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Identification of a new *Sarcocystis* sp. in marsh deer (*Blastocerus dichotomus*) from wetlands of Argentina

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ABSTRACT

The marsh deer (*Blastocerus dichotomus*) is the largest South American native deer species and is listed as "Vulnerable" by IUCN due to the population reduction. As part of a conservation and disease surveillance program, muscle samples from 14 marsh deer found dead in 2016 and 2017 in northeast Argentina were obtained at necropsy. Samples from each animal were processed as pooled muscles (heart, diaphragm, tongue and hindlimb) by homogenization and direct microscopical observation to detect intracellular *Sarcocystis* spp. cysts. Sarcocysts were observed in six samples, and several cysts recovered from two samples were processed by transmission electron microscopy. The cysts were thin-walled and showed a cyst-wall ultrastructure with ribbon-like protrusions similar to other species using cervids as intermediate host and canids as definitive hosts. Genomic DNA from individual sarcocysts from three marsh deer were successfully amplified by PCR of *18S rRNA* and *COI* gene fragments and further sequenced. Sequence comparison revealed a 99.3–100% identity among them and only 93.7–96.6% and 88.8–89.7% identity at *18S rRNA* and *COI* markers, respectively, with other *Sarcocystis* spp. Despite morphological similarities, the high sequence divergence at *18S rRNA* and *COI* fragments allowed the assumption that *Sarcocystis* sp. from marsh deer is a different species from others using cervids as intermediate hosts. Therefore, we propose the name *Sarcocystis blastoceris* n. sp. for the species infecting marsh deer.

1. Introduction

The marsh deer (*Blastocerus dichotomus*) is the largest native deer in South America, classified as "Vulnerable" by the International Union for the Conservation of Nature (IUCN) (Duarte et al., 2016) and the Red List of Mammals of Argentina (Pereira et al., 2019). The species naturally inhabits wetlands and marshy areas. Argentina's two largest populations are located in the Iberá Wetlands and the Lower Delta of the Parana River (Pereira et al., 2019). Wetlands in Argentina have suffered significant ecosystem changes due to intensive forestry activity and growing livestock expansion, affecting marsh deer populations (Neiff and de Neiff, 2006; Quintana, 2011; Orozco et al., 2013; Guillemi et al., 2019). During 2016 and 2017, extraordinary floods occurred in Lower Delta and Iberá Wetlands, respectively. In 2016, more than 200 dead marsh deer were recorded, while the number peaked at 400 in 2017 (Orozco et al., 2017, 2020). The development of participatory surveillance of marsh deer morbidity and mortality was launched in Argentina, where an association between unfavorable environmental conditions, high parasite load, and reduction of body score with mortality was identified (Orozco et al., 2020). In the study, muscle cysts of *Sarcocystis* spp. were observed in cardiac and skeletal muscles; however, no further identifications were performed. *Sarcocystis* spp. are intracellular

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protozoan parasites with heteroxenous life cycles, forming muscle cysts (sarcocysts) on intermediate hosts in which the infection is generally chronic and asymptomatic. Most species use herbivores as intermediate host and carnivores/omnivores as definitive host, and predator-prey relationships favor protozoans' life cycle (Dubey et al., 2016).

The proper differentiation of *Sarcocystis* spp. could be achieved by morphological features based on ultrastructure of cyst walls along with molecular characterization mainly based on sequencing from different genetic markers, such as *18S rRNA*, *cytochrome C oxidase subunit I gene (COI)* and *internal transcribed spacer 1 (ITS1)* (Gjerde, 2013; Gjerde, 2014a; Dubey et al., 2016; Gjerde et al., 2017a, b). During the last years, several studies have demonstrated the richness of *Sarcocystis* spp. present in muscles from different cervids; for example, *Cervus elaphus* harbored up to 13 different species (Dahlgren et al., 2008; Dahlgren and Gjerde, 2010; Gjerde, 2014a; Dubey et al., 2016; Gjerde et al., 2017a, b; Rudaitytė-Lukošienė et al., 2021). Recently, studies on the identification of *Sarcocystis* spp. in exotic red deer (*C. elaphus*) (Chang Reissig et al., 2016) and the native deer species pudu (*Pudu puda*) and huemul (*Hipocamelus bisulcus*) have been performed in Argentina (Chang Reissig et al., 2020). In the mentioned studies, *S. taeniata* was identified in *C. elaphus,* and three different species were recorded in the native deer species. *Sarcocystis* spp. identified in pudu and huemul were morphologically and molecularly similar from other species previously identified in cervids (Chang Reissig et al., 2020). Until now, there is no specific description of the *Sarcocystis* spp. using marsh deer as intermediate hosts. The investigation of parasitic and infectious agents is crucial for health surveillance and identifying potential transmission cycles of wildlife pathogens. This study aimed to identify and describe *Sarcocystis* spp. in muscles from free-ranging marsh deer from two wetlands in Argentina.

