borate 0.045M; EDTA 1 mM) and a 100bp (GeneRulerTM 100 pb DNA Ladder) ladder as a ruler. The gel was stained with ethidium bromide and observed under an UV transiluminator at 365 nm.

For sequencing, the PCR products for *cox1* and *28S* were excised from the gel and purified using a commercial kit (GE Healthcare, Illustra, GFX PCR DNA and GEL Band Purification Kit) according to instructions provided by the manufacturer. The purified DNA was stored at −20 °C until sequencing.

2.3. Cloning of amplicons

Amplicons derived from *18S* and ITS1 were cloned into plasmids using the kit InsTAclone PCR Cloning Kit (Thermo Scientific), according to the manufacturer. The recombinant colonies of the transformed bacteria were directly toothpicked from Petri dishes and amplified with a PCR based on the primers M7 and M13, which anneal to the insertion site of the target fragment, to obtain an expected product 800-1000bp long. After PCR confirmation, the successfully recombined colonies were directly sequenced as described in section 2.4.

2.4. Sequencing reactions and analysis

The agarose gel purified PCR products and the residues of the cloned colonies were sequenced using a commercial kit for this purpose: ABI PRISM BigDye® Terminator v3.1 (Ready Reaction Cycle Sequencing, Applied Biosystems), according to the manufacturer's instructions. Each amplicon was sequenced in two directions and in duplicate with the same primers employed in their original PCR. The reaction mixture consisted of 2 μl of Premix Ready Reaction (ABI PRISM BigDye®), 1 μl of sequencing buffer (ABI PRISM BigDye®), 0.5 μl of Primer (10 pmol/μl), 6.5 μl of amplified amplicon, totalizing 10 μl.

The sequencing cycle was performed in a Veriti 96-well Thermal Cycler (Applied Biosystems) with the following conditions: (i) initial denaturation at 96 °C for 1 min; 25 repetitions of (ii) denaturation at 96 °C for 10 s; (iii) annealing at 50 °C for 5 s; (iv) extension at 60 °C for 4 min. Then, the samples were stored at 4 °C and protected by aluminum paper until precipitation. DNA was precipitated in ethanol and submitted to capillary electrophoresis system in an automatic 3500 Genetic Analyzer (Applied Biosystems®), according to instructions provided in the equipment. The quality of sequenced products, as well as the assembled sequences were assessed using the programs Phred-Phrap, included in the software Codoncode Aligner v. 4.2.1 (Codoncode Corp. Dedham, MA, USA).

The sequences from this study were compared with others reported in GenBank using nucleotide BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). For *cox1* and *18S* analysis, molecular phylogenies were inferred with the program MEGA- Molecular Evolutionary Genetics Analysis, version 10.2.6 (Kumar et al., 2018) by using the Maximum Likelihood method. The evolutionary model of nucleotide substitution for each analysis were determined with the software MEGA X and the sequence alignments were done with the software Aliview version 1.27 (Larsson, 2014).

2.5. Bioassay in rodents

Bioassay was conducted in three lineages of mice [Balb/c, Balb/c Nude and interferon gamma knockout (IFN-γ-KO)] and in a lineage of rat (Wistar). The use of animals was allowed by the Ethical Committee of the School of Veterinary Medicine and Animal Science of the Federal University of Bahia (License number: 105/2018).

Sporocysts were orally administered to mice and rats by gavage using different doses of purified sporocysts (Table 2), according to inocula reported in previous studies (Paperna, 2002; Verma et al., 2016). Animals were inoculated under general anesthesia by inhalation with isofluorane in a glass container. The rodents were maintained in sterilized cages, with autoclaved food and water ad libitum. Cages were enriched with autoclaved wood shavings and with plastic igloos.

At the end of experiment, animals were euthanized by general anesthesia with isoflurane, followed by cervical dislocation. Tissues were collected for histopathological analysis. Tissues collected from Balb/c mice included tongue, diaphragm, brain, hindlimb muscles, heart and liver. Tissues collected from Nude and INF- γ-KO mice, and rats, consisted of the above mentioned tissues and additional ones, including abdominal muscles, esophagus, lungs and masseter.

2.6. Statistical analysis

Measurements of sporocysts were evaluated using the Kruskal-Wallis test followed by pairwise analysis and Bonferroni correction. Values of higher than 0.05 were considered as statistically significant.

3. Results

3.1. Frequency and quantitation of sporocysts

Out of 65 examined common boas, sporocysts of *Sarcocystis* sp. were observed by sucrose flotation in the feces of a single animal (1.53%). The initial classification of the sporocysts in the *Sarcocystis* genus was reached on the basis of its peculiar morphology (Fig. 1). The snake that shed sporocysts was kept in the Clinics of Wild and Exotic Animals for nine months and five additional fecal samples of this snake could be evaluated for sporocysts. Of the six examined fecal samples, sporocysts were detected in four of them. The entire volume of each fecal sample was used for maximum recovery and quantitation of the excreted sporocysts. The highest number of sporocysts was observed in the first time

Table 2
Bioassay in rodents using *Sarcocystis* sp. sporocysts shed by the common boa (*Boa constrictor*) in Bahia, Brazil.

Rodent	Number of rodents	Inocolum (sporocysts)	Sample	Euthanasia (DPI)
M. musculos Balb/c	02	1,000	02	116
M. musculus Balb/c	01	10,000	02	116
M. musculus Balb/c Nude	01	2,500	06	87
M. musculus Balb/c Nude	01	25,000	02	87
M. musculus IFN-γ-KO	01	2,500	06	87
M. musculus IFN-γ-KO	01	25,000	02	87
R. norvergicus Wistar	01	10,000	06	111
R. norvergicus Wistar	01	50,000	02	111

IFN- γ-KO = interferon-gamma knockout mice.
DPI:Days post inoculation.

