Parasitology Research (2019) 118:1601–1608
https://doi.org/10.1007/s00436-019-06291-0

PROTOZOOLOGY - ORIGINAL PAPER

Sarcocystis species identification in the moose (*Alces alces*) from the Baltic States

Petras Prakas[1] · Viktorija Kirillova[2] · Rafael Calero-Bernal[3] · Muza Kirjušina[2] · Eglė Rudaitytė-Lukošienė[1] · Miguel Ángel Habela[4] · Inese Gavarāne[2] · Dalius Butkauskas[1]

Received: 1 December 2018 / Accepted: 12 March 2019 / Published online: 21 March 2019

Abstract

Various muscle tissue samples from 60 moose (*Alces alces*) in the Baltic region were examined for *Sarcocystis* species. Sarcocysts were detected in 49 out of 60 (81.7%) moose investigated. Six species, *Sarcocystis alces*, *Sarcocystis hjorti*, *Sarcocystis linearis*, *Sarcocystis silva*, *Sarcocystis ovalis*, and *Sarcocystis* sp., were identified using light microscopy (LM), and DNA sequence analysis (*cox1* and 18S rDNA). Sarcocysts of *S. alces*, *S. ovalis*, and *S. hjorti* were studied using transmission electron microscopy (TEM); sarcocyst walls of *S. alces*, *S. ovalis*, and *S. hjorti* were type 25, type 24, and type 7a, respectively. *Sarcocystis linearis* previously found in roe deer and red deer was also shown to use moose as an intermediate host for the first time. The unknown *Sarcocystis* sp. was rare and might employ another main intermediate host. Phylogenetic results demonstrated that *Sarcocystis* sp. was most closely related to *Sarcocystis tarandivulpes*, using canids as definitive hosts.

Keywords *Alces alces* · 18S rDNA · *cox1* · *Sarcocystis* · Ultrastructure · Phylogeny

Introduction

The representatives of the genus *Sarcocystis* are tissue cyst-forming apicomplexan protozoans characterized by an obligatory prey-predator two-host life cycle. Asexual multiplication with tissue cyst formation occurs in the extra-intestinal locations of the intermediate host (mainly the striated muscle and the central nervous system), while sexual multiplication stages (oocysts/sporocysts) develop in the small intestine of a definitive host (Dubey et al. 2016).

Members of the family Cervidae act as intermediate hosts of a remarkably high species diversity of the genus *Sarcocystis*. In Europe, investigations on *Sarcocystis* infecting wild ruminants allowed the identification of six different species in reindeer (*Rangifer tarandus*) and roe deer (*Capreolus capreolus*), seven in sika deer (*Cervus nippon*) and moose (*Alces alces*), and 11 in red deer (*Cervus elaphus*) (Dahlgren et al. 2007; Gjerde 2014a, b; Gjerde et al. 2017a, b; Prakas et al. 2016, 2017; Rudaitytė-Lukošienė et al. 2018). Dissimilarities in *Sarcocystis* species composition were observed in various cervids in different European countries. For instance, recently *Sarcocystis linearis* has been described in roe deer from Italy (Gjerde et al. 2017a). Previously, this *Sarcocystis* species was not found in roe deer during researches carried out in Lithuania, Norway, and Poland (Gjerde 2012; Kolenda et al. 2014; Prakas et al. 2017). Therefore, complex epidemiology of the genus in Cervidae hosts has to be further investigated to fully reveal the variety of the *Sarcocystis* species in a wide geographical area.

Firstly, sarcocysts found in the muscles of moose in Germany, Lithuania, Norway, Canada, and the USA were examined using microscopical methods (Dubey 1980; Mahrt and Colwell 1980; Colwell and Mahrt 1981; Sedlaczek and Zipper 1986; Blakstad 1997; Kutkienė 2002). With the aid of molecular methods, seven *Sarcocystis* species from moose have been characterized in Norway and Canada (Dahlgren and Gjerde 2008; Gjerde 2012, 2014a). Five *Sarcocystis*

Section Editor: Daniel K. Howe

✉ Petras Prakas
prakaspetras@gmail.com

[1] Nature Research Centre, Akademijos 2, LT-08412 Vilnius, Lithuania

[2] Institute of Life Sciences and Technology, Daugavpils University, Parādes Street 1A, Daugavpils LV-5401, Latvia

[3] SALUVET Group, Animal Health Department, Complutense University, Avda. Complutense s/n, 28040 Madrid, Spain

[4] Animal Health Department, University of Extremadura, Avda. Universidad s/n, 10071 Cáceres, Spain

species, *Sarcocystis hjorti*, *Sarcocystis scandinavica*, *Sarcocystis silva*, *Sarcocystis alces*, and *Sarcocystis ovalis* have been identified in Norway (Dahlgren and Gjerde 2008), whereas *Sarcocystis alceslatrans*, *Sarcocystis taeniata*, *S. alces*, and *S. ovalis* were later found in the muscles of two Canadian moose (Gjerde 2014a). Hence, comprehensive ultrastructural and DNA analyses of sarcocysts obtained from moose in various geographical regions need to be carried out to fully disclose the diversity and distribution of *Sarcocystis* species within this host.