2. Material and methods

2.1. Samples

In the frame of a participatory surveillance of marsh deer morbidity and mortality, tissue samples were collected from dead marsh deer during the years 2016 and 2017 (Orozco et al., 2020). A total of 14 animals, 10 from Iberá Wetland (five adults, four yearlings and one fawn) and four from Lower Delta (one adult, two yearlings and one fawn) were necropsied and samples of heart, diaphragm, tongue and skeletal muscle (hindlimb) were collected. The samples were preserved frozen at −20 °C until laboratory analysis.

2.2. Direct microscopic examination

All the muscles for each animal were processed as a pooled muscles sample following the methodology of direct microscopic examination previously described (Moré et al., 2011; Chang Reissig et al., 2020). Briefly, 5–10 g of muscles were ground in a tissue homogenizer with the addition of 50 ml Phosphate Buffered Saline (PBS - pH 7.2), filtered and centrifuged. Approximately 2 ml of homogenate sediment were placed in a Petri dish, diluted with PBS and observed in an inverted microscope at 40× magnification (Nikon, TMZ). For molecular studies, individual cysts or cyst portions were collected with a micropipette with a filtered tip along with 20 µl PBS and frozen at −20 °C in 1.5 ml DNase-free microtubes. When several cysts were observed, they were collected (around 10 per sample) and fixed in 2.5% glutaraldehyde for transmission electron microscopy (TEM), carried out by the Electron Microscopy Central Service "Shin-Ichi Itagaki" from the FCV-UNLP using a JEM 1200 EX II (JEOL) transmission electron microscope and proceeding as previously described (Moré et al., 2011).

2.3. Molecular analyses

DNA extraction was performed from individual sarcocysts collected during the microscopic examination, using a commercial kit according to the manufacturer's instructions (Wizard genomics, Promega, USA). Each routine of DNA extraction was performed along with a process control sample (as negative control using only the kit solutions). A fragment of *Sarcocystis* spp. *18S rRNA* gene was amplified by PCR using the primer combinations *SarcoF-SarcoR, ERIB1-PrimerB* and *SarcoFext-SarcoRext,* as previously described (Moré et al., 2011, 2013). Additionally, fragments of *COI* were amplified using the *SF1* forward primer and two different reverse primers (*SRD8* and *SR9*), according to protocols previously described by others (Gjerde, 2013; Gjerde et al., 2017a). Cyst DNA samples with positive results in the *18S rRNA* PCR were further amplified with primers *SU1F* and *5.8SR2*, targeting the *ITS1* according to a previously described protocol (Gjerde, 2014b). Table 1 provide the sequence and reference for primers successfully used. All the PCR were conducted using a commercial Taq enzyme (Invitrogen, USA) in a conventional thermocycler (T18, IVEMA, Argentina). Each PCR routine was conducted using a positive control (*Sarcocystis cruzi* DNA), a negative control (process control sample) and a non-template control (NTC).

Amplification products with an estimated concentration of at least 40 ng/µl were purified using a commercial kit according to manufacturer instructions (Wizard SV clean-up system, Promega). Purified amplicons were submitted for sequencing to Macrogen Inc., South Korea (http://www.macrogen.com), with both primers used for each amplification. Sequences obtained were aligned and analyzed using the Geneious software (R9 version). Consensus sequences obtained were compared with others reported in GenBank by BLAST analysis.

The *18S rRNA* and *COI* sequences obtained were aligned with 38 and 37 other sequences from *Sarcocystis* spp., respectively, most of them corresponding to species using cervids and ruminants as intermediate host. Phylogenetic trees were constructed using the Neighbor-Joining method based on the Tamura-Nei genetic distance model, with 1000 bootstraps and using a *T. gondii* sequence as outgroup to root the tree.