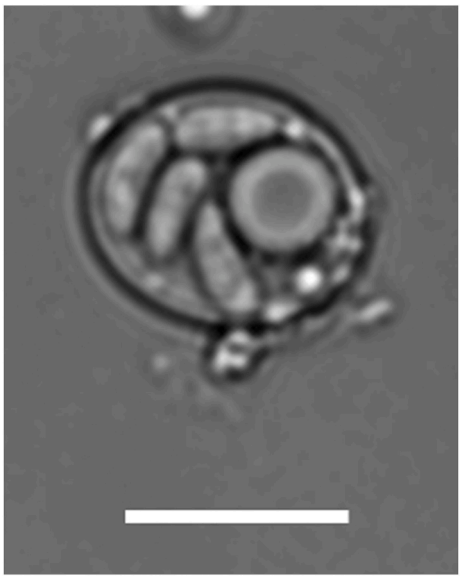

Fig. 1. Sporocyst of *Sarcocystis* sp. shed by a common boa (*Boa constrictor*) in Bahia, Brazil. Four elongated sporozoites and a round residual body are observed inside the sporocyst. Bar = 10 μm.

when parasites were detected. The numbers of sporocysts shed in each day are summarized in Table 3.

3.2. Morphometric analysis of sporocysts

Sporocysts shed in four different days were measured and presented significant differences for the minimum and maximum values for length and width; the differences were observed for the same fecal sample and among different days. The sizes of sporocysts for each fecal sample were as follows: 9.2–12.5 μm ($\bar{x}$ = 10.78) x 7.0–10.11 μm ($\bar{x}$ = 8.49) (sample 02); 9.23–12.53 μm ($\bar{x}$ = 10.88) x 6.68–9.45 μm ($\bar{x}$ = 8.49) (sample 03); 9.63–13.71 μm (11.48) x 7.35–11.22 μm ($\bar{x}$ = 9.28) (sample 05); and 9.67–12.85 μm (10.95) x 7.5–10.79 μm ($\bar{x}$ = 8.67) (sample 06). Statistical analysis revealed significant differences ($p < 0.05$) in the lengths of sporocysts between day 05 and days 02 and 06. Regarding the width of sporocysts, differences were observed between day 05 and days 02, 03 and 06. No differences in lengths or widths of sporocysts were observed in the remaining days.

3.3. Molecular analysis

Four genetic markers (*18S*, *cox1,* ITS1, and *28S*) were employed for identification of the species of *Sarcocystis* shed by the common boa. Molecular analyses were performed with sporocysts obtained from the sample number 6. Sequencing of *cox1* generated a chromatogram with well-defined peaks and high PHRED (Phil's Read Editor) scores (>19), whereas sequencing of *18S* and ITS1 showed low quality chromatograms with double peaks; it was necessary to clone these PCR products into plasmids to obtain high quality consensus sequences.

Table 3
Sporocysts of *Sarcocystis* sp. detected in 4/6 fecal samples excreted by a common boa (*Boa constrictor*) in Bahia, Brazil.

Sample	Date	Number of sporocysts		Fecal volume (g)
		Per μL	Total	
01	June 5, 2018	-		-
02	July 17, 2018	129.8	6.49×10^5	10
03	September 21, 2018	15.4	4.62×10^4	17.17
04	November 02, 2018	-		-
05	December 19, 2018	23.4	1.48×10^5	39.7
06	March 12, 2019	37.4	3.06×10^5	54.34

The *cox1* amplicon of the boa sporocysts (accession number OQ985176) was 983 bp long and presented 100% identity with *Sarcocystis* sp. found in tissues from the rodent *Lagostomus maximus* in Argentina (OP936996– OP937000). High identities with boa sporocysts *cox1* (99.09%) were also found with other *Sarcocystis* spp., such as those from the snake *Pantherophis alleghaniensis* (KU891603) and from the rodent *Mus spretus* (MT418689). The *cox1*-based phylogeny was reconstructed with the 100 hits obtained with the highest identity in BLAST search, using the megablast tool (Fig. 2). After concatenating titles from identical sequences, 33 sequences remained in the dataset. The *cox1*-based phylogeny clustered the 100 sequences within four clades, one formed by species in which birds are definitive hosts, the second formed by species in which didelphid opossums are definitive hosts, a third clade formed by one single taxon (*Sarcocystis strixi*) and a fourth clade formed majorly by species in which lizard and snakes serve as definitive hosts. The sequence from this study is included in the last group that is characterized by long branches, indicating high evolutionary distance among their forming taxons.

Sequences of two *18S* clones were obtained from sporocysts (accession numbers: OR004698 and OR004699). The amplicons of each clone were 911 and 913 bp long and had 99.10% identity with each other. The *18S* phylogeny was reconstructed based on the 45 hits with the highest identity in BLAST search, using both clones as query and two different program selection: highly similar sequences (megablast) and more dissimilar sequences (discontiguous megablast) (Fig. 3). After removing duplicates, 69 sequences remained in the dataset. Two programs were selected because *Boa constrictor 18S* sequences have long segments that allow poor quality alignments with homologues in the Genbank. Several clades were inferred from *18S* phylogeny and the clade composed by the sequences of this study along with *18S* sequences derived from *L. maximus* was demonstrated to be equally divergent from any known species of *Sarcocystis*. The two *18S* clones presented identities between 98.8% and 99.2% with *18S* sequences from *L. maximus* in Argentina (OP937321– OP937324).

Sequences of six ITS1 clones were obtained from sporocysts (accession numbers: OQ991184-OQ991189); three of them (OQ991184, OQ991186, and OQ991188) were identical to each other. The degree of identity between each pair of non-identical ITS1 clones varied from 97.81 to 98.57%. None of the ITS1 clones present correspondence with any sequence deposited in Genbank. No ITS1 sequence was found for the Argentinian *L. maximus*, whose *18S* and *cox1* had high identities with those from the Brazilian common boa.