Up till now, fragmentary investigations on *Sarcocystis* species diversity in moose were carried out in Lithuania (Kutkienė 2002; Prakas 2011). Under the light microscopy (LM), Kutkienė (2002) found two types of sarcocysts in skeletal muscles. Taking into account a detailed morphological and molecular examination of *Sarcocystis* species from moose in Norway and Canada performed later (Dahlgren and Gjerde 2008; Gjerde 2014a), sarcocysts Type I and Type II distinguished by Kutkienė (2002) resembled *S. alces* and *S. hjorti*, respectively. Subsequently, Prakas (2011) confirmed *S. hjorti* in one animal using 18S rDNA sequence analysis. Whereas, *Sarcocystis* diversity from moose in Latvia has not been examined before. In the present study, the identification of six *Sarcocystis* species, including an unknown parasite, in moose from Latvia and Lithuania and a new host record for *S. linearis* is achieved.

Materials and methods

Muscle sample collection

Various muscle samples, mainly the diaphragm ($n = 56$), also the heart ($n = 17$), the leg ($n = 7$), the neck ($n = 2$), the tongue ($n = 2$), and the esophagus ($n = 1$), of 60 free-ranging moose were collected over the period 2015/2017. Muscle samples were collected by hunters during legal hunts. Overall, 43 moose came from Lithuania and 17 were from Latvia (54–57°N, 21–27°W).The muscle samples obtained were delivered to the laboratories in Lithuania and Latvia and kept frozen (− 20 °C) until a microscopical examination for the presence of sarcocysts was conducted.

Morphological analysis

Sarcocystis infection prevalence and intensity were evaluated in stained muscle samples. For this purpose, 1 g (± 0.1) of muscles was cut into pieces, stained with 0.2% methylene blue solution, lightened with 1.5% acetic acid solution, pressed into a glass compressor, and examined under LM. The infection intensity was estimated by counting sarcocysts per gram of a sample.

Sarcocysts were morphologically differentiated in fresh-squashed preparations. A total of 45 sarcocysts with a small amount of host muscles were successfully isolated from 33 moose, placed in individual microcentrifuge tubes containing 96% ethanol, and stored at − 20 °C until DNA extraction. Most of sarcocysts used for a molecular examination were morphologically characterized according to the size and shape of the cyst and the structure of the cyst wall. In order to evaluate the cyst wall appearance a fragment of sarcocyst was released from muscle fibers with the help of two fine preparation needles. Some sarcocysts were isolated without examining the cyst wall as we failed to remove completely a part of the cyst from muscle fibers without damaging it.

Six excised sarcocysts (two of each species), morphologically identified by LM according to cyst size, shape, and cyst wall protrusions as *S. alces*, *S. ovalis*, and *S. hjorti*, were fixed in 2.5% glutaraldehyde and processed for transmission electron microscopy (TEM). Briefly, fixed sarcocysts were post-fixed in 1% buffered osmium tetroxide, sections cut on a Leica UC6 ultramicrotome, and contrasted with 4% uranyl acetate and 3% lead citrate, as suggested by Trupkiewicz et al. (2016). Semi-thin sections were stained with toluidine-blue and examined under LM for the presence of cysts and selection of the study area. Finally, ultra-thin sections were examined at the Spanish National Centre for Electron Microscopy (Madrid, Spain) using the JEOL JEM 1400 Plus device at 80 kW.

Molecular analysis

Genomic DNA was extracted from individual sarcocysts using the QIAamp® DNA Micro Kit (Qiagen, Hilden, Germany) according to the manufacturer's tissue protocol. Known species were genetically identified with the help of *cox1* sequences, while in this study the unknown *Sarcocystis* sp. (isolate AaLT16.5) was characterized using *cox1* and 18S rDNA sequence analysis. Partial *cox1* sequences were obtained with SF1 forward and SR8D/SR9/SR5 reverse primers (yielding 1072/1085/1103 bp) (Gjerde 2013, 2014b) depending on *Sarcocystis* species, whereas two primer pairs SarAF/SarBR and SarCF/SarDR were used for almost complete 18S rDNA sequence amplification (yielding 1815–1881 bp) (Kutkienė et al. 2010). The Dream-Taq PCR Master Mix (Thermo Fisher Scientific Baltics, Vilnius, Lithuania) was used for PCR reactions. The cycling conditions began with one cycle at 95 °C for 5 min, followed by 35 cycles at 94 °C for 45 s, at 54–60 °C depending on the primer pair for 60 s, at 72 °C for 80 s, and ended at 72 °C for 10 min. The PCR products were visualized using 1.5% agarose gel electrophoresis and purified with exonuclease ExoI and alkaline phosphatase FastAP (Thermo Fisher Scientific, Vilnius, Lithuania). PCR products were sequenced directly with the 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) using the same forward and reverse primers as for the PCR.

Phylogenetic and statistical analyses

The newly obtained sequences were compared with those of various *Sarcocystis* spp. using the Nucleotide BLAST program (megablast option). A multiple alignment was performed using the MUSCLE algorithm (Edgar 2004) loaded into the MEGA7 software (Kumar et al. 2016). The phylogenetic trees were constructed using the TOPALi v2.5 software (Milne et al. 2004). In the present study the sequences of *Sarcocystis* species obtained from moose were deposited in GenBank with accession numbers MK224430 and MK234125–MK234169.