Table 1
Description of primers used for conventional PCR and sequencing of the 18S rRNA gene, cytochrome oxidase subunit I gene (COI) and internal transcribed spacer 1 (ITS1) region from *Sarcocystis* spp.

Primer name	Orientation	Target Gen	Amplicon length (bp)	Primer sequence (5′- 3′)	Reference
SarcoFext	Foward	18S rRNA	850–900	GGT GAT TCA TAG TAA CCG AAC G	Moré et al. (2013)
SarcoRext	Reverse	18S rRNA		GAT TTC TCA TAA GGT GCA GGA G	Moré et al. (2013)
SF1	Foward	COI	1000–1100	ATG GCG TAC AAC AAT CAT AAA GAA	Gjerde (2013)
SRD8	Reverse	COI		CAT TGC CCA TDA CTA CGC C	Gjerde (2013)
SR9	Reverse	COI		ATA TCC ATA CCR CCA TTG CCC AT	Gjerde et al., 2017b
SU1F	Foward	ITS1	800–1200	GAT TGA GTG TTC CGG TGA ATT ATT	Gjerde, 2014b
5.8SR2	Reverse	ITS1		AAG GTG CCA TTT GCG TTC AGA A	Gjerde, 2014b

3. Results

3.1. Direct microscopic examination and TEM

Sarcocystis spp. infection was identified by direct microscopic examination in pooled muscles from six animals (43%; 6/14). The positive animals were 5/10 from Iberá Wetlands (three adults and two yearling deers) and 1/4 from the Lower Delta region (adult deer). All positive samples presented microscopical fusiform sarcocysts. A total of 18 sarcocysts or cysts portions were observed at optical microscopy (five from each sample with several cysts and two from each of the four remaining positive pooled muscle samples). The few complete cysts observed measured 500–650 μm long and 45–70 μm wide, while the sarcocyst wall was thin (≤1 μm), and no clearly visible protrusions were observed at optical microscopy (Fig. 1A and B).

From two samples (17-12 CDP and 17-14 CDP), several cysts were observed and further recovered for TEM processing. A total of four cysts from these two marsh deer samples were observed by TEM and presented a thin cyst wall consisting of a ground substance layer and the outer membrane with small invaginations (undulated appearance), from which arise bent ribbon-like protrusions folded over the cyst surface (Fig. 2). The ground substance layer was smooth and measured approximately 0.4 μm thick. The cyst wall ultrastructure was similar to other *Sarcocystis* spp. using cervids as IH like *S. cervicanis, S. grueneri*, *S. linearis, S. morae*, *S. taeniata, S. wapiti* and the observed in sarcocysts from pudu from Argentina (Chang Reissig et al., 2020). The bradyzoites contained amylopectin granules and were vacuolated, and no proper descriptions were achieved (Fig. 2).

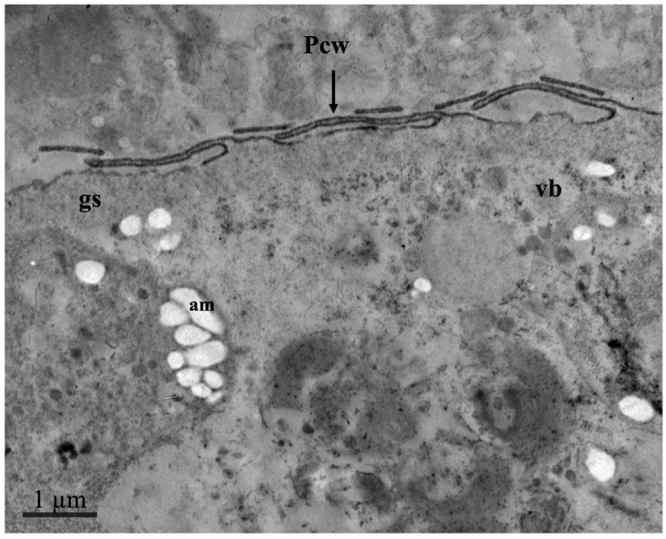

Fig. 2. Transmission electron microscopy (TEM) image of the cyst wall from a microscopical sarcocyst in a marsh deer muscle (17-12CDP). Note the primary cyst wall (**Pcw**) from which arise bent ribbon-like protrusions folded over the cyst surface. The ground substance layer (**gs**) showed no granules nor microtubules. Electron lucid amylopectin granules (**am**) appear irregularly distributed within vacuolated bradyzoites (**vb**). Both, bradyzoites and host muscle cell are decomposed.