The *28S* amplicon was 850 bp long. Portions of the 5′ and 3' ends could not be sequenced and the final sequenced fragment was 747 bp (OR483803). It did not present correspondence with any sequence deposited in Genbank, similarly as found for the ITS1 sequences. The best match was the sequence KU891601.1 (*Sarcocystis* sp. ex *Pantherophis alleghaniensis 28S* ribosomal RNA gene, partial sequence; query coverage = 61%; % identity = 80.39%; e-value = 5e-55).

3.4. Bioassay in rodents

All mice and rats that were inoculated with sporocysts of the parasites excreted by the boa did not present any clinical sign until euthanasia, that was conducted between 87 and 116 days post inoculation. Tissue fragments of the rodents stained by HE were examined and did not reveal any parasitic stages.

4. Discussion

In this study, sporocysts of *Sarcocystis* sp. were detected in four fecal samples of a common boa in Salvador, Bahia that was rescued from the streets and kept in captivity for nine months. During captivity, the boa was fed rodents provided by commercial breeders or fed laboratory-raised rodents, such as Swiss mice, Wistar rats and hamsters. Although

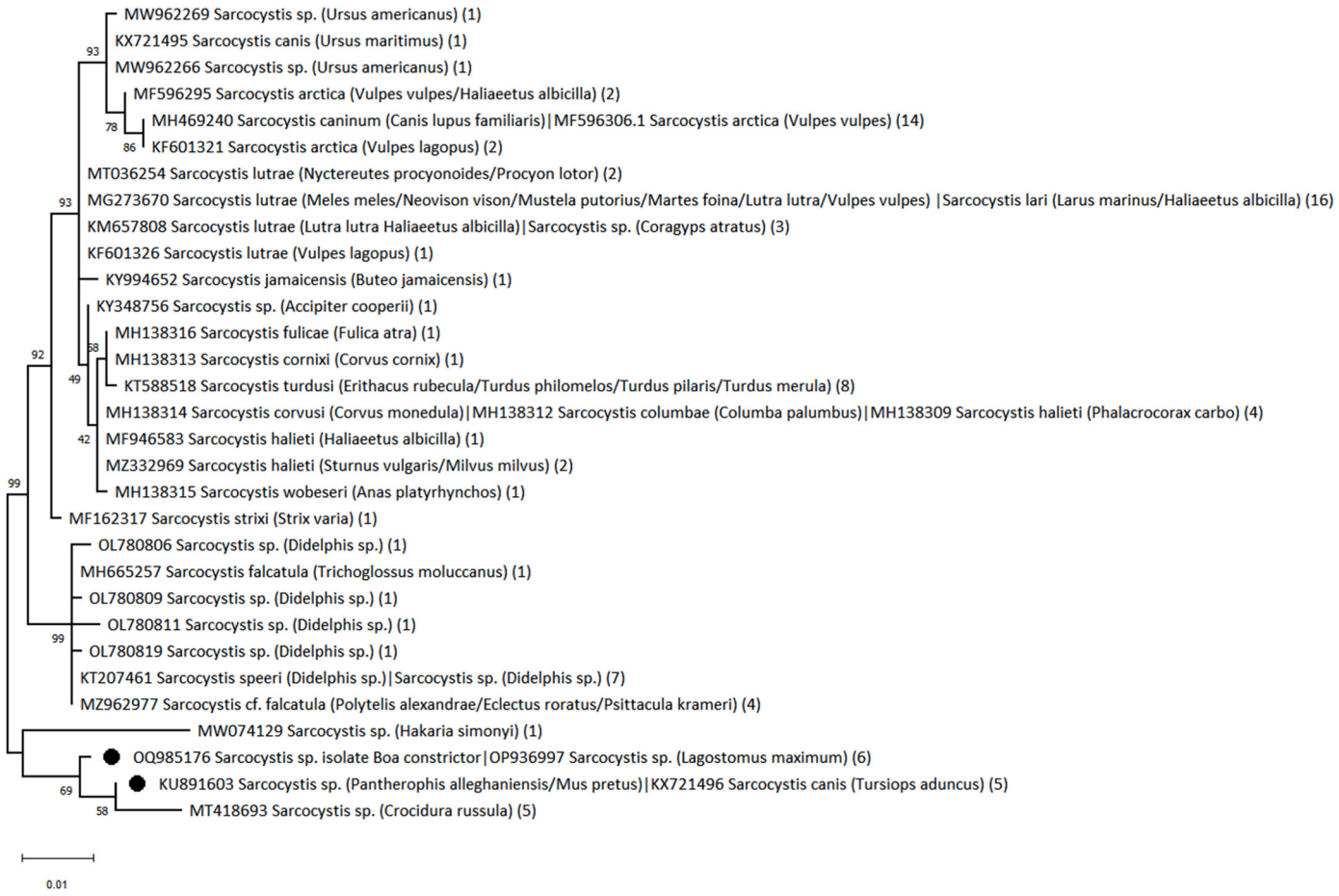

Fig. 2. *Cox1*-based evolutionary analysis of *Sarcocystis* spp.: The tree was inferred by using the Maximum Likelihood method and Tamura 3-parameter model. A discrete Gamma distribution was used to model evolutionary rate differences among sites. The tree with the highest log likelihood is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. This analysis involved 31 nucleotide sequences. All positions containing gaps and missing data were eliminated (complete deletion option). Branches marked with (•) contain sequences detected in snakes. The numbers between parenthesis represent the number of identical sequences at each terminal node. The names between parenthesis represent the hosts in which each sequence at terminal node was found. There were 807 positions in the final dataset. Evolutionary analyses were conducted in MEGA X.

intestinal sections of the snake were not available for histological demonstration of sporocysts in intestinal epithelium, shedding of sporocysts in four fecal samples within an interval of eight months is highly suggestive that the *B. constrictor* served as definitive host of *Sarcocystis* sp. Duration of sporocysts' shedding by snakes is variable, ranging from three to 13 months (Jakel, 1995; Slapeta et al., 1999; Modry et al., 2000). However, a short sporocysts' shedding by a snake was reported in literature and corresponded to 35 days (Morsy et al., 2012). A putative ingestion by the snake of sporocysts inside the intestinal tract of a rodent is improbable. The rodents which were offered to the boa were fed commercial dry food and had no access to any potential source of tissue cysts.