Results

Infection rates and *Sarcocystis* species identified in the moose from the Baltic States

Sarcocysts were detected in 81.7% (49/60) of the moose collected in the Baltic States. Prevalence of sarcocyst infection was not significantly different between the two countries (82.4% in Latvia and 81.4% in Lithuania). Cysts were found in all types of muscles (the diaphragm, the heart, the leg, the neck, the tongue, and the esophagus) examined. Very similar rates of sarcocysts infection prevalence were observed in the diaphragm (75.0%) and the heart (76.5%), with the average infection intensity degree of 14.0 cysts/g in the heart, and 4.5 cysts/g in the diaphragm ($p = 0.11$).

Under LM, four morphological types were observed among the 45 sarcocysts isolated. Three of them were microscopic, with either a smooth cyst wall ($n = 38$), or with hair-like ($n = 3$) or finger-like ($n = 2$) protrusions, whereas the single sarcocyst of the fourth type was macroscopic and oval in shape ($n = 1$). When comparing DNA sequences five known *Sarcocystis* species (*S. alces*, *S. hjorti*, *S. linearis*, *S. silva*, *S. ovalis*) and an unknown *Sarcocystis* sp. were identified. Five *Sarcocystis* species, *S. alces*, *S. hjorti*, *S. silva*, *S. ovalis*, and *Sarcocystis* sp., were confirmed in the moose form Lithuania ($n = 43$), while *S. alces* and *S. linearis* were identified in the moose from Latvia ($n = 17$). The sarcocyst of *Sarcocystis* sp. was found only in the diaphragm muscle of one moose (isolate AaLT16.5) and was subjected to DNA investigations. The sarcocyst was spindle-shaped, measured 1800 × 160 μm, and was filled with banana-shaped 11.5 × 3.9 (10–13 × 3.5–4.3; $n = 10$) μm bradyzoites. Unfortunately, during the excision of the sarcocyst, we did not succeed in releasing the cyst wall from muscle tissues and detecting sarcocysts of *Sarcocystis* sp. repeatedly. For this reason, no detailed morphological description of the parasite under investigation is given.

Transmission electron microscopy examination

Ultrastructural details on *S. alces* (Fig. 1a, b), *S. ovalis* (Fig. 1c, d), and *S. hjorti* (Fig. 1e, f) are summarized in Table 1.

Molecular results and phylogeny

Out of the 38 excised sarcocysts showing a smooth cyst wall under the LM, 36 were identified as *S. alces*, and two remaining ones as *S. linearis*. Cysts of *S. alces* were found in the diaphragm ($n = 25$), the leg ($n = 2$), the heart ($n = 7$), the tongue ($n = 1$), and the esophageal muscle ($n = 1$). Three sarcocysts with hair-like, two cysts with finger-like protrusions, and one macroscopic oval-shaped cyst, all isolated from the diaphragm muscles, were assigned to *S. hjorti*, *S. silva*, and *S. ovalis*, respectively.

Out of 36 *S. alces* sequences analyzed, nine *cox1* haplotypes were ascertained, sharing 99.4–99.9% identity. The *cox1* sequences of *S. alces* obtained in this study shared 98.6–100% identity with other sequences of *S. alces* from Canada (KF831244–KF831247) and Norway (KC209578–KC209587, KF241309–KF241310). The average number of nucleotide differences (K_{XY}) between European and Canadian isolates of *S. alces* was 8.275, whereas between Norwegian and the Baltic States isolates K_{XY} accounted for 1.667.

Three new *cox1* sequences of *S. hjorti* differed slightly (0–0.2%) from one another and shared 99.4–100% identity with other sequences of *S. hjorti* from moose (KC209640–KC209643, KF241353–KF241354) and red deer (KC209635–KC209639, KF241342–KF241352, KY973288). Two sequences of *S. linearis* obtained differed by 1.1% and showed 98.6–99.3% identity with those of *S. linearis* from two other known hosts, roe deer (KY018969–KY018987) and red deer (KY973296–KY973312). The two sequences of *S. silva* differed from each other at 12 of 1029-nt positions (98.8% identity). These sequences shared 98.5–99.7% identity with other sequences of *S. silva* from roe deer (KC209686–KC209687, KF241410, KF898110–KF898113, KX643344–KX643345, KY018988–KY019014) and moose (KC209688–KC209689). In the present study, the established sequence of *S. ovalis* demonstrated 98.8–100% similarity with other sequences of this species from moose (KC209644–KC209647, KC209650, KF241367–KF241382), red deer (KC209651–KC209655, KF241355–KF241366), sika deer (MF596210–MF596211), and sporocysts shed by common magpie (*Pica pica*) (KC209648–KC209649).