3.2. PCR and sequencing

DNA from 12 individual cysts from the six positive deer pooled muscles samples (one single cyst from three samples and three individual cysts from the remaining three samples) were amplified by *18S rRNA* PCR. Out of all samples, eight resulted positive or weak positive, and only three cysts produced amplicons with proper concentration for sequencing using the primers combination *SarcoFext-SarcoRext*. These cysts were from different animals: marsh deer 17-12 and 17-14 CDP (Iberá wetland) and 17-6 CDP (Lower Delta). The *18S rRNA* gene fragment sequences obtained (449, 474 and 733bp) showed a 99.8–100% identity among them with one single nucleotide polymorphism (SNP) in 17–12 CDP sequence (Table 2). After that, these DNA samples were further amplified by *COI* PCR, obtaining amplicons with both primer combinations (*SF1/SR8D* and *SF1/SR9*) and submitted for sequencing. By *ITS1* PCR, these samples gave weak products, and only from the 17-12 CDP was the amplicon sequenced, obtaining a poor-quality result; consequently, no consensus was achieved. The *COI* consensus sequences (ranging from 999 to 1039 bp) from 17-12 and 17-14 CDP were identical and had a 99.3% identity with the one from 17 to 6 CDP (7 SNPs). All the obtained consensus sequences were uploaded in the GenBank, and the sequencing results and BLASTn comparison are summarized in Table 2.

In the phylogenetic tree, the *18S rRNA* sequences from *Sarcocystis* sp. in marsh deer muscles were positioned in a branch with a *S. grueneri* sequence. Several sister clades contained sequences from *S. cervicanis*, *S. cruzi*, *S. hjorti*, *S. iberica*, *S. linearis, S. morae, S. rangi, Sarcocystis* sp.

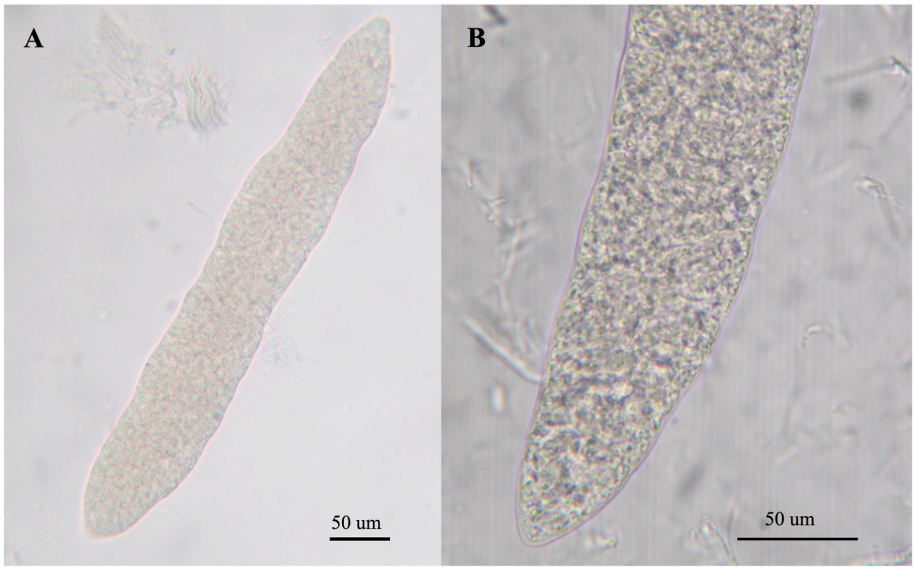

Fig. 1. A and B: Microscopical sarcocyst detected in marsh deer muscles. **A:** Optical microscopy image from a complete cyst. **B**: higher magnification of a portion from the cyst displayed in **A**. Note the thin cyst wall without apparent protrusions.

Table 2
Sequencing and BLAST comparison results from marsh deer individual sarcocysts samples.