In our study, the common boa probably ingested one or more intermediate hosts containing encysted bradyzoites of the parasite. It is unknown whether the snake was infected in wild or in captivity, as well as the time that *Sarcocystis* sp. was ingested. It is also possible that the snake was infected in wild and re-infected when in captivity.

Molecular analysis of the sporocysts shed by the boa was performed using PCR targeted to four genetic markers (*cox1*, *18S*, ITS1 and *28S*). Sequencing of *cox1* revealed a high quality consensus sequence, which had 100% identity with *Sarcocystis* found in the rodent *L. maximus* in Argentina (Canova et al., 2023). On the other hand, sequencing of PCR amplicons derived from *18S* and ITS1 yielded chromatograms with several double picks and ambiguous results, suggesting that the snake probably shed sporocysts of more than one *Sarcocystis* spp. or a species that contain different alleles for each loci. The PCR products for *18S* and ITS1 were cloned and generated suitable consensus sequences. Interestingly, the two *18S* clones from the boa sporocysts also had high identities with *Sarcocystis* sp. from *L. maximus* in Argentina (Canova et al., 2023), coorroborating the results obtained with *cox1* analysis.

Six clones were obtained for ITS1, which had between 908 and 910 bp. No significant producing alignment was observed after querying the ITS1 sequences derived from the Brazilian boa in BLAST search. Similarly as for ITS1, the *28S* generated sequence of the boa sporocysts did not show any correspondence with deposited sequences in GenBank. Unfortunately, ITS1 and *28S* sequences were not available for the *L. maximus* sarcocysts from Argentina. Analyses of *cox1* and *18S* show that the *Sarcocystis* species found in *B. constrictor* is new and probably identical to that found in *L. maximus* in Argentina.

Sporocysts shed by the boa were employed in bioassays using four lineages of rodents, as this snake species frequently prey on rodents when in wild. Besides rodents, birds, reptiles and several small mammals are also food sources for boas (Pizzatto et al., 2009). No parasitic stage was found in any tested rodent. However, the role of mice and rats as intermediate hosts of this *Sarcocystis* sp. cannot be completely excluded. Sporocysts shed by the snake were not administered to rodents as fresh parasites; instead, they were stored at 4 °C in antibiotic/antimycotic solution for 13–21 months before administration to the rodents. Direct assessment of biological viability of the parasite is a difficult task, as parameters such as motility and the use of certain dyes are not feasible