In the present work, *cox1* sequence of *Sarcocystis* sp. obtained from moose showed the greatest similarity to *S. tarandivulpes* (92.4–92.9%) from reindeer (KC209712–KC209722) and to *S. mehlhorni* (92.1–92.2%) from the black-tailed deer (*Odocoileus hemionus*) (KT378044–KT378045). By contrast, 18S rDNA sequence of unknown

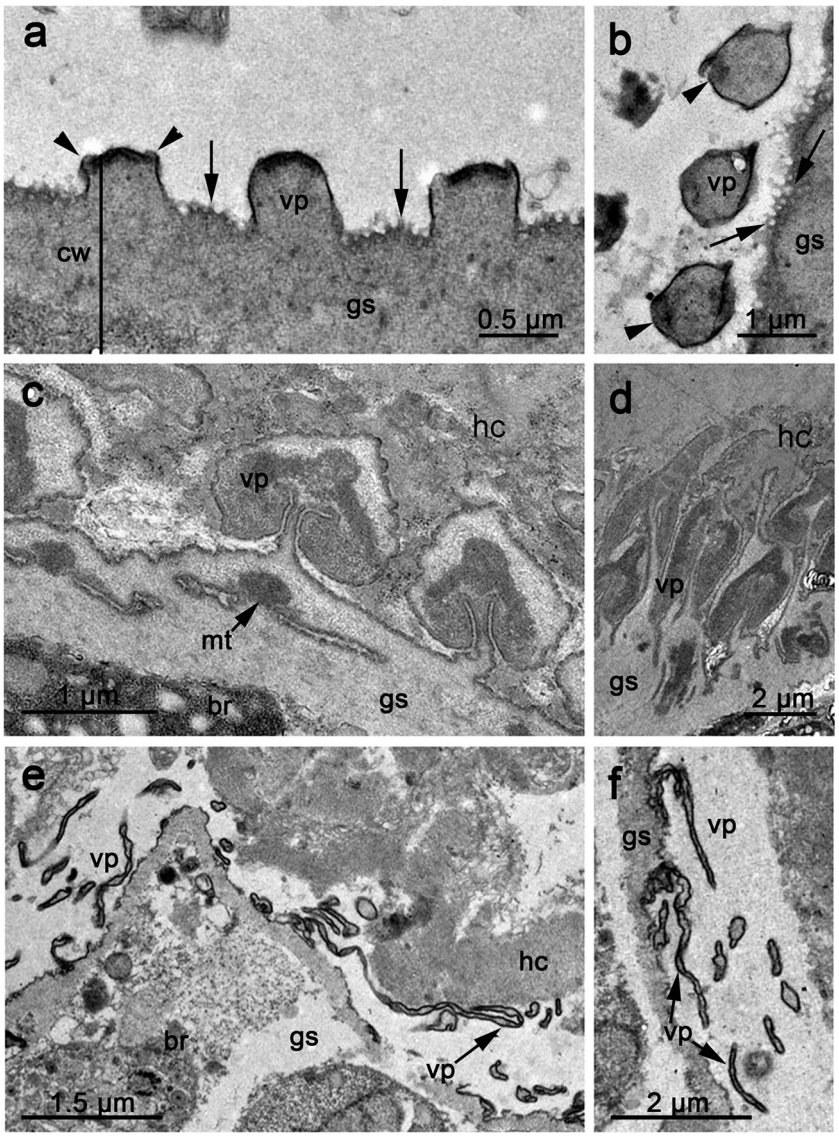

Fig. 1 TEM micrographs of *Sarcocystis* spp. from the moose (*Alces alces*). Note protrusions (vp), cyst wall (cw), ground substance (gs), host cell (hc), microtubules (mt), bradyzoites (br). **a**, **b** *S. alces*, note blebs (arrows) and disk-shaped plaques (arrowheads). **c**, **d** *S. ovalis*. **e**, **f** *S. hjorti*

Sarcocystis sp. differed from the sequences of *S. tarandivulpes* (EF056012, EF467656–EF467657) and *S. mehlhorni* (KT378042) only by 0.2–0.3% and 0.3%, respectively.

The phylogenetic analysis showed close relationship of *Sarcocystis* sp. from moose with numerous *Sarcocystis* species using members of the family Cervidae and Bovidae as intermediate hosts, and canids as definitive hosts. On the basis of *cox1* sequences, *Sarcocystis* sp. from moose was most closely related to *S. tarandivulpes* and *S. mehlhorni* (Fig. 2). The latter three species were placed in a separate clade with a maximum support value.

Discussion

In the present work, sarcocysts were found in 35 moose from Lithuania and 14 moose from Latvia out of 60 animals examined (81.7%) in the Baltic area. High infection prevalence was previously reported not only in moose in Lithuania (44/53, 83.0%) (Malakauskas and Grikienienė 2002) but also in Norway (28/34, 82.3%) and Canada (196/205, 96.0%) (Mahrt and Colwell 1980; Dahlgren and Gjerde 2008). Under the LM, sarcocysts with a smooth cyst wall prevailed in the moose examined in the Baltic States. A molecular analysis of 38 sarcocysts without clearly visible protrusions showed that 36 of them were assigned to *S. alces* and two to *S. linearis*. It was also demonstrated that *S. alces* was predominant *Sarcocystis* species in the diaphragm and the esophagus of the Norwegian moose (Dahlgren and Gjerde 2008). Other *Sarcocystis* species (*S. hjorti*, *S. linearis*, *S. silva*, *S. ovalis*, and *Sarcocystis* sp.) detected in the moose from the Baltic States were relatively rare. Based on transmission experiments and/or phylogenetic data, canids are definitive hosts of *S. alces*, *S. hjorti*, and *S. linearis* (Dahlgren and Gjerde