ID	Age/Sex	Region	Cyst ID	Molecular analysis			GenBank Accession number
				Target gene	Bp	BLASTn identity, sequences with the highest scores (#)	
17-6 CDP	adult/F	Delta	17-6 CDP A	18S rRNA	733	96.7% *S. grueneri* (EF056010), 96.1% *S. tarandivulpes* (EF467657) and *Sarcocystis* sp. from huemul (MT137767 and MT137769), 95.4% *S. wapiti* (MH167456)	ON911503
				COI[1]	999	88.8% *S. grueneri* (KC209624 among others)	ON932790
				COI[2]	NR		–
17-12 CDP	adult/M	Ibera	17-12 CDP C	18S rRNA	474	94.7% *S. grueneri* (EF056010), 93.7% *S. tarandivulpes* (EF467657) and *Sarcocystis* sp. from huemul (MT137767 and MT137769)	ON911504
				COI[1]	1025	88.8% *S. grueneri* (KC209624 among others)	ON932791
				COI[2]	1016	88.9% *S. grueneri* (KC209624 among others)	ON932792
17-14 CDP	yearling/ F	Ibera	17-14 CDP B	18S rRNA	449	95.2% *S. grueneri* (EF056010), 94.3% *S. tarandivulpes* (EF467657) and *Sarcocystis* sp. from huemul (MT137767 and MT137769)	ON911505
				COI[1]	973	89.7% *S. grueneri* (KC209624 among others)	ON932793
				COI[2]	1039	88.8% *S. grueneri* (KC209624 among others)	ON932794

References: F = female, M = male. Bp = Sequence base pairs. (#) = accession numbers of sequences retrieved by BLAST alignment. COI = mitochondrial cytochrome oxidase subunit I gene; [1] = amplification with primers SF1 and SRD8; [2] = amplification with primers SF1 and SR9.
NR: no results after several attempts.

from huemul and pudu in Argentina, *S. taeniata S. tenella*, *S. venatoria* and *S. wapiti*, suggesting a common ancestor for all this species (Fig. 3). The tree inferred from *COI* sequences positioned all obtained sequences from marsh deer *Sarcocystis* sp. cysts together and in a branch (with high consensus support) along with *S. grueneri* sequences. A sister clade contained sequences from *S. cervicanis*, *S. linearis, S. morae, Sarcocystis* sp. from pudu in Argentina and *S. taeniata* (Fig. 4).

3.3. Taxonomic summary

Etymology: *Sarcocystis blastoceris* n. sp. Named after the record in marsh deer muscles (*Blastocerus dichotomus*).

Sarcocysts morphology: thin-walled (≤1 μm) without visible protrusions at optical microscopy. Measured up to 650 μm long and 70 μm wide. Cyst wall ultrastructure by TEM formed by a layer of ground substance and the outer unit membrane which form small invaginations into the ground substance giving an undulating appearance, from which