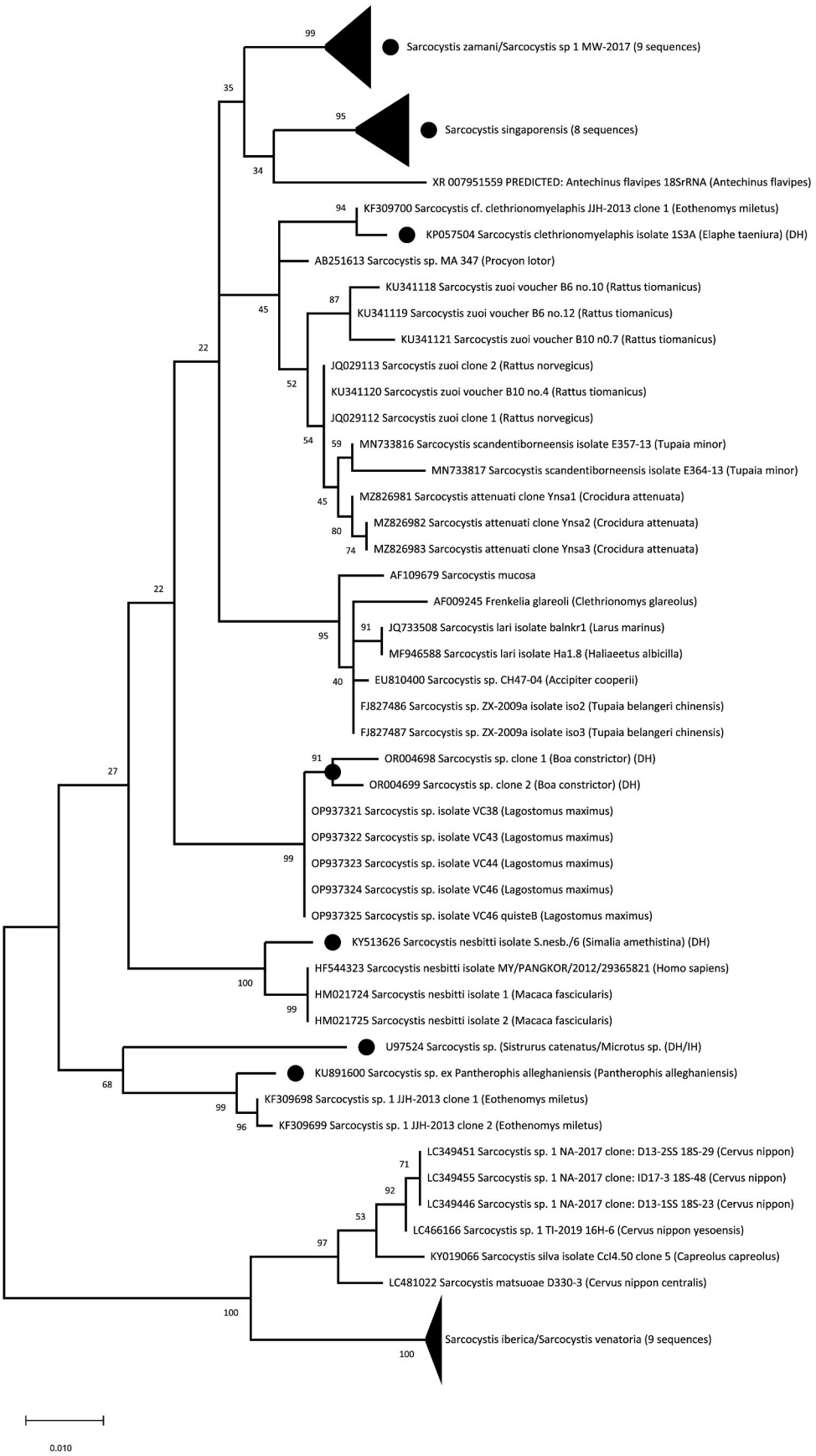

Fig. 3. *18S*-based evolutionary analysis of *Sarcocystis* spp.: the tree was inferred by using the Maximum Likelihood method and Tamura 3-parameter model. A discrete Gamma distribution was used to model evolutionary rate differences among sites. The tree with the highest log likelihood is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. This analysis involved 69 nucleotide sequences. All positions containing gaps and missing data were eliminated (complete deletion option). Branches marked with (●) contain sequences detected in snakes. The names between parenthesis represent the hosts in which each sequence at terminal node was found. There was a total of 624 positions in the final dataset. Evolutionary analyses were conducted in MEGA X.

for sporocysts, whose resistant sporocyst wall protects sporozoites from insults from the environment and hampers the visualization of any movement of the sporozoites inside sporocysts (Savini et al., 1996; Elsheikha and Mansfield, 2004). Bioassay is a useful tool to test infectivity of the parasite (Savini et al., 1996; Cheadle et al., 2001), but has limitations when its life cycle is unknown. Excystation of sporozoites from sporocysts would be another alternative for examining viability of the parasites, although not tested in this study (Elsheikha et al., 2004).

No information is available about infectivity of stored *Sarcocystis* sp. sporocysts from *B. constrictor*. Viability of other *Sarcocystis* spp. shed by snakes stored at refrigerator, such as *S. villivillosi, S. zamani* and *S. singaporensis,* were of six, 14, and 24 months, respectively. Therefore, viability of sporocysts seem to depend on the species of the parasite (Beaver and Maleckar, 1981).

In the current study, sporocysts from fecal sample n. 02 were stored for 21 months until bioassay, and sporocysts from fecal sample n. 06 for 13 months, both kept in refrigerator, before oral inoculation in the rodents. Therefore, the sporocysts might have their infectivity compromised by the prolonged storage time.

The inoculum size for mice used in the present experiment was based on a previous bioassay of *S. pantherophisi* in IFN-γ-KO (Verma et al., 2016); these authors used 800 sporocysts and observed neurological signs in the infected mice and schizonts of the parasite in the CNS of the animals. For the bioassay in Wistar rats, the inoculum of sporocysts was based on a study by Paperna (2002) that induced infection by *S. singaporensis* in these rodents by administration of at least 2,000 sporocysts, causing death in the rats after inoculation of 22,000 sporocysts.

In the present study, the period that rodents were observed after inoculations (87–116 DPI), as well as the selected rodent tissues for analysis (at least, tongue, diaphragm, brain, hindlimb muscles, heart and liver), would be suitable to detect parasites in these samples, in case infections had occurred in the animals. Immature sarcocysts of *S. atheridis* were observed in the skeletal muscle of CD-1(ICR)BR mice at 19 DPI (Slapeta et al., 1999). In bioassay using *S. muriviperae,* initial formation of sarcocysts were identified from 36 DPI in abdominal and thoracic muscles of the mice (Matuschka et al., 1987). Sarcocysts of *S. pantherophisi* were observed at 48 DPI in abdominal, tongue, masseter and limb muscles of IFN-γ-KO (Verma et al., 2017).

The frequency of common boas shedding sporocyst in the current study was low (1.53%) when compared with other studies. It is worth to note that only fecal samples from live animals were employed in this study. The sensitivity of sporocysts detection in fecal samples is much lower than the use of intestinal scraping (Scioscia et al., 2017). In two studies conducted in Brazil, fecal samples were obtained from different species of snakes in captivity and examined for parasites. In the first study, *Sarcocystis* sp. was found in 6/56 (10.7%) snakes, but none of them was of the species *Boa constrictor* (Souza et al., 2014); no molecular tests were used to identify the species of *Sarcocystis*. In a second study, fecal samples from 48 snakes from different species (only one *B. constrictor*), were examined and no sample was positive for *Sarcocystis* sp. (Marques et al., 2020).

In conclusion, sporocysts shed by the Brazilian boa were confirmed to belong to a new and unreported species of *Sarcocystis*, which had high identities with *Sarcocystis* sp. found in tissues from the rodent *L. maximus* in Argentina. The *Sarcocystis* sp. here described seems to have *B. constrictor* as definitive host and *L. maximus*-related rodents as intermediate hosts. Considering that *L. maximus* has not been found in Northeastern Brazil, we suspect that other small mammals closely related to *L. maximus* should serve as intermediate hosts for this *Sarcocystis* species in Brazil.

Author contributions

Conceptualization, L.F.P.G.; formal analysis and investigation, T.L.B., R.F.J., W.B.S. and R.M.S.; resources, L.F.P.G., R.M.S.; original draft preparation, T.L.B.; review and editing, L.F.P.G.; project administration and funding acquisition, L.F.P.G. All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

The authors declare no conflict of interest.