Table 1 Summarized information regarding TEM observations of sarcocysts of *S. alces*, *S. ovalis*, and *S. hjorti* infecting moose (*Alces alces*) in Baltic area

Structure	*Sarcocystis* species		
	S. alces	*S. ovalis*	*S. hjorti*
Metrocytes	Not seen	Not seen	3.8 × 3.1 μm ($n = 2$)
Bradyzoites	13.0 × 3.5 μm ($n = 6$)	6.8 × 1.5 μm ($n = 6$)	11.0 × 3.6 μm ($n = 5$)
Secondary cyst wall	Absent	Apparently fibrous material	Absent
Primary cyst wall type[a]	25	24	7[a]
Total thickness	Up to 1.2 μm	Up to 15 μm	Less than 1 μm
Ground substance	Up to 0.7 μm wide, with visible electrondense mt	1.2–1.6 μm wide, with visible groups of tightly packed mt scattered underneath each vp	Remarkable thin, about 280 nm
Septae (thickness)	380 nm	380 nm	Not seen
Villar protrusions	Regularly arranged/aligned up to 0.5-μm-long vp, blebs-vesicles present in interspaces, polyedric to dome shaped or platform-like, up to four electron-dense disk-shaped plaques on top.	Up to 12-μm-long (1.4 × 1.7 μm in section), mushroom-like or ace of spades vp with a core of tightly packed mt at their bases; some vp may arise from the surface of other protrusions. In some views they appear tongue-shaped and flattened towards the tip. Tight peduncle that joins vp to the gs. Irregular vesicles between vp. Also may resemble types 26 and 39.	Up to 5 μm (probably up to 10 μm) long, slender hair-like vp, apparent absence of mt. vp arise from apparently narrow peduncles, and due to length they are folded over the surface of the cysts and they seem to be entangled. Also resemble types 6, 7b, and 12.

vp villar protrusions, mt microtubules, gs ground substance

[a] Dubey et al. (2016)

2010b; Gjerde et al. 2017a). Thus, canids, precisely red fox (*Vulpes vulpes*), raccoon dog (*Nyctereutes procyonoides*), and gray wolf (*Canis lupus*) abundant in Lithuania and Latvia (Baltrūnaitė 2010; Kampe-Pērsone 2017), are most likely to be main predators responsible for *Sarcocystis* infection in moose. Whereas in Norway, sarcocysts of *S. ovalis* were found in 16 of 35 moose examined (Dahlgren and Gjerde 2008). The definitive hosts of this *Sarcocystis* species are corvid birds (Gjerde and Dahlgren 2010). Hence, differently from the Baltic States, corvids play a significant role in transmitting *Sarcocystis* from moose in Norway. Here we provided TEM-based morphology of *S. alces*, *S. ovalis*, and *S. hjorti*. The initial morphological description of *S. alces* (Dahlgren and Gjerde 2008) was given in the moose from Norway by SEM. The authors showed closely arranged squared and short platform-like protrusions; images of SEM resembled a chessboard. TEM micrographs of *S. alces* were first provided by Blakstad (1997) in Figs. 4 and 5. It is interesting to note that morphology of *S. alces* (Blakstad 1997, and Fig. 1a, b in present study) is similar to that of *S. gracilis* from the roe deer (Sedlaczek and Wesemeier 1995). Dahlgren and Gjerde (2008) named *S. ovalis* when studying animals from Norway and Canada; under the SEM, the surface of sarcocyst has tongue-like, slanting protrusions clearly aligned in rows and small invaginations. Same morphology was observed in the *S. ovalis* detected in the Norwegian red deer (Dahlgren and Gjerde 2010a). In the present study, sarcocysts had tongue-like protrusions at longitudinal sections (Fig. 1d), whereas they seemed mushroom-like at cross sections (Fig. 1c). Under TEM, sarcocyst type B in the moose from Canada reported by Colwell and Mahrt (1981) corresponded to *S. ovalis*. Gjerde (1985) described sarcocysts of *S. hardangeri* from reindeer (*Rangifer tarandus*) with numerous slanting tongue-like protrusions in TEM micrographs. Hence, ultrastructurally *S. ovalis* and *S. hardangeri* share very similar sarcocyst morphology under the TEM. Slender and very long hair-like protrusions of *S. hjorti* observed under the SEM (Dahlgren and Gjerde 2010a) were clearly identified in the present study under the TEM (Fig. 1e, f). Moose acts as an intermediate host for two *Sarcocystis* species, *S. hjorti*, and *S. alceslatrans* with indistinguishable sarcocyst morphology under the SEM (Gjerde 2014a). Furthermore, both species displayed quite similar hair-like protrusions under the TEM (Colwell and Mahrt 1981).