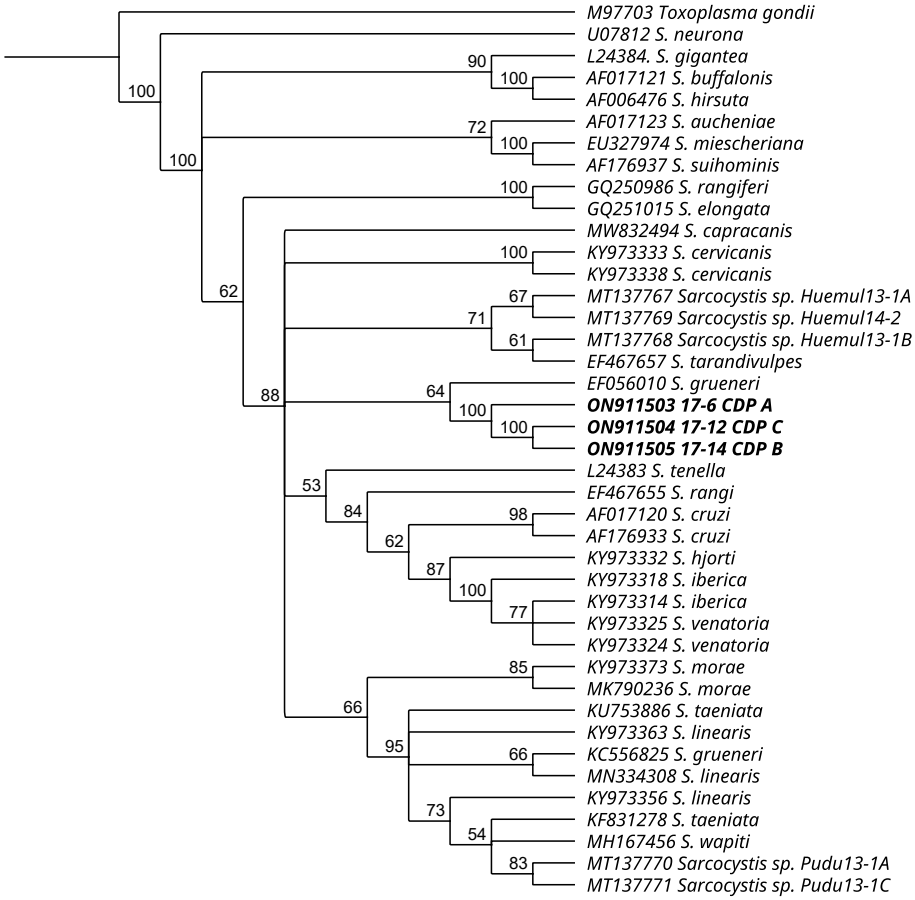

Fig. 3. Neighbor-Joining consensus phylogenetic tree. Phylogenetic tree based on an alignment of 41 *Sarcocystis* spp. 18S rRNA sequences performed with GENEIOUS software (Version R9), using a Tamura-Nei genetic distance model. The three sequences from marsh deer obtained in the present study are in bold. Branch consensus support is expressed as % from 1000 bootstraps. Sequence M97703 from *T. gondii* used as outgroup.

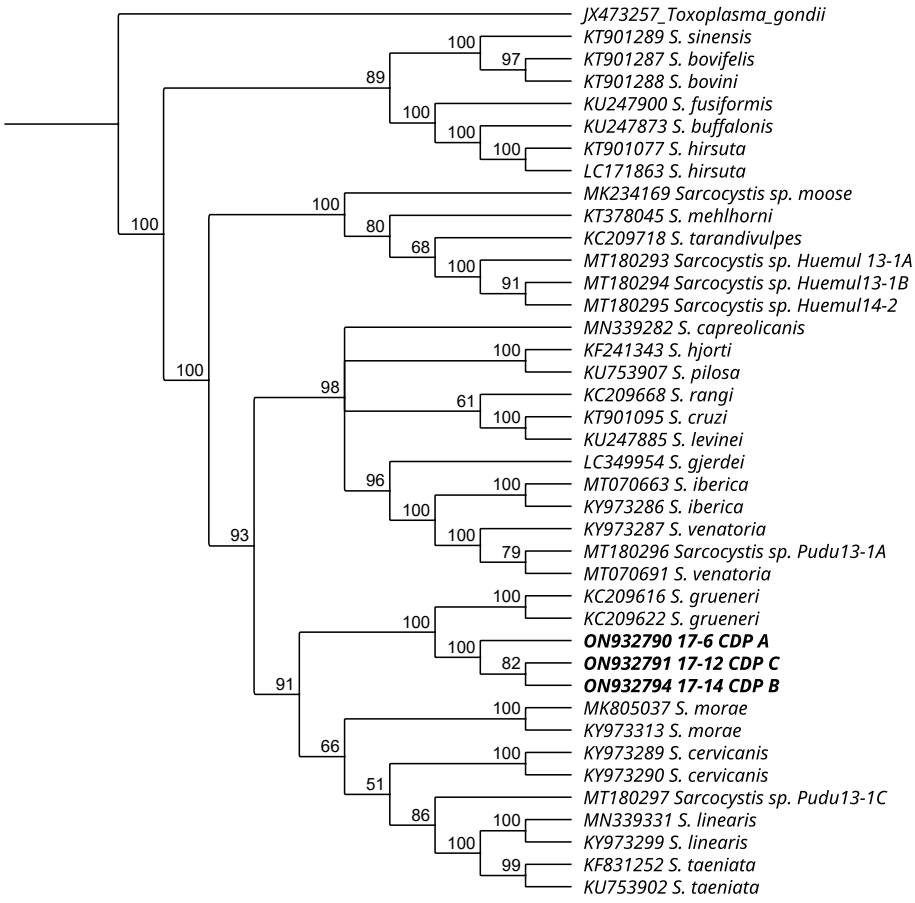

Fig. 4. Neighbor-Joining consensus phylogenetic tree. Phylogenetic tree based on an alignment of 40 *Sarcocystis* spp. *COI* sequences performed with GENEIOUS software (Version R9), using a Tamura-Nei genetic distance model and no outgroup. The three sequences from marsh deer (one from each animal) are in bold. Branch consensus support is expressed as % from 1000 bootstraps. Sequence JX473257 from *T. gondii* used as outgroup.

arise bent ribbon-like protrusions folded over the cyst surface. Smooth ground substance layer measured 0.4 μm thick.