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References

Abe, N., Matsubara, K., Tamukai, K., Miwa, Y., Takami, K., 2015. Molecular evidence of *Sarcocystis* species in captive snakes in Japan. Parasitol. Res. 114, 3175–3179.

Abubakar, S., Teoh, B.T., Sam, S.S., Chang, L.Y., Johari, J., Hooi, P.S., Lakhbeer-Singh, H. K., Italiano, C.M., Omar, S.F., Wong, K.T., Ramli, N., Tan, C.T., 2013. Outbreak of human infection with *Sarcocystis nesbitti*, Malaysia. Emerg. Infect. Dis. 19, 1989–1991, 2012.

Beaver, P.C., Maleckar, J.R., 1981. *Sarcocystis singaporensis* Zaman and Colley, (1975) 1976, *Sarcocystis villivilliso* sp. n., and *Sarcocystis zamani* sp. n.: development, morphology, and persistence in the laboratory rat, Rattus norvegicus. J. Parasitol. 67, 241–256.

Brehm, H., Frank, W., 1980. [The life-history of *Sarcocystis singaporensis* Zaman and Colley, 1976 in the definitive and intermediate host (author's transl)]. Z. Parasitenkd. 62, 15–30.

Canova, V., Helman, E., Robles, M.D.R., Abba, A.M., More, G., 2023. First report of *Sarcocystis* spp. (Apicomplexa, sarcocystidae) in *Lagostomus maximus* (desmarest, 1917) (rodentia, chinchillidae) in Argentina. Int. J. Parasitol. Parasites Wildl. 20, 180–186.

Carreno, R.A., Schnitzler, B.E., Jeffries, A.C., Tenter, A.M., Johnson, A.M., Barta, J.R., 1998. Phylogenetic analysis of coccidia based on *18S* rDNA sequence comparison indicates that Isospora is most closely related to *Toxoplasma* and *Neospora*. J. Eukaryot. Microbiol. 45, 184–188.

Cheadle, M.A., Tanhauser, S.M., Scase, T.J., Dame, J.B., Mackay, R.J., Ginn, P.E., Greiner, E.C., 2001. Viability of *Sarcocystis neurona* sporocysts and dose titration in gamma-interferon knockout mice. Vet. Parasitol. 95, 223–231.

Costa, C.C., 2015. Percepção dos policiais do Pelotão Ambiental do Estado de Sergipe sobre Agenda Ambiental na Administração Pública. Rev. Eletrônica Gest., Educ. Tecnol. Ambient. 19 (3), 395–407.

Dubey, J., Calero-Bernal, R., Rosenthal, B., Speer, C., Fayer, R., 2016. Sarcocystosis of Animals and Humans. CRC Press, Boca Raton, FL.

Ellis, J.T., Morrison, D.A., Liddell, S., Jenkins, M.C., Mohammed, O.B., Ryce, C., Dubey, J.P., 1999. The genus *Hammondia* is paraphyletic. Parasitology 118, 357–362.

Elsheikha, H.M., Mansfield, L.S., 2004. Assessment of *Sarcocystis neurona* sporocyst viability and differentiation between viable and nonviable sporocysts using propidium iodide stain. J. Parasitol. 90, 872–875.

Elsheikha, H.M., Murphy, A.J., Mansfield, L.S., 2004. Viability of *Sarcocystis neurona* sporocysts after long-term storage. Vet. Parasitol. 123, 257–264.

Fayer, R., 2004. *Sarcocystis* spp. in human infections. Clin. Microbiol. Rev. 17, 894–902 table of contents.

Fischer, S., Odening, K., 1998. Characterization of bovine *Sarcocystis* species by analysis of their *18S* ribosomal DNA sequences. J. Parasitol. 84, 50–54.

Gjerde, B., 2013. Phylogenetic relationships among *Sarcocystis* species in cervids, cattle and sheep inferred from the mitochondrial cytochrome c oxidase subunit I gene. Int. J. Parasitol. 43, 579–591.

Gjerde, B., Josefsen, T.D., 2015. Molecular characterisation of *Sarcocystis lutrae* n. sp. and *Toxoplasma gondii* from the musculature of two Eurasian otters (*Lutra lutra*) in Norway. Parasitol. Res. 114, 873–886.

Gjerde, B., Schulze, J., 2014. Muscular sarcocystosis in two arctic foxes (*Vulpes lagopus*) due to *Sarcocystis arctica* n. sp.: sarcocyst morphology, molecular characteristics and phylogeny. Parasitol. Res. 113, 811–821.

Gondim, L.F.P., Soares, R.M., More, G., Jesus, R.F., Llano, H.A.B., 2021. *Sarcocystis neurona* and related *Sarcocystis* spp. shed by opossums (*Didelphis* spp.) in South America. Rev. Bras. Parasitol. Vet. 30, e006521.

Gondim, L.F.P., Soares, R.M., Tavares, A.S., Borges-Silva, W., de Jesus, R.F., Llano, H.A. B., Gondim, L.Q., 2019. *Sarcocystis falcatula*-like derived from opossum in Northeastern Brazil: in vitro propagation in avian cells, molecular characterization and bioassay in birds. Int. J. Parasitol. Parasites Wildl. 10, 132–137.

Hebert, P.D., Cywinska, A., Ball, S.L., deWaard, J.R., 2003. Biological identifications through DNA barcodes. Proceedings. Biological sciences 270, 313–321.