The molecular analysis of sarcocysts isolated from the moose harvested in the Baltic States revealed six *Sarcocystis* species. Four of them, *S. alces*, *S. hjorti*, *S. silva*, and *S. ovalis*, were previously identified in moose. Meanwhile, recently *S. linearis* characterized in roe deer and red deer (Gjerde et al. 2017a, b) was confirmed in moose for the first time. On the basis of *cox1* sequence comparison, it can be stated that *Sarcocystis* sp. found in the present study differed from

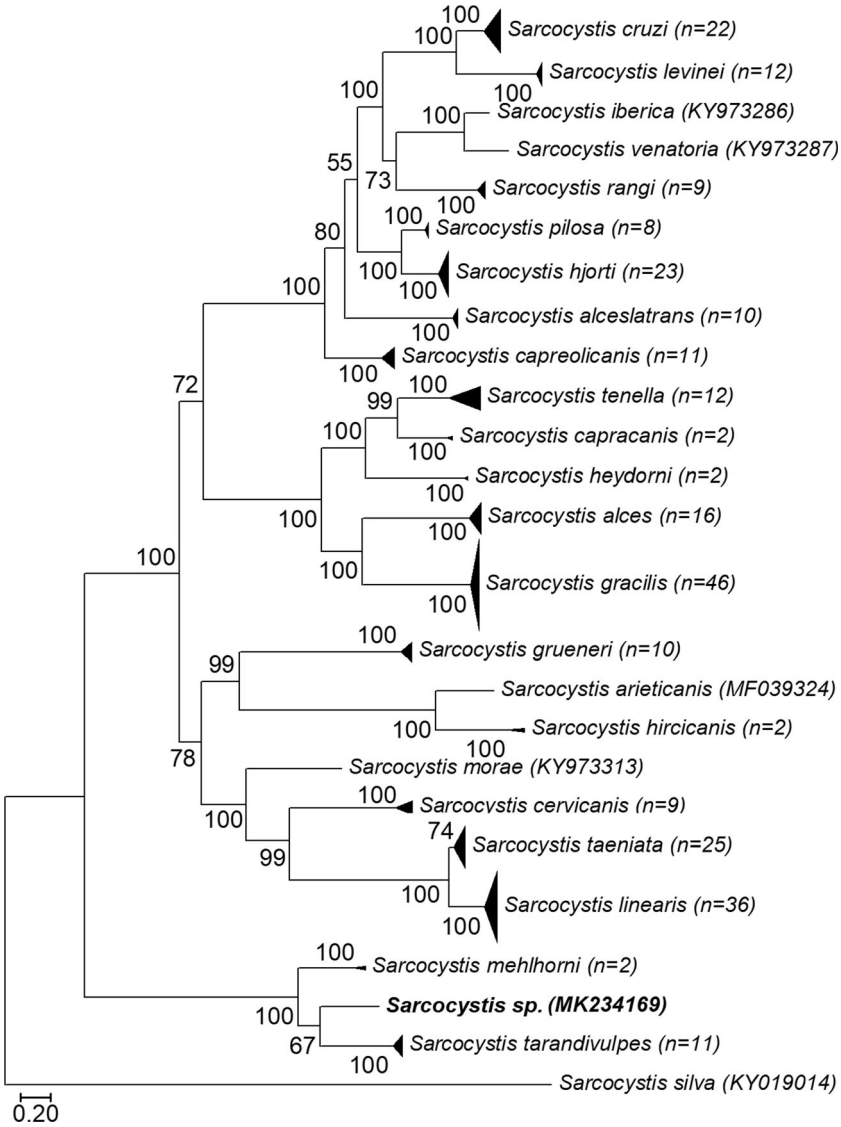

Fig. 2 Phylogenetic tree for selected *Sarcocystis* species based on *cox1* sequences. The tree was constructed using Bayesian methods scaled according to the branch length and rooted on *S. silva*. The final alignment contained 272 sequences and 1002 aligned nucleotide positions. The K80 + I + G evolutionary model was set for the phylogenetic analysis. GenBank accession numbers or the number of sequences for corresponding taxon are given behind the *Sarcocystis* species name. The sequence of unnamed *Sarcocystis* sp. obtained in this study is in boldface

other *Sarcocystis* species characterized at this locus. A phylogenetic analysis carried out (Fig. 2) suggests that members of the family Canidae are likely to be definitive hosts of *Sarcocystis* sp. Previously, 12 *S. alces* isolates from Norway (KC209578–KC209587, KF241309–KF241310) and four *S. alces* isolates from Canada KF831244–KF831247) were characterized at *cox1* (Dahlgren and Gjerde 2008; Gjerde 2014a). In the present study, additionally 20 *cox1* sequences of *S. alces* from Lithuania (MK234125–MK234134, MK234141–MK234144, MK234150–MK234153, MK234159–MK234160) and 16 *cox1* sequences of *S. alces* from Latvia (MK234135–MK234140, MK234145–MK234149, MK234154–MK234158) were obtained. We determined significantly greater genetic distances between European and Canadian isolates than between Norwegian and the Baltic States ones. Consequently, at *cox1* genetic differences of *S. alces* were observed according to geographical distances. However, more sampling areas should be covered and more samples from North America examined to draw robust conclusions on population genetics pattern of *S. alces*.