Intermediate hosts: Marsh deer (*Blastocerus dichotomus*).

Distribution: Argentina and probably South America following distribution of intermediate host.

Definitive host: based on the phylogenetic results and predator-prey geographical distribution, highly probable to be wild native canids (i.e. *Chrysocyon brachyurus*, *Lycalopex gymnocercus*, *Cerdocyon thous*) and domestic dogs.

Molecular characteristics: sequences registered in GenBank from *18S rRNA* gene fragment (Accession numbers ON911503-ON911505) and cytochrome oxidase subunit I gene (COI) fragment (Accession numbers ON932790-ON932794).

4. Discussion

The current study presents a morphological and molecular characterization of an undescribed *Sarcocystis* sp. found in muscles from marsh deer from Argentina. A moderate frequency was observed by optical microscopy, observing sarcocysts in muscles from 6/14 animals. Some of the samples were in advanced autolysis. Therefore, the true prevalence of *Sarcocystis* spp. in marsh deer may be even higher than reported here due to cyst disruption prior to or during homogenization (Moré et al., 2011, 2013). *Sarcocystis* sp. infected animals were observed in both sampled regions, being 5/10 in Iberá and 1/4 in Delta. In Brazil, a frequency of *Sarcocystis* spp. infection of 3/31 was observed in musculoskeletal system of marsh deer, also by optical microscopy (Navas-Suárez et al., 2018). This lower proportion observed in Brazil could be due to a lower interspecific contact or deer predation. However, to confirm such a hypothesis, more representative samplings are required.

All the sarcocysts observed were microscopic and thin-walled. At optical microscopy, no evident protrusions were observed, similar to features from other *Sarcocystis* spp. detected in cervids in Argentina (Chang Reissig et al., 2016, 2020). The cyst wall ultrastructure from four cysts obtained from two marsh deer from Iberá Wetlands showing ribbon-like protrusions resembled other species in cervids muscles like *S. cervicanis S. grueneri, S. linearis, S. morae, S. taeniata* and *S. wapiti* (Gjerde, 2014a; Dubey et al., 2016; Gjerde et al., 2017a, b; Cerqueira-Cézar et al., 2018; Delgado de Las Cuevas et al., 2019; Rudaitytė-Lukošienė et al., 2021). A similar image was obtained previously from cysts in pudu muscles from Argentina (Chang Reissig et al., 2020). Altogether, the morphological information suggested that marsh deer are frequently infected with a single species, similar to other *Sarcocystis* spp. affecting cervids worldwide and the *Sarcocystis* sp. identified in pudu muscles in Argentina.

The molecular results reinforce the hypothesis of a single species present in marsh deer from Argentina. However, sequence identities (especially the ones from the *COI* gene) were below 90% with other *Sarcocystis* spp. reported sequences. Other cervid species showed a greater diversity of *Sarcocystis* spp. in muscles (Gjerde, 2014a; Dubey et al., 2016; Gjerde et al., 2017a, b; Rudaitytė-Lukošienė et al., 2020a; Rudaitytė-Lukošienė et al., 2020b; Rudaitytė-Lukošienė et al., 2021), possibly a restricted predator-prey relationship is occurring with the marsh deer in South America, resulting in a reduced species variety. On the other hand, from 12 individual cysts or cyst portions, only eight resulted positive by *18S rRNA* PCR, and from only three samples, a sequence shorter than expected was obtained. Some of the samples were in an advanced autolysis process, which may have resulted in DNA