Hebert, P.D., Gregory, T.R., 2005. The promise of DNA barcoding for taxonomy. Syst. Biol. 54, 852–859.
Hu, J.J., Liu, Q., Yang, Y.F., Esch, G.W., Guo, Y.M., Zou, F.C., 2014. *Sarcocystis eothenomysi* n. sp. (Apicomplexa: sarcocystidae) from the large oriental vole eothenomys miletus (thomas) (cricetidae: microtinae) from anning, China. Syst. Parasitol. 89, 73–81.
Hu, J.J., Liu, T.T., Liu, Q., Esch, G.W., Chen, J.Q., Huang, S., Wen, T., 2016. Prevalence, morphology, and molecular characteristics of *Sarcocystis* spp. in domestic goats (*Capra hircus*) from Kunming, China. Parasitol. Res. 115, 3973–3981.
Hu, J.J., Meng, Y., Guo, Y.M., Liao, J.Y., Song, J.L., 2012. Completion of the life cycle of *Sarcocystis zuoi* , a parasite from the Norway rat, Rattus norvegicus. J. Parasitol. 98, 550–553.
Italiano, C.M., Wong, K.T., AbuBakar, S., Lau, Y.L., Ramli, N., Syed Omar, S.F., Kahar Bador, M., Tan, C.T., 2014. *Sarcocystis nesbitti* causes acute, relapsing febrile myositis with a high attack rate: description of a large outbreak of muscular sarcocystosis in Pangkor Island, Malaysia. PLoS Neglected Trop. Dis. 8, e2876, 2012.
Jakel, T., 1995. Cyclic transmission of *Sarcocystis gerbilliechis* n. sp. by the Arabian saw-scaled viper, *Echis coloratus*, to rodents of the subfamily gerbillinae. J. Parasitol. 81, 626–631.
Jakel, T., Burgstaller, H., Frank, W., 1996. *Sarcocystis singaporensis*: studies on host specificity, pathogenicity, and potential use as a biocontrol agent of wild rats. J. Parasitol. 82, 280–287.
Jakel, T., Promkerd, P., Sitthirath, R., Guedant, P., Khoprasert, Y., 2019. Biocontrol of rats in an urban environment in Southeast Asia using *Sarcocystis singaporensis*. Pest Manag. Sci. 75, 2148–2157.
Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., 2018. Mega X: molecular evolutionary genetics analysis across computing platforms. Mol. Biol. Evol. 35, 1547–1549.
Larsson, A., 2014. AliView: a fast and lightweight alignment viewer and editor for large datasets. Bioinformatics 30 (2014), 3276–3278.
Lau, Y.L., Chang, P.Y., Subramaniam, V., Ng, Y.H., Mahmud, R., Ahmad, A.F., Fong, M. Y., 2013. Genetic assemblage of *Sarcocystis* spp. in Malaysian snakes. Parasites Vectors 6, 257.
Lau, Y.L., Chang, P.Y., Tan, C.T., Fong, M.Y., Mahmud, R., Wong, K.T., 2014. *Sarcocystis nesbitti* infection in human skeletal muscle: possible transmission from snakes. Am. J. Trop. Med. Hyg. 90, 361–364.
Li, Q.Q., Yang, Z.Q., Zuo, Y.X., Attwood, S.W., Chen, X.W., Zhang, Y.P., 2002. A PCR-based RFLP analysis of *Sarcocystis cruzi* (Protozoa: sarcocystidae) in Yunnan Province, PR China, reveals the water buffalo (*Bubalus bubalis*) as a natural intermediate host. J. Parasitol. 88, 1259–1261.
Mandour, A.M., 1969. *Sarcocystis nesbitti* n. sp. from the rhesus monkey. J. Protozool. 16, 353–354.
Marques, S.M.T., Meyer, J., Bilhalva, L.C., Fernandes, L.S., Natal, A.C.C., Alievi, M.M., 2020. Avaliação parasitológica de serpentes das famílias Boidae, Elapidae, Pythonidae e Viperidae no sul do Brasil. Rev. Agr. Acad. 3, 150–156.
Matuschka, F.R., Heydorn, A.O., Mehlhorn, H., Abd-Al-Aal, Z., Diesing, L., Biehler, A., 1987. Experimental transmission of *Sarcocystis muriviperae* n. sp. to laboratory mice by sporocysts from the Palestinian viper (*Vipera palaestinae*): a light and electron microscope study. Parasitol. Res. 73, 33–40.
Modry, D., Koudela, B., Slapeta, J.R., 2000. *Sarcocystis stenodactylicolubris* n. sp., a new sarcosporidian coccidium with a snake-gecko heteroxenous life cycle. Parasite 7, 201–207.
More, G., Pantchev, N., Herrmann, D.C., Vrhovec, M.G., Ofner, S., Conraths, F.J., Schares, G., 2014. Molecular identification of *Sarcocystis* spp. helped to define the origin of green pythons (*Morelia viridis*) confiscated in Germany. Parasitology 141, 646–651.
Morsy, K., Bashtar, A.R., Abdel-Ghaffar, F., Mehlhorn, H., Al Quraishy, S., Al-Ghamdi, A., Koura, E., Maher, S., 2012. *Sarcocystis acanthocolubri* sp. n. infecting three lizard species of the genus *Acanthodactylus* and the problem of host specificity. Light and electron microscopic study. Parasitol. Res. 110, 355–362.
Mugridge, N.B., Morrison, D.A., Jakel, T., Heckeroth, A.R., Tenter, A.M., Johnson, A.M., 2000. Effects of sequence alignment and structural domains of ribosomal DNA on phylogeny reconstruction for the protozoan family sarcocystidae. Mol. Biol. Evol. 17, 1842–1853.
Munday, B.L., Hartley, W.J., Harrigan, K.E., Presidente, P.J., Obendorf, D.L., 1979. *Sarcocystis* and related organisms in Australian wildlife: II. Survey findings in birds, reptiles, amphibians and fish. J. Wildl. Dis. 15, 57–73.
Olias, P., Olias, L., Krucken, J., Lierz, M., Gruber, A.D., 2011. High prevalence of *Sarcocystis calchasi* sporocysts in European Accipiter hawks. Vet. Parasitol. 175, 230–236.