Three *Sarcocystis* species, *S. alceslatrans*, *S. scandinavica*, and *S. taeniata*, previously described in moose, have not been detected in this study. According to the phylogenetic placement, *S. alceslatrans* and *S. taeniata* use canids as definitive hosts (Gjerde 2014a), and experimental studies revealed that thin-walled sarcocysts (probably *S. alceslatrans*/*S. taeniata*) from the moose in Alberta (Canada) and Montana (USA) were spread by dogs (*Canis familiaris*) and coyote (*Canis latrans*) (Dubey 1980; Colwell and Mahrt 1983). It is possible that *S. alceslatrans* uses such specific definitive hosts as coyotes, absent in the area under investigation. Sarcocysts of *S. taeniata* have been found in Lithuania, however, in another intermediate host, the sika deer (Prakas et al. 2016; Rudaitytė-Lukošienė et al. 2018). The introduced sika deer is mainly raised on farms, and therefore, widespread environment contamination with sporocysts of *S. taeniata* is unlikely.

According to Dahlgren and Gjerde (2008), *S. scandinavica* was found in the esophagus of one out of 34 Norwegian moose investigated. Thus, the distribution of this species could also be limited. Further surveys will be necessary to ascertain whether a wide variety of *Sarcocystis* species detected in moose is due to the distribution of different definitive hosts or sampling bias caused by a small number of individuals examined.

Acknowledgments This research was supported by the Open Access to research infrastructure of the Nature Research Centre under Lithuanian open access network initiative. The authors are grateful to Marisa García for the excellent technical assistance during TEM operations. Our thanks also go to the reviewers for their valuable comments and suggestions, which contributed to the improvement of the manuscript.

Funding This study was funded from the project of the Research Council of Lithuania (grant no. S-MIP-17-45). R. Calero-Bernal is a receipt of a Complutense University postdoctoral grant (CT65/16).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Baltrūnaitė L (2010) Winter habitat use, niche breadth and overlap between the red fox, pine marten and raccoon dog in different landscapes of Lithuania. Folia Zool 59:278–284. https://doi.org/10.25225/fozo.v59.i4.a2.2010

Blakstad M (1997) Untersuchungen zum Vorkommen und zur Morphologie von Sarkosporidien beim Elch im Raum Ost-Agder, Südnorwegen. (Thesis), Tierärztliche Hochschule, Hannover.

Colwell DD, Mahrt JL (1981) Ultrastructure of the cyst wall merozoites of *Sarcocystis* from moose (*Alces alces*) in Alberta, Canada. Z Parasitenkd 65:317–329. https://doi.org/10.1007/bf00926727

Colwell DD, Mahrt JL (1983) Development of *Sarcocystis alceslatrans* Dubey, 1980, in the small intestine of dogs. Am J Vet Res 44:1813–1818

Dahlgren SS, Gjerde B (2008) *Sarcocystis* in moose (*Alces alces*): molecular identification and phylogeny of six *Sarcocystis* species in moose, and a morphological description of three new species. Parasitol Res 103:93–110. https://doi.org/10.1007/s00436-008-0936-1

Dahlgren SS, Gjerde B (2010a) Molecular characterization of five *Sarcocystis* species in red deer (*Cervus elaphus*), including *Sarcocystis hjorti* n. sp., reveals that these species are not intermediate host specific. Parasitology 137:815–840. https://doi.org/10.1017/S0031182009991569

Dahlgren SS, Gjerde B (2010b) The red fox (*Vulpes vulpes*) and the arctic fox (*Vulpes lagopus*) are definitive hosts of *Sarcocystis alces* and *Sarcocystis hjorti* from moose (*Alces alces*). Parasitology 137:1547–1557. https://doi.org/10.1017/S0031182010000399

Dahlgren SS, Gjerde B, Skirnisson K, Gudmundsdottir B (2007) Morphological and molecular identification of three species of *Sarcocystis* in reindeer (*Rangifer tarandus tarandus*) in Iceland. Vet Parasitol 149:191–198. https://doi.org/10.1016/j.vetpar.2007.08.015

Dubey JP (1980) *Sarcocystis* species in moose (*Alces alces*), bison (*Bison bison*) and pronghorn (*Antilocapra americana*) in Montana. Am J Vet Res 41:2063–2065

Dubey JP, Calero-Bernal R, Rosenthal BM, Speer CA, Fayer R (2016) Sarcocystosis of animals and humans, 2nd edn. CRC Press, Boca Raton

Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Acids Res Nucleic 32:1792–1797

Gjerde B (1985) Ultrastructure of the cysts of *Sarcocystis hardangeri* from skeletal muscle of reindeer (*Rangifer tarandus tarandus*). Can J Zool 63:2676–2683. https://doi.org/10.1139/z85-400

Gjerde B (2012) Morphological and molecular characterization and phylogenetic placement of *Sarcocystis capreolicanis* and *Sarcocystis silva* n. sp. from roe deer (*Capreolus capreolus*) in Norway. Parasitol Res 110:1225–1237. https://doi.org/10.1007/s00436-011-2619-6

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. https://doi.org/10.1016/j.ijpara.2013.02.004

Gjerde B (2014a) Morphological and molecular characteristics of four *Sarcocystis* spp. in Canadian moose (*Alces alces*), including *Sarcocystis taeniata* n. sp. Parasitol Res 113:1591–1604. https://doi.org/10.1007/s00436-014-3806-z