degradation. Regarding the *ITS1* fragment, a low level of amplification was also observed, and no consensus sequence was achieved. Similar difficulties in amplifying and sequencing the *18S rRNA* and *ITS1* fragments from *S. wapiti* from North American *C. elaphus* have been mentioned by Cerqueira-Cézar et al. (2018). Probably, the priming regions from *S. wapiti* and the species detected in marsh deer are not conserved, as previously assumed from most *Sarcocystis* spp. (Dahlgren et al., 2008; Moré et al., 2013). Additionally, an intraspecific sequence variation (both *18S rRNA* and *ITS1*) could also result in unintelligible sequences when the PCR products are sequenced directly. Despite the morphological similarity, the *18S rRNA* gene sequences obtained from *Sarcocystis* sp. in marsh deer showed a low homology (93.7–96.7%) with sequences from *S. cervicanis* in red deer in Spain (Gjerde et al., 2017b) *S. grueneri* from reindeer in Norway (Dahlgren and Gjerde, 2007), *S. linearis* in roe deer from Spain and Lithuania (Rudaityté- Lukošienė et al., 2020b), *S. morae* in fallow deer in Lithuania (Rudaityté- Lukošienė et al., 2020a); *Sarcocystis* sp. from pudu from Argentina (Chang Reissig et al., 2020), *S. taeniata* in sika deer in Lithuania (Prakas et al., 2016), *S. tarandivulpes* from reindeer from Iceland (Dahlgren et al., 2007) and *S. wapiti* from elk (Cerqueira-Cézar et al., 2018). The differences at this target suggested that the *Sarcocystis* sp. in marsh deer could be a different species. In addition, the sequences from the present study were phylogenetically positioned in the same branch with only a *S. grueneri* sequence. Several other sequences from *S. cervicanis*, *S. cruzi*, *S. hjorti*, S. iberica, *S. linearis, S. morae, S. rangi, Sarcocystis* sp. from huemul and pudu in Argentina*, S. taeniata S. tenella*, *S. venatoria* and *S. wapiti* were distant related, however, according to the positioning it is possible that all these species shared a common ancestor. Since most of these species use canids as definitive hosts, the *Sarcocystis* sp. in marsh deer may use canids too.

Aiming further characterization, different PCR (using two reverse primers) to obtain *COI* gene fragments were performed as previously reported for species using cervids as intermediate hosts (Gjerde, 2013; Gjerde et al., 2017a). Both combinations resulted in proper amplicons for sequencing, and good quality sequences were obtained from all three cysts previously analyzed by *18S rRNA*. These results reinforce the idea of priming failure or intraspecific sequence variation of *18S rRNA* and *ITS1* fragments. All the *COI* sequences obtained in the present study showed a high homology among them and a low homology with others reported in the GenBank, being up to 89.7% with several *S. grueneri* sequences, with the highest score with the sequence KC209624 from *Rangifer tarandus* from Norway (Gjerde, 2013).

As far as we know, there are no reports of *COI* sequences from *S. wapiti* (Cerqueira-Cézar et al., 2018), so proper comparisons are not possible. Despite morphological similarities, the high sequence divergence at *18S rRNA* and *COI* allowed the assumption that *Sarcocystis* sp. from marsh deer is a different species from *S. wapiti* and other *Sarcocystis* spp. producing cysts in cervids muscles. Thus, we propose the name *Sarcocystis blastoceris* n. sp. for the species producing sarcocysts in marsh deer. In the phylogenetic tree, all *S. blastoceris COI* sequences are positioned along with sequences from *S. grueneri* from Norway (Gjerde, 2013) and with a sister clade containing sequences from *S. cervicanis*, *S. linearis, S. morae, Sarcocystis* sp. from pudu in Argentina and *S. taeniata*. Several of these species are known to use canids as definitive hosts and suggest a potential common ancestor for these species (Dubey et al., 2016; Tuska-Szalay et al., 2021). Additionally, these native cervids are part of an alimentary chain being consumed (as prey or carrion) by domestic dogs (*Canis familiaris*) and different native canid species like maned wolf (*Chrysocyon brachyurus*), pampas fox (*Lycalopex gymnocercus*) and crab-eating fox (*Cerdocyon thous*) (Canevari et al., 2007).

In summary, the sarcocyst morphology, the positioning on the phylogenetic trees, and that marsh deer are frequently predated by dogs and foxes, allowed the assumption that *S. blastoceris* could have canids as definitive hosts.

Further studies should be conducted to properly identify naturally infected definitive hosts for *S. blastoceris* and its potential role on marsh deer health status.

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Availability of data and material

The sequences obtained in the frame of this study are reported and available at the GenBank (Accession numbers ON911503-ON911505 and ON932790-ON932794).

Ethics approval

Biosafety and animal processing procedures were performed according to approved protocols (Argentinean Institutional Committee For The Care And Use Of Experimental Animals; Protocol N° 2014–40), issued by Faculty of Veterinary Sciences, University of Buenos Aires. Transit permits of the samples were obtained from the provincial governments.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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