Paperna, I., 2002. Ultrastructural study of the development of *Sarcocystis singaporensis* sarcocysts in the muscles of its rat host. Parasite 9, 161–166.
Pizzatto, L., Marques, O.A.V., Facure, K., 2009. Food habits of Brazilian boid snakes: overview and new data, with special reference to *Corallus hortulanus*. Amphib. - Reptil. 30, 533–544.
Porter, T.M., Golding, G.B., 2012. Factors that affect large subunit ribosomal DNA amplicon sequencing studies of fungal communities: classification method, primer choice, and error. PLoS One 7, e35749.
Prakas, P., Oksanen, A., Butkauskas, D., Sruoga, A., Kutkiene, L., Svazas, S., Isomursu, M., Liaugaudaite, S., 2014. Identification and intraspecific genetic diversity of *Sarcocystis rileyi* from ducks, Anas spp., in Lithuania and Finland. J. Parasitol. 100, 657–661.
Rubiola, S., Chiesa, F., Zanet, S., Civera, T., 2018. Molecular identification of *Sarcocystis* spp. in cattle: partial sequencing of Cytochrome C Oxidase subunit 1 (COI). Ital. J. Food Saf. 7, 7725.
Savini, G., Robertson, I.D., Dunsmore, J.D., 1996. Viability of the sporocysts of *Sarcocystis cruzi* after exposure to different temperatures and relative humidities. Vet. Parasitol. 67, 153–160.
Scioscia, N.P., Gos, M.L., Denegri, G.M., More, G., 2017. Molecular characterization of *Sarcocystis* spp. in intestine mucosal scrapings and fecal samples of Pampas fox (*Lycalopex gymnocercus*). Parasitol. Int. 66, 622–626.
Slapeta, J.R., Kyselova, I., Richardson, A.O., Modry, D., Lukes, J., 2002. Phylogeny and sequence variability of the *Sarcocystis singaporensis* Zaman and Colley, (1975) 1976 ssrDNA. Parasitol. Res. 88, 810–815.
Slapeta, J.R., Modry, D., Koudela, B., 1999. *Sarcocystis atheridis* sp. nov., a new sarcosporidian coccidium from Nitsche's bush viper, Atheris nitschei Tornier, 1902, from Uganda. Parasitol. Res. 85, 758–764.
Slapeta, J.R., Modry, D., Votypka, J., Jirku, M., Lukes, J., Koudela, B., 2003. Evolutionary relationships among cyst-forming coccidia *Sarcocystis* spp. (Alveolata: Apicomplexa: Coccidea) in endemic African tree vipers and perspective for evolution of heteroxenous life cycle. Mol. Phylogenet. Evol. 27, 464–475.
Soares, R.M., Lopes, E.G., Keid, L.B., Sercundes, M.K., Martins, J., Richtzenhain, L.J., 2011. Identification of *Hammondia heydorni* oocysts by a heminested-PCR (hnPCR-AP10) based on the *H. heydorni* RAPD fragment AP10. Vet. Parasitol. 175, 168–172.
Souza, J.L., Barbosa Ada, S., Vazon, A.P., Uchoa, C.M., Nunes, B.C., Cortez, M.B., Silva, V.L., Mas, L.B., Melgarejo, A.R., Bastos, O.M., 2014. Parasitological and immunological diagnoses from feces of captive-bred snakes at Vital Brazil Institute. Rev. Bras. Parasitol. Vet. 23, 123–128.
Tenter, A.M., 1995. Current research on *Sarcocystis* species of domestic animals. Int. J. Parasitol. 25, 1311–1330.
Tenter, A.M., Johnson, A.M., 1997. Phylogeny of the tissue cyst-forming coccidia. Adv. Parasitol. 39, 69–139.
Tian, M., Chen, Y., Wu, L., Rosenthal, B.M., Liu, X., He, Y., Dunams, D.B., Cui, L., Yang, Z., 2012. Phylogenetic analysis of *Sarcocystis nesbitti* (Coccidia: sarcocystidae) suggests a snake as its probable definitive host. Vet. Parasitol. 183, 373–376.
Verma, S.K., Lindsay, D.S., Mowery, J.D., Rosenthal, B.M., Dubey, J.P., 2017. *Sarcocystis pantherophisi* n. sp., from eastern rat snakes (*Pantherophis alleghaniensis*) as definitive hosts and interferon gamma gene knockout mice as experimental intermediate hosts. J. Parasitol. 103, 547–554.
Verma, S.K., Lindsay, D.S., Rosenthal, B.M., Dubey, J.P., 2016. Ancient, globally distributed lineage of *Sarcocystis* from sporocysts of the Eastern rat snake (*Pantherophis alleghaniensis*) and its relation to neurological sequalae in intermediate hosts. Parasitol. Res. 115, 2697–2704.
Wassermann, M., Raisch, L., Lyons, J.A., Natusch, D.J.D., Richter, S., Wirth, M., Preeprem, P., Khoprasert, Y., Ginting, S., Mackenstedt, U., Jakel, T., 2017. Examination of *Sarcocystis* spp. of giant snakes from Australia and Southeast Asia confirms presence of a known pathogen - Sarcocystis nesbitti. PLoS One 12, e0187984.
Yang, Z.Q., Zuo, Y.X., Yao, Y.G., Chen, X.W., Yang, G.C., Zhang, Y.P., 2001. Analysis of the *18S* rRNA genes of *Sarcocystis* species suggests that the morphologically similar organisms from cattle and water buffalo should be considered the same species. Mol. Biochem. Parasitol. 115, 283–288.
Zaman, V., Colley, F.C., 1975. Light and electron microscopic observations of the life cycle of *Sarcocystis orientalis* sp. n. in the rat (*Rattus norvegicus*) and the Malaysian reticulated python (*Python reticulatus*). Z. Parasitenkd. 47, 169–185.