Gjerde B (2014b) *Sarcocystis* species in red deer revisited: with a redescription of two known species as *Sarcocystis elongata* n. sp. and *Sarcocystis truncata* n. sp. based on mitochondrial *cox1* sequences. Parasitology 141:441–452. https://doi.org/10.1017/S0031182013001819

Gjerde B, Dahlgren SS (2010) Corvid birds (Corvidae) act as definitive hosts for *Sarcocystis ovalis* in moose (*Alces alces*). Parasitol Res 107:1445–1453. https://doi.org/10.1007/s00436-010-2017-5

Gjerde B, Giacomelli S, Bianchi A, Bertoletti I, Mondani H, Gibelli LR (2017a) Morphological and molecular characterization of four *Sarcocystis* spp., including *Sarcocystis linearis* n. sp., from roe deer (*Capreolus capreolus*) in Italy. Parasitol Res 116:1317–1338. https://doi.org/10.1007/s00436-017-5410-5

Gjerde B, Luzón M, Alunda JM, de la Fuente C (2017b) Morphological and molecular characteristics of six *Sarcocystis* spp. from red deer (*Cervus elaphus*) in Spain, including *Sarcocystis cervicanis* and three new species. Parasitol Res 116:2795–2811. https://doi.org/10.1007/s00436-017-5590-z

Kampe-Pērsone G (2017) Latvijas zīdītāji. Pilnīgs sugu apraksts. Zvaigzne ABC, Riga

Kolenda R, Ugorski M, Bednarski M (2014) Molecular characterization of *Sarcocystis* species from Polish roe deer based on ssu rRNA and *cox1* sequence analysis. Parasitol Res 113:3029–3039. https://doi.org/10.1007/s00436-014-3966-x

Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Mol Biol Evol 33:1870–1874. https://doi.org/10.1093/molbev/msw054

Kutkienė L (2002) On the investigations of *Sarcocystis* (Protista: Coccidia) fauna in moose (*Alces alces*) in Lithuania. Acta Zool Litu 12:82–85

Kutkienė L, Prakas P, Sruoga A, Butkauskas D (2010) The mallard duck (*Anas platyrhynchos*) as intermediate host for *Sarcocystis wobeseri* sp. nov. from the barnacle goose (*Branta leucopsis*). Parasitol Res 107:879–888. https://doi.org/10.1007/s00436-010-1945-4

Mahrt JL, Colwell DD (1980) *Sarcocystis* in wild ungulates in Alberta. J Wildl Dis 16:571–576. https://doi.org/10.7589/0090-3558-16.4.571

Malakauskas M, Grikienienė J (2002) *Sarcocystis* infection in wild ungulates in Lithuania. Acta Zool Litu 12:372–380. https://doi.org/10.1080/13921657.2002.10512527

Milne I, Wright F, Rowe G, Marshall D, Husmeier D, McGuire G (2004) TOPALi: software for automatic identification of recombinant

sequences within DNA multiple alignments. Bioinformatics 20: 1806–1807

Prakas P (2011) Diversity and ecology of *Sarcocystis* in Lithuanian game fauna. PhD thesis, Vilnius University, Vilnius, Lithuania.

Prakas P, Butkauskas D, Rudaitytė E, Kutkienė L, Sruoga A, Pūraitė I (2016) Morphological and molecular characterization of *Sarcocystis taeniata* and *Sarcocystis pilosa* n. sp from the sika deer (*Cervus nippon*) in Lithuania. Parasitol Res 115:3021–3032. https://doi.org/10.1007/s00436-016-5057-7

Prakas P, Rudaitytė E, Butkauskas D, Kutkienė L (2017) *Sarcocystis entzerothi* n. sp. from the European roe deer (*Capreolus capreolus*). Parasitol Res 116:271–279. https://doi.org/10.1007/s00436-016-5288-7

Rudaitytė-Lukošienė E, Prakas P, Butkauskas D, Kutkienė L, Vepštaitė-Monstavičė I, Servienė E (2018) Morphological and molecular identification of *Sarcocystis* spp. from the sika deer (*Cervus nippon*), including two new species *Sarcocystis frondea* and *Sarcocystis nipponi*. Parasitol Res 117:1305–1315. https://doi.org/10.1007/s00436-018-5816-8

Sedlaczek J, Wesemeier H-H (1995) On the diagnosis and nomenclature of *Sarcocystis* species (Sporozoa) in roe deer (*Capreolus capreolus*). Appl Parasitol 36:73–82

Sedlaczek J, Zipper J (1986) *Sarcocystis alceslatrans* (Apicomplexa) bei einem paläarktischen Elch (Ruminantia). Angew Parasitol 27:137–144

Trupkiewicz JG, Calero-Bernal R, Verma SK, Mowery J, Davison S, Habecker P, Georoff TA, Ialeggio DM, Dubey JP (2016) Acute, fatal *Sarcocystis calchasi*-associated hepatitis in roller pigeons (*Columba livia* f. *dom.*) at Philadelphia zoo. Vet Parasitol 216:52–58. https://doi.org/10.1016/j.vetpar.2015.11